THE UNIVERSITY
of EDINBURGH

The Challenges of Tuberculosis Prevention through Early Detection of Latent Tuberculosis Infection in New Immigrants to the State of Kuwait

Adel Mohanna AL-Harbie

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for the degree of

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Declaration

I declare that the research described within this

thesis is my own work

and that this thesis is my own composition

and certify that it has never been submitted for any

other degree or professional qualification

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Adel Mohanna AL-Harbie

Edinburgh, 2012

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Acknowledgement

Abstract

Introduction: Despite management advances worldwide, tuberculosis still remains a serious uncontrolled disease. The absence of either a 'gold' standard diagnostic test, or a conventional rapid 'reference' laboratory test for asymptomatic *Mycobacterium tuberculosis* (MTB) carriers complicates disease control. Through mandatory screening of high-risk groups, early diagnosis of latent tuberculosis infection (LTBI) cases allows recognition and better control of the tuberculosis pandemic.

Materials and Methods: The current tuberculosis screening guidelines as recommended by the World Health Organization, chest X-ray and tuberculin skin test were assessed and revealed rises in TB morbidity and fatality trends in the Kuwait population (low incidence country). In order to evaluate options for LTBI diagnosis, the current work implemented a 4-month prospective, observational, repeated-measure and randomly implemented survey on 180 new immigrants to Kuwait using a structured risk factor questionnaire whilst, simultaneously evaluating the performance of the two standard diagnostics (chest X-ray and tuberculin skin test) with the new biomarker interferon gamma release assays (T-SPOT *.TB* test and QuantiFERON Gold In-Tube test (QNF-GIT)); which detect the release of interferon gamma (INF-γ) released from sensitization to specific MTB antigens.

Results: Associations between various epidemiological risk factors - such as socio-demographic status, smoking and environmental exposure-contact - were associated in the laboratory diagnosed LTBI participants. Positive identification of LTBI prevalence detected by two radiologists was 10.1% having 'moderate' inter-reader agreement (Kappa = 0.505), compared to no positives being detected by three pulmonologists. TST results were negative (less than 10-mm 'cut-off') even in the 86.1% Bacillus Calmette-Guérin vaccinated expatriates. Estimated LTBI using QNF-GIT was 28.3% compared to 41.1% positive T-SPOT *.TB* test. Both interferon gamma assays revealed concordant 'abnormal' results in 26.1% with 'good' agreement (kappa = 0.627).

Conclusion: Detection of latent tuberculosis infection can be facilitated by introducing evidence-based diagnostic classification depending on history taking of epidemiological-related risk factors and chest X-ray plus either interferon gamma assays.

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Abstract of this: **Abbreviations used**

Active TB	Active tuberculosis disease
AFB	Acid fast bacilli
BCG	Bacillus Calmette-Guérin
CD4+	T-lymphocyte CD4 (T-helper cell)
CDC	Centers for Disease Control and Prevention
CFP-10	Culture filtrate protein 10
CXR	Chest X-ray
Date of access	DOA
DM	Diabetes mellitus
DOTS	Directly observed treatment, short
DTH-IV	Delayed hypersensitivity reaction-type 4
EPTB	Extrapulmonary tuberculosis
ESAT-6	Early secretory antigenic target 6
FDA	Food and Drug Administration
HPA	Health Protection Agency
HIV	Human immunodeficiency virus
HCW	Health care workers
IGRAs	Interferon gamma release assays
INF-γ	Interferon gamma
QNF-GIT	QuantiFERON Gold In-Tube test

Abbreviations used

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LTBI	Latent tuberculosis infection
MDR-TB	Multidrug resistant *Mycobacterium tuberculosis*
MTB	*Mycobacterium tuberculosis*
NICE	National Institute for Health and Clinical Excellence
NTM	Non-*Mycobacterium tuberculosis*
PTB	Pulmonary tuberculosis
RD-1	'Region of Difference'-1
TB	Tuberculosis
TST	Tuberculin skin test
WHO	World Health Organization

History of Tuberculosis in Edinburgh

A history of the Craigleith Hill District in Scotland (Edinburgh)

Evidence of another historical item is found in 01/08/2011

100 year old TB book found

A BOOK written more than 100 years ago about tuberculosis has been found at a city hospital.

The document was prepared for a conference in Washington DC about the illness, devising ways on how to stop the spread of the disease.

It was found in the Royal Victoria hospital in Craigleith, and is now being restored by the Lothian Health Services Archive.

Staff described it as "part report, part scrapbook", and sheds light on how Edinburgh medics were consulted about challenges across the world.

Authors of the booklet cited the "filthy habit of spitting" as a chief cause of the spread of the illness, and the messages were translated in the book into several different languages.

The Royal Victoria was established in 1894 as a TB hospital by the famous medic Sir Robert Philip. A spokeswoman for the Archives said: "It is likely that he or another doctor from the Royal Victoria attended the congress and brought this item back to the hospital."

EDINBURGH EVENING NEWS 1ST AUGUST 2011

(http://www.craigleithhill.co.uk/craigleith_house.html)

The Edinburgh Scheme - Rules for consumptive patients and those looking after them [Royal Victoria Hospital Annual Report (1891-1989), courtesy of Special Collections, Centre for Research Collections, Ref: SD3851-3856]

Rules for Consumptive Patients and Those Looking after Them.

(As issued to Out-Patients at the Victoria Hospital.)

Consumption is a communicable disease. It may pass from person to person. It may pass from one lung to the other, or from one organ to another.

The chief source of infection is the expectoration of the consumptive. The great danger lies in the drying of the expectoration, and the blowing about of the dried infectious material.

The spread of consumption can be largely prevented. *If the succeeding directions be obeyed, there need be no serious danger in ordinary intercourse with patients. The breath of the consumptive is not directly infectious.*

The patient should expectorate into a jar or cup containing a tablespoonful of carbolic acid (1 to 20) or other disinfectant.

The vessel should be changed once in twelve hours, or oftener. It should be cleansed by being filled up with *boiling* water. The combined contents should be poured down the w.c. The vessel should then be washed with *boiling* water.

When the patient is out of doors, he should carry a pocket spitting flask (such as Dettweiler's, or the Victoria Hospital simpler model). The flask should be used and cleansed like the jar. The patient should never spit on the streets.

The patient should not use handkerchiefs for expectoration. If this ever has to be done, the handkerchief should be of an inexpensive material, that it may be burned after use. Squares of rag or paper, which may be used for convenience, should be similarly treated.

(http://www.lhsa.lib.ed.ac.uk/exhibits/tales/tb_x.htm)

The expectoration should on no account be swallowed, for thereby the disease may pass to other organs.

Consumptive patients should avoid kissing.

Consumptive mothers should not suckle.

If expectoration has been accidentally deposited on the floor or other object, it should be wiped up and burned, and the surface of the object cleansed with strong antiseptic.

Rooms which have been long occupied by a consumptive patient should, before occupation by someone else, be carefully disinfected, as after other infectious disease.

FRESH AIR is the food of the lungs. Therefore, see that the lungs be not starved.

A.—By Day.—The patient should occupy as airy a room as possible. It must be scrupulously dry, and preferably removed from the ground. The window should be *freely* open. When able, the patient should be out of doors once or several times during the day. He must avoid over-effort, and damp, or chill, which would counteract the benefit.

B.—By Night.—He should sleep alone. The bedroom should be large and airy. The window should be kept open, less or more according to the season.

Copies of these, on card, can be had for distribution, price 2s. 6d. per 100, on application to the Physician, Victoria Hospital for Consumption, Craigleith, Edinburgh.

(http://www.lhsa.lib.ed.ac.uk/exhibits/tales/tb_x.htm)

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1 Chapter one

Tuberculosis

1.1 Introduction

1.1.1 Disease definition

Tuberculosis (TB) is a contagious bacterial infectious disease that is considered to be one of the deadliest infectious diseases worldwide. Infection with tuberculosis is fatal and TB had a major impact on global health, grasping international attention with an increase in number of cases worldwide, critically including both developed and developing countries. It is estimated that one third of the world's population are infected with the causal agent of TB, *Mycobacterium tuberculosis* (MTB), an aerobic pathogenic bacillus which establishes infection in the lungs. Two billion people are believed to be carrying non-eradicated intra-granulomatous TB bacilli as latent tuberculosis infection (LTBI) and one in 10 of them will become sick with active TB during their lifetime.

The *Mycobacterium tuberculosis* complex consists of seven organisms: *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, *M. canetti*, *M. caprae* and *M. pinipedi*. The majority of TB in humans is caused by *M. tuberculosis* with a small proportion of TB disease caused by *M. bovis* and *M. africanum* (LoBue *et al.*, 2010). TB remains one of the biggest global human killers accounting for 9.4 million new cases of active TB (3.6 million of which are in women) and 3 million TB-related deaths with an incidence of 140 cases per 100,000 inhabitants. TB today is a disease of poverty, with more than 90% (up to 8 million) of total TB cases occurring in developing countries and more than half of all deaths (2 million) occurring in Asia. People living with human mmune deficiency virus (HIV) (approximately 1.4 million) are at a greater risk of contracting TB and TB is the leading cause of death in HIV-positive people. HIV individuals infected with TB are 20 to 40 times more likely to develop active TB (WHO, 2010c).

The observed decline in the global TB pandemic during the last century is considered to be largely due to improved living standards and the use of antibiotics in the 20th Century. But while TB has gradually disappeared from the health agenda of countries in the developed world, TB has increased in the developing south and has become the most important cause of death among the infectious diseases (estimated death rate

of 50/100,000 during 2010, with more than 95% of deaths in developing countries). In 1993, TB was declared by the World Health Organization (WHO) a global public health emergency and WHO established TB in the General Program of Work for 2002-2005 as a major communicable, notifiable (but now curable) disease that posses a serious threat to health and economic development (WHO, 2010c; WHO, 2010g).

Among the world population of 5,288 billion people in 1990, the global case detection rate for TB was estimated at 55% with the incidence of new cases at 6, 800 million (incidence rate 128/100,000 population in 2010). In 2009 the global case detection rate was 63% with the incidence of new cases at 9,300 million (incidence rate 137/100,000 population) translating to about 4,700 deaths a day among 6,825 billion people (WHO, 2010d). The detected incidence in China was 4.5 million active TB cases with 80% were living in rural areas (WHO, 2008a). India (the 1st highest TB nation) and China (2nd highest TB nation) together account for 35% of the annual new cases globally and 43% of global TB prevalence (WHO, 2009a).

Most of the global burden of new cases come from South East Asia, with up to 70% being detected in India (1,824,400 new cases) and Indonesia (540,000 new cases). The African continent contributes up to 18% of the worldwide new cases, mostly concentrated in Nigeria (373,682) and Ethiopia (267,147), followed by Latin America and the Caribbean (Brazil 110,000 new cases and Mexico 34,000 new cases). Asia–Pacific region accounts for one-fifth of worldwide TB incidence (Figure 1.1).
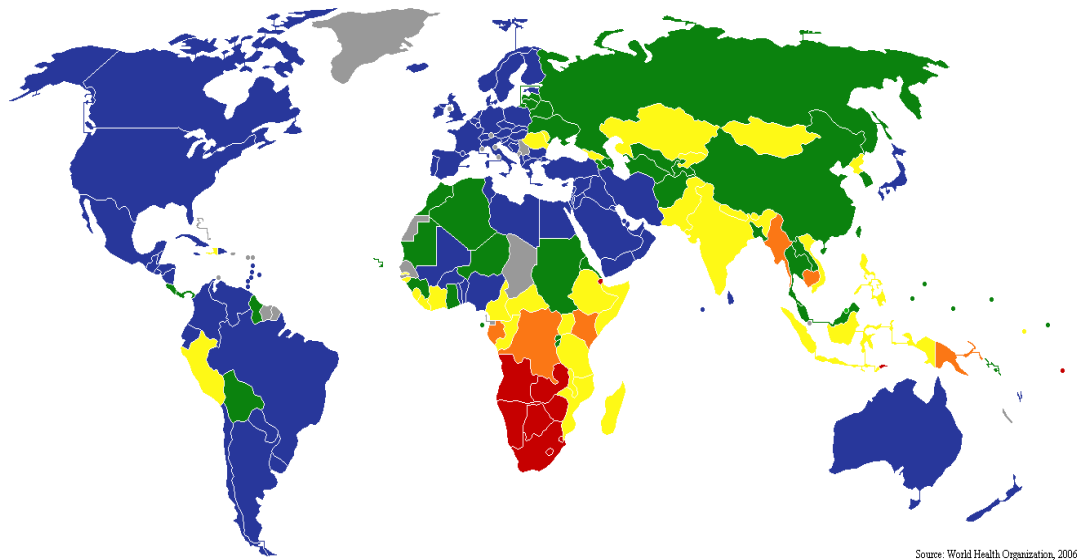
Figure 1.1: World map showing reported cases of tuberculosis per 100,000 citizens in 2006. Red = >300, orange = 200-300; yellow = 100-200; green 50-100; blue = <50 and grey = N/A) (USAID, 2008) (Date of access (DOA): 27/06/2008)

1.1.2 The history of TB and present day importance

Tuberculosis is considered one of scientific, medical, social, and political failures since TB remains one of the leading causes of mortality and morbidity (WHO, 2010d). Tuberculosis disease has been known as 'Consumption disease' (because TB consumes people from the major disease symptoms of bloody cough, fever, pallor and long relentless wasting or cachexia) after 'phthisis' (Greek for consumption) or 'Wasting disease' since without treatment, patients would waste away or Pott's disease (gibbus of the spine and joints) or 'Koch's Disease' (after Robert Koch who discovered the tuberculosis bacillus) (Gupta *et al.*, 2009).

Early evidence of TB has been found in pre-historic skeletal human remains (7000 BC) and skeletal bone spots and tubercular decay have been observed in Egyptian mummies dating from 3000-2400 BC (Zink *et al.*, 2003). Severe TB disease was mentioned around 2000 BC in both India and the Americas, and there are references to the human 'White Plaque and White Death' around 460 BC where Hippocrates believed that phthisis as fatal hereditary widespread disease.

Until the mid-1800s, tuberculosis was still considered a hereditary disorder and symptomatic people who were considered contagious and difficult to treat, were usually sent to sanatoriums to breathe fresh air and required routine follow-ups to assess their general health and nutrient progress. On March 24, 1882 a German scientist named Robert Koch discovered the tuberculosis bacterium bacillus that causes TB (Figure 1.2). Until 1940s and 1950s, there was no antibiotic treatment for TB but, after anti-tuberculosis (anti-TB) drugs were discovered, many infected people were treated and death rates dropped dramatically.

Figure 1.2: Robert Koch (1843–1910), Nobel Laureate 1905 discovered *Mycobacterium tuberculosis* in 1882 (http://www.erj.ersjournals.com/cgi/reprint/29/3/423.pdf)

The discovery that TB was a bacterial infection led to a plethora of research activity, the development of the Bacille Calmette-Geurin (BCG) vaccine and development of a therapeutic regimen (Migliori *et al.*, 2007) following the emergence of 'germ

theory'. A major breakthrough came in 1944, when Selman Waksman and Albert Schatz, discovered streptomycin an antibiotic that killed the TB bacteria and over the following three decades two further treatments were licensed for treatment of TB isoniazid (INH) in 1952 and rifampicin (R) in the early 1970's.

During the 20th century, globally tuberculosis has killed approximately 100 million people. However following the discovery that TB was a bacterial disease, public health measures were implemented to combat its spread and during the 2nd half of 20th century in developed countries the total number of TB cases was observed to have declined due to raised socio-economic levels and greater public health awareness.

However, in the mid-1980's, the number of global TB cases started rising. The increase in TB cases in developed nations was associated with the immigration of workers from highly endemic TB countries (foreign-born immigrants) and in globally with the emergence of TB/HIV syndemic co-infection arising from the HIV/AIDS pandemic, TB drug resistance, and the use of immune-suppressive agents (WHO, 2009a).

1.1.3 Epidemiology of tuberculosis

The magnitude of the global TB burden is expressed as the number of incident cases per 100,000 population. Asia (South-East Asia and Western Pacific regions) account for 55% of global cases, the African subcontinent for 31%, and the Americas, European and Eastern Mediterranean regions accounting for the remainder. The WHO 2008 report shows the South-East Asia region carrying one third (36%), five million, of the total annual incidence (USAID, 2008).

Tuberculosis incidence from surveillance and survey data collated by WHO estimates 9.27 million new cases of TB in 2007 (139 per 100,000 population), compared with 9.24 million new cases (140 per 100,000 population) in 2006. Of these 9.27 million new cases, an estimated 44% or 4.1 million (61 per 100,000 population) were diagnosed having smear- and culture-positive samples (WHO, 2009a).

The latest estimates of the global burden of TB disease in 2009 were 9.4 million incident cases, 14 million prevalent cases, 1.3 million deaths among normal HIV-negative people and 0.38 million deaths among HIV-positive people. Most cases were in the South-East Asia (35%), African (30%) and the Western Pacific (20%) regions. An estimated 11–13% incident cases were HIV-positive with cases from the African Region accounting for around 80% of cases (WHO, 2010d).

An individual's geographical history is important when considering diagnoses particularly in the context of rising immigration and foreign travel. In 2004, a high percentage of Asians (95%) and Hispanics (75%) born outside the US were reported to have TB, nine times higher than among people born in the United States. In 2006, the Centers for Disease Control and Prevention (CDC) reported 13,799 cases of active TB and estimated 10-15 million people to have latent TB in the US (NIAID, 2006).

In 2006, WHO reported the prevalence of active TB infection at 14 million with a prevalence of 219/100,000 persons (WHO, 2009a; Yew *et al.*, 2007). Tuberculosis in 22 countries accounted for more than 95% of new cases and mortality rates with disease burdens in South and East Asia (India, China, Indonesia, Bangladesh, Philippines, Pakistan, Thailand, Russian Federation) followed by Africa (Nigeria, Ethiopia, Uganda, South Africa) and the Western Pacific regions (Brazil, Mexico, Peru).

The incidence of TB has been estimated by the WHO to be 139/100,000 per healthy people based on the number of new cases of TB reported in 2006 (WHO, 2009a). South Africa has one of the highest worldwide incidences with more than 200 new cases/100,000 country population and 35 deaths daily (Mathema *et al.*, 2006). Almost 9 million new cases were estimated worldwide in 2004, but this figure is believed to be subject to gross underreporting due to missing cases and increasing difficulties in TB control (Dye, 2006; WHO, 2009a). Tuberculosis incidence rates can vary even between neighboring countries due to different community risk factors and differences in health care systems. Even countries with endemic TB face difficulties' in case registration and WHO notification gaps e.g. miss-diagnosed cases (Dye, 2009; Frieden *et al.*, 2003) (Figure 1.3).
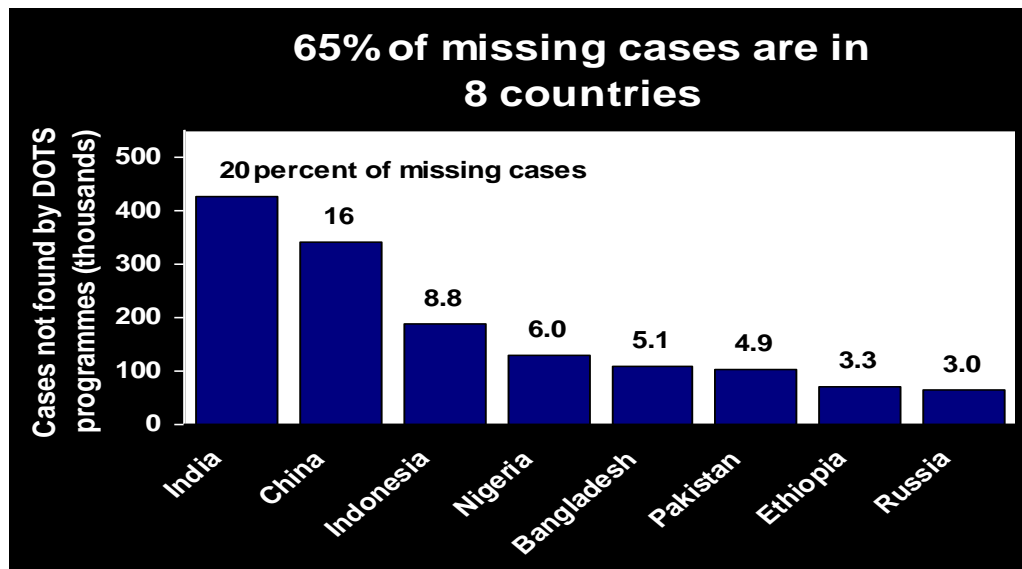
Figure 1.3: Missed cases in common eight tuberculosis-endemic countries (http://app.who.int/tb/surveillanceworkshop) (DOA: 27/06/2008)

In India TB remains one of the leading infectious causes of mortality, in 2006 estimated to have caused more than 325,000 deaths. There were nearly 2 million new TB cases in 2006 (representing more than 21 % of all TB cases worldwide) with an estimated incidence rate of 168 new cases per 100,000 in population.

China has the second largest number of TB patients in the world, 80% of which are living in rural areas with restricted access to health services (Long *et al.*, 2008). China is also one of the countries with high levels of drug resistant TB and accounts for approximately 24% of the global burden of multi-drug resistant *Mycobacterium tuberculosis* (MDR-TB) (He *et al.*, 2010). A recent national anti-tuberculosis drug resistance survey estimated approximately 120,000 new multi-drug resistant TB cases emerging annually in China, including 9,000 extensively drug-resistant *Mycobacterium tuberculosis* (XDR-TB) cases.

In comparison in the Americas there are only 41 reported cases per 100,000 in population compared to 356 per 100,000 populations in Africa.

Case detection rates fell globally in 2006 but began rising in China and India (due to WHO support) compared to continued low detection rates in the African Region (Dye, 2009). Funding for TB control reached U.S.$3.3 billion across 90 countries in 2008, with only 5 out of the 22 high-burden countries reporting no data and funding

gaps (despite, U.S.$385 million being provided for registration and notification system purpose in 2008) (WHO, 2009a).

TB incidence and prevalence has dropped in nations with advanced socio-economic status compared to persistence in areas with disparities between socio-political-economic growth and community instability (demographic shifts). Increasing globalization brings sustained risks of introduction of active TB and potential re-activation of LTBI in nations who have achieved domestic TB control (Figure 1.4).
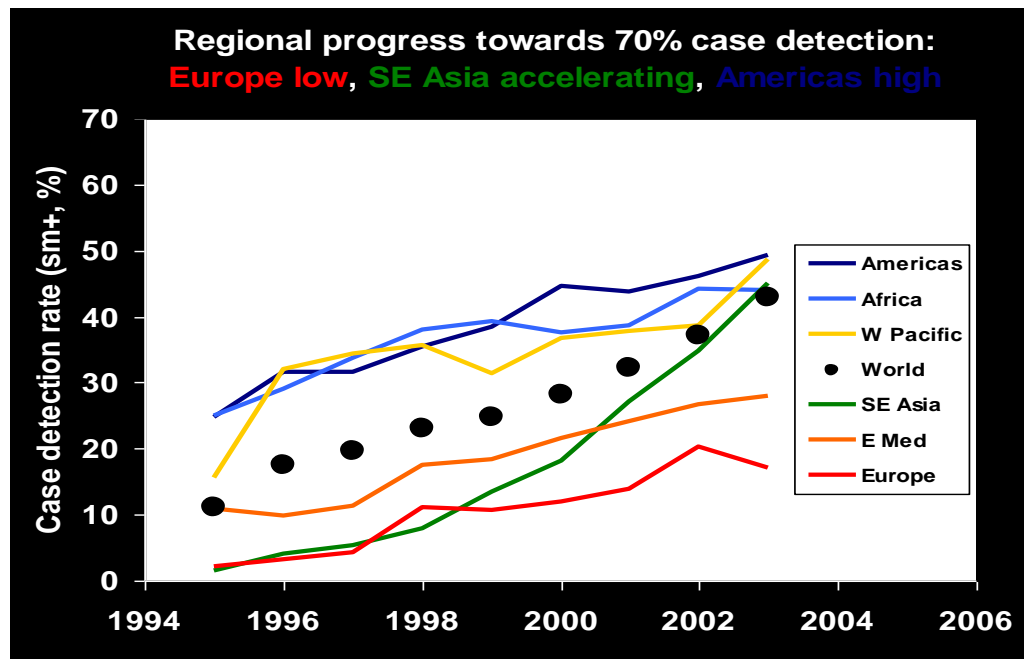
Figure 1.4: Worldwide progress toward TB case detection (Dye, 2009) (DOA: 26/01/2012)

Nigeria has the highest number (449,558 in 2006) of detected TB cases in sub-Saharan Africa (largely due to its enormous population) and has been placed among the top 5 of WHO's 22 high-TB burden countries (Iliyasu *et al.*, 2009). In Europe, USA and Canada, rates of TB detected by screening range from 0.80 - 4.9% and for latent tuberculosis infection (LTBI) from 7.6 - 47%.

Tuberculosis epidemiology and the efficacy of control activities are known complicated by the emergence of drug-resistant bacilli and by the synergism of TB with HIV co infection and these factors present major difficulties for TB control

especially in the developing world. In Geneva all cases of TB identified are in individuals born outside Switzerland (Langenskiold *et al.*, 2008).

1.1.4 Global burden and TB impact

Since 1993 WHO has considered TB as an uncontrolled, fatal contagious, epidemic and global health emergency. Tuberculosis is a highly infectious but curable disease with 5-10% latency in infected immune-competent individuals (LTBI or reservoirs of dormant *M. tuberculosis* bacilli that re-activate during a persons life time). Even though prevalence has decreased in the developed nations e.g. West Europe, North America, Japan, still, it remains a major disease burden in tropical and developing countries (Dye *et al.*, 1999). Tuberculosis is responsible for 25% of adult deaths in the developing world, more than caused by malaria, diarrhea or autoimmune deficiency syndrome (AIDS). The estimated peak of the global incidence rate was 143 cases in 2004 but this has fallen slowly to 139 cases per 100,000 populations in 2007 in the five WHO regions (WHO, 2009a).

1.1.5 Infectious agent

The bacteria from the *Mycobacterium tuberculosis* complex that are found primarily in humans and are known to cause TB include *M. tuberculosis*, *M. bovis*, *M. africanum, M. microti and M. canetti*. The Mycobacterium species belong to the order actinomycetales, family mycobacteriaceae and genus Mycobacterium. There are more than 70 species of mycobacterium of which two are considered major human pathogens; *Mycobacterium tuberculosis* (Tuberculosis or Koch's disease, Koch, 1882) and *Mycobacterium leprae* (leprosy or Hansen's disease, Hansen, 1874).

The remaining Mycobacterium's are considered environmental and are known collectively as Mycobacterium Other Than Tuberculosis (MOTT) organisms. MOTTs are responsible for various opportunistic infections that can occur without person-to-person transmission and are generally resistant to drugs. *Mycobacterium avium-intracellulare* (MAI) or *-complex* (MAC) for example, is diagnosed by blood

and/or organ tissue culturing and is frequently seen in immune-compromised individuals and requires life-long antibiotic therapy (Abe *et al.*, 1999).

Mycobacteria are aerobic, slender, non-motile, non-encapsulated, non-spore forming and rod-shaped bacilli (2-4 micrometers (μ) in length and 0.2-0.5 μ in width). These intra-cellular pathogens infect alveolar macrophages (i.e. are macrophage inhabitants) and are found within membrane-bound particles or 'phagosomes'. Mycobacterias are resilient bacilli capable of adapting to different environmental conditions, they are resistant to phagocytosis and remain dormant for decades within phagocytic or non-phagocytic tissue cells with the ability to re-activate. Their lipid rich (lipoid) cell wall makes it impermeable and resistant to bacterial standard stains e.g. Gram stain. Acid-fast stains depend on the ability of mycobacteria to retain dye when treated with mineral acid or an acid-alcohol solutions such as the Ziehl-Neelsen (ZN stain) for acid fast bacilli (AFB) that is used for *M. tuberculosis* (Glickman *et al.*, 2001; Hernandez-Pando *et al.*, 2000) (see Figure 1.5).
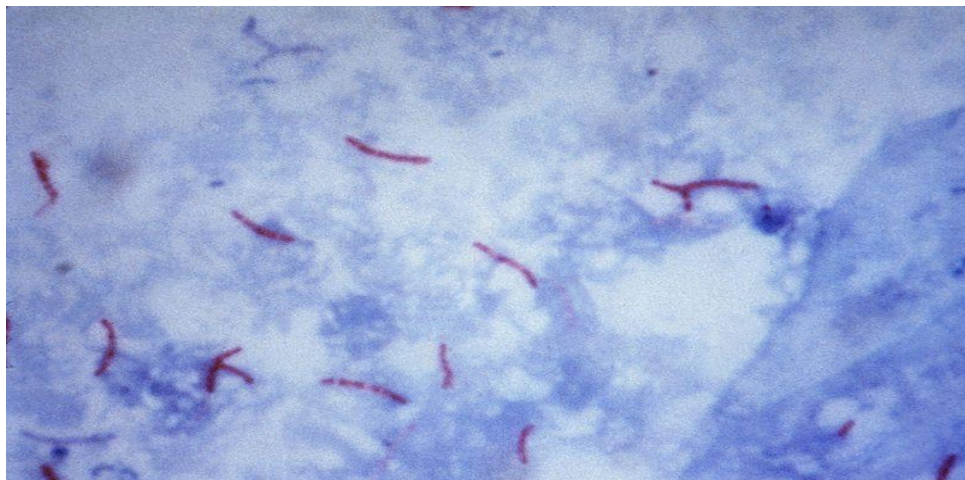
Figure 1.5: *Mycobacterium tuberculosis* **(red bacilli) visualization using the Ziehl-Neelsen stain for acid fast micro-organisms in a sputum sample** (Magnified with light microscope x1000) (Image of a work of the Centers for Disease Control and Prevention, http://en.wikipedia.org/wiki/File:Mycobacterium_tuberculosis_Ziehl-Neelsen_stain_02.jpg) (DOA: 26/01/2012)

The thickness of the bacterial cell wall induces mycobacterium intra-granulomatous necrosis, interrupting specific immunity forcing the bacilli to remain in a stationary phase inside necrotic tissues. MTB is a slow-growing micro-organism with a slow

generation time of 15 and 20 hours. MTB forms obligate aerobic complexes in well-aerated body tissues (Figure 1.6).
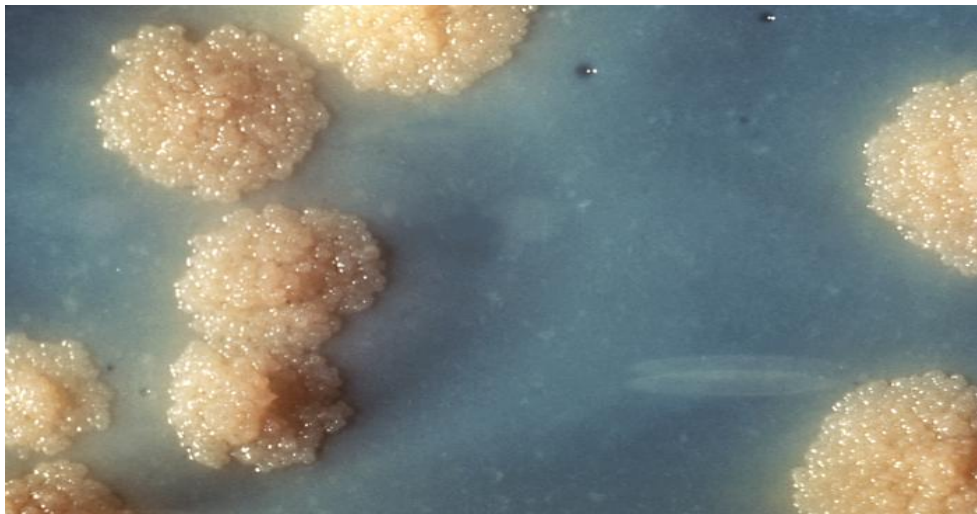
Figure 1.6: *Mycobacterium tuberculosis* **culture showing colourless rough colonial morphology (**Adopted from CDC's Public Health Image Library (PHIL) ID number 4428, http://phil.cdc.gov/phil/details.asp?pid=4428) (DOA: 26/01/2012)

Externally expelled MTB can survive in soil for 18-24 hours, from sputum for 20-30 hours and in droplet nuclei for 8-10 days. Bacilli are sensitive to heat and are killed at high temperatures and in ultra-violet light e.g. direct sunlight (Hussein, 2007).

A single released airborne droplet nucleus from an infected individual may contain between 1-3 MTB organisms. Organisms can remain suspended in the air for several hours, sufficient to infect alveolar macrophages and establish respiratory pulmonary tuberculosis (PTB) infection and TB disease in humans (Tang *et al.*, 2006). The main determinants for TB transmission include the number of inhaled bacilli, duration of exposure to the contaminated infection source and organism virulence.

Infectiousness is quantitatively estimated as the amount/number of bacilli. This route of transmission is more critical in the developing country environments due to poverty-related direct close contact and longer periods of exposure to an infectious TB case (Lienhardt *et al.*, 2002b).

An active TB case or an infectious un-diagnosed and un-treated case for example an individual with a positive sputum smear sample (SSS+) can infect ten and twenty

persons over the course of a year, and contagiousness is increased in adults compared with a child's' weak cough (Escombe *et al.*, 2007; Maddineni *et al.*, 2008).

1.1.6 Modes of transmission

Tuberculosis is mainly transmitted from human to human by inhalation of the contagious, infectious droplets containing TB organisms that are expelled through coughing by an infected person into the air via the respiratory tract pathways, except in the case of *M. bovis* which is contacted from cattle, commonly by ingestion of contaminated raw milk. An infected person with active TB disease e.g. pulmonary or laryngeal TB can transmit bacilli through coughing, sneezing, spitting, shouting, laughing, talking and expectorating the infectious airborne droplets. Other modes of mycobacterial transmission such as occupational inhalation of droplets during laboratory-personnel mucus manipulations or might be from oral infection of bovine tuberculosis. There is no evidence for TB transmission from shaking hands or from drinking fluids or sharing towels (Mathema *et al.*, 2006).

The inhaled bacilli lodge in the lung terminal air spaces and replicate within host macrophages. Bacillary clearance from affected tissues or sputum is a sign of an effective host response (resistance) and cure in response to antibiotic treatment, but any recent transmissions may complicate resolution after MTB infection.

The period of tubercle communicability is unlimited for as long as infectious bacilli are being discharged. 'Time delay' (the period from onset of symptoms to reporting the case to a health care facility) is a significant factor that serves to aggravate the severity of TB epidemics and undermines TB control programs (Gele *et al.*, 2009). In Tanzania, for example, endemic for TB, the mean time delay was 266 days from the first visit to a traditional healer compared with 94 days for individuals who consulted a health facility (Wandwalo *et al.*, 2000).

Bovine tuberculosis presents a significant zoonotic risk to humans through uncommon inhalation of infectious aerosols and/or by ingestion of raw milk (un-pasteurized or non-boiled) and dairy products from infected cattle reservoirs (LoBue *et al.*, 2010). The probability of transmission can be determined by several factors:

1)- infectiousness of the source patients, 2)- susceptibility of contacts, duration of exposure and 3)- the exposure environment (CDC, 2005b).

1.1.7 Tuberculosis pathology & pathogenesis

Understanding the pathogenesis of TB may provide a means for its control. The stages in progression of TB termed are defined in terms of the 'three E's'; 1)- Elimination (immune detection and destruction of phagocytic cells harbouring tubercle bacilli), 2)- Equilibration (latent infection with MTB), and 3)- Escape (development of primary or post-primary tuberculosis with/without lymph node or distant sites involvement) (Grange *et al.*, 2011). Following entry of contagious tubercle organisms into inside the body of the host, the bacilli have three potential fates; i) they may be killed by the immune system, ii) they may multiply and cause primary TB or iii) they can become dormant and remain asymptomatic as a latent tuberculosis infection (LTBI) (Figure 1.7).
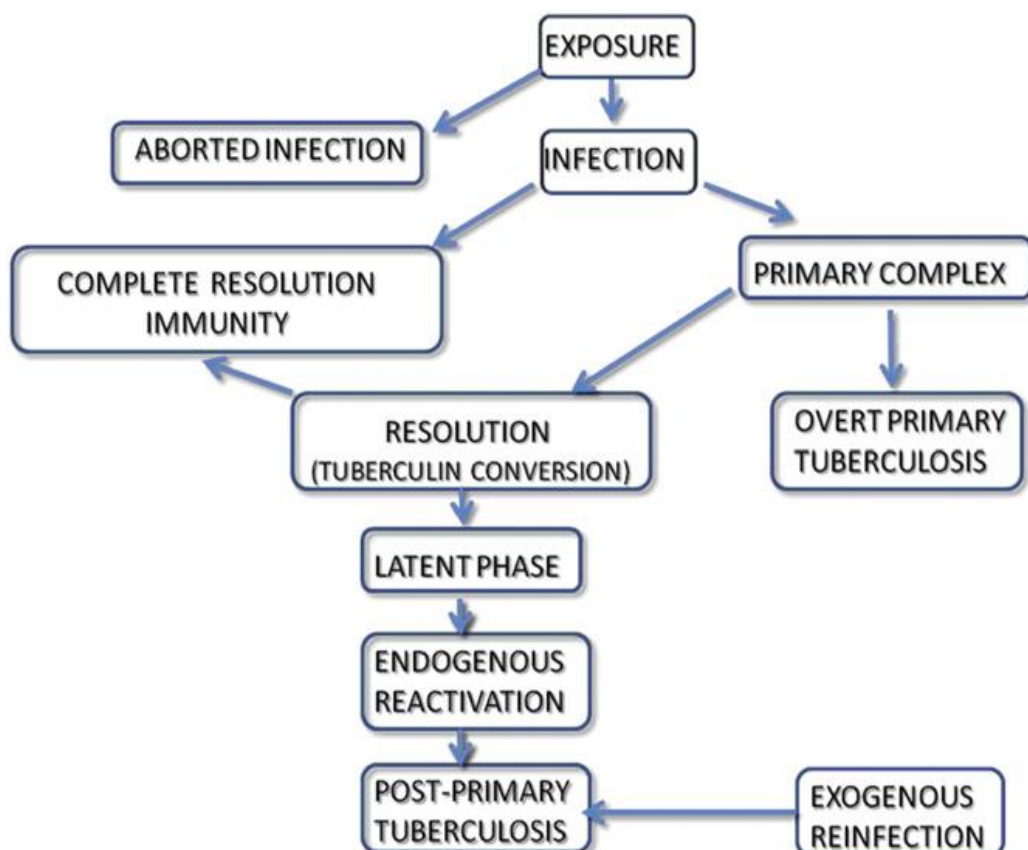
Figure 1.7: Flow chart of the 'timetable' of tuberculosis (Grange *et al.*, 2011)

Dormant MTB can proliferate after a latency period in relation to endogenous re-activation or exogenous re-infection phenomenon. The immunological response of MTB to maintain LTBI is a complex phenomenon, the host is in constant battle with the microbe at the granuloma level to control infection and prevent activation (Tufariello *et al.*, 2003). Re-activation of TB disease may occur following either (B) or (C) as described by Richeldi (2006) and supported by Grange *et al.* (2011) (Figure 1.7).

Tuberculosis disease has three main disease phases:

A- Minor infection with no symptoms. The patient is in good health with mild or no symptoms (in most cases). The bacterium, MTB, has invaded the body, changes its chemical signature, and lives in a dormant state. A small scar on the lung may be observed on a chest X-ray (CXR). This is considered as latent tuberculosis infection (LTBI). It is estimated that one-third of the world population have LTBI representing a large reservoir for TB disease. In the absence of active TB disease, infected people cannot spread the infection to other people.

B- Infection may progress to active, symptomatic TB. This occurs in less than 5% of cases. Tuberculosis bacilli can multiply and spread within the body, the high bacillary concentration may be enough to generate spontaneous mutations and induce drug resistance. Common examples are those at risk of being affected are immuno-compromized people or malnourished people and those living in poverty or poor health environments. The immune response is directed at the growing bacilli (Cardona, 2007). Depending on risk factors, an 'infectious index case' occurs when an active TB case becomes acute, infectious and expels high numbers of TB bacilli (Uys *et al.*, 2007).

C- Re-activated (secondary) infection due to dormant bacilli can cause active TB symptomatic disease months or years after the initial infection. The ''walled off'' bacteria within the formed granulomatous scar, multiply, and re-circulate within the host. Post-dormancy re-activations occur commonly in the lungs (main route of entry) and less within other organs (Glickman *et al.*, 2001). Re-activation is probably associated with a failing immune system commonly found in a number of high risk

groups e.g. HIV/AIDS, extreme ages, malnourished or taking immune-suppressive drugs (Blanco *et al.*, 2002). Tuberculosis virulence is directly affected by the rate of re-activation from latency among hosts (Basu *et al.*, 2009).

The pathology of TB may be observed from the defences of the host immune system reacting against TB bacilli. Bacillary clearance from affected tissues or sputum is a sign of response to antibiotic treatment (and cure). The human immune system cells, such as lung alveolar or epithelioid macrophages, Langhans giant cells, with lymphocytes or plasma cells, can resist TB infection. This inflammatory immune response is a delayed hypersensitivity reaction-type 4 (DTH-IV) and is used as the basis of the tuberculin skin test (TST) (see section 1.4; Laboratory diagnosis of tuberculosis; 1.4.1 TST).

Defender cells are able to: 1. Surround TB bacilli forming a granulomatous scar lesion around it, 2. Isolate it from the rest of body tissues and 3. Halt the bacterial multiplication and localize the infection.

Virulence factors for MTB include: 1. A thick hydrophobic cell wall (filled with mycolic acids); 2. Ability to induce intra-granulomatous necrosis and 3. Slow metabolism (x 100 times slower than Escherichia coli).

In 90% of immune-competent individuals, the host cellular immune response is elicited through cytokines and chemokines that contain or limit infection to the primary invasion site by forming granulomatous lesions named ''Ghon complex or Ghon focus'' (Figure 1.8).
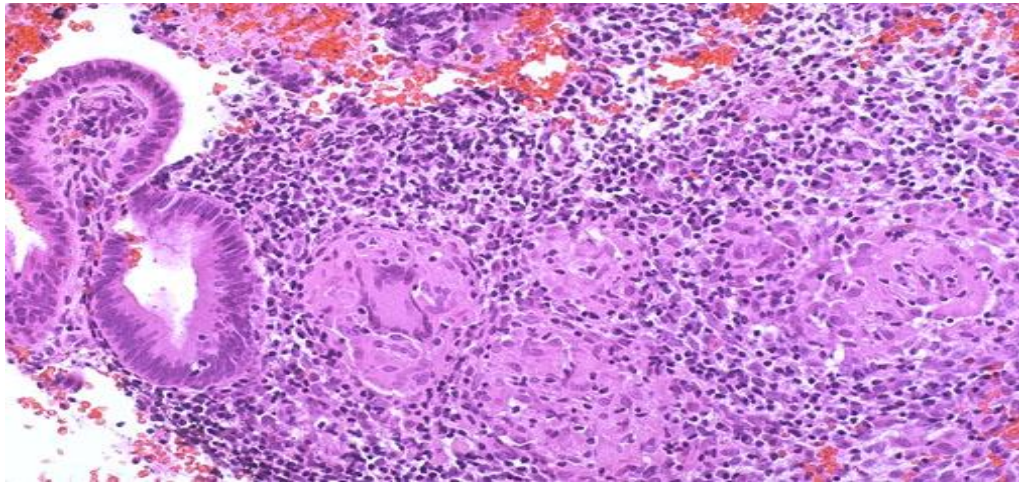
Figure 1.8: Histopathology section showing multiple *Mycobacterium tuberculosis* bacillary 'Ghon complex' granulomas (pale pink) containing multinucleated Langhans giant cells (http://library.med.utah.edu/WebPath/TUTORIAL/MTB/MTB011.html) (DOA: 26/01/2012)

The presence of epithelioid cells denoting granulomatous chronic lesions with or without caseation (caseous necrotic tissue) is the best diagnostic criteria for a MTB infection. In 10% of cases un-contained infection and bacillary replication result in TB lesions including tissue necrosis and cavitations. Infected tissue maintains stable latent bacilli (LB) populations, and prolongs production of foamy macrophages (FM) which facilitate LB escape to re-grows within near alveolar spaces inducing new granulomas once freed up from old granulomas. Cavitations' are well recognized to correlate with high colony forming unit (CFU) levels and estimated bacillary loads in the cavitary wall from 10^7 to 10^{10} in contrast with only 10^2 to 10^4 in caseous necrosis lesion areas. TB pathogenesis is associated with bacillary loads which increases both infectiousness and disease severity (Palaci *et al.*, 2007).

After four weeks post-infection, neutrophils accumulate and then surrounded by lymphocytes forming an external ring followed by external accumulation of FM to clean the alveolar spaces. These macrophages ingest mycobacterial bacilli and are filled internally with respiratory cells, surfactant, bacilli and lipoid vacuoles, they then leave the alveolar spaces via the main respiratory airway tracts and are removed by the stomach swallowing reflex or by expectoration (coughing). Re-infection may occur during the chronic phase once the FM leave the granulomatous tissue and

move toward the alveolar spaces, continuously producing low levels of interferon gamma (INF-γ) and targeting only TB growing bacilli.

During active immunity, macrophages that are already infected and the newly arrived macrophages become foamy and filled with acid fast bacilli which are easier to detect than in the chronic phase (Cardona, 2007). Vitamin D deficiency plays a role in the dysfunction of macrophages and is a risk factor for tuberculosis (Gupta *et al.*, 2009).

1.1.8 Incubation period

The incubation period (IP) is defined as the time period from bacillary entry and infection until appearance of TB disease symptoms and signs. Incubation periods are variable and range from few weeks to several years or even for life if the infection is not treated but most commonly between four and twelve weeks from primary lesion(s) exposure. It is commonly accepted that the IP is within the first five years of TB infection and the risk of infection declines as this time interval increases (Lienhardt *et al.*, 2002b) i.e. acquired infection at early age (e.g. in children) is associated with longer incubation periods than adulthood acquired infection (Lillebaek *et al.*, 2002). During this incubation period, bacillary organisms can reach and infect other body systems and organs such as the lymph nodes, kidney and meninges and cause extra-pulmonary tuberculosis (EPTB).

Epidemiological control programs aim to detect infected TB cases before the 'threshold time' (maximum time period from starting infectiousness until TB diagnosis and treatment) and reduce progression to active TB disease (NICE, 2006).

The elimination phase is achieved when the incidence of all forms of active tuberculosis fall below 1 per 100,000 population per year in the high incidence population group (Rieder *et al.*, 1994).

Recent infection is defined as a significant skin test reaction after documentation of a negative tuberculin skin test within the preceding two years. This is used synonymously with "tuberculin skin test conversion" (Rieder *et al.*, 1994).

1.1.9 Tuberculosis immunology

Pathogenesis strategies for tuberculosis bacilli include:

1. Resistance (or modification) of the host immune response; 2. Arrest of phagosome maturation and inhibition of phago-lysosome fusion (considered a hostile compartment for TB bacilli); 3. Persistence (inactive dormant form but retaining re-activation potential) after which the bacilli can 4. Replicate within host macrophages.

Dormant live bacilli divide slowly, liberating secreted proteins to maintain their cytoplasm proteins, evading the immune system recognition and controlling the host environment. *Mycobacterium tuberculosis* is potent inducer of apoptosis in host macrophages through pore formation in the phagosomal membranes to allow the exchange of molecular cytoplasm nutrients to MTB and promote antigen translocation to major histocompatability complex (MHC) class II pathway. A strong and specific protective immunity against these cytoplasmic proteins (liberated from dying or dead bacilli) can prevent the transition from dormancy to active disease (Wiker, 2001).

Bacterial containment of the protective immune response is focused on the granulomatous-resisting different participating T cells as follows;

1. CD4 T cells (CD4+) or T-helper type (Th1) recognizes antigen peptides encoded by the major histocompatibility complex (MHC) class II
2. CD8 T cells (CD8+) or cytotoxic T cells recognize cytoplasmic antigens (e.g. tumor cell or viral cell) or peptides bound to MHC class I cells
3. CD1 restricted T cells recognize MTB cell wall-abundant glycolipids.

Both CD8+ and CD1 secrete perforin and granulysin that directly kill the mycobacterium within the infected macrophages (Kaufmann, 2002).

CD4+ T-cells also play a principal immune response against invading mycobacterium by producing lymphotoxin alpha (LT-α). Inflammatory cytokines like interferon gamma (INF-γ), tumor necrosis factor-alpha (TNF-α), interleukin-2 (IL-2) and LT-α help in macrophage activation which reduce MTB replication and,

then together with dendritic cells and T-cell populations will cause the formation of organized productive TB-containing granuloma's. Missing such cellular balance lead to non-containing caseous lesions with/without MTB re-activations and TB disseminations.

Granuloma in response to chronic infection requires CD4+ to regulate the protective function, restrict pathogen dissemination from the inflammation site and protect the surrounding healthy tissues from immune-pathology associated with chronically activated macrophages. With CD4+ T cells quantitative depletions, MTB re-activate at the injury site and with the presence of INF-γ from CD8+ T cells e.g. T cells less than 200 cells/ml is risk factor for TB/HIV flare-up (Hogan *et al.*, 2007). The median CD4+ count is significantly higher in patients with *M. tuberculosis* than those with mycobacteria other than tuberculosis (MOTT) (Tamhane *et al.*, 2009).

Proportions of INF-γ, IL-2 and TNF-α secreting CD4+ T cells that are re-stimulated with MTB usually higher in TB infected children as compared with LTBI or other study groups and this increase is indicative of active TB infection. T cell-derived cytokines like INF-γ are indicators of efficient immune memory and protection. The effecter mechanisms that kill MTB are taken up by macrophages activated from T cell lymphocytes. Bacilli inhibit phagosome maturation, lysosome fusion and INF-γ mediated activation and so, blocks the classical active macrophage killing. Th1 INF-γ producing lymphocytes are found to be in balance with type 2 helper (Th2) stimulated by parasitic infections, atopy or asthma.

1.1.10 Tuberculosis and interferon-gamma

Interferon gamma's (INF-γ) are natural proteins or soluble cytokines released from the immune system T cells Th1 as a host defensive mechanism against foreign organisms. INF-γ has anti-bacterial, anti-viral and anti-tumor properties. INF-γ belongs to the class of cytokine glycoprotein's that act directly by inhibiting viral replication, stimulating natural killer cells (NK) and as a central mediator for macrophages activation either alone or in synergy with TNF-α. Host cell resistance against foreign organisms is stimulated by INF-γ by increasing antigen presentation

to lymphocytes (T and B cells) acting as potent mediator of activation to macrophage bactericidal activity against MTB (Herrmann *et al.*, 2009).

INF-γ has also has a crucial protective role against various pathogens through stimulation of normal cells to increase the expression of MHC complex type I and immunoglobulin receptors of antigens presentation for macrophage, activating lysosome activity within macrophages, and recruiting bactericidal T lymphocyte. This is carried out in synergy with TNF-α and both activate infected macrophages by initiating effecter mechanism of cell-mediated immunity (CMI).

MTB infection inhibits the INF-γ signalling pathway to evade the human immune response and defects in INF-γ genes or receptors predispose individual to mycobacterial infections. After antigen exposure, INF-γ or INF-γ-producing cells are used as a marker for effecter cell activity, and lymphocytic cell absence or antigens of remote infection are not related to the boosting effects of INF-γ (Choi *et al.*, 2008).

MTB re-infection begins during the chronic phase once FM leaves the granulomatous tissue toward alveolar spaces. MTB–infected alveolar epithelial cells release sustainably cytokine INF-γ at high concentrations post-infection at 48 hours (33 +/- 3 pg/ml) and 72 hours (111 +/- 4 pg/ml) compared to non-infected cells (2.6 +/- 1.6 pg/ml) as measured by enzyme linked immunosorbant assay (ELISA). This INF-γ plays a role in the regulation of the intra-cellular innate immune response and internalizes only live TB pathogen rather than cell surface-attached dead bacilli (Sharma *et al.*, 2007). INF-γ responses are more common in latent TB subjects as compared to those without TB infection (Lahey *et al.*, 2009).

Advances in genomics and immunology for MTB detection has lead to the development of several promising alternative tests to the TST. In-vitro interferon gamma release assays (IGRAs) tests measure the released INF-γ specifically produced by MTB-stimulated T (Th1) cells (section 1.4; Laboratory diagnosis of tuberculosis; 1.4.2 IGRAs).

An evaluation of 130 children in France using IGRAs QuantiFERON test has shown a difference in INF-γ level values between healthy control children, LTBI and cases

of active TB, particularly in the significant difference between LTBI (INF-γ median value 0.85 IU/ml) and the higher median value in active MTB (INF-γ median value 3.28 IU/ml). Peripheral blood INF-γ levels have also been observed to differ between treated and untreated patients, which are also related to the individual's immunity against TB and also can be useful to monitor INF-γ levels before/after anti-TB treatment (Tiwari *et al.*, 2007).

A decline in INF-γ values during treatment allows the clinician to monitor effect of curative or preventive chemotherapy (Herrmann *et al.*, 2009). Therapeutic INF-γ binds and then activates the cell-surface INF-γ receptor, stimulating antibody-dependent cytotoxicity and enhances NK cell attachment to tumor cells, and thereby inducing apoptosis in malignant cells.

1.1.11 Latent tuberculosis infection (LTBI)

The WHO recommends effective TB control, but diagnosis and control are dependent on early detection and identification of LTBI individuals at high risk of re-activation and progression to active TB disease (Hussein, 2007). Identification of a primary source or suspicion of LTBI or active TB disease is a crucial step in targeting investigation(s) but communities worldwide face difficulties in TB control due to the absence of any gold standard diagnostic tests.

Areas highly endemic for TB disease suffer from negative consequences of the disease, for example economic costs related directly or indirectly to TB. WHO estimated the total direct cost of TB control to be U.S.$2.0 billion by WHO in 2006 while TB screening costs were estimated at U.S.$72.1 million (Nolte, 2006). WHO recommends governments worldwide to continuously co-operate towards TB and LTBI elimination.

1.1.12 Difference between LTBI and active TB disease

Latent tuberculosis infection occurs when the number and activity of tuberculosis bacterial germs becomes enough to cause symptoms in normal healthy looking individuals. Active TB cases are commonly presented with suspect symptoms such

as a chronic cough for more than 3 weeks, fever with hemoptysis or weight loss, and/or fatigue and night sweats (see Table 1.1).

Table 1.1 The major differences between latent tuberculosis infection and active tuberculosis disease (CDC, 2011e)

LBTI	Active TB disease
Asymptomatic	Symptomatic : bad cough > 3weeks, chest pain, hemoptysis, weakness, weight loss >10% normal body weight, fever/chills relieved by night sweating
Not feel sick	Feel sick
Cannot spread TB bacilli to others	Spread TB bacteria to others
Usually variable TST and blood test result (not indicative result)	Usually has positive TST and blood test results indicative of TB infection
Normal chest X-ray (common), sputum and culture	Higher chances than LTBI to have positive CXR, sputum and culture
Need prophylactic treatment for 6-9 months (prevent progression to active TB disease)	Need urgent curative treatment (6 months) +/- isolation

The mechanism of evolution of LTBI granulomatous lesions can be summarized as follows; 1)- LTBI diagnosis is complicated by the fact that slow growing resilient bacilli are able to resist stressful conditions and survive in necrotic tissues with ability to grow within the granuloma's periphery; 2)- Growing TB bacilli are resisted by the host immunological response mainly through active immunity (CD4+) and 3)- Post-accumulation of necrotic tissues and dead MTB, foamy macrophages harbor latent TB bacilli but exert local immune-suppression by reducing antigen presentation of actively growing TB bacilli.

1.1.13 Disease susceptibility and resistance

Susceptibility to TB is multifactorial and host genetic factors are believed to play a major role in disease development and progression. Host susceptibility and resistance are directly associated with human leukocyte antigens (HLA) and non-HLA genes serve as genetic markers of TB pre-disposure and have been established in different ethnic groups e.g. for Asian PTB, ethinic Indians present HLA-DR2 and HLA–DQ1 (Selvaraj, 2004) and HLA-DRB1*14 as susceptibility alleles for MTB disease (Duarte *et al.*, 2011).

Human genetic variation is an important determinant for MTB infection outcome. Bellamy and colleagues conducted a study of the two-stage genome-wide linkage, searching for regions in the human genome containing tuberculosis-susceptibility genes, they disproved monogenicity and identified two regions on chromosome 15q and Xq for TB susceptibility. X chromosome susceptibility may contribute to male predominance observed in some populations (Bellamy *et al.*, 2000).

Anaerobic and acidic conditions within closed lesions reduce bacterial metabolism but the existence of 'persisters' or resuscitation-promoting factors (rpfs) genes, isolated from tissues MTB DNA actively promote emergence from dormancy and low susceptibility to drug treatment (Davies *et al.*, 2008).

Tuberculosis may present as a resurgent disease in immune-suppressed individuals such as those with diabetes mellitus, cancer patients and renal dialysis or organ transplant patients. MTB infection is among the most important of HIV-related opportunistic infections due to compression of the host's immune defenses, leading to failure to control latent MTB infection with subsequent development of active 'symptomatic' tuberculosis (Raviglione *et al.*, 1992).

Culture-positive isolates can be matched with outbreak strains using genotyping. Genome-wide studies identify macrophage response genes and different clinical outcomes due to variations of innate immune response genes that regulate susceptibility and resistance to TB (Balamurugan *et al.*, 2004; Thuong *et al.*, 2008). In Korong district, northern Malawi, homozygosity for the complement receptor 1

(CR1) polymorphism has been associated with susceptibility in healthy population but was not observed among HIV positive (+ve) cases due to overriding genetic influences. Heterozygosity was also associated with protection against TB in both HIV+ve and HIV−ve cases suggestive of influences on the innate immune response even in immune-compromised individuals (Fitness *et al.*, 2004). Genotyping studies of HIV infection support the concept of LTBI progression to active TB status from remote re-activation rather than recent TB infection (Driver *et al.*, 2006). TB/HIV co-infected patients soon become asymptomatic and non-infectious usually within 10-14 days post–anti-TB treatments, having a low transmission rate (due to weak-type of cough) even if still having a TB positive sputum smear (Campbell *et al.*, 2006).

Kibiki and colleagues showed that one third of Tanzanian's (TB endemic African country) were TB/HIV co-infected and nearly 11% of tested strains using spoligotyping were resistant to anti-TB drugs (Kibiki *et al.*, 2007).

1.1.14 Morbidity and mortality

Worldwide, TB is the highest leading cause of morbidity and mortality from a curable contagious infectious disease (CDC, 2008a). Untreated, it can be deadly. More people died during the last decade from TB than perhaps any other decade in history. In 1999 WHO reported a global TB prevalence of 32% (1.86 billion people), with the total new and existing cases between 7.96 and 16.2 million, and 1.67 million deaths, representing a global case fatality rate of 23%. The WHO published reports of 2005 on the last World TB Day held on 24th March 2008 estimated that all TB parameters were increased (doubled) in a global pattern (see Table 1.2).

Table 1.2: Estimated tuberculosis incidence, prevalence and mortality, 2005 (WHO, 2008a)

WHO region	Incidence				Prevalence		TB mortality	
	All forms		Smear-positive[b]					
	number (1000s) (% global total)	Per 100,000 population[*]	Number (1000s)	Per 100,000 population	Number (1000s)	Per 100,000 population	Number (1000s)	per 100,000 population
Africa	2, 529 (29)	343	1, 088	147	3, 773	511	544	74
The Americas	352 (4)	39	157	18	448	50	49	5.5
Eastern Mediterranean	565 (6)	104	253	47	881	163	112	21
Europe	445 (5)	50	199	23	525	60	66	7.4
South-East Asia	2, 993 (34)	181	1, 339	81	4, 809	290	512	31
Western Pacific	1, 927 (22)	110	866	49	3, 616	206	295	17
Global	8, 811 (100)	136	3, 902	60	14, 052	217	1, 577	24

[a] Incidence = defined as new disease cases arising in given period; prevalence = defined as the number of cases which exist in the population at a given point in time
[b] Smear-positive cases are those confirmed by smear microscopy, and are the most infectious cases (http://www.who.int/mediacentre/factsheets/fs104/en/index.html) (DOA: 26/01/2012)

TB infection or morbidity progresses rapidly particularly in early adulthood (within the first few years of infection). Nearly 14-17 million older children and adults die each year in developing countries. Mortality is related to the disease severity or systemic disseminations of disease, such as meningeal or miliary tuberculosis. Children born to HIV positive parents, whether infected or not, are at high risk for death. A study of 'healthy adults' in Mumbai, India, to detect LTBI showed that 80% of residents were already infected with TB and were harboring MTB-exposed T-cells compared with non-exposed UK healthy adults (Lalvani *et al.*, 2001a).

1.1.15 Risk factors

Screening of TB and LTBI can be facilitated for identification through combination of the aggravating-associated risk factors. The risk of acquiring infection depends on the infectiousness of the index case, the duration and degree of exposure to surrounding environments, and organism virulence (CDC, 2005b; Maddineni *et al.*, 2008). Risk factors for TB can be directly or indirectly related to social, behavioral, biological, physiological, environmental/occupational, and multi-factorial combined variables.

1.1.15.1 Social factors

Tuberculosis disease is directly related to community socio-economic conditions. The WHO report published in 2008 considers TB as one of the top 10 leading causes of death in 2004 in relation to an individual's income; the seventh cause in low-income groups, the ninth cause in middle-income groups (but not in high-income populations) (WHO, 2004). Tuberculosis threatens the poorest and low-income community groups and decreases developments of economic-public health systems such as emergence of TB drug-resistant strains.

The prevalence of health care workers (HCWs) having LTBI in these two population groups ranges between 33-79% compared with 5-55% in high-income regions (Topic *et al.*, 2009). Parent education and employment are other significant risk factors for TB infection and detected more in BCG positively vaccinated compared with non-vaccinated people (Soysal *et al.*, 2005).

To halt the disease's advance and globalization, the WHO considers the first requirement to be accurate and rapid diagnosis of all suspected TB individuals, followed by control measures and treatment administration. For example, any subject aged between 12-14 years and living in a country with a TB incidence rate of more than (≥) 20 new cases per 100,000 people (as identified by WHO) is considered as a suspected TB case and tested using the tuberculin skin test. Improved living conditions both in country of origin and newly occupied regions (especially in the first few years) decrease the incidence of TB (Scotto *et al.*, 2009).

The prevalence increases with close contacts to a TB index case. Age is known infection social marker for children and is directly affected by both community internal exposures e.g. close contact to same household and parent TB index case with/ without external exposures due to mobility outside households e.g. inside the school or playground (Langenskiold *et al.*, 2008).

Increased age is also at risk for TB infection, with a 100-fold higher than non-exposed healthy people (Pai *et al.*, 2005). Older age reveals significant results of positive interferon gamma release assays (IGRAs) testing due to waning of anti-tuberculosis immunity (Horsburgh *et al.*, 2010).

Tuberculosis is considered an infectious killer of women of reproductive age worldwide due to less protective sex steroid hormones (Weiss, 2008).

Anaemia with significantly low hemoglobin levels is prevalent among adults with pulmonary tuberculosis, and is associated with severe infection (Gupta *et al.*, 2009).

1.1.15.2 Behavioral factors

Smoking is the major behavioural and occupational risk factor, which is increasing throughout the world. Passive smoking is significantly associated with increased risk for clinical TB, and children exposed to passive smoking exhibit more positive TST results than non-exposed subjects (Kolappan and Gopi, 2002; Lin *et al.*, 2007). Individuals living in the same household together with smoking parents are significantly at higher risk (odds ratio (OR) 4.6) of developing TB disease than non-smoking infected households (OR 1.2). Coughing in heavy smokers can delay a TB diagnosis due to an increase in the infectious stage and longer exposure intensity facilitating bacillary transmission (den Boon *et al.*, 2007). Both smoking crack with cocaine addiction and history of alcohol abuse increase LTBI environmental risk exposure (Grimes *et al.*, 2007; Lin *et al.*, 2007).

Tobacco neutralization of TNF-α in pulmonary macrophages is also associated with excess iron in broncho-alveolar macrophages in the smoker's lung. Low immunity can also be related to other factors such as decrease muco-ciliary activity and decrease clearance of inhaled substances, abnormal vascular and epithelial

permeability, change in epithelial mucus amount and consistency, increase number of non-functional phagocytosis versus pathogenic organisms, and decrements of T-helper cells, NK cells, T-cytotoxic cells fighting capacity and innate immunity (den Boon *et al.*, 2007; d'Arc Lyra Batista *et al.*, 2008).

Stopping smoking and close monitoring of adherence for six-month anti-TB therapy reduces the risks of LTBI re-activation. Relapse phenomenon with the bulk (up to 65%) occurring within the first year of follow-up after completing anti-TB therapeutic course was related to living in areas with non functional family health programs e.g. inadequate drug supply, wrong prescriptions/dosages, or adverse effects, related with independent causation to smoking (OR 2.53) (d'Arc Lyra Batista *et al.*, 2008).

1.1.15.3 Biological factors

Host-related genetic factors vary between cases and community controls, which continuously been validated through family-based association studies to improve understanding of biology and MTB transmission. Valid associations can explain various risk factors like; 1- source of infectivity, 2- intensity of TB infection, and 3- infection susceptibility (Lienhardt *et al.*, 2005).

Longer duration of exposure and daily exposures in TB high-prevalence regions present an additive risk (Soysal *et al.*, 2005).

Diagnostic positive test concordance is greater in monozygotic twins than dizygotic twins, and is associated with human leukocyte antigen (HLA) and immunological differences between infected and healthy controls in the same households for their pattern of clinical disease (Lienhardt *et al.*, 2002a). Diabetes mellitus (DM) directly impairs the innate and adaptive immune responses needed to resist MTB proliferation after reduction in Th1 cells, IFN-gamma levels, and reduced neutrophil chemotaxis, oxidative killing and leukocyte bactericidal activity (Jeon *et al.*, 2008).

1.1.15.4 Physiological factors

Impaired cellular immunity is associated with; 1- young age less than 5 years old, 2- pathological factors (e.g. HIV and related-opportunistic infections, diabetes mellitus, chronic renal failure on hemo-dialysis), and 3- iatrogenic immune-suppression (e.g. organ transplant, TNF-α agents, systemic steroids).

Although changes in the immune system during aging lead to increased susceptibility to mycobacterial infections and re-activation of latent foci, TB remains associated with younger age groups in developing countries (independently of HIV status). This may be explained by the higher proportion of young people, a higher annual risk of infection and shorter life expectancies in developing countries (Solari *et al.*, 2008).

TNF-α cytokines control TB infection by maintaining an immunological balance between infected host and tubercle bacilli to avoid MTB re-activation, therefore anti-TNF-α therapeutic agents (e.g. anti-rheumatoid treatment) or steroids (e.g. prednisolone), and lack of compliance to anti-TB treatment would arrest TNF-α activities and ameliorate the immune response (Cardona, 2007; Kobashi *et al.*, 2008).

Other indirectly associated risk factors, related to other conditions, include silicosis (Risk ratio [RR] = 30), immuno-suppressive therapy (RR = 12), hemo-dialysis (RR = 10-15), jejunoileal bypass (RR = 27-39), and carcinoma of head and neck (RR = 16) (Pai *et al.*, 2005).

1.1.15.5 Environmental/occupational factors

There is always considerable heterogeneity in the risk of LTBI between different occupations. Occupational exposure to TB infected case noticed in nosocomial infections is acquired within the community which usually includes health care workers (HCWs) and their relatives (Freeman *et al.*, 2010). He and colleagues concluded that LTBI prevalence in medical staff (56%) was significantly higher than that in administrative/logistic staff (42%) within the same TB centre (He *et al.*, 2010), in addition to varying degrees of patient contact such as with a nurse, doctor, laboratory technician or physical therapist (Eisenberg and Pollok, 2010; Joshi *et al.*, 2007).

Overcrowding and using small transport vehicles with longer exposure-time of public employees are a variety known risk factor combinations that are difficult to control (Horna-Campos *et al.*, 2011). Health service providers and HCWs can be related with inadequate data availability can increase transmission risks e.g. nurse in TB hospital ward, doctors admitting TB infectious cases are associated with delayed diagnosis of acute or chronic TB patients (Schablon *et al.*, 2009; Schablon *et al.*, 2010b).

In industrialized countries, populations at high risk are those comprising: 1)-individuals exposed to or infected from MTB close contacts, foreign-born immigrants and recent arrivals within five years from TB high prevalence region, elderly, special racial and ethnic minority population, residents of long-term care facilities e.g. HCWs in nursing homes, drug abusers, medically under-served, homeless, occupational exposure in comparison to, 2)- individuals developing TB disease once recently re-infected within two years, HIV-co-infected, immuno-suppressive conditions or medications, age extremes, history of inadequate anti-TB treatment with appearance of drug-resistance strains; multi-drug resistance (MDR-TB) and extreme-drug resistance (XDR-TB) (Maddineni *et al.*, 2008).

The variability of job category reflects variation in exposure frequency and intensity. A study revealed 104 out of total 160 healthy looking nurses with OR of 1.55 having positive TST and IGRAs test (Pai *et al.*, 2005). Close contact and proximity of jail inmates raise HIV and TB possibilities due to overcrowding, shared respiratory ventilation buildings (> 12 hours) and poor living conditions facilitate MTB transmission to others from undetected active TB cases. Environmental conditions and proximity to a contagious index case (> 8 hours) complicates TB control programs e.g. hospital wards, aircraft (Al-Jahdali *et al.*, 2003; Demkow *et al.*, 2008).

Proximity exposure (OR 3.5) and/or past history of TB infection (OR 4.3) are highly associated risk factors with positive TB diagnostic tests. Regular investigation is needed to identify even the contacts of treated-TB index case where less than 30-40% is only completing LTBI prophylactic therapy (Bur *et al.*, 2003).

1.1.15.6 Risk factor combination

TB exposure is affected by combined internal and external occupational-environmental risk factors. Combined socio-economic factors are complicating TB elimination and affecting assessments of the implemented control programme outcomes and are commonly noticed in the developing countries (Lienhardt *et al.*, 2002b). Low social class, overcrowding, illiteracy, poverty (low income and property ownership), under/malnutrition are all related to immune-suppression (CD4+ counts < 200 cells/mm^3) with anergic DTH-IV immune responses against TB concomitant infections and LTBI re-activations (Pai *et al.*, 2005).

The relative frequency of exogenous re-infection against endogenous re-activation is still ambiguous and undetermined. For example endogenous re-activations are commonly related to impairment of the immune system such as poverty and alcohol and drug abuse are known risks leading to other related TB risk factors e.g. smoking or medical re-activation risk factors - such as DM, HIV/AIDS and other related debilitating medical conditions, poor eating habits and inadequate nutrition with vitamin deficiency (LoBue *et al.*, 2010).

Environmental effects such as nutritional status or living on traditional farms or parasitic (helminth) infections are known risk factors that affect the immune system in early (neonatal) life with consequences for later disease outcomes or for responses to BCG vaccination in infancy (Djuardi *et al.*, 2010). Vitamin D deficiency due to poor nutrition and low socio-economic status also increases the probability of LTBI and TB (Talat *et al.*, 2010).

Large case control study including 820 cases done in 3 different countries sharing similar socio-economic environmental conditions and geographic borders in West Africa; Gambia, in Conakry and Bissau, revealed that TB control difficulties can be due to various risk factors. Multifactorial combination of host–related and environmental-related risk factors such as male gender (due to migration for job and crowding with others), HIV infection (due to LTBI re-activation), smoking habits (related to dose–effect relationship and smoking duration), history of asthma (due to MTB eliciting Th1 immune mechanism and production of IL-12, INF-γ, TNF-α but inhibiting Th2 activity), family history of TB, marital status (widow and divorced subjects), adult overcrowding per living room and closeness/direct contact (first-

degree social proximity) between household occupants and low socio-economic status (poverty and absence of ownership) (Lienhardt *et al.*, 2005).

Risk factor combinations increase the difficulty of distinguishing TB from other respiratory disorders. Shared risk factors are targeted by TB control programmes in reducing TB globalization and health system overburden. A comparative study of 404 treated PTB in group 1 during the period between (1986-1995) against other PTB treated 414 patients in group 2 during the period between (1996-2005) revealed a significant increase in atypical chest findings in group 2 related to non-respiratory conditions and with lower lung field predominance e.g. cancer, collagen vascular disease or use of immune-suppressive medication, compared with positive microbiological findings e.g. sputum smear and culture positive results in group 1 (Kobashi *et al.*, 2008).

Previous history of TB infection is a common risk factor and integrated as pulmonary tuberculosis with higher suspicion than CXR performances (Wu *et al.*, 2009). Common additional risks are prevalence of TB infection within exposed populations, presence of an infectious source, air density carrying TB droplet nuclei, duration of exposure to infected polluted air and quality of indoor/outdoor air ventilation (filtration).

In 2004, a retrospective study done on 2,508 TB cases between 1996-7 in Catalonia, Spain, found a TB incidence of 41 per 100,000 person-years with detection of combined behavioural changes; 19.4% (487/2,506) were co-infected with HIV, and 35.6% (893/2,508) presented with positive sputum smear, 16% (401/2,508) were alcohol abusers and 13% were intravenous drug abusers (IVIG) (327/2,508) (Godoy *et al.*, 2004).

1.1.16 Impact of migration on LTBI & TB

Human migration is a well-documented increasing phenomenon and immigrants are in particular need of close monitoring and screening programmes to tackle TB transmission and to improve public health. Migration is a major risk factor with an impact on all infectious diseases and population-demographic changes due to

contact-exposure phenomenon and importation of LTBI and active TB disease from high- toward low-prevalence regions (McCarthy, 1984). An individual's geographical history is important in any assessment (immigration and foreign travel, destinations, travel-exposure durations), as well as consideration of the age and general health of travelers including the risks of work type (Freeman *et al.*, 2010).

Since the 1980's, human migration has reached an un-precedented scale, with more than 150 million people estimated to be long-term residents in a country other than that of their country of birth. In addition, the numbers of short-term travellers exceed those seeking permanent resident status by 50 times (7,500 million). Immigration demographics show that Asians, Blacks, and Hispanics bear the greatest burden of TB and screening of immigrants can protect community public health in nations of low TB incidence. In 2003, the total number of reported TB cases in Australia was 1,013 cases with 80% being foreign born. In the UK there were 6,400 reported cases, with 64% cases being in foreign-born residents. Western Europe faces irregular migration of TB cases - phenomena that has increased during the past two decades and is investigated using routine medical examination and migrant screening (MacPherson and Gushulak, 2006). The incidence of TB in the country of birth is crucial to predict variation in demographic changes, and this can be used to focus and target TB elimination and prevention efforts toward the high-risk TB groups (Watkins *et al.*, 2002).

The increase in TB incidence and prevalence due to immigration was observed in 4-year universal genotyping of TB patient isolates from the Almeria region of Spain, which had high immigration from TB endemic areas in North and Sub-Saharan Africa and has showed a rise in number of TB cases from 19% in 1997 to 48.9% in 2006 (Miguel *et al.*, 2008).

Tuberculosis notification rate been rising quickly in Eastern Europe countries (5% per year, 1997-2002), and in African countries due to high HIV prevalence (East and West African countries; 7% per year), but a slowing of rate was detected since the mid 1990s (Figure 1.9). The case notification rate is stable or in mild decline in the rest of the world.
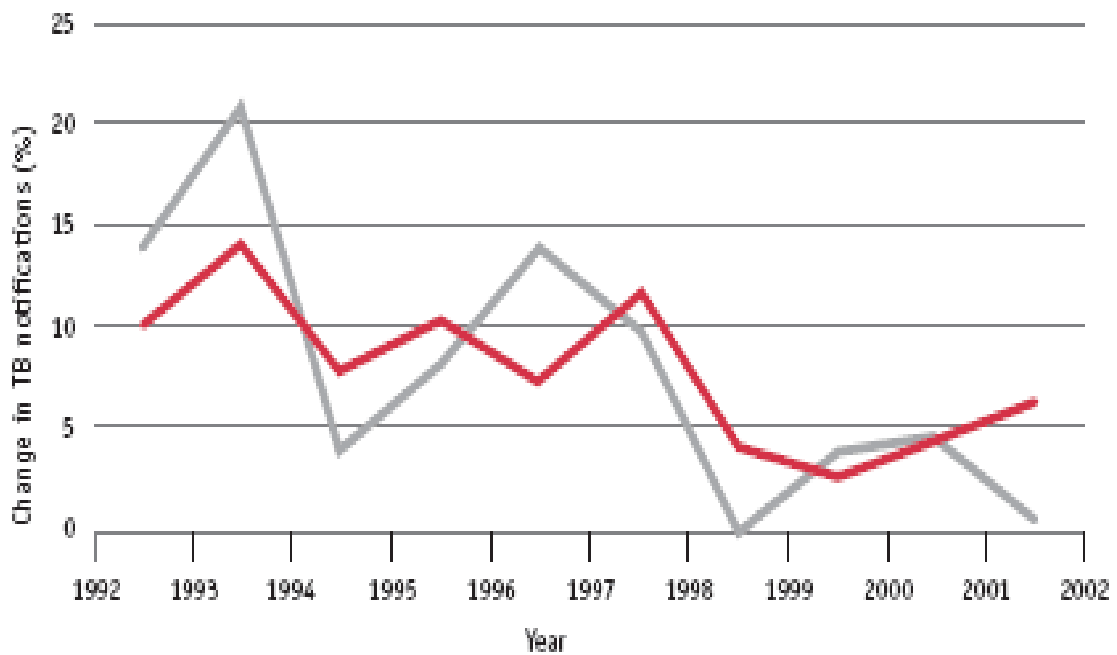
Figure 1.9: Annual changes in TB notification rates 1992–2002 (average percent change on previous year) in notification rates (all forms, DOTS and non-DOTS) between consecutive years for two groups of countries; Africa – high HIV (red) and eastern European countries (grey) (http://www.who.int/tb/publications/global_report/2004/06_results1/en/index1.html) (DA 26/7/2008)

The total reported TB cases in England between 1999 and 2003 were 31,290 and 37% (n = 11,404) were from Indian, Pakistani and Bangladeshi ethnic origins, 29% were from white ethnic groups and 19% (n = 5,665) were from black African ethnic groups. Nearly 66 % (n = 17,987) of all TB cases were from non-UK born residents. In London, the TB incidence rate and tuberculosis cases increased from 32.3 to 41.3 per 100,000 healthy populations compared with only mild increases outside London (from 7.7 to 8.4 per 100,000). After adjustment of all other variables, most cases of TB were shown to belong to non-UK born population groups that had arrived in UK within five years prior to their diagnosis. These cases were also associated with increased number of INH-resistant strains and MDR-TB resistance (Crofts *et al.*, 2008). Treating drug-resistant TB is expensive costing between £50,000 and £70,000 per patient and has serious financial implications for National Health Boards.

In Germany, the influx of BCG-vaccinated immigrants since 1998 from TB high-incidence countries represents a potential pool for new TB infection and highlights the need for LTBI early detection (reducing TB reservoirs) especially in young

children, and lowering the raised frequency of non-*Mycobacterium tuberculosis* (NTM) infection by differentiation from other mycobacterial types by using INF-γ levels and IGRAs test (Pai and Menzies, 2007). A screening study in Northern Germany on 270 HCWs concluded that LTBI prevalence with IGRA positive results reached 3.5% in 115 participants under 30 years of age and 22% in the 41 participants that were over 50 years. A higher prevalence was associated with job-exposure risk e.g. physicians and nurses than among other professional workers e.g. radiologists (Schablon *et al.*, 2009).

Risk factors associated with delays in diagnoses pose a significant barrier to TB diagnosis and control e.g. low public awareness and inadequate health service funding (Kariminia *et al.*, 2009; Long *et al.*, 2008). Worldwide, it is essential to have adequate knowledge as regards drug susceptibility for *M. tuberculosis*, especially in the low TB prevalence regions, due to the risks of high travel of hosts and uncontrolled immigration (Saif Alfaresi and Hag-Ali, 2010).

In Victoria, the second most populous state of Australia, 80% of TB new cases develop in foreign-born individuals (109/100,000) accounting for 18 times more TB incidence than rates of the total population (6/100,000), and were common during the first five years post-migration resettlement and from South-East Asia regions related to migration displacements (MacIntyre *et al.*, 1999). There was however a contradictory result from a comparison between Canada and Australia of TB patients diagnosed after sputum smear stratification that did not show an association between TB incidence in immigrants and country of birth (due to in-appropriate, in-accurate available data about historical exposure in their origin or destination region (Watkins *et al.*, 2002).

A study done between 1994-2005 in Norway to assess immigration impact on the 3,131 notified TB cases over the 12-year period showed that 705 of the strains isolated from TB high-incidence expatriates (the majority from Somalia, Pakistan, the former Yugoslavia, Ethiopia, Vietnam, Thailand, India) were associated with a steady increased number of notified TB cases from 4.7 to 7.2 per 100,000 population due to importation and spreading of *M. tuberculosis* bacilli. The majority of MDR-TB cases were also isolated from migrants (MacPherson and Gushulak, 2006).

Importation of immigrant pathogenic strains can influence local population strains over extended time periods (Dahle *et al.*, 2007). In 2008, total immigrants present in Geneva, Switzerland represented 45% population with 84% of TB cases being foreign-born, which can be related to increased immigration of close contacts harbouring or acquired LTBI and history of BCG vaccination e.g. approved TST more than 5-mm 'cut-off' size (rather than > 10-mm) was significantly associated with previous BCG (Brodie *et al.*, 2008).

Between 5 and 10% of total infected immune-competent individuals turn out to be asymptomatic LTBI cases, where then 50% can progress to active TB within the first one and two years post-exposure.

An Australian historical cohort study done on 24,610 refugees reaching Sydney between 1984-1998 reveals that most TB cases already came from South Asian countries and 29% of LTBI re-activation within the 1st year and 65% within five years post-arrival (Marks *et al.*, 2001).

1.1.17 Impact of smoking on LTBI & TB

TB and tobacco usually are regarded as two colliding epidemics of public health importance (Pai and Menzies, 2007). Tobacco smoking increased substantially in the past two decades with a pandemic of 1.3 billion smoker's worldwide. The risk of prevalence, incidence, recurrences and mortality of clinical TB infection with male predominance is more among current than ex-smokers and non-smokers, and in turn, more with the duration of smoking than the number of cigarettes smoked daily (Gajalakshmi *et al.*, 2003; Jee *et al.*, 2009). Susceptibility and exposures to smoking of school students in South Asian families and low-income countries was observed by Guindon *et al.* (2008). Smoking is responsible for more than one-third of TB deaths (38%) in the Taiwanese population (Wen *et al.*, 2010).

Both tuberculosis (LTBI/TB) and tobacco dependence, which is common in low- and middle-income countries are regarded as two colliding epidemics of public health importance. Smoking is associated with treatment non-adherence and tuberculosis infection relapses and delays in recovery (Hsien-Ho *et al.*, 2008; Jianming *et al.*,

2009). A casual association also showed positive dose-response relationship with daily number of cigarettes smoked of estimated OR 2.5 (95% confidence interval (C.I.): 1.42 to 4.37) (Kolappan *et al.*, 2002). Tobacco delays the recovery of pulmonary tuberculosis and induces pulmonary tissue sequels despite correctly applied anti-tuberculosis treatment due to treatment failures, defaults, and relapses.

Smoking is perhaps one of the risk factors that should be most amenable to change. The number of active tuberculosis cases increases with numbers of smokers due to increases in miss-management practices, treatment failure and relapses, MTB transmission and control difficulties (d'Arc Lyra Batista *et al.*, 2008; Kolappan *et al.*, 2002; Ng *et al.*, 2008; Pradeepkumar *et al.*, 2008; Santha *et al.*, 2002).

Smoking influences the clinical progress of TB lesions and delays LTBI/TB diagnosis. Exposure to smoking produces alterations in both natural and acquired cell immunity, affecting macrophages and leukocytes. It induces apoptosis in both activated and non-activated macrophages, leading to bacillary multiplication. On a biological basis, smoking increases TB risk through a decreased immune response, mechanical disruption of ciliary function, defects in macrophage immune responses, and/or tissue CD4+ lymphopenia. Similar to HIV/AIDS pathology, smoking alters the mucociliary apparatus such as cell dysfunction and destruction and lysozyme activity, resulting in great numbers of germs and toxic substances reaching the alveolar tissues.

Cigarette exposure has been shown to increase MTB bacillary burden in mice infected with *M. tuberculosis*, and increased weight loss and mortality in mice infected with influenza virus. This study provides the first direct evidence that cigarette smoke exposure increases bacillary burden through direct inhibition of the pulmonary T-cell response versus MTB in a mouse animal model increasing susceptibility to MTB pathogen (Arcavi *et al.*, 2004; Feng Y. *et al.*, 2010).

1.1.18 Impact of BCG vaccine on LTBI & TB

The Bacille Calmette-Guérin vaccine (or Bacillus Calmette-Guérin, BCG) was prepared from an attenuated live bovine tuberculosis strain (*Mycobacterium bovis*)

cultured in humans. The BCG vaccine is injected intra-dermally as a single injection into the upper arm (deltoid muscle) and injection is preceded by tuberculin skin test, performed by a skilled nurse and not having a false positive result. BCG stimulates the immune system to produce antibodies against MTB and tuberculosis infection. The indicated age range and vaccination frequency varies between different countries. For example: WHO's new BCG policy (applied in Kuwait) recommends vaccination of all children born in TB-endemic regions at three months of age. India introduced BCG mass immunization in 1948 recommending 2 and/or 3 immunizations during life. In the UK, BCG vaccination, which was a compulsory since 1954, was withdrawn as a routine immunization in 2005 because it was considered as not cost-effective. BCG vaccination can have obvious public health advantages. It can lessen the burden of TB-related disorders such as LTBI prevalence and active TB incidence in children (Soysal *et al.*, 2005; Trunz *et al.*, 2006).

The latest WHO policy for BCG immunization rules out vaccination in HIV positive children but recommends vaccination for travelers to TB endemic areas. The initial absence or low predominance of emerging antigens for sensitizing immunity, added to the gradual degeneration and necrosis of antigen-specific T cells in infected lungs, would physically prevent cellular penetration and promote bacillary resistance (Henao-Tamayo *et al.*, 2009). BCG lacks a group of genes (Rv3971 to rv3879) including the genes for antigenic proteins 'Region of Difference' (RD-1) made by MTB early during culture growth but with unclear precise action (discussed further in section 1.4.2 IGRAs). Data show that non-mutant bacilli expressing RD-1 causes dramatic necrosis in mice lungs unable to make INF-γ. Under in vivo conditions, the RD-1 encoded proteins cause direct or indirect progressive necrosis secondary to interactions between host cells and bacterial proteins, but not due to bacterial overloads (Junqueira-Kipnis *et al.*, 2006).

The tuberculosis skin test (TST) cannot distinguish between previous BCG vaccination and current TB infection due to poor specificity in BCG negative people and poor sensitivity in children. Meta-analysis proves that previous BCG administration increases the likelihood of TST false positive results up to 15 years

post-vaccination (Richeldi, 2006), while, IGRAs results are independent of BCG vaccination (discussed further in chapter 6).

Absence of an immunization scar can determine the likelihood for TB infection and is significantly associated with TB disease severity, which can be related to the level of exposure to MTB (Soysal *et al.*, 2005).

The impacts of BCG on the tuberculin skin test and LTBI results will be further discussed in chapter 5; section 5.3.

1.1.19 Impact of HIV/AIDS on TB & LTBI

The human immunodeficiency virus (HIV) is the strongest risk factor for the development of human tuberculosis among individuals infected with MTB. High prevalence of TB/HIV co-infection in various geographical areas and in specific population groups has made TB the most common AIDS-diagnostic disease in the world, and is compulsory to test for *M. tuberculosis*.

The impact of the HIV pandemic is felt all globally and particularly in the many resource-poor countries in Sub-Saharan Africa, where two thirds of the world's TB infected population lives. Between 40-80% of TB+/HIV+ patients have pulmonary disease in Africa and South America (Raviglione *et al.*, 1992).

Although TB re-emerged as a major public health problem in the developed and industrialized countries due to international migration and the breakdown of health services, including TB services, HIV infection is the strongest risk factor with a significant increase in the rate of re-activation (rate ratio [RR] 57; 95% C.I.: 26-120) and among those older than 50 years of age (RR, 3.8; 95% C.I.: 1.3-11) (Horsburgh *et al.*, 2010). TB/HIV co-infection impairs the immune system and increases morbidity and mortality rates. The spread of HIV makes TB diagnosis difficult and HIV/AIDS epidemics are significantly influencing TB epidemiology due to the emergence of immune-suppression and increased susceptibility to TB infection in addition to rapid MTB progression of recently acquired infection and/or LTBI re-activation.

TB high-prevalence areas are also suffering from the HIV/AIDS epidemic creating the TB/HIV syndemic and the emergence of drug-resistant strains to anti-TB treatment. Evolution of TB strains continues as human demography changes with HIV (Basu *et al.*, 2009). For example TB patients in African countries are predominantly co-infected with HIV and are disproportionately spreading TB over sub-populations associated with new TB epidemics. A total of 36.1 million people were living with HIV/AIDS at the end of 2000, 25.3 million (70.1%) living in sub-Saharan Africa and 5.8 million (16.1%) from South East Asia, with half million people dying in 2001 (WHO, 2006), and 1.37 million co-infected new cases estimated in the WHO 2007 report (WHO, 2009a).

Concurrent TB/HIV positive (+ve) co-infection makes the patient less infectious, however, a single positive smear sample is considered an important risk factor for LTBI progression to the active TB form, and lower lifetime annual risk reaches 7-10% compared with higher risk reaching 10% among TB+/HIV- (TB positive/HIV negative) patient (Balcells *et al.*, 2008; Driver *et al.*, 2006).

TB/HIV co-infection raises the risk of LTBI re-activation 100-fold, which can be qualified using the tuberculin skin test (TST) response and quantified according to T cell lymphocyte count. In the industrialized countries HIV+ patients are 60 times at higher risk of developing TB from dormant LTBI re-activation compared with being six times more likely in TB endemic countries.

Worldwide, the incidence of TB remains high among the HIV-infected populations and this mandates for early diagnosis and treatment. Even though anti-retroviral therapy slows the rate of latent re-activation and increases lifespan of hosts, HIV increases the rate of recurrence, even in TB-treated patients, and TB/HIV co-infection complicates patient management causing diagnostic difficulties, drug interactions, adverse drug events, host immune re-constitution (T cell immune response), and emergence of drug resistance. MDR-TB cases were detected in 200,000 out of total 8.8 million newly infected TB patients and in 1.6 million of TB-related deaths.

HIV is associated with depletion of circulating CD4+ cells and their interferon gamma levels. Worldwide, the increment in HIV prevalence raises TB re-activations, and early case detection to prevent MTB mutant virulent species and epidemic mortalities (Basu *et al.*, 2009). Large effects of HIV infection significantly increase the proportion of diagnostic test non-reactors such as TST and IGRAs (Dodd *et al.*, 2010). HIV/AIDS has a statistically significant association with a low CD4+ T-cell count less than 200 cells /µL and is more likely to have normal or minimal CXR findings, and requiring other diagnostic tests (chapter 5; sections 5.1; Old diagnostic test of tuberculosis).

Periodic skin testing in primarily negative TST anergic individuals is useful to identify high risk groups before active TB disease develops. HIV sero-positive cases with/without acid fast bacilli (AFB) positive smear are commonly associated with absence or lack of detectable CXR cavitations and are considered less infectious due to weak type of cough and age > 40 years with less immunologic resistance compared with to HIV sero-negative individuals (Samb *et al.*, 1999).

A study done in North Nigeria reviewed patient records and revealed that out of 1,320 HIV/AIDS, those TB co-infected were 10.5% and have predictors of late clinical presentations according to WHO classifications and baseline lymphocyte CD4+ cell count was less than 200 /µL thus recommending IGRAs diagnostic tests (Iliyasu *et al.*, 2009). Another study done on 247 HIV positively diagnosed patients with a decline in CD4+ cell count revealed a decrease in indeterminate results using Enzyme-linked immunospot (ELISPOT) assay (Karam *et al.*, 2008).

In Vietnam, ranked by WHO as 13th among the first 22 high TB-prevalence countries, a study of 21,000 TB positive (+ve) patients revealed that urban populations are more HIV +ve than rural areas. Another conclusion was that HIV +ve patients have 50 % lifetime risk of progressing from LTBI to active disease form, compared with only 5-10% of HIV–/TB+ infected patients, and face higher mortality rates reaching 34% in those HIV+/TB+ sputum smear compared with 3% for HIV -ve infected persons. This was the only study done in South East Asia that revealed a significant reduction in mortality (up to 46%) for HIV+/TB+ patients

prescribed co-trimoxazole (CTX) antibiotic as preventive therapy recommended by WHO after noticing Vietnam TB/HIV syndemic spread (Trinh *et al.*, 2007).

Non-*Mycobacterium tuberculosis* infections reduce sputum smear microscopy specificity and late clinical stages with increase in risk of opportunistic infections and recurrences in individuals previously exposed to TB that have a low CD4+ cell count suggestive of immune-suppression states like diabetic mellitus (Iliyasu *et al.*, 2009).

In Cuba, both DM and alcohol abuse are specific risk factors facilitating re-activation of previous TB infection in close contacts (Matthys *et al.*, 2009). One contradictory multivariate analysis concludes that past history of TB was independently associated with positive TST and high CD4+ cell count (mean CD4+ > 550 +/- 220) but further surveillance studies are required to assess similar results (Britton *et al.*, 2005).

1.1.20 Tuberculosis diagnosis

Infectious disease diagnosis is important for differentiation between TB and other common infectious and mainly respiratory disorders and between active TB against LTBI where both are not only differentiated by INF-γ levels. For the diagnostic guideline and criteria's of TB described in the diagnosis of tuberculosis see chapter 1 section 1.2, section 1.3 and section 1.4.

1.1.21 Differential diagnosis

Any organ can be infected with MTB forming the characteristic 'tubercle' granulomatous lesion, but TB infection is associated with the respiratory system or lungs as the main route of entry (> 80% of occasions). Pulmonary tuberculosis should always be included in the differential diagnosis of persons with pulmonary signs or symptoms and appropriate diagnostic measures should be instituted (ATS, 1992).

1.1.21.1 Differential diagnoses of patchy lesions (Table 1.3)

Most of the lower respiratory tract infections (e.g. pneumonia) are causes of fever with productive/ purulent sputum. Similar diagnosis to TB, majority of the

underlying malignancy are causing weight loss, night sweats with suppressed immunity and pre-disposition to infections such as leukemia and lymphomas. HIV infection is associated with opportunistic chest infections and non-*Mycobacterium tuberculosis* manifested as cervical lymph adenopathy (see Table 1.3).

Table 1.3: Differential diagnoses of pulmonary against extra-pulmonary patchy lesion (Medical manual, 2010)

Pulmonary TB	Extra pulmonary TB
Lung infections: patchy lesion of pneumonia or abscess caused by various causative organisms (bacterial, viral, fungal, parasitic); e.g., *Streptococcus pneumonae , Staphylococcus aureus, Mycoplasma pneumonae, Pnumocystis carinii pneumonae* (PCP), viral, e.g. RSV, fungal mycosis e.g., histoplasmosis, aspergillosis, parasitic e.g. pulmonary echinococcosis, Bronchial carcinoma, Other non-infectious diseases e.g. silicosis, sarcoidosis, COPD	TB always considered in any extra pulmonary differential diagnoses

1.1.21.2 Differential diagnosis of other-than patchy lesions (Table 1.4)

Abnormal lung findings can be due to pulmonary tuberculosis or related differential diagnosis for other causes (Table 1.4).

Table 1.4: Differential diagnoses of pulmonary lesion(s) (Arthritis Center, 2009)

Pulmonary Nodule(s)	Pleural Effusion (exudative)
Infection (TB, fungi, pyogenic abscess)	Infection (TB, fungi)
Malignancy (bronchogenic, metastasis, lymph proliferative diseases)	Malignancy (bronchogenic, metastasis)
Arteriovenous malformations	Pulmonary embolism
	Gastrointestinal disease
Rheumatic disorders (rheumatoid arthritis, sarcoidosis, amyloidosis)	Rheumatic conditions (systemic lupus erythematosus)

1.1.22 Tuberculosis treatment

The principal goals for tuberculosis treatment are: 1)- to cure the individual patient, and 2)- to reduce the transmission of *Mycobacterium tuberculosis* to the community population, therefore, successful treatment has public health benefits through prescribing appropriate regimens and follow-up completion of therapy.

1.1.22.1 Drug category/choice of therapy

The aims of tuberculosis therapy are to cure the patient's symptoms and to minimize the possibility of transmission of the bacillus to healthy subjects. Adverse effects and drug interactions of anti-tuberculosis drugs may result in modification or discontinuation of treatment. Successful anti-TB management can restore the quality of life, reduce mortality from active TB, prevent case relapse and drug resistance development.

Studies on mycobacterium cell wall bio-synthesis such as mycolic acids and mycobactins have facilitated understanding of the mechanism of drug action required to design and test new specific anti-mycobacterial agents and to overcome resistance problems. Optimization of available therapeutic medication is used to improve drug quality and develop new regimens. MTB lipoid cell walls (containing mycolic acids and mycolate synthetase enzyme complex) are targeted by specific tuberculostatics (Table 1.5).

Table 1.5: Origin of anti-tuberculosis drugs in therapeutic use and their mode of action (Yew *et al.*, 2007)

Drug	Year of discovery	Mode of action
Streptomycin (S)	1944	Inhibition of protein synthesis
Pyrazinamide (P)	1952	Converted to pyrazinoic acid (inhibit protein and RNA synthesis)
* Isoniazid (INH) or (I)	1952	Inhibit mycolic acid synthesis (reduce NAD content)
Ethambutol (E)	1961	Slow inhibition of RNA synthesis (affect lipid and cell wall metabolism)
* Rifampicin (R)	1965	Inhibition of RNA synthesis (bind to bacterial DNA-dependent RNA polymerase)

 * means the most effective first line anti-tuberculosis treatment

1.1.22.2   Treatment schedule

The primary aim of chemotherapy in pulmonary tuberculosis is to render the sputum bacteriologically negative for tubercle bacilli and to effect a bacteriological 'cure'. Human and animal studies demonstrated that TB transmission and host infectiousness dramatically decreased within days to weeks from instituting effective treatment.

Active TB cases are treated using the standard short course regimen as prescribed (Table 1.5): For the first 2 months a combination of 4 drugs (P), (E), (R), and (I) then, for the next 4 months receive (R) and (I).

The treatment regimen for majority of TB cases is given as '2H3R3Z3E3/4H3R3', i.e. 2-month initial phase of HRZE given 3 times a week, followed by a 4-month of continuation phase of HR given 3 times a week (Table 1.6).

Table 1.6: Standard regimens for new TB patients (presumed, or known, to have drug-susceptible TB) (Medical manual, 2010; WHO, 2010f)

Intensive phase treatment	Continuation phase
2 months: HRZE(S)	4 months: HR

H = isoniazid, R = rifampicin, Z = pyrazinamide, E = ethambutol, S = streptomycin

Any patient is considered sensitive and cured after completion of prescribed therapeutic regimen and shows relief from disease complaints (even though relapse rates can reach of 2-3%). Prescribing INH for 9 months or as the new regimen of both INH plus rifampicin for 3 months, either is considered as LTBI preventive/prophylactic therapy especially for the high-risk groups. Even with free treatment prescriptions (45-88%) in the U.S the anti-tuberculosis (anti-TB) results were disappointing due to low treatment completeness ratio (16-79%) (Langenskiold *et al.*, 2008).

Age dose-dependent usage of anti-tuberculosis treatment differs worldwide according to national guidelines. WHO recommendations for newly diagnosed active TB are shown in Table 1.7.

Table 1.7: Recommended doses of first-line anti-tuberculosis drugs for adults (WHO, 2010f)

Chemotherapy agents	Recommended dose			
	Daily		3 times per week	
	Dose and range (mg/kg)	Maximum (mg)	Dose and range (mg/kg)	Daily maximum (mg)
Isoniazid (INH)	5 (4–6)	300	10 (8–12)	900
Rifampicin (R)	10 (8–12)	600	10 (8–12)	600
Pyrazinamide (P)	25 (20–30)	–	35 (30–40)	–
Ethambutol (E)	15 (15–20)	–	30 (25–35)	–
Streptomycin[a] (S)	15 (12–18)		15 (12–18)	1000

Prolonged anti-TB treatment causes a deficiency in serum vitamin D levels and an increase in the probability of LTBI and TB re-activation (Talat *et al.*, 2010).

1.1.22.3 Treatment evaluation

Successful treatment has been achieved for more than 36 million patients since 1995 via the WHO strategy for tuberculosis control. Evaluations consider the therapeutic outcomes and benefits against adverse effects e.g. INH-induced hepatitis against TB clinical improvement need regular follow-up even after therapy completion and reduced patient's anxiety (CDC, 2003; Tissot *et al.*, 2005) (Figure 1.10).
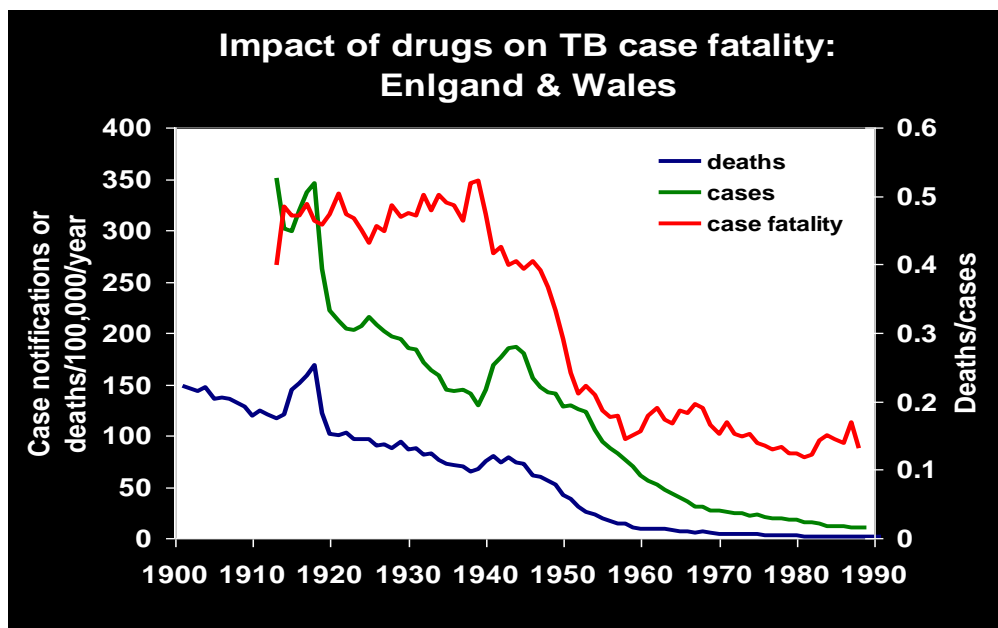
Figure 1.10: Example of drug impacts on TB case fatality in England and Wales (Dye, 2009) (DOA: 26/07/2008)

Infectiousness does not always end with diagnosis, and PTB relapses are related to risk factors such as smoking, non-adherence to regular treatment, and prolonged infectiousness. An inadvertent side-effect of expanding TB therapy is an increase in TB virulence (Basu *et al.*, 2009). False positive results lead to inappropriate initiation of chemotherapy, adverse effects and emergence of resistant MTB strains while false negative results lead to miss-diagnosis, facilitating transmission and LTBI progression. Both are related to unnecessary resource wastage and overburdening of health-system (Al Zahrani *et al.*, 2000).

Although combination drug therapy has cured sensitive- and resistant-MTB, still some patients fail to respond with serious clinical and epidemiological consequences e.g. treatment defaults and failure, with continuous uncontrolled MDR/XDR-TB cases in endemic regions such as India (WHO, 2009a).

1.1.23 Prevention of tuberculosis infection

The two clinically indistinguishable forms of TB disease entail different prevention strategies: primary TB is prevented by strict contact tracing with treatment of infected contacts, whereas prevention of re-activation TB is accomplished by screening for, and treatment of, latent tuberculosis infection (LTBI). Successful preventive measures can be implemented by assigning responsibilities for the control programmes, conducting regular TB risk assessments through developing written control plans e.g. early detection and treatment of TB-related cases, personal protection through population education, laboratory and treatment availability, and screening of high risk groups.

Any control programme must ensure early detection e.g. airborne precautions and treatment of both suspected and confirmed diagnosed TB cases. Emphasis is needed on periodic guideline revision for each community high-risk group including Group 1 - indirect exposure to TB patients e.g. HCWs in hospital departments or Group 2 - indirect exposure e.g. travellers and preventing occupational TB (CDC, 2008a).

The setting of goals for future implementation over the 10 years, 2006 – 2015, was a crucial step toward TB elimination by assessment of all Stop TB Strategy steps such as the goal of revising the TB incidence globalization in 2015 or the role of reducing TB prevalence and mortality rates by 50% relative to 1990. The WHO report on Global TB Control in 2007 concluded that 2005 targets were met by the Western Pacific Region and 26 other countries including high-burden areas; China, Philippines, and Vietnam.

Preventive chemotherapy is defined as the treatment of subclinical, latent infection with *M. tuberculosis* to prevent progression to active TB (Rieder *et al.*, 1994).

Treatment follow-up is represented by the directly observed treatment, short course (DOTS) strategy, as an international recommended step for treatment completion since the mid-1990's for implementation in any TB control programme, especially for individuals having new smear-positive or combined smear–negative and with extra-pulmonary complications (WHO, 2006).

Cost-effectiveness facilitates DOTS implementation in 182 countries (cost per disability-adjusted life-year (DALY)) implemented in African countries was U.S.$1-3 and East Asia about less than U.S.$2 and having five essential components: political commitment, diagnosis by sputum smear microscopy, reliable drug supply, short course treatment with standard first line drug regimens, and TB control offices for a recording/notification system allowing assessment of individual patient outcomes and overall programme performance (Baltussen *et al.*, 2005) (Figure 1.11).
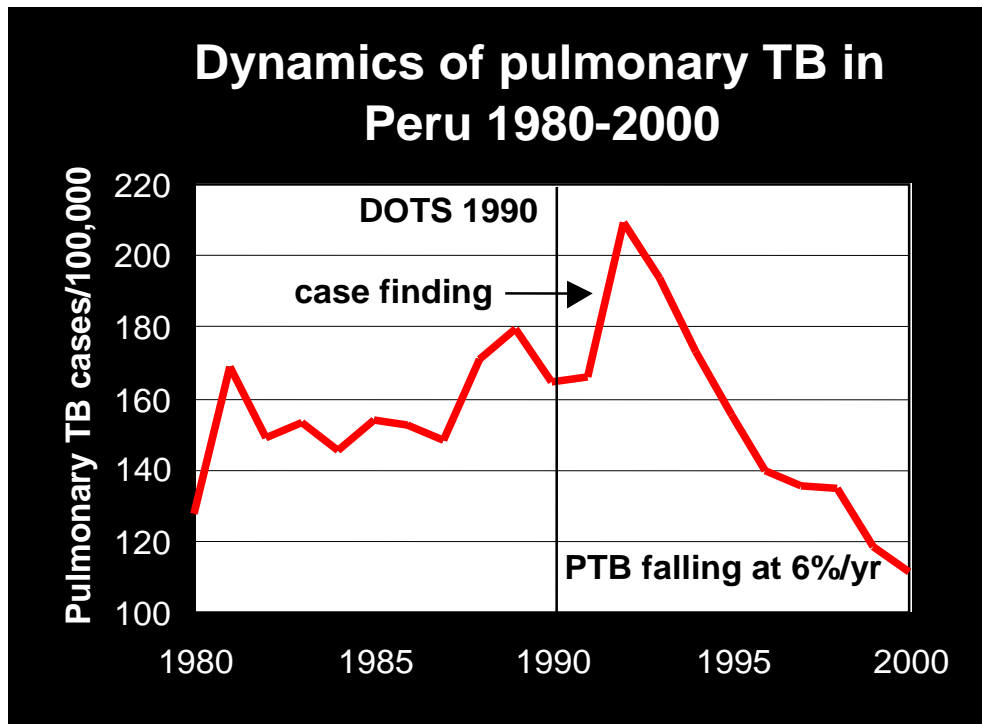
Figure 1.11: Pulmonary tuberculosis in Peru, in the 1980's and 1990's (http://apps.who.int/tb/surveillanceworkshop/) (DOA: 26/01/2012)

Successful application of DOTS can achieve TB elimination and LTBI prevention toward further prevention of LTBI progress (WHO, 2007a).

In 2006, WHO addressed the new Stop TB Strategy with the following strategic steps:

1. High-quality services of DOTS expansion (free availability of anti-TB treatments with direct follow-ups) e.g. in 2004, more than 22 million TB patients treated in various 183 countries

2. Address the issues of TB/HIV co-infection and appearance of severe resistant strains treated by DOTS as target elimination by 2015

3. Strengthen the health system through contribution of national TB control programme e.g. planning, management and supply systems

4. Engage all care providers from public, private, and health-care workers e.g. ensure TB case notification and high-quality care service

5. Empower people afflicted with TB e.g. applying community TB care projects to ensure public education and sustained political support, and

6. Promote research for disease prevention.

1.1.24 Tuberculosis infection control

1.1.24.1 Preventing latent TB progressing to TB disease

Normally people with LTBI never develop active TB disease but some people are more likely to do so due to the presence of TB-related risk factors e.g. HIV +ve individual, re-infected with TB within last two years, young children, the elderly or those that have been improperly treated for TB. High risk groups require preventive treatment prescribed by health care facilities for those holding lower bacterial levels (or latent tuberculosis cases).

1.1.24.2 Infection control within health care settings

Health care settings need a well designed infection-control programme mainly to detect airborne infectious and highly contagious organisms and to provide treatment of all persons at high risk of exposure e.g. HCW of all health departments. Periodic

review of guidelines' and evaluation of programme effectiveness are also needed to minimize TB transmission risks.

1.1.24.3 Preventing exposure to TB while traveling abroad

Level of knowledge about LTBI/TB disease for traveller's should be raised through regular administrative procedures to limit close contacts or prolonged duration of exposures' to crowded endemic regions, and consulting travel clinics, and personal protective devices e.g. facial mask (CDC, 2005a).

1.1.25 Public health control

All diagnosed TB cases should be notified to health-related authorities e.g. Ministry of Health departments and WHO TB control programs (WHO, 2009a):

Case - All infectious cases (mainly pulmonary or laryngeal TB) should be; 1- early isolated, 2- interviewed, 3- collecting consecutively a three-day sputum samples post-discharge, and 4- ensure adequate treatment completion. All these step help restriction of index infectiousness.

Contact - All contacts with an active TB index case should be screened using CXR and TST according to the country policy guidelines and repeated for inconclusive results before a prophylactic therapy decision is taken.

Prevention and control - The advice given by the UK Department of Health remains similar to that advocated by Sir Robert Philip over 100 years ago: ''The most important part of controlling TB is identifying and treating those who already have the disease, to shorten their infection and to stop it being passed on to others'' (Figures 1.12 A and B):

A: Tuberculosis education in school lesson   .   B: Tuberculosis patients inside cover shelter

Figure 1.12: Tuberculosis prevention and control programmes in UK, 1940's
(http://www.lhsa.lib.ed.ac.uk/exhibits/tales/tuberculosis.html) (DOA: 26/01/2012)

Diagnosed active TB cases should have follow-up precautions and strict instructions to limit transmission of TB contagious bacilli. The WHO Stop TB Control programme for TB elimination is an essential target to be reached before 2015, in addition to poverty eradication, universal education, reduction of maternal/child mortalities, and combating common infectious diseases like HIV/AIDS and malaria. Figures 1.13 and 1.14 show the similarities in approach between different centuries.

Figure 1.13: American Red Cross poster campaigning against tuberculosis-Fight tuberculosis on the front door (http://www.zazzle.com/fight_tuberculosis_us00212_poster-228486111885147844) (DOA: 26/01/2012)

Figure 1.14: World TB Day 2011 TB-Elimination: Together We Can!

(http://www.cdc.gov/tb/events/WorldTBDay/PosterLg_2011.htm) (D0A: 26/01/2012)

Common examples of control guidelines for TB elimination against spread of infection are shown in Table 1.8.

Table 1.8: CDC tuberculosis classification for immigrants and refugees (CDC, 2008a)

Tuberculosis classification of United States immigrant/refugee			
Disease class	**CXR**	**AFB smear**	**Restrictions**
A (TB, infectious)	Active TB	Positive	No entry to U.S. until treated and smears are negative
B1 (TB, clinically active, not infectious)	Active TB	Negative	Report to local health department for further medical evaluation within 30 days of arrival in U.S.
B2 (TB, not clinically active)	Inactive TB (LTBI)	Not required unless symptomatic	Same as above
No class (Normal)	Normal	Not required	None

AFB = acid fast bacilli

Researchers are applying state-of-the-art genomic and post-genomic techniques to identify key molecular pathways that could be exploited to develop improved TB

interventions and preventive vaccines. For example risk factor for studies is associated with strain clustering was linked to country of origin and previous city of residence (Franzetti *et al.*, 2010).

1.1.26 Diagnosis of tuberculosis-related disorders

Worldwide, tuberculosis exists at various scales from low incidence to epidemic and the disease still remains difficult to diagnose, especially in regions with limited resources. The WHO priorities for a TB-free-future stress on improvements of diagnostic accuracy with reliable integration of human tuberculosis. National control programmes in high prevalence regions are driven towards reducing the burden of TB and helping to define end-points in trials of new TB vaccines. Missed diagnoses of active TB or that treated prophylactically as LTBI are associated with appearance of drug resistant strains (Balabanova *et al.*, 2005).

Timely diagnosis and treatment is important for the patient morbidity and mortality, Tuberculosis diagnosis is 100% accurate for the gold standard test of culturing bacilli for 4 to 8 weeks for a definitive bacteriological diagnosis. However, TB still can be suspected among any asymptomatic individual that has no specific clinical symptom/or sign. Thus identifying a quick and efficient TB diagnostic tool is a major global public health priority.

Clinical interpretation combined with assessment of cost-effectiveness plays an important role in LTBI detection and TB control. Significant disagreement between diagnostic tests results would necessitate an evidence-based medicine (EBM) for a comprehensive technique to support before taking medical decision. False-positive diagnoses result in medical service wastage e.g. inefficient resource use and false-negative diagnoses threaten the public health e.g. by risking the spread of TB infection. Differentiated from other TB-related disorders a complete medical evaluation for tuberculosis must include a medical history, a physical examination, and a radiographic examination (e.g. chest X-ray) and/or microbiological examination (e.g. IGRAs) (described in the following sections: 1.2, 1.3 and 1.4).

1.2 Tuberculosis clinical diagnosis and case definition

A retrospective analysis of respiratory medicine in 2003 concluded that the clinical practice with scientific evidence-based medicine (EBM) test results was the major goal for early diagnoses and management to improve community health. Various diagnostic and disease monitoring tools are not supported by a high quality of evidence testing's e.g. subjective reading of TST positive reaction differs between different countries according to TB endemicity which necessitates improvements in the diagnostic accuracy of TB and PTB-related clinico-laboratory-radiography combinations (Borrill *et al.*, 2003).

1.2.1 Medical history

The WHO recommends TB investigations any individual having persistent cough more than 3 weeks until proven otherwise, similarly to any chest X-ray cavity as probable LTBI until proven otherwise.

1.2.1.1 Direct history (un-structured interview, face-to-face)

TB can be diagnosed through passive case detection i.e. detection via symptoms. History taking from the subject includes obtaining symptoms of TB and PTB disease such as prolonged cough more than three weeks associated with/without chest pain. Other generalized symptoms include low-grade remittent fever, chills, night sweats, appetite loss, weight loss, easy fatigability. Expectorations such as those producing mucoid sputum changing to purulent or hemoptysis (blood) can be considered as a clinical predictor in smear-negative TB patients (Kanaya *et al.*, 2001). Other directly related medical history includes prior TB exposure or past TB treatment and searching for other epidemiological TB-related risk factors such as HIV. For example smear-negative PTB cases have lower experience of cough for more than three weeks than smear-positive PTB cases (45% vs. 72%) (Tamhane *et al.*, 2009).

1.2.1.2 Patient screening questionnaire (structured interview)

A screening tool facilitates LTBI epidemiological case definition and diagnosis (discussed in detail in chapter 4).

1.2.2 Physical examination

Medical/ clinical examination looking for physical signs performed to detect subject experienced symptoms. Any shared or common finding(s) to other health-related disorder¸ like fever, chest pain or productive cough, will complicate TB diagnosis. Pulmonary presentations are common in adults, whereas, systemic complications (TB dissemination) are noticed in children or immuno-compromized individuals.

PTB thoracic sequelae involve all parts of the chest and respiratory tract system; acute respiratory distress syndrome (ARDS), multiple cystic lesions, extensive consolidations, pleural effusions, pneumothorax or empyema.

Other symptom-based characteristics proposed for active TB diagnosis in children are the following predictors: 1) persistent, non-remittent coughing or wheezing; 2) failure to thrive despite food supplementation; and 3) fatigue or reduced playfulness.

Comparative studies testing immune-competency in controls reveal that TB cases lose more than 10% of total body weight within short time period, night sweating, hypoxemia, abnormal sputum and sometimes auscultation abnormalities. Systemic or organ/tissue evaluation and lung examinations by auscultation and percussion help limiting TB differential diagnoses and reaching final correct diagnosis. Case finding depends on the various clinical presentation(s) to the health service. Mean duration of symptoms (287 days) was longer in subjects reporting coughing with other chest symptoms compared to relatively short durations (146 days) from reporting a cough alone. The longer the period of symptomatic complaints (> 52 weeks) is associated with having high sputum positive grading (3+) and the presence of a sick contact within the same household. Smear positive grading was not associated with systemic symptom combination compared with significant findings in patients having only symptomatic chest complaints (El-Sony *et al.*, 2002).

1.2.3 Laboratory diagnostic testings for tuberculosis

WHO Stop TB Strategy recommends an evidence-based laboratory identification of MTB in TB diagnosis. Result discrepancies reach 58% between clinical findings, laboratory results and treatment outcomes, by which international treatment regimens can be accurately prescribed after the discovery of reliable diagnostic technique. For example, reduced awareness of inconsistent laboratory errors leads physicians to rely on radio-graphical findings in TB management resulting in over-diagnosis and improper treatment of false positive (FP) TB case (Joshi *et al.*, 2005).

Nowadays, laboratory case definition depends on the demonstration and isolation of MTB from clinical specimens (respiratory or non-respiratory), but is dependent on the diagnostic criteria for each country and their health resources.

1.2.3.1 Microbiological studies

Microscopic investigation are performed after obtaining samples of body tissue or fluid samples using different procedures: Acid Fast Bacilli (AFB) are stained with e.g. Ziehl-Neelsen (ZN stain) or Auramine stain (AS), and bacteria can be grown in TB growth culture using liquid media (Middle Brook) or solid media (Lowenstein-Jeensen: LJ). Microbiological diagnosis requires a concentration technique to maximize organism numbers and a decontamination procedure to kill other contaminating micro-organisms.

The following examples are commonly accepted international diagnostic tests;

1.2.3.1.1 Sputum smear sample (SSS)

Sputum comprises secretions expectorated (the Latin ''to spit'') from respiratory airway tracts e.g. mucus and saliva and is abnormally associated with lung diseases e.g. coughing blood (hemoptysis) in tuberculosis disease.

Bacteriology is the smear status of pulmonary cases by which identification of *M. tuberculosis* is made for or any case by culture or other newer methods. Smear-positive cases are the most infectious states, likely to transmit MTB and TB disease

in their surroundings i.e. considered as the focus for infection targeted for control measures and contact investigations. A new sputum smear-positive pulmonary TB case is defined by the presence of at least one acid-fast bacillus (AFB+) in at least one sputum sample (WHO, 2009a).

The WHO recommends staining for microscopy (M/S) using ZN stain as the primary diagnostic strategy for TB diagnosis in the developing countries (Sadaphal *et al.*, 2008). Microscopic examination of a sputum smear with ZN stain for AFB is a 100-year-old method using special bacteriological stain that identifies TB acid-fast organisms. It is simple, inexpensive, accessible and cost-effective, and can be used as the primary and relatively rapid test for prediction of culture outcomes.

The limitations of the test are that the test requires a minimum number of 10,000 organisms/ ml as resolved by detergent concentrations, shows lower sensitivity (63%) and specificity (97%), results in pauci-bacillary samples e.g. HIV +ve smear sample, and cannot differentiate between MTB and NTM (Tiwari *et al.*, 2007). Obtaining insufficient sputum will delay TB diagnosis and requires repeating SS or using a forced sputum collection (induced-type collection) (Greenberg *et al.*, 1994).

A positive sputum sample will denote infectiousness even in an asymptomatic case. Infectiousness is also correlated with sputum bacillary numbers (sputum grading) and duration of infectiousness e.g. higher grading by sputum smear is usually coincident with an individual who has been symptomatic for a longer period.

Case detection significantly increases with longer SS examination time (10 minutes instead of 2 minutes) (Cambanis *et al.*, 2007).

If sensitivity rose to more than 85% then it is estimated that an additional 400, 000 individuals could be saved each year globally. Reduction of ZN-phenol decreases staining sensitivity and TB detection (Selvakumar *et al.*, 2005). AFB stain of pre-bronchoscope sputum requires concentration (using sodium hypochlorite) by a trained technician to increase SS positivity (Aderaye *et al.*, 2007).

Any suspected individual is considered non-infected after three consecutive negative samples. A definite or relapsed TB case requires two repeated positive specimens or at least one positive culture result before final diagnosis (Keeler *et al.*, 2006).

Risk factors for SS +ve samples are age (15-44 years), alcohol (delays in seeking medical care and TB diagnosis) and male gender (longer exposure) (Godoy *et al.*, 2004). HIV patients become less infectious in later disease stages of their disease, complicating TB detection having SS –ve sample and obtaining MTB culture growth (Apers *et al.*, 2003). Such difficulties were revealed in another comparative study between four African countries sharing common geographic borders and social life due to different HIV prevalence's of more than 33% e.g. Kenya, Zaire, Zambia and South Africa (Samb *et al.*, 1999).

1.2.3.1.2 Culture and antibiotic sensitivity

The microbiological culture method is the multiplication of microbial organisms using specific growth culture media under controlled laboratory conditions to determine the type of infectious organism, and is commonly used for respiratory sample pathologies. Microscopic observation of broth culture is rapid, less expensive than the new TB diagnostics such as IGRAs and laborious but would be suitable in the developing countries (Tiwari *et al.*, 2007).

The isolation and culture of tubercle bacilli remains the 'gold standard' for definitive diagnosis of tuberculosis with 100% specificity and no missing of any PTB case, but, requires four to eight weeks duration for achieving the final results due to slow growth of the MTB. Culture is also useful in differentiating MTB from other mycobacteria's. Other limiting factors for isolation are paucibacillary samples mainly in childhood lesions and the requirement for more than 100 bacilli to be present in 1 ml of specimen compared with 5,000-10,000 bacteria of 1 ml SS sample (Aderaye *et al.*, 2007). Collection of gastric aspirate or broncho-alveolar lavage (BAL) for smear and culture is the most practical method.

Examples of commonly used MTB culture methods include:

BACTEC 460 radiometric TB systems (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA) widely used and considered as reference standard test in developed countries especially for EPTB and SS negative samples.

Anti-microbial drug susceptibility (ADS) has short MTB detection rate (mean reaching 14 days), but there are disadvantages in the high costs and the requirement for safe disposal of radioactive waste products (Rodriguez *et al.*, 2007). In a study of 337 samples examined in Spain comparing BACTEC 460 test and LJ media, BACTEC 460 showed higher sensitivity and negative predictive values, respectively, (97% versus (vs.) 90%, and 92% vs. 81%) with 100% similar specificities and shorter detection time in both SS positive samples (7 days vs. 24 days) and SS negative samples (11 days vs. 29 days for LJ media) (Casal *et al.*, 1997).

The **MGIT 960 mycobacterium detection system** (Becton Dickinson) is a new automated non-radioactive technique that identifies mycobacterium growth using a sensor to detect oxygen utilization for intensifying dye fluorescence present in detection tubes (Tiwari *et al.*, 2007).

1.2.3.1.3 Bronchoscopy

Bronchoscopy is a visualizing technique that can directly examine the inside of airways. Bronchoscopy is an invasive test used to view airway abnormalities e.g. vascular bleeding, lung tumour biopsy and for therapeutic purposes (e.g. tumour surgical therapy, foreign body removal). The procedure shows a high level of accuracy level in diagnosing respiratory infections and missed focal abnormal CXR findings and guides broncho-alveolar lavage (BAL) to obtain samples confirming TB diagnosis even in normal CXR to rule out other possible diagnoses (Rosenthal, 2003). For example, bronchoscopy diagnosis of complicated endo-bronchial TB cases in patients having high C-reactive protein levels (CRP) and small TST induration size less than 5-mm higher than undiagnosed EPTB cases (Araz *et al.*, 2008).

Fluids obtained by bronchoscopy are expensive and the procedure can be risky, but many lung-based immunological studies rely on bronchoscopy (Breen *et al.*, 2007). New hospitals reserve bronchoscopy as a reference diagnostic test for all suspected respiratory complaints, and in addition to performing two gastric washes (GW) it is regarded as the optimal choice for PTB diagnosis in a SS negative sample in a patient

presenting with a difficult diagnosis (Dickson *et al.*, 2003). Rare disadvantages that have been noticed include airway tears, bleeding, pneumothorax and laryngospasm.

1.2.3.2 Molecular diagnosis

1.2.3.2.1 Interferon gamma release assays (IGRAs)

IGRAs involve two immunological blood tests; QuantiFERON test and T-SPOT .*TB* test using specific antigens to detect LTBI in those previously exposed with MTB (Schablon *et al.*, 2009). IGRAs are discussed in section 1.4.2.

1.2.3.2.2 Nucleic acid amplification test (NAA)

The newest laboratory tools for the diagnosis of TB are nucleic acid amplification (NAA) tests which are performed on direct specimens in several hours. Nucleic acid sequences tests are heterogeneous tests using the polymerase chain reaction (PCR) technique to amplify and detect mycobacterium NA with superior accuracy. The test can be used for respiratory samples and is quick and sensitive. Tests are however labour-intensive and specific tests for MTB (e.g. PCR, DNA and RNA probes and IGRAs) have a limitation in low-income resource countries due to the expensive cost of equipment and consumable reagents. NA targeted sequencing reveals a highly accuracy result and replaces other diagnostic tests such as insertion sequence (IS) 6100 specific for MTB (Campbell *et al.*, 2006).

Two commercial NAA tests are available that are approved by FDA in 2006 for organism identification, speciation, and drug mutational resistance. These include the Amplified *Mycobacterium tuberculosis* direct test (AMTDT, Gen-Probe) with an overall sensitivity of 92% and specificity of 99%, and the Amplicor test (Roche Diagnostics) (Nolte, 2006). AMTDT can predict culture outcome before making anti-TB treatment decision, due to positive concordance with AFB staining and increased PPV, where both tests show positivity with 99% accuracy (Gallina *et al.*, 2000).

In Egypt, a prospective study evaluated 50 children at risk from families having a positive history of TB and concluded that the Gen-Probe technique could rapidly detect MTB in respiratory samples (sensitivity 96.7%, specificity 100%, and

accuracy 98%) (el-Sayed Zaki *et al.*, 2008). Amplicor MTB detects minimal active TB disease with 100% specificity but lower sensitivity (73%) (Al Zahrani *et al.*, 2000).

1.2.3.2.3 Polymerase chain reaction (PCR)

PCR is a selective direct amplification technique targeting specific DNA sequences and generating millions of copies of particular DNA sequences relying on thermal recycling. Primers (short DNA fragments) are complementary to their target region enabling selective and repeated amplification. PCR has revolutionized the field of infectious diseases as a diagnostic molecular technique since 1984 (e.g. real-time PCR) proven with highly sensitive and specific parameters relative to other conventional diagnostic methods and as an aid in rapid diagnosis of EPTB patients; sensitivity 89 % (75–100 %), specificity and PPV are 100%, NPV 96 % (91–100 %) and test accuracy 97 % (93–100%) (Queipo-Ortuno *et al.*, 2009).

PCR is rapid, easy to perform with high sensitivity > 80% and high specificity > 99%. Better results are also revealed in SS positive smear (96%) than SS negative (53%) smear (Roos *et al.*, 1998). Post-sample collection, PCR amplification is completed within 2-4 hours and the result detection assay requires another 2-24 hours.

Direct PCR methods were targeted at 11 regions of MTB HE7Rv (and absent from *M. bovis*) based on DNA amplification tests applied to 273 sputum samples revealed agreement with LJ culture media in comparing DNA purification/DNA amplification; 94% with culture results, sensitivity (93%), specificity (96%) and perfect concordance between both tests (Pinto *et al.*, 2007).

Comparative results of sensitivity and mean detection time to other MTB diagnostic procedures were respectively; PCR (sensitivity 74%, MDT < one day), LJ culture (49%, MDT = 24 days) and BACTEC culture (59%, MDT = 12.89 days). Comparison of other 145 samples test sensitivities (sensitivity & specificity) of suspected MTB cases for early PTB and EPTB diagnosis were respectively; PCR (73% & 76%), BACTEC test (54% & 55%), LJ media (51% & 47%), and ZN stain

(50% & 20%) (Negi *et al.*, 2005). PCR can also enhance diagnostic ability in pauci-bacillary infection and atypical tuberculosis presentations.

A study performed on 120 Tanzanian HIV positive patients testing collected from BAL, the sensitivity of both AFB smear (66%) and serology (zero%) against real time-PCR higher sensitivity (57-85%) and specificity (91%) in diagnosing active TB disease. Another conclusion for the low CD4+ cell count patients is that real-time PCR reduces level of contamination, reduces false positive and false negative, and diagnoses active MTB in BAL with high sensitivity (85%) and specificity levels (91%) (Kibiki *et al.*, 2007).

In Uganda, sputum PCR (targeting secA1 gene specific to MTB) on 127 HIV -ve subjects was shown to be highly sensitive (99%) and specific (88%) for PTB detection in comparison with oral wash PCR (73% sensitivity and 88% specificity) (Davis *et al.*, 2009).

A comparison between PCR, AFB staining and AFB growth culture methods on 1,134 pulmonary samples and 1,839 non-pulmonary samples using estimated sensitivities as follows; PCR 77.5%, AFB culture 80%, AFB stain 49% and specificity as follows; PCR 100%, AFB culture 100%, AFB stain 99% (Kim *et al.*, 2008).

PCR is not useful for testing drug sensitivity and NTM diagnosis. PCR use is limited due to the high cost of the test (U.S.$15 per test) and is not feasible at present in most clinical laboratories, is unable to differentiate viable from non-viable AFB, and is not useful for drug sensitivity testing and NTM diagnosis, and common false positive results are associated with laboratory-introduced contamination (Tiwari *et al.*, 2007). Another limiting factor is contamination of samples or the presence of inhibitors leading to false-negative or false-positive results.

1.2.3.3 Other diagnostic tests requiring laboratory analysis
1.2.3.3.1 Complete blood count (CBC)

Blood tests such as full blood count, erythrocyte sedimentation rate (ESR) and urine and saliva samples also can be used for indirect MTB detection. CBC is never a

definitive diagnostic test but can be used to limit other differential diagnoses e.g. iron deficiency normocytic anemia with neutrophilia and lymphopenia are common blood findings in cases of active TB disease. New DNA tests are diagnostic for MTB bacilli.

1.2.3.4 Radiological diagnostic tests

Radiology is the branch of medicine by which the interior of the human body is visualized using various forms of radiation. Radiologists utilize an array of imaging technologies to diagnose or treat diseases such as ultrasound (US), computerized tomography (CT), and magnetic resonance imaging (MRI). Interventional radiology is performing medical procedures (usually minimally invasive) with the guidance of imaging technologies. Medical imaging is carried out by the radiographer or radiological technologist and read by the specialist radiologist.

Radiological screening is considered as another major diagnostic tool for identifying respiratory abnormalities. LTBI detected as post-active pulmonary disease using the regular revision guidelines of WHO recommendations, the American College of Radiology and UK National Institute of Health and Clinical Excellence (NICE) to improve diagnostic test accuracy (MacPherson and Gushulak, 2006).

1.2.3.4.1 Chest X-ray (CXR)

See below - section 1.3.1 Radiographical diagnostic test for tuberculosis.

1.2.3.4.2 Computerized tomography scan (CT scan)

CT scans or computed axial tomography (CAT) scans were introduced in the 1970s as medical imaging methods to generate three-dimensional images and are considered as rapid tests for diagnosing and screening of multiple disease entities supplementing X-ray results and detecting both acute and chronic changes in lung parenchyma. Cavitation is a common sign of LTBI and TB disease activity or miliary TB spread (hematogenous-dissiminated small nodules occurs in 2-6% of primary TB) (Nakanishi *et al.*, 2009). Other common CT findings of PTB re-activation are centri-lobular small nodules, large nodule more than 8 mm, branching linear and

nodular opacities (tree-in-bud sign), and patchy or lobular opacities of consolidation. The main advantage is achieved by reducing super-imposition of structures outside the lungs, but hazards of radiation doses and the expensive cost of the computing machine limit CT usage.

CT is indicated for MTB detection in normal or inconclusive CXR and determines TB disease activity and complications e.g. 1-3 mm small size nodules. CT is valuable for a clinically ill patient having in-conclusive CXR findings for management guiding. A study done on 139 HIV +ve patients, CT was superior (69%) more than CXR (10%) in detection and exclusion of TB pulmonary complications e.g. severe pneumonias or atypical manifestations (Jeong and Lee, 2008). High-resolution CT (HRCT) predicts the risk of PTB in SS –ve sample with good reproducibility in probable TB cases.

1.2.3.4.3   Other radiological diagnostic tests

Magnetic resonance imaging (MRI) has the highest specificity and sensitivity indicated according to the country health resource budgets and cost/ benefit ratio.

Comparison between tuberculosis radiologic and laboratory diagnostic tests are detailed accordingly;

1.3 Radiological diagnostic test for tuberculosis
1.3.1 Chest X-ray (CXR)

The WHO recommends in imaging studies using chest radiography to detect chest and lung lesion(s) when any 'suggestive appearance of chest TB finding(s)' is considered as probable TB case until proven otherwise.

Chest radiographic findings and patterns might be most accurately interpreted in conjunction with clinical history and specifically prior TB treatment. Even though, approximately half of normal healthy individuals do not show a common "expected" radiographic pattern of LTBI. Recent primary TB is characterized by lymph adenopathy, pleural effusion, lower or mid lung zone infiltrates, whereas, lesions due

to TB re-activation(s) are typically located in the lower part of upper lobes or the upper part of middle and/ or lower lobes with characteristic cavitatory lesion.

CXR images are taken for all body anatomy e.g. chest wall, heart, bones, and is still considered as the standard rapid test (usually completed within 15 minutes), simple (no special preparation), commonly performed, cheap (inexpensive machine), excluding other salaries e.g. in a Cambodian survey the total costs of screening 30,000 subjects was U.S.$550,000 (U.S.$25 per person), non-invasive (painless), rare side effects, suitable for medical surveillance and diagnosing and evaluating lung response to medical treatment. CXR images expose the body to small doses of ionizing radiation to produce an internal image (Glaziou *et al.*, 2008).

CXR equipment has a fixed box-like apparatus containing X-ray film or an image-recording digital plate and X-ray producing tube. X-rays are small-burst radiations pass through objects to record images. Various films depend on the body part's degree of absorption e.g. bone is more absorbable (appears white) than soft tissue or muscle (appears gray) while lung air (appears black) against lung tissue (dark) (Figure 1.15).

Two chest views are taken from the back (poster-anterior; PA) and from lateral side (lateral; L). PA is more informative but still using both views show higher diagnostic accuracy (OR 3.7) relatively than taking separate PA view (OR 3.09) or lateral view (OR 2.4) (Parimon *et al.*, 2008) (Figure 1.15).

Figure 1.15: Procedure of chest X-ray radiography taking postero-anterior (PA) view (http://www.radiologyinfo.org/en/pdf/chestrad.pdf) (DOA: 26/01/2012)

CXR is indicated for any suspected TB chest complaint, such as cough, fever, hemoptysis, and shortness of breath but with contra-indications in pregnant women (fetus radiation exposure), chest metals (image interference) or risk of cancer (with repeated and high radiation dose exposure). In 2000, the American Thoracic Society (ATS) and CDC recommend using a single PA radiograph for all TST positive adults and children more than 5 years of age, with limitation to lateral radiographs due to economics saving U.S.$18 per case and U.S.$72,000 per year and reduction in annual case load of 4,000 radiographs and radiation exposure (Meyer *et al.*, 2003).

Characteristics

Chest radiographic classifications for TB abnormalities are categorized into five broad groups:

1. Normal or minor findings (non-related to TB)

2. Granuloma's with or without calcification considered likely due to remote TB infection (LTBI) and/or is defined as possible active TB. Another three findings are considered as consistent with probable active TB:

3. Apical fibro-nodular disease or multiple non-calcified nodules

4. Pleural disease or mass in the parenchyma, hilum, or mediastinum, and

5. Parenchymal lung multiple infiltrates with or without cavitations'.

CXR can detect chest lesions e.g. cavitatory or nodular lesions, even in the absence of another superior diagnostic test like a SS +ve sample, but still cannot exclude TB particularly in immuno-suppressed patients, as category (Figure 1.16).
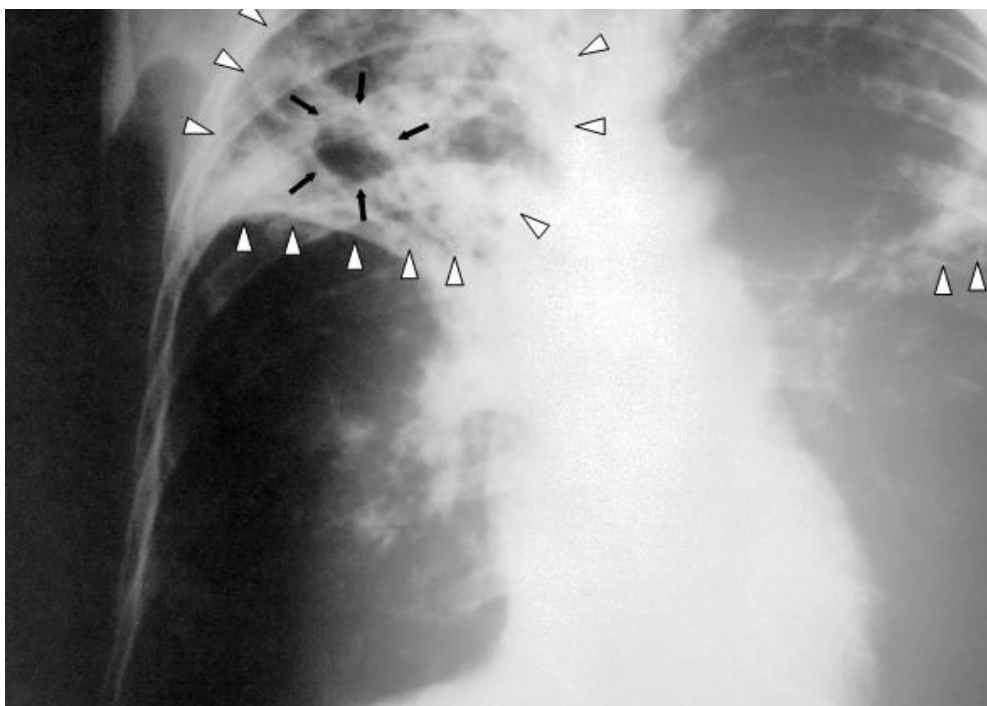
Figure 1.16: Chest X-ray of patient diagnosed with advanced bilateral pulmonary tuberculosis showing bilateral pulmonary infiltrate and cavitatory formation (http://emedicine.medscape.com/article/230802-diagnosis) (DOA: 26/07/2009)

Radiological differences between outbreak and non-outbreak cases reported that cavitations were not characteristic of outbreak cases. For example the extent of cavitations in HIV-positive patients has been directly correlated with CD4+ cell counts, and the radiographic changes in the outbreak cases may reflect severe immuno-suppression at the time of *M. tuberculosis* infection. Outbreak patients of

nosocomial exposure usually share several characteristics. HIV-positive and significantly immuno-compromised and HIV-positive patients, having average mean CD4+ cell counts of 119 cells/mm^3 usually presented with common acute febrile illnesses and might harbor of MDR-TB super infection (Sacks *et al.*, 1999).

Radiological findings of patchy consolidation, nodular opacity, mass-like lesion, fibro-calcified lesion and pleural effusion are typical PTB findings and there is a significant positive association with foreign-born population from TB endemic region (high TB incidence > than 20 cases per 100,000 population) compared to TB low endemic areas (low TB incidence < than 20 per 100,000 population) (ACHA, 2011).

Periodic CXR screening of LTBI-infected HCWs and for patients previously treated is crucial and has been recommended since 1983 by FDA and WHO (Mangura *et al.*, 1999). Fibrotic lesions are defined as well-delineated radiographic lesions compatible with healed tuberculosis (Rieder *et al.*, 1994).

CXR a routine test for screening immigrants in suspected mid-adolescence having pauci-bacillary sputum smear with low risk of TB secondary transmission (MacPherson and Gushulak, 2006). Suspected PTB in children is better diagnosed by pediatrician's with a higher specificity and diagnostic accuracy (diagnostic OR 8.3) than primary care clinicians (diagnostic OR 2.5) (Swingler *et al.*, 2005). Prevalence of radiologically active TB disease was 33 per 1,000 individuals and higher than previous estimates of community radiographic surveys undertaken between 1955-1957 in India (13.5 to 26.6 per 1,000 populations) (Joshi *et al.*, 2007).

Statistical parameters

Chest X-rays show variable statistical characteristics. Usually CXR-tested TB suspects and those having 'any pathology detection' showed lower sensitivity and specificity than those radiographic CXR-tested and diagnosed within the 'consistent for TB' class.

CXR statistical finding in scoring 'TB' among those suspects having sputum smear negative was sensitivity 80% and specificity 67% compared with score suspect of

'any pathology' respectively; 92% and 63%. Sensitivity (77%) of scoring 'highly consistent for TB' among suspected HIV negative was higher than among HIV positive suspects (49%) (van Cleeff *et al.*, 2005).

Poor inter-reader agreements' of pulmonary parenchymal abnormalities on chest radiographs suggests offering a list of differential diagnoses instead of an actual diagnosis. Researches in radiographic interpretations should focus on the potential lack of consistency in CXR interpretation reporting abnormalities, which can be improved with involvement of more radiography readers'. For missing of specific difficult radiographic findings, inter- and intra-observer reading is recommended to be assessed using kappa (k) agreement value (Bloomfield *et al.*, 1999).

Overall good intra-reader agreement (k = 0.621) and moderate for inter-reader agreement (k = 0.439) improved with repeated training on reading and following reporting system of standardized CXR films (Graham *et al.*, 2002). For example, experienced radiologists show high intra-observer agreement for reporting presence or absence of CXR findings with fewer errors (Tudor *et al.*, 1999).

Costs

CXR is a relatively cheap procedure. In Canada 2000, a programme prospectively screened for inactive TB tested 250,000 immigrants and showed consistent low-cost preventive intervention for young immigrants with 50% high probability of TB infection and 10% HIV sero-positivity. The total cost saving per 1,000 populations was U.S.$398,870 against U.S.$492,840 using TST screening and the incremental cost per case prevented was U.S.$21,580 (Schwartzman *et al.*, 2000).

In 1979, estimated total expenditure was U.S.$8,200,575 (U.S.$25 per film) if all hospitals in USA took radiographs of TST positive employees (Mangura *et al.*, 1999).

CXR screening is cost-effective compared to TST and is applied in most countries worldwide compared to TST. A study on pilgrims coming from high endemic areas travelling annually to Mecca, Saudi Arabia, revealed that the final results of TST

(performed within one month before travel to Mecca) were impractical follow-up test for TB abnormalities instead of CXR and having higher costs, time consuming of administrative infrastructure's (Al-Jahdali *et al.*, 2003).

Advantages

CXR remains the major diagnostic and screening tool for health disorders particularly for pulmonary chest abnormalities or for examining lesions, and can deliver a rapid test result. A CXR atypical film of an immune-suppressed individual e.g. HIV+ve patient is best interpreted by two qualified readers guided by a standardized recording policy system and reported from third radiographic reader for difficult lesions and discrepant results (van der Werf *et al.*, 2008).

Characteristic PTB re-activation is commonly manifested as focal or patchy heterogeneous consolidation involving the apical and posterior segments of upper lobes and either/or both superior segments of lower lobes (Figure 1.17).
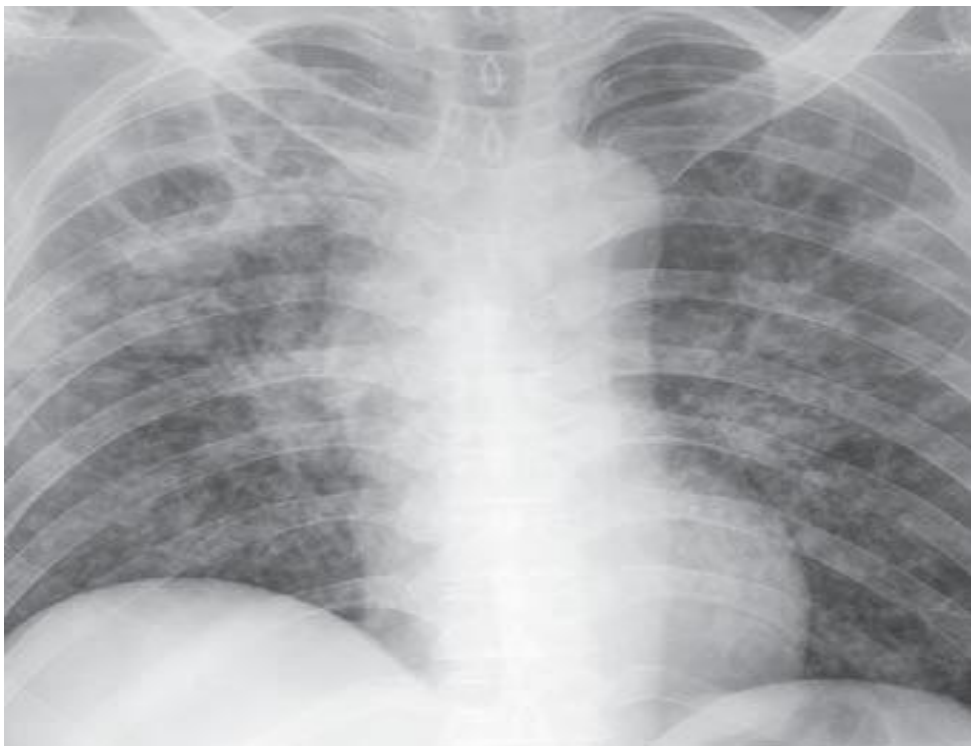
Figure 1.17: Postero-anterior CXR of tuberculosis re-activation with cavitations (right lung) and ill-defined nodules in both lungs (Jeong and Lee, 2008) (DOA: 26/07/2009)

Other typical findings include the occurrence of lesion like nodular or linear opacities (25%), cavitations (20-45%), hilar or mediastinal lymph adenopathy (5-10%) and unilateral pleural effusions (15-20%).

Characteristic 'tuberculoma' is only observed in 5% of PTB cases (Figure 1.15). Tuberculoma lesion size is often about 0.5-4 cm diameter with round or oval sharp margins and histological central caseous necrosis composed of peripheral histiocytes, multinucleated giant cells, and collagen fibers' (Jeong and Lee, 2008). Large cavities are common in right lobes (average 40%) and left lower lobes (30%).

A past history of PTB can help in differentiating between MTB and NTM pulmonary cavitory lesions. Factors predicting PTB cavitations with environmental mycobacterias are age less than 50 years history of malignancy large upper lobe cavity lesion confined to one lobe and/ or multiplicity of lymph nodes more than two (> 2) (Yang *et al.*, 2007).

High prevalence of abnormal radiographic finding(s) reflect combinations of previous and recent exposure to MTB infection, and so CXR can still identify subjects at risk of LTBI progression to active TB until specific INF-γ levels measured. For example number of calcified nodules (> 2-5 lesions) and fibrotic lesion size of more than two (> 2) mm in 80% suspected cases are suggestive of inactive TB at risk for re-activation within 10 years period compared with contained primary lesions due to repeated exposures (van der Werf *et al.*, 2008).

Another LTBI finding is non-calcified nodular lesion(s) present in mid and/or upper lung zones or as pulmonary infiltrates with subsequent PTB (sensitivity 66% and specificity 82%) (Linh *et al.*, 2007).

Reliable risk factor indicators of lung abnormal changes are smokings, ethnic origin (African and East Europeans, 90%), symptomatic sputum smear positive sample (60%) than negative smears (46%) (Mathez *et al.*, 2007).

CXR imaging done on any suspected patchy pneumonia and help in epidemiological surveys, but usage of un-necessary antibiotics is associated with diagnostic delays

leading to prolonged morbidity, increased mortality and mycobacterium transmission.

Antibiotic prescription (e.g. macrolides treating community-acquired pneumonias) without taking CXR during the first health care visit was independently associated with health care delays (mean delay = 69 days) compared with CXR imaging (mean delay = 15 days) (Golub *et al.*, 2005).

Limitations

CXR is an objective method with subjective reading that depends on readers' experience for differentiation between image findings. CXR results can provide diagnostic clues with ambiguous analysis e.g. atypical findings in immune-compromised subjects and with inter-and intra-observer image interpretation (Kobashi *et al.*, 2008; Tiwari *et al.*, 2007).

Limitations in interpretation of various health professionals. For example poor inter-observer agreement in localizing abnormalities usually detected between TB specialists (k = 0.473) and respiratory specialists (k = 0.277) (Jeong and Lee, 2008). A study of 805 chest films reveals inter-reader good agreement (k = 0.69) for normal CXR finding with excellent intra-reader agreement (k = 0.90). For TB-consistent abnormalities, both agreements were good (k = 0.69) and higher for parenchyma more than for central structure abnormalities (den Boon *et al.*, 2007).

Suspected CXR findings only detect high-risk groups but not TB disease activity. Other high penetration diagnostic tests e.g. CT scan, detects pulmonary findings and can replace CXR.

Miss-diagnosis is crucial for difficult findings. For example, failure to recognize any hilar or mediastinal lymph-adenopathy of primary PTB lesions or over-looking of parenchymal abnormalities of dormant bacillary re-activation with failure to detect any characteristic LTBI upper lobe nodule opacity or scar can instead be diagnosed by CT scan. Initial correct diagnosis of CXR findings in primary TB disease is achieved only in 49% and 59% for secondary TB re-activation TB to CT scan correct diagnosis (91%) and TB exclusion (76%) (Breen *et al.*, 2007).

Lateral X-ray film is frequently performed for LTBI evaluation without additional benefit in detection of any missed abnormal X-ray findings by PA view, but is still useful to rule out other differential diseases like malignancy and NTM. LA adds no information in altering TB management e.g. missed calcified granuloma makes no treatment changes (Meyer *et al.*, 2003; Swingler *et al.*, 2005).

CXR performance depends on TB 'severe' presentations in addition to co-morbid diseases. HIV radiological findings are a significantly independent predictor of TB disease and are commonly associated with atypical findings (Geng *et al.*, 2005) (Figure 1.18).

Figure 1.18: Chest radiograph of atypical pulmonary tuberculosis (Geng *et al.*, 2005)

Atypical radiographic findings are associated with a paucity of cavitations, pleural effusion, miliary TB, and interstitial lesion patterns. The developing countries are facing the spread of a TB/HIV syndemic. In Ethiopia and Uganda, HIV-positive patients usually have normal CXR findings are sputum sample smear–negative for AFB with a CD4+ cell count less than 200 cells/mm^3 and reduced mycobacteriological colony count in comparison with HIV-negative cases (Aderaye *et al.*, 2004).

Disadvantages

CXR film (machine, reagents) and inability to relate radiographic findings to results of sputum smear results, which add more problems of under- and over-reading difficulties. The degree of reader's agreement depends on the reader professional experience and complex interpretation. In a Canadian study, where 973 films were read twice by three independent observers, for TB abnormal versus normal findings, the intra-reader agreement using five diagnostic categories was moderate to good (k = 0.59-0.72, mean 0.636) compared with fair inter-reader agreement (k = 0.44-0.56, mean 0.526) (Zellweger *et al.*, 2006).

Radiographic appearance cannot be relied on directly to assess presence of pulmonary lesions or PTB activity for treatment decision. Out of a total of 8, 995 immigrants, 630 with demonstrated abnormalities, only 125 subjects with CXR findings had suspected TB lesions and 107 subjects proven active TB disease (90% were from African and East European origin) (Mathez *et al.*, 2007).

Radiographic accuracy is another limiting factor with interpretation errors related directly to reporting facilities (poor information communication) and radiology experience. CXR is invaluable with low diagnostic accuracy in certain conditions e.g. mediastinal lymph adenopathy or other extra pulmonary sites e.g. pleural lesion.

Radiographic reader complacency can be directly and indirectly related to daily reading of plain radiographs, over-reading and missing abnormal findings, reporting time and personality type, lack of knowledge, and under-reading/wrong interpretations (Tudor *et al.*, 1999).

Extra pulmonary tuberculosis is considered more infectious than pulmonary tuberculosis case regardless of CXR failure to detect EPTB which can be diagnosed by history taking of clinical complaint such as severe weight loss, related socio-economic risk factors and laboratory testing for bacteriological identifications of positive sputum smear examination and culture (OR 4.3) (Parimon *et al.*, 2008; Zellweger *et al.*, 2006).

Further clarification for chest X-ray advantages, disadvantages, and limitation are further discussed in chapter 5 (section 5.1).

1.4 Laboratory diagnostic tests for tuberculosis

1.4.1 Tuberculin skin test (TST)

TST has existed without any changes since the late 19th century. The test is essentially as described first by Robert Koch in 1890 and as modified by Charles Mantoux a French physician who developed Koch's work for use in diagnosis of both active and latent TB infections.

Definition

TST is a traditional in vivo skin test to measure an individual's immune inflammatory response delayed type hypersensitivity reaction-4 (DTH-IV) against multiple mycobacterial antigens.

Procedure

TST is performed using intra-dermal injection of the tuberculin (glycerin extract of tubercle bacilli) of a purified protein derivative (PPD); a standardized killed extract from cultured TB or mixture of precipitates of more than 200 non-species-specific molecules of mycobacterial antigens including *M. bovis* and non-*Mycobacterium tuberculosis* strains.

TST is performed by injecting 0.1 ml of tuberculin (100 units/ml) intra-dermally into the volar surface of the left forearm followed by a water pool ink mark drawn around the injection site for easy reading after 48-72 hours (but not more than one week). Sub-cutaneous injection (instead of intra-dermal injection) gives false-negative results and requires an experienced operator nurse to perform the different stage patterns; Mantoux screening test; TST (tuberculin sensitivity) and two-step testing (which is less common).

TST results are recorded by measuring the transverse diameter of cutaneous indurations (swelling) in millimetres (but not the red erythema) (Zellweger *et al.*, 2006) (Figure 1.19 and Figure 1.20).
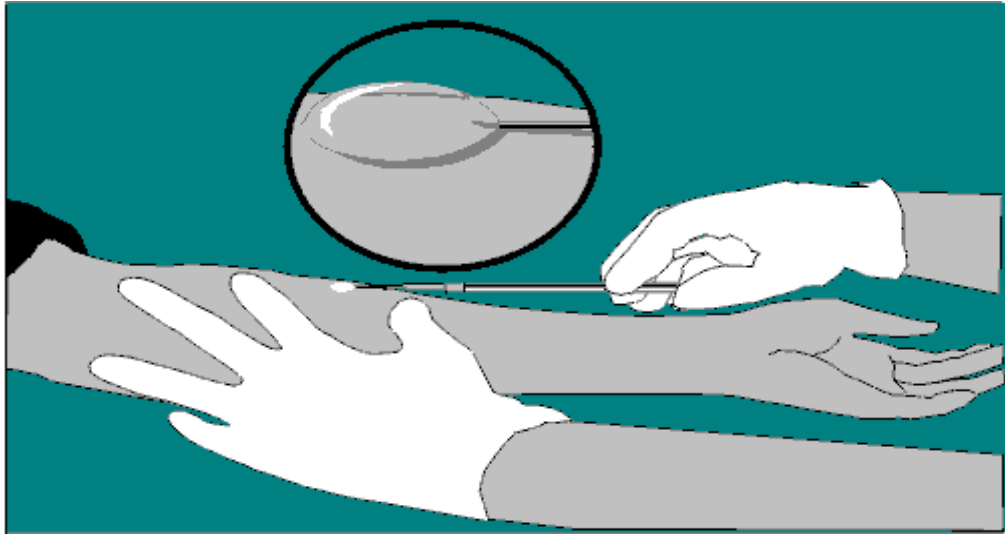
Figure 1.19: Mantoux tuberculin skin test showing intra-dermal injection of PPD into the forearm (http://www2.cdc.gov/PHTN/tbmodules/modules1-5/m3/3-m-02a.htm)
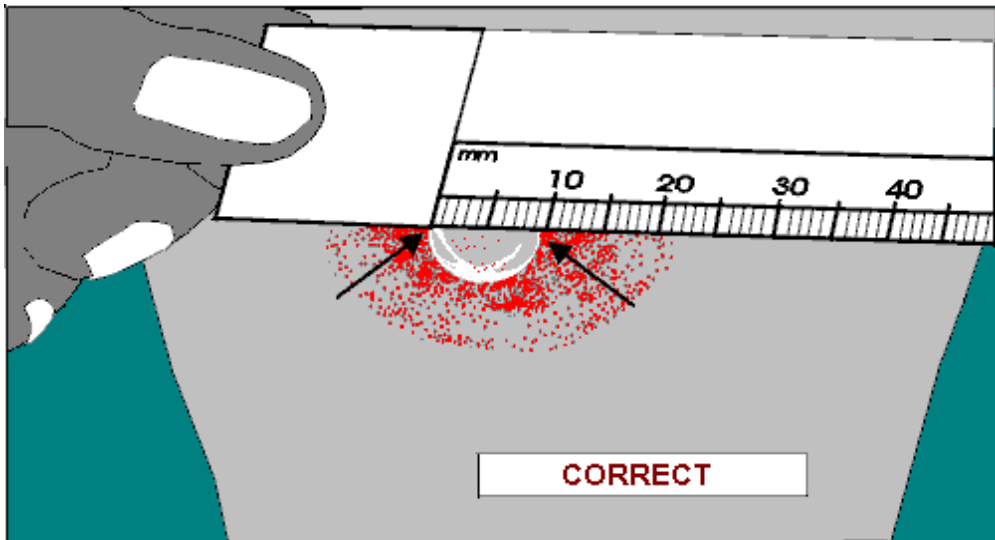
Figure 1.20: Tuberculin skin result measure induration (swelling) size in millimetres (http://www2.cdc.gov/PHTN/tbmodules/modules1-5/m3/3-m-02a.htm) (DOA: 26/07/2008)

TST results are recorded and reported according to national guideline policy. For example, any indurations' size more than 5-mm diameter is considered as a positive reaction in BCG non-vaccinated individuals such as those in the UK or the U.S. compared with TB endemic regions as positive more than 10-mm e.g. China or even more than 20-mm in India.

Two common PPD manufactured Aplisol and Tubersol types were injected intra-dermally in 116 subjects and were then compared with IGRAs to conclude agreement of 93% at day 2 (k = 0.80) and 94% at day 7 (k = 0.76). Overall agreement for Tubersol was 89% and 86% for Alpisol recorded during same time period. Seventh day TST reading can be compared with IGRAs INF-γ level (Tat *et al.*, 2005).

Cost

TST is cheap and is manufactured from low cost materials. Cost of a single test is U.S.$9.79 (£5).

Advantages

TST is in widespread use and is the preferred test in developing countries e.g. India and Indonesia and worldwide remains the second choice for LTBI diagnosis. TST is a simple, cost-effective and confirmatory test in regions having BCG +ve vaccination regimens particularly in close contacts to TB infectious index cases. Such tuberculin converters to positive result have 5-10% risk of developing active TB after 2-5 years, and another 5-10% risk during lifetime period (Al-Orainey, 2009).

Among 1,885 Tanzanian screened HIV +ve cases, 13 had active TB disease, 1,206 subjects looked healthy, but all 635 of LTBI subjects (100%) showed a TST response more than 5-mm compared with active TB patients (84.6%) and negative results in healthy subjects. TST indurations' is higher in HCWs with radiological lesions suggestive of possible active TB e.g. non-calcified nodules and indication for periodical TST re-testing (Joshi *et al.*, 2007).

The mean TST diameter was significantly higher in both active PTB and inactive PTB patients compared with healthy individuals (Akbulut *et al.*, 2009).

For LTBI screening, both NICE and HPA guidelines also recommend performing TST during BCG immunization against infectious diseases. CDC guidelines consider any TST induration size more than 15-mm as diagnostic sign of LTBI in low-risk populations, even without past history of BCG.

Screening for LTBI in new expatriates following the 1st positive TST is recommended by HPA, but NICE guidelines are limited to compulsory testing in large screening programmes linked to health service (HPA, 2008).

Baseline TST testing is recommended for HCWs to track acquired occupational TB and/or tuberculin converter's associated with previous TB exposure. TST is repeated as standard compulsory test for initial negative TST result (e.g. false negative in immune-suppression condition). A study on 527 internationally adopted children (IAC) at risk of severe TB disease/and LTBI re-activation having low body weights and initial negative TST results could detect LTBI in 20% converters to TST positivity compared with 47% after two-step repeated TST. BCG scar and/or documented immunization (OR 15) are associated with initial TST positive, but positivity risk decreases after repeating TST (OR 7) (Trehan *et al.*, 2008).

Disadvantages/ limitations

TST subjective reading reduces test specificity and impacts on LTBI detection. Diameter results must be read with caution, particularly in vaccinated individuals and in endemic regions.

One-step TST tuberculin reactivity is less reliable in those having induration size more than 18-mm even in endemic areas due to BCG positive vaccination and MTB shared antigens reducing TST specificity (Tissot *et al.*, 2005). PPD negative result is down-regulated in immune-suppressed individuals or those highly anergic with 'Koch phenomenon' post-vaccination.

In Australia 2004, a prospective study tested the recently culture-proven TB cases revealed that both TST induration and specific IFN-γ release were higher in MTB infection compared with NTM cases and to other risk factor combinations e.g. protein malnutrition, HIV co-infection (Britton *et al.*, 2005). Practical difficulties in administration and interpretation give false results. PPD mycobacterium antigen mixtures induce painful or scarred indurations' skin reactions. Biological variability may affect TST results between inter-subject e.g. BCG boosting and intra-subject immune response e.g. response against NTM antigens (HPA, 2008). TST of different

indurations' sizes; more than 5-mm and more than 10-mm are correlated to BCG immunization and false positive results (Diel *et al.*, 2008).

Meta-analysis of TST specificity relating with the injected PPD concentration and preparation heterogeneity, reader subjectivity, and variable international cut-off point achieve LTBI miss-diagnosis and active TB case loss up to 20-25%. Negative AFB smears have negative PPD reactions (induration < 5-mm) compared with positive AFB smears (RR = 2.2) (Samb *et al.*, 1999).

Further clarification for tuberculin skin test advantages, disadvantages, and limitation are further discussed in chapter 5 (section 5.2).

1.4.2 Interferon gamma release assay tests (IGRAs)

Various TB diagnostic methodologies address the absence of 'gold' standard diagnostic tests in relation to: 1)- comparison of blood test results to identify true positives with active TB, 2)- testing TB true negatives and LTBI free individuals (from TB non-endemic areas and without risk factors), 3)- degree of concordance with TST results, 4)- positive IGRAs with LTBI exposure risks, and 5)- longitudinal follow-up studies for positive IGRAs individuals to ascertain LTBI progression and predict PPV validity in diagnosing LTBI (HPA, 2008).

LTBI screening includes taking a history of past TB infection and management e.g. exposure to index case, incomplete anti-TB course, TB relapse, partial follow-ups or radiographic residual changes with calcified nodular granuloma and laboratory MTB isolations with rapid IGRAs or PCR diagnostic superiorities (Lalvani *et al.*, 2008).

In clinical settings, 24 hours is considered a practical period between results reporting in relation to established diagnostic accuracy (mean range 85%). Assessment of consistent diagnostic accuracy is calculated through measuring the kappa agreement value between different procedures using longitudinal studies comparing IGRAs with other diagnostic tests like CXR and TST.

Definition

IGRAs are two new diagnostic (blood) tests and are implemented at present in the developed countries for detection of LTBI and active tuberculosis. Both tests detect the cytokine interferon gamma released from MTB previously sensitized T-lymphocytic cells. IGRAs use antigenic proteins present in all MTB-complex species having peptides; early secretory antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), which are encoded by a unique genomic segment of MTB (stretch of DNA) termed as 'Region of Difference' or RD-1 present specifically in MTB and *M. africanum* but not in BCG (*M. bovis*) or majority of environmental non-mycobacterium (with exception for *M. kansasii*, *M. marinum* and *M. szulgai*) (Andersen *et al.*, 2000; Richeldi, 2006) (Figure 1.21). The loss of RD-1 region from *M. bovis* during virulence attenuation permits production of BCG vaccine. Another highly specific antigen TB7.7 (Rv2654) was newly mapped to RD-11 genome by which can maximize MTB detection sensitivity, in which TB7.7 unique phage insertion was added to the latest version of QuantiFERON (QuantiFERON Gold In-Tube) test.
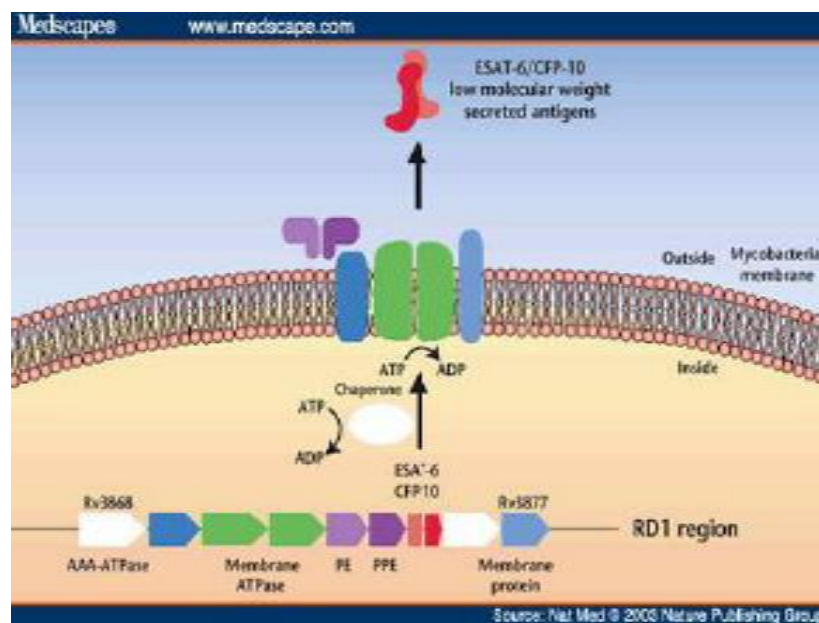
Figure 1.21: Demonstration of IGRA specific antigen
(http: //www.rsm.ac.uk/tbscreening07/catanzaro.pdf) (DOA: 26/07/2008)

Genetic studies reveal both ESAT- 6 and CFP-10 antigens are peptides of 15 amino acids long and overlapped to adjacent peptides by 10 residues. Peptide (protein)

sequences are uniquely restricted to ESAT and CFP proteins of MTB complex (Lalvani *et al.*, 2001a).

Combination of both IGRAs antigens reactivity is significantly higher in active TB disease than LTBI (Yew *et al.*, 2009).

Under in vivo conditions, the RD-1 encoded proteins can cause direct or indirect progressive necrosis secondary to interactions between the host cells and bacterial proteins but not due to bacterial overloads. Data supports that non-mutant MTB bacilli expressing RD-1 can cause dramatic necrosis in mice lungs which is unable to make INF-γ (Junqueira-Kipnis *et al.*, 2006). Molecular diagnosis and analysis of MTB strains investigated to compare between the past and new TB infections according to the country source of origin is the future recommendation.

Evidence-based study results attest to IGRAs superiority in LTBI detection compared with other TB standard diagnostic tests. In Norway, 155 HCWs compared with 48 healthy controls using both TST and T-SPOT *.TB* test to assess risk of acquiring TB after recent exposure to PTB positive sputum sample at three different University hospitals reveal LTBI prevalence was 3% with low risk of transmission frequency (2%) within short duration and IGRAs improve this approach (Storla *et al.*, 2009).

IGRAs based on RD1-specific antigens are correlated better with exposure intensity and BCG independence, and accurately detect LTBI better than PPD-based assays.

BCG lacks groups of genes (Rv3971 to rv3879) including genes for antigenic proteins ESAT-6 and CFP-10 of RD-1 made by MTB early during culture growth but with unclear precise action.

IGRAs assess risk factors of BCG vaccinated individuals. Available commercial IGRAs are independent of any previous BCG vaccination and test the immune responses against specific TB antigens missing from other mycobacterias' or BCG attenuated bovine TB and are not confounded by the BCG vaccine.

Indications

The WHO and National Tuberculosis Program (NTP) recommend international TB elimination using IGRAs for the following: suspicion of LTBI, discrimination of TB

from NTM infections, diagnosis of case problems in any clinically-related differential diagnosis e.g. acute life threatening TB meningitis, diagnosis of suspected TB in TST negative result e.g. anergy, organ transplant, repeating IGRAs for variable INF-γ level results, and as a marker of treatment cure post-active TB treatment or LTBI prophylaxis. IGRAs can be used as a confirmatory test for positive or indeterminate TST results read by TB specialist (Al-Orainey, 2009).

Worldwide examples of implementation of IGRAs

The UK Health Protection Agency (HPA) recommends the use of IGRAs for LTBI detection undertaken at the time of TST reading and for HCWs or those in direct contact with immune-compromised patients and those at risk of developing active TB disease e.g. testing nurses, laboratory technicians. Other HPA indications for IGRAs include use for primary diagnosis of active TB, to confirm diagnosis of highly suspected with negative culture result cases, individuals lacking radiological and histo-pathological tests, and to help confirmation of final decision after starting anti-TB treatment (HPA, 2008). Guidelines of NICE (UK) recommended IGRAs for HCWs without known history of BCG vaccination even if free of active TB disease or used for active TB diagnosis and ruling out MTB infection. The U.S. CDC recommended QuantiFERON testing for all TST positive and negative results including evaluation of recent immigrants or sequential test surveillance programmes and suggests TST replacement by QuantiFERON test.

IGRAs can be used as primary tests to rule out LTBI in immuno-compromised individuals showing a false negative TST result and in public health investigations.

Advantages

IGRAs evaluate TB infection by differentiating LTBI and active TB positive result from other differential diagnoses, and excluding non-infected immune-compromised individuals having negative or indeterminate results (Madariaga *et al.*, 2007).

LTBI was detected using the ESAT-6 ELISPOT test in 80% of healthy adults for contact tracing in high-prevalence areas and this is helpful in assessing LTBI prevalence (Lalvani *et al.*, 2001b).

IGRAs maintain a higher diagnostic sensitivity and specificity for LTBI than TST in subjects taking disease modifying anti-rheumatic drugs (DMARDs) for TNF-α blockade e.g. rheumatic disease (Lalvani *et al.*, 2008). A follow-up study of 42 participants in a Dutch supermarket for employee contact investigation in 2005 and 2006 showed 59% (17/29) as having TST negative result has converted after one year to TST positive result. On the other hand, 82% (23/28) have primarily T-SPOT *.TB* test positive result which reverted to negative results compared to only 3% (1/29) reversal of QNF-GIT participants. According to the Netherland epidemiology, these converted TST results in association with IGRAs negative reversion are not related to MTB re-exposure or BCG boosting effects of past TB infection.

CFP-10 antigen response indicates active bacterial replication, whereas, ESAT-6 antigen persists as an immunological 'scar' indicating higher T-SPOT *.TB* test specificity for LTBI detection (Franken *et al.*, 2007).

ESAT-6 assay detects more positive results in MTB high-risk groups than TST, distinguishes asymptomatic NTM infection, and accurately detects recent MTB infections in close contacts to index cases and require long term follow-up to estimate predictive value of active TB progression (Lalvani *et al.*, 2001b).

IGRAs reduce false-positive results and avoid LTBI preventive prophylaxis among children and the adult population. T-SPOT *.TB* test is correlated more with the degree of exposure (closeness and duration of exposure) than TST. A two-step screening approach of contact tracing using TST followed by QNF-G test increases LTBI detection and test accuracy (LTBI missing reduced by 30-40% of new case expectation because of recent transmission) (Al-Orainey, 2009).

IGRAs tested on 383 subjects detected positive results in all close contacts irrespective of their BCG vaccination. PTB close contacts scoring ELISPOT positive results (34%) higher than QNF-GIT scoring (22%), in addition to fewer

indeterminate results in those taking immune-suppressive treatments (Ferrara *et al.*, 2006).

In Turkey, another study focused only on 908 pediatrics cases recently exposed to household TB index cases, ELISPOT positive children with high INF-γ levels were significantly associated with 3-4 fold progression to active TB compared with non-significant positive TST results of 2.7 fold (Bakir *et al.*, 2008).

A large prospective study done on 626 hospital patients followed up OPDs in the TB Prevention and Control Unit in Barcelona, Spain, T-SPOT .*TB* test positive results were significantly higher than QNF-GIT results. Agreement between both IGRAs (T-SPOT.*TB*+/QNF-GIT+) was the highest 83.2% (k = 0.66) compared with lower T-SPOT.*TB*+/TST+ agreement of 64.5% (k = 0.34) or QNG-GIT+/TST+ agreement of 58% (k = 0.26 (Dominguez *et al.*, 2008).

TB infection control with careful follow-up screening is crucial to reduce nosocomial infection transmission. Prevalence of LTBI was 77% in Georgian HCWs, detected by using combination of QNF test and TST, and was significantly higher than single use test (QNF +ve detect LTBI prevalence 66 % and 60 % for TST +ve result). Length of employment > 5 years was associated with TST +ve result (OR 5) against QNF +ve test (OR 2.26) with moderate agreement of 73% with positive TST > 10-mm (k = 0.43) (Mirtskhulava *et al.*, 2008).

LTBI screening in high risk groups using TST and/or IGRAs is cost–effective even in low TB incidence areas, but a hybrid strategy is more economical. A study in San Francisco on homeless, refugees, intravenous drug users (IDU's), and immigrants implementing QNF test including costs of phlebotomy, laboratory and personal costs revealed IGRA cost was only U.S.$33.67 per patient tested for undertaking routine TB control programmes (Dewan *et al.*, 2006).

Excluding indeterminate results, the estimated specificity of IGRAs for LTBI detection in low-risk population is still high, ranging between 96-99% compared with standard study test parameters TST-/QNF+ result, were detected more in subjects born in TB endemic countries (OR 5) than those having both tests negative (Luetkemeyer *et al.*, 2007). In Finland, 99 pediatrics' cases aged less than 18 years

were tested, comparing modified versions of both T-SPOT *.TB* test (sensitivity 85%, specificity 100%) and QNF-GIT (sensitivity 92%, specificity 91%) revealed excellent agreement (0.89-0.90) between both IGRAs and each test separately with TST, without interfering with delayed type hypersensitivity reaction (Tavast *et al.*, 2009).

Limitations

The main limitation is that IGRAs are expensive (cost; U.S.\$37.9, £20-40).

Indeterminate results can occur when the positive mitogen control fails to react, and needs to be interpreted with caution due to absence of stimulable lymphocytes either due to pre-analytic error with freezing of blood samples during transportation or due to a real absence of living T-lymphocytes in immune defects (Madariaga *et al.*, 2007; Zellweger, 2008). In advanced HIV +ve patients with having low CD4+ cell count less than 100 cells/mm^3 both TST and QNF-GIT tests are less sensitive and significant relation with indeterminate results e.g. QNF -ve result association (OR 4.2) (Luetkemeyer *et al.*, 2007).

The absence of sufficient data to assess serial testing (IGRAs reversion and conversion phenomenon) limit IGRAs assessment. Longitudinal (time-period) follow-up screening studies are required to approve IGRAs repeatability and accuracy assessment.

The IGRAs test technology has been designed to replace the 115-year-old TST and this is the first approved diagnostic tool with superior accuracy to assist in elimination of TB globally. The two commercially available IGRAs measure ex vivo interferon gamma released from circulating MTB-sensitized T-cell lymphocytes correlated with TB exposure. Both IGRAs similarly explore the immune cell response against MTB specific antigens and provide quantitative and dynamic measurement of immune reaction than TST, in which IGRAs can be added to serial screening studies (Richeldi, 2006).

Meta-analysis studies of published articles revealed IGRAs high statistical reproducible parameter than TST (Pai *et al.*, 2006).

IGRAs detect both active TB and act as surrogate diagnostic test for LTBI in TB high-risk group populations. TST specificity is only higher than IGRAs in BCG non-vaccinated population (pooled specificity = 0.97 (0.95–0.99), p = 0.032). The overall meta-analysis conclusion recommends IGRAs implementation (especially QNF test) in BCG vaccinated individuals particularly for infants or in TB endemic countries having immunization guidelines of repeating BCG (Veerapathran *et al.*, 2008).

Newly diagnosed TB cases detected by ELISPOT test (sensitivity 78%) - better than QNF-GIT (64%) and with a similar sensitivity detection of TB contacts (Adetifa *et al.*, 2007).

Both IGRAs increase sensitivity and specificity for LTBI detection either combined with, or, replacing TST (Pai *et al.*, 2005).

A study of 425 subjects detected high sensitivity using a combination of TST and either IGRA test. A negative result combination (QNF-GIT–/TST–) can exclude active TB by 14-fold-time. IGRAs rapid immune-diagnosis of active TB is especially required for sputum smear negative and EPTB cases (Goletti *et al.*, 2008).

IGRAs test help detection of LTBI prevalence and assess TB-related health disorders in low-incidence countries and help identification of TB cases in high-risk groups.

In Gambia - a poor-resource TB-endemic country - both IGRAs respond to MTB exposure gradient and are positively associated with increased TST indurations' size. Both IGRAs also diagnose LTBI in TB contacts with similar agreement and significant concordance 75% (k = 0.43) (Schablon *et al.*, 2009).

IGRAs tests are recommended as baseline screening tests for HCWs or new immigrants before, and annually after, employment (Bakir *et al.*, 2008).

IGRAs indeterminate result denotes that the test finding gives no fixed-type diagnosis (neither positive nor negative) e.g. 1- insufficient response of the mitogen positive control, 2- nonspecific background staining in the wells, 3- nonspecific INF-γ release by the mononuclear cells in the well (result in high nil-control spot count) commonly occurs in immune-compromised individuals (children, old age > 60 year,

pregnant). In clinical practice on such occasions, subjects should be screened using other available diagnostic tests e.g. sputum routine microscopy or TB growth culture.

1.4.2.1   QuantiFERON Gold In-Tube test

Definition

In vitro and indirect laboratory tests aid in detection of MTB infection using peptide cocktail of simulating antigens; QuantiFERON test (QNF), an enzyme-linked immunosorbant assay (ELISA) tests uses whole blood involving two specific stimulating antigens: 1)- early secretory antigenic target-6 (ESAT-6) and 2)- culture filtrate protein-10 (CFP-10). Both antigenic proteins encoded within the 'Region of Difference-1' (RD-1) are specific to MTB genome.

The IGRAs test accuracy is improved by choosing antigens absent from BCG vaccine and are able to stimulate specifically INF-γ responses.

The first IGRA version was the QuantiFERON (QNF) test followed by the QuantiFERON GOLD (QNF-G) test.

The latest version replacing the old generation is QuantiFERON Gold-InTube (QNF-GIT) test and another third specific antigen TB7.7 (Rv2654) encoded by a phage-inserted region, 'Regional of Difference-11' (RD11) is added.

Assay technology depends on incubating heparinized whole blood over 16-24 hours to detect TB infection based on measurement of INF-γ levels (Lalvani *et al.*, 2008). The three antigenic proteins have no cross-reaction with PPD and are used in a surveillance programme with the two-step TST testing or as screening test for TST boosting phenomenon.

Separated or isolated ESAT-6 and CFP-10 antigens that are used for active TB detection reveal lower sensitivity (84%) and specificity (89%) in comparison with QNF combined-antigen test (sensitivity 93% and specificity 100%) with 90% concordance of positive results (k = 0.82) (Sauzullo *et al.*, 2008).

Procedure

One ml whole blood is withdrawn and placed in each of two separate heparin tubes; one coated with specific antigens (ESAT-6 and CFP-10) and the other serves as negative control. Tubes are sealed and incubated at 37 C for 16-24 hours, preparatory to centrifugation. During this period, MTB antigens recognized by T-cell lymphocytes subsequently secrete INF-γ into the cell supernate against no secretion from non-infected T cells. Plasma removal followed by assay of released IFN-γ from sensitized T cells and previously exposed to MTB antigens, ESAT-6 and CFP-10. ELISA determines INF-γ levels using 200-microlitre plasma sample. After stimulation, the plasma supernate can be kept at 2-8 C for 4 weeks or at -20 C for three months.

In the ELISA, monoclonal antibodies determine quantitatively released INF-γ concentration levels and are read as international units (IU) using a special Software package for result evaluation and interpretation. After correction for negative control tube, any value of more than 0.35 IU/ml is considered as the cut-off point for a positive result according to the manufacturer guidelines.

Non-specific INF-γ concentrations found in negative control tube are first subtracted from the test result (Kunst, 2005; Pai *et al.*, 2006).

Characteristics

The QNF test was approved first by Federal Drug Administration (FDA) in 2001 and QNF-G test in 2005 to aid in MTB diagnosis by measuring cell-mediated immune reactivity and the latest QNF-GIT approved finally on 10/10/2007.

The test reported as a comparable diagnostic test, having characteristic high sensitivity and specificity in diagnosing both PTB and EPTB, is less reactive to atypical MTB, is conveniently administered, has a more objective result and interpretation, and is used as baseline test in surveillance health systems allowing reliable reporting and research comparison.

A cross-sectional study done on 800 navy recruits (TST, QNF, and QNF-G) revealed estimated TST specificity (98%) at induration size more than 10-mm cut-off value

compared with estimated QNF (92%) and QNF-G (99.8%). Those having positive TST result of more than 10-mm and more than 15-mm were 20–40 times likely having discordant results for soldiers born in TB high-prevalence countries due to resolved or past old infections, and lower TST specificity and lower QNF sensitivity. For example, TST +ve response was more with larger indurations' size and so a combination of both QNF and TST is more specific to detect LTBI than separate testing (Mazurek *et al.*, 2007).

Advantages

The QNF test is valuable diagnostic test (highly sensitive and specific) and detects 70% of truly infected active TB and 100% predictor of LTBI at risk of progression (Sauzullo *et al.*, 2008).

The challenge of IGRA positive result without using an invasive procedure permits the QNF test to be performed as adjunctive tool for rapid detection of PTB and EPTB. Diagnostic accuracy for LTBI detection among exposed contacts is higher than TST +ve results. A Norwegian study, performed on 904 asylum seekers, detected LTBI prevalence defined by TST as 50% and only 29% by using QNF test. Predictors of positive results using QNF and TST more than 15-mm tests are associated with ages between 18-29 years, female gender, country of origin and TB country background (Asian's then African's), and accumulated exposure time. Adjusting TST cut-off value to lower indurations' than 10 or 15-mm will increase LTBI prevalence (Winje *et al.*, 2008).

In Bussan, South Korea, a study done on three TB risk groups concluded that the QNG test can be considered as diagnostic tool for LTBI, particularly in BCG +ve vaccinated and differentiates vaccinated healthy individual from active TB case compared to TST difficulties. Measured INF-γ levels in TB patients was higher (INF-γ = 15.5 +/- 4.5 IU/ml) than nurses (high TB risk group) (INF-γ = 11.7 +/- 5.5 IU/ml) and medical students (low TB risk group) (INF-γ = 1.3 +/- 0.9 IU/ml). Results were also significantly correlated with the duration of employment (intensity and frequency of exposure to MTB) and noso-comial infection (Eum *et al.*, 2008).

Multivariate analysis concluded the presence of a significant correlation with working duration but contradictory to BCG vaccination. These differences can be related to biological factors (participant immunology), BCG vaccination, and laboratory-related errors (Ozdemir *et al.*, 2007).

The QNF test can detect LTBI in BCG +ve individuals with low TB exposure risks (Lee *et al.*, 2009; Taggart *et al.*, 2001). In Hamburg, Germany, a study followed 601 contacts diagnosed as having AFB +ve smear and MTB culture-positive, 40% were TST −ve at 5-mm cut-off against 11% QNG test −ve result. LTBI progression rate to active TB disease was detected better by QNF test (14%) than TST (2%). BCG vaccinated subject were 12 times higher to have TST +ve (OR 12.5) and 6 times to be foreign–born (OR 5.8) whereas QNF test positivity is BCG vaccination independent (OR 1.14) (Diel *et al.*, 2008).

Limitations

QNF test results revealed low sensitivity for LTBI/TB diagnosis in those with low CD4+ cell count e.g. advanced immune-suppressed individual or HIV/AIDS (Balcells *et al.*, 2008). Processing of whole blood for 12 hours is considered a major limitation, weakening the QNF test with un-acceptable positive INF-γ levels.

In a study done on 270 active TB subjects, the QNF-GIT indeterminate result rate was 3.5% after treatment associated with risk factors e.g. age > 60 years, being female gender, and ethnic origin due to decreased T cell response (Chee *et al.*, 2008). In another multivariate analysis, QNF-G positivity had no significant association with exposure durations due to the masking effect of age but still detected accurately all true LTBI infections in healthy individuals (Harada *et al.*, 2006).

Disadvantages

QNF test repeatability and accuracy need longitudinal re-assessment screening studies on large sample sizes. Among 63 subjects tested in California using two-step QNF-GIT study design for three months with assumed sensitivity 70% and

specificity 98%, unexpected false-reversion (32%) and false–conversion rates (6.95%) were revealed (Perry *et al.*, 2008).

Further clarification for QNF-GIT advantages, disadvantages, and limitation are further discussed in chapter 6 (section 6.1).

1.4.2.2   T-SPOT *.TB* test

Definition

ELISPOT (enzyme-linked immunospot assay) test was first developed by Cecil Czerkinsky in 1983 based on modified version of ELISA immunoassay to enumerate B cells secreting antigen-specific antibodies with identification and enumeration of cytokine-producing cells at the single cell level. ELISPOT assay used to measure released IFN-γ.

The second IGRA test is T-SPOT *.TB* assay produced only by Oxford Immunotec Ltd. (Oxford, UK) and approved by FDA in 2006. T-SPOT *.TB* test is an in vitro diagnostic assay measures specifically IFN-γ released from sensitized T-cell lymphocytes (LMCs) following isolation, washing and exposure of peripheral blood monomorphonuclear cells to MTB antigens, ESAT-6 and CFP-10. Appearance of spots in the test well represents MTB-sensitized T-cell and is interpreted after comparison with negative and positive control wells (Kunst, 2006; Pai *et al.*, 2006). Each visualized spot represents secretory products of responding reactive cells. Unlike QuantiFERON ELISA test, T-SPOT *.TB* test does not determine INF-γ level.

Procedure

T-SPOT *.TB* assay is based on ELISPOT methodology and requires isolation and incubation of standardized 250,000 LMCs in each of the test wells.

In vivo sensitized T-cells from blood circulation are incubated ex-vivo overnight to re-encounter MTB antigens to release the INF-γ cytokine from the sensitized T-lymphocytes and give dark spot or 'footprint'.

The manufacturer recommends 4 ml blood withdrawal (2 ml in children) and assay performed within the first eight hours after sampling. LMCs are separated from blood on Ficoll gradient then placed in microtitre wells already surface-coated with antibody against INF-γ. Antigens are then added for comparison with a positive control (phythaemagglutinin; or PHA) followed by incubating the plate at 37 C for 16-20 hours and adding of a second enzyme-conjugated antibody against INF-γ and chromogenic substrate where bound enzyme cleaves the substrate [translator's correction] and a coloured spot forms. Each counted spot is read visually or using magnifying glass, or rarely by using specific plate-reader. The accepted cut-off point for positive result is more than > 5 spots. The positive mitogen control normally gives at least 20 spots. If the negative control plate gives an apparent positive result (non-specific stimulation) then the positive control spots plate should show at least twice higher than the number of negative control spots.

Characteristic

The T-SPOT .*TB* test is a new technology designed to specifically and quantitatively detect activated T effecter cells. It is highly sensitive, provides a rapid result (< 24 hours), is convenient, objective and accurate in assessing the cellular immune response against MTB infection, and is used as screening tool to identify community TB and LTBI risk groups.

Advantages

The T-SPOT .*TB* test was introduced to increase TB and LTBI diagnostic rates and as a useful screening tool with immune-epidemiological evidence of high diagnostic accuracy to detect TB-related disorders at community level.

ELISPOT responses in advanced HIV positive patients are independent of CD4+ cell count.

The T-SPOT .*TB* assay has negative association with history of anti-TB treatment. After two weeks of therapy in 270 patient culture +ve proven results, a statistical difference is detected between both IGRAs performance. The T-SPOT .*TB* test

positive assay show higher sensitivity in 254 cases (94%) compared with positive QNF-GIT test in 224 patients (83%) (Chee *et al.*, 2008).

In UK, February 2001, one student in a secondary school diagnosed as being sputum smear positive and PTB CXR positive cavitations after which 1,128 other contact students tested with TST only detected 69 active TB secondary cases and 254 LTBI infected students. Between May and June in 2001, another school outbreak involved 535 students from one infectious index case and diagnosis was based on conventional criteria (clinical assessment, radiological CXR and Heaf test yield LTBI of 24%). A combination of TST and ELISPOT tests revealed high agreement (k = 0.72). ELISPOT +ve result was significantly associated with direct time-exposure to the index case (OR 2.5). ELISPOT positive results in LTBI-presumed students have higher Heaf grades with longer exposure durations to MTB than ELISPOT negative students (Ewer *et al.*, 2003).

Positive reversion to negative result occurred post anti-TB curative and preventive chemoprophylaxis (Chee *et al.*, 2008). A silicosis patient is an indication for T.SPOT *.TB* testing, especially in TB endemic countries due to aggravating risk factor combinations (Leung *et al.*, 2008). Similar indications for use in hemo-dialysis patients (chronic renal failure) or cutaneous anergy and insensitive TST (Passalent *et al.*, 2007).

Close contacts of recently MTB-infected patients require follow-up to estimate predictive values of developing active TB disease. Screening study on close contacts a jail in Texas, USA, found LTBI prevalence rates of 19% using T-SPOT *.TB* test against 8.5% using TST and lower false-positive results (Porsa *et al.*, 2007).

Disadvantages/limitations

There are difficulties in LTBI detection and discrimination between LTBI and active TB disease due to an increase in immune-compromised disorders. The discordance reflects the limitation of the IGRAs tests for implementation. The UK National Institute for Health and Clinical Excellence (NICE) guidelines 2006 approve a discordance between T-SPOT.*TB*+/TST- (10-24%) and similar value for

T.SPOT.*TB*-/TST+ (10-27%). IGRAs are considered an inadequate replacement to TST for LTBI detection (Janssens *et al.*, 2007).

Further clarification for T-SPOT .*TB* test advantages, disadvantages, and limitation are further discussed in chapter 6 (section 6.2).

1.5 Aim and outline of the thesis

The overall aim of the thesis is to study the challenges in tuberculosis prevention in the State of Kuwait during 2010, through early detection and management of latent tuberculosis infection and tuberculosis disease suspicion. The magnitude of the TB disease problem (morbidity, resistance and death) prompts more investment in clinical, epidemiologic and operational research in TB. Failure and weakness of the health care system to suspect and early diagnose TB-related suspects to reduce re-activation risks and active TB case development argues for a need for revising case-finding strategies which can directly result in reduction of non-infectious (LTBI) and infectious (active) TB cases and better TB control. Identification of latent tuberculosis infection cases (as 'suspect TB') can be facilitated by building a classification tree using epidemiological-radiographic-laboratory test results (epidemiological suspect and diagnostic test results). The approach adopted in the thesis is outlined below;

Chapter I introduces an overview discussion of latent tuberculosis and tuberculosis disease definition, diagnosis, and management.

Chapter II assesses the trends of tuberculosis morbidity and mortality representing TB health-related status of the community residents of both Kuwaitis' and non-Kuwaitis' in the State of Kuwait.

Chapter III describes the thesis methodology of the project steps: A- new immigrant interview using questionnaire, and B- describe implementation of the four tuberculosis diagnostic tests on the same immigrant: two old tests; chest X-ray and tuberculin skin test, and two new tests; interferon gamma release assays (IGRAs).

Chapter IV discusses LTBI-epidemiological suspicion and investigates risk factors through clarification of the questionnaire epidemiological variables with estimations of LTBI point-prevalence.

Chapter V discusses LTBI-laboratory diagnostics and evaluates the performance of the two old tuberculosis diagnostic tests; chest X-ray and tuberculin skin test, and the effect of BCG vaccination on Mantoux diagnostic test results.

Chapter VI evaluates the performance of the two new tuberculosis IGRAs diagnostic tests; QuantiFERON Gold In-Tube and T-SPOT *.TB* tests for diagnosis of LTBI cases.

Chapter VII building a classification tree of evidence-based laboratory diagnostic criteria for detection of LTBI and 'suspect tuberculosis' cases.

Chapter VIII identifies evidence-based predictors for LTBI.

Chapter IX discusses the cost effectiveness of immigrant's chemo-preventive screening.

Chapter X describes general discussion and recommendations.

Thesis Output: Recommendations to modify institutional infection control and occupational health policy with comparison of the effectiveness of a new model versus the old screening policy to speedily predict highly 'suspect TB' (secondary case re-activations), TB prevention using prophylactic chemotherapy and save human and economic costs.

Thesis Conclusion: The project result finally permits development of a screening algorithm aimed at achieving 100% sensitivity for confirmed LTBI and 'suspect TB' case with the highest possible positive predictive value (PPV) for physician-suspensions and early diagnosis by which it is able to prevent re-activation risk disease.

2 Chapter Two

Assessment of tuberculosis-related health status of all residents in Kuwait community

Summary

This chapter presents the health system and standard health policy for diagnostic era and control of tuberculosis in Kuwait. This work assesses retrospectively the epidemiological trends of tuberculosis morbidity, mortality and related fatality by gender and nationality associated with international migration into the State of Kuwait, and the policy measures followed to control migration patterns. The work described in this chapter presents a descriptive analysis of the trends in TB morbidity and mortality, and risk factors-tuberculosis disease association, in the current demographic infrastructure of Kuwait residents according to nationality and gender. Demographic data were obtained from the Kuwait national records over a 26-year-period (1984-2009).

2.1 Introduction

2.1.1 The State of Kuwait

Kuwait lies at the northwestern corner of the Arabian Gulf and covers an area of 17,820 square kilometres (Km^2) (11,073 square miles). It shares a 222-kilometre (138-mile) border with Saudi Arabia to the south and southwest, and a 242-kilometre (150-mile) borderline with Iraq to the north and west. Kuwait has a population density of 171.21 persons per Km^2 with an urban population rate of 97 percent allocated all over the country towns, including the capital city, Kuwait City, were majority of people are located along the Arabian Gulf Sea, toward the south (Figure 2.1). The currency is the Kuwaiti Dinar (K.D.). Following decades of in-house family disputes and on-going conflicts with the Ottoman Empire, in 1899 Britain agreed to manage foreign relations until Kuwait gained independence in 1961. Kuwait's agricultural development is very limited, having an extremely hot climate and a dominant desert topography and with the exception of fish. It is almost entirely dependent on imported food. During the late 1940s, the extent of Kuwait's oil resources became obvious and the long-term potential of the petroleum industry was realized, now having a modern and well-developed infrastructure. Kuwait has several major electric power-generating plants providing the country with drinking water through desalination operations and removing salt from seawater. The chief exports are oil, oil-related products and fertilizers whereas imports are food and livestock, construction materials, vehicles and parts, and clothing.

Figure 2.1: International map of the State of Kuwait

(http://www.worldatlas.com/webimage/countrys/asia/kw.htm) (DOA: 26/01/2012)

2.1.2 Kuwait population residents

The population of Kuwait refers to the total number of inhabitants, of which a third are natives, divided unequally across the country's six governorates: Al 'Asimah, Hawalli, Al Farwaniya, Mubarak Al-Kabeer, Al Jahra and Al Ahmadi (Figure 2.1). Immigrants represent 70% of the total population. The total population of Kuwait (nearly 3.5 million) is composed of Kuwaitis: non-Kuwaitis in ratio of 1:2.5, and is considered a good example of a non-endemic country with a TB incidence of 35 per 100,000 populations (WHO, 2010e). The population growth rate (PGR) is estimated to be 3.44 per 1000 person. The annual crude birth rate of Kuwait is 21.88% while the annual crude death rate is 2.42 deaths per 1000 persons. According to the estimation census made on December 2004, the total population was 2,644,777 with a total of 942,892 Kuwaiti's compared with 1,118,911 Kuwaiti's out of 3,484,881 when counted on December 2009 (HVS, 1984-2009). A similar distribution has been observed for the last three decades (Figure 2.2).
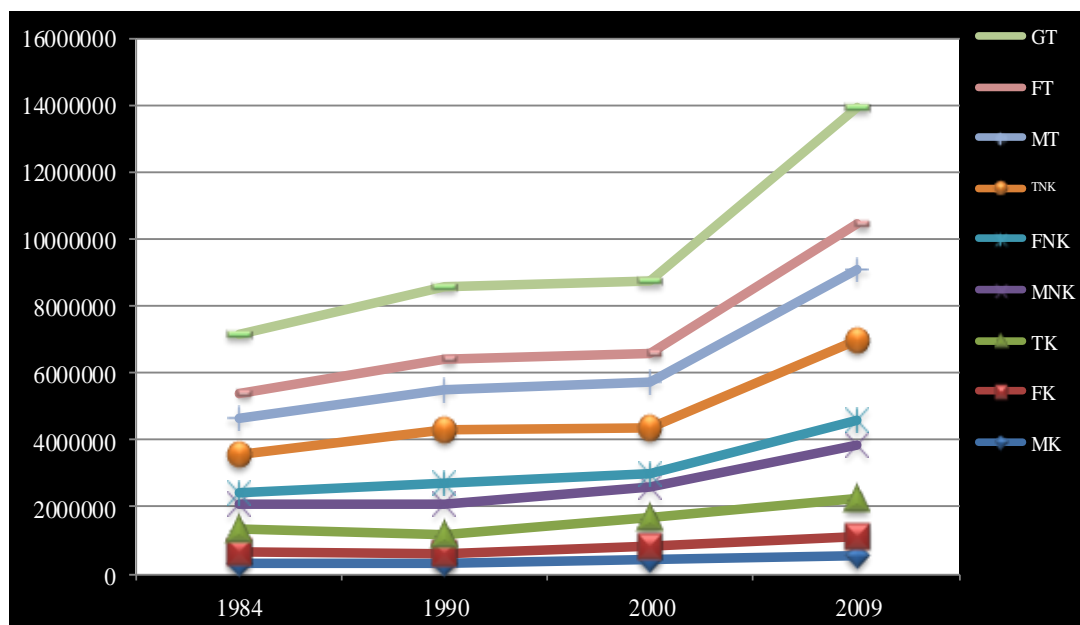
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MK = Kuwaiti males, FK = Kuwaiti females, TK = total Kuwaiti's, MNK =non-Kuwaiti males, FNK = non-Kuwaiti females, TNK = total non-Kuwaiti's, MT = total males, FT = total females, GT = grand total

Figure 2.2: Chart represents the distribution of Kuwait residents by gender and nationality through three different decades (1980's, 1990's and 2000's) (HVS, 1984-2009)

Migration is increasing annually all over the world. Immigration having travel-associated negative health impacts and in particular affecting TB non-endemic regions. The net migration rate (NMR) of Kuwait is around 15.65%, ranking third worldwide in 2010 (Factbook, 2011).

Table 2.1: Net Migration Rate of Kuwait (2000-2011) estimated) (Factbook, 2011)

Year	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Net Migration Rate	14.77	14.31	13.88	14.04	14.18	14.96	15.66	16.05	16.39	16.02	15.65	0.65

The main immigration 'pulling' or 'in-migration' factor is the need for a labour force which is similar to all developed and non-endemic low incidence countries (Figure 2.3) (Shah, 2007).

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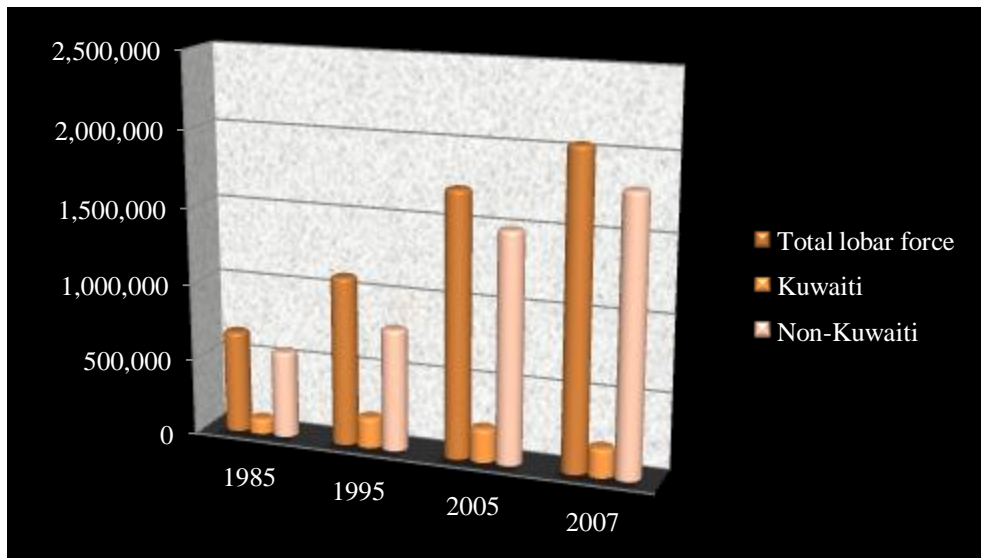
Figure 2.3: Chart represents the total labour force (age 15 years and above) and distribution by nationality in Kuwait, 1985-2007 (Shah, 2007)

The second driver for immigration is family re-unions/visits, followed on third by business and marketing (Figure 2.4) (Shah, 2007).
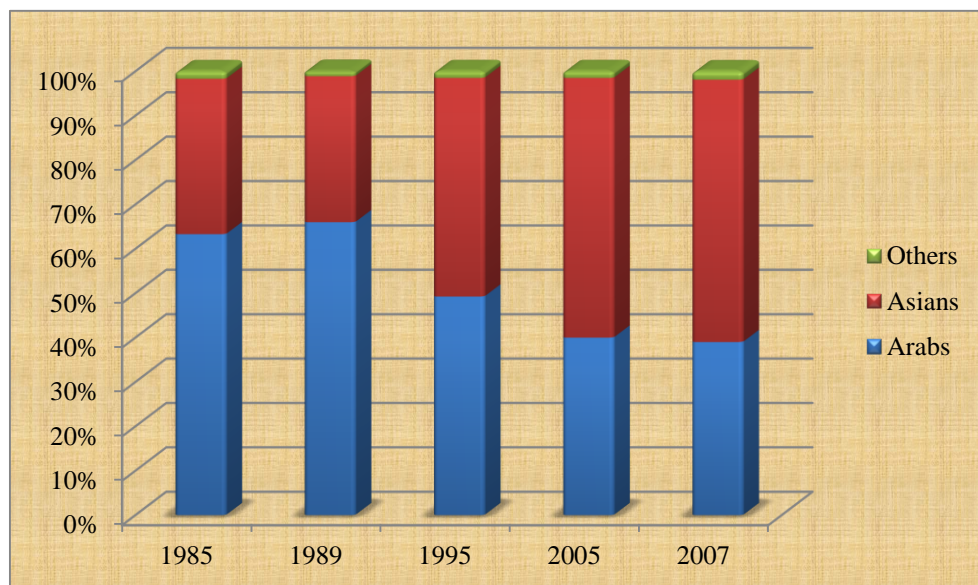
Asians = includes all Asians, Arabs = includes all Arab nationalities

Figure 2.4: The distribution of non-Kuwaiti population by nationality or broad category of origin in Kuwait, 1985-2007 (Shah, 2007)

2.1.3 Impact of migration on LTBI & TB

With the increase in migration, immigrants are in particular need of close monitoring and screening programmes to tackle TB transmission and to ensure effective general public health. Migration is a major risk factor with public health risk on infectious diseases epidemiology. Population-demographic changes are related to contact-exposure and importation of LTBI/active TB from high- toward low-prevalence regions such as return visits and re-exposure risks (McCarthy, 1984). An individual's geographical history (immigration and foreign travel, destinations, travel-exposure durations) is important in any assessment, as well as consideration of age and general health of traveler's including risk of type of job/work (Freeman *et al.*, 2010). Immigration demographics indicate that Asians, Blacks, and Hispanics bear the greatest burden of TB and screening of immigrants can protect community public health in nations of low TB incidence. Western Europe faces irregular migration of TB cases a phenomenon that has increased during the past two decades and is investigated using routine medical examination and migrant screening (MacPherson and Gushulak, 2006). The incidence of tuberculosis in the country of birth is crucial to predict variation in demographic structure changes which can be used to focus and target TB elimination and prevention efforts toward the high-risk TB groups (Watkins *et al.*, 2002). Worldwide, and in Kuwait in particular (where the number of immigrants has doubled the Kuwaiti indigenous population), it is essential to understand the drug susceptibility of *M. tuberculosis*, especially in low TB prevalence regions due to the risks of uncontrolled immigration and high travel of hosts (Saif Alfaresi and Hag-Ali, 2010).

Despite national and WHO international recommended guidelines for the diagnosis and treatment of both LTBI and TB disease, health care workers still lack alertness and have poor knowledge of TB added, to which can be added under-estimation of the risk of progression of LTBI to the active disease (Karakousis *et al.*, 2007).

2.1.4 Arabian Gulf countries' health policies

Countries have different responsibilities and legal obligations towards various types of international migrants, these responsibilities being restricted by international human rights laws, national laws and labour organizations. Regardless of the reason for migration, fears of the consequences of uncontrolled migration on national public health has led to restrictive immigration procedures been followed, as a compulsory requirement for immigration e.g. obtaining a visa requires prior health examination from an immigrant's mother country, and such workers are only admitted on a temporarily for specified periods.

2.1.5 Kuwait's health and anti-tuberculosis policy

A global expansion of TB control programmes for immigrant populations in Kuwait has achieved a significant decrease in TB-harboring immigrants from countries of high TB incidence. International collaboration between TB immigration screening programmes and national TB programmes within the countries of origin offer benefits to both immigrant-source and immigrant-receiving countries (Alvarez *et al.*, 2011). Kuwait Ministry of Health has been proactive in committing support and financial resources for health system maintenance for example funding TB epidemic control programmes utilizing both national governmental and non-governmental efforts in collaboration with the Stop TB Partnership through adequate follow-ups, sufficient information handling procedures, and authorities' coordination (Oxlade *et al.*, 2009). Kuwaiti governmental support to the Ministry of Health has successfully improved the health care system and increased the quality of human life, for example offering a free and accessible health care service with compulsory notification of any infectious disease within the country. An increased movement of people through travel and immigration has stressful effects on public health responses and associated with transmission threats of international communicable diseases (Coker, 2004). The budget for the National Programme for Tuberculosis (NPT) in Kuwait was increased to U.S.$6 million for 2011 (compared with U.S.$4 million in 2006) (WHO, 2010b). Worldwide, TB screening programmes for immigrants can take place in three ways: first, pre-departure screening in the country of origin; secondly, on arrival screening

occurs at the point of entry into the host country and at airports or other transit migrant centres; and, thirdly, through post-arrival screening after entry to the host country in transitioning centres such as Immigration Centres. In Kuwait, the Department of Public Health guidelines specify infectious diseases control through both steps one and three for TB screening before official entry of immigrants to the State.

Policy measures and governmental efforts in Kuwait manage migration, family re-unification and permit authorized movements through obligatory legislation and serious measures that serve to safeguard workers' rights. Immigrants have no right to remain in Kuwait in the destination country beyond the period of authorized employment. A balanced migration policy means bringing the immigration level down towards the emigration level. Kuwait operates an 'entry points based system' that includes a compulsory visa known in Arabic language as 'Kafalah' followed by employment-limited authorization and issue of the obligatory civil identification card, which is is provided only after the person has been awarded a certificate reporting a post-examination 'normality report' issued by the Tuberculosis Control Unit (TCU), a sub-division from Department of Public Health. The entry report includes results of six diagnostic tests: HIV/AIDS, hepatitis B and C, malaria, filariasis and a chest X-ray (CXR). New immigrants are tested at one of four immigration centres; Al Asma'a, Al Farwaniya, Al Jahra or Al Fahaheel, and test results are transferred to the Department of Public Health for final approval of entry. A positive test result is used as an indication for isolation of the immigrant and treatment before deportation. Within the Tuberculosis Control Unit, any suspected CXR tuberculosis case is further tested with the tuberculin skin test; positivity is an indication for further diagnostic testing such as sputum culture and antibiotic sensitivity to rule out active TB prior to admission for treatment and rejection.

2.1.6 Kuwait notification system

In 2009, Kuwait's population totaled 3,484,881 with an increase of 47% of human immigration, compared with 1,786,616 in 1984 (Kuwait Times, December 07, 2009). Almost 60% are foreign residents looking for jobs, with an estimated 70% being under the age of 24 years (HVS, 1984-2009). Kuwait has on average 500 to 700

expatriates and new immigrants coming into the country per day. Each immigrant should be registered within one month after been offered a temporary viza before entry to the country.

A strict notification system provides accurate assessments of the national health system and care services, and missed or lost cases are not an issue. By law, any suspected infectious diseases, including TB cases, are only diagnosed and treated in a governmental hospital. The overall case detection rate for TB in Kuwait is 100%, compared with only 70% in the South East Asia Region (WHO, 2010c). In European industrialized countries such as Norway, Ireland and Great Britain, a detected rise in incidence of TB since 2001, among young foreign-born subjects belonging to immigrant families from high-incidence countries, is due to increases in national TB notification rates (Dahle *et al.*, 2007).

All tuberculosis death and hospital discharge rates can be safely used for comparative purposes of the health status and communicable diseases trends either national (within Kuwait) and/or international (worldwide). Surveillance systems can determine causes of death (mortality) and discharge (morbidity) as potential sources for detecting improvements in TB diagnosed case rates (Home, 1984). With high coverage of the Ministry of Health, Kuwaitis' regular national surveillance and establishment of tuberculosis reference centres (e.g. TCU) add elements of control to both diagnosis and management. Accurate registration data are available and accessible without the need for morbid rate estimations. The slowing of the decrease in TB rates all over the world and in Kuwait can be largely attributed to persistence of LTBI/TB in foreigners' and foreign-born populations. The rate of TB is usually nearly 2 to 5 times higher among non-Kuwaiti residents than among the Kuwaiti population, and TB is most prominent in the Asian populations (HVS, 1984-2009; Jassal and Bishai, 2010).

2.1.7 Strengths and limitation of health policy system

Strict notification of any diagnosed and admitted TB individual to the Ministry of Health authorities in Kuwait is essential. Any suspect and diagnosed TB cases should be admitted. No admission, diagnostic testing or management is allowed except in the three authorized governmental hospitals; 1)- temporarily in any general hospital

or Infectious Disease hospital, and then transferred to or admitted directly to, 2)-Tuberculosis Curative Unit. All admissions represent all the cases of tuberculosis in the country, and thus incidence and prevalence can be calculated from these hospital-based data.

Limitations appear because of the absence of a central database able to differentiate newly diagnosed (incident) cases from old re-admitted cases i.e. re-admission is considered as a new case. TB stigmatization may lead to the preference of some residents, even Kuwaitis, to be managed outside the country. Such cases can be missed with absent diagnosis in the issued death certificate. Wrong diagnosis due to language barriers and miss-diagnosis may occasionally occur e.g. in prison or field workers in the desert due to case accessible difficulties.

2.2 Aims and objectives

Assess the morbid trends of tuberculosis disease of all residents in Kuwait during the last three decades.

2.3 Methodology

Tuberculosis data between 1984 and 2009 were collected from the annual publications of the Department of Health Information and Vital Statistics (HVS). Morbidity and mortality rates were calculated accordingly using Microsoft Office Excel 2003 (©Microsoft Corp etc), and figures were drawn using Microsoft Office Excel 2007 (©Microsoft Corp etc).

2.4 Results

2.4.1 Trends in tuberculosis morbidity (1984-2009)

Population morbidity and mortality in Kuwait can be identified through study of all residents who are influenced by infectious diseases and by the endemic tuberculosis of the foreign migrant's according to the geographic distributions and related socio-demographic backgrounds. Tuberculosis case admission is taken according to the disease defining characteristics for any individual within a time period, where the

hospital occurs for implementation of diagnostic-related-procedures. Tuberculosis morbidity rates representing tuberculosis prevalence over a 26-year-period (1984-2009) in Kuwait were calculated as percentages of tuberculosis hospital discharges per 1,000 population (almost equal to prevalence) and tuberculosis hospital discharges per 1,000 hospital discharges (which represents the burden on hospitals). All rates been tested for two demographic population characteristics (gender and nationality), and were calculated as represented as the following:

2.4.1.1 Tuberculosis hospital discharges per 1,000 total population during a 26-year-period (1984-2009) (Figure 2.5, Appendix 1 - Table A)

The overall morbidity trends of TB discharges per 1,000 populations have declined gradually from 0.49 in 1984 to 0.29 in 1989. Post Iraqi invasion a steady continuous fluctuation trend reaching average of 0.26 until 1999, then declined to 0.08 per 1,000 populations until 2004 is observed. A sudden increase in the TB morbidity trend has occurred since 2005 (0.22 per 1,000 populations) and continued almost at the same rate until 2009. The highest calculated rate after invasion was 0.29/1000 population in 1993, 1996 and 1997, whereas, the lowest value rate of 0.07/1000 population was demonstrated in 2001, 2003 and 2004. In general, males and non-Kuwaitis' rates were predominantly higher than those of their counterparts (Figure 2.5).
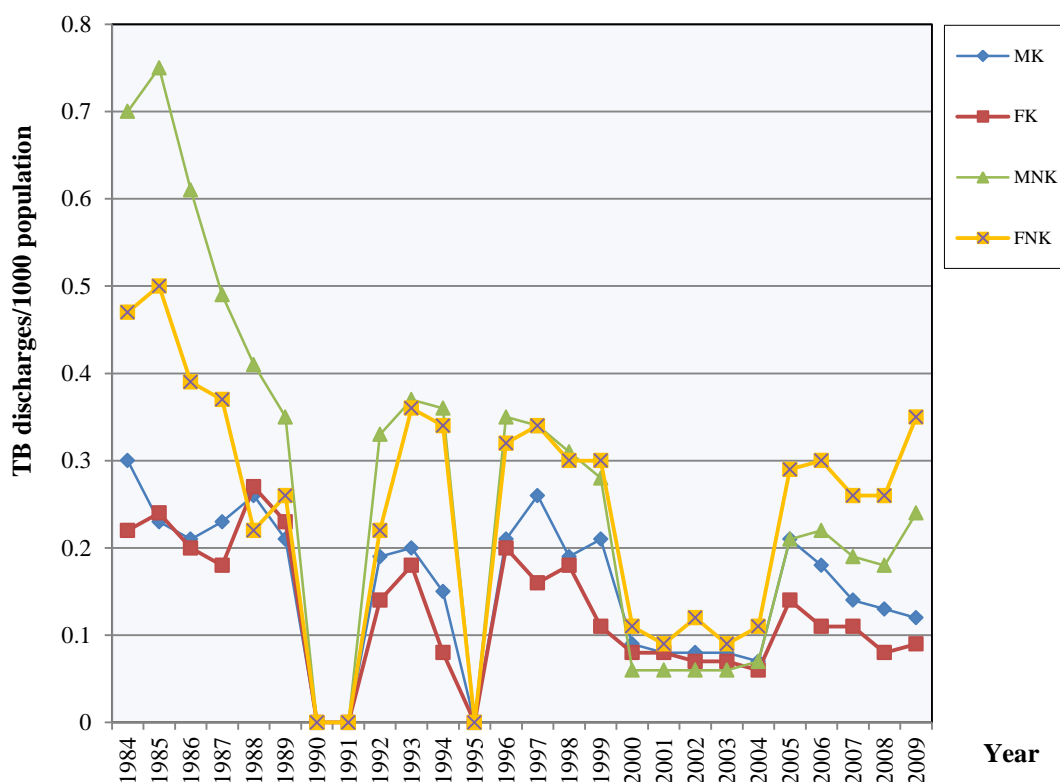
Figure 2.5: Trends in tuberculosis hospital discharges per 1,000 population by gender and nationality reported in Kuwait, 1984-2009 (MK = Kuwaiti male, FK = Kuwaiti female, MNK = non-Kuwaiti male, FNK = non-Kuwaiti female, N/A = data not available)

2.4.1.2 Tuberculosis hospital discharges per 1,000 total hospital discharges during a 26-year-period (1984-2009) (Figure 2.6, Appendix 2 - Table B),

In general there was a decline between 1984 and 2004, with two steady periods; the first was between 1989 and 1996 and the second one, at a lower level, between 2000 and 2004. Then the trend sharply increased until 2009. All non-Kuwaitis' rates have been continuously rising since 2005, where males reached 7.59 and females 4.61 in 2009, compared with rates of only 1 for Kuwaitis. Non-Kuwaitis always had higher rates (nearly double) than those of Kuwaitis and also male rates were comparatively higher than those of females. The highest calculated rates after the invasion were 4.54 in 1997 compared with the lowest rate of 0.89 in 2001 (Figure 2.6).
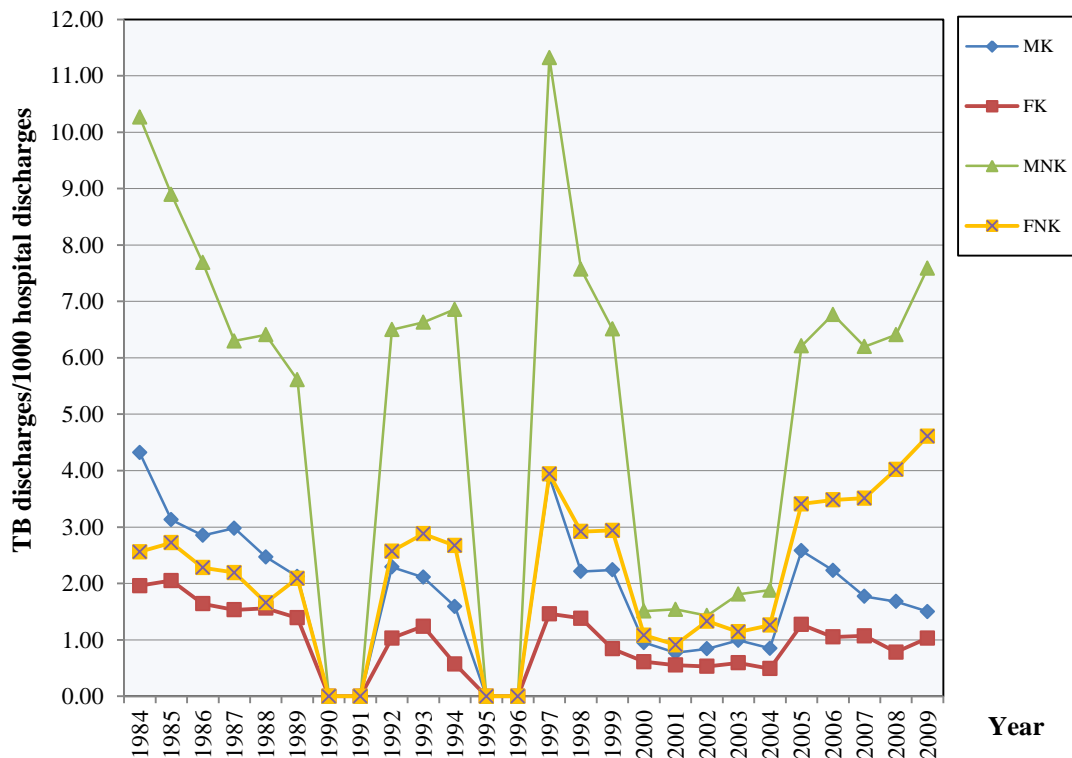
 Figure 2.6: Trends in tuberculosis hospital discharges per 1,000 hospital discharges by gender and nationality reported in Kuwait, 1984-2009 (MK = Kuwaiti male, FK = Kuwaiti female, MNK = non-Kuwaiti male, FNK = non-Kuwaiti female, N/A = data not available)

2.4.2 Trends in tuberculosis mortality (1984-2009)

Tuberculosis mortality status representing the tuberculosis deaths over a 26-years-period in Kuwait. All rates been tested also for two demographic population characteristics (gender and nationality), and were calculated as represented as the following:

2.4.2.1 Tuberculosis cause-specific mortality rate per 100,000 populations (TB CSMR/100 000 population) during a 26-years-period (1984-2009) (Figure 2.7, Appendix 3 - Table C)

Tuberculosis cause-specific death rate is defined as the annual total number of deaths from tuberculosis per 100,000 mid-year population at risk.

The overall mortality trends reveal tremendous sharp declines of TB cause-specific mortalities per 100,000 populations in all CSMR annual rates from 2.63 in 1984 to 0.64 in 1989. Post-1990 a steady continuous fluctuating trend reached averages of less than 1 until 2009. A sudden reversal with double increase in TB morbidity trends has occurred since 2008 from 0.56 to 1.16 for Kuwaitis' mortalities and 0.54 to 0.85 for non-Kuwaitis per 1,000 populations. The highest calculated rate after invasion was 1.17/100 000 populations in 1995, whereas the lowest value of 0.39/100 000 populations was calculated in 1998. Non-Kuwaiti females rates averaged around one/100 000 populations after no cases in 1993. In general, male and Kuwaitis' rates were predominantly higher than those of their counterparts (Figure 2.7).
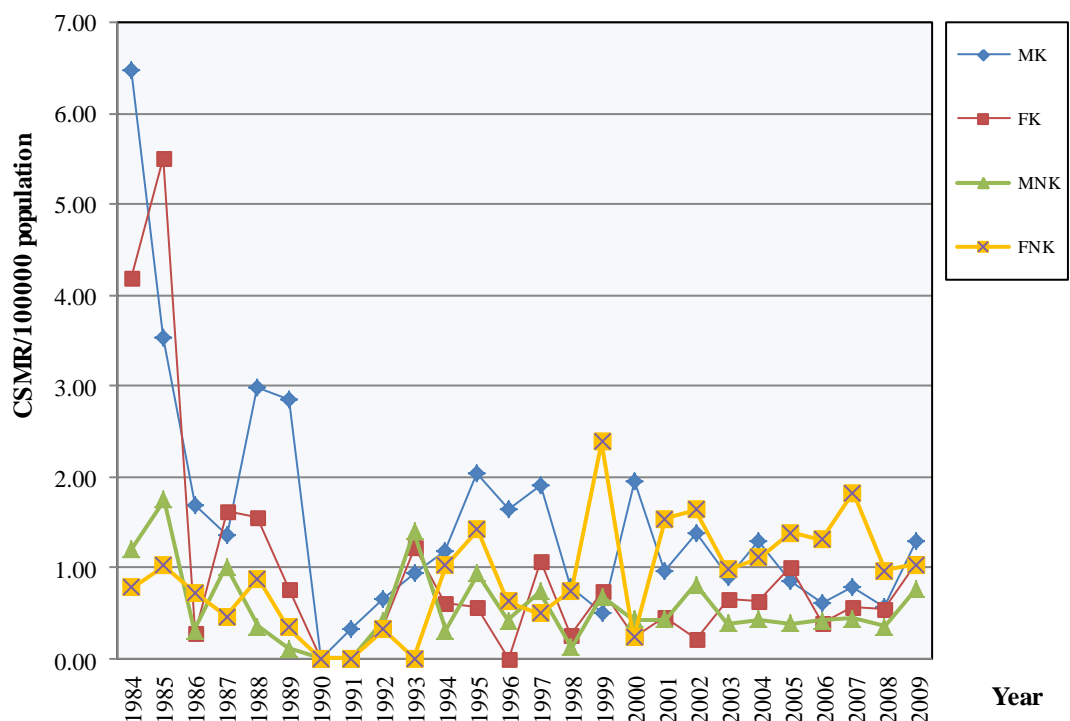
Figure 2.7: Trends in tuberculosis cause-specific mortality rates (CSMR) by gender and nationality reported in Kuwait, 1984-2009 (MK = Kuwaiti male, FK = Kuwaiti female, MNK = non-Kuwaiti male, FNK = non-Kuwaiti female, N/A = data not available)

2.4.2.2 Tuberculosis case fatality rate (tuberculosis deaths per 100 tuberculosis hospital discharges (TB deaths/100 TB hospital discharges) (Figure 2.8, Appendix 4 - Table D)

On average, the overall trends of TB case fatality rates were below 5% of total TB discharges during the 26-years-period, with intervene by a sharp incline of average 10% between 2000 and 2004, followed by decline to rates below 5%. Kuwaiti's suffered higher case fatalities than non-Kuwaitis. There was a sharp increase in Kuwaiti fatalities from 5.56% in 2008 to 11.02% in 2009, compared with a 0.5% increase for non-Kuwaitis (from 2.65% in 2008 to 3.06% in 2009). Males Kuwaiti's have markedly higher case fatalities than females (Figure 2.8).
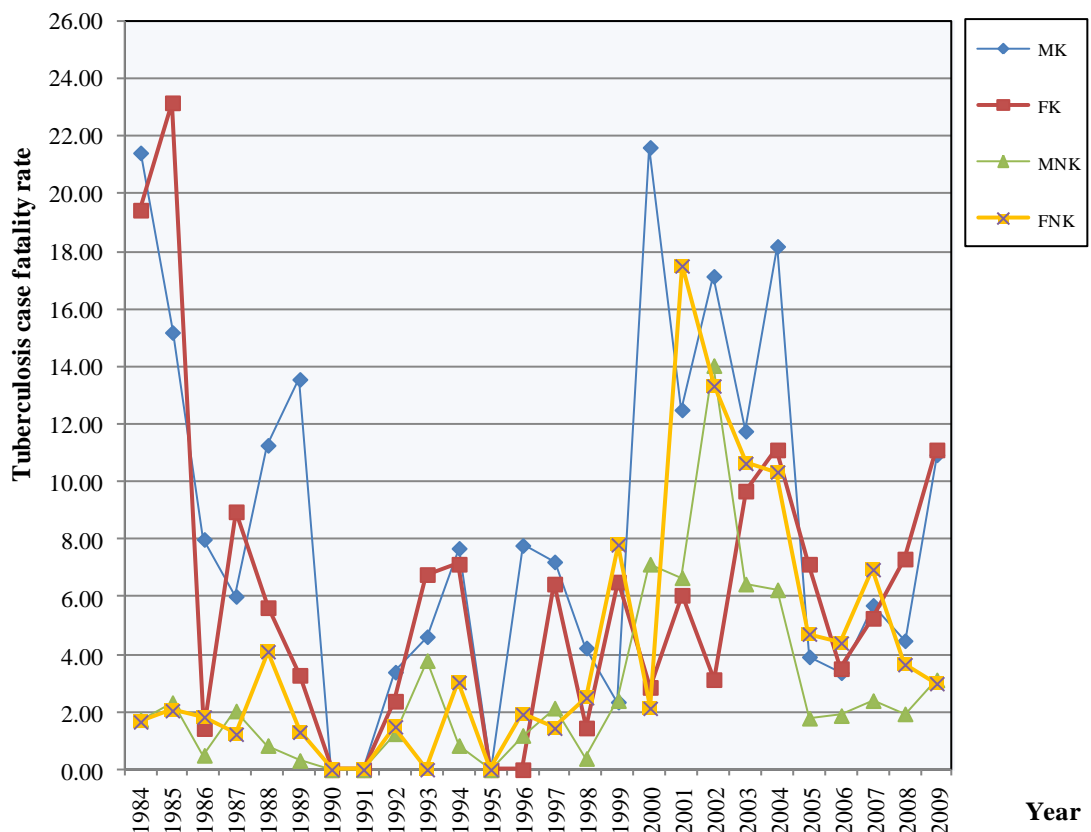
Figure 2.8: Trends in tuberculosis case fatality rates by gender and nationality reported in Kuwait, 1984-2009 (MK = Kuwaiti male, FK = Kuwaiti female, MNK = non-Kuwaiti male, FNK = non-Kuwaiti female, N/A = data not available)

2.5 Discussion

International economic migration flows are continuously increasing all over the world from low socio-economic regions towards richer countries, where the majority of immigrants are looking for economic opportunities and economic globalization. Mobility of the world's population is rapidly becoming a key determinant of infectious disease epidemiology. Prevalent screening using definitive intervention and cost-effective procedures for agreed diagnostic criteria toward health restoration are pre-requisites.

Tuberculosis, hepatitis and HIV/AIDS are predominantly detected in Asian and African immigrants. Tuberculosis epidemiology differs according to the infection pressures in different countries, with consequent variable probability of development of tuberculosis disease: the greater duration of exposure and the length of time spent in close contact with high-risk populations, the greater the risk of latent TB infection and late-appearing clinical disease (McCarthy, 1984). Immigrants from TB-endemic countries play a role as sources of tuberculosis infection, particularly within migrant communities due to contact patterns of the migrant communities (Lillebaek *et al.*, 2002; Rieder *et al.*, 1994). Follow-up screening of high-risk groups at entry for the burden of immigrant-associated tuberculosis (active infectious and inactive non-infectious) (Coker *et al.*, 2006) is needed, together with efficient intervention programmes (Liu *et al.*, 2009) and follow up targeting of TB contacts (Mulder *et al.*, 2009) for anti-TB and preventive therapy. Screening among migrants will lead to earlier case detection, shortened symptom duration, and reduction by 33% of MTB infectiousness and hospitalization. Cooperation among countries should reduce political differences and facilitate the development of improved screening programmes.

The demographic infrastructure and population health status of Kuwait has been changing over the last four decades and is associated with the global changes in trends of communicable diseases and the overloads of health care services. There are common worldwide TB-associated demographic risk factors such as gender, place of birth, occupation, nationality and ethnic origin (Kruijshaar and Abubakar, 2009). A

gender difference exists, men showing a higher reported case notification rate of tuberculosis than women, necessitating active case finding and strengthening of primary health care (Buu *et al.*, 2010; Sharma and Kumar, 2010).

The direct indicators of the burden of tuberculosis are prevalence (indicating the number of people suffering from the disease at a given point of time) and annual mortality (indicating the number dying each year). Both of these rates respond quickly to improvements in control, as timely and effective treatment reduces the average duration of disease (thus decreasing prevalence) and the likelihood of dying from TB (thus reducing mortality rate e.g. cause-specific and proportionate mortality rate defined as TB death cases per 100%). Worldwide there were 5.8 million notified cases of TB in 2009 and a case detection rate of 63% (ranges, 60 - 67%) compared with 61% in 2008 (WHO, 2010d).

Kuwait shows a similar worldwide picture for all TB mortality and morbidity trends. These rates represent TB burden and overloads on Ministry of Health and Kuwait population. Substantial rate declines of mortality and morbidity burden of TB in Kuwaitis relative to non-Kuwaitis could be due to improved socio-economic conditions and life expectancy and disappearance of old, healed but untreated TB (Horsburgh *et al.*, 2010). This reflects advances in the general hygiene which can justify the implications of improvements in TB control and having lower drug-resistant MTB strains compared with those regions under continued health overloads (Hickson, 2009; Mangtani *et al.*, 1995; Oxlade *et al.*, 2009).

A mild and slow annual rise of 100,000 was achieved in the total population below two millions until 1998 (total 2,027,103), but since 2005, a total growth increments have reached an annual growth of 300,000. There has been a large increase of immigrants into Kuwait (the majority from low-social class migrants) since 2004. In 2005, out of total 1,893,602 non-Kuwaitis, 38.6% were of TB endemic Asian origin e.g. India, Indonesia, Bangladesh, the Philippines (HVS, 1984-2009).

Disproportionate over-estimation of hospital discharges and morbidity can be due to recurrent discharges for the same TB case and comparison between different nationalities should be cautious. Similarly, international mobility and long-term travelers and tourists to areas of high endemicity for tuberculosis (incidence more

than >40 cases/100000 population/year) are potentially at risk of contracting TB because Kuwaiti people have high exposure probabilities to MTB and infectious cases, which have raised the Kuwaitis' case fatality ratios. This has been associated with re-exposure and spread of other external infectious diseases to Kuwait community e.g. malaria or HIV/AIDS (McCarthy, 1984).

The relative frequency of exogenous re-infection against endogenous re-activation is still ambiguous and undetermined. Kuwaitis' higher case fatalities can be related to combinations of multiple morbid risk factors for affection by both internal and external environmental factors, which are complicating TB elimination and weakening assessment of control programme outcomes (Lienhardt *et al.*, 2002b).

2.5.1 Common ecological factors

2.5.1.1 Biological factors

2.5.1.1.1 Age

Higher trends of TB mortality and morbidity in the elderly than younger adults is an increasing problem in many countries, associated with age-related decline in immunity and increasing longevity of LTBI occurs particularly in the developed world, whereas, other statistically proved risk factors predominating in the developing countries such as poverty, malnutrition and tobacco smoking (WHO, 2009).

A strong association between aging and TB development with excess/rise of TB morbid indicators is usually noticed in younger women and older men due to decline in macrophages, which are the host's anti-TB defence mechanism. Older ages, and usually in males above 45 years, show higher re-activation risk of dormant LTBI, as immunity weakens and BCG vaccine efficiency flare-ups (Gajalakshmi *et al.*, 2009; Kolappan *et al.*, 2007). Clinical improvement in response to treatment was less satisfactory (~50%) due to adverse effects (Gust *et al.*, 2011).

2.5.1.1.2　Gender

In Kuwait, the reversal shift to female predominance since 1999, having the highest rate values for all tuberculosis discharge per 1,000 population rates, might be due to predominance of female immigrants employed for in-house jobs e.g. housemaids and cooks, with overcrowding and prolonged exposure-contacts and difficulty in hiding TB morbidity symptoms due to stigma-social anti behaviors and public ignorance (Courtwright and Turner, 2010; Juniarti and Evans, 2010; Svensson *et al.*, 2011) in contrast to male predominance all over the world revealed by the study of Neyrolles and Ouintana-Murci (2009), which should be encountered for research recommendations according to the needs of Kuwaiti's environment to household immigrants. The significance of household crowding and associated-risk of prolonged contact is observed in Kuwaiti's household disease transmission. The impact of stigma on social behaviours and TB diagnosis and treatment has serious socio-economic consequences, particularly for women, and is commonly associated with fear of infection, TB diagnostic delay and treatment non-compliance (Courtwright and Turner, 2010; Juniarti and Evans, 2010). For example, in 2009, tuberculosis discharge per 1,000 population rate of female non-Kuwaitis was 0.35% compared with 0.24%, 0.12% and 0.09% for the non-Kuwaiti males, Kuwaiti males and Kuwaiti females respectively which could be a specific recommendation for further research about the needs of Kuwaiti's environment to household immigrants.

Waning of anti-tuberculosis immunity in old age (above 50 years) compared with younger groups with LTBI is a potential biological mechanism for an increase in LTBI re-activation (Horsburgh *et al.*, 2010).

2.5.1.1.3　Sex hormones

Sex differences are based on infectious disease epidemiology. Worldwide, TB is a leading cause of mortality among women, but higher morbidity rates and worse outcomes are reported more inr men. Protection/susceptibility to MTB and related-mortality rates is correlated with sex hormones. High sex steroids are less protective in females during their reproductive years (Holmes, 1998; Shetty *et al.*, 2006; Weiss,

2008). Special care for high risk groups of old and reproductive ages is crucial for a successful TB control programmes to be considered.

2.5.1.1.4　Host genetics

Tuberculosis is a disease with a genetic predisposition. Pre-existing differences in strain genetic background, rural-urban cluster strains and innate immune response differences are related to compensatory changes of morbid trends which been found to play a role in the emergence and spread of acquired drug-resistant MTB strains, and which is a man-made problem (Borrell and Gangeux, 2011; Crampin *et al.*, 2009). Host genetic factors determine differential susceptibility to infection and disease outcome because of human's genetic variation associated with sex-specific effects and complex susceptibility to pulmonary TB (Neyrolles and Quintana-Murci, 2009; WHO, 2009).

2.5.1.2　Socio-economic-cultural factors
2.5.1.2.1　Income

Combined socio-economic factors are complicating TB elimination and are affecting control programme outcomes commonly noticed in the developing countries (Lienhardt *et al.*, 2002b). Low social class, overcrowding, illiteracy and under/malnutrition are all related to immune-suppression (CD4+ counts less than 200 cells/mm^3) with anergic DTH-IV immune responses against TB-concomitant infections and LTBI re-activations (Pai *et al.*, 2005).

2.5.1.2.2　Stigmatization

Cultural epidemiological approaches are useful for TB control, such as treatment adherence, determinants of default and impacts on behaviour of gender-specific features of stigma (Weiss *et al.*, 2008). TB stigma is an important barrier to health-seeking behavior, delays in TB diagnosis, treatment non-compliance that necessitates health intervention instruments and consequently hinders the control and management of all infectious diseases. Identification and evaluation of causes and impact of TB stigmatization on TB diagnosis and treatment has been systemically

reviewed by Courtwright and Turner (Coutwright and Turner, 2010). Perception of TB infection and self-isolation from TB diagnosis due to secrecy and shame are barriers for males in seeking care due to risks of unemployment and income loss (Wieland *et al.*, 2010). Reversed rate percentages of tuberculosis discharges per 1,000 populations toward feminization such as females and marital status or male and sexual contact (Kip *et al.*, 2011; Weiss *et al.*, 2008).

2.5.1.2.3   Behavioural factors

Population mobility is a major factor in globalization of public health threats. Internationally, significant increases in travel-related respiratory and vector-borne infections are potential risks for introduction of airborne droplet transmission into non-endemic countries, and numbers of travellers' deaths are still under-estimated. Importation of immigrant pathogenic strains can influence local population strains over extended time periods (Dahle *et al.*, 2007).

Smoking with exposure to toxic substances is the major behavioural and occupational risk factor that is increasing all over the world including Kuwait (Rao *et al.*, 2011). Tobacco smoking/addiction can be the cause of LTBI re-activations, and the three-fold-rise in TB case fatalities due to smoking addiction since the last decade (Jeon and Murray, 2008; Wen *et al.*, 2010), which is also related to different ethnic backgrounds (Wang and Shen, 2009).

2.5.1.2.4   Socio-economic factors

Socio-economic risk factors have direct and indirect impacts on TB transmission, which consequently add more barriers toward the health care sectors. Risk factors including age, gender, daily contacts and various genetic polymorphisms should be targeted by any surveillance programme.

Although changes in the immune system during aging lead to increased susceptibility to mycobacterial infections and re-activation of latent foci, TB is still associated with younger ages in developing countries, independently of HIV status. This may be explained by the higher proportion of young people, a higher annual risk of infection

and shorter life expectancies in comparison with industrialized countries (Solari *et al.*, 2008).

2.5.1.2.5 Virulence of *Mycobacterium tuberculosis* infecting strain

Emergence of drug resistant tuberculosis is a serious public health problem newly appearing in non-endemic countries, which requires strengthening tuberculosis control and improving monitoring of therapy. MTB spoligotyping is suitable for the broad evidence-based epidemiological investigation of tuberculosis patients.

Linkage between immigrant geographic origin and MTB genotypic strains varied significantly with clinical picture presentations and raised TB mortality and case fatality rates in non-endemic countries (Thuong *et al.*, 2008). The predominance of foreign-born genotypic and drug-resistant strains varies significantly with differences in severity and clinical picture presentations, to which Kuwaitis are sensitive, and in which more pulmonary TB develops in males compared with females, in contrast to which there are more extra-pulmonary complications in females than in males (Ladefoged *et al.*, 2011; Svensson *et al.*, 2011).

2.5.1.2.6 Nutritional factors

Environmental effects such as nutritional status or living on traditional farms or parasitic (helminthes) infections are known risk factors to affect the immune system in early (neonatal) life with consequences for later disease outcomes or for responses to BCG vaccination in infancy (Djuardi *et al.*, 2010). Vitamin D deficiency and related anemia (e.g. iron deficiency anemia) are common in females all over the world due to poor nutrition and high susceptibility to MTB with low antimicrobial immunity increases LTBI and TB probabilities' (Neyrolles and Quintana-Murci, 2009; Talat *et al.*, 2010).

Re-activations are commonly related to impairment of the immune system, caused by poverty (low income and property ownerships), and alcohol and drug abuse, leading to smoking or medical re-activation risk factors – such as DM, HIV/AIDS and other

related debilitating medical conditions, poor eating habits and inadequate nutrition with vitamin deficiency (LoBue *et al.*, 2010b).

Predominance of diabetus disease and cardiovascular diseases in Kuwait is related to under-nutrition according to age and gender (Dye *et al.*, 2011). Diabetics have more severe clinical TB infections, which require large controlled and longer treatment, and are more likely to develop MDR-TB than non-diabetics (Chang *et al.*, 2011). Overweight as important risk factor and related-drug resistance is an important outcome for the binomial TB-DM in which diabetics are unaware of DM and TB risks (Jeon and Murray, 2008).

2.5.1.3 Risk factor combinations

The relative frequency of exogenous re-infection against endogenous re-activation is still ambiguous and undetermined.

Tuberculosis exposure is affected by internal and external environmental risk factors. Previous history of TB infection is a common risk factor, which can be integrated as 'PTB suspicion', which is also help TB diagnosis higher than the CXR performances (Wu *et al.*, 2009). Common additional risks are prevalence of TB infection within exposed populations, presence of infectious source, air density carrying TB droplet nuclei, duration of exposure to infected polluted air and quality of indoor/outdoor air ventilation (filtration).

Close contact and proximity of jail inmates raise HIV and TB possibilities due to overcrowding, shared respiratory ventilation buildings (> 12 hours) and poor living conditions, facilitating MTB transmission to others from undetected active TB cases. Environmental conditions and proximity to contagious index cases (> 8 hours) can be considered as barriers for TB control programmes e.g. hospital wards, aircraft (Al-Jahdali *et al.*, 2003; Demkow *et al.*, 2008).

Variability in the median period from immigrant arrival until diagnosis of TB is related to both immigrant origin and site of TB lesion e.g. Asians infected with extra-pulmonary disease have a shorter median time-period before diagnosis, which can be due to delay in diagnosis or detecting TB post-immigration due to increased risk of

exposure to other immigrants during or after travel (Cowei and Sharpe, 1998). Public transportation of overcrowded immigrants and increased exposure-time represents an additional occupational risk factor for LTBI re-activation and TB infection.

For example a Kuwaiti family living in overcrowded place (close-contact exposure) and having housemaid coming from TB endemic country (long exposure durations with new LTBI/TB suspect immigrant) in addition to poor eating habits or under/malnutrition such as vitamin D deficiencies (Talat *et al.*, 2010), and having immune-suppression (weak immune responses) against MTB facilitate LTBI re-activations mainly in smokers' and diabetic patient or complaining from other debilitating conditions (LoBue *et al.*, 2010b; Pai *et al.*, 2005).

2.6 Conclusions

Notification rates of tuberculosis in Kuwait, standardized for gender and nationality, have declined between 1984 and 1990, but the decline has decreased since 2009. Kuwait infrastructure has changed substantially and awareness of socio-demographic changes may speed-up diagnosis of tuberculosis. The annual rise and TB burdens of non-Kuwaiti population are overloading the health care system and medical service. Overcoming higher rates of TB morbidity of non-Kuwaitis in comparison with those of the Kuwaitis population constitute a serious public health issue that warrants urgent evaluation and re-structuring of the tuberculosis control programme through highly-qualified surveillance system and strict quantification and reduction of TB stigmatization. Fluctuation of declining and increments in the overall national TB rates, coupled with the persistence of disproportionately high TB rates among foreign-born non-Kuwaitis, suggest the need for a re-doubling of public health efforts aimed at the eradication of TB in Kuwait. Tuberculosis-associated immigration can be limited through verification of new screening guidelines for diagnostic testing to detect LTBI early, reduce TB misdiagnoses, and limit MTB transmissions.

2.7 Recommendations

1- To control and further reduce TB incidence and prevalence, both nationally and locally, public health professionals must urgently and effectively target high-risk sub-populations through:

2- Adequate surveillance systems allowing the identification of population segments having a particular excess risk of tuberculosis compared with the general population, and the screening for tuberculosis and infection with *Mycobacterium tuberculosis;*

3- Applying diagnostic guidelines to improve immigration and related communicable disease control/prevention strategies can contribute to a reduction in TB morbid trends due to breaking of *Mycobacterium tuberculosis* transmission chain.

4- Improvement in screening of immigrants for TB upon arrival to Kuwait, public health efforts and re-enforcing awareness of TB risks, transmission, diverse manifestations to limit diagnostic delays in the health services;

5- Availability of cost effective new diagnostic tests (interferon gamma release assays; IGRAs) which assist in screening of high-risk groups for LTBI, enabling effective preventive chemo-prophylaxis;

6- Reduction of structural and behavioural barriers in health systems by filling the gap of lack of knowledge regarding migrants' exposure to risk factors, morbidity, and psychosocial needs;

7- Careful planning, pilot testing, enhanced information sharing, practitioner training, and ongoing programme evaluation;

8- Using Kuwait electronic health records (EHRs) evolution of prospective surveillance detection of latent TB infection;

9- Facing the challenge of detection of new MTB resistant strains.

3 Chapter three

Materials and Methods

3.1 Ethics and responsibilities

A field trip was undertaken to Kuwait in August-November 2008 to achieve agreement for the project implementation. Project approval was guided by the Permanent Coordination Committee for Medical and Health Researches in their 11th meeting on Tuesday, 28/10/2008, where they discussed and approved the methodology for the research protocol: 'The Challenges of Tuberculosis Prevention through Early Detection of Latent Tuberculosis Infection in New Immigrants to The State of Kuwait'. The Scientific Research Committee approval is considered as ethical clearance for conducting the research and was followed by official documents sent to the involved ministry departments for permission and facilitating the researcher's mission.

Scientific requirements involved the followings;

- Recommendation official letter from the University of Edinburgh external supervisor: Professor Sue Welburn
- Agreement of head of Public Health department
- Project methodology (the preliminary methodology agreed on)
- Data collection sheet (questionnaire) of participants in English language (Appendix 5)
- Screening tool result sheets; chest X-ray (Appendix 6), tuberculin skin test (Appendix 7) and both IGRAs; QuantiFERON Gold In-Tube test (Appendix 8) and T-SPOT .*TB* test (Appendix 9)
- Participant informed consent; both in English (Appendix 10) and Arabic (Appendix 11) languages
- Researcher adoption pledge; both in English (Appendix 12) and Arabic (Appendix 13) languages and
- Letter signed by researcher, detailing that the Ministry of Health will not be financially supporting the project.

Permission has been granted to facilitate with allowance for data collection and implementation of all diagnostic tests for the eligible new immigrants to Kuwait

during registration Al' Farwaniya Immigration Centre and TB Control Unit (two different departments of Ministry of Health in Kuwait).

3.2 Role of authority

The project responsible authorities; Ministry of Health in the State of Kuwait and the University of Edinburgh in Scotland have thoroughly facilitated and organized all the steps of research implementation with no interference with project data. The author declares no competing interests. The author follows direct administrative supervision in the project field to improve quality of work and has no authority to interfere with the entry to Kuwait of any immigrant having positive result(s) and/or latent tuberculosis infection diagnoses. The researcher provided research staff with basic background information about the study and project step follow-ups. Each potential participant in the project screening was adequately informed of the aims, methods and sources of funding of the screening, any possible conflicts, the institutional affiliations of the researcher and the study benefits and potential risks. On request, immigrant has all human rights to be informed about his/her research extra- (non)- compulsory test results of both interferon gamma release assays and/or tuberculin skin test but not chest X-ray (the ordinary test) results.

3.3 Study methodological design

A review on methodology of various published studies employed for detection of latent tuberculosis infection and active tuberculosis disease cases was carried out in order to overview the general picture of tuberculosis worldwide. The findings of the review formed the basis and context for the development of the methodological design and the screening of the new immigrants to Kuwait as follows.

3.4 Study design

The research followed an observational, randomized and repeated-measure, prospective cross-sectional study (Chiang *et al.*, 2009; Knapp and Miller, 1992).

3.5 Study duration

The study period was four months from February to May 2010, research being conducted during four working days (Sunday to Wednesday).

3.6 Study population

The study permitted involvement of any new immigrant entering through Al Farwaniya Immigration Centre in Kuwait during February and May 2010. Project implementations were preceded by a preliminary clarification of all research steps and the four tuberculosis diagnostic test procedures. An adoption pledge was signed by the researcher (Appendix 12; English or Appendix 13; Arabic) followed by participants' verbal or signature of written participant consent (Appendix 10: English or Appendix 11; Arabic).

3.7 Sample size calculation

Sample size was calculated using the basic national records statistics of Kuwait and systemic review of international latent tuberculosis infection (LTBI) statistics and annual reports of the World Health Organization as follows (Horsburg, 2004; HVS, 1984-2009; WHO, 2008b; WHO, 2010d):

- Average total daily immigrants entering Kuwait = 400 - 700 (average 500) individuals per day

- Average total immigrants entering Kuwait per year = 500 x 25 days/month x 12 months/year = 150,000 immigrants per year

Excluding non-working days and holidays, the average of 120,000 (between 80,000 and 150,000) immigrants are legally entering the country as a newly immigrants and the majority enter occupied in the labour force as employee workers more than family re-unions. The prevalence of latent tuberculosis infected individuals in the six billion populations infected with *Mycobacterium tuberculosis* worldwide is five to ten percent (two billion) individuals and these cases are always at risk of re-

activation to the active tuberculosis disease form and further distributing tuberculosis infection. The mid-point average is seven and a half percent (7.5%), and was taken to represent LTBI prevalence. Using the Win EpiScope statistical package (Thrusfield *et al.*, 2001), the estimated sample size required for project implementation reveals the following values (Table 3.1):

Table 3.1: Sample size calculation using Win Episcope 2.0 statistical package programme (Thrusfield *et al.*, 2001)

Average immigrants entering Kuwait per year (80,000-150,000)	Average expected prevalence of LTBI (%) (International prevalence 5-10%)	Accepted precision /error (%)	Level of confidence (%)	Sample size (n)
120,000	7.5	2	95	667
120,000	7.5	3	95	297
120,000	7.5	4	95	**167**

The lowest accepted and calculated sample size was one hundred and sixty seven participants to estimate prevalence with a specified 95% confidence interval of 'True' implies correction for a test's sensitivity and specificity of 3.51% and 11.49%. The size was increased to one hundred and eighty immigrants with the availability of screening diagnostic tests (n = 180).

3.8 Study selection

During their compulsory registration at the Immigration Centre, new expatriates were randomly chosen as one in every thirty four new immigrants without selection bias. All eligible participants were approached to enroll in the study after accepting the informant consent and after being briefed on their interviewee rights.

3.9 Inclusion criteria

Any healthy adult new immigrant aged between eighteen and sixty years of age was simultaneously eligible for inclusion and invited to participate. Other epidemiological or tuberculosis risk factor differences were not considered as limiting factor such as gender, nationality, ethnic origin, past history of TB infection or metabolic disease(s).

3.10 Exclusion criteria

Any immigrant was excluded from the study if:

1. He/she refused to participate and/or refused consent agreement,
2. There was an absolute language barrier with difficulty in contact even with the help of a third party (e.g. a nurse capable of translating),
3. He/she was newly diagnosed as having acute infectious disease within the last six months before entry to Kuwait e.g. presenting signs and/or symptoms of active TB, acute hepatitis or HIV/AIDS,
4. He/she was at increased risk of adverse reaction(s) to tuberculin skin test e.g. skin allergy or venepuncture e.g. blood disorders like hemophilia or sickle cell anemia, or was a severely malnourished (thin wasted) individual,
5. He/she had a history of previous positive tuberculin skin test result less than 6 months prior to entry and enrollment, and
6. He/she missed follow-up for a tuberculin skin test reading result after five days.

3.11 Tuberculosis screening tools

For each new immigrant, all of the following screening diagnostic tools were performed including an interview for data collection using a structured questionnaire (Appendix 5) followed by applying another three tuberculosis diagnostic tests on top of the ordinary chest X-ray; tuberculin skin (prick) test and both interferon gamma release assay (IGRAs) tests: 1- QuantiFERON Gold In-Tube test (QNF-GIT) and 2- T-SPOT .*TB* test.

3.12 Study setting

3.12.1 Al Farwaniya Immigration Centre

Out of the four immigration centres covering the whole of Kuwait (Al 'Asimah, Al Fahaleel and Al Jahra), Al Farwaniya Immigration Centre had been chosen randomly for immigrant screening. Al Farwaniya Immigration Centre is located in Al Farwaniya governorate and is considered to be the most populous region in the State

of Kuwait (covering 907,321 out of the 3,442,945 total population in 2009) (HVS, 2009), with a non-Kuwaiti predominance and with up to 200-300 worldwide new immigrants being tested daily during five working days per week.

Immigrant registration and numbering (standard daily procedure) described as;

Project procedure's

After expatriate ordinary registration and following WHO guidelines for diagnostic test implementation (Chiang *et al.*, 2009; WHO, 2002a), all new immigrants were:

- Freely allowed to choose whether or not to participate in the study,
- Informed of the research procedures and risks,
- Protected from any harm related to testing procedures,
- Protected from stigma associated with tuberculosis,
- The only one able to know the results of his/her own diagnostic tests, and
- His/her right to abstain from participating or to withdraw consent at any time without reprisals.

The following Figure 3.1, Figure 3.2 and Figure 3.3 clarified the project steps. After their ordinary administrative registration (Step 1), all participants were randomly selected and invited to participate after they provided informed consent (written or verbal; Appendix 10 and Appendix 11) followed by (Step 2) were data gathering through confidential face-to-face interview using epidemiological and LTBI risk factor assessment questionnaire (Appendix 5). An expert and highly-qualified data collector speaking four languages was trained for data extraction to obtain tuberculosis-related answers (Step 2). One interviewer was used to obviate inter-rater variation. When there was a complete language barrier the immigrant was excluded.

Then participant will enter the nursing room (Step 3) to perform the routine post-entry and ordinary diagnostic tests for any new immigrant including; 1- withdrawal of routine blood sample for screening by the virology polymerase chain reaction (PCR) for HIV, hepatitis B & C, 2- finger prick and slide microscopy for malaria and

filariasis, and 3- intramuscular (IM) anti-meningitis chemoprophylaxis versus meningococcal disease.
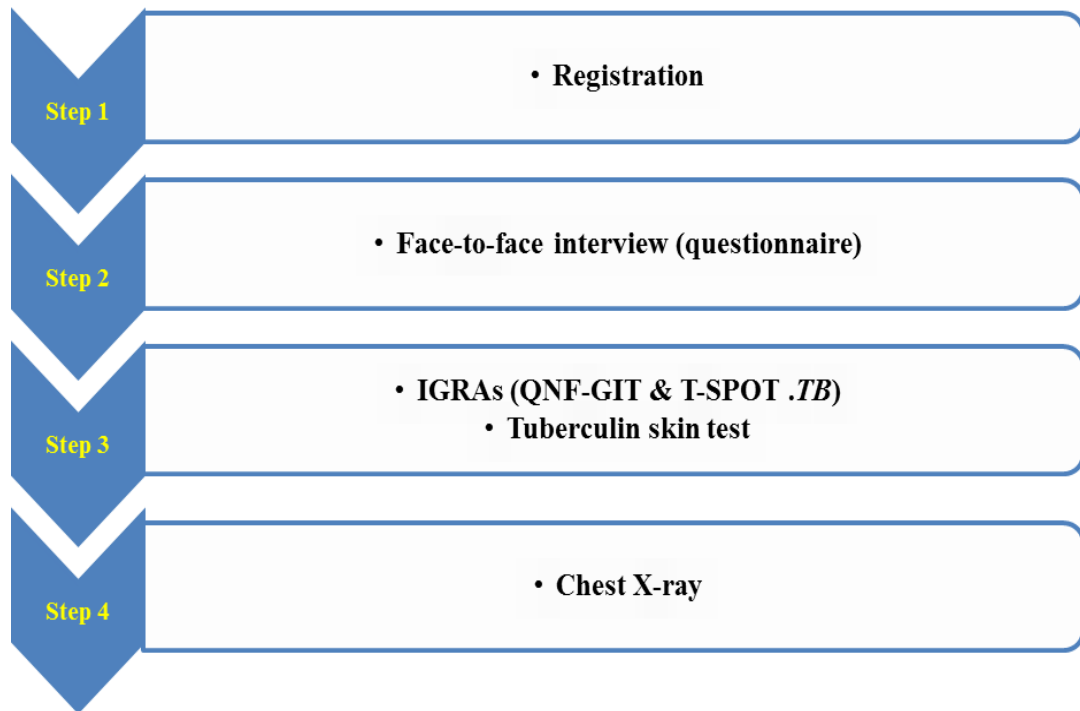
Figure 3.1: Flow diagram representing the strategic plan for screening the 180 new immigrants to Kuwait in Al Farwaniya Immigration Centre (Step 1: immigrant registration), (Step 2: data collection), (Step 3: nursing room), (Step 4: Chest X-ray room), IGRAs = interferon gamma release assay tests including both QuantiFERON Gold In-Tube test (QFT-GIT), T-SPOT .*TB* test)

Apart from the listed compulsory tests in the nursing room and (Step 3) started by the following: first measurement of both body weight and height, followed secondly by withdrawal of both IGRAs blood samples on the same PCR prick, then thirdly administration of the tuberculin skin test and fourthly checking for scar of BCG vaccination. Finally, in (Step 4) immigrants were referred to radiography for taking ordinary compulsory chest X-ray. As usual all radiographic pictures were electronically transferred to Tuberculosis Control Unit (TCU) for the medical staff to read and comment (Figure 3.2 and Figure 3.3).
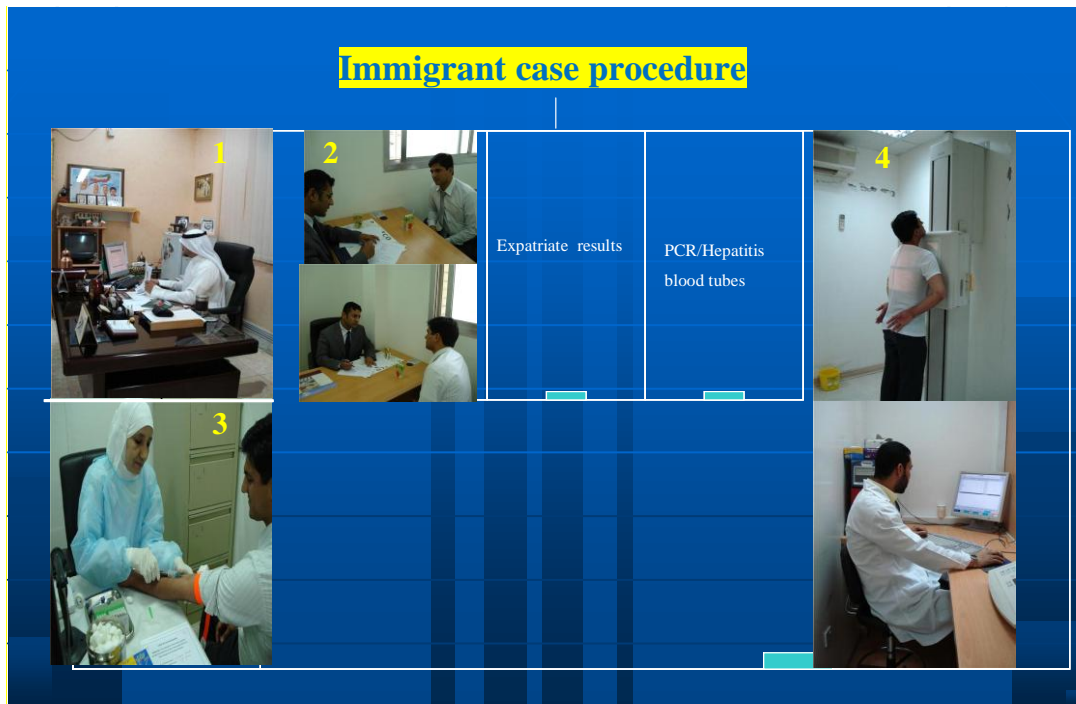
Figure 3.2: Case management of new immigrants at Steps (1 to 4) in Al Farwaniya Immigration Centre in Kuwait

Case management during (Step 3) in the nursing room are shown in Figure 3.3.
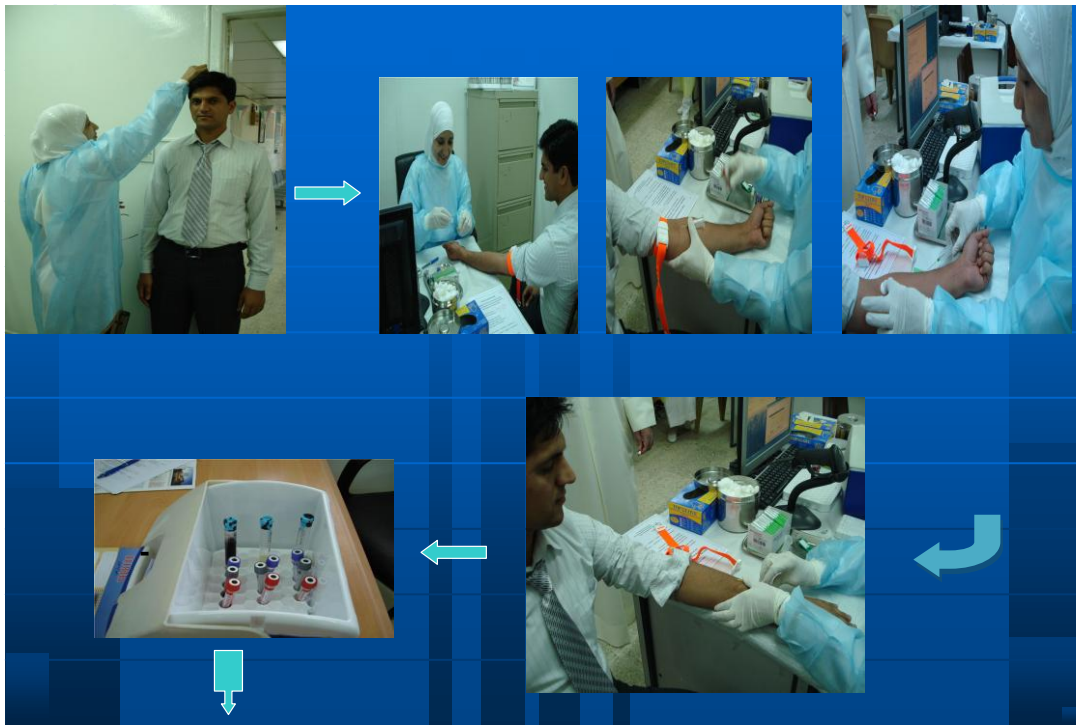
Figure 3.3: Immigrants case management in the nursing room; 1- measurement of body height/weight, 2- blood withdrawal of interferon gamma release assay (IGRAs) samples, 3- tuberculin skin test administration (prick test) and BCG scar detection

IGRAs blood samples were transferred within 30 minutes to TCU. The total time for management of each case was between 45 and 60 minutes and three to four participants were interviewed per day within four working days during the four months of the study.

Step 1

This began with random selection of any registered immigrant and invitation to participate after he/she provided informed consent and preliminary agreement for participation (written or verbal; Appendix 10 and Appendix 11) after being given an explanation of all field examination testing, and adoption pledge primarily signed by the researcher (Appendix 12 and Appendix 13).

Step 2

This involved contact between the data interviewer and participant using as pre-designed and pre-tested identical questionnaire developed following a literature review of all potential risk factors of LTBI/TB disease (Appendix 5). The questionnaire will be discussed in thorough in chapter 4.

Step 3

In the nursing room the following examinations and test procedures were performed:

1. Body weight (in Kilogram) and height (in centimetre) were measured and recorded on the tuberculin skin sheet (Appendix 7)

2. Peripheral venipuncture and venous blood of IGRAs tests were withdrawn as: one sample of 8 ml heparinized blood tube for the T-SPOT *.TB* test (further discussed in 3.12.2.1.1) and three samples of 1 ml heparinized blood tubes for the QuantiFERON-TB Gold In-Tube test (QFT-GIT) (further discussed in section 3.12.2.1.2) and

3. Tuberculin skin (prick) test using the Mantoux technique was performed according to recommendations of the WHO-Kuwait tuberculosis standards for mass screening of *Mycobacterium tuberculosis* infection, where:

The testing nurse administered exactly five Tuberculin Units of 0.1 ml of purified protein derivative (PPD) as 5TU of tuberculin (Tubersol: Sanofi Pasteur Ltd, Toronto, Ontario, Canada), which was intra-dermally slowly injected in the superficial skin layers (Mantoux technique) using one-ml disposable tuberculin syringes, on the volar surface of the mid-anterior left forearm. The left forearm was chosen by convention to avoid errors in allocating the test site during reading. However, in case of scarring (e.g. previous BCG) on the left forearm, the test was given on the right forearm (Figure 3.3, Figure 3.4, and Figure 3.5). Each participant was carefully examined for the presence of a Bacille de Calmette Guerin (BCG) scar and recorded on the TST sheet (Appendix 7).

Figure 3.4: Injecting five tuberculin units (5TU) of a Mantoux skin test (http://en.wikipedia.org/wiki/Tuberculosis_diagnosis) (DOA: 26/01/2012)

Figure 3.5: Measuring the size of induration using flexible calliper after 46-72 hour post-application of 5 tuberculin units (http://en.wikipedia.org/wiki/Tuberculosis_diagnosis) (DOA: 26/01/2012)

Within 48 and 72 hours, both readers (the administrative nurse and researcher) double checked and identified the margins of transverse induration of the TST site by carefully palpating the edges of the reaction for measurement. A small flexible caliper (15 cm length ruler calibrated in millimetre) was used to measure any firm and well circumscribed induration or soft well-defined margin swellings. Care was taken not to measure erythematous skin reaction. The readers also asked and examined the immigrant for the presence of any adverse reactions in the test site such as skin hematomas', bullae, vesicles, and necrosis. Presence of BCG scar determines

individual's vaccination status and was looked for on both arms from the shoulder region to the wrist, because of differences in international vaccination schedule.

In accordance with the American Thoracic Society and Centers for Disease Control and Prevention guidelines (ATS and CDC, 1999; CDC, 2005c; CDC, 2010b, Farhat *et al.*, 2006) and Department of Public Health (Ministry of Health in Kuwait), either with or without history of prior BCG, positive tests were defined as those for which were transverse diameters of the palpable hardened areas with an induration size of 10 millimetre ($\geq$ 10-mm) (further discussed in chapter 5) and finally,

Step 4

Chest X-ray was performed in the radiography room by expert radiographers'. Postero-anterior (PA) graphs were taken as an ordinary test and copied by CXR printer machine. Excluding one Syrian female due to pregnancy contraindication, chest radiographs of the 179 participating individuals were then read by three different pulmonologists and re-read by another two qualified reader radiologists' guided by a standardized recording policy, for purpose of interpretation, recording and reportation for the presence of discrepant abnormal findings' in a specific chest X-ray recording sheet (Appendix 6) which is further discussed in chapter 5 (van der Werf *et al.*, 2008).

After collection of blood samples, both IGRAs heparinized tubes were transported within 30 minutes of collection in an insulated covered icebox to the tuberculosis central laboratory of Kuwait in Tuberculosis Control Unit for rapid sample processing after collection.

3.12.2 Tuberculosis Control Unit (TCU)

The Public Health department, through the Tuberculosis Control Unit belongs to Sabah Health Area within Al 'Asimah governorate and is considered as a central office for directing the national tuberculosis programme (NTP) working hard to bring tuberculosis under control where TB screening in the chosen policy using CXR and TST or CT scan as further testing for abnormal CXR finding. Suspected cases are

usually ascertained by the TB Curative Unit and Al-Rashed Centre for Respiratory diseases and Allergy. Further detailed management procedures are taken such as repeating several CXR's, taking three consecutive morning sputum samples for acid fast bacilli (AFB) examination, culture and drug sensitivity and other advanced investigations for complicated cases e.g. broncho-alveolar lavage (BAL), lung biopsy, lung bronchoscopes.

The function of the TB Control Unit includes the following:

- Screening referrals from: A- The centre of examination of expatriates, B- The general Medical Council, C- The centre for examination of food handlers, D- Contacts from the TB Curative clinic, E- Contacts of diagnosed and confirmed TB case in hospitals
- Laboratory examination of sputum samples referred from all clinics and hospitals.
- Screening of referrals of suspected TB, thoracic and extra thoracic TB before they are referred to the TB Curative Unit
- Receives and process all TB disease notifications from all sources of notification in the country, register all cases and ensure application of the direct observational therapy, short course (DOTS) strategy
- Processes TB notifications and implements contact investigation.

In addition to daily interpretation of average 1,000 to 1,500 chest radiographic interpretations over the six different governorate medical centre's, TCU has the central laboratory for tuberculosis interferon gamma assays (IGRAs) testing in Kuwait.

3.12.2.1 Tuberculosis central laboratory

Manipulation and analysis of IGRAs blood samples was performed according to the manufacturer's instructions for tube preparation at the central and only tuberculosis laboratory in Kuwait (Figure 3.6).
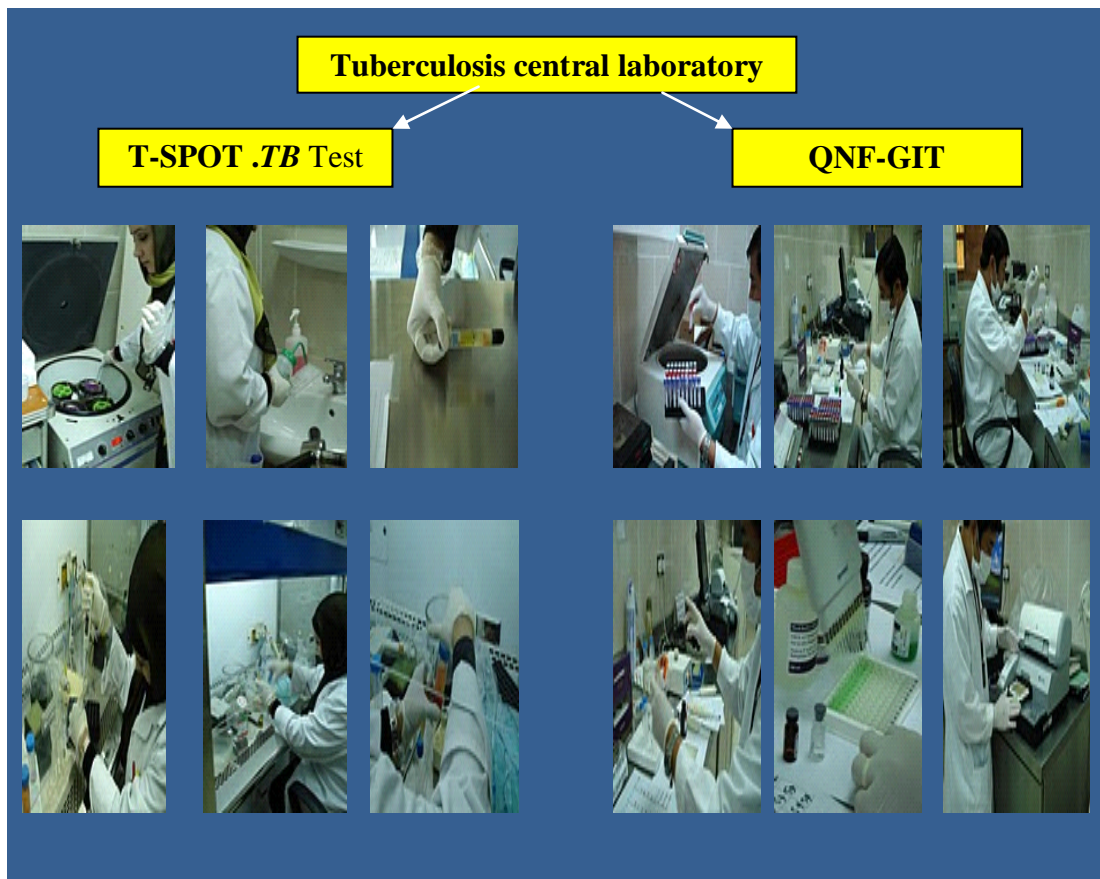
Figure 3.6: Laboratory processing of interferon gamma release assay (IGRAs) samples in the central tuberculosis laboratory of Kuwait (QNF-GIT = QuantiFERON Gold In-Tube test)

Both ex vivo IGRAs were employed to identify *Mycobacterium tuberculosis* infection and described accordingly:

3.12.2.1.1   T-SPOT *.TB* test

The commercial T-SPOT *.TB* assay (Oxford Immunotec Ltd, Oxford, United Kingdom) used the collected peripheral venous blood into cell preparation tubes (Becton Dickinson, Franklin Lakes, NJ). A new biomarker test using ESAT (early-secreted antigenic target 6-kDa protein) and CFP-10 (culture filtrate protein 10), stand out as suitable antigens inducing an interferon-gamma (IFN-γ) secreting, T-cell-mediated immune response to MTB infection.

Principle procedure of T-SPOT *.TB* test (Oxford Immunotec Ltd, Oxford)
(Figure 3.3, Figure 3.6, Figure 3.7, Figure 3.8, Figure 3.9);

1) A precoated INF-γ cytokine in ELISPOT plate was seeded with peripheral blood mononuclear cells (PBMCs) per well and incubated with media with no antigen (as a negative control), peptide antigens derived from ESAT-6 (labeled panel A), peptide antigens derived from CFP-10 (labeled panel B), *M. tuberculosis* H37Rv PPD, or phytohemagglutinin (PHA as a positive control) in a 5% CO_2 atmosphere at 37°C for 16 to 24 hours

2) Peripheral blood mononuclear cells (PBMC's) were separated from a whole blood sample and washed to remove any sources of background interfering signal.

3) PBMC's were incubated with the antigens to allow stimulation of any sensitized T-lymphocytic cells present in the blood sample.

4) Secreted INF-γ cytokine was captured by specific antibodies on the layer membrane (which forms the base of the well), and the cells and other unwanted materials were removed by washing.

5) A second antibody, conjugated to alkaline phosphatase (ALP) and directed to a different epitope on the cytokine molecule, which is able to bind to the INF-γ cytokine captured on the membrane surface, was added.

6) Any unbound conjugate was removed by a second washing.

7) Then a soluble substrate was added to each well; this was cleaved by bound enzyme able to form a spot of insoluble precipitate at the reaction site. Each read spot represents a footprint of an individual cytokine-secreting T-cell. Evaluating the number of spots obtained provides a measurement of the abundance of *M. tuberculosis* sensitive effecter CD4+ T-cells in the participant peripheral blood (Figure 3.7).
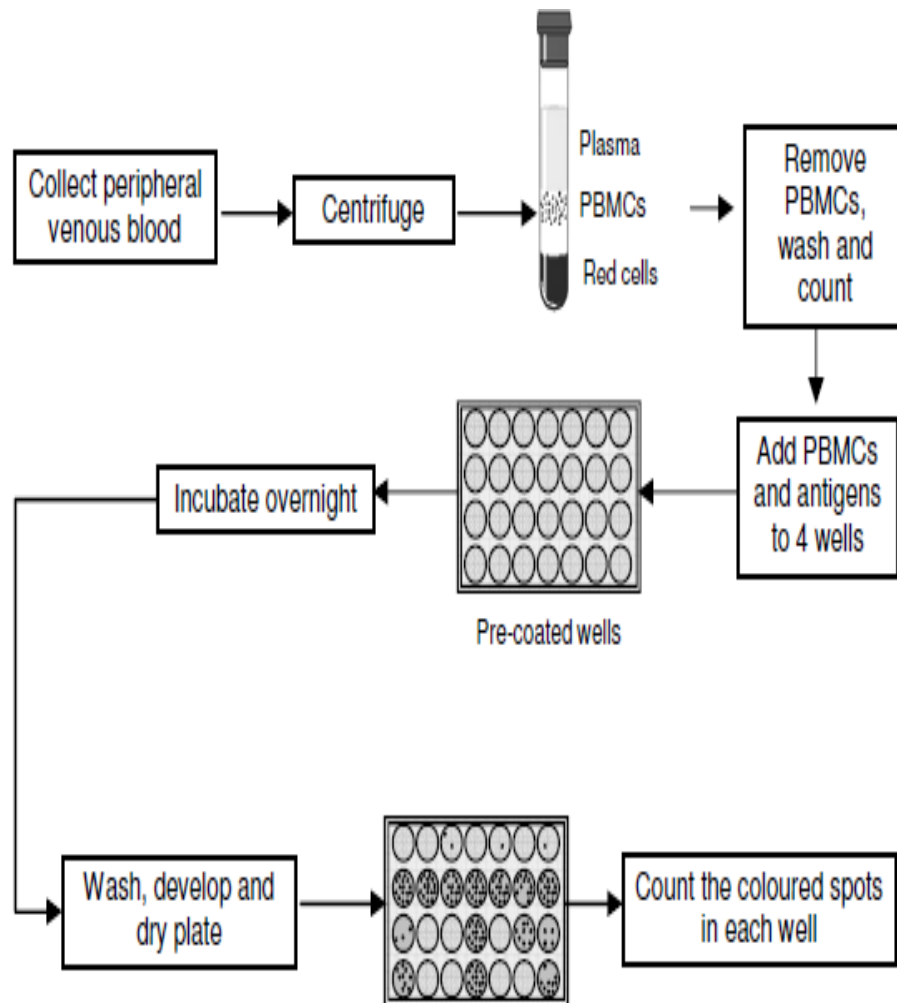
Figure 3.7: The main laboratory steps of T-SPOT .*TB* test (http://www.oxfordimmunotec.com/96-UK) (DOA: 26/01/2012)

T-SPOT .*TB* test result was counted as spots from T-lymphocytes sensitized to *M. tuberculosis* antigens and delineated captured interferon gamma. Following the manufacturer's recommendations, results were then interpreted by subtracting the spot count in the negative (NIL) control from the spot count in Panel A (containing ESAT-6 antigens) and Panel B (contain CFP-10 antigens) (Figure 3.8).
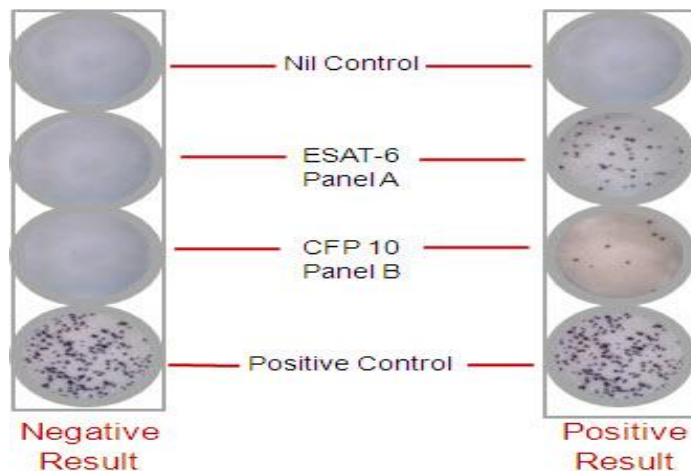
Figure 3.8: Interpretation of T-SPOT .TB test; ESAT-6 (early secretory antigen target-6), CFP 10 (culture filtrate protein-10) (http://www.tbtestingservices.com/TB%20Testing) (26/01/2012)

The test was interpreted as positive if individual displayed response as either or both panel A or panel B had six or more spots more than the negative control ($\geq$ 6 CFU; colony forming unit), and this number was at least twice the number of spots in the negative control. The test was considered to have failed if the negative control spot count was more than ten spots (> 10 spots) or if there were less than twenty spots (< 20 spots) in the positive control and both panels A and B was nonreactive according to the criteria above. Spots were counted manually and with ELISPOT magnifying glass reader (Figure 3.8, Figure 3.9).
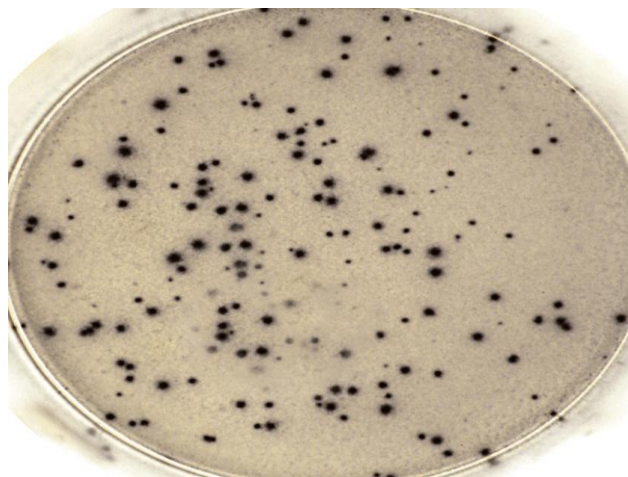
Figure 3.9: A positive T-SPOT .*TB* test result of either panel A or panel B or both had six or more spots than the negative control (spot forming unit represents sensitized T-lymphocyte to previous *M. tuberculosis* infection) (http://newenglandtb.pbworks.com/f/T-Spot+TB.pdf)

Spots were enumerated and double-checked by two observers (the laboratory technician and laboratory head manager) using a stereomicroscope apparatus and spots manual counting, and the mean spot count was interpreted as the final test result.

3.12.2.1.2 QuantiFERON-TB Gold In-Tube test (QFT-GIT)

The QFT-GIT is an indirect, bimolecular, blood test able to measure the cell-mediated immune response of MTB-infected (LTBI case) individuals. Stimulation of effector T cells in the whole blood with a specific antigen(s) or mitogen, followed by simple quantification of the resulting IFN-γ in the plasma is the basis of QuantiFERON test.

Principle procedure of QuatiFERON Gold In-Tube test (Cellestis Limited, Carneige, Melbourne, Victoria, Australia) (Figure 3.3, Figure 3.6, Figure 3.10)

The test specially used the designed three blood collected tubes (one aliquots ml blood each) that had been coated with *Mycobacterium tuberculosis* specific antigens (ESAT-6, CFP-10 and TB7.7), along with a negative and a positive control tubes. The previous three TB-specific antigens are encoded within two regions of the *M. tuberculosis* genome, which are deleted from all BCG strains and most non-tuberculous mycobacterial (NTM) species (with the exception of *M. kansasii* , *M. marinum* and *M. szulgai*), and for which QNF-GIT is highly specific for detecting MTB infection. Stimulation of T-lymphocytes in whole blood with the three highly specific antigens results in cytokine gamma interferon (IFN-γ) production only in those infected with *M. tuberculosis* (Figure 3.10);

1) Three evacuated blood collection tubes (one ml blood each) were used for the ex vivo QuantiFERON-TB Gold In-tube assay and testing was performed according to the manufacturer's instructions (Cellestis Ltd, Carnegie, VIC, Australia): the first tube contained heparin alone (Nil tube, negative control), the second tube contained the T-cell mitogen, phytohemagglutinin (mitogen tube, positive

control), and the third tube contained *M. tuberculosis*-specific antigens, including ESAT-6, CFP-10, and TB7.7 (TB antigen tube).

2) After mixing, the tubes were incubated upright for 20 hour at 37 ° C before plasma was harvested and stored frozen at -20 ° C until further analysis. All analyses were performed within five days.

3) An enzyme linked immunosorbent assay (ELISA) was then used to measure quantitatively the amount of IFN-γ present in each of the three tubes (Nil control, TB-Antigen, Mitogen control) (Figure 3.10).

4) The results were calculated using specific QFT-GIT Software provided by manufacturer (Cellestis Limited, Carnegie, Australia).
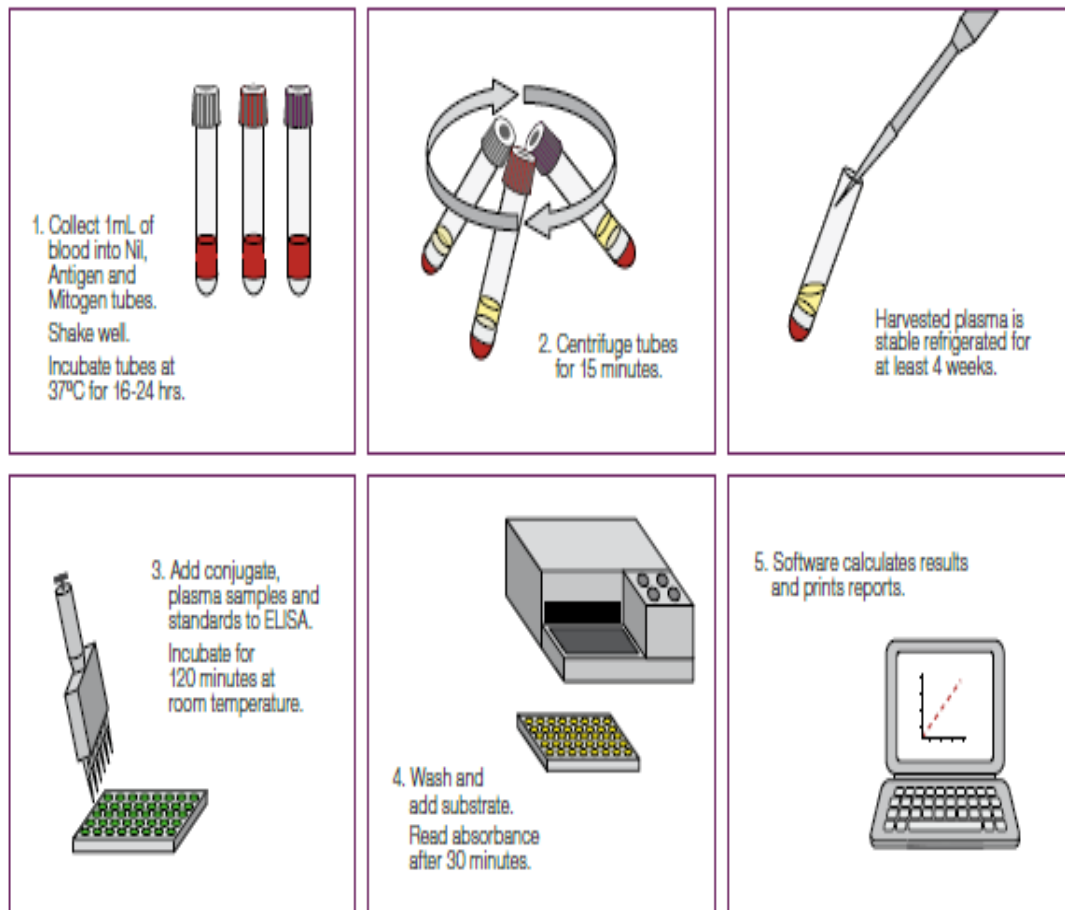
Figure 3.10: The main laboratory steps of QuantiFERON Gold In-Tube test (QFT-GIT) (http://www.cellestis.com/IRM/Company/ShowPage.aspx?CPID=1414) (DOA: 26/01/2012)

According to the manufacturer's specifications, any immigrant blood sample was considered highly likely to have previous *M. tuberculosis* infection if containing the IFN-γ concentration of the TB-Antigen tube above the test cut-off, which is more than or equal 0.35 international unit per millilitre (≥ 0.35 IU/ml), or more than or equal $\geq 25\%$ in the unstimulated negative control INF-γ value sample. Indeterminate results were defined by the ELISA apparatus as either: 1)- a negative control (unstimulated sample) INF-γ level of more than 8.0 IU/ml or 2)- a positive control INF-γ response of 0.5 IU/ml or less with a TB antigen minus a negative control INF-γ response of either less than 0.35 IU/ml or less than 25% of the negative control value.

3.13 Work quality control

Quality assurance in LTBI prevalence screening aims at: 1- ensuring the highest sensitivity and specificity in case detection, 2- ensuring the quality of the data collected, and 3- reducing potential selection and information bias.

All screening step components require particular attention to ensure that safety was paid to immigrants' health and test procedure safety through strict follow-up and completeness of documented data. The research investigator was responsible for provincial coordination, fieldwork organization and quality control through coordination of the implementation process, and manipulated and reviewed daily all collected data to ensure legible, complete and consistent recording (Chiang *et al.*, 2009). The investigator and helping staff adhered rigorously to the study. Direct close monitoring was the responsibility of the researcher.

3.14 Safety warnings and precautions

Laboratory technicians conducting IGRAs tests followed strict precautions when handling material of human origin and all blood samples were considered potentially infectious. Handling of blood samples and assay components, their use, storage and disposal were also in accordance with defined procedures in appropriate national

biohazard safety guidelines or regulations. Care was also taken when working with chemicals considered as potentially hazardous.

3.15 Identification of latent tuberculosis infection

From a systemic literature review, a new laboratory categorization was invented for defining latent tuberculosis infection using a scoring system for the results of four tuberculosis diagnostic tests and epidemiological associated risk factors predicting suspect tuberculosis carriers.

3.16 Laboratory-related diagnosis of LTBI

Based on a literature review, the researcher constructed a definition terminology based on a new scoring system as the basic backbone to predict asymptomatic LTBI cases with a high suspicion of TB before active TB development. The following descriptive table describes a new laboratory classification of diagnostic testing's, which can be considered as a new categorized diagnostic criterion for LTBI case definition and as the 'paradox of the gold standard', in addition to TB risk factors combination, instead of absence of a gold standard diagnostic test (Table 3.2).

Table 3.2: Categorization criterion for latent tuberculosis infection case definition using a combination of four-tuberculosis diagnostic tests and results scores

Tuberculosis diagnostic test result				Test score	LTBI case definition*
Old (Standard)		New			
CXR	TST	QFT-GIT	T-SPOT *.TB*		
-	-	-	-	1	**Negligible**
+	-	-	-	2	**Low**
-	+	-	-	3	**Low**
-	-	+	-	4	**Low**
-	-	-	+	5	**Low**
+	+	-	-	6	**Average**
+	-	+	-	7	**High**
-	+	+	-	8	**High**
+	-	-	+	9	**High**
-	+	-	+	10	**High**
-	-	+	+	11	**Extremely high**
+	-	+	+	12	**Extremely high**
-	+	+	+	13	**Extremely high**
+	+	+	+	14	**Extremely high**

CXR = chest X-ray, TST = tuberculin skin test, QFT-GIT = QuantiFERON Gold In-Tube test, T-SPOT *.TB* = T-SPOT *.TB* test, LTBI = latent tuberculosis infection

***Risk defined according to Thrusfield, M. (2007)**

Categorization of the case-risk of LTBI (Table 3.3) and the case diagnosis definitions following Thrusfield (2007).

Table 3.3: Definition of risk (Thrusfield, 2007)

LTBI case diagnosis	Definition
Negligible (of 'likelihood)	Need not to be considered, Insignificant (of 'likelihood'), means unimportant (c.f. 'significant)
Low (of 'likelihood')	Less than average, coming below the normal level
Average (of 'likelihood')	The usual amount or extent
High (of 'likelihood')	Extending above the normal or average level
Extremely (of 'likelihood')	Outermost, further from the centre, situated at either end; the highest or most extreme degree of anything

'Likelihood' means probability, the state or fact or being likely, 'Likely' means probable, such as might will happen or be true, to be reasonably expected

Positivity of tuberculosis diagnostic test results was defined accordingly, and a negative test result meant exclusion of LTBI case as not fulfilling the positive-result criteria (Table 3.4).

Table 3.4: Definition of test positivity of the four-tuberculosis diagnostic tests for latent tuberculosis infection

LTBI diagnostic test	Positive result definition[1]
Chest X-ray[2]	Abnormal result consistent with LTBI (probable TB)
Tuberculin skin test[3]	Induration size more than or equal 10 millimetre ($\geq$10-mm)
QuantiFERON Gold In-tube test[4][¥]	INF-γ value cut-off more than or equal 0.35 international unit per millilitre ($\geq$ 0.35 IU/mL)
T-SPOT .*TB* test[5][¥]	Either or both panel A or panel B had six or more spots than the negative control (> 6 CFU; colony forming unit)

[1] **Positive test definition will be further explained for each test in chapters five and six (comparison of diagnostic testing)**

3.17 Epidemiological-related diagnosis of LTBI

Epidemiological variable- and socio-demographic-questionnaire was structurally performed before gathered and tested for identification of statistical associations between exposure risk factors of LTBI and 'suspect TB' cases (see chapter 4).

3.18 Data management and statistical analysis

Data were collected prospectively and then entered into a computerized SPSS database for analysis using the Statistical Package for Social Sciences Software, version 17.0 (SPSS 17.0, SPSS Inc., Chicago, IL, USA) (SPSS 17.0, 2008). All recorded questionnaire data were double entered on daily basis by two data entry specialist and revised finally by the researcher checking for discrepancies between the two entries to ensure accuracy. Data were expressed and entered as number for the quantitative variable and as interval number for qualitative variables. Some

quantitative variables were also given an interval value to determine qualitative variable, for example immigrant's age of 27 years was entered differently as: a- quantitatively (exact age 27 years) and/or b- qualitatively as (25- which means lie between 25 and 30 years).

The sensitivity of a test is defined as the proportion of true positives detected by the test, which represents the probability of the test to produce a positive result in true positives, classified using a 'Gold Standard' test (this is the internationally accepted, but slow, demonstration of growth of *Mycobacterium tuberculosis* in culture). On the other hand, the specificity of a test is the proportion of true negatives that are detected by the test; which represents the probability of the test to produce true negative result with using the 'Gold Standard' test to classify true status (Knapp and Miller, 1992).

The positive predictive value (PPV) of a LTBI diagnostic test defines the probability of LTBI in new immigrant with positive result (T+IB+), therefore represents a proportion of immigrants with positive test result in total 180 immigrants with that the positive result is a true positive, whereas, the negative predictive value (NPV) describes the probability of not having LTBI in immigrant with a negative LTBI diagnostic test results (T-IB-), therefore also represents a proportion of immigrants without LTBI with a negative diagnostic result in total of 180 immigrants with negative test results (TN/TN+FN). Both predictive values vary and largely dependent on the prevalence of LTBI in the examined 180 recent immigrants (Simundic, 2008; Thrusfield, 2007).

Table 3.5 shows the outcomes of a test in relation to the gold standard true positive status, and the equations to calculate the sensitivity, specificity, positive predictive value and negative predictive values.

Table 3.5: Outcomes of a diagnostic test and equations (Simundic, 2008; Thrusfield, 2007)

		Subjects with LTBI	Subjects without LTBI	
		True Positive	True Negative	Total
Test	Test Positive	TP	FP	TP+FP
	Test Negative	FN	TN	FN+TN
	Total	TP+FN	FP+TN	TP+FP+FN+TN
Equations	Sensitivity = TP/(TP+FN)			
	Specificity = TN/(FP+TN)			
	Positive predictive value (PPV) = TP/(TP+FP)			
	Negative predictive value (NPV) = TN/(TN+FN)			

True positive (TP) - with LTBI with the value of diagnostic test results occurring above the cut-off,

False positive (FP) - without LTBI with the value of diagnostic test results occurring above the cut-off,

False negative (FN) - without LTBI with the value of diagnostic test results occurring below the cut-off, and

True negative (TN) - with LTBI with the value of diagnostic test results occurring below the cut-off.

Frequencies and percentages were used in chapter 4 to summarize the socio-demographic characteristics and the estimated prevalence of diagnosed LTBI was calculated using all the four trial diagnostics. The prevalence of LTBI was estimated by dividing the number of participants with positive result of the diagnostic test by

the total number of study participants who had undergone the research study. The combined prevalence of LTBI was estimated by dividing the number of participants who tested positive using all the four diagnostic tests by the total number of study participants who had the results for all the four tests.

Categorical variables were analyzed using a Likelihood ratio (LLR χ2) when Pearson's chi square is inappropriate for small sample [defined as the ruling-in diagnosis or the ratio of expected test probability result in subjects with LTBI to the subjects without LTBI; [LR+ = (T+ıB+) / (T+ıB-) = sensitivity / (1-specificity)], to measure the diagnostic accuracy and to compare the proportions of target outcomes in which can link the pre-test and post-test probability of LTBI in each immigrant. Likelihood ratio for negative test result (LR-) defined as the ruling-out diagnosis [LR= (T-ıB+) / (T- ıB-) = (1-sensitivity) / specificity)] and denotes the ratio of the probability that a negative result will occur in subjects with the disease to the probability that the same result will occur in subjects without the disease. Non-parametric measure of correlation statistics of non-normally distributed continuous variables were analyzed using a Kruskal-Wallis (KW χ^2) test, which corresponds to one-way analysis of variance, and it is related to the median and interquartile ranges (IQR) (Thrusfield, 2007).

Agreement between diagnostic test results (positive or negative) was assessed using Cohen's Kappa (k) coefficient (Altman, 1991) and implemented in SPSS version 17.0 (SPSS 17.0, 2008). Agreement of test results was based on the analysis of the Kappa statistics using the computer programme SPSS version 17.0.

Kappa value = (A-E)/ (N-E)

A = ∑all positives and negatives common to both techniques

E = (∑positives)/N + (∑negatives/N)

N = the total number of immigrant participants tested by the four techniques

A Kappa value can lay between 0 and 1, where 0.80-1 means very good agreement between tests; 0.61-0.80: good agreement; 0.41-0.60: moderate agreement; 0.21-

0.40: fair agreement; and < 0.20: poor agreement. A negative Kappa value indicates that the two tests agreement is less than expected by chance (Altman, 1991).

Confidence interval (CI) is a measure of location (or an interval estimate of sample parameter) that indicates an estimated reliability. Also defined as a range of values has a specified probability of including the true value of the risk factor variable, if the research studies are repeated. C.I. created at the 95% level indicates that 95% of the time properly constructed confidence intervals should contain the true value of the tested variable using Confidence Interval Analysis Software (C.I.A., 2000). This corresponds to hypothesis testing with p-values, with a conventional cut-off for p of less than 0.05 (Knapp and Miller, 1992). The p-value is the probability of obtaining an actually observed test results which often rejects the null hypothesis when the p-value is less than 0.05 and 0.1 (or the significance level), by which the result is statistically significant due to rejection of null hypothesis. P-value allows assessment of the role of chance (or whether or not the findings are 'significantly different' or 'not significantly different' from the reference value) (Thrusfield, 2007).

The range of data set is simply a possible limit of spread and is defined as the difference between the highest and lowest values. Concentrating more on the middle portion of distribution, is the interquartile range (IQR), also called midspread, which is defined as the difference between the upper (Q3 or the 75th percentile) and the lower (Q1 or the 25th percentile) quartile values. IQR uses the middle 50% of data that equals to the length of the box in a box plot and is not affected by outliers or the extremes. The IQR is used to build box plots, simple graphical representations of a probability distribution. Semi-interquartile range (SIQR) is half the interquartile range (Knapp and Miller, 1992, Thrusfield, 2007).

Questionnaire quantitative variables were drawn as box-and-whiskers plots diagrams using SPSS version 17.0 (SPSS 17.0, 2008), whereas, the spread sheet were visualized as figures and charts of the qualitative variables were drawn using Microsoft Office Excel 2007 (Microsoft Office; USA, 2006).

3.19 Pilot study

The researcher coordinated all study steps having direct supervision on all involved project staff (screening team) to identify any practical difficulties and how to tackle them. The researcher and involved project staff companion carried out a pilot study to clarify the field steps using only the project questionnaire and tuberculin skin test followed by ordinary chest X-ray reading (Step 1-4; described in Figure 3.1) and to test the study protocol form on a random sample (five new immigrants) over two days in Al Farwaniya Immigration Centre. Major limitation in administration and data collection for future facilitations were identified. Participant agreement and follow-up for TST reading result were the main addressed difficulties tackled during the project implementation and diagnostic interventions.

3.20 Study outcome

The objective of the study is to assess the accuracy of tuberculosis diagnostic tools in diagnosing latent tuberculosis infection and to identify major epidemiological risk factors of latent tuberculosis infection and re-activation transmission. Consequently, a new guideline for LTBI identification/diagnosis using results of the four anti-tuberculosis diagnostic tests was proposed.

3.21 Study output

To envisage the study outputs being published in medical journals such as The Lancet, British Medical Journal (bmj), eMedicine, European Respiratory Journal (ERS), PLoS ONE, BMC Infectious Diseases and American Thoracic Society (ATS).

3.22 Study data review and source

The project study searched identical strategy and selection criteria for previous data identified from Pubmed and other reviews such as the Lancet, bmj, PLoS ONE, BMC Infectious Diseases, eMedicine, The International Journal of Tuberculosis and Lung Disease, Chest, Indian Journal of Bacteriology, Annuals of Thoracic Medicine,

the American College of Chest Physicians, The New England Journal of Medicine, The Journal of Infectious Diseases, National Insititute for Health and Clinical Excellence (NICE), and the American Thoracic Society and Center of Disease Control and Prevention (ATS and CDC), from 1928 to 2011 (including keywords: tuberculosis diagnosis, latent tuberculosis infection, interferon gamma release assay (IGRAs) , QuantiFERON test, T-SPOT .*TB* test, tuberculin skin test, chest X-ray). Epidemiological and tuberculosis risk factors were searched from 1920 to 2011 (including keywords: tuberculosis diagnostic criteria, latent tuberculosis infection, migration, BCG, smoking, socio-economic condition). The search includes only studies published in English and relevant to tuberculosis diagnostic testing.

3.23 Study strength

All enrolled new immigrants were completely screened in officially authorized tuberculosis Immigration Centre (Department of Public Health-Ministry of Health in Kuwait) they are legally and compulsory registered before entry to Kuwait. Random sample selections from recent expatriates assumed to be healthy immigrants on entry, who come from many countries (14 different nationalities) with different TB risk exposures thereby increasing external validity. Testing four different TB diagnostic tests, on the same subject, at the same time, strengthens the research in an area that had not previously been investigated. The central and only TB laboratory of TCU in the country was responsible for IGRAs processing. Follow-up and immigrant compliance was 99%.

3.24 Limitations of the study

The absence of a gold standard test to compare the four diagnostic tests can be considered as a limiting factor. Another limitation is the small sample size which might constrain generalization of the results to a larger population and wider community. Thirdly, there were no previous data on LTBI prevalence in immigrants or Kuwait residents for comparison. Fourthly, all questionnaire answers and obtained data were based on immigrants' memory or hiding past history, and related confounding effects of potential risk factors were difficult to be controlled; this might

result in under- or over-estimation of the actual effects of the selected risk factors (Saqib *et al.*, 2011). Fifthly, refusal in research participation was high for the family re-union immigrants who account for 10-20% of Kuwaitis' expatriates, and limit result comparisons to other immigrants.

3.25 Study plan and key results

The basic laboratory of the four different latent tuberculosis infection diagnostic tests and their results been implemented on the 180 new expatriates arriving to Kuwait during February and May 2010 is described in the following Figure 3.11.
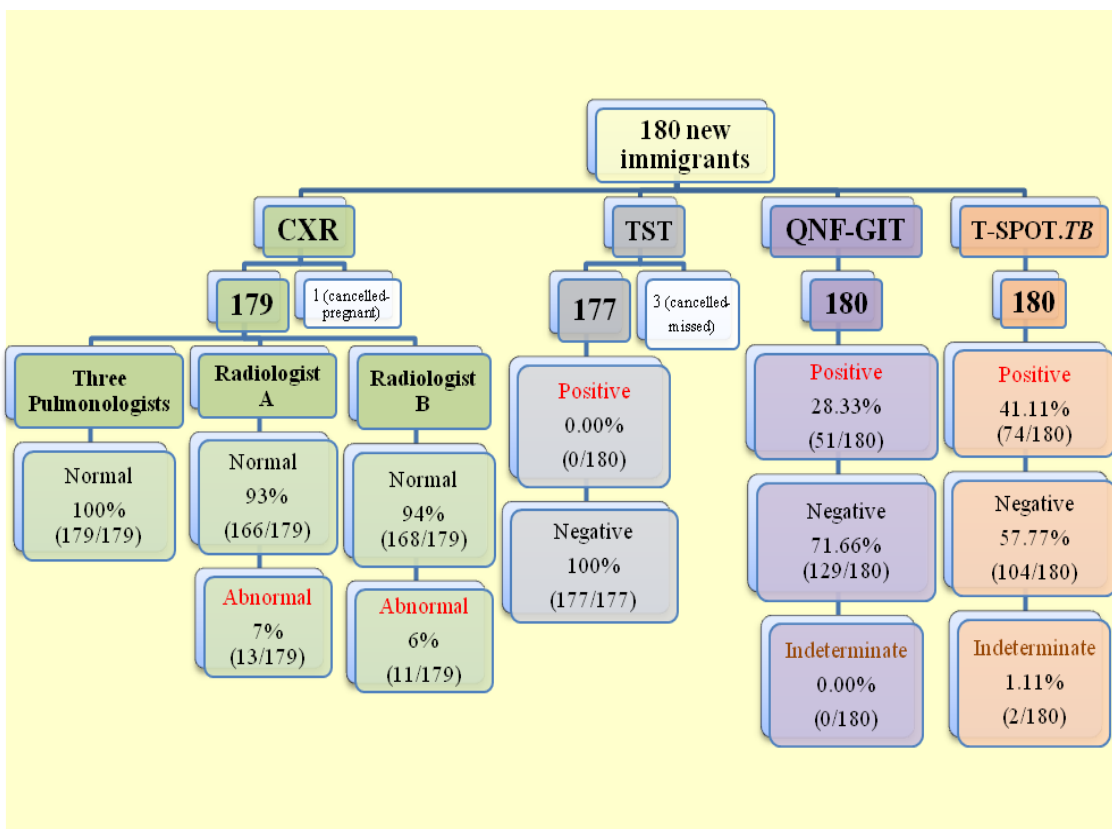
Figure 3.11: Flow diagram representing the four diagnostics of latent tuberculosis infection and their results after implemented on 180 new immigrants to Kuwait during February and May, 2010

3.26 Conclusion

Prevention of tuberculosis-related health disorders depend on early case detection and rapid intervention. Absence of a gold standard diagnostic tool for both LTBI and TB cases complicates tuberculosis prevention. This is a research study that can be used to design both qualitative and quantitative mass screening programme, supported with evidence-based statistical findings able to confirm the research hypotheses in strengthening the health system and medical care services.

3.27 Study recommendation and future plans

Regular literature reviews evaluating IGRAs and tuberculosis diagnostics, in addition to raising health resources for TB elimination requires multiple shared tasks between the Kuwaiti community and public health system through facilitating a new diagnostic strategy of TB interventions using IGRAs tests as compulsory tests would help in *Mycobacterium tuberculosis* control and TB elimination.

4 Chapter four

Assessment of the evidence-based epidemiological characteristics and predictive risk factors for detection of latent tuberculosis infection and 'suspect tuberculosis' cases

4.1 Introduction

The convergence of several infectious disease epidemics, such TB, HIV, influenza and non-communicable health epidemics such as tobacco smoking, during the 21st century have promoted worsening outcomes for TB (Jianming *et al.*, 2009). Tuberculosis must be considered as an inter-related risk factors-disease that needs to be addressed through combinations of social, economic, environmental interventions.

The continuous rises in notifications and incidence of TB in non-endemic countries (including Kuwait) are predominantly observed in foreign-born immigrants from TB endemic regions. Inadequate screening due a lack of data on LTBI prevalence and related risk factors complicates TB control as illustrated by a recent UK study (Pareek *et al.*, 2011).

Since there is no gold standard for the diagnosis of LTBI, other measures such as using an epidemiological gradient of risk factors can be used to evaluate laboratory diagnostic tests (Brodie *et al.*, 2008, Ewer *et al.*, 2003; Lalvani *et al.*, 2001b). Studies concerning the performance of such epidemiological characteristics are needed to assess diagnostics validity, build public acceptance and implementation by clinicians, impacting on the decision to isolate LTBI prior to progression to active TB disease (Solari *et al.*, 2008). Tuberculosis diagnostics and screening can be supported by high quality evidence-based epidemiological (risk factor assessment) screening tools to improve LTBI case definition. Careful screening and identifying symptoms by the medical staff raises the performance of chest X-rays performed for routine admission and reduces re-activation of TB cases (Barnes *et al.*, 1988; Feingold, 1977). A structured interview is a common screening tool which can correlate exposure to TB contacts as surrogate markers for LTBI, and explores the differences between treated, against missed, TB cases (Chee *et al.*, 2008; El-Sony *et al.*, 2002; WHO, 2007b).

Multi-factorial epidemiological (disease) models consider the interactions of biological, cultural, ecological, and politico-economic factors in explaining TB infection and its resurgence. Similarly, multi-factorial explanation model of the

resurgence of tuberculosis, including the interaction between biomedical, political, cultural, and economic factors which point to the analytical challenges achieved by combination of variable disease transmissions with global addressing of environmental conditions such as exposure risk and the socio-cultural surroundings (Dean and Fenton, 2010).

Social determinants and tuberculosis risk factors

Measures of socio-economic status (SES) and exposure to various risk factors for tuberculosis are included in the screening study for two main reasons; first to establish the prevalence in different socio-economic groups, and secondly to estimate the prevalence of exposure and determine the association between specific risk factors and LTBI/TB disease. Ecological studies based on the analysis of populations have shown wide-ranging differences that explain causal relationships associating common risk factors to the prevalence of LTBI/TB (Shetty *et al.*, 2006). Even with 95% curative rates of MTB bacilli there are still TB resurgences', spreading this disease in socially crowded and disadvantaged adults, such as immigrants from endemic countries (Elender *et al.*, 1998; Ho, 2004). Monitoring risk factors facilitates our understanding of the distribution and causes of TB prevalence/epidemics in high-risk groups and helps target interventions (Glaziou *et al.*, 2008; Salazar-Vergara *et al.*, 2003; WHO, 2007b).

4.2 Aim

To examine and describe the epidemiological risk factors for suspects harbouring latent tuberculosis infection through sample-based screening.

4.3 Objective

To identify the exposure risk(s) for latent tuberculosis infection through comparative evaluation of socio-demographics and health-related risk behavior in healthy new immigrants to Kuwait.

4.4 Methodology

All of the selected 180 new immigrants were interviewed over a period of four months (February to May 2010). Written informed consent was obtained from all participants, which was preceded by adoption pledge signed by the investigator.

4.5 Data collection

A standard risk factor questionnaire interview in English (Appendix 5) was designed that comprised twenty five quantitative variables and forty three qualitative variables to obtain information as regards socio-economic status and to collect data such as BCG vaccination status, previous exposure to TB patients and clinical conditions related to chest/lung diseases. Such data facilitates understanding of the distribution and causes of TB prevalence/epidemics in Kuwait and facilitating future control (Glaziou *et al.*, 2008; Salazar-Vergara *et al.*, 2003; WHO, 2007b).

4.6 Reliability and validity

Questionnaires were pre-tested on a small pilot study (five immigrants), before starting surveillance to resolve any identified difficulties such as language barrier. The information gained was used to improve immigrants' communication and follow-up cooperation.

4.7 Statistical analysis

Association of socio-demographic data and risk factors with the results of LTBI screening were measured by the Likelihood ratio (LLR $\chi^2_{(d.f.)}$) test. The Kruskal-Wallis (KW $\chi^2_{(d.f.)}$) test was used to calculate and compare non-normally distributed variables for overall significance of risk factors. Excel 2007 was used to draw the qualitative variables using the graph and chart of data percentages and SPSS 17.0 Software programme (SPSS 17.0, 2008) to build box-and-whiskers plots diagrams of the quantitative variables. Analyses of risk factor variables and diagnostic test variants (uni- or multivariate) results were analyzed under 95% confidence intervals

using C.I.A. Software (2000) and a p-value of less than 0.05 was considered as the level of statistical significance. Statistical formulas are detailed in chapter 3; section 3.18 (Materials and Methods).

4.8 Results

The questionnaire was divided into nine divisions (A to E) according to the epidemiological characteristics comprising both the socio-economic status and the tuberculosis-related risk factors (data collection questionnaire; Appendix 5).

4.8.1 Socio-demographic characteristics

The socio-economic variables of the 180 immigrant participants according to latent tuberculosis infection categories and statistical analysis are shown in Table 4.1.

Table 4.1: The distribution of socio-demographic characteristics according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

Demographic variables		Negligible (n=90)		Low (n=36)		Average (n=0)		High (n=4)		Extremely High (n=46)		Can not be judged (n=4)		Total (n=180)		Likelihood ratio	p-value
		n	%	n	%	n	%	n	%	n	%	n	%	n	%		
Age group (years)	20-	18	20.00	5	13.89	0	0	0	0.00	2	4.35	2	50.0	27	15.00	LLR $\chi^2_{(24)}$= 38.244	0.033
	25-	26	28.89	9	25.00	0	0	1	25.00	7	15.22	1	25.0	44	24.44		
	30	22	24.44	12	33.33	0	0	2	50.00	9	19.57	1	25.0	46	25.56		
	35-	14	15.56	6	16.67	0	0	0	0.00	10	21.74	0	0.0	30	16.67		
	40-	8	8.89	2	5.56	0	0	1	25.00	8	17.39	0	0.0	19	10.56		
	45-	2	2.22	1	2.78	0	0	0	0.00	6	13.04	0	0.0	9	5.00		
	50+	0	0.00	1	2.78	0	0	0	0.00	4	8.70	0	0.0	5	2.78		
Age	Median (IQR)	30.00 (11)		31.00 (10)		- (-)		32.50 (13)		37.00 (11)		24.00 (10)		31.5 (11)		KW $\chi^2_{(4)}$= 25.741	<0.001
Gender	M	76	84.44	25	69.44	0	0	1	25.00	34	73.91	1	25.00	137	76.11	LLR $\chi^2_{(4)}$= 14.008	0.007
	F	14	15.56	11	30.56	0	0	3	75.00	12	26.09	3	75.00	43	23.89		
Nationality	Egypt	20	22.22	6	16.67	0	0	0	0.00	0	0.00	0	0.00	26	14.44	LLR $\chi^2_{(52)}$= 72.522	0.032
	Syria	1	1.11	0	0.00	0	0	0	0.00	0	0.00	1	25.00	2	1.11		
	India	35	38.89	8	22.22	0	0	0	0.00	19	41.30	1	25.00	63	35.00		
	Pakistan	1	1.11	0	0.00	0	0	0	0.00	0	0.00	0	0.00	1	0.56		
	Sri Lanka	3	3.33	2	5.56	0	0	1	25.00	0	0.00	0	0.00	6	3.33		
	Philippines	8	8.89	12	33.33	0	0	2	50.00	13	28.26	0	0.00	35	19.44		
	USA	1	1.11	0	0.00	0	0	0	0.00	0	0.00	0	0.00	1	0.56		
	Poland	1	1.11	0	0.00	0	0	0	0.00	0	0.00	0	0.00	1	0.56		
	Indonesia	0	0.00	1	2.78	0	0	0	0.00	0	0.00	0	0.00	1	0.56		
	China	0	0.00	1	2.78	0	0	0	0.00	0	0.00	0	0.00	1	0.56		
	Ethiopia	5	5.56	1	2.78	0	0	1	25.00	6	13.04	1	25.00	14	7.78		
	Nepal	14	15.56	5	13.89	0	0	0	0.00	7	15.22	1	25.00	27	15.00		
	Venezuela	1	1.11	0	0.00	0	0	0	0.00	0	0.00	0	0.00	1	0.56		
	North Korea	0	0.00	0	0.00	0	0	0	0.00	1	2.17	0	0.00	1	0.56		
Marital status	single	39	43.33	13	36.11	0	0	0	0.00	11	23.91	1	25.00	64	35.56	LLR $\chi^2_{(12)}$= 13.368	0.343
	married	48	53.33	22	61.11	0	0	4	100.00	35	76.09	3	75.00	112	62.22		
	widow	1	1.11	0	0.00	0	0	0	0.00	0	0.00	0	0.00	1	0.56		
	divorce/separate	2	2.22	1	2.78	0	0	0	0.00	0	0.00	0	0.00	3	1.67		

Continued Table 4.1

		n	%	n	%	n	%	n	%	n	%	n	%	n	%		
Ethnicity	Asian	61	67.78	29	80.56	0	0	3	75.00	40	86.96	2	50.00	135	75.00	LLR χ²$_{(16)}$= 26.478	0.028
	Black	6	6.67	1	2.78	0	0	1	25.00	6	13.04	1	25.00	15	8.33		
	White	1	1.11	0	0.00	0	0	0	0.00	0	0.00	0	0.00	1	0.56		
	Caucasian	21	23.33	6	16.67	0	0	0	0.00	0	0.00	1	25.00	28	15.56		
	Other	1	1.11	0	0.00	0	0	0	0.00	0	0.00	0	0.00	1	0.56		
Education	illiterate	7	7.78	0	0.00	0	0	0	0.00	4	8.70	0	0.00	11	6.11	LLR χ²$_{(20)}$= 25.245	0.192
	primary	8	8.89	2	5.56	0	0	0	0.00	7	15.22	1	25.00	18	10.00		
	Intermediate	7	7.78	4	11.11	0	0	0	0.00	6	13.04	2	50.00	19	10.56		
	secondary	25	27.78	10	27.78	0	0	0	0.00	13	28.26	0	0.00	48	26.67		
	diploma	14	15.56	6	16.67	0	0	1	25.00	3	6.52	0	0.00	24	13.33		
	university & above	29	32.22	14	38.89	0	0	3	75.00	13	28.26	1	25.00	60	33.33		
duration of employment experience (years)	Median (IQR)	5.00 (7)		7.00 (7.8)		- (-)		5.00 (15)		10.00 (11.3)		2.00 (12.3)		6.5 (9)		KW χ²$_{(4)}$= 7.276	0.122
Total income in mother country (U.S.$)	Not working	3	3.33	2	5.56	0	0	1	25.00	6	13.04	1	25.00	13	7.22	LLR χ²$_{(24)}$= 31.551	0.139
	<50	9	10.00	0	0.00	0	0	0	0.00	1	2.17	1	25.00	11	6.11		
	50-	18	20.00	7	19.44	0	0	1	25.00	9	19.57	1	25.00	36	20.00		
	100-	35	38.89	19	52.78	0	0	1	25.00	23	50.00	1	25.00	79	43.89		
	250-	16	17.78	3	8.33	0	0	0	0.00	2	4.35	0	0.00	21	11.67		
	500-	7	7.78	2	5.56	0	0	0	0.00	3	6.52	0	0.00	12	6.67		
	1000 and more	2	2.22	3	8.33	0	0	1	25.00	2	4.35	0	0.00	8	4.44		
Income (U.S. $)	Median (IQR)	134.00 (196)		188.50 (128)		- (-)		146.00 (-)		133.50 (110)		68.00 (-)		136 (174)		KW χ²$_{(4)}$= 3.532	0.473
Family size (person)	1-4	31	34.44	11	30.56	0	0	2	50.00	14	30.43	1	25.00	59	32.78	LLR χ2$_{(8)}$= 6.586	0.582
	5-10	52	57.78	20	55.56	0	0	2	50.00	31	67.39	3	75.00	108	60.00		
	>10	7	7.78	5	13.89	0	0	0	0.00	1	2.17	0	0.00	13	7.22		
Total family size living in the same place	Median (IQR)	5.00 (3)		5.00 (3)		- (-)		4.00 (3)		5.00 (3)		6.00 (5)		5 (3)		KW χ²$_{(4)}$= 2.962	0.564

Continued Table 4.1											
Total living room	**Median (IQR)**	2.00 (1)	3.00 (2)	-	-	1.50 (2)	2.00 (2)	4.00 (3)	2 (2)	KW $\chi^2_{(4)}$= 4.281	0.369
Total sleeping room	**Median (IQR)**	2.00 (1)	2.00 (1)	-	-	1.50 (1)	2.00 (2)	4.00 (2)	2 (1)	KW $\chi^2_{(4)}$= 8.977	0.062
General crowding index (GCI)	**Median (IQR)**	2.17 (2.03)	2.08 (2.25)	-	-	2.25 (1.13)	2.58 (2.38)	1.68 (0.6)	2.08 (2)	KW $\chi^2_{(4)}$= 2.731	0.604
Sleeping crowding index (SCI)	**Median (IQR)**	2.33 (1.06)	2.00 (1.33)	-	-	2.50 (0.75)	2.67 (1.88)	1.88 (0.63)	2.33 (1.25)	KW $\chi^2_{(4)}$= 5.906	0.206

Averag category = zero (no cases were detected), (-) means cannot be computed by SPSS Software due to zero cases, IQR = interquartile range

The overall median age of the immigrants was observed to be 31.5 years with an interquartile range (IQR) of 11 years and the semi-interquartile range (SIQR) of 5.5 years. The minimum age of participants was 20 years and the oldest immigrant in the study was aged 56 years. Older median ages were observed in the 'extremely high' LTBI group at 37 years (IQR = 11 years) and in the 'high' LTBI at 32.5 years (IQR = 13 years), compared with the 'negligible' LTBI at 30 years (IQR = 11 years) and 'low' LTBI at 31 years (IQR = 10 years), with a statistically significant difference between all age classes for the five different LTBI groups (KW $\chi^2_{(4)}$ = 25.741, p < 0.001) (Figure 4.1, Table 4.1).
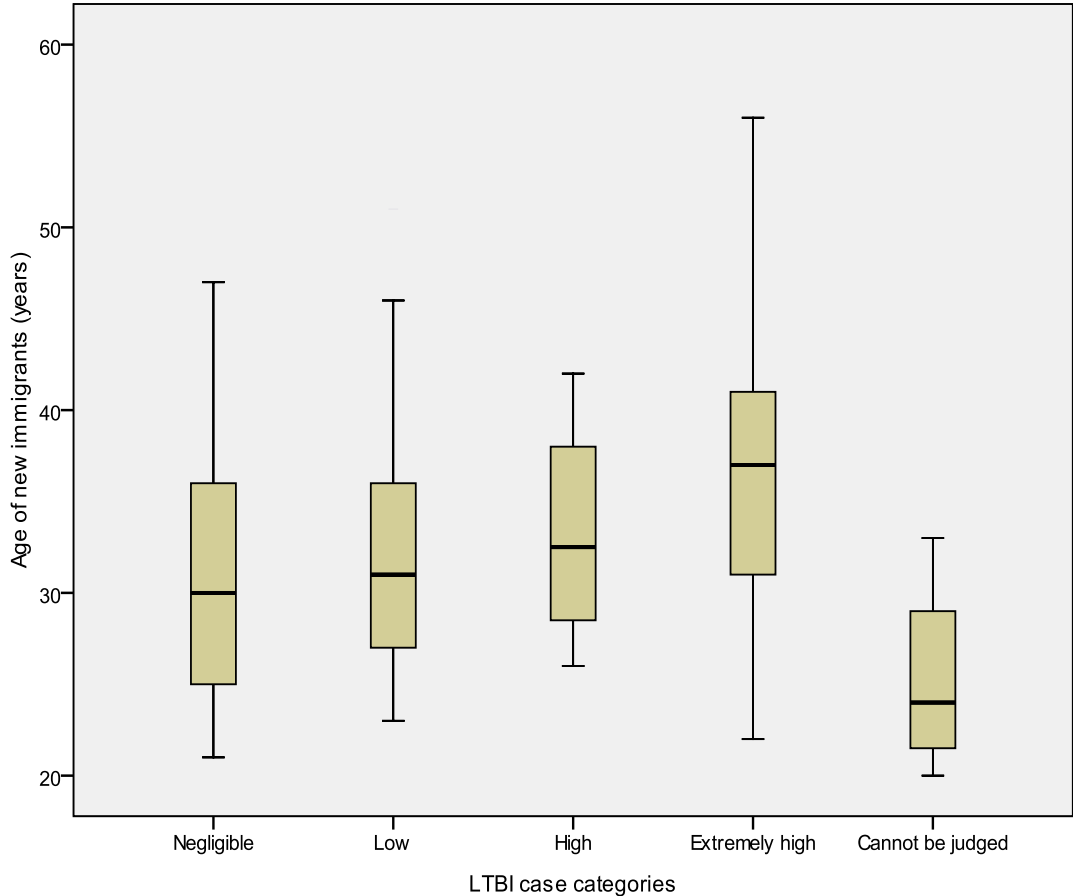
Figure 4.1: Box-and-whiskers plots show an increasing of the median age (years) in terms to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

The majority of participants were aged between 20 and 45 years (92.22%). Similarly, members of the 'negligible' LTBI group were aged between 20 years and 40 years 88.89% (80/90). The majority of the 'extremely high' classifications were aged between 25 and 50 years 86.95% (40/46) and between 20 and 40 years for the 'low' classification of LTBI 88.89% (32/36). These differences were statistically significant (LLR $\chi^2_{(24)}$ = 38.244, p = 0.033). (Figure 4.2, Table 4.1).
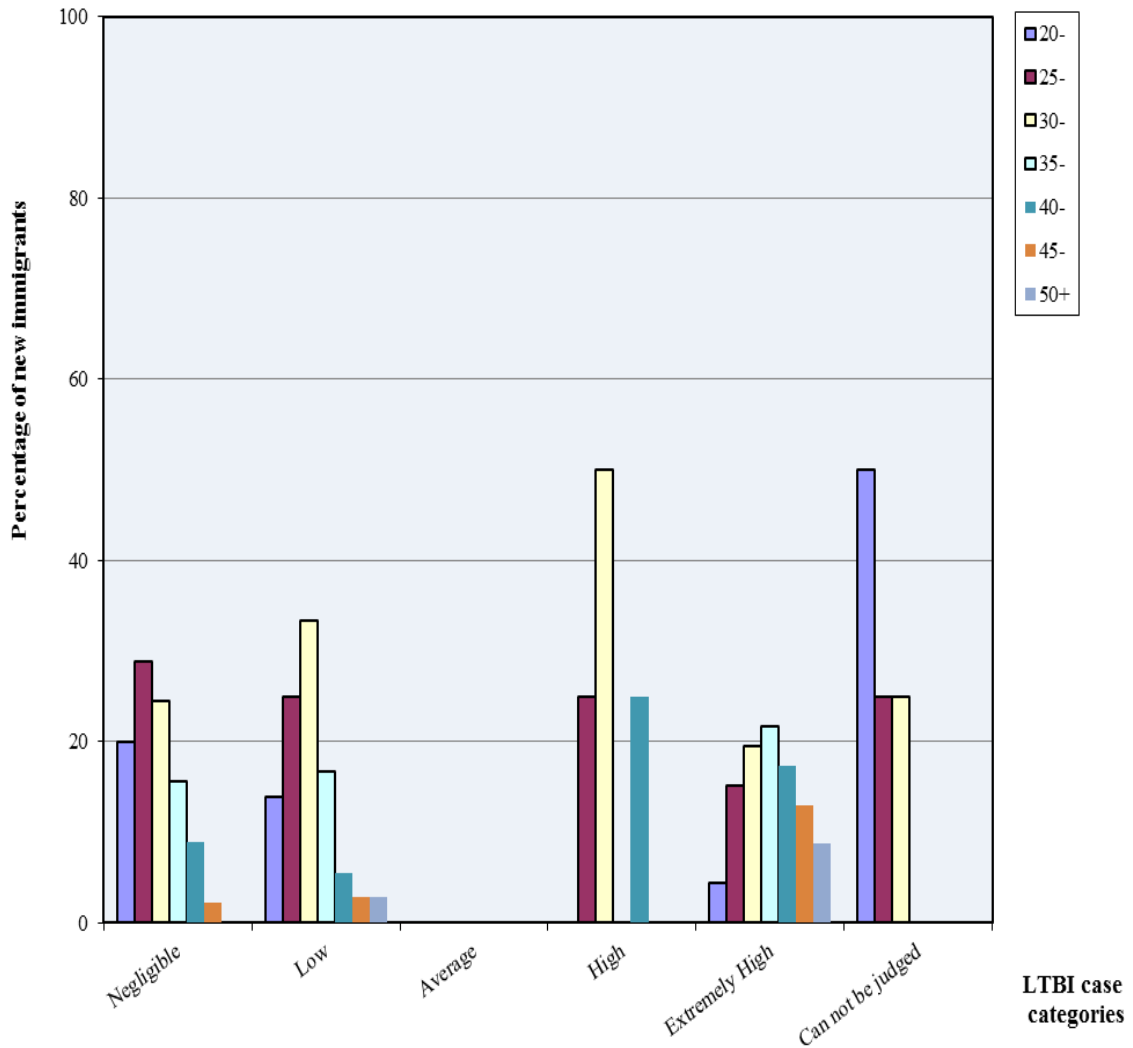
Figure 4.2: Percent distribution of the age groups (years) according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

The response rate of participation in the study was higher for male immigrants 76.11% (137/180), with 'negligible' LTBI as 84.44% (76/90) followed by the 'extremely high' LTBI immigrants at 73.91% (34/46) and 'low' LTBI participants in 69.44% (25/36). The gender difference was statistically significant (LLR $\chi^2_{(4)}$ = 14.008, p = 0.007) (Figure 4.3, Table 4.1).
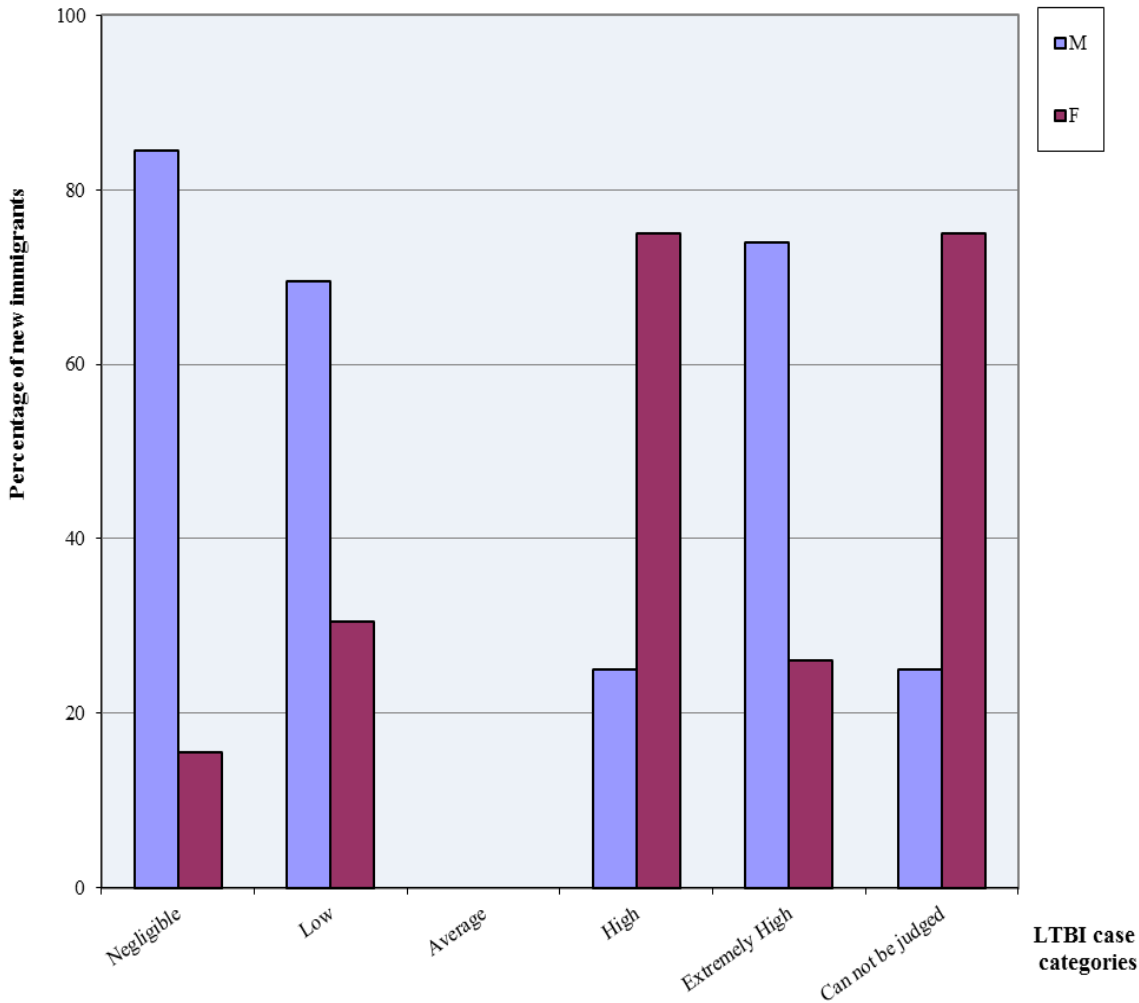
Figure 4.3: Percent distribution of gender according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

Statistically significant differences were detected between nationality and LTBI case categories. Most of the immigrants in this study 77.22% (139/180) came from counties endemic of TB, broken down as follows; Indians 35%, Filipinos 19.44%, Nepalis 15%, Ethiopians 7.78% and Sri Lankans 3.3%. Egyptians represented the majority of immigrants coming from non-endemic countries: 14.44% (26/180) (LLR $\chi^2_{(52)}$ = 72.522, p = 0.032) (Figure 4.4, Table 4.1).
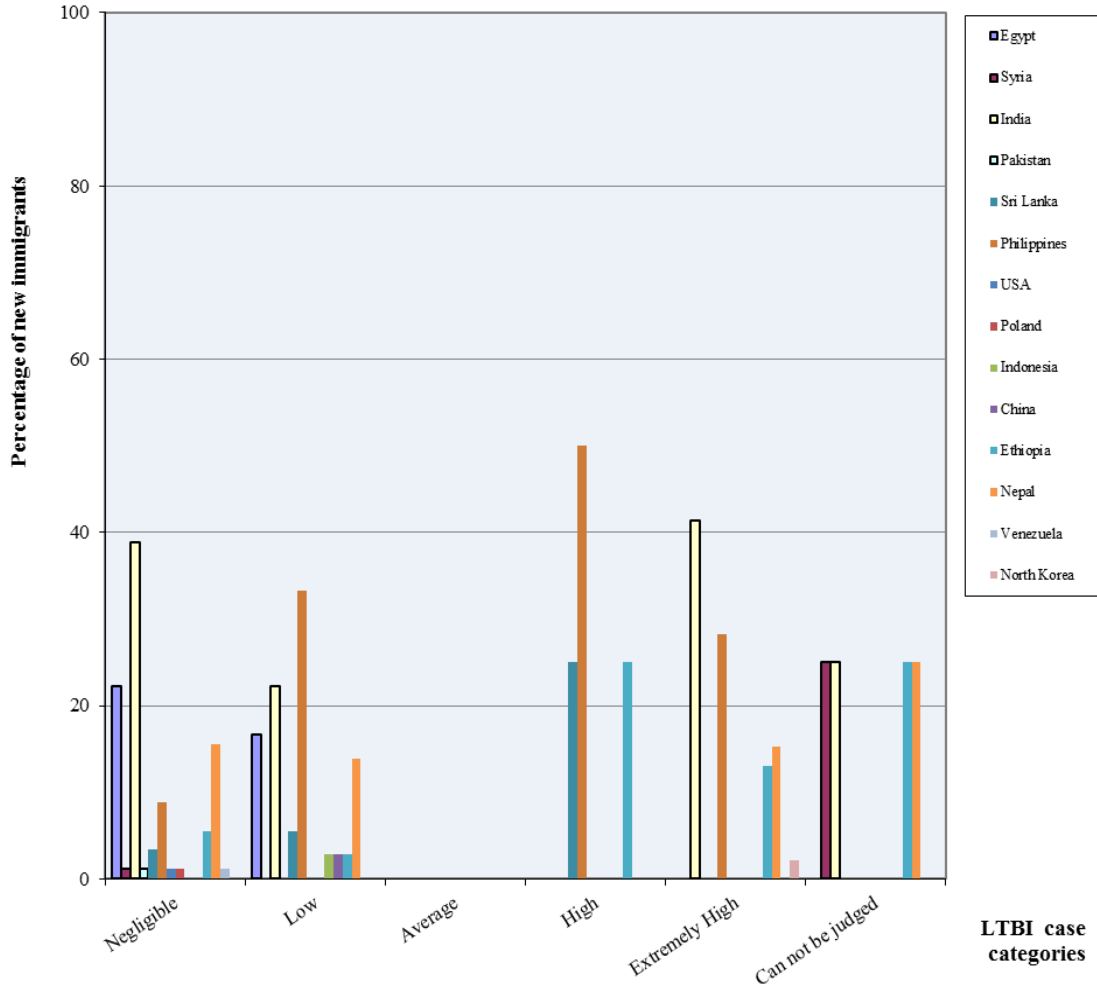
Figure 4.4: Percent distribution of the nationality according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

Marital status was not observed to be significantly associated with LTBI-defined immigrants, the composition being: married 62.22% (112/180) and single 35.56% (64/180) (LLR $\chi^2_{(12)}$ = 13.368, p = 0.343) (Figure 4.5, Table 4.1).
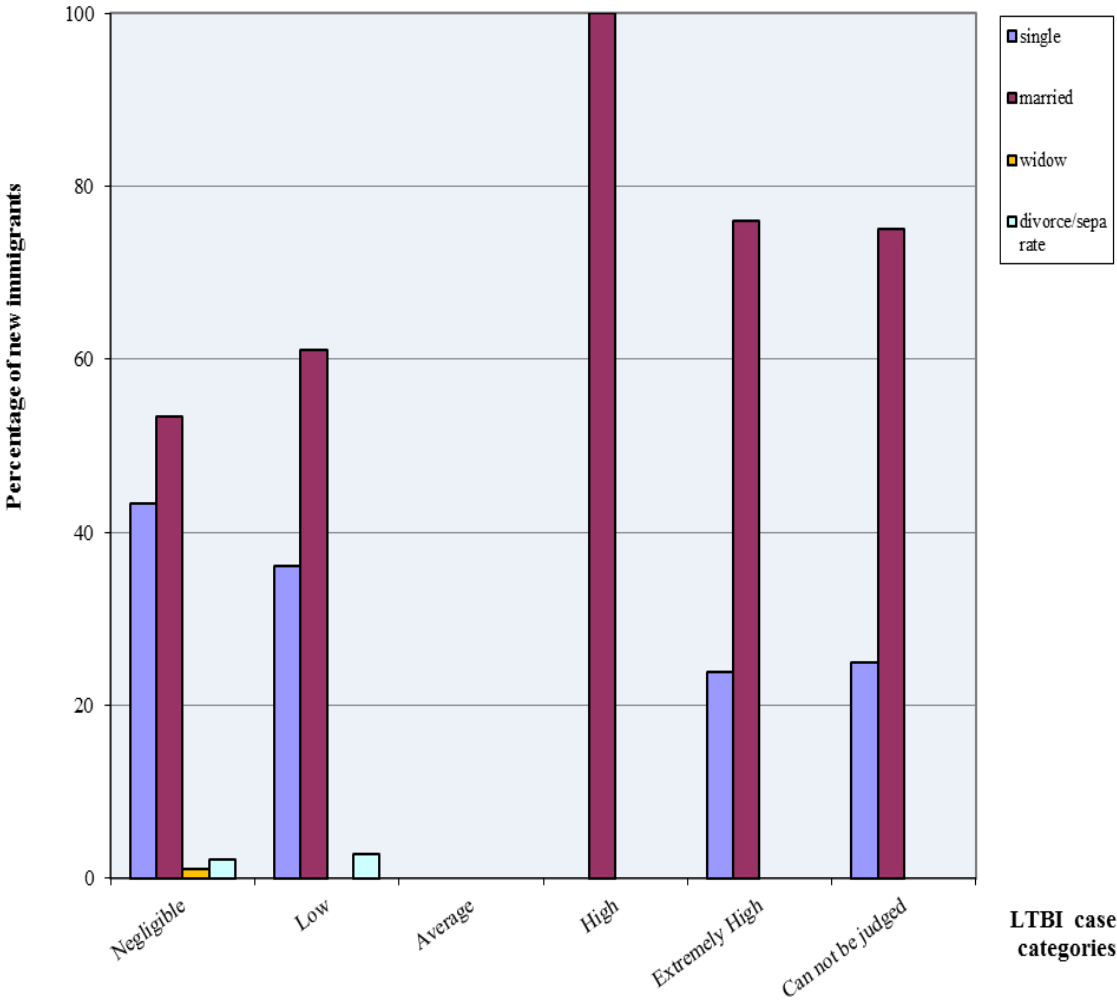
Figure 4.5: Percent distribution of the marital status according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

A statistically significant association was found between ethnic groups and LTBI defined categories. The majority of LTBI defined immigrants were Asians 75% (135/180), classified as 'extremely high' for LTBI in 86.96% (40/46) and 'negligible' LTBI in 67.78% (61/90). There were 15.56% (28/180) Caucasians, comprising 'negligible' LTBI in 23.33% (21/90) and 'low' LTBI in 16.67% (6/36). There were 8.33% (15/180) Blacks, divided equally between 'negligible' in 6.67% (6/90) and 'extremely high' in 13.04% (6/46) (LLR $\chi^2_{(16)}$ = 26.478, p = 0.028) (Figure 4.6, Table 4.1).
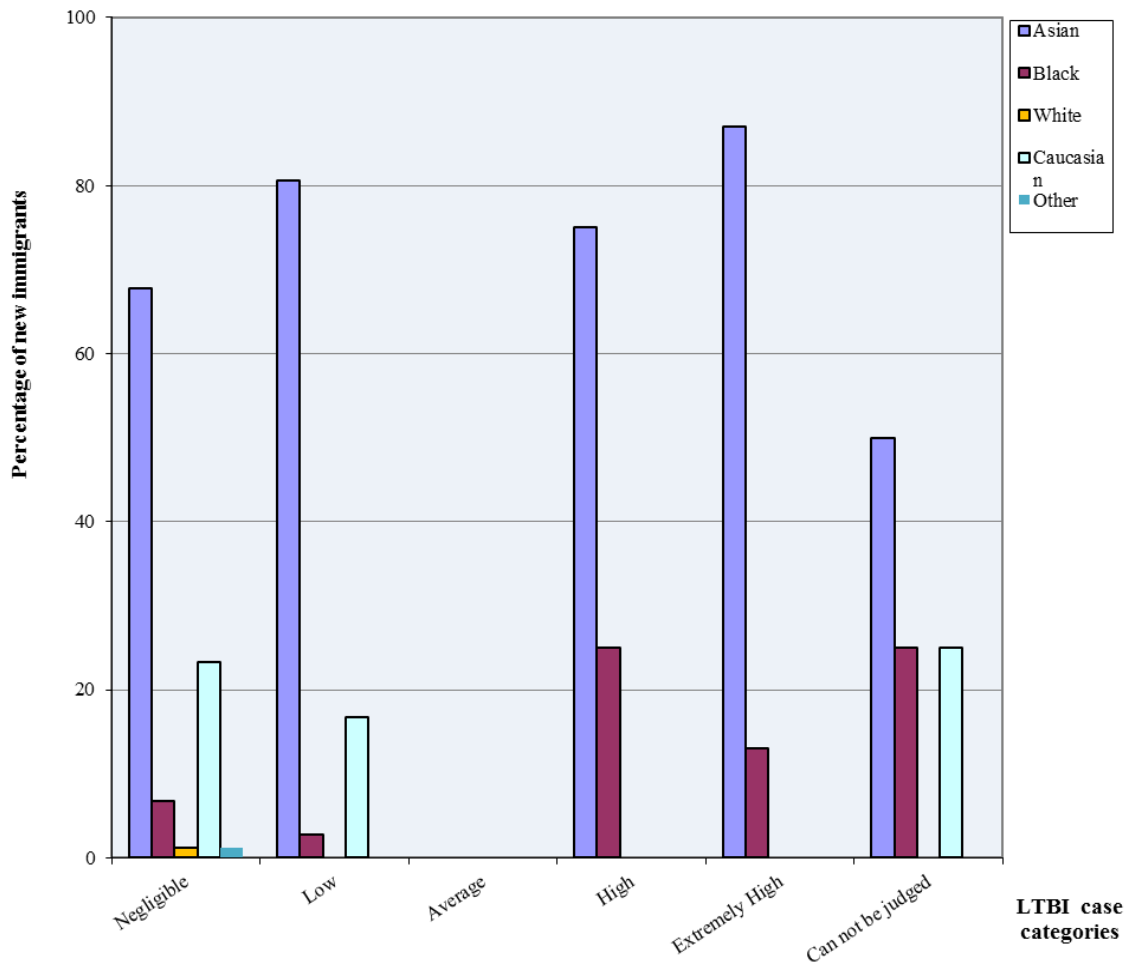
Figure 4.6: Percent distribution of the ethnic origin according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

The level of education was not a statistically significant risk factor for LTBI and 'suspect TB' cases. The majority of participants had graduated from secondary school and higher levels of education 73.33% (132/180), compared to 26.67% (48/180) of those graduated from intermediate school and lower levels (which was divided equally in all LTBI participant groups). For example Filipino participants commonly had diploma and university qualifications 81.11% (31/36) compared to Nepali's 74.07% (20/27) that were graduated and had intermediate school qualifications and lower levels (LLR $\chi^2_{(20)}$ = 25.245, p = 0.192) (Figure 4.7, Table 4.1).
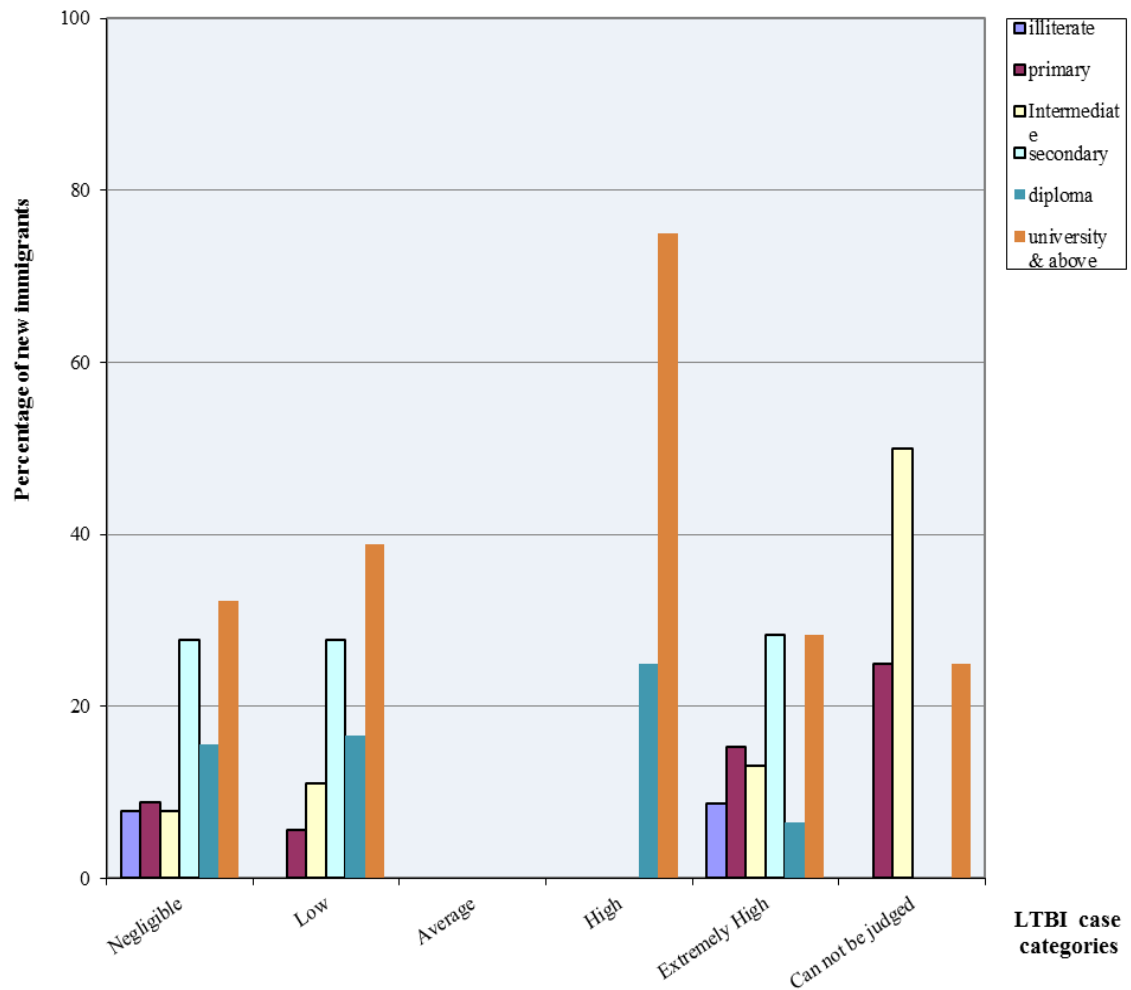
Figure 4.7: Percent distribution of the education status according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

Total immigrants occupied in their mother country were 90.55% (163/180), with overall median duration of total employment was 6.5 years (IQR = 9, SIQR = 4.5). The longest duration of work experience was for the 'extremely high' LTBI classification as 10 years (IQR = 11.3) compared to lower number of years of work experience for the 'negligible' LTBI as 5 years (IQR = 7) or the 'low' LTBI as 7 years (IQR = 7.8) with no statistical significant difference (KW $\chi^2_{(4)}$ = 7.276, p = 0.122) (Figure 4.8, Table 4.1)
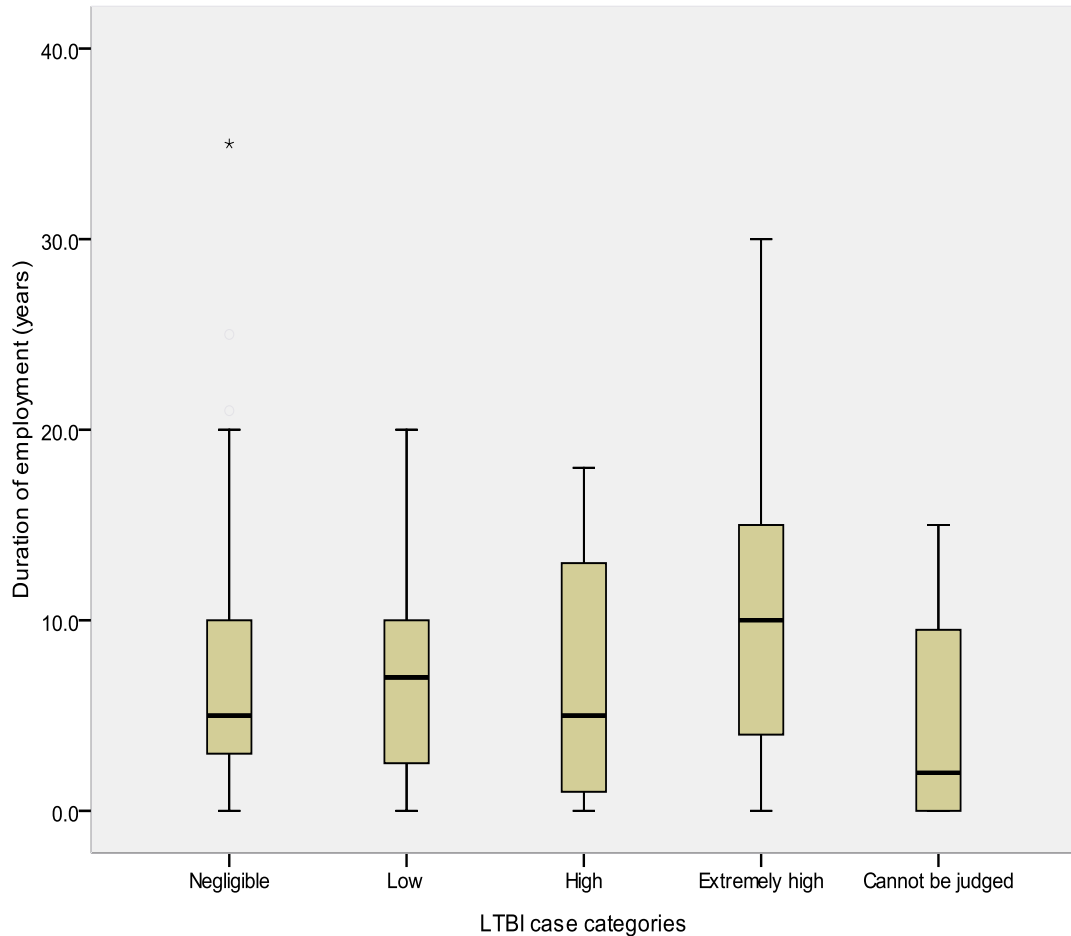
Figure 4.8: Box-and-whiskers plots of the average duration of employment (years) in the mother country according to latent tuberculosis infection categories of 163 employed new immigrants to Kuwait during February and May, 2010

LTBI categories were not statistically significantly different with the average total income per month in the mother country for those employed immigrants in 92.78% (167/180). The majority of employed immigrants (20%) received between U.S.$50 and U.S.$100, and 43.89% received between U.S.$100 and U.S.$250. Only 6.11% of immigrants received less than U.S.$50, compared with 4.44% who received more than U.S.$1000 (LLR $\chi^2_{(24)}$ = 31.551, p = 0.139) (Figure 4.9, Table 4.1).
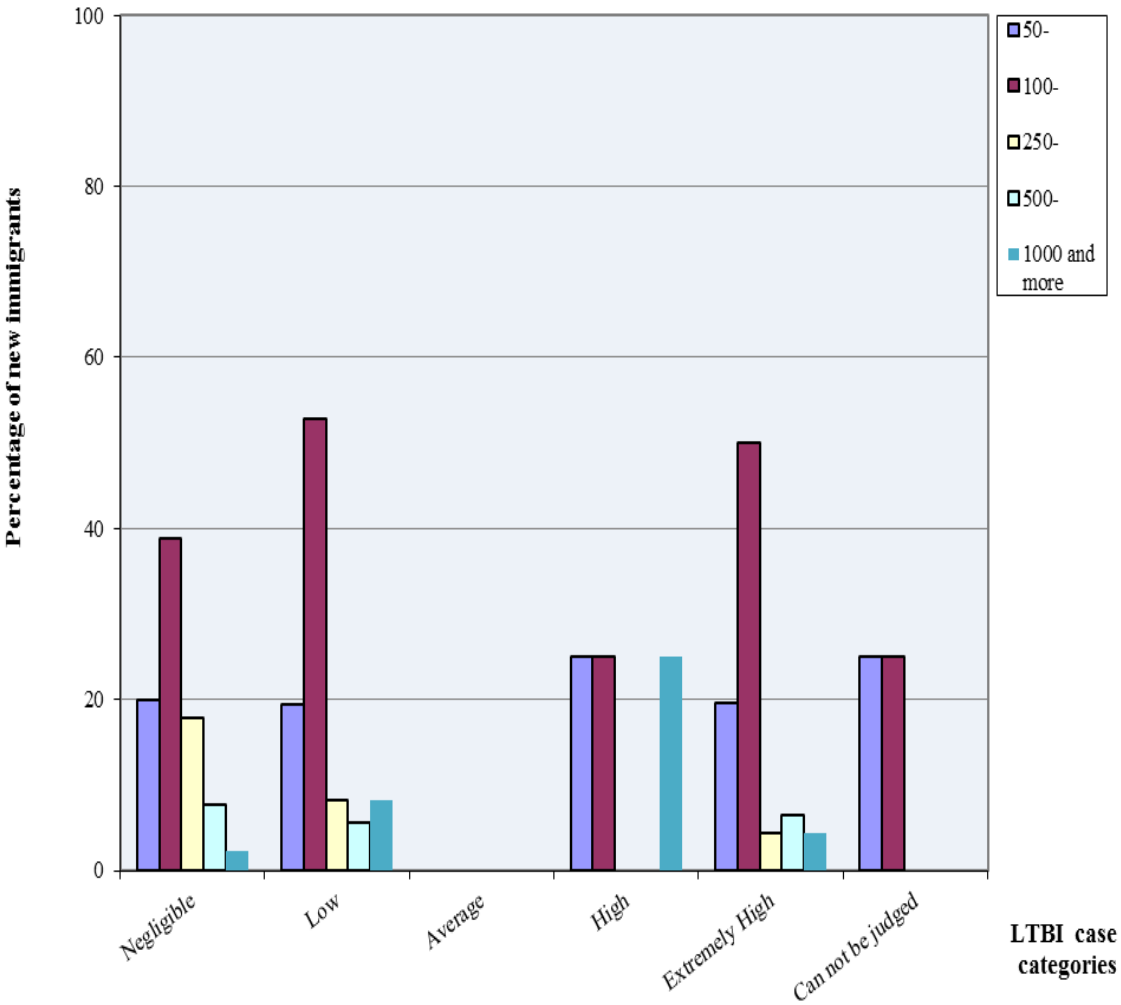
Figure 4.9: Percent distribution of the total income (U.S.$) in the mother country according to latent tuberculosis infection categories of 163 employed new immigrants to Kuwait during February and May, 2010

The overall median income of the employed participants in their mother country was U.S.$136 (IQR = 174, SIQR = 87). The lowest salary was U.S.$22 and the highest U.S.$4,452. The highest overall median income was in the 'low' LTBI group: U.S.$188.50 (IQR = 128) versus U.S.$l35 (IQR = 110) in those with 'extremely high' LTBI, and U.S.$134 for 'negligible' LTBI participants (IQR = 196) (KW $\chi^2_{(4)}$ = 3.532, p = 0.473) (Table 4.1).

Sixty percent (108/180) of immigrant homes in their mother country contained one to ten persons, 32.78% (59/180) had family sizes of between one and four persons and 7.22% had family sized in excess of more than ten individuals there was no statistically significant differences (LLR $\chi 2_{(8)}$ = 6.586, p = 0.582). The majority 67.39% (31/46) of immigrants in five to ten person households belonged to the 'extremely high' category, followed by 57.78% (52/90) were belonging to the 'negligible' LTBI and by 55.56% (20/36) were from the 'low' LTBI group (Figure 4.10, Table 4.1).
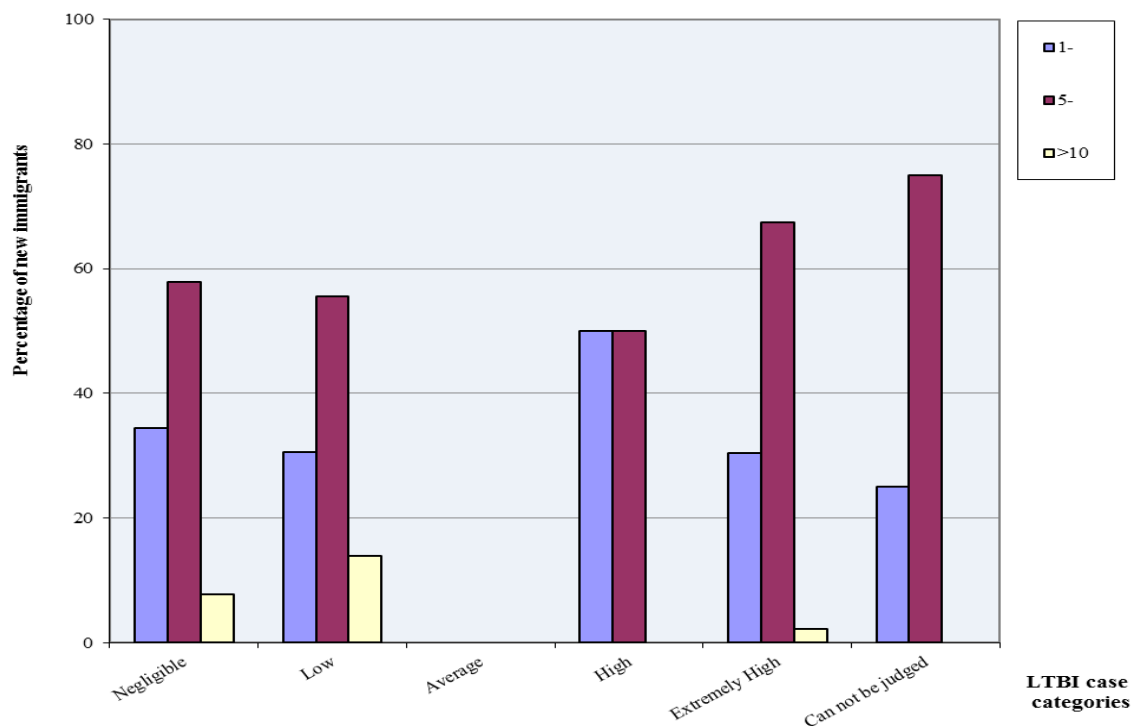
Figure 4.10: Percent distribution of the total family size (subjects) according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

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The overall median number of family individuals living within the same place in the mother country was five (IQR = 3) and was the same for all classified LTBI groups except for 'high' LTBI, which had a median of four (IQR = 3). The minimum number of individuals was two, and the maximum was 30 (KW $\chi^2_{(4)}$ = 2.962, p = 0.564) (Figure 4.11, Table 4.1).
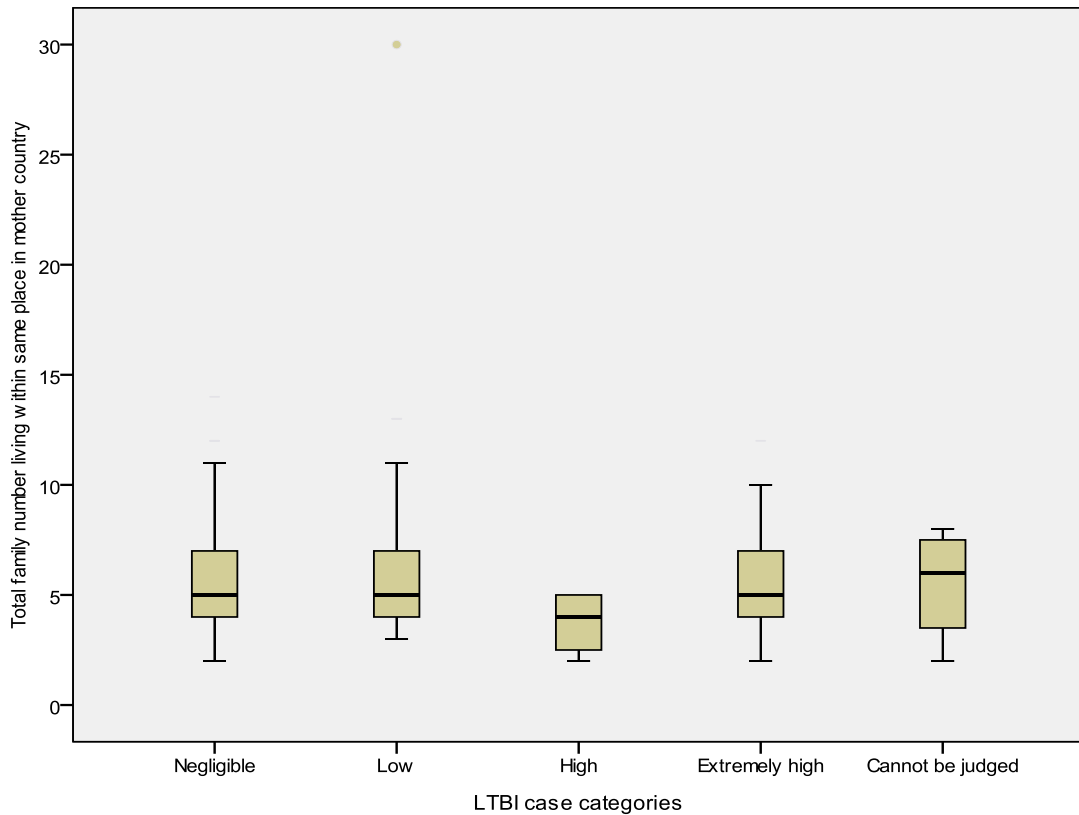
Figure 4.11: Box-and-whiskers plots of the total family size living within the same house in the mother country according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

The overall median number of total living rooms within the same living place in the mother country was two (IQR = two, SIQR = one), and the lowest number of living rooms was one versus the highest number of ten, but with no statistically significant difference (KW $\chi^2_{(4)}$ = 4.281, p = 0.369) (Table 4.1). The overall median number of

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bedrooms in the same house in the mother country was two (IQR = two, SIQR = one). The lowest bedroom number was one versus the highest sleeping rooms of seven in all LTBI groups with statistical significance at 10% (KW χ^2 (4) = 8.977, p = 0.062) (Table 4.1).

The median number of general crowding index (G.C.I.) (denoted by the number of co-residents per living room (Baker *et al.*, 2000)) of immigrants was 2.083 (IQR = 2, SIQR = 1), and the highest was found in the 'extremely high' LTBI as 2.58 (IQR = 2.38) and the lowest index in the 'low' LTBI group as 2.08 (IQR = 2.25). The lowest household crowding index was 0.50 versus the highest G.C.I. value was 13.0, but without statistical significant difference (KW χ^2 (4) = 2.731, p = 0.604)   (Figure 4.12, Table 4.1).
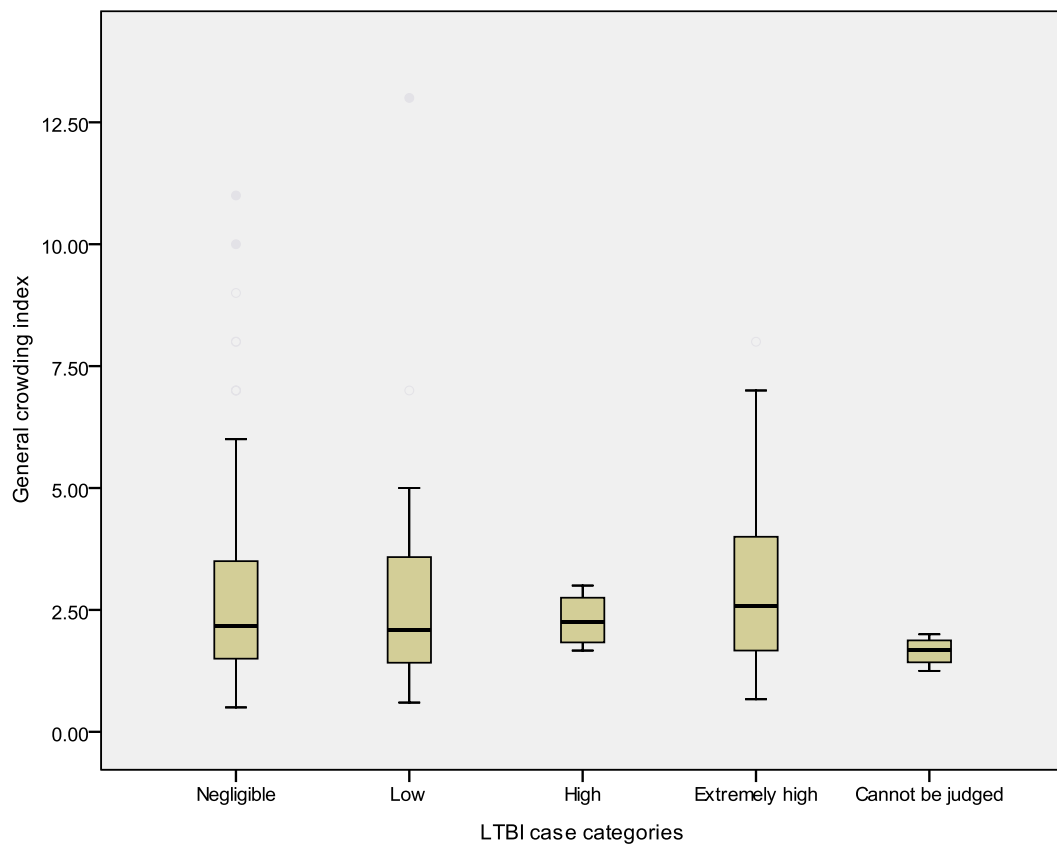
Figure 4.12: Box-and-whiskers plots of the general crowding index (G.C.I.) according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

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The overall median number of sleeping crowding index (S.C.I.) (denoted by the number of co-residents per sleeping bedroom (Baker *et al.*, 2000)) of immigrants was 2.333 (IQR = 1.25, SIQR = 0.625), and the highest S.C.I. was found in the 'extremely high' LTBI group, with a value of 2.67 (IQR = 1.88) and the lowest index was found in the 'low' LTBI group, with a value of 2.00 (IQR = 1.33). The minimum S.C.I. number was 1.00, and the highest was 13.0 (KW $\chi^2_{(4)}$ = 5.906, p = 0.206). No statistical significant difference was observed (Figure 4.13, Table 4.1).
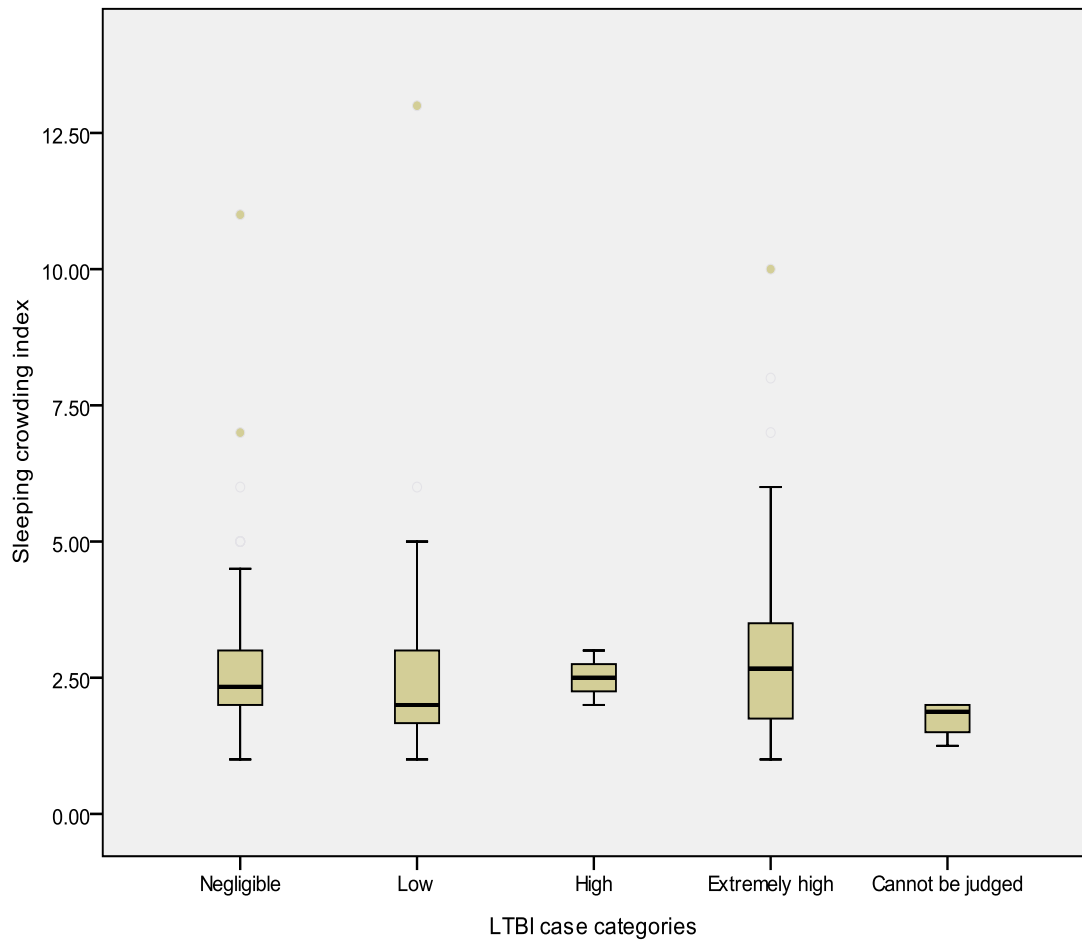
Figure 4.13: Box-and-whiskers plots of the sleeping crowding index (S.C.I.) according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

4.8.2 Risk factors for *Mycobacterium tuberculosis* infection

The susceptibility of immigrants to *Mycobacterium tuberculosis* infection in their mother countries and relation with transport routes and sanitary systems according to latent tuberculosis infection defined categories are shown in Table 4.2.

Table 4.2: Distribution of transport routes and sanitary systems in the mother country according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

Tuberculosis disease knowledge		Negligible (n=90)		Low (n=36)		Average (n=0)		High (n=4)		Extremely High (n=46)		Can not be judged (n=4)		Total (n=180)		Likelihood ratio	p-value
		n	%	n	%	n	%	n	%	n	%	n	%	n	%		
Feasible access to medical health care service		90	100.00	35	97.22	0	0	4	100.00	46	100.00	4	100.00	179	99.44	LLR $\chi^2_{(4)}=$ 3.241	0.518
Duration to medical health care service (minute)	<60	80	84.2	3	75.0	0	0	3	100.0	42	91.3	4	100.0	155	86.1	LLR $\chi^2_{(12=)}$ 8.079	0.779
	60-	12	12.6	1	25.0	0	0	0	0.0	3	6.5	0	0.0	18	10.0		
	120-	1	1.1	0	0.0	0	0	0	0.0	0	0.0	0	0.0	3	1.7		
	>120	2	2.1	0	0.0	0	0	0	0.0	1	2.2	0	0.0	4	2.2		
Duration access to medical health care service (minute)	Median (IQR)	15.00 (20)		15.00 (29)		-	-	30.00 (8)		15.00 (14)		17.50 (5)		15 (20)		KW $\chi^2_{(4)}=$ 5.970	0.201
TB exposure in mother country: Transport routes																	
Walking	Yes	89	98.89	36	100	0	0	4	100.00	46	100.00	4	100.00	179	99.44	LLR $\chi^2_{(4)}=1.392$	0.846
Bicycle/ tricycle/mo torcycle	Yes	48	53.33	15	42	0	0	0	0.00	18	39.13	2	50.00	83	46.11	LLR $\chi^2_{(4)}=8.052$	0.090
Microbus/ bus/train	Yes	66	73.33	29	81	0	0	4	100.00	37	80.43	0	0.00	136	75.56	LLR $\chi^2_{(4)}=14.884$	0.005
Car vehicle	Yes	37	41.11	9	25	0	0	1	25.00	19	41.30	2	50.00	68	37.78	LLR $\chi^2_{(4)}=3.858$	0.426
Other e.g. ship, airplane	Yes	2	2.22	0	0	0	0	0	0.00	2	4.35	0	0.00	4	2.22	LLR $\chi^2_{(4)}=2.728$	0.604

Continued Table 4.2																

Presence of sanitary system (sanitary condition of living area in mother country)
Water supply in mother country:

Water tap/gallon	Yes	61	67.78	29	81	0	0	4	100.00	30	65.22	3	75.00	127	70.56	
Water pump/well	Yes	23	25.56	6	17	0	0	0	0.00	15	32.61	1	25.00	45	25.00	LLR $\chi^2_{(4)}$= 1.392
Imported water storage	Yes	6	6.67	1	3	0	0	0	0.00	1	2.17	0	0.00	8	4.44	
Presence of sewage system in mother country	Yes	86	95.56	36	100	0	0	4	100.00	41	89.13	4	100.00	171	95.00	LLR $\chi^2_{(4)}$= 7.110
	No	4	4.44	0	0	0	0	0	0.00	5	10.87	0	0.00	9	5.00	

(p-value column): 0.846 ; 0.130

Average LTBI category is zero (means no detected cases), (-) means cannot be computed by SPSS Software programme, IQR = interquartile range

4.8.2.1   Access to health care services

Medical care systems in the participants' mother countries were accessible to almost all 99.44% participants except for one 'low' LTBI case, but with no statistical significant difference (LLR $\chi^2_{(4)}$ = 3.241, p = 0.518). Duration time taken to reach the health care services in mother country within less than one hour was answered by 86.1% immigrants', compared to 10% of participants who were able to reach medical services between one and two hours. 3.9% of immigrants have difficulties to reach health care services for more than two hours (LLR $\chi^2_{(12)}$ = 8.079, p = 0.779). Average median duration (minutes) of access to medical care services was 15 minutes (IQR = 20, SIQR = 10) for all participants, and those defined as 'high' LTBI cases scored the longest time to reach within 30 minutes (IQR = 8) (KW $\chi^2_{(4)}$ = 5.970, p = 0.201). The minimum duration of accessibility was 2 minutes and the maximum time to reach health services was 150 minutes (Figure 4.14, Table 4.2).
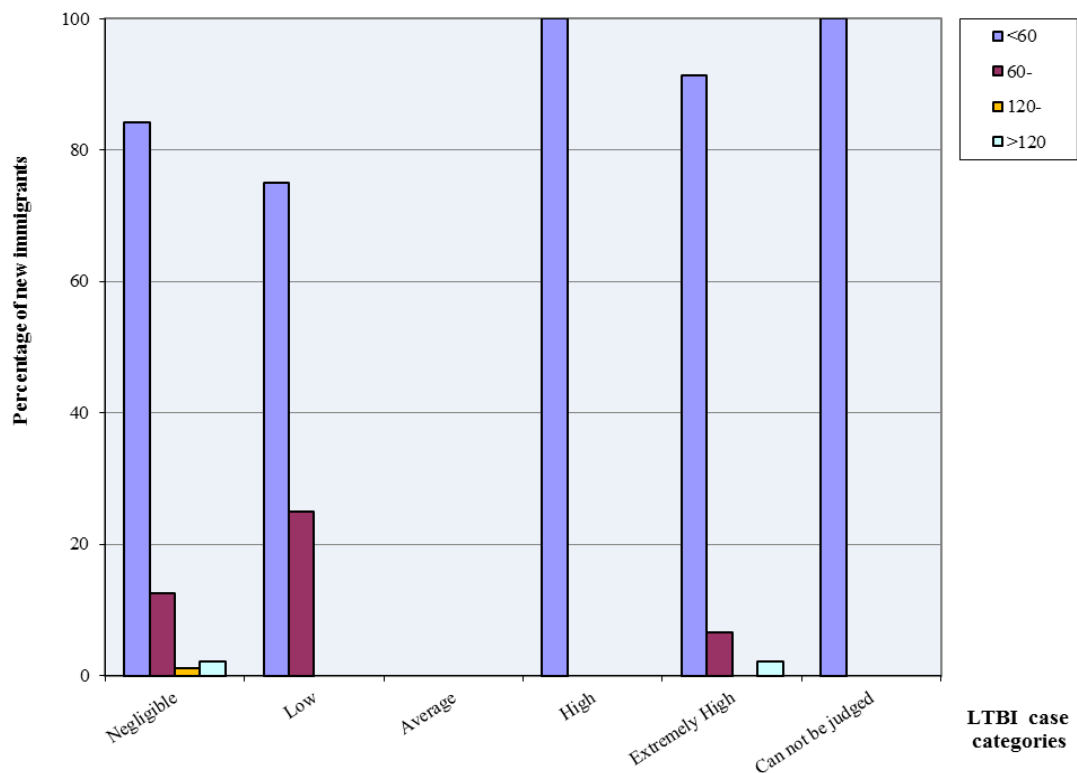
Figure 4.14: Percent distribution of the average duration (minutes) to health care services in the mother country according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

4.8.2.2   Transport routes

The greatest (99.44%) of transport routes used by immigrants in the remote country was on foot which was similar to all LTBI participant groups but without statistical significant difference for LTBI development (LLR $\chi^2_{(4)}$ = 1.392, p = 0.846). 46.11% of immigrants rode a bicycle and/or tricycle and/or motorcycle, and these were usually found in the 'negligible' LTBI category in 53.33% and the 'extremely high' LTBI in 39.13%, which showed statistical significance at 10% level (LLR $\chi^2_{(4)}$ = 8.052, p = 0.090). 75.56% of immigrants were using microbuses (e.g. Chapini in the Philippines) and/or bus and/or train, separated more in the 'high' LTBI 100% and the 'extremely LTBI' 80.43% versus 73.33% of the 'negligible' group. Exposure and closed environmental contact revealed statistical significant association (LLR $\chi^2_{(4)}$ = 14.884, p = 0.005). Only 37.78% of participants also used cars (LLR $\chi^2_{(4)}$ = 3.858, p = 0.426) (Table 4.2).

4.8.2.3   Sanitary systems

The presence of sanitary systems and proper sanitary conditions within houses in the mother country can be determined by the water supply system. The majority of participants 70.56% (127/180) utilized the water tap and water gallon (small tanks) in their home country and 25% of immigrants were and/or also using a water pump or pump well. Only 4.44% of participants were utilizing imported water storage in their living places (LLR $\chi^2_{(8)}$ = 7.836, p = 0.450) (Figure 14.5, Table 4.2). Also 95% of immigrants reported having proper sewage systems in their living places and houses. However, significant association between sanitary system was not significant (LLR $\chi^2_{(4)}$ = 7.110, p = 0.130) (Table 4.2).
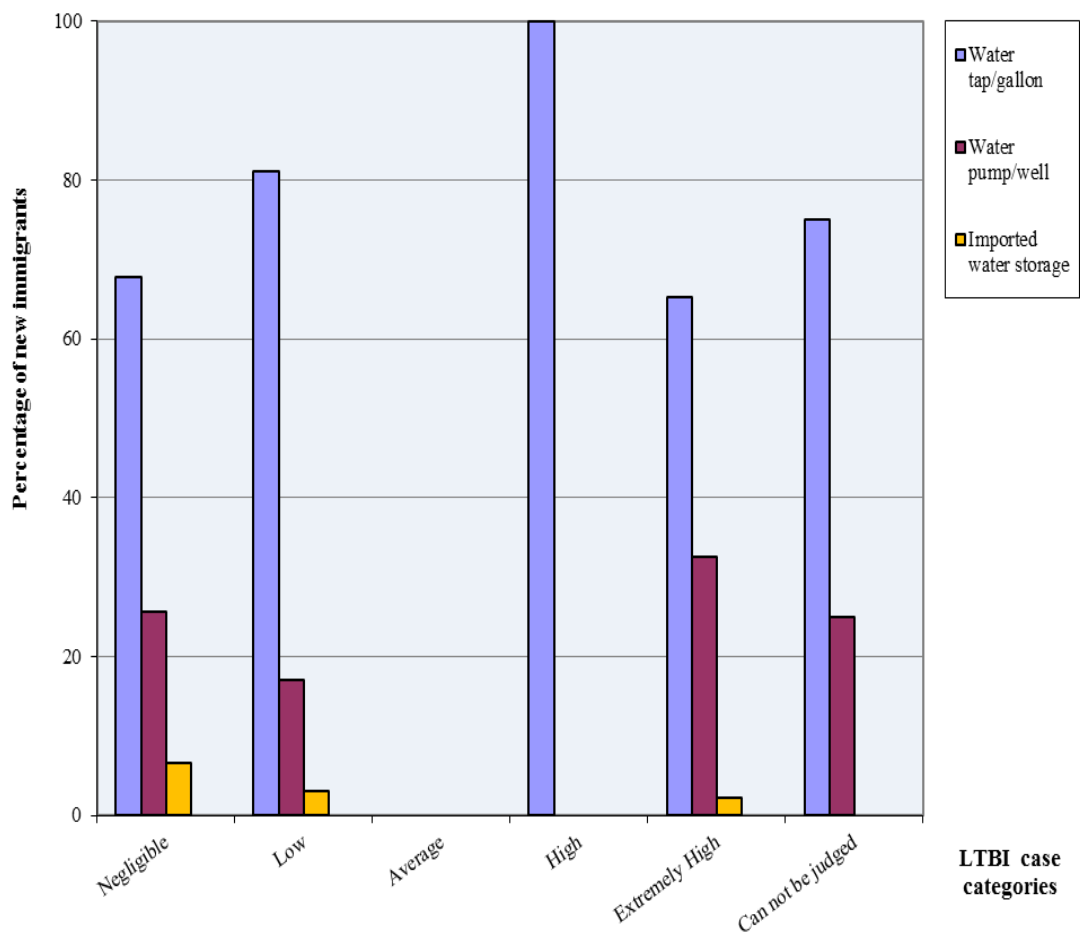
Figure 4.15: Percent distribution of the water supply systems used in the mother country according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

4.8.3 Entry to and living conditions in the State of Kuwait

Study immigrants living conditions pre- and post-entry to Kuwait are presented in Table 4.3.

Table 4.3: Distribution of immigrants' living conditions in Kuwait according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

Entry to Kuwait		Negligible (n=90)		Low (n=36)		Average (n=0)		High (n=4)		Extremely High (n=46)		Can not be judged (n=4)		Total (n=180)		Likelihood ratio	p-value
		n	%	n	%	n	%	n	%	n	%	n	%	n	%		
Proposed occupation in Kuwait	**House-related job**	40	44.44	14	38.89	0	0	2	50.00	27	58.70	2	50.00	85	47.22	LLR $\chi^2_{(12)}$= 20.923	0.052
	Manual worker	28	31.11	14	38.89	0	0	1	25.00	17	36.96	1	25.00	61	33.89		
	Non-manual worker	22	24.44	8	22.22	0	0	1	25.00	2	4.35	0	0.00	33	18.33		
	Other	0	0.00	0	0.00	0	0	0	0.00	0	0.00	1	25.00	1	0.56		
Previous residence in Kuwait	**Yes**	13	14.44	3	8.33	0	0	0	0.00	7	15.22	0	0.00	23	12.78	LLR $\chi2_{(4)}$= 3.353	0.501
Since when from last visit to Kuwait (year)	**median (IQR)**	2.00 (2.50)		3.00 (--)		-- (--)		-- (--)		-- (--)		-- (--)		2.00 (3)		KW $\chi^2_{(10)}$= 14.258	0.162

Continued Table 4.3

Test performed before entry to Kuwait	**Chest X-ray**	87	96.67	35	97.22	0	0	4	100.00	46	100.00	4	100.0	176	97.78	LLR $\chi^2_{(4)}$= 2.919	0.572
	Sputum (M/S)	1	1.11	5	13.89	0	0	1	25.00	3	6.52	0	0.00	10	5.56	LLR $\chi^2_{(4)}$= 10.562	0.032
	TST (PPD test)	5	5.56	5	13.89	0	0	0	0.00	4	8.70	0	0.00	14	7.78	LLR $\chi^2_{(4)}$= 3.578	0.466
	Forced cough test	5	5.56	4	11.11	0	0	2	50.00	6	13.04	0	0.00	17	9.44	LLR $\chi^2_{(4)}$= 7.667	0.105
Sleeping condition/area in Kuwait (ventilation condition)	**Presence of sleeping closed-room with good ventilation**	75	83.33	35	97.22	0	0	4	100.00	39	84.78	3	0.75	156	86.66	LLR $\chi^2_{(5)}$= 6.131	0.294
	Presence of sleeping closed-room without good ventilation	15	16.67	1	2.78	0	0	0	0.00	7	15.21	1	0.25	24	13.33		

Continued Table 4.3									
Total individual living in same room in Kuwait	**Median (IQR)**	3.00 (2)	3.00 (3)	-- (--)	1.00 (2)	2.50 (2)	3.00 (6)	3.00 (2)	KW $\chi^2_{(4)}$= 5.786	0.216
Duration of post-entry registration in Kuwait (day)	**Median (IQR)**	11.00 (18)	11.50 (16.5)	-- (--)	19.00 (21.5)	12.50 (12.25)	8.50 (13.5)	11.5 (17)	KW $\chi^2_{(4)}$= 1.551	0.818

Average LTBI category is zero (means no detected cases), (-) means cannot be computed by SPSS Software programme, IQR = interquartile range

12.78% (23/180) of participants had past history of previous residence in Kuwait, with male predominance (73.91%) and the majority were belonging to the 'negligible' LTBI 14.44% (13/90) and the 'extremely high' LTBI groups 15.22% (7/46) (LLR $\chi^2_{(4)}$ = 3.353, p = 0.501) (Table 4.3). The overall median duration since the last entry/visit to Kuwait was two years (IQR = 3). The minimum duration was before one year and the maximum was 23 years, but was not significantly different between groups (KW $\chi^2_{(10)}$ = 14.258, p = 0.162). The median duration of registration post-entry to Kuwait was 11.5 days (IQR = 17), which was nearly the same for the 'negligible' and 'low' LTBI, but was longer for those 'high' LTBI immigrants 19 days (IQR = 21.5) and 'extremely high' 12.5 days before registration (IQR = 12.25) (KW $\chi^2_{(4)}$ = 1.551, p = 0.818) (Table 4.3). Only 2.2% (4/180) immigrants had entered the country without the ordinary CXR testing having been performed in their mother countries (LLR $\chi^2_{(4)}$ = 2.919, p = 0.572). Additional tests including sputum smear and microscopy (M/S) were performed on 5.55% (LLR $\chi^2_{(4)}$ = 10.562, p = 0.032), tuberculin skin test (PPD test) performed for 7.8% (LLR $\chi^2_{(4)}$ = 3.578, p = 0.466), and only 9.4% immigrants did a forced cough test (LLR $\chi^2_{(4)}$ =7.667, p = 0.105) (Table 4.3).

The purpose of in-put migration according to the proposed occupation in Kuwait was subdivided into the following jobs: First came to work in house-related jobs (working as housemaid, driver, cooker and guardian/gardener) were 47.22%, and the majority of new immigrants belong to the 'extremely high' LTBI group in 58.7% (27/46). Second immigrants entered to join manual working jobs were 33.89%, with the majority also belongs to the 'extremely high' in 36.96% (17/46), and thirdly those came to work as non-manual workers were 18.33% (33/180), and the majority belong to the 'negligible' LTBI group in 24.44% (22/90). Statistical significant difference was associated between immigrant's occupational jobs in the country and LTBI development (LLR $\chi^2_{(12)}$ = 20.923, p = 0.052) (Figure 4.16, Table 4.3).
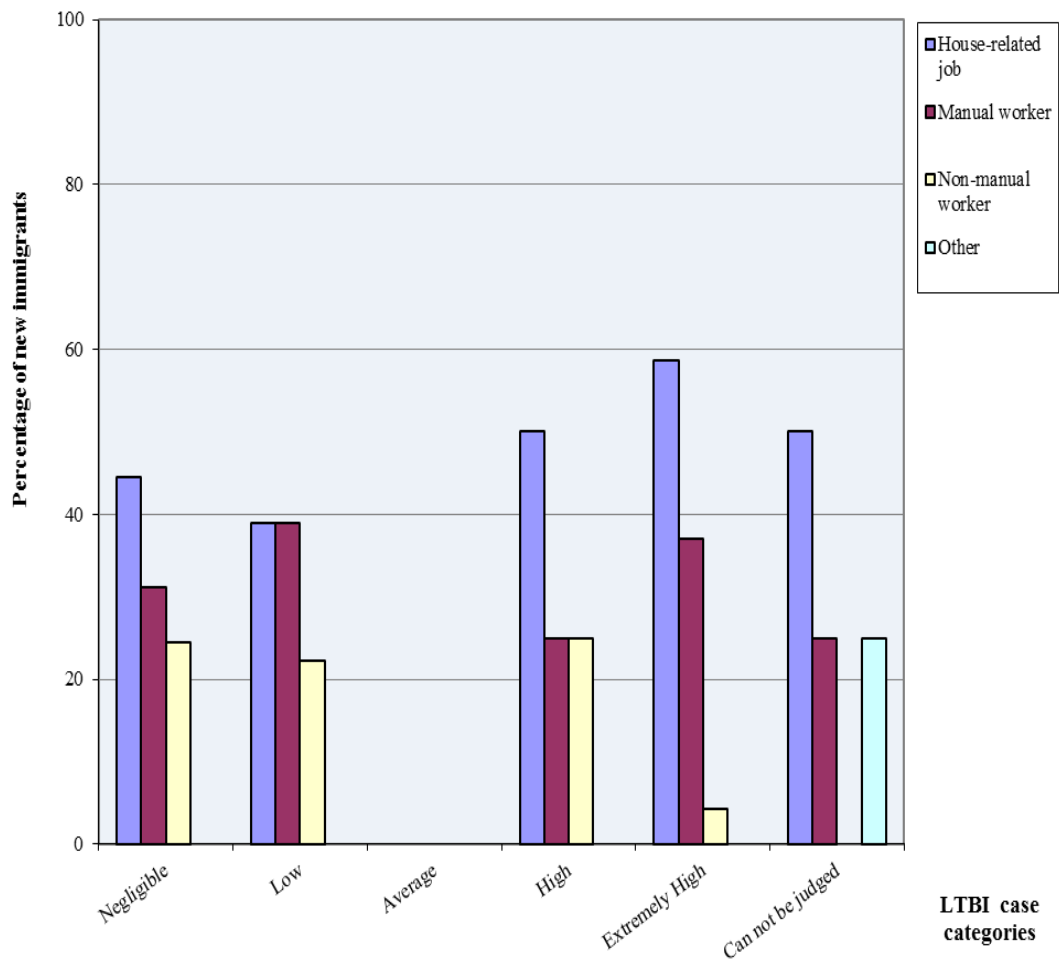
Figure 4.16: Percent distribution of proposed stratum occupation in Kuwait according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

The overall median number of individuals living in the same room as the participating immigrant in Kuwait was three individuals (IQR = two, SIQR = one). The minimum number of accompanying people was one with a maximum of 12 (KW $\chi^2_{(4)}$ = 5.786, p = 0.216) (Table 4.3).

86.66% of immigrants reported closed, well-ventilated bedrooms, compared with 13.33% of immigrants living with poor ventilation (LLR $\chi^2_{(5)}$ = 6.131, p = 0.294) (Figure 4.17, Table 4.3).
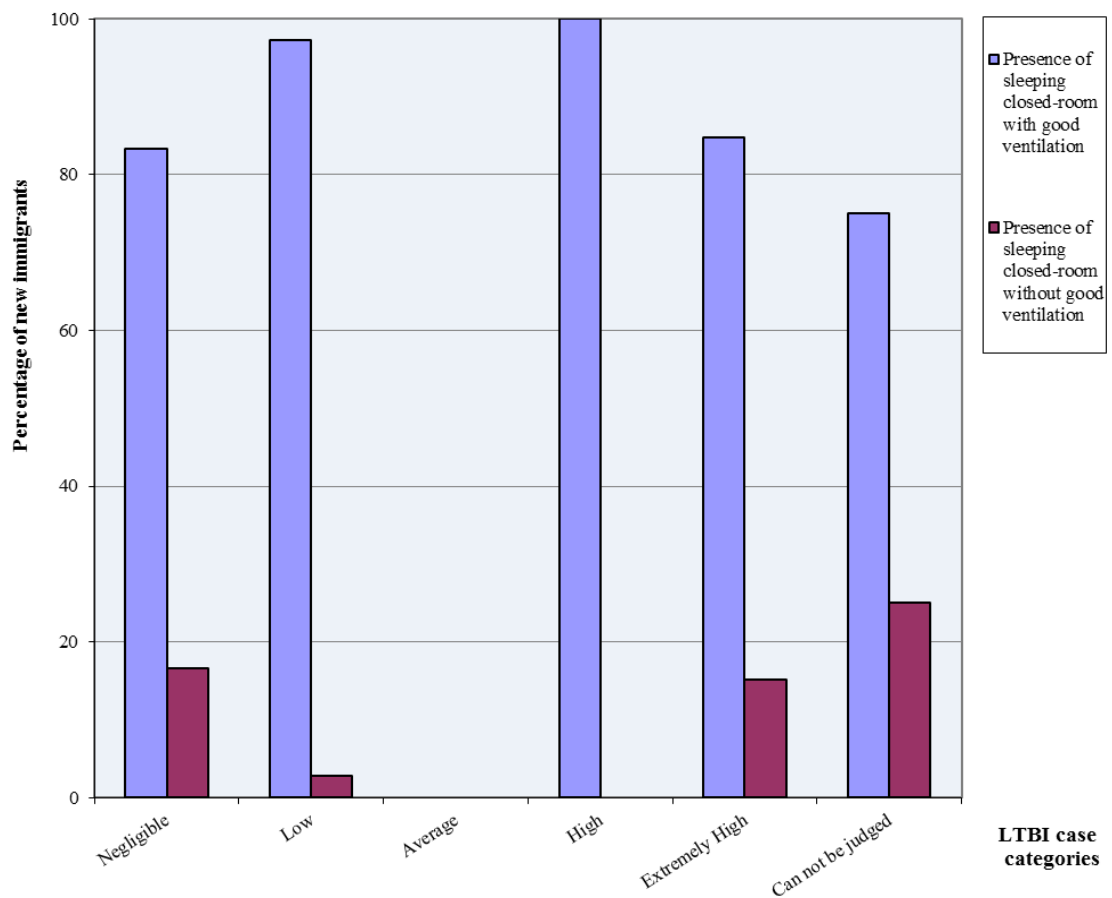
Figure 4.17: Percent distribution of the ventilation condition within the living region in Kuwait according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

4.8.4 Knowledge about tuberculosis

High levels of TB awareness is crucial for the success of treatment and prevention/control efforts in high risk populations, and represents a key challenge for public health initiatives. Association of presentations with chest TB in patients remains controversial, even more so in asymptomatic LTBI cases. Table 4.4 shows the immigrants' clinical knowledge about TB disease and describes the general knowledge of tuberculosis.

Table 4.4: Distribution of immigrant's tuberculosis knowledge according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

| General tuberculosis knowledge		Negligible (n=90)		Low (n=36)		Average (n=0)		High (n=4)		Extremely High (n=46)		Can not be judged (n=4)		Total (n=180)		Likelihood ratio	p-value
		n	%	n	%	n	%	n	%	n	%	n	%	n	%		
General knowledge	Correct	68	75.56	28	77.78	0	0	4	100.00	45	97.83	3	75.00	148	82.22	LLR $\chi^2_{(4)}$=16.103	0.003
	Incorrect	22	24.44	8	22.22	0	0	0	0.00	1	2.17	1	25.00	32	17.78		
Cough	Yes	68	75.56	28	77.78	0	0	4	100.00	44	95.65	2	50.00	146	81.11	LLR $\chi2(8)$=24.553	0.002
	No	2	2.22	3	8.33	0	0	0	0.00	2	4.35	1	25.00	8	4.44		
	Unknown	20	22.22	5	13.89	0	0	0	0.00	0	0.00	1	25.00	26	14.44		
Hemoptysis	Yes	55	61.11	24	66.67	0	0	3	75.00	38	82.61	3	75.00	123	68.33	LLR $\chi2(8)$=22.590	0.004
	No	14	15.56	7	19.44	0	0	1	25.00	8	17.39	0	0.00	30	16.67		
	Unknown	21	23.33	5	13.89	0	0	0	0.00	0	0.00	1	25.00	27	15.00		
Fever	Yes	57	63.33	26	72.22	0	0	3	75.00	40	86.96	2	50.00	128	71.11	LLR $\chi2(8)$=22.032	0.005
	No	12	13.33	5	13.89	0	0	1	25.00	6	13.04	1	25.00	25	13.89		
	Unknown	21	23.33	5	13.89	0	0	0	0.00	0	0.00	1	25.00	27	15.00		
Night sweating	Yes	32	35.56	15	41.67	0	0	1	25.00	21	45.65	1	25.00	70	38.89	LLR $\chi2(8)$=15.726	0.046
	No	37	41.11	15	41.67	0	0	3	75.00	24	52.17	2	50.00	81	45.00		
	Unknown	21	23.33	6	16.67	0	0	0	0.00	1	2.17	1	25.00	29	16.11		
Generalized weakness	Yes	56	62.22	26	72.22	0	0	2	50.00	36	78.26	3	75.00	123	68.33	LLR $\chi2(8)$=24.435	0.002
	No	13	14.44	5	13.89	0	0	2	50.00	10	21.74	0	0.00	30	16.67		
	Unknown	21	23.33	5	13.89	0	0	0	0.00	0	0.00	1	25.00	27	15.00		

Continued Table 4.4															
Weight loss/wasted	**Yes**	54	60.00	25	69.44	0	0	2	50.00	39	84.78	3	75.00	123	68.33	LLR χ2(8)=24.824	0.002
	No	15	16.67	6	16.67	0	0	2	50.00	7	15.22	0	0.00	30	16.67		
	Unknown	21	23.33	5	13.89	0	0	0	0.00	0	0.00	1	25.00	27	15.00		
Vomiting/diarrhea	**Yes**	38	42.22	17	47.22	0	0	1	25.00	21	45.65	2	50.00	79	43.89	LLR χ2(8)=17.087	0.029
	No	31	34.44	13	36.11	0	0	3	75.00	24	52.17	1	25.00	72	40.00		
	Unknown	21	23.33	6	16.67	0	0	0	0.00	1	2.17	1	25.00	29	16.11		
Body pain (bone/joint)	**Yes**	28	31.11	8	22.22	0	0	1	25.00	11	23.91	1	25.00	49	27.22	LLR χ2(8)=18.626	0.017
	No	41	45.56	21	58.33	0	0	3	75.00	34	73.91	2	50.00	101	56.11		
	Unknown	21	23.33	7	19.44	0	0	0	0.00	1	2.17	1	25.00	30	16.67		
Other symptom(s)/sign(s)	**Yes**	3	3.33	1	2.78	0	0	0	0.00	3	6.52	0	0.00	7	3.89	LLR χ2(8)=22.038	0.005
	No	66	73.33	30	83.33	0	0	4	100.00	43	93.48	3	75.00	146	81.11		
	Unknown	21	23.33	5	13.89	0	0	0	0.00	0	0.00	1	25.00	27	15.00		

Average LTBI category is zero (means no detected cases), (-) means cannot be computed by SPSS Software programm

Immigrants were asked indirectly about TB to prevent stigma and misperceptions (Wieland *et al.*, 2010). Not a single participant was able to answer all TB questions correctly. However, out of 180 immigrants interviewed, general knowledge about TB was high with statistical significant difference (LLR $\chi^2_{(4)}$ = 16.103, p = 0.003). A total of 82.22% (148/180) of immigrants heard about TB disease and at least correctly defined tuberculosis as a 'dangerous infectious respiratory disease' and these were distributed more in the 'high' and 'extremely high' LTBI groups. Common clinical symptoms of TB answered as 'yes'/'unknown' were distributed as follows: cough 81.11%/14.44% (LLR $\chi^2_{(8)}$ = 24.553, p = 0.002), fever 71.11%/15% (LLR $\chi^2_{(8)}$ = 22.032, p = 0.004), generalized weakness and weight loss (or wasted) 68.33%/15% (LLR $\chi^2_{(8)}$ = 24.435, p = 0.002), and hemoptysis 68.33%/15% (LLR $\chi^2_{(8)}$ = 22.590, p = 0.004).

Tuberculosis symptoms were less frequently identified as vomiting/diarrhea 43.98% (LLR $\chi^2_{(8)}$ = 17.087, p = 0.029), night-sweats 38.89% (LLR $\chi^2_{(8)}$ = 15.726, p = 0.046), and body pains (bone/joints) (LLR $\chi^2_{(8)}$ = 18.626, p = 0.017) or other complaints such as TB orbital swelling (LLR $\chi^2_{(8)}$ = 22.038, p = 0.005) (Table 4.4).

4.8.5 Sources of tuberculosis knowledge

General information about tuberculosis was explored through immigrants' answers about their various sources of the infection and disease knowledge, as shown in Table 4.5. Common sources of knowledge were associated with the defined LTBI categories, and distributed as TB knowledge in the mother countries within the study/learning areas (e.g. school/class and close friends) (LLR $\chi^2_{(4)}$ = 11.512, p = 0.021), and/or public shared talks and external communications (community and social networking) (LLR $\chi^2_{(4)}$ = 11.236, p = 0.024). LTBI categories were also defined for immigrants who stated that television and reading advertisements in newspapers/magazines as sources of knowledge with significant association (LLR $\chi^2_{(4)}$ = 15.275, p = 0.004). Medical campaigns and posters were also important sources of TB knowledge dissemination, which were significantly different across the LTBI categories (LLR $\chi^2_{(4)}$ = 16.627, p = 0.002) (Table 4.5).

Table 4.5: Distribution of tuberculosis knowledge sources according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

| Tuberculosis Knowledge Source		Negligible (n=90)		Low (n=36)		Average (n=0)		High (n=4)		Extremely High (n=46)		Can not be judged (n=4)		Total (n=180)		Likelihood ratio	p-value
		n	%	n	%	n	%	n	%	n	%	n	%	n	%		
Family/Relative	Yes	37	41.11	16	44.44	0	0.00	1	25.00	26	56.52	1	25.00	81	45.00	LLR $\chi2_{(4)}$=4.380	0.357
Study area (e.g. school/friend subject)	Yes	30	33.33	16	44.44	0	0.00	4	100.00	23	50.00	1	25.00	74	41.11	LLR $\chi2_{(4)}$=11.512	0.021
Work peer/close contact	Yes	32	35.56	17	47.22	0	0.00	3	75.00	22	47.83	1	25.00	75	41.67	LLR $\chi2_{(4)}$=4.887	0.299
Public shared talks	Yes	67	74.44	29	80.56	0	0.00	3	75.00	44	95.65	3	75.00	146	81.11	LLR $\chi2_{(4)}$=11.236	0.024
TV/Newspaper advertisement	Yes	61	67.78	27	75.00	0	0.00	4	100.00	43	93.48	3	75.00	138	76.67	LLR $\chi2_{(4)}$=15.275	0.004
Medical campaign/Poster	Yes	41	45.56	24	66.67	0	0.00	4	100.00	34	73.91	3	75.00	106	58.89	LLR $\chi2_{(4)}$=16.627	0.002
Other source(S)	Yes	10	11.11	5	13.89	0	0.00	0	0.00	8	17.39	1	25.00	24	13.33	LLR $\chi2_{(4)}$=2.555	0.635
Example(s) of other knowledge source	Friend/Relative	1	1.11	0	0.00	0	0.00	0	0.00	1	2.17	0	0.00	2	1.11	LLR $\chi2_{(28)}$=24.124	0.675
	Hospital	5	5.56	0	0.00	0	0.00	0	0.00	1	2.17	1	25.00	7	3.89		
	Internet	0	0.00	3	8.33	0	0.00	0	0.00	2	4.35	0	0.00	5	2.78		
	Internet & journal	1	1.11	1	2.78	0	0.00	0	0.00	1	2.17	0	0.00	3	1.67		
	Internet & public	0	0.00	1	2.78	0	0.00	0	0.00	0	0.00	0	0.00	1	0.56		
	Journal	1	1.11	0	0.00	0	0.00	0	0.00	1	2.17	0	0.00	2	1.11		
	Public	2	2.22	0	0.00	0	0.00	0	0.00	2	4.35	0	0.00	4	2.22		

Average LTBI category is zero (means no detected cases)

On the contrary, association of the defined LTBI groups and the TB knowledge from close contacts of immigrant's families or relatives were not statistically significant (LLR $\chi^2_{(4)}$ = 4.380, p = 0.357) or occupational close contacts such as work peer (LLR $\chi^2_{(4)}$ = 4.887, p = 0.299), which might be due to TB stigma and symptoms hiding. Networking sources such as internet also revealed insignificant association (LLR $\chi^2_{(4)}$ = 24.124, p = 0.675) (Table 4.5).

4.8.6 Risk of tuberculosis exposure and contact

Using degree of exposure to TB as a surrogate for LTBI, epidemiological risk factors correlate with TB exposure.

4.8.6.1 Risk of travel or work outside

Risks of tuberculosis-associated exposure-contact transmissions of MTB bacilli for the 180 new immigrants according to latent tuberculosis infection defined categories are shown in Table 4.6.

Table 4.6: Distribution of tuberculosis travel and work risk factors according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

| Tuberculosis Disease History		Negligible (n=90)		Low (n=36)		Average (n=0)		High (n=4)		Extremely High (n=46)		Can not be judged (n=4)		Total (n=180)		Likelihood ratio	p-value
		n	%	n	%	n	%	n	%	n	%	n	%	n	%		
Have work or travel outside mother country?	Yes	43	47.78	18	50.00	0	0	3	75.00	20	43.48	2	50.00	86	47.78	LLR $\chi2_{(4)}$ = 1.654	0.799
	No	47	52.22	18	50.00	0	0	1	25.00	26	56.52	2	50.00	94	52.22		
Work outside mother country	Yes	37	41.11	17	47.22	0	0	3	75.00	18	39.13	2	50.00	77	42.78	LLR $\chi2_{(4)}$= 2.440	0.655
	No	53	58.89	19	52.78	0	0	1	25.00	28	60.87	2	50.00	103	57.22		
Work outside in :	Endemic	10	11.11	1	2.78	0	0	1	25.00	3	6.52	0	0.00	15	8.33	LLR $\chi2_{(12)}$= 10.541	0.569
	Endemic & non-endemic	2	2.22	2	5.56	0	0	1	25.00	1	2.17	0	0.00	6	3.33		
	Non-endemic	25	27.78	14	38.89	0	0	1	25.00	14	30.43	2	50.00	56	31.11		
Work duration in outside countries (years)	Median (IQR)	0.00 (3)		0.00 (3)		--(-)		3.34 (7)		0.00 (4)		1.25 (4)		0.00 (3)		KW $\chi^2_{(4)}$= 2.173	0.704

Continued Table 4.6

		n	%	n	%	n	%	n	%	n	%	n	%	n	%	Statistic	p
Travel outside mother country	Yes	8	8.89	1	2.78	0	0	0	0.00	2	4.35	0	0.00	11	6.11	LLR $\chi^2_{(4)}$= 3.220	0.522
	No	82	91.11	35	97.22	0	0	4	100.00	44	95.65	4	100.00	169	93.89		
Travel and/or visit to :	Endemic	4	4.44	0	0.00	0	0	0	0.00	1	2.17	0	0.00	5	2.78	LLR $\chi^2_{(12)}$= 8.334	0.759
	Endemic & non-endemic	3	3.33	0	0.00	0	0	0	0.00	0	0.00	0	0.00	3	1.67		
	Non-endemic	1	1.11	1	2.78	0	0	0	0.00	1	2.17	0	0.00	3	1.67		
Travel duration in outside countries (weeks)	Median (IQR)	3.00 (5)		-- (--)		-- (--)		-- (--)		6.00 (--)		-- (--)		4.00 (5)		KW $\chi^2_{(2)}$= 1.244	0.537

Average LTBI category is zero (means no detected cases), (-) means cannot be computed by SPSS Software programme

Out of the total 180 participants, 42.78% (77/180) had worked outside their mother countries for at least one year. 31.11% of immigrant workers employed in non-endemic regions, such as Gulf countries, and were distributed mainly in the 'low' LTBI group 38.89% and 'extremely high' LTBI 30.43%. Immigrants working previously in endemic regions constituted only 8.33%, and were distributed mainly in the 'negligible' LTBI 11.11% (LLR $\chi^2_{(12)}$ = 10.541, p = 0.569) (Figure 4.18, Table 4.6).
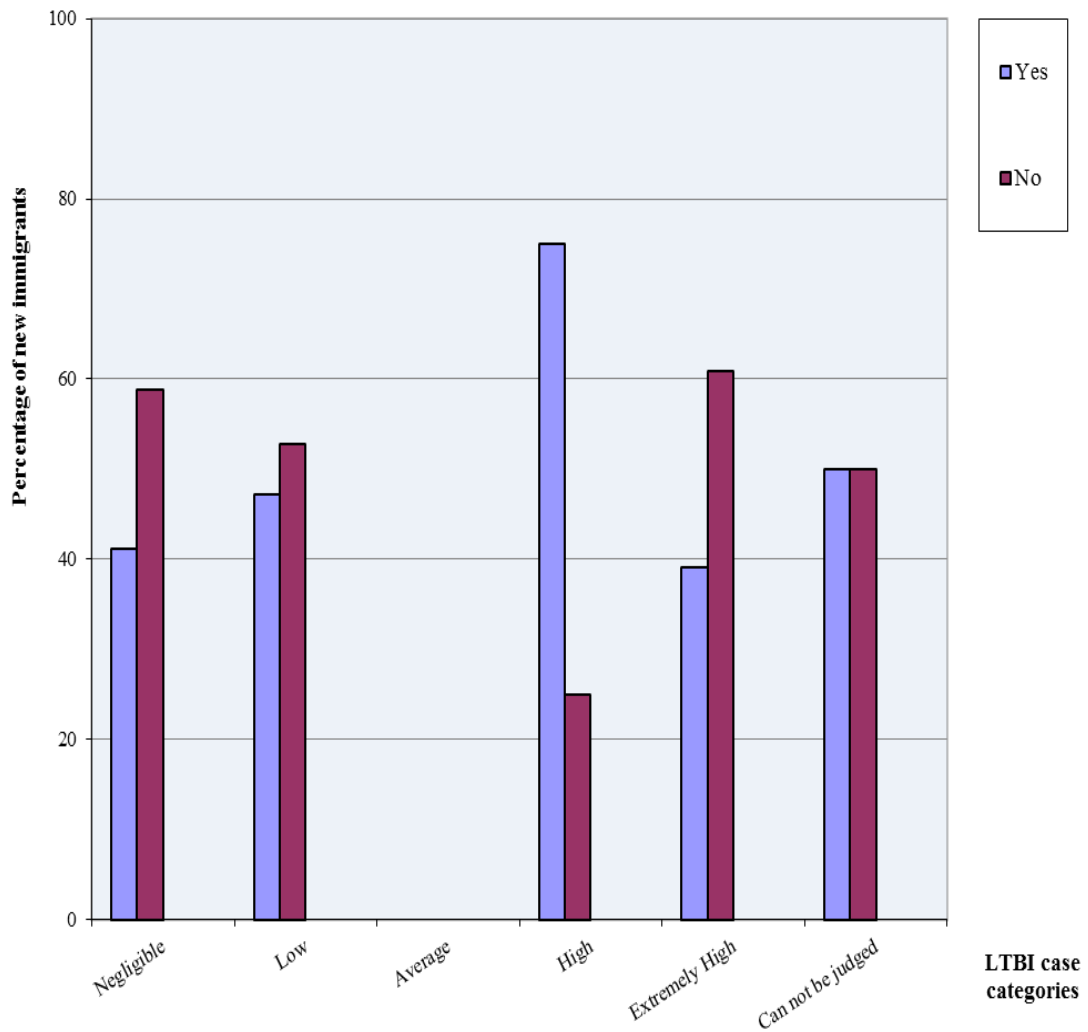
Figure 4.18: Percent distribution of working history outside the mother country according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

Only 6.11% (11/180) of immigrants had travelled outside their mother countries, and all fell the 'negligible' LTBI group 8.89% (LLR $\chi^2_{(4)}$ = 3.220, p = 0.522). Immigrants travelled to both endemic and non-endemic regions, but only 5% been travelled to endemic countries versus 3% travelled to non-endemic regions only (LLR $\chi^2_{(12)}$ = 8.334, p = 0.759). The overall median duration of travel or visit was less than 4 weeks (IQR = 3, SIQR = 1.5). The minimum travel duration was one week and the longest was 20 weeks. The differences were not statistically significant between LTBI categories (KW $\chi^2_{(2)}$ = 1.244, p = 0.537) (Figure 4.19, Table 4.6).
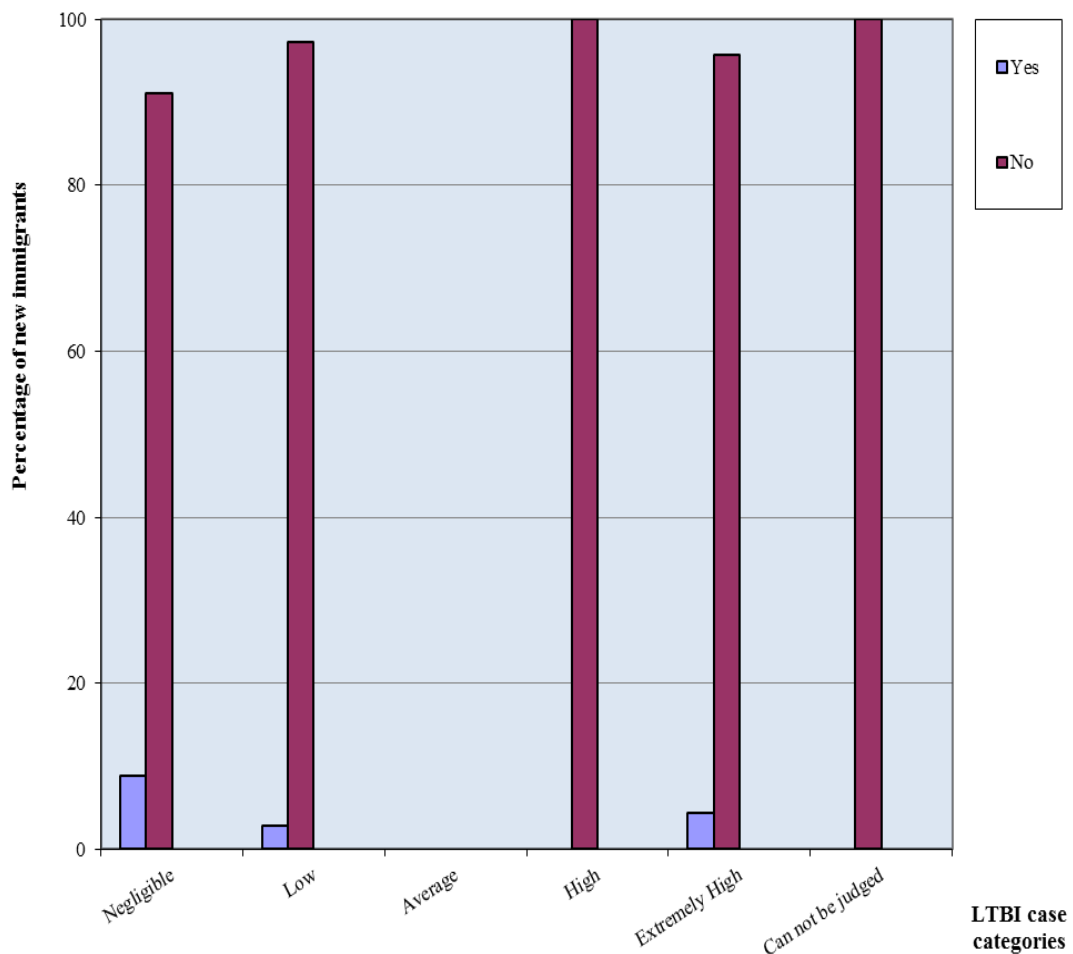
Figure 4.19: Percent distribution of travel history outside the mother country according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

4.8.6.2 History of environmental (inside household and outside household) contacts

Screening of household contacts, which has been prioritized in industrialized countries, merits serious consideration as a means of interrupting transmission in high-burden settings. The distribution of environmental contacts inside- and outside households of the immigrants in their mother countries according to latent tuberculosis infection categories is shown in Table 4.7.

Table 4.7: Distribution of environmental exposure contacts in the mother country according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

Tuberculosis-associated Risk Factor: Tuberculosis Exposure		Negligible (n=90)		Low (n=36)		Average (n=0)		High (n=4)		Extremely High (n=46)		Can not be judged (n=4)		Total (n=180)		Likelihood ratio	p-value
		n	%	n	%	n	%	n	%	n	%	n	%	n	%		
Average number of daily contact to inside same households	1-2	52	57.78	21	58	0	0.00	4	100.00	25	54.35	2	50.00	104	57.78	LLR $\chi^2_{(12)}$= 10.869	0.540
	3-5	33	36.67	11	31	0	0.00	0	0.00	20	43.48	2	50.00	66	36.67		
	6-10	5	5.56	3	8	0	0.00	0	0.00	1	2.17	0	0.00	9	5.00		
	>10	0	0.00	1	3	0	0.00	0	0.00	0	0.00	0	0.00	1	0.56		
Duration of contact to inside same households (hours)	1-5	1	1.11	1	3	0	0.00	0	0.00	4	8.70	0	0.00	6	3.33	LLR $\chi^2_{(8)}$= 11.361	0.182
	6-10	30	33.33	8	22	0	0.00	1	25.00	17	36.96	0	0.00	56	31.11		
	>10	59	65.56	27	75	0	0.00	3	75.00	25	54.35	4	100.00	118	65.56		
Average number of daily contact to outside non-households	1-2	14	15.56	4	11	0	0.00	1	25.00	4	8.70	1	25.00	24	13.33	LLR $\chi^2_{(16)}$= 22.613	0.124
	3-5	23	25.56	7	19	0	0.00	3	75.00	16	34.78	1	25.00	50	27.78		
	6-10	38	42.22	12	33	0	0.00	0	0.00	14	30.43	0	0.00	64	35.56		
	>10	15	16.67	13	36.1	0	0.00	0	0.00	12	26.09	2	50.00	42	22.78		
Duration of contact to outside non-households (hours)	<1	2	2.22	2	6	0	0.00	1	25.00	4	8.70	2	50.00	11	6.11	LLR $\chi^2_{(12)}$= 20.285	0.062
	1-5	4	4.44	2	6	0	0.00	0	0.00	2	4.35	1	25.00	9	5.00		
	6-10	49	54.44	21	58	0	0.00	2	50.00	17	36.96	1	25.00	90	50.00		
	>10	35	38.89	11	31	0	0.00	1	25.00	23	50.00	0	0.00	70	38.89		

The average number of daily contacts within households was not significantly associated with LTBI categories. 57.78% of immigrants had daily contacts to either one and/or two individuals or 36.67% between three and five subjects. The details of those exposed to three and/or five subjects were distributed as follows; 'extremely high' LTBI in 43.48%, 'negligible' LTBI in 36.67% and 'low' LTBI in 31% (LLR $\chi^2_{(12)}$ = 10.869, p = 0.540) (Figure 4.20, Table 4.7).
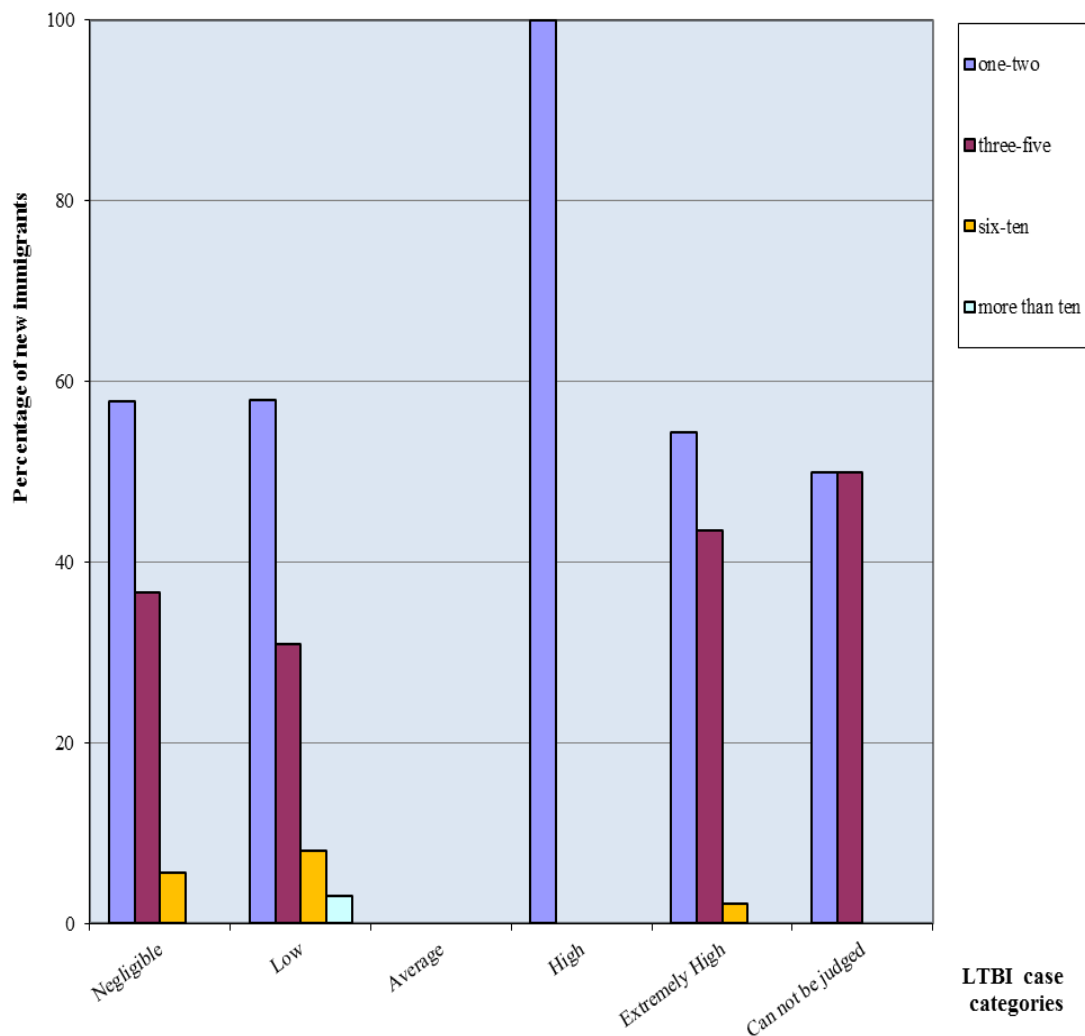
Figure 4.20: Percent distribution of average number of daily contacts with inside household individuals in the mother country according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

In all LTBI categories, the average duration of contact to individuals inside households for more than 10 hours was recorded in 65.56% of immigrants and between six and ten hours was observed in 31.11%. No significant differences were detected between LTBI categories and duration of contact inside households (LLR $\chi^2_{(8)}$ = 11.361, p = 0.182) (Figure 4.21, Table 4.7).
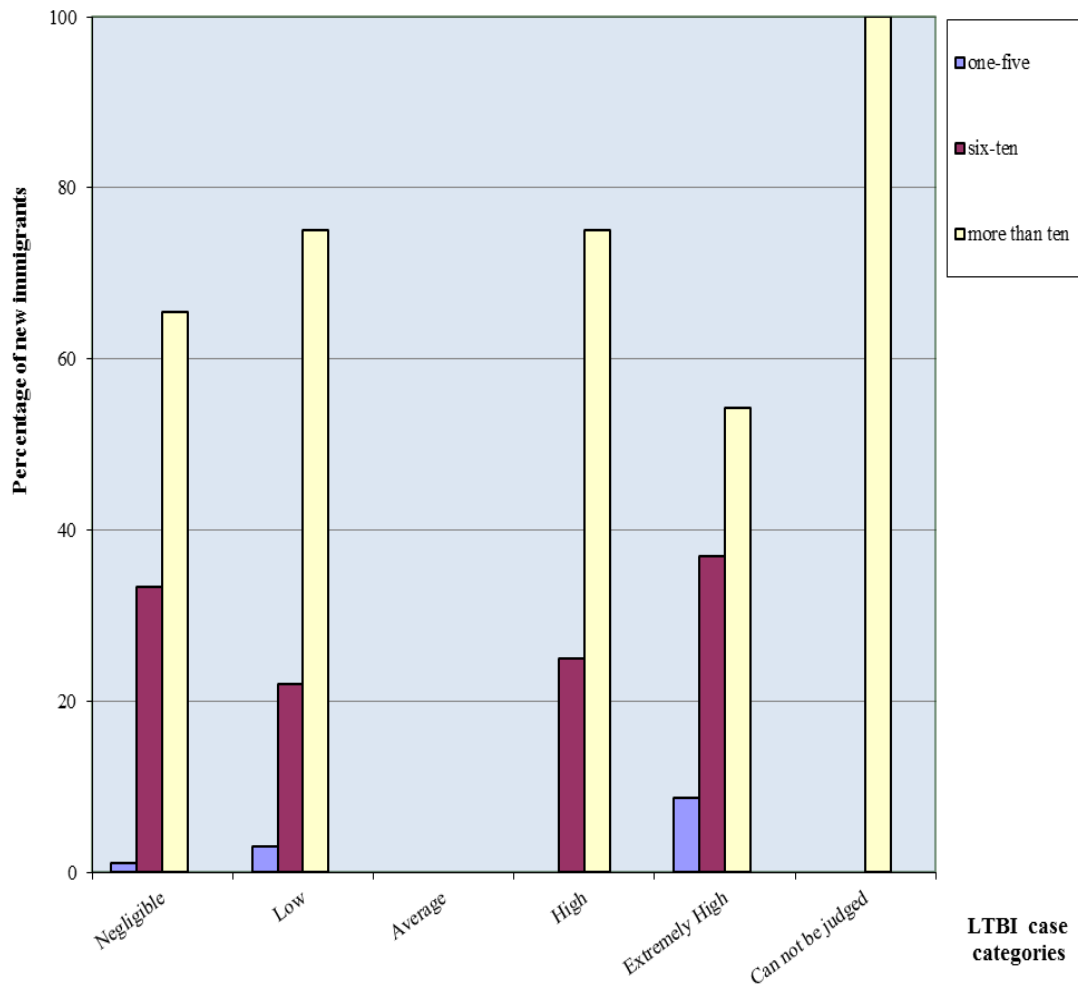
Figure 4.21: Percent distribution of duration (hours) of daily contacts with inside household individuals in the mother country according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

The average number of immigrants' daily contact to outside (non-household) environment was not significantly associated with LTBI development. 35.56% of immigrants had daily contacts to either six and/or ten individuals outside homes, or 27.78% contacted between three and five subjects. The details of those 22.78% participants who were exposed to more than ten individuals were divided as follows; 'negligible' LTBI in 16.67%, 'low' LTBI in 36.1% and 'extremely high' LTBI in 26.09% (LLR $\chi^2_{(16)}$ = 22.613, p = 0.124) (Figure 4.22, Table 4.7).
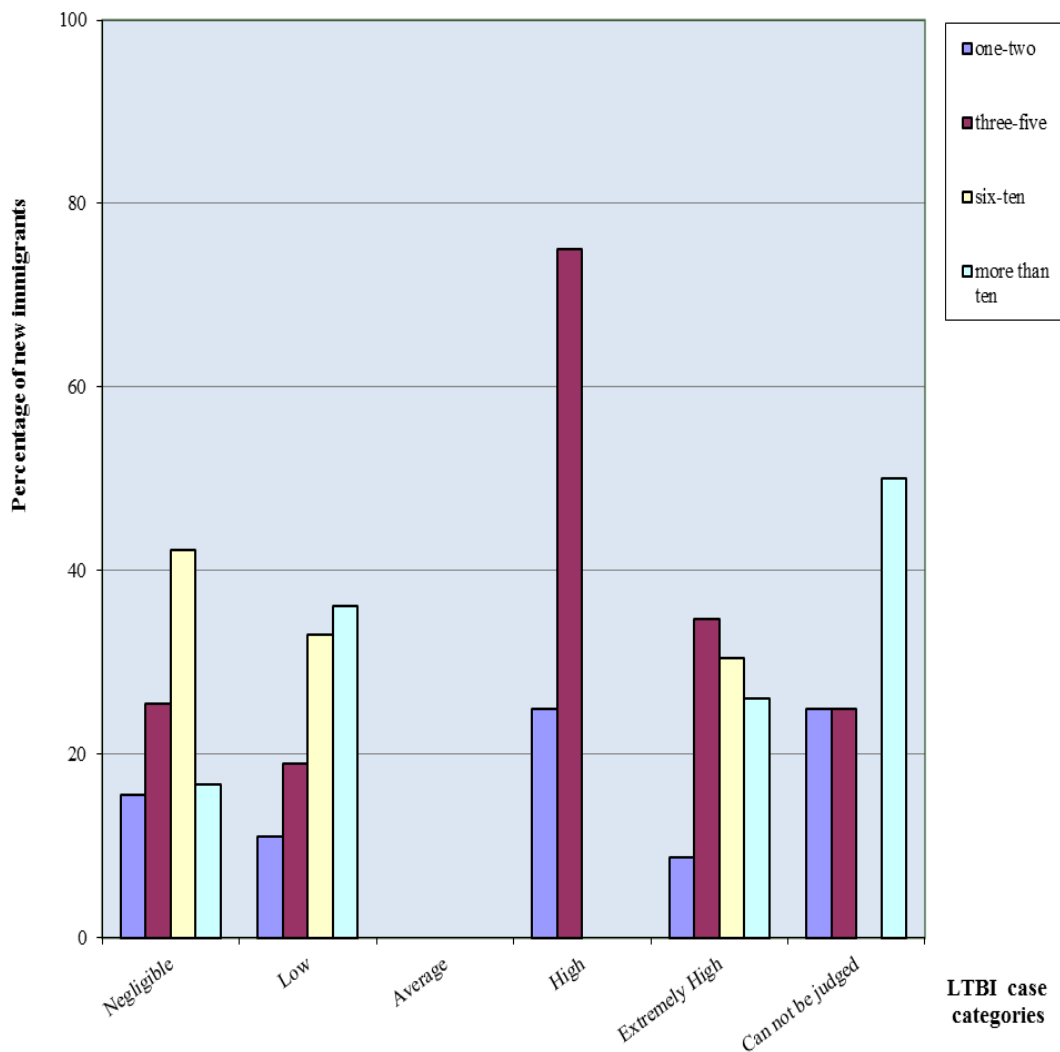
Figure 4.22: Percent distribution of average number of daily contact to outside households according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

For all LTBI categories, the duration of contact to individuals outside households was significantly associated at the 10% significance interval. 50% of participants had contacts to outside environment between six and ten hours and other 38.89% of them spent more than ten hours outside their households (LLR $\chi^2_{(12)}$ = 20.285, p = 0.062) (Figure 4.23, Table 4.7).
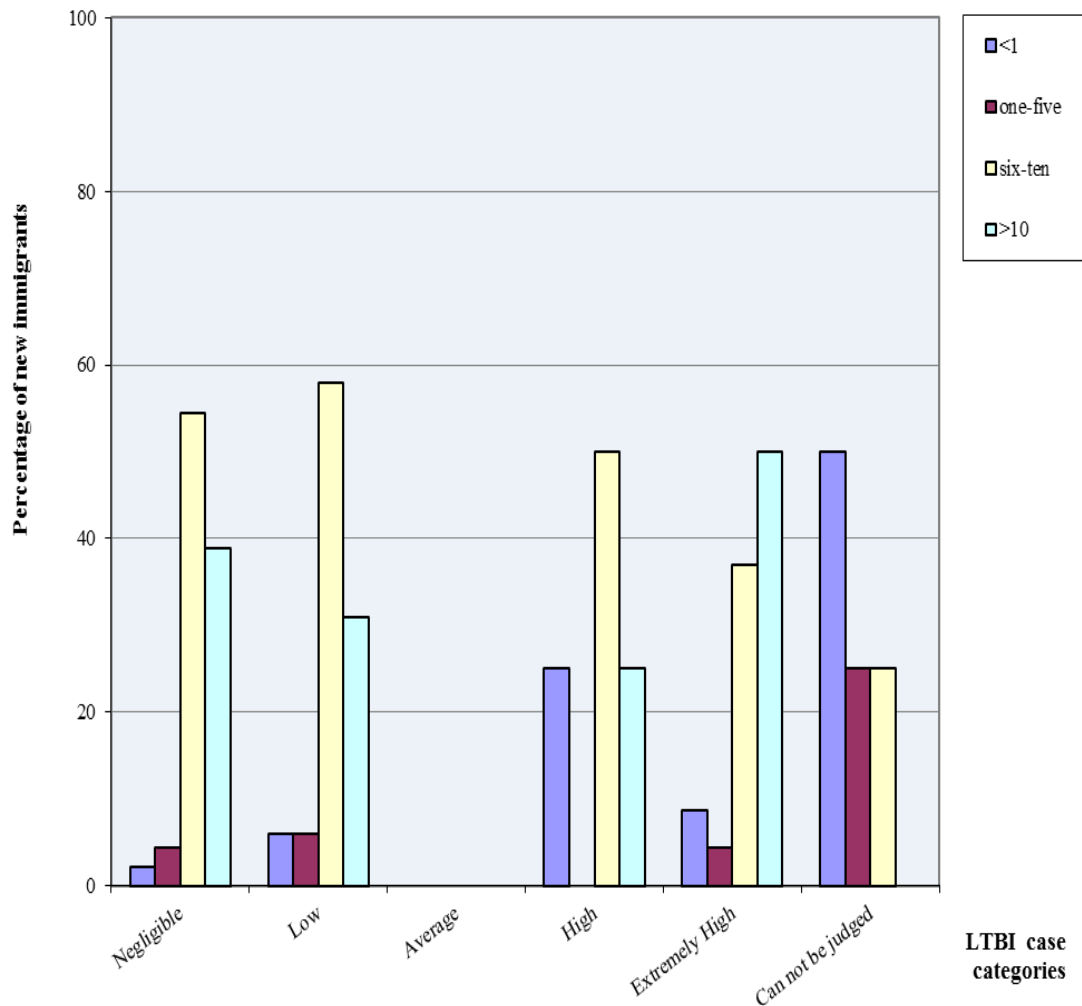
Figure 4.23: Percent distribution of average duration (hours) of daily contact to outside households according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

4.8.6.3   History of direct contact to diagnosed tuberculosis patient

Table 4.8 lists the direct contact of immigrants to diagnosed tuberculosis patients in their mother countries according to latent tuberculosis infection categories.

Table 4.8: Distribution of direct contact with diagnosed tuberculosis patient according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

History of contract with TB infection		Negligible (n=90)		Low (n=36)		Average (n=0)		High (n=4)		Extremely High (n=46)		Can not be judged (n=4)		Total (n=180)		Likelihood ratio	p-value
		n	%	n	%	n	%	n	%	n	%	n	%	n	%		
Have been infected or contacted with TB case	Yes	5	6	0	0	0	0	0	0	6	13	0	0	11	6.11	LLR $\chi^2_{(8)}$= 13.799	0.087
	Unknown	10	11	4	11.11	0	0	0	0	1	2	0	0	15	8.33		
If Yes, TB disease was diagnosed by medically qualified staff	Yes	5	6	0	0	0	0	0	0	6	13	0	0	11	6.11	LLR $\chi^2_{(4)}$= 8.561	0.073
Duration of complaint before tuberculosis diagnosis (month)	Median (IQR)	12.00 (24)		-- (--)		--	(--)	--	(--)	13.50 (21)		-- (--)		13 (12)		KW $\chi^2_{(1)}$= 0.077	0.782
Prescribed anti-TB therapy	Yes	5	6	0	0	0	0	0	0	5	11	0	0	10	5.56	LLR $\chi^2_{(4)}$= 6.993	0.136
Complete	Yes	3	3	0	0	0	0	0	0	3	7	0	0	6	3.33	LLR $\chi^2_{(8)}$= 8.671	0.371
	Unknown	2	2	0	0	0	0	0	0	3	7	0	0	5	2.78		
Cured	Yes	3	3	0	0	0	0	0	0	4	9	0	0	7	3.89	LLR $\chi^2_{(8)}$= 7.476	0.486
	Unknown	2	2	0	0	0	0	0	0	1	2	0	0	3	1.67		
Default	Unknown	4	4	0	0	0	0	0	0	6	13	0	0	10	5.56	LLR $\chi^2_{(4)}$= 8.890	0.064

Continued Table 4.8																
Failure	**Yes**	1	1	0	0	0	0	0	0	2	4	0	0	3	1.67	LLR $\chi^2_{(8)}=$	0.637
	Unknown	3	3	0	0	0	0	0	0	2	4	0	0	5	2.78	6.088	
Relapse	**Yes**	1	1	0	0	0	0	0	0	2	4	0	0	3	1.67	LLR $\chi^2_{(8)}=$	0.345
	Unknown	3	3	0	0	0	0	0	0	4	9	0	0	7	3.89	8.971	
Resistance	**No**	86	96	36	100.00	0	0	4	100.00	40	87	4	100.00	170	94.44	LLR $\chi^2_{(4)}=$	0.064
	Unknown	4	4	0	0	0	0	0	0	6	13	0	0	10	5.56	8.890	
DOTS	**Yes**	0	0	0	0	0	0	0	0	1	2	0	0	1	0.56	LLR $\chi^2_{(8)}=$	0.266
	Unknown	4	4	0	0	0	0	0	0	5	11	0	0	9	5.00	9.985	
Rifampicin	**Yes**	3	3	0	0	0	0	0	0	3	7	0	0	6	3.33	LLR $\chi^2_{(8)}=$	0.371
	Unknown	2	2	0	0	0	0	0	0	3	7	0	0	5	2.78	8.671	
Pyrizanamide	**Yes**	3	3	0	0	0	0	0	0	3	7	0	0	6	3.33	LLR $\chi^2_{(8)}=$	0.371
	Unknown	2	2	0	0	0	0	0	0	3	7	0	0	5	2.78	8.671	
INH	**Yes**	3	3	0	0	0	0	0	0	3	7	0	0	6	3.33	LLR $\chi^2_{(8)}=$	0.371
	Unknown	2	2	0	0	0	0	0	0	3	7	0	0	5	2.78	8.671	
Ethambutol	**Yes**	3	3	0	0	0	0	0	0	3	7	0	0	6	3.33	LLR $\chi^2_{(8)}=$	0.371
	Unknown	2	2	0	0	0	0	0	0	3	7	0	0	5	2.78	8.671	
Other treatment e.g. streptomycin	**Unknown**	4	4	0	0	0	0	0	0	6	13	0	0	10	5.56	LLR $\chi^2_{(8)}=$ 13.799	0.064

Continued Table 4.8

Presence of anti-TB adverse reaction	**Unknown**	3	3	0	0	0	0	0	0	5	11	0	0	8	4.44	LLR $\chi^2_{(8)}$= 13.799	0.111
Fever	**Unknown**	3	3	0	0	0	0	0	0	5	11	0	0	8	4.44	LLR $\chi^2_{(8)}$= 13.799	0.111
Vomiting/diarrhea	**Unknown**	3	3	0	0	0	0	0	0	5	11	0	0	8	4.44	LLR $\chi^2_{(8)}$= 13.799	0.111
Orange urine discoloration	**Unknown**	3	3	0	0	0	0	0	0	5	11	0	0	8	4.44	LLR $\chi^2_{(8)}$= 13.799	0.111
Hepatitis	**Unknown**	3	3	0	0	0	0	0	0	5	11	0	0	8	4.44	LLR $\chi^2_{(8)}$= 13.799	0.111
Other side effects	**Unknown**	3	3	0	0	0	0	0	0	5	11	0	0	8	4.44	LLR $\chi^2_{(8)}$= 13.799	0.111

Average LTBI category is zero (means no detected cases), (-) means cannot be computed by SPSS Software programme

6.11% (11/180) of participants admitted a previous history of direct contact with a diagnosed TB patient(s), 13% (6/46) in the 'extremely high' LTBI group and 6% (5/90) in the 'negligible' LTBI group. 85.55% (154/180) denied any contact, and 8.33% (15/180) reported 'unknown' contact. Differences were not statistically significant (LLR $\chi^2_{(8)} = 13.799$, p = 0.087) (Table 3).

There were no statistically significant difference in relation to LTBI categories as follows; all contacted TB patients were diagnosed by a medically-qualified health care service in their mother country (LLR $\chi^2_{(4)} = 8.561$, p = 0.073), and 90.9% (10/11) had received prescribed anti-tuberculosis chemotherapy (LLR $\chi^2_{(4)} = 6.993$, p = 0.136). The overall median duration of disease complaints of their infected contacts before tuberculosis diagnosis was 13 months (IQR = 12 months) (KW $\chi^2_{(1)} = 0.077$, p = 0.782) (Table 3).

The anti-TB therapy was reported for the following regimen courses in relation to LTBI categories; completed treatment course 3.33% (LLR $\chi^2_{(8)} = 8.671$, p = 0.371), complaints cured 3.89% (LLR $\chi^2_{(8)} = 7.476$, p = 0.486), treatment default 5.56% (LLR $\chi^2_{(4)} = 8.890$, p = 0.064), treatment failure (LLR $\chi^2_{(8)} = 6.088$, p = 0.637), treatment relapse (LLR $\chi^2_{(8)} = 8.971$, p = 0.345), and DOTS follow-up 0.56% (LLR $\chi^2_{(8)} = 9.985$, p = 0.266). No history of TB treatment resistance was recalled by the immigrants (LLR $\chi^2_{(4)} = 8.890$, p = 0.064). 3.33% of participants know about the treatment of TB and recalled using a full course of anti-TB drugs (rifampicin, pyrizanamide, INH, ethambutol). 2.78% answered 'unknown' about treatment (LLR $\chi^2_{(8)} = 8.671$, p = 0.371). Only 4.44% of immigrants reported absence of adverse effects of anti-TB treatment (such as fever, vomiting/diarrhea, hepatitis, orange urine discoloration) of their TB patients (LLR $\chi^2_{(8)} = 13.799$, p = 0.111) (Table 3).

4.8.6.4 The stigma of tuberculosis

The distribution of TB stigma and risk factors of immigrant's exposure to tuberculosis patients in the mother countries is given in Table 4.9.

Table 4.9: Distribution of tuberculosis exposure risk factor according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

Tuberculosis-associated Risk Factor: Tuberculosis Exposure		Negligible (n=90)		Low (n=36)		Average (n=0)		High (n=4)		Extremely High (n=46)		Can not be judged (n=4)		Total (n=180)		Likelihood ratio	p-value
		n	%	n	%	n	%	n	%	n	%	n	%	n	%		
Previous history of contact with TB diagnosed patient	Yes	30	33.33	15	42	0	0	2	50.00	24	52.17	2	50.00	73	40.56	LLR $\chi^2_{(8)}$= 7.877	0.446
	No	48	53.33	17	47	0	0	2	50.00	20	43.48	2	50.00	89	49.44		
	Unknown	12	13.33	4	11	0	0	0	0.00	2	4.35	0	0.00	18	10.00		
In-door close contact	Yes	4	4.44	1	3	0	0	0	0.00	9	19.57	0	0.00	14	7.78	LLR $\chi^2_{(4)}$= 11.047	0.026
	No	86	95.56	35	97	0	0	4	100.00	37	80.43	4	100.00	166	92.22		
Out-door close contact	Yes	19	21.11	9	25	0	0	1	25.00	16	34.78	1	25.00	46	25.56	LLR $\chi^2_{(4)}$= 2.906	0.574
	No	71	78.89	27	75	0	0	3	75.00	30	65.22	3	75.00	134	74.44		
Average number of daily contact to inside same household diagnosed TB	1-2	52	57.78	21	58	0	0	4	100.00	25	54.35	2	50.00	104	57.78	LLR$\chi^2_{(12)}$= 10.869	0.540
	3-5	33	36.67	11	31	0	0	0	0.00	20	43.48	2	50.00	66	36.67		
	6-10	5	5.56	3	8	0	0	0	0.00	1	2.17	0	0.00	9	5.00		
	>10	0	0.00	1	3	0	0	0	0.00	0	0.00	0	0.00	1	0.56		
Average number of daily contact to inside HH diagnosed TB	Median (IQR)	1.00 (1)		1.00 (1)		-- (--)		-- (--)		1.00 (1)		1.50 (1)		1.00 (1)		KW $\chi^2_{(4)}$= 2.958	0.565

Duration of contact to inside same households (hours))	**1-5**	1	1.11	1	3	0	0	0	0.00	4	8.70	0	0.00	6	3.33	LLR $\chi^2_{(8)}=$ 11.361	0.182
	6-10	30	33.33	8	22	0	0	1	25.00	17	36.96	0	0.00	56	31.11		
	>10	59	65.56	27	75	0	0	3	75.00	25	54.35	4	100.00	118	65.56		
Average number of daily contact to outside non-households	**1-2**	14	15.56	4	11	0	0	1	25.00	4	8.70	1	25.00	24	13.33	LLR $\chi^2_{(16)}=$ 22.613	0.124
	3-5	23	25.56	7	19	0	0	3	75.00	16	34.78	1	25.00	50	27.78		
	6-10	38	42.22	12	33	0	0	0	0.00	14	30.43	0	0.00	64	35.56		
	>10	15	16.67	13	36.1	0	0	0	0.00	12	26.09	2	50.00	42	22.78		
Average number of daily contact with NHH diagnosed TB	**Median (IQR)**	1.00 (1)		1.00 (1)		-- (--)		1.50 (1)		1.00 (1)		1.50 (1)		1.00 (1)		KW $\chi^2_{(4)}=$ 4.150	0.386
Duration of contact to outside non-households (hours)	**<1**	2	2.22	2	6	0	0	1	25.00	4	8.70	2	50.00	11	6.11	LLR $\chi^2_{(12)}=$ 20.285	0.062
	1-5	4	4.44	2	6	0	0	0	0.00	2	4.35	1	25.00	9	5.00		
	6-10	49	54.44	21	58	0	0	2	50.00	17	36.96	1	25.00	90	50.00		
	>10	35	38.89	11	31	0	0	1	25.00	23	50.00	0	0.00	70	38.89		

Average LTBI category is zero (means no detected cases), (-) means cannot be computed by SPSS Software programme

All immigrants were re-questioned about history of previous contacts with TB diagnosed patients. The results revealed statistical insignificant difference for association between LTBI stigma and those immigrants answered 'yes' in 40.56% (73/180) compared to those immigrants who answered as 'no' 49.44% (89/180) or those answered as 'unknown' in 10% (18/180) (LLR $\chi^2_{(8)}$ = 7.877, p = 0.446). 50% of immigrants belonging to the 'high' and 'extremely high' LTBI groups were daily exposed to infected subjects compared to less percentage of the rest groups (Figure 4.24, Table 4.9).
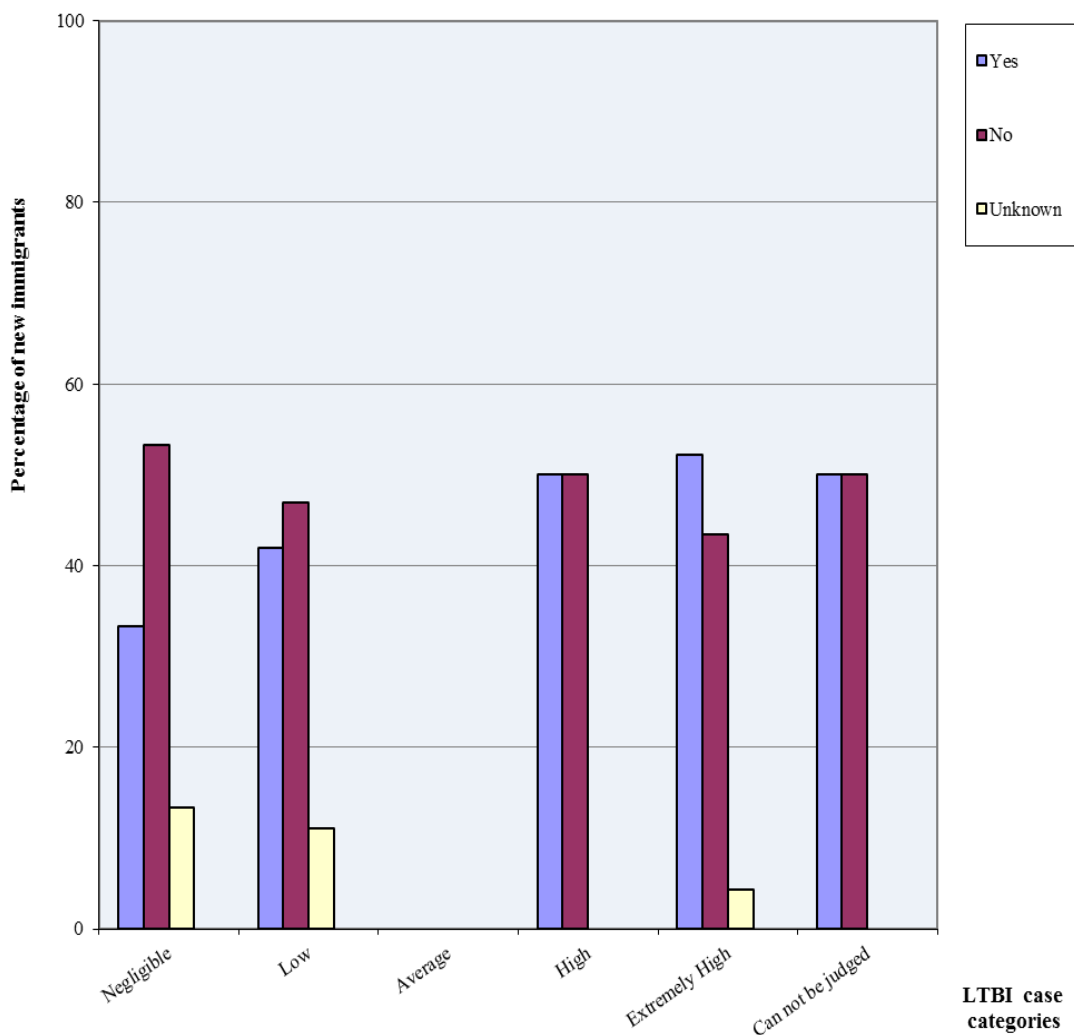
Figure 4.24: Percent distribution of 'previous history' contacts with diagnosed tuberculosis patients in the mother country according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

Indoor close contacts to diagnosed TB patients were significantly associated with LTBI risk development in 7.78% (14/180) of participants (LLR $\chi^2_{(4)}$ = 11.047, p = 0.026). Those participants were belonging to the 'extremely high' LTBI group 19.57% (9/46) than those of the 'negligible' LTBI group 4.44% (4/90) (Figure 4.25, Table 4.9).
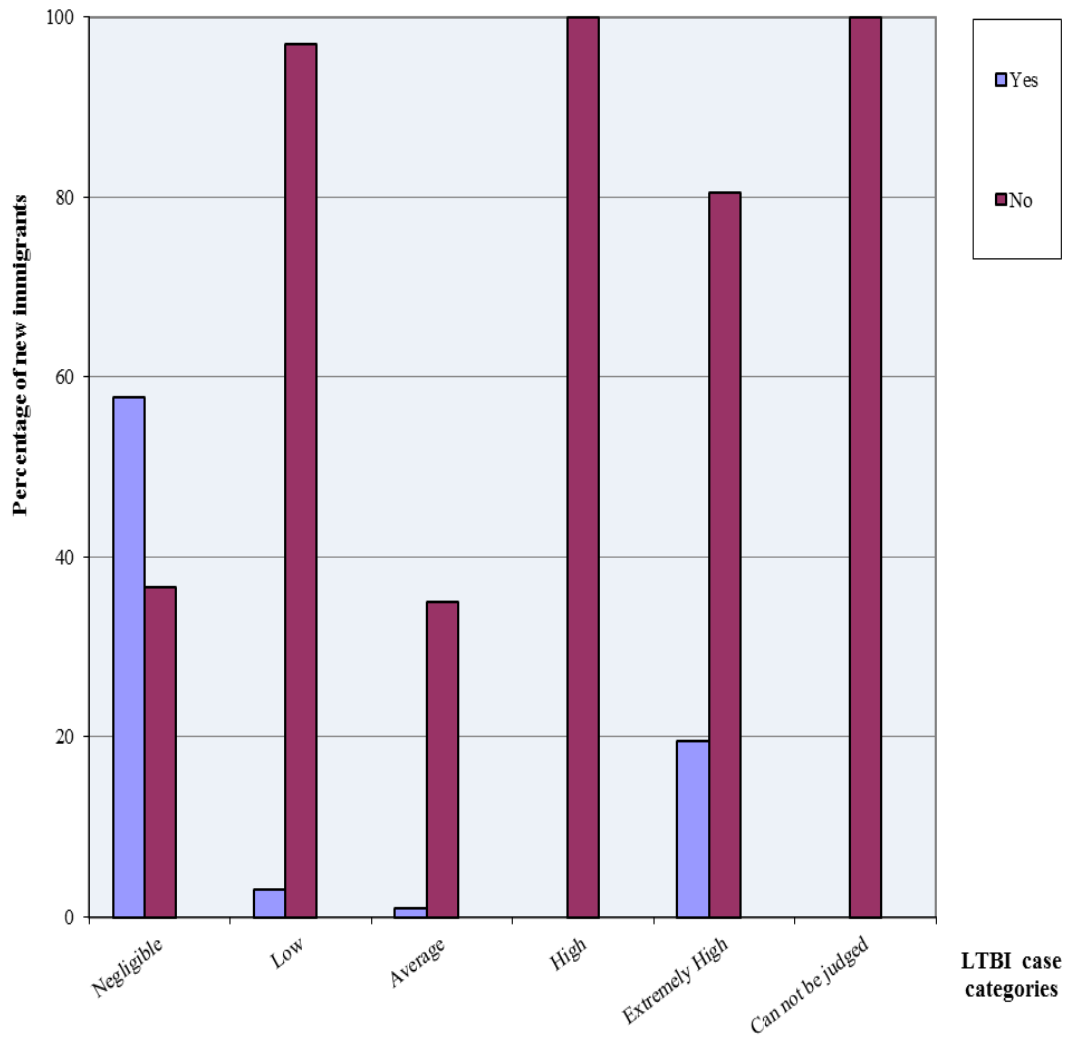
Figure 4.25: Percent distribution of indoor close contacts to diagnosed household tuberculosis patients in the mother country according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

On the other hand outdoor close contacts to diagnosed TB patients were not significantly associated with LTBI suspicion in 25.56% (46/180) of participants, the majority belonging to the 'extremely high' LTBI group in 34.78% and 25% of participants in each of the 'low' and 'high' LTBI groups (LLR $\chi^2_{(4)}$ = 2.906, p = 0.574) (Figure 4.26, Table 4.9).
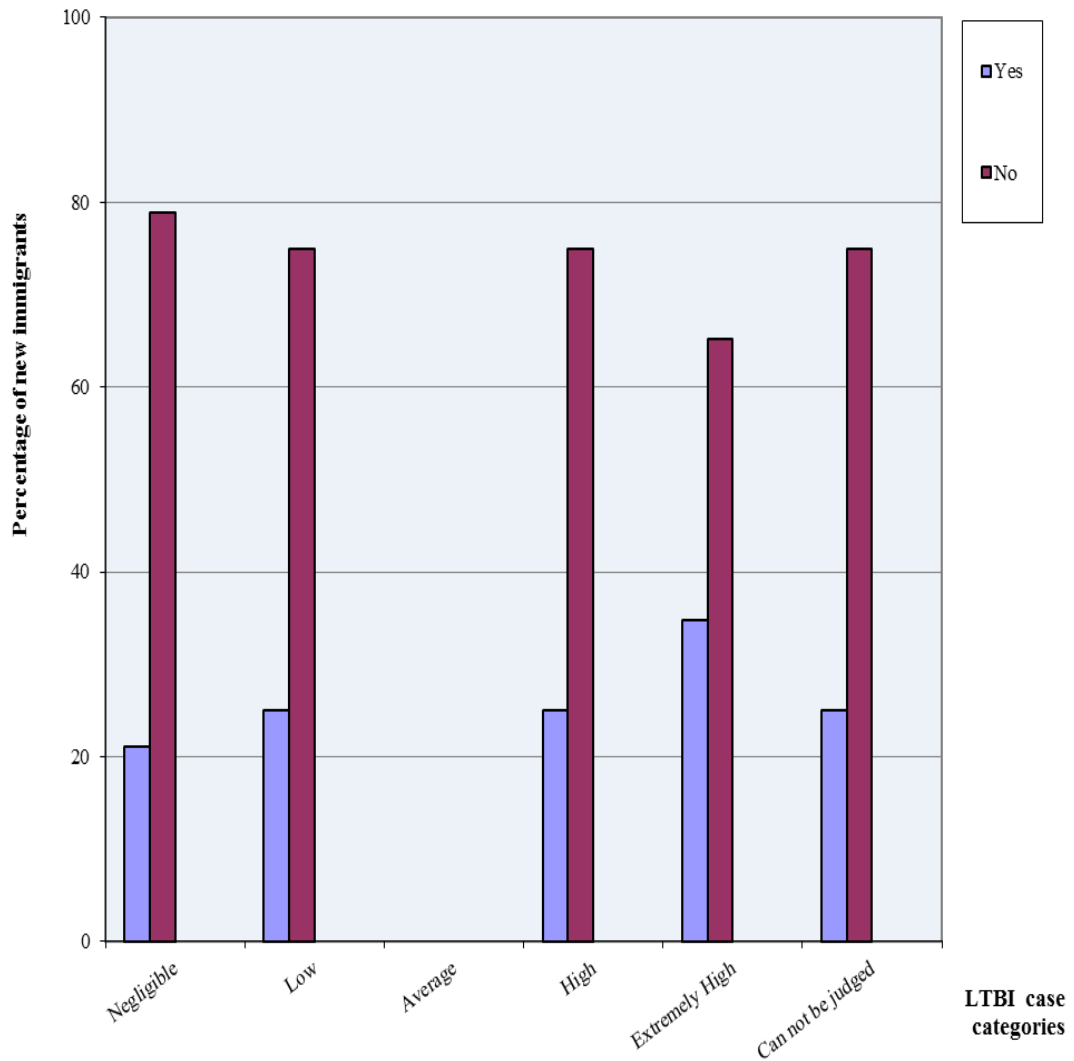
Figure 4.26: Percent distribution of previous history of outdoor contacts with tuberclosis diagnosed patients in the mother country according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

The average number of daily contacts to household (inside) diagnosed patient in the mother country was not significantly associated with development of LTBI. Contact with one TB patient to inside the living sites was answered by 36.67% (66/180) of immigrants, and the majority was belonging to the 'extremely high' LTBI in 43.48% and 'negligible' LTBI in 36.67%. Comparatively, 5% had contact with two TB patients and only 0.56% of immigrants had contact with three TB patients (LLR $\chi^2_{(12)}$ = 10.869, p = 0.540) (Figure 4.27, Table 4.9).
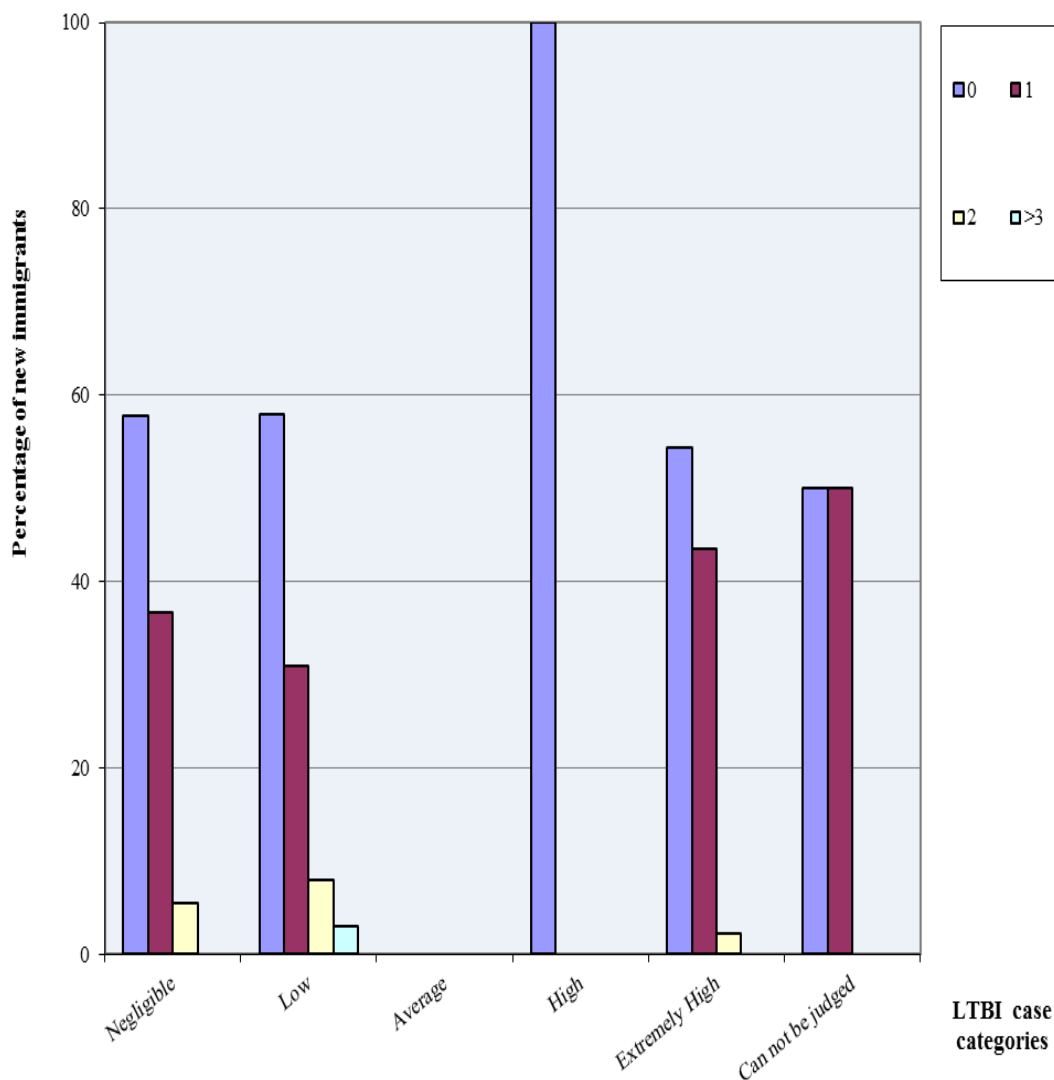
Figure 4.27: Percent distribution of the average number of daily contacts with diagnosed tuberculosis patients inside household individuals in the mother country according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

The overall median number of daily contacts to inside household diagnosed TB patients in the mother country was one subject (IQR = one). The lowest number of contacts with TB diagnosed patients was one, whereas the maximum was four (KW $\chi^2_{(4)}$ = 2.958, p = 0.565) (Table 4.9).

The average period of contact with those having TB patients inside households was not significantly associated with LTBI categories. Including immigrants without history of exposure to TB patients and participants who have daily contact of less than one hour were both composed 90% (162/180) of sample size, and were represented equally for all LTBI group participants (LLR $\chi^2_{(12)}$ = 14.630, p = 0.262). Immigrants had average duration of exposure of one hour duration to an inside TB contact (IQR = 1, SIQR = 0.5) (KW $\chi^2_{(4)}$ = 2.958, p = 0.565) (Figure 4.28, Table 4.9).
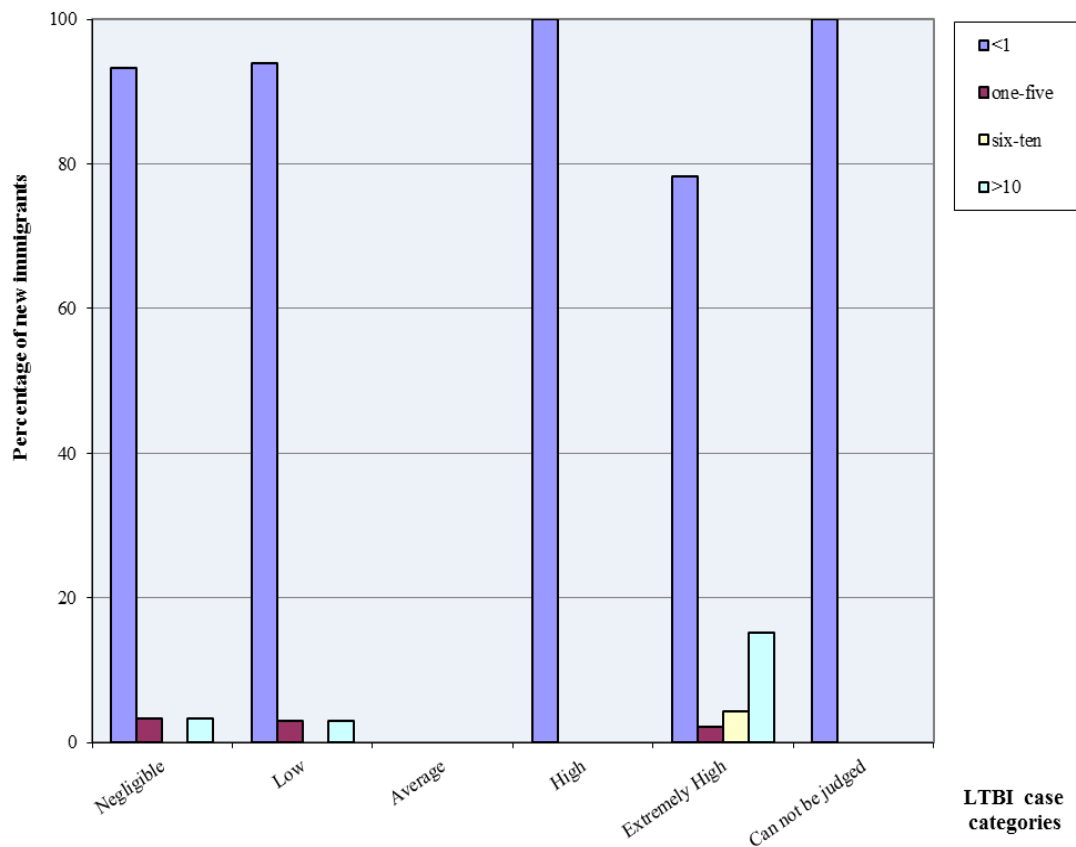
Figure 4.28: Percent distribution of average duration of daily contacts with diagnosed tuberculosis patients to inside household individuals in the mother country according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February-May, 2010

Average number of daily contacts to diagnosed patient outside households in the mother country was not significantly associated with development of LTBI and TB suspicion. 29.44% (53/180) of participants had external contact to one non household TB patient, compared to 6.11% of immigrants had contact with more than one TB patients. 'Extremely high' LTBI group was the predominant group had external contacts to various TB patients (LLR $\chi^2_{(12)}$ = 7.808, p = 0.800) (Figure 4.29, Table 4.9).
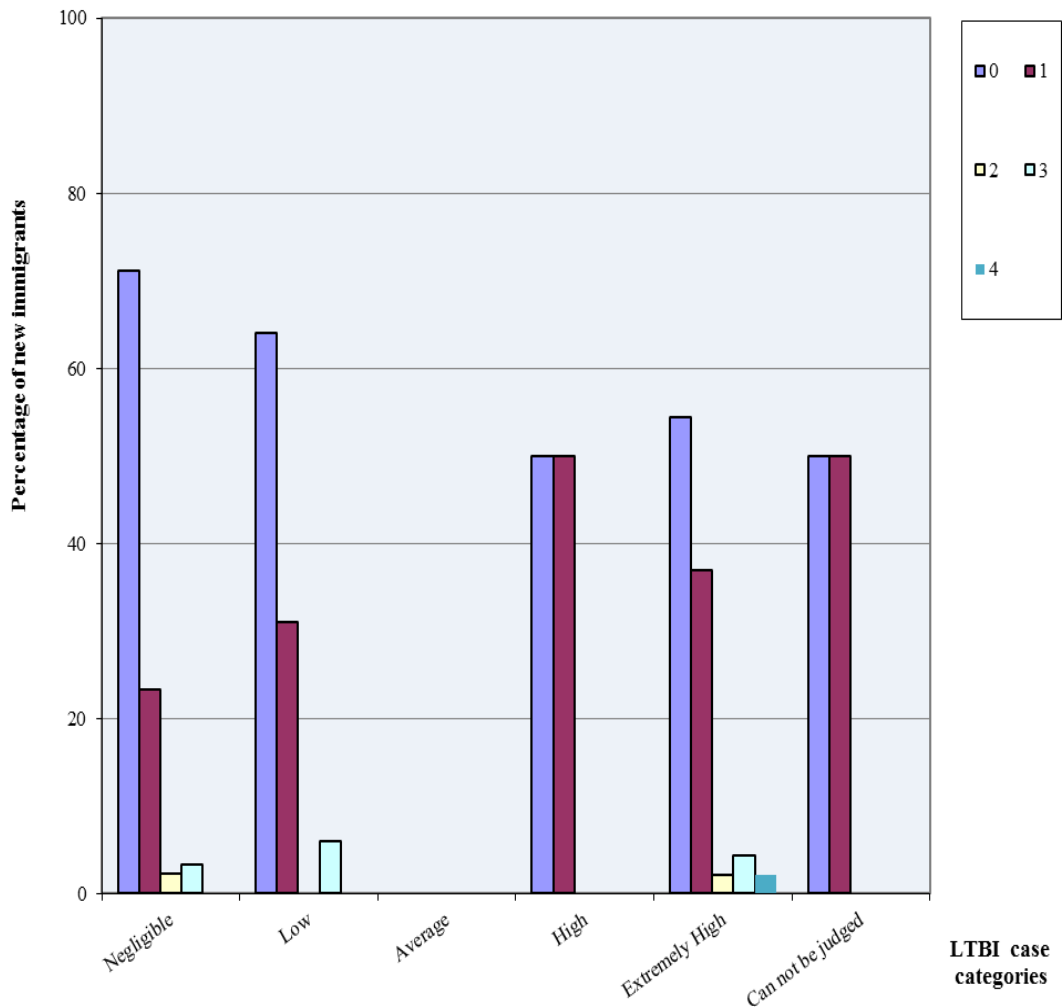
Figure 4.29: Percent distribution of the average number of daily contacts with diagnosed tuberculosis patients to outisde household individuals in the mother country according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

Including immigrants with no history of external contacts and those had daily external contacts with non-household TB patients of less than one hour were detected in 96.11% (173/180), compared to only 3.33% who spent daily between one and five hours with predominance of the 'extremely high' LTBI group (LLR $\chi^2_{(8)}$ = 7.285, p = 0.506) (Figure 4.29, Table 4.9). The overall median number of daily external contacts to non-household diagnosed TB patient in the mother country was one TB case (IQR = one contact). Lowest number of contacts was one, and highest number was four (KW $\chi^2_{(4)}$ = 4.150, p = 0.386) (Table 4.9).

4.8.6.5 Risk factors for progression of infection to active tuberculosis

Multiple risk factors associated with progression of *M. tuberculosis* toward the active form of tuberculosis disease can be sub-divided into the categories detailed below.

4.8.6.5.1 History of infectious and non-infectious (chronic) disorders

The past history of catching infectious and/or chronic non-infectious disorders according to latent tuberculosis infection defined categories is described in Table 4.10.

Table 4.10: History of infectious and chronic non-infectious disorders according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

Past history of Infectious and Non-infectious Diseases (chronic) disorders		Negligible (n=90)		Low (n=36)		Average (n=0)		High (n=4)		Extremely High (n=46)		Can not be judged (n=4)		Total (n=180)		Likelihood ratio	p-value
		n	%	n	%	n	%	n	%	n	%	n	%	n	%		
Skin allergy	Yes	5	5.56	4	11.11	0	0	1	25.00	1	2.17	0	0.00	11	6.11	LLR $\chi^2_{(4)}$=4.934	0.294
Malnutrition	Yes	0	0.00	1	2.78	0	0	0	0.00	1	2.17	0	0.00	2	1.11	LLR $\chi^2_{(4)}$=3.203	0.525
Malaria	Yes	3	3.33	2	5.56	0	0	0	0.00	4	8.70	0	0.00	9	5.00	LLR $\chi^2_{(8)}$=12.777	0.120
	Unknown	9	10.00	1	2.78	0	0	0	0.00	0	0.00	1	25.00	11	6.11		
Asthma	Yes	2	2.22	4	11.11	0	0	0	0.00	0	0.00	0	0.00	6	3.33	LLR $\chi^2_{(8)}$=12.394	0.134
	Unknown	2	2.22	0	0.00	0	0	0	0.00	3	6.52	0	0.00	5	2.78		
HIV/AIDS	No	90	100.00	36	100.00	0	0	4	100.00	46	100.00	4	100.00	180	100.00	-	--
Hepatitis	Yes	1	1.11	1	2.78	0	0	0	0.00	2	4.35	0	0.00	4	2.22	LLR $\chi^2_{(8)}$=6.954	0.542
	Unknown	4	4.44	4	11.11	0	0	0	0.00	6	13.04	0	0.00	14	7.78		
Silicosis	Yes	0	0.00	1	2.78	0	0	0	0.00	0	0.00	0	0.00	1	0.56	LLR $\chi^2_{(8)}$=12.433	0.133
	Unknown	82	91.11	26	72.22	0	0	2	50.00	41	89.13	3	75.00	154	85.56		
Alcoholism	Yes	26	28.89	12	33.33	0	0	2	50.00	9	19.57	0	0.00	49	27.22	LLR $\chi^2_{(4)}$=5.705	0.222
Diabetus mellitus	Yes	3	3.33	0	0.00	0	0	0	0.00	1	2.17	0	0.00	4	2.22	LLR $\chi^2_{(4)}$=2.422	0.659

Continued Table 4.10

Variable	Category	n	%	n	%	n	n	n	%	n	%	n	%	n	%	LLR χ²	p
Cardiovascular disease	Yes	3	3.33	0	0.00	0	0	0	0.00	4	8.70	0	0.00	7	3.89	LLR $\chi^2_{(8)}$=8.546	0.382
	Unknown	0	0.00	0	0.00	0	0	0	0.00	1	2.17	0	0.00	1	0.56		
Blood disorder	Yes	0	0.00	1	2.78	0	0	0	0.00	1	2.17	0	0.00	2	1.11	LLR $\chi^2_{(8)}$=6.479	0.594
	Unknown	0	0.00	1	2.78	0	0	0	0.00	0	0.00	0	0.00	1	0.56		
Other disease	Yes	6	6.67	4	11.11	0	0	0	0.00	2	4.35	0	0.00	12	6.67	LLR $\chi^2_{(4)}$=2.518	0.641
Past history of surgical procedure	Yes	20	22.22	7	19.44	0	0	0	0.00	6	13.04	1	25.00	34	18.89	LLR $\chi^2_{(4)}$=3.522	0.475
Past history of pharmaceutical/medical drugs	Yes	9	10.00	3	8.33	0	0	0	0.00	9	19.57	0	0.00	21	11.67	LLR $\chi^2_{(4)}$=5.039	0.283

Average category = means zero (no detected cases)

Most of the immigrants in the study described different past histories of disease exposure but with no statistical significant differences between past history of contacting previous chronic disorders (infections/non-infectious) and LTBI categories. The majority of complained of skin allergies 6.11% (LLR $\chi^2_{(4)}$ = 4.934, p = 0.294), malaria 5% (LLR $\chi^2_{(8)}$ = 12.777, p = 0.120), cardiovascular disorders 3.89% (LLR $\chi^2_{(8)}$ = 8.546, p = 0.382), asthma 3.33% (LLR $\chi^2_{(8)}$ = 12.394, p = 0.134), DM 2.22% (LLR $\chi^2_{(4)}$ = 2.422, p = 0.659) and 2.22% admitted catching hepatitis during childhood (LLR $\chi^2_{(8)}$ = 6.954, p = 0.542). All immigrants denied any past history of a diagnosis of HIV/AIDS.

72.22% of immigrants (predominantly male) admitted to drinking alcohol in their TB endemic and mother countries; Philippinos 48.57% (17/35), Nepali's 38.26% (11/28) and Indians 14.28% (9/63). Those participants were distributed without significant difference as 'negligible' LTBI 28.89% (26/90), 'low' LTBI group 33.33% (12/36) and 'extremely high' LTBI 19.57% (9/46) (LLR $\chi^2_{(4)}$ = 5.705, p = 0.222) (Table 4.10).

Up to 11.66% (21/180) of immigrants had a current history of taking pharmaceutical agents, and 6.66% (12/180) had a history of cardiovascular disease and diabetes mellitus constituted. Participants having past history of surgical procedure were 18.89% (34/180) (LLR $\chi^2_{(4)}$ = 3.522, p = 0.475). Participants with a current history of taking regular pharmaceutical therapy (mainly skin anti-allergic steroids) were 11.67% (21/180) (LLR $\chi^2_{(4)}$ = 5.039, p = 0.283) (Table 4.10).

4.8.7 Body mass index (weight-for-height measure)

Body Mass Index (BMI) is a nutritional indicator and measure of health and body composition. BMI is calculated by dividing a person's weight in kilograms (kg) by the height in metres squared (m^2). The normal BMI lies between 18.50 and 24.99, representing a healthy range. A BMI lower than 18.50 is considered underweight, compared with overweight subjects (BMI 25.00-30.00) and obese individuals (BMI above 30.00).

A strong association between extremes of relative body weight and the TB burden level has been well characterized all over the world. Common inverse relationship is detected between BMI and TB incidence, and within the BMI normal and overweight ranges 18.50 and 30.00 kg/m^2, respectively (Lonnroth *et al.*, 2010). The distribution of BMI according to the latent tuberculosis infection classification of the 180 foreigner participants is detailed in Table 4.11.

Table 4.11: Distribution of body mass index (BMI) according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

Body mass index		Negligible (n=90)		Low (n=36)		Average (n=0)		High (n=4)		Extremely High (n=46)		Can not be judged (n=4)		Total (n=180)		Likelihood ratio	p-value
		n	%	n	%	n	%	n	%	n	%	n	%	n	%		
Body mass index score	<18.50 (Underweight)	7	7.78	2	5.56	0	0	0	0.00	4	8.70	0	0.00	13	7.22	LLR $\chi_{2(12)}=$ 8.722	0.726)
	18.50-24.99 (Normal weight)	50	55.56	21	58.33	0	0	1	25.00	28	60.87	4	100.00	104	57.78		
	25.00-30.00 (Overweight)	27	30.00	11	30.56	0	0	2	50.00	12	26.09	0	0.00	52	28.89		
	>30.00 (Obesity)	6	6.67	2	5.56	0	0	1	25.00	2	4.35	0	0.00	11	6.11		
BMI	Median (IQR)	24.12 (6)		24.06 (5.23)		-	-	27.09 (6.16)		23.04 (4.71)		22.51 (5.78)		24.017 (5.48)		KW $\chi^2_{(4)}=$ 3.912	0.418

Average LTBI category is zero (means no detected cases), (-) means cannot be computed by SPSS Software programme

The majority of new immigrants 57.78% (104/180) were lying within the normal weight-for-height measures and BMI ranges (18.50-24.99 kg/m^2) in comparison to 28.89% (52/180) been overweight immigrants (25.00-30.00 kg/m^2) and 6.11% were obese (more than 30.00 kg/m^2). Only 7.22% (13/180) of participants were underweight and the majority belongs to the 'extremely high' LTBI group 8.70% (4/46) and 'negligible' LTBI 7.78% (7/90) (LLR $\chi^2_{(12)}$ = 8.722, p = 0.726) (Figure 4.30, Table 4.11).
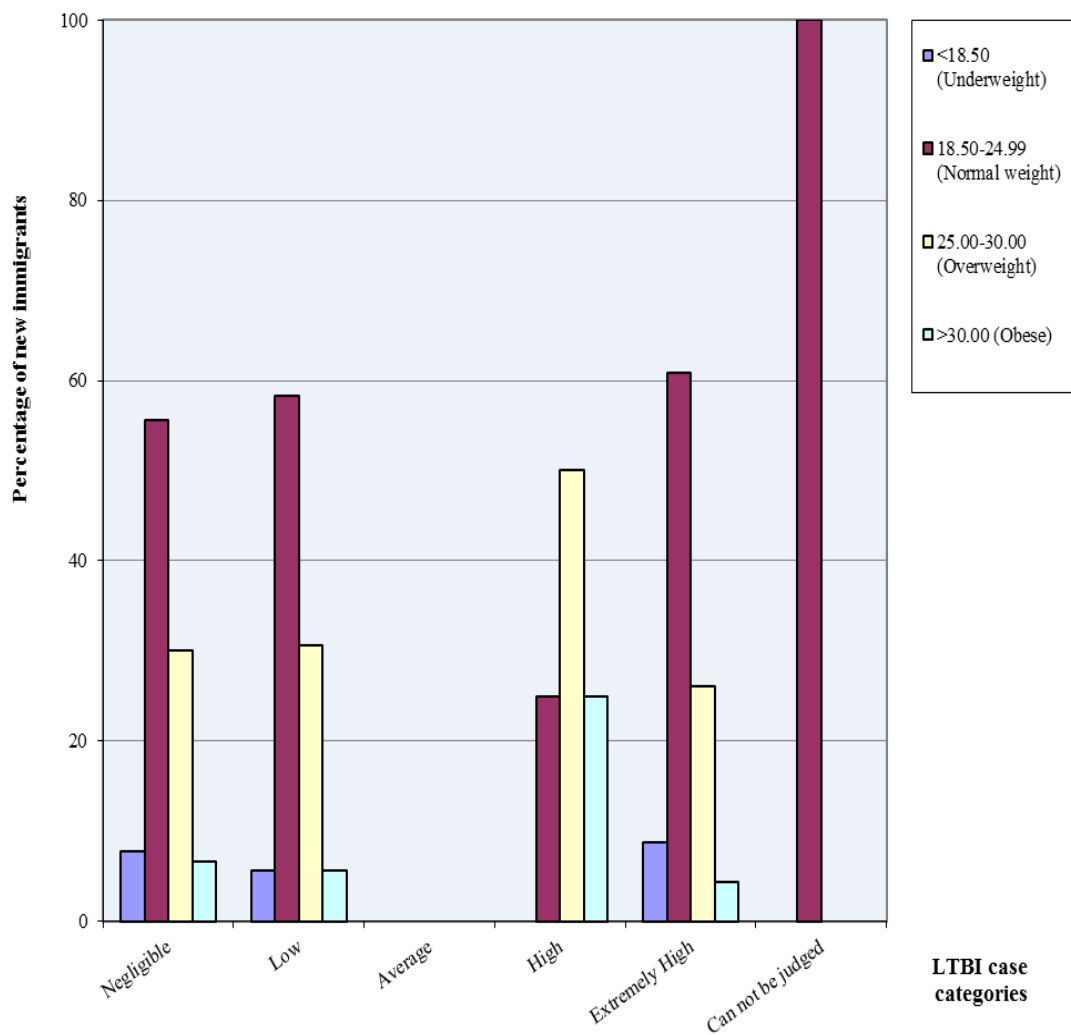
Figure 4.30: Percent distribution of body mass index on the latent tuberculosis infection case categories of 180 new immigrants to Kuwait during February and May, 2010

The overall median BMI of all immigrants was normal and equals 24.017 kg/m^2 (IQR = 5.48, SIQR = 2.74) but with no statistical significance difference toward LTBI development (KW $\chi^2_{(4)}$ = 3.912, p = 0.418), except for associated high BMI for the 'high' LTBI group as 27.09 (IQR = 6.16). The minimum BMI was 14.70 kg/m^2 versus the highest BMI value was 36.83 kg/m^2 (Figure 4.30, Figure 4.31, Table 4.10).
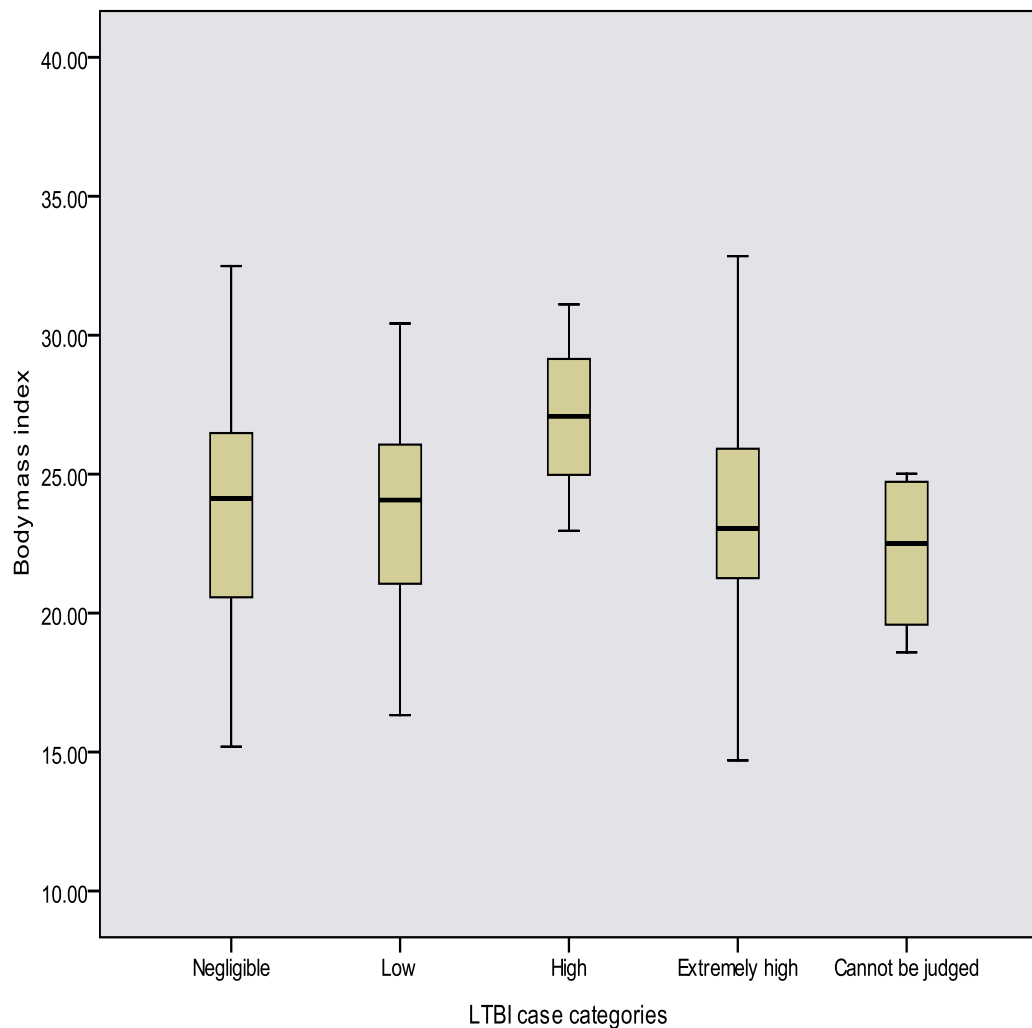
Figure 4.31: Box-and-whiskers plots of the distribution of body mass index (BMI) according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

4.8.8 History of smoking and smoking behavioral habits

Assessment of smoking as a risk factor, according to latent tuberculosis infection is described in Table 4.12.

Table 4.12: Distribution of smoking according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

History of Smoking & Smoking Behaviors		Negligible (n=90)		Low (n=36)		Average (n=0)		High (n=4)		Extremely High (n=46)		Can not be judged (n=4)		Total (n=180)		Likelihood ratio	p-value
		n	%	n	%	n	%	n	%	n	%	n	%	n	%		
Smoking history	Current smoker	40	44.44	9	25.00	0	0	1	25.00	14	30.43	0	0.00	64	35.56	LLR $\chi^2_{(8)}$ =13.303	0.102
	Ex-smoker	12	13.33	7	19.44	0	0	0	0.00	9	19.57	0	0.00	28	15.56		
	Never smoke	38	42.22	20	55.56	0	0	3	75.00	23	50.00	4	100.00	88	48.89		
Positive history of smoking	Smoke or not during life period	52	57.78	16	44.44	0	0	1	25.00	23	50.00	0	0.00	92	51.11	LLR $\chi^2_{(4)}$ =9.135	0.058
	Never smoke	38	42.22	20	55.56	0	0	3	75.00	23	50.00	4	100.00	88	48.89		
Starting age of smoking (years-range)	Never smoke	38	42.22	20	55.56	0	0	3	75.00	23	50.00	4	100.00	88	48.89	LLR $\chi^2_{(24)}$ =29.286	0.210
	<20	15	16.67	5	13.89	0	0	0	0.00	8	17.39	0	0.00	28	15.56		
	20-	22	24.44	6	16.67	0	0	0	0.00	2	4.35	0	0.00	30	16.67		
	25-	9	10.00	3	8.33	0	0	1	25.00	5	10.87	0	0.00	18	10.00		
	30-	3	3.33	1	2.78	0	0	0	0.00	5	10.87	0	0.00	9	5.00		
	35-	1	1.11	1	2.78	0	0	0	0.00	3	6.52	0	0.00	5	2.78		
	>40	2	2.22	0	0.00	0	0	0	0.00	0	0.00	0	0.00	2	1.11		

Continued Table 4.12

Variable	Category	n	%	n	%	n	%	n	%	n	%	n	%	Total n	Total %	Statistic	p
Starting age of smoking (years)	Median	21.50		22.00		-	(--)	-		25.00		-		22		$KW\ \chi^2_{(3)}=2.313$	0.510
	(IQR)	(8)		(7)			(--)		(--)	(13)		(--)		(10)			
Age of stop smoking (years-range)	Never smoke or still current smoker	78	86.67	29	80.56	0	0	4	100.00	37	80.43	4	100.00	152	84.44	$LLR\ \chi^2_{(24)}=13.875$	0.949
	<20	3	3.33	0	0.00	0	0	0	0.00	0	0.00	0	0.00	3	1.67		
	20-	3	3.33	1	2.78	0	0	0	0.00	1	2.17	0	0.00	5	2.78		
	25-	3	3.33	4	11.11	0	0	0	0.00	5	10.87	0	0.00	12	6.67		
	30-	2	2.22	1	2.78	0	0	0	0.00	1	2.17	0	0.00	4	2.22		
	35-	1	1.11	1	2.78	0	0	0	0.00	1	2.17	0	0.00	3	1.67		
	>40	0	0.00	0	0.00	0	0	0	0.00	1	2.17	0	0.00	1	0.56		
Age of stop smoking (years)	Median	25.50		28.00		-	(--)	-		29.00		-		28		$KW\ \chi^2_{(2)}=2.363$	0.307
	(IQR)	(12)		(8)			(--)		(--)	(9)		(--)		(9)			
Duration of current smoking (year-range)	Never smoke	38	42.22	20	55.56	0	0	3	75.00	23	50.00	4	100.00	88	48.89	$LLR\ \chi^2_{(12)}=10.073$	0.610
	<1	4	4.44	3	8.33	0	0	0	0.00	1	2.17	0	0.00	8	4.44		
	1-2	9	10.00	1	2.78	0	0	0	0.00	2	4.35	0	0.00	12	6.67		
	3-5	15	16.67	3	8.33	0	0	0	0.00	4	8.70	0	0.00	22	12.22		
	6-10	8	8.89	5	13.89	0	0	0	0.00	6	13.04	0	0.00	19	10.56		
	>10	16	17.78	4	11.11	0	0	1	25.00	10	21.74	0	0.00	31	17.22		
Duration of current smoking (years)	Median	3.00		4.00		-	(--)	-		4.00		-		4		$KW\ \chi^2_{(3)}=4.227$	0.238
	(IQR)	(3)		(3)			(--)		(--)	(2)		(--)		(2)			

Continued Table 4.12

																Statistic	p-value
Duration of ex-smoking (years)	Median (IQR)	4.00 (8)		2.00 (8)		- (--)		- (--)		4.00 (18)		- (--)		3.5 (7)		KW $\chi^2_{(2)}$= 1.261	0.532
Smoking Habits (preferred)	Cigarette (manufactured)	40	44.44	14	38.89	0	0	1	25.00	18	39.13	0	0.00	73	40.56	LLR $\chi^2_{(4)}$= 5.228	0.265
	Tambacco	12	13.33	1	2.78	0	0	0	0.00	7	15.22	0	0.00	20	11.11	LLR $\chi^2_{(4)}$= 6.525	0.163
	Birri	1	1.11	0	0.00	0	0	0	0.00	0	0.00	0	0.00	1	0.56	LLR $\chi^2_{(4)}$= 1.392	0.846
	Shisha/Qedo	4	4.44	1	2.78	0	0	0	0.00	0	0.00	0	0.00	5	2.78	LLR $\chi^2_{(4)}$= 3.828	0.430
	Cigar	1	1.11	0	0.00	0	0	0	0.00	0	0.00	0	0.00	1	0.56	LLR $\chi^2_{(4)}$= 1.392	0.846
Frequency number of cigarette per day	Never smoke	49	54.44	22	61.11	0	0	3	75.00	28	60.87	4	100.00	106	58.89	LLR $\chi^2_{(16)}$= 19.446	0.246
	<5	15	16.67	4	11.11	0	0	0	0.00	7	15.22	0	0.00	26	14.44		
	5-10	14	15.56	7	19.44	0	0	0	0.00	5	10.87	0	0.00	26	14.44		
	>10-20	5	5.56	1	2.78	0	0	1	25.00	6	13.04	0	0.00	13	7.22		
	>20	7	7.78	2	5.56	0	0	0	0.00	0	0.00	0	0.00	9	5.00		
Frequency number of other preferred smoking (excluding cigarrette) per day	Never smoke	72	80.00	34	94.44	0	0	4	100.00	39	84.78	4	100.00	153	85.00	LLR $\chi^2_{(12)}$= 8.322	0.759
	<5	9	10.00	1	2.78	0	0	0	0.00	3	6.52	0	0.00	13	7.22		
	5-10	6	6.67	1	2.78	0	0	0	0.00	3	6.52	0	0.00	10	5.56		
	>10-20	3	3.33	0	0.00	0	0	0	0.00	1	2.17	0	0.00	4	2.22		

Smoking habits were significantly associated with those participants having positive history of smoking during life period in 51.11% (92/180) compared to those who never smoke in 48.89% (88/180). The relationship between smoking and LTBI categories was close to significance at 5% (LLR $\chi^2_{(4)}$ = 9.135, p = 0.058) (Figure 4.32, Table 4.12).
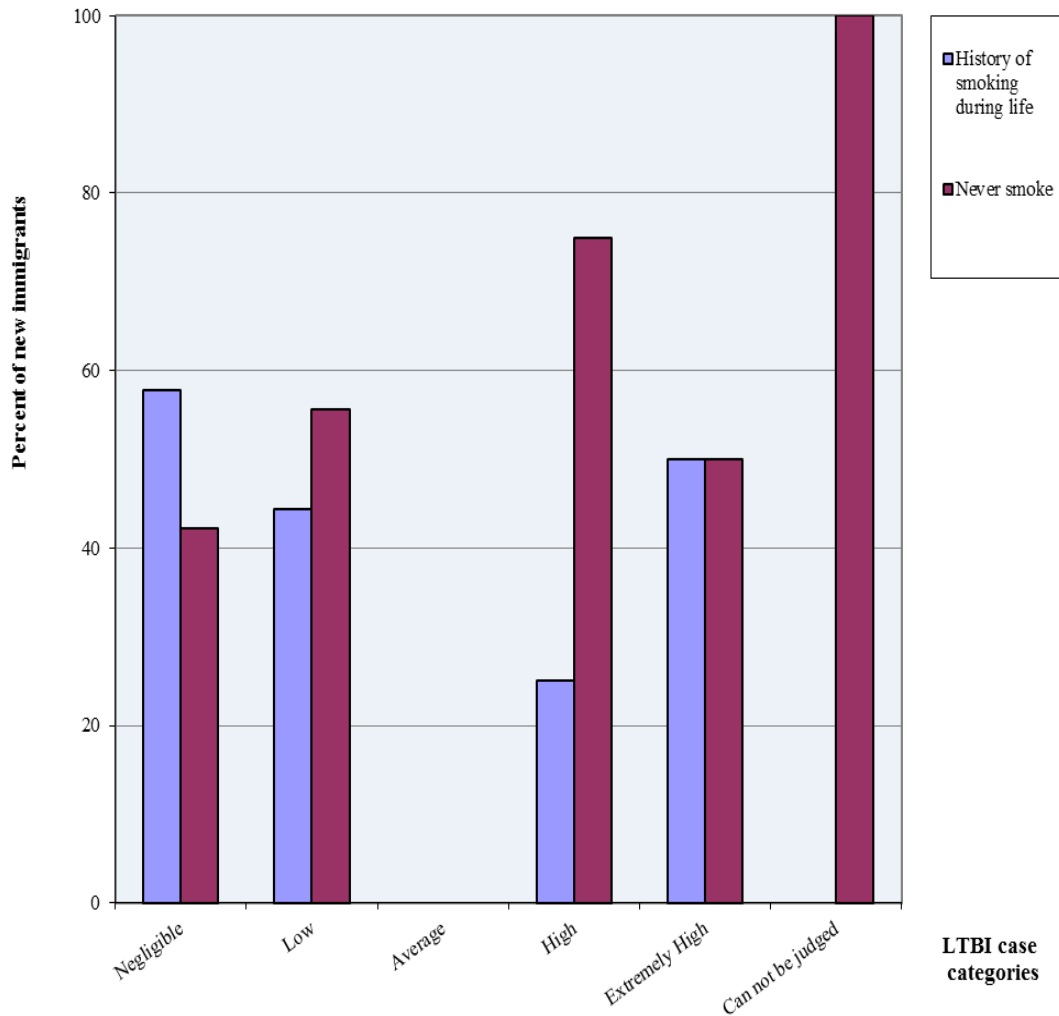
Figure 4.32: Percent distribution of past history of smoking during life according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

51.11% of participants had positive history of smoking, in which current smokers were 35.56% (64/180) and ex-smokers were 15.56% (28/180). The majority of current smokers belonged to the 'negligible' LTBI 44.44% and 'extremely high' LTBI group 30.43%. On the other hand, the majority of ex-smokers were belonged to 'extremely high' LTBI 19.57% and 'low' LTBI group 19.44% (LLR $\chi^2_{(8)}$ = 13.303, p = 0.102) (Figure 4.33, Table 4.12).
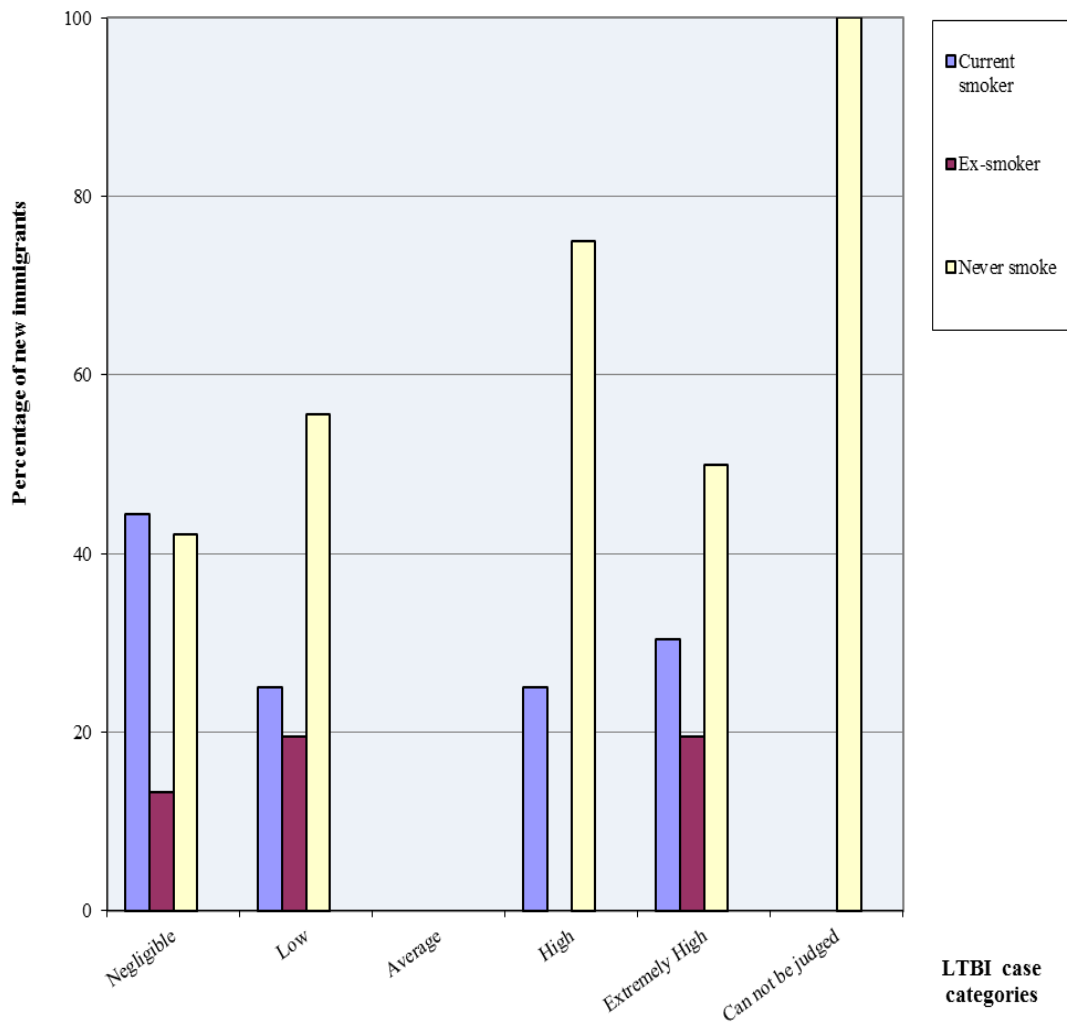
Figure 4.33: Percent distribution of smoking history according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

The starting age for smoking was not significantly associated with LTBI categorization, where the majority 32.2% (58/180) had started smoking below the age of 25 years. The majority of 'extremely high' LTBI started smoking at young ages below 20 years and older ages above 30 years (LLR $\chi^2_{(24)}$ = 29.286, p = 0.210) (Figure 4.34, Table 4.12).
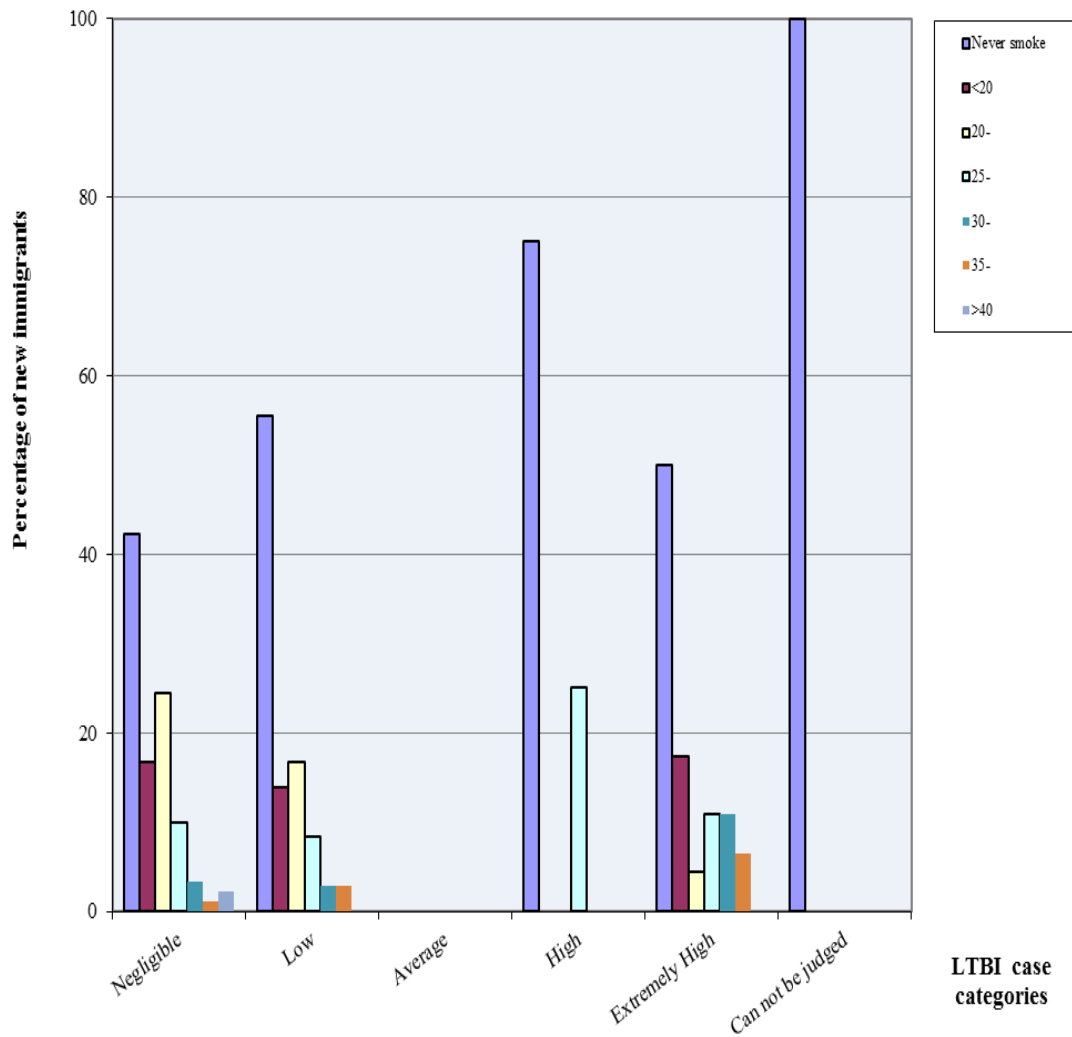
Figure 4.34: Distribution of starting age groups by smoking history according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

The overall median age of starting smoking was 22 years (IQR = 10, SIQR = 5). The median starting age for the 'negligible' LTBI group was 21.5 years (IQR = 8 years, SIQR = 4), and was 25 years for the 'extremely high' LTBI. The minimum age of

starting smoking was 8 years, versus the maximum of 40 years. However the differences for LTBI categorization were not statistically significant (KW $\chi^2_{(3)}$ = 2.313, p = 0.510) (Table 4.12).

The age at which smoking stopped was not significantly associated with LTBI categorizations. 6.67% of ex-smoker participants had stopped smoking between the ages between 25 and 30 years, and predominantly were from the 'low' and 'extremely high' LTBI groups (11%) (LLR $\chi^2_{(24)}$ = 13.875, p = 0.949) (Figure 4.35, Table 4.12).
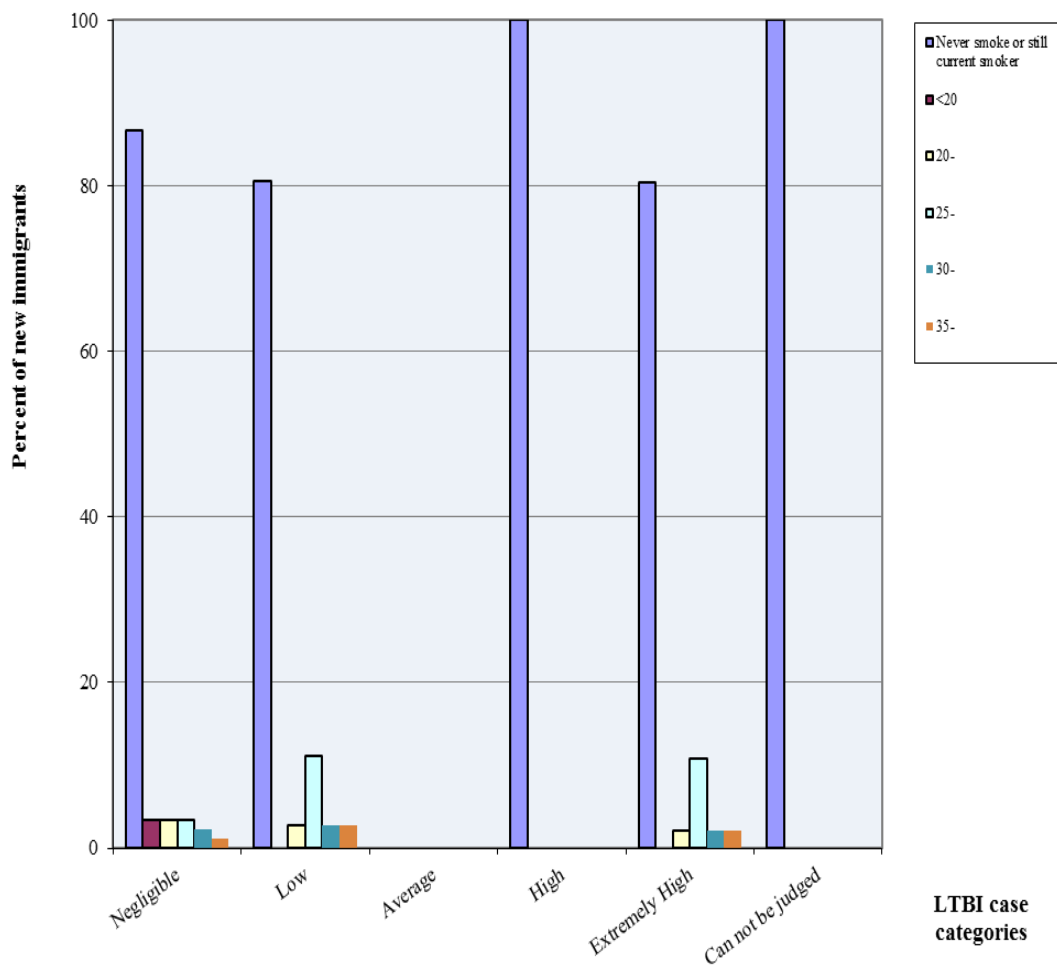
Figure 4.35: Percent distribution of stopping age groups by smoking history according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

The overall median age at which smoking stopped for all immigrants was 28 years (IQR = 9, SIQR = 4.5), and the stopping age for the 'extremely high' LTBI was 29 years (IQR = 9) and similarly for the 'low' LTBI group at 28 years (IQR = 8). The minimum age of stop smoking was 19 years and the maximum 43 years (KW $\chi^2_{(2)}$ = 2.363, p = 0.307) (Table 4.12).

Excluding non-smokers, current smokers were used to smoke for longer durations of more than 10 years in 17.22%, and were high (longer duration) for the 'extremely high' LTBI in 21.74% and the 'low' LTBI group in 17.78%. Immigrants smoked between 6-10 years were answered by 10.56%, which was detected in the 'extremely high' LTBI 13.04% and the 'low' LTBI group in 13.89% (LLR $\chi^2_{(12)}$ = 10.073, p = 0.610) (Figure 4.36, Table 4.12).
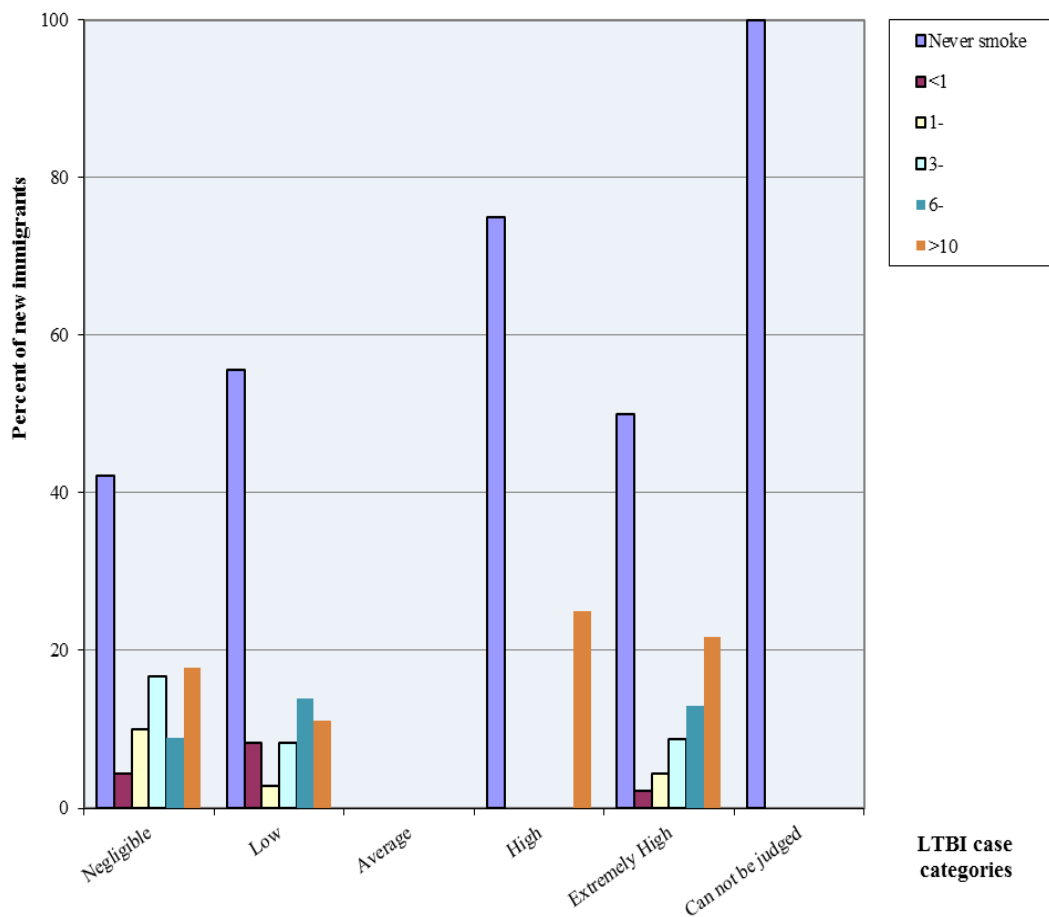
Figure 4.36: Percent distribution of duration of current smoking by smoking history according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

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The overall median duration of current smoking was 4 years (IQR = 2, SIQR = 1), and was the same for all LTBI categories. The minimum duration of smoking was one year versus the maximum duration of five years started at older ages (KW $\chi^2_{(3)}$ = 4.227, p = 0.238) (Table 4.12). The overall median duration of ex-smoking was 3.5 years (IQR = 7, SIQR = 3.5). The minimum duration of ex-smoking was less than one year versus the maximum duration of 29 years before stopping at older ages (KW $\chi^2_{(2)}$ = 1.261, p = 0.532) (Table 4.12).

Smokers showed the following preferences (Table 4.12):

'Manufactured' cigarettes in 40.56% (predominantly by the 'negligible' LTBI group in 44.44% and 'extremely high' LTBI in 39.13%), with no significant differences (LLR $\chi^2_{(4)}$ = 5.228, p = 0.265),

Chewing 'tambacco tobacco' in 11.11% and were preferred by 'extremely high' LTBI in 15.22% (LLR $\chi^2_{(4)}$ = 6.525, p = 0.163),

'Birri' or 'cigar' 0.56% each was represented by 'negligible' immigrant case (LLR $\chi^2_{(4)}$ = 1.392, p = 0.846), and smoking 'Shisha' (or 'Qedo') in 2.78% and majority were from the 'negligible' LTBI group in 4.44% (LLR $\chi^2_{(4)}$ = 3.828, p = 0.430).

The number of cigarettes smoked per day was similar for those who smoked up to 10 cigarettes per day (14.44% of participants). The 'negligible' LTBI participants prefer to smoke less than 5 cigarettes were 16.67% compared to 7.78% who preferred to smoke more than 20 cigarettes per day. The 'low' LTBI group prefers to smoke between 5-10 cigarettes 19.44%, whereas those 'extremely high' LTBI participants preferred smoking between 10 and 20 cigarettes per day were 13.04% and were higher than the rest LTBI groups. However, statistical significance between number of smoking cigarettes and LTBI development was absent (LLR $\chi^2_{(16)}$ = 19.446, p = 0.246) (Figure 4.37, Table 4.12).
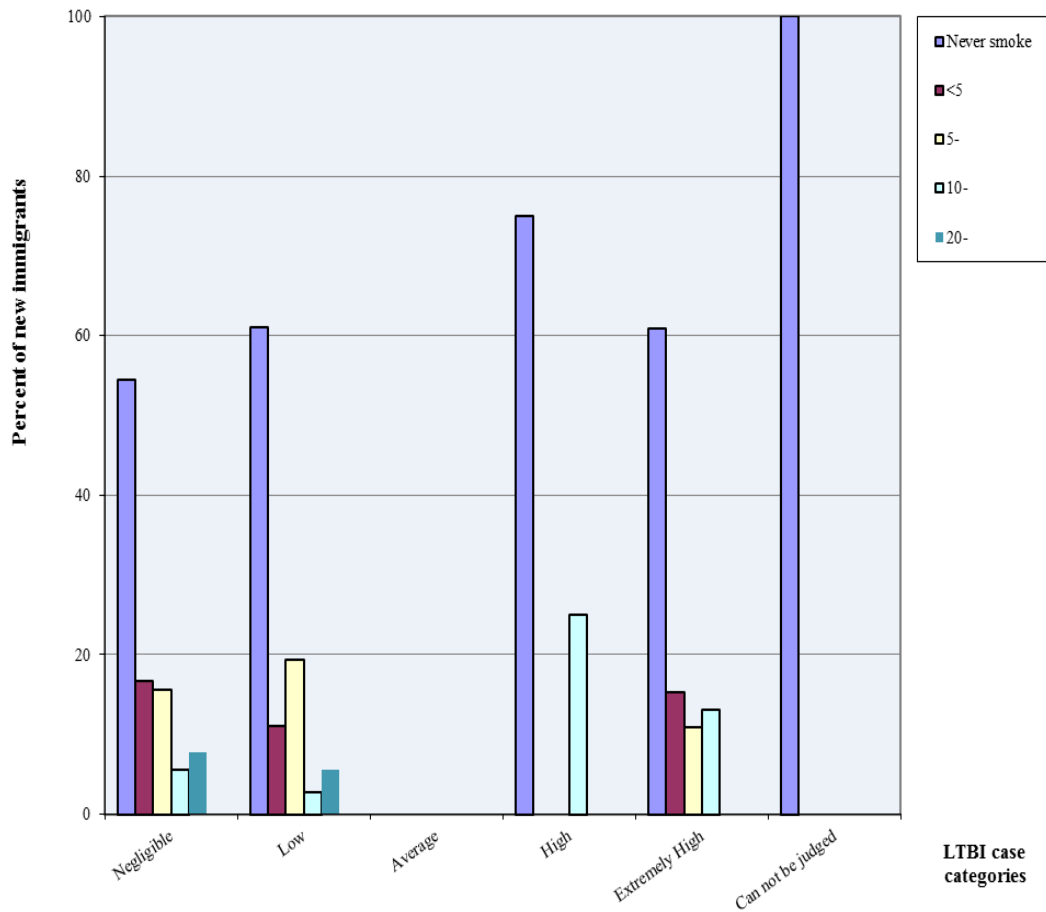
Figure 4.37: Percent distribution of the frequency number of smoking cigarettes per day according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

For those who smoked other than cigarettes, the frequency number of smoking habits was mainly less than 5 times per day. The 'negligible' LTBI group was 20% and higher among the other LTBI groups and the 'extremely high' LTBI participants were 15.21%. However, the frequency of smoking other than cigarettes has insignificant statistical difference with LTBI diagnosis (LLR $\chi^2_{(12)}$ = 8.322, p = 0.759) (Figure 4.38, Table 4.11). Cigarette smoking was preferred by 79.34% (73/92) of smokers versus chewing 'tambaco tobacco' and/or smoking 'birri' were preferred by Indians 21.73% (20/92), whereas, 'shisha' smoking was preferred by Egyptians 5.43% (5/92). A preference of other smoking habits such as 'pedis' (manual cigarettes), 'pipe', 'hukka', or other type of smoking habits were not preferred.
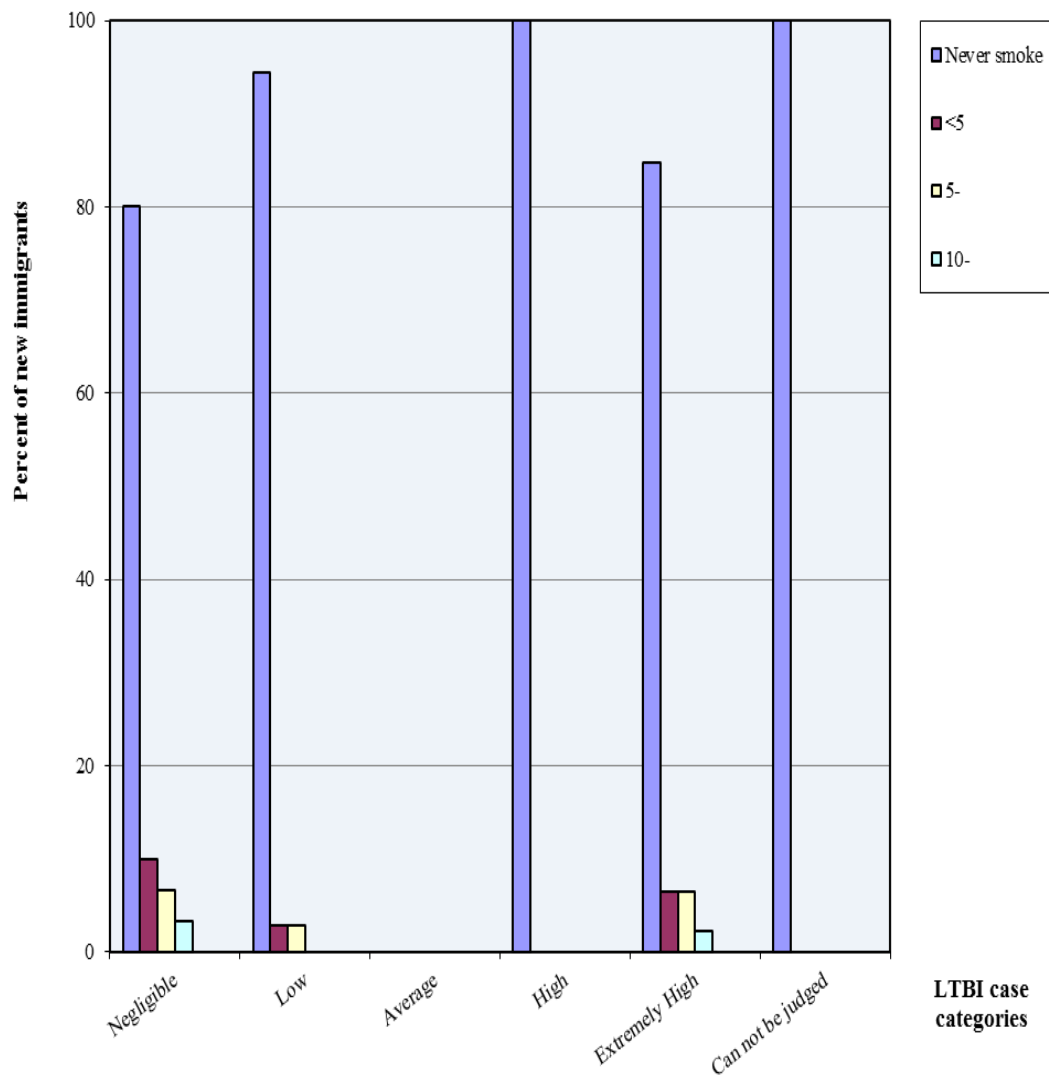
Figure 4.38: Percent distribution of the frequency number of smoking habits other than cigarettes per day according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

4.9 Discussion

4.9.1 Study screening questionnaire (structured interview)

Questionnaires are a research screening tool, pioneered by Sir Francis Galton (1882-1911), consisting of multiple questions designed to gather information for statistical analysis of respondents' responses and early detection of all health-related disorders (Mellenbergh, 2008). In 1968, WHO published guidelines for screening to detect and

assess TB chronic disease re-surgence due to latency re-activation, and to help in continuous case-finding using acceptable examination tests and ensuring confidentiality as important component of management strategies (WHO, 2007b).

Questionnaire should be universally arranged, then can be used as a rapid, cheap and objective (verbal) screening tool for data collection and preceded by participant's ethical agreement. A well designed, standardized, confidential, case sensitive should be carefully arranged with unbiased questionnaires to describe the epidemiological and demographic variables and related diagnostic test results. Final statistical analysis also facilitates public interaction between the community and health systems (Glaziou *et al.*, 2008; Long *et al.*, 2008).

There are common worldwide TB-associated demographic risk factors, in addressing epidemiological determinants of health in the prevention and control of tuberculosis,. Global TB health can be determined by relating occupational variables (e.g. occupation and job category, years of exposure) and non-occupational variables (e.g. age, gender, place of birth, education level, nationality, ethnic origin) to the immune response indicators (e.g. BCG scar, TST indurations, and IGRAs results). Individual-level determinants, including high-risk behaviors, are major drivers of disease transmission. The patterns and distribution of infectious diseases in the population are influenced by a dynamic interplay between the prevalence of the infectious agent, the effectiveness of preventive and control interventions, and a range of social and structural environmental factors (Dean and Fenton, 2010; Kruijshaar and Abubakar, 2009).

Modified questionnaire construction from previous population surveys is crucial for screening success, and should be designed using clear and precise questions. Questionnaire should also be culture-oriented, reasonable, administered with responsibility, avoiding re-call bias, hierarchically ordered (simple questions followed by complicated issues), and links between TB with other socio-economic factors (van der Werf *et al.*, 2008). Using a previously validated questionnaire help to improve research/study/test accuracy (Minodier *et al.*, 2010).

The importance of the questionnaire data and screening re-in forces the aims and objectives, and the likelihood of associations will provide useful information for interpreting the results for detecting the future trends of TB (WHO, 2007b).

Descriptive analysis of questionnaire data facilitates knowledge of characteristics of immigrants that are sampled, by which precise population- and individual-level factors that are associated with LTBI and risks of re-activation progressions can be identified. This knowledge assists in the development of targeted diagnostic guidelines before testing immigrants and high risk groups (Freeman *et al.*, 2010).

4.9.2 Epidemiological risk factors for latent tuberculosis infection

Tuberculosis prevalence screening in a representative sample of the population offers a unique opportunity for collecting additional data on socio-economic status and risk factors for TB and health care-seeking behaviours. Information about these factors will contribute to wide arrays of epidemiological knowledge and estimating of the prevalence of TB more precise. Additional questions on the socio-economic status and epidemiological risks supported with another feasibility study to assess the additional time and resource requirements (WHO, 2007b).

Communities' tuberculosis rates are heavily influenced by the social, environmental and epidemiologic context, emphasizing that socio-economic status and related risk factors are important determinants of epidemiologic trends. Seven main components of access to which factors can be assigned: availability, adequacy, acceptability, accessibility, affordability, health beliefs and medical/non-medical costs (Mackenbach *et al.*, 2008; Wieland *et al.*, 2010). Risk factor variables were grouped into demographic, socio-economic, life style behavioral factors and personal health-related factors/living conditions.

Accordingly risk factor variables and socio-economic-demographic, and life style behavioral factors and personal health-related factors for *Mycobacterium tuberculosis* infection among new immigrants to Kuwait was grouped in our arranged questionnaire.

4.9.2.1 Socio-demographic characteristics

Socio-economic status is a significant risk for LTBI/TB susceptibility. TB has enormous public health and economic implications in high-burden countries. There is therefore an urgent need to provide targeted interventions, particularly for individuals most at risk of TB, to gain better knowledge about the distribution of TB in the population, as well as a better understanding of what factors are driving the TB epidemic in a given setting.

4.9.2.1.1 Age

Aging and process of increasing in TB incidence are positively associated. As growing older then populations are at higher risks of TB infection and complications (Dye *et al.*, 2011; Mahomed *et al.*, 2011; Wen *et al.*, 2010). The prevalence of infection increased with age in both men and women in the middle reproductive ages (Gopi *et al.*, 2005). Latent TB was statistically significantly associated with older ages due to longer duration of exposures and co-morbidities of other diseases. In addition to atypical presentations and delayed diagnosis of LTBI, elderly people are also associated with LTBI/TB and even deterioration of successful TB control (Abuaku *et al.*, 2010; Dye, 2006; Shetty *et al.*, 2006). Old age is a major risk factor for LTBI due to the longer duration of direct or indirect contact to MTB, associated with unemployment and malnutrition or longer consumption of unpasteurized dairy products (Bradshow *et al.*, 2011; Gomes *et al.*, 2011; Kip *et al.*, 2011; Sia *et al.*, 2010). Older age groups have higher rates of LTBI prevalence and positive IGRAs than younger groups, possibly due to waning immunologic reactivity (Winston and Navin, 2010), associated with negative/indeterminate results in active TB patients (Hang *et al.*, 2011). TB risk is positively associated with increasing adult age most strongly in individuals from low-income geographic countries e.g. Africa and Asia (Goldhaber-Fiebert *et al.*, 2011; Li *et al.*, 2010; Mahomed *et al.*, 2011).

The median age (31.5 years) of our research findings was of the average age risk for LTBI development, which is also similar to similar age ranges (more than 35 years) concluded by Rafiza *et al.* (2011) and Rutherford *et al.* (2010).

On the contrary Kik *et al.* (2009) showed no association between increasing age or gender as risk factors for positive diagnostic testing's of LTBI using TST, and both IGRAs. Similarly, Hill and colleagues (2008) also found no significant association between various age groups and gender or ethnicity with positive IGRAs in 2,348 Gambian contacts to diagnosed TB patients (Hill *et al.*, 2008).

4.9.2.1.2 Gender

Males bears a higher burden of TB; this is supported by higher prevalence rates in our study, which could be explained by a variety of other predisposing factors, such as higher case notification or smoking (Abuaku *et al.*, 2010; Feng *et al.*, 2011b; Goldhaber-Fiebert *et al.*, 2011; Gopi *et al.*, 2005; Mahomed *et al.*, 2011; Rafiza *et al.*, 2011; Wen *et al.*, 2010). Being a female 'housewife' with a low level of schooling education was a significant explanatory variable for East Asian endemic countries such as the Philippines, Bangladesh and India (Kip *et al.*, 2011; Rutherford *et al.*, 2010; Weiss *et al.*, 2008; Winje *et al.*, 2008). Other studies did not find any correlation between age or gender and LTBI (Boccia *et al.*, 2009; Caley *et al.*, 2010; Carvalho *et al.*, 2005; Kik *et al.*, 2009). The QFT-GIT-based prevalence of LTBI was not significantly associated with gender or age (Legesse *et al.*, 2011). Kik and others (2009) also found no association between age and gender in immigrants from endemic countries (Kik *et al.*, 2009).

4.9.2.1.3 Nationality

Country of origin is important in determining risk of latent TB (Mankia *et al.*, 2011). LTBI screening and intensive contact tracing, both followed by compulsory LTBI prophylaxis is needed to reduce TB in high-risk ethnic groups (O'Donnell *et al.*, 2010). Similar to the findings here, the immigrants' country region of birth is considered as a high influential explanatory factor (independent variable) for LTBI cases, which is related to MTB exposures in their geographic residence, in addition to poor regions within endemic countries (Carvalho *et al.*, 2005; Lavigne *et al.*, 2006; Sun *et al.*, 2010). TB exposed immigrant close contacts were influenced by prior and/or recent exposure of immigrants to TB in the mother country of origin in the Indian subcontinent, Asia and sub-Saharan Africa (Kik *et al.*, 2009; Pareek *et al.*,

2011). Our results relating LTBI and the country of origin and being born in high incidence (endemic) countries has been observed in other studies (Kik *et al.*, 2009; Saracino *et al.*, 2009; Winje *et al.*, 2008).

4.9.2.1.4 Civil status/gender

Being married was a predominant common risk factor for having LTBI/TB in our study. Married females with excessive housework commonly experience TB diagnosis/treatment delays (Yimer *et al.*, 2009). An Italian study on new immigrants revealed a similar gender risk association (Carvalho *et al.*, 2005). No association between LTBI and gender was detected by Rafiza *et al.* (2011).

4.9.2.1.5 Ethnic and religious status

Ethnicity provides important clues to genetic and cultural differences that predispose to LTBI and needs further exploration using MTB genotypes. Ethnicity is defined biologically and culturally (lifestyle) factors, and is related to country of origin and is strongly associated with TB in which further studies of genome-linkage MTB susceptibility among non-endemic countries residents are warranted (Ladefoged *et al.*, 2011; WHO, 2010i).

Certain ethnic groups are more susceptible to TB infection than others. LTBI prevalence among blacks and mixed race groups is significantly higher than other races, and is associated with environmental and host factors and co-morbid conditions (Mahomed *et al.*, 2011; Rutherford *et al.*, 2010). Muslim, black and non-Hispanic white races are predisposed to LTBI, re-activations and HIV (Kip *et al.*, 2011; O'Donnell *et al.*, 2010). Other ethnic groups are less vulnerable to LTBI, associated with genetic and cultural differences.

Cultural beliefs and attitudes can determine the differences between positive compliance toward anti-TB treatment and negative responses against LTBI prophylaxis (Carvalho *et al.*, 2005). Additionally, educational levels and ethnicity are reported predictors of positive TST and LTBI suspicion (Minodier *et al.*, 2010). Carvalho *et al.* (2005) and Rafiza *et al.* (2011) found no association between race,

religion and LTBI development in immigrant populations. LTBI screening and intensive contact tracing, both followed by compulsory LTBI prophylaxis, is needed to reduce TB in highly risk ethnic groups (O'Donnell *et al.*, 2010).

4.9.2.1.6   Educational level

Education offers a practical strategy for helping society to deal with TB. Higher levels of education and health are significantly protective against TB morbid trends, observed from the differences among people access/interpret/benefit/respect knowledge about LTBI/TB disease (Mackenbach *et al.*, 2008; Shetty *et al.*, 2006). Education highlights the misperceptions about TB transmission and severity, helps the distinction between asymptomatic LTBI and active TB form (Geng *et al.*, 2005; Wieland *et al.*, 2010). Low levels of education are predictive for positive TB tests (Mahomed *et al.*, 2011; Wen *et al.*, 2010) and are also related to HIV stigma (Kip *et al.*, 2011). Choosing alternative self-treatment behaviors and seeking allopathic medicine is related to time delays for diagnosis particularly in low-income endemic countries, due to compromised health system operation and referral patterns (Portero *et al.*, 2002; Weiss *et al.*, 2008). In contrast to previous findings, and consistent with our results, higher levels of various education were not associated with LTBI suspicion due to short delays in TB patient diagnosis/management (Jurcev-Savicevic and Kardum, 2011). Lack of such associations was also found by Carvalho *et al.* (2005) and Rafiza *et al.* (2011).

4.9.2.1.7   Occupation

Individuals coming in direct contact with TB infected people are at high risk of LTBI, which is commonly seen in the occupational nosocomial infection in health care workers, such as nurses in hospital wards and TB treatment units (Rafiza *et al.*, 2011). The type of hospital job category and duration of years in the health care profession are significantly associated with latent tuberculosis infection (Cagalayan *et al.*, 2011). On the other hand, inability to work is a known major cause of increased poverty and low socio-economic status (Mauch *et al.*, 2011). Unemployment is strongly associated with LTBI/TB risk (Sia *et al.*, 2010) and consequent increases in TB prevalence (Mackenbach *et al.*, 2008; Mangtani, *et al.*,

1995; Shetty *et al.*, 2006), mainly in unemployed or homeless individuals. On the opposite, both poverty and low SES in low resource countries increase MTB propagation and disease poor conditions which reduce working opportunities and raise poverty levels (Geraldes Santos Mde *et al.*, 2007).

4.9.2.1.8   Employment

In contrast the research results presented here, longer duration of exposure(s) to an index case for more days was significantly associated with carrying MTB bacilli and showing positive IGRAs (Bradshow *et al.*, 2011). Years served in the health care profession were significantly associated with positive QNF-GIT but not TST (Cagalayan *et al.*, 2011). Furthermore, length of time in employment or profession can be used as a proxy measure for increased likelihood of occupational exposure to TB in immigrants (Eum *et al.,* 2008). Pai and colleagues (2005) reported that the years served in the health care services (more than 5 years versus less than one year) had a statistically significant effect on both positive TST and QTF results.

A health system delay of more than seven weeks was positively related to unemployment status and illiteracy (Pantoja *et al.*, 2009). High prevalence of LTBI was detected among Georgian HCWs, and a longer duration of employment increased MTB carrier risks (Mirtskhulava *et al.*, 2008). Our findings show that the duration of employment was not significantly associated with LTBI diagnosis because risk of infection depended primarily on the duration of exposure to a person with infectious TB disease inside/outside and concentration of infectious droplet nuclei in the air. A similar finding was also reported by Cagalayan *et al.* (2011) and Rafiza *et al.* (2011).

4.9.2.1.9   Income and social status

Even though there still no internationally agreed standard definition, accepted indicators of socio-economic status are median household income and expenditure, crowding, level of education, and housing quality. Household income in private occupied dwellings is a measure of total household income adjusted for household composition, below which a particular country-related line, deprivation can be

defined. Income-health inequalities and residents of resource-poor neighborhoods are at greater risk of adverse health outcomes, compared with residents of more affluent neighborhoods (Mackenbach *et al.*, 2008).

Total household income is reflected in socio-economic status through the type of housing and LTBI exposure risks (Shetty *et al.*, 2006). Low-income and poverty with social inequalities determines and threatens TB outcomes, and are closely associated with the socio-economic status of the population (Geraldes Santos Mde *et al.*, 2007; Mahomed *et al.*, 2011; Weiss *et al.*, 2008). Geographical economic barriers add risks to vulnerable people for MTB transmission and disease progression, such as charges for health services and lost incomes. Financial barriers and access to TB health care are of immigrant's concern in the developing countries, in which the service policy makers are requested for facilitated interventions (Dean and Fenton, 2010; Long *et al.*, 2008; Sia *et al.*, 2010; Wei *et al.*, 2009). The length of health-seeking and total anti-TB therapeutic costs in poor communities are directly related to the length (delays) of health seeking behaviour and catastrophic health expenditure (Pantoja *et al.*, 2009). A direct relationship between SES factors and TB treatment outcomes exists because of the financial burden and the quality of case management. Challenges of facilitated health system to equalize the poverty with the country SES inequalities (Muniyandi and Ramachandran, 2008). Income level is associated with the choice of health care provider (Charles *et al.*, 2010) and factors affecting treatment success (Xu *et al.*, 2010). In contrast, high income and wealthier household socio-economic conditions were associated with higher levels of education about TB knowledge (Portero *et al.*, 2002) and prevalence of TB infection in urban regions (Boccia *et al.*, 2009). The participants in this study were considered to be of average income and more educated, and fit these associations.

4.9.2.1.10 Total individuals living in the same place (family size)

The low SES is ecologically related to household crowding and is a sign of low income/poverty linked with deprivations and high TB incidence. The proportion of the population living in overcrowded housing (households requiring one or more

additional bedrooms) has association with the prevalence of certain infectious diseases and tuberculosis (Baker *et al.*, 2000).

The number of house residents is significantly associated with the presence of self-reported latent or active TB, either currently or during their past household residence (Larcombe *et al.*, 2011). Average family income defines economic status differently, depending on the number of family members. Crowding in country of birth and related housing quality of migrants is an important variable for tuberculosis risk (Mangtani *et al.*, 1995; WHO, 2007b). Overcrowding is independently associated with TB infection (Boccia *et al.*, 2009), and poor standards of housing conditions (Muniyandi and Ramachandran, 2008; Pantoja *et al.*, 2009). High rates of TB infection are observed in household contacts with longer duration of intense exposure (infectiousness of two years) in high prevalence countries (Lin *et al.*, 2008; Sia *et al.*, 2010). Family size is associated with CXR cavitation status in TB cases (WHO, 2007a). Similarly, treatment delays of TB patients were independently associated with an increase in the household size (Sabawoon *et al.*, 2011).

These research findings on the contrary revealed no significant associations between family member size and LTBI development, because the majority of immigrants were coming from average income conditions in their mother countries.

4.9.2.1.11 Urbanization (general crowding index/sleeping crowding index)

Household density is known as both an indicator of low socio-economic status and as a risk factor associated with high morbidity and mortality risks. Social discrimination and poverty, overcrowded housing, poor sanitation and malnutrition are all major risk factors combinations for LTBI development and facilitating MTB re-activations' during the initial years of residency after migration in urban and industrialized countries (McKenna *et al.*, 2005). Immigration of young foreign-born adults is the dominant factor of urbanization and TB epidemiology in low-incidence and high-income countries. TB prevalence is higher in urban overcrowded (social mixing with infected case) populations, associated with low housing quality compared with housing characteristics of well ventilated rural regions (Gopi *et al.*, 2005). Excess in

TB risks and household crowding was strongly detected in Europe and adverse negatively associated with schooling and urban location (Goldhaber-Fiebert *et al.*, 2011). Similar findings were also noticed in Philippino's immigrants due to their living in high-density urban areas having high socio-economic status, still are at higher risks of LTBI than rural areas because of closed-ventilation environments (Boccia *et al.*, 2009). In India, even with presence of access to health services, still the risk of TB infection is expected to double between 2010 and 2030 due to increased urbanization to nearly 600 million people (Lalvani *et al.*, 2001a). Recent similar findings of raised TB rates due to urbanization associated with rise in body mass index and spreading of diabetes mellitus in Korea (Dye *et al.*, 2011), and rises in body weight as concluded by Goldhaber-Fiebert *et al.* (2011). Effective socio-economic markers and overcrowding indices to identify those at risk of LTBI/TB can be observed in residence of shared cooking facilities in urbanizing community (Shetty *et al.*, 2006).

The profile of social determinants-of-health framework in public health should be verified and able to identify suspected immigrants for screening which proved significantly by Satcher D. (2010). Our data demonstrated that household crowding, a correlate of low socio-economic status, might be associated with LTBI development (Baker *et al.*, 2000) which needs further analytic studies. Even though all medical centres were accessible in mother countries to all sample participants, still risk factor predictors of LTBI need further assessment.

4.9.2.2   Risk factors for *Mycobacterium tuberculosis* infection
4.9.2.2.1   Access to health care services

Health-seeking behaviors are associated with knowledge of key symptoms and/or signs, perceived curability, and to perception of TB services without barriers and ethical confidentiality and cultural-related stigma. Health-system barriers in accessing tuberculosis care cause delays in timely diagnosis/treatment, resulting in continued MTB transmission. Access is a pre-requisite for utilization of health care services. Lack of TB infrastructure and financial resources is crucial and a reason for failure to seek treatment access, especially for foreign-borne immigrants (Xu *et al.*,

2010). Lower accessibility and difficult access to the health care services and follow-up programmes, with lack of resources (mainly in rural areas), should be managed through improvements of the nearest residence to free public health centre services (Ladefoged *et al.*, 2011).

Health care systems in the mother country were accessible to almost all participants because the majority were coming from urban settings with few obstacles to reaching public and/or private medical centres to treat patient health disorders and tested by TB diagnostics and managements. Income-related inequalities are important in health self-assessment and promotion of seeking health care (Mackenbach *et al.*, 2008). Despite educational background and family income the immigrants' in this study showed intentional health-seeking behavior for TB symptoms and discouragement of self-treatment toward better health care services. A similar conclusion was also concluded by Portero *et al.* (2002). Illness-costs of immigrants were identified as the main barrier from health care seeking for symptoms management (Aye *et al.*, 2010).

Poor access to health facilities and limited awareness of TB within most communities and health seeking behavior was shown by Gele *et al.* (2009), which is not a problem for recent expatriates in Kuwait. Short travel distances and distance times are positively associated with attendance at, and satisfaction in, public health services with various reasons of attendance (Nteta *et al.*, 2010), in addition with non-adherence/loss of follow-up due to social stigma and adverse effects (Gust *et al.*, 2011). This can be due to inefficient diagnostic facilities and from incompetent health care providers (Saqib *et al.*, 2011).

4.9.2.2.2 Transport routes

The research results support the qualitative possibility of MTB transmission and LTBI development on transfer close overloaded machine such as cars, buses or trains, but without defining risks of LTBI development quantitatively (Edelson and Phypers, 2011; Ling *et al.*, 2011). The risk of contact transmission during transportation when persons with active tuberculosis travel on public transport routes should be further studied mainly in Philippino and Indian immigrants and their

contacts because of the use of small travel tools e.g. chapini (small buses) and related positive IGRAs.

4.9.2.2.3   Sanitary systems

Socio-hygienic conditions should be regularly studied to analyze associations with related morbid disorders (both infectious and non-infectious conditions). Better sanitation using centralized water supply through sewer chlorinated system limits public health disorders. Poor sanitation is associated with TB mainly in low-income countries due to poverty, over-crowding, homelessness of irreversible increasing population growths (Baker *et al.*, 2008; Lienhardt *et al.*, 2005) with no town resettlement/poor living conditions and without access to running water, bathrooms or flushing toilets (Ladefoged *et al.*, 2011). Re-emergence of tuberculosis imposes public health threats and had received global attention. Inadequate sanitary infrastructure and an improper sewage system can continue to be a problem necessitates raising the population hygiene through supplement of a new water supply system and disinfection of sewage water.

McKenna *et al.* revealed significantly a correlation between MTB re-activation and poor sanitary conditions of new immigrants to urban areas (McKenna *et al.*, 2005). However, our findings revealed no significant relationships between the sanitary system and development of LTBI risks. Similarly, Grafein *et al.* (2011) recently found no association, using IGRA (QNF-GIT), between LTBI and age, gender, years of schooling, fieldwork, mobility/travel duration and access to health care.

4.9.2.3   Entry into and living conditions in the State of Kuwait

Return visits to Kuwait concerning re-exposure risks added to the country pre-entry regular screening can prove immigrants remained uninfected (McCarthy, 1984). Early immigrants' registration within short period after entry (median duration was 11.5 days), proved no missing of recent expatriates and representing strict notification system in Kuwait in comparison to worldwide countries having weak registration systems and TB control practices. Then majority of those coming back to

Kuwait were from endemic (Asian) countries and immigrants targeted with easy follow-up should be considered.

Longer duration of registration post-entry increases the risks of exposure-contact and risk of developing LTBI and TB disease within first 12 to 24 months of exposure, especially from those highly suspicious TB immigrants (Ling *et al.*, 2011). Compared with Kuwait short duration of post-entry registration, Richard and colleagues (2005) results detected delay of 35 days from entry to the reply through mailed letter and 70 days until immigrant's first clinic visit in Canada (an example of developed country with high immigrants). Even with no significant association, new immigrants had high risk of exposure contact due to sharing a bedroom with other subjects (which can be an index case) in Kuwait and co-habitation in small closed-room environments, aggravating LTBI re-activation/transmission, and which was also observed by Sia *et al.* (2010).

Positive association can be detected if repeat LTBI diagnostics after period of exposure time. The majority of proposed occupations in Kuwait was significantly associated with longer duration of exposures within house-related jobs and closed communities. Also the new living trends of Kuwaiti's such as renting small flats have more risks of contacting and infection from the 'extremely high' LTBI groups. In-migration pulling factors to Kuwait such as house-related jobs and manual workers constituted the major daily expatriates which belong significantly to the 'extremely high' group and TB suspicious cases. On the contrary few immigrants entered as non-manual occupants were belonging to the 'negligible' LTBI group. Consequently populations of non-endemic countries are at greater risk of acquiring TB due to repeated contacts with LTBI and MTB transmission from the immigrant workers and MTB carriers (Edelson and Phypers, 2011; Fronteira and Ferrinho, 2011).

Ventilation frequency and airflow influence respiratory infections and TB spread in the TB mother countries. Consequently, after entry to Kuwait, air handling and ventilation are important to prevent airborne transmission. Larcombe and colleagues (2011) found a significant association between crowding in permanent house

residents and the presence of latent and active TB disease. Thesis necessitates investigation of the environmental factors surrounding the epidemiological characteristics followed by educating the community population regarding the importance of air ventilation especially in urban developed countries.

4.9.2.4   Knowledge about tuberculosis disease

A number of factors, including socio-economic and lack of information concerning TB, play a key role in governing the health seeking behaviour of patients with TB symptoms (Yimer *et al.*, 2009). The relevance of distress patterns with respect to TB non-specific symptoms is a common public interest (Weiss *et al.*, 2008). Poor knowledge of TB prevents migrant patients voluntarily seeking TB care post-entry to non-endemic countries, which is the case in the majority of admitted TB foreign-born patients (Wei *et al.*, 2009). Immigrants were primarily asked indirectly about latent TB to ignore barriers of misperceptions and low awareness of TB infection (Wieland *et al.*, 2010). On the contrary, the majority (95%) of the study subjects had contact with the nearest medical provider within a reasonable period of time following any complaints. A common feature in urbanized regions is the health seeking behaviours and looking for medical opinion from the facilitated medical care services (Charles *et al.*, 2010; Yimer *et al.*, 2009).

Absence of typical respiratory- and non-respiratory symptoms reduces clinical suspicion with consequent delayed diagnosis of LTBI/TB and increases in TB morbidity/mortality due to probable re-activation risks (Feng *et al.*, 2011).

The symptoms/signs of PTB are usually non-specific but typical presentations, such as unexplained body weight loss, remain as important factors to remind medical care physicians to consider a diagnosis of tuberculosis. Accurate education about tuberculosis was defined as having knowledge about respiratory MTB airborne transmission when coughing or sneezing (Khandoker *et al.*, 2011), which was significantly related to the level of immigrants' education (Portero *et al.*, 2002), as confirmed by our findings.

4.9.2.4.1   Respiratory symptoms

The presence of a chronic cough more of than three weeks, dyspnoea and hemoptysis are clear TB symptoms, and are independently associated with overall PTB mortality (Feng *et al.*, 2011; Portero *et al.*, 2002). Delay in diagnosis due to non-considered TB symptoms by the people and/or health care providers is proved in endemic countries such as Pakistan (the 8th TB burden country). Diagnostic delays (up to median delays of 8 weeks) related to lower detection rates can be significantly improved through early suspicion and accurate diagnostic tests (Saqib *et al.*, 2011). A longer diagnostic delay is associated with under-estimated TB symptoms such as complaints of coughing and losing body weight (Jurcev-Savicevic and Kardum, 2011). Immigrants (~54%) sought treatment from governmental hospitals for coughing of more than three weeks were missed, mainly due to symptomatic under-estimations and knowledge of coughing as a mode of transmission, and was correlated with medical and community education levels (Kar and Logaraj, 2011; Portero *et al.*, 2002). However Garfein *et al.* (2011) concluded absence of a significant association between LTBI development and TB knowledge (symptoms and transmission), but not due to difficult access to health care service.

4.9.2.4.2   Constitutional symptoms

The presence of constitutional symptoms represents higher disease severity and is associated with increased risk of PTB death. Weight loss (wasting), prolonged fever, anorexia, and malaise, generalized weakness, and hyperhidrosis were clear constitutional complaints that are significantly associated with clinical complaints of PTB mortality, and which force the immigrant community toward health-seeking behavior were all significantly associated. Similar findings were shown by Feng *et al.* (2011), Garfein *et al.* (2011) and Portero *et al.* (2002).

Gaps in the knowledge of *M. tuberculosis* biology and its interaction with the human host need further research. Aye *et al.* (2010) proved the community members' belief in TB as an inherited and normally incurable disease. Background knowledge on tuberculosis and social support could show a positive impact on the perception of self-health care and LTBI screening adherence such as presence of a health-care

worker with the same native language as the immigrant to raise the educational level about LTBI/TB. Level of TB knowledge was 66% and the majority usually answered 'I do not know' about previous TB vaccination unless asked about the reason of BCG scar presence (Ailinger *et al.*, 2004). TB knowledge is correlated with delay of TB diagnosis according to geographical location, e.g. median delay was 74 days in India compared to 60 days in Bangladesh and 33.5 days in Malawi (Weiss *et al.*, 2008).

4.9.2.5   Sources of tuberculosis knowledge

Worldwide, the media is a source of health education and information about TB, especially in endemic countries suffering from the disease burden's consequences (Khandoker *et al.*, 2011; Wandwalo and Morkve, 2000). Communication and social mobilization activities in TB control result in multiple benefits including bridging pre-existing gaps between the health system and the community through support and coordination of general health services stakeholders. Community settlement provided with social support and raised TB knowledge permit positive self-perceptions of health care even for undocumented immigrants (Carvalho *et al.*, 2005).

Enhancement of TB knowledge, attitudes and behaviors build-up community efficacy to combat TB. Also socio-economic factors should be supported by the design of TB information campaigns and by prioritizing public health interventions about TB (Portero *et al.*, 2002). Standardized data collection and the presence of a health-care workers with the same native language as the immigrant assist in identifying the gaps in knowledge between immigrant communities and providers of TB care for medical campaigns and implemented projects with successful engagement of treated patients (Carvalho *et al.*, 2005; Kamineni *et al.*, 2011).

Time delays to diagnosis are the most important obstacles to the control of the infectious cases and TB epidemic, and need to be shortened (Uys *et al.*, 2007). Total delay includes time delay to TB diagnosis, patient delay and health care system delay (Sreeramareddy *et al.*, 2009; Yimer *et al.*, 2009). Factors contributing to delayed access to medical care include socio-economic status and related personal health such as poverty and type of health seeking behaviours (Aye *et al.*, 2010). To reduce LTBI/TB diagnostic delay, efforts should always be made to increase TB knowledge,

especially in the less-educated segments of the population and within endemic countries, in particular (Jurcev-Savicevic and Kardum, 2011).

Factors, such as access to media, improve correct knowledge about TB, especially in daily viewers of television, and by daily reading of newspaper/magazines and listening to radio (Khandoker *et al.*, 2011). A main source of TB knowledge is radio messages and television advertisements (Portero *et al.*, 2002). Television is reported to be the main source of information supporting TB knowledge and teaching (Kar and Logaraj, 2011).

Designing and implementing interventional programmes, based on levels of community education and using the media to disseminate TB knowledge, is urgently needed, particularly among women. For example the belief that TB is inherited is significantly associated with a non-formal education. Advice from family members, relatives and/or friends were not significant in this study but has been shown to be significant in endemic countries e.g. Philippines (Portero *et al.*, 2002).

4.9.2.6 Risk of tuberculosis exposure

Public health authorities are considering attention to the travel clinics for LTBI screening of international travelers. Immigrants use multiple transport facilities in low-income and TB endemic regions. Understanding the overlapping nature of TB epidemics, and their social and structural determinants, is key to designing and implementing effective prevention campaigns. TB infection is frequent among travelers to high incidence-countries, especially in those long-term travelers and/or those involved in work due to longer exposures, such as health care providers. Association with travel across borders suggests the potential for TB transmission, which can be acquired in their mother countries or recently from exposure during travel. Contact tracing of public transport routes for travel considering the proximity to TB index cases and the duration of exposure and other confounding risk factors (Edelson and Phypers, 2011).

Close-contacts are considered a high-risk setting for transmission of MTB bacilli, emphasizing the need for environmental-control measures able to reduce these risks.

Commuting transport difficulties and longer distances from health care services and missing follow-up visits such as using multiple buses can lead to incomplete or failures of INH-prophylaxis in those LTBI diagnosed close-contacts to PTB (Machado *et al.*, 2009).

Immigrants arriving from high TB incidence countries are at higher risk than the general population for developing active TB disease, particularly in the first two to five years after arrival (McKenna *et al.*, 2005). However, risks of TB exposure cannot be indicative of those immigrant 'minorities' able to travel or work outside their low-income mother countries. Similar findings by Grafein *et al.* (2011) indicate the risk of direct exposure to MTB and spreading TB during migration. On the opposite arrivals from TB endemic region was not significantly associated with MTB carrying or LTBI suspicion (Mendez-Echevarria *et al.*, 2011). Our research results also revealed no significant associations between LTBI presence and risks of travel or working outside in endemic countries.

LTBI and false-positive TST's were apparent in travelers in high-incidence countries. There remains uncertainty of which travelers' might benefit from pre- or post-travel TST screening. Mantoux test conversion is likely to over-estimate the risk of LTBI and MTB infection in the low-prevalence populations due to low positive predictive values (Freeman *et al.*, 2010).

4.9.2.6.1 History of environmental (inside household and outside household) contacts

The burden of TB disease among household contacts is always studied and represents an opportunity to target transmission-interruption interventions from the index cases. Individual risk behaviors influence the probability of contact with other infected or infectious individuals and affect delays in TB diagnosis with further MTB transmission. Higher prevalence of latent infection due to cumulative exposure in high burdens of TB is still unclear and is the topic for future researches to detect risks of exposure (Pareek *et al.*, 2011).

Contact tracing is integral in tuberculosis management and usually begins with the nearest first-degree relatives such as the household contacts to peripheral contacts previously diagnosed to have LTBI or active TB. Common factors significantly associated with latent TB infection are history of living in the same house with close family members or friends who had active tuberculosis (Rafiza *et al.*, 2011). Diagnosis of LTBI and TB among immigrants and their contacts can prevent TB transmission among communities in rural with low-incidence areas that have limited resources for contact investigations. Other studies performed among close contacts in different settings have shown a better correlation of both IGRAs with recent exposure and closeness of contact, in comparison with TST unable to detect risks of previous MTB infection (Adetifa *et al.*, 2007; Brodie *et al.*, 2008; Ewer *et al.*, 2003; Lalvani *et al.*, 2001b).

High- and medium-priority contact tracing and diagnostic investigation to identify cases of active and latent TBI is an important component of the WHO recommendations for tuberculosis control all over the world. National policies and guidelines can be facilitated through evaluation and revision of diagnostic interventions. LTBI/TB is highly prevalent in TB households (Sia *et al.*, 2010). Contact screening using approximate risk-based assessment depends on infectiousness of TB index cases, susceptibility of contacts and intensity of exposure. Based on exposure time among tuberculosis contacts, IGRAs conversion to abnormal results can be detected after exposure. IGRAs can accurately and more specifically discriminate LTBI compared with the less sensitive TST, based on risk of TB exposure (Jong Lee *et al.*, 2010).

Even with absence of significance in these research findings for environmental exposure either inside or outside households, the longer duration of daily exposure to outside contacts in endemic countries can add risk factors to LTBI development and MTB carrying which was significantly associated in those exposed outside for more than 10 hours. A similar association of duration-contacts in adults was commonly found to be latently infected with MTB in a high TB burden endemic regions where demographic and poverty-related socio-economic factors are risk predictors of 'TB

suspicion' (Mahomed *et al.*, 2011). The same findings were also recently elsewhere (Edelson and Phypers, 2011; Fronteira and Ferrinho, 2011; Ling *et al.*, 2011).

In contrast, our study's final results revealed that previous contacts and exposures to the index case did not appear to increase the positive likelihood of TB diagnostic testing. Risk factors of positive IGRAs and TST were not associated with TB infection in the past and previous or recent household close contacts (high risk of exposure) to index cases. The previous conclusions were similar in other publications (Bradshow *et al.*, 2011; Kik *et al.*, 2009; Rafiza *et al.*, 2011). Also Shanaube and colleagues recently revealed lack of strong associations between either positive TST or QFT-GIT result with risk factors related to household infectivity and MTB transmission (Shanaube *et al.*, 2011).

4.9.2.6.2 History of 'direct contact' with diagnosed tuberculosis patient

MTB airborne transmission plus crowding are associated risks of close and intimate family contact with an infectious open TB patient and consequent LTBI development in the lower age groups. Intimate family close-contact with a household TB case represents a significant risk factor for young children (less than five years) mortality in a low-income country due to longer exposure duration (Gomes *et al.*, 2011). Household crowding can be related to the number of people residing in a household, and exceeding any adequate services provided to the occupancy members such as absent additional bedrooms. Elevated risk of TB morbid trends and incidence can be reduced through improvements in measures of household crowding in endemic countries. Higher levels of direct close contact with diagnosed and treated active TB case are significantly increases with risk of contracting MTB infection. A closer relationship, such as indoor households and mixing of students in the same class room, increase risks of both MTB infections and LTBI diagnosis (Caley *et al.*, 2010; Ewer *et al.*, 2003; Sia *et al.*, 2010).

Previous history of TB infection is a common risk factor which can be integrated as suspected LTBI even without performing chest X-ray (Crampin *et al.*, 2009; Wu *et al.*, 2009). Past history of household contacts exposed to TB patients are likely to

develop LTBI which necessities mandatory follow-up and monitoring to provide treatment and reduce the risk of active TB occurrence. For example positive IGRAs are more likely to develop re-activation active TB disease which can be prevented (Huang *et al.*, 2010; Mendez-Echevarria *et al.*, 2011).

In-door activity related to housing condition and resident health are correlated. Winter (cold) seasonality of increased humidity and low airflow is positively associated with high rates of TB case notifications during summer and spring (Fares, 2011). MTB transmission is associated with crowding and lacking of adequate ventilated housing. The health of immigrants within overcrowded living places reflects householder's health, where the small, overcrowded rooms increase the risk of LTBI and re-activations (Larcombe *et al.*, 2011). Living in the same house with TB patients is a known risk factor due to shared ventilation air space and close (direct or indirect) contact with each other where high and low LTBI risk groups identified. Contacts of known TB cases are known to contribute greatly to TB disease burden in certain settings. It is possible that the true number and prevalence of LTBI cases was underestimated due to death of active TB disease after infected from contact-exposure achieved by Hill and colleague findings (Hill *et al.*, 2008). For example, in West Africa, up to 45% of newly diagnosed TB cases have a known TB contact (Lienhardt *et al.*, 2005). Positive ELISPOT results were significantly associated with direct exposure within weeks of exposure to index case, which results in LTBI-presumed students having higher TST grades due to longer durations of exposure to MTB than ELISPOT negative school students (Ewer *et al.*, 2003).

Pertero and others showed no correlation between knowledge about the level of contagiousness (length of exposure) to the socio-economic and demographic data (Portero *et al.*, 2002). In endemic countries alternative solutions to allopathic medicine are found and are related to time delay, complicating help-seeking behavior in urban regions (Weiss *et al.*, 2008).

This research showed an absence of a significant association between past history of contacting previous TB infection, which is usually a major cause of resurgence or recurrent TB. Similar findings were also reported in other publications, which

recommend further MTB genotyping. On the contrary, no association was found between previous TB history and clinical presentation of PTB (Feng *et al.*, 2011b).

Regarding the standard chemotherapy of TB (four different drugs plus the length duration of 9 months) proved in the interviewed participants without significant association due to incomplete knowledge about infected patients, except answering correctly about treatment default and/or resistance. Both Portero *et al.* (2002) and Rafiza *et al.* (2011) found no associations with having previous anti-tuberculosis treatment - similar to our findings.

Unfavorable factors can lead to adverse outcomes of TB treatment, and complicate MTB transmission and the appearance of resistant strains. Socio-demographic risk factors such as age, gender, migration status, alcoholism, smoking, lower educational and income levels, unemployment, non-supervised treatment, medical staff attitude towards patients, treatment of other concomitant diseases and HIV infection, presence of hemoptysis or other side effects of anti-TB drugs can complicate treatment (Abuaku *et al.*, 2010; Santha *et al.*, 2002). A treatment delay of 30 days was considered as a performance indicator for TB control programmes in high-TB-burden countries (ATS and CDC, 1999; Lin *et al.*, 2008; Yimer *et al.*, 2009).

4.9.2.6.3   Stigma of tuberculosis

Recent exposure surrogate measures such as gradient of recent exposure have been used to evaluate IGRAs, which correlate better with an exposure gradient (Brodie *et al.*, 2008, Ewer *et al.*, 2003; Lalvani *et al.*, 2001b). Exposure to TB patient can estimate the probability of having a positive QNF-GIT result and can be function of related risk factors (Mendez-Echevarria *et al.*, 2011). For example positive IGRAs can determine latent TB infection in foreign-born immigrants and also reflect prior TB exposure in the country of origin (Kik *et al.*, 2009).

Stigma is assumed to be due to its airborne transmission and the close link to poverty and general community rejection (Cramm *et al.*, 2010; Wei *et al.*, 2009; WHO, 2005). The increased level of indirect exposure usually did not translate to increased risk of infection from contacting the TB index case. For example 'near' direct

exposure to an open active TB case such as student exposure in a classroom has more risk than 'far' direct exposure to teachers (Caley *et al.*, 2010; Ewer et al., 2003). On the contrary Garfein *et al.* (2011) findings revealed no prediction of significant association between LTBI detection or ever contact to TB patient.

Sleeping in proximity to a TB case in a different house or different room was not significantly associated with an positive ELISPOT test or Mantoux test, even though five to six percent of positive TB diagnosis case contacts can develop TB within two years (Hill *et al.*, 2008).

Stigmatization of TB is a multi-targeted barrier to TB control, having direct relevance to health-seeking behavior and negative impacts at three levels; 1)- individual level (shame, afraid from death penalty, employment), 2)- family level (transmission stigma, blamed rejection, isolation) and 3)- society level (geographic TB endemicity marking, cultural perception and attitudes associations such as TB/HIV 'the dirty disease' association) (Courtwright and Turner, 2010; Cramm *et al.*, 2010; Kipp *et al.*, 2011; Wieland *et al.*, 2010). In this study private secrecy was lessened during deep questioning in face-to-face interviews.

TB stigma is associated with community avoidance of seeking care to treat their symptoms/complaints and consequent high morbidity/mortality burdens. The presence of TB stigma in both patients and community members, worldwide (and Asian endemic countries in particular), suggests that intervention is required to combat TB stigma by simply improving the knowledge and raising the education levels about TB disease and co-infection consequences (Kip *et al.*, 2011). Richard and colleagues (2005) in a Canadian study on 760 new immigrants revealed 22% reported previous adequate TB treatment that reflects incomplete documentation at the initial pre-immigration evaluation and delays in clinical data completion.

34.44% of immigrants felt the need to maintain confidentiality even though they already had contacted TB patients due to stigma in the mother countries and more important is unemployment risks in high-income countries and Kuwait (Kamineni *et al.* 2011; Kar and Logaraj, 2011). Fear of infected with and/or dying from incurable complaints of 'dirty disease', family stigma from spreading the disease to others, and

the social isolation coming from TB diagnosis are manifestations of community perception experiences with TB in their native countries and directly related to TB stigma in answering the interview questions (Courtwright and Turner, 2010; Cramm *et al.*, 2010; Kip *et al.*, 2011; Wieland *et al.*, 2010).

Engagement with new immigrants showed barriers to TB testing, including low awareness and lack of knowledge about latent TB, in addition to miss-perceptions related to TB including secrecy, shame, fear, and isolation. Similar findings were observed by Wieland *et al.* (2010).

Community education providing scientific understanding of TB disease and consequent LTBI development should be an international request toward TB control. Similar results were recommended by Juniarti and Evans (2010) to implement stigma-reducing programmes and strategies able to stop the spread of MTB to the family and community. Patient diagnostic delays were found associated with an increase in the household size, TB social isolation stigma, diagnostic barriers with a longer time to reach a private health-care facility and default chemotherapy (incomplete treatment), initial seeking of alternative services and self-treatment through traditional health providers (Courtwright and Turner, 2010; Cramm *et al.*, 2010; Dean and Fenton, 2010; Kip *et al.*, 2011; Sabawoon *et al.*, 2011; Wieland *et al.*, 2010).

Reduction of tuberculosis-associated stigma, encouraging public-private healthcare services, encouraging LTBI case-finding with recording of symptoms and screening of contacts, improving diagnostic capabilities is requested. Worldwide general education for a better understanding and acceptance of TB with its associated infection risks can improve the 'social isolation process', and should be done at individual, family, and community levels.

Cultural factors associated with TB stigma among TB patients e,g, being an old female and Muslim is different and cannot be compared within the same community members such as old female with low education levels without the effects of low TB knowledge (Kipp *et al.*, 2011).

Stigma limitation

TB stigma is considered to be a major limitation of the thesis study, but reflecting the barriers and involved difficulties in carrying out a high-profile and large-scale screening of new immigrants in the future. Important collected variables such as past history of TB infection or contact to infected TB patient or duration of exposures are completely relied on immigrant's recall bias. Similar to Kipp *et al.* (2011) our research findings would recommend to document and grade the level of TB stigma and accurately identify risk factors related with stigma. These are important steps towards developing interventions to reduce TB stigma.

4.9.2.7 Risk factors for progression of infection to active tuberculosis
4.9.2.7.1 History of infectious and non-infectious (chronic) disorders

Trends in TB burden can be determined by TB case detection and cure rates, and also by numerous other biological, social and economic factors. To ensure rapid declines in TB burden, countries should consider the risk factors or range of health-related outcomes associated with TB prevalence such as HIV, smoking, malnutrition, cardiovascular diseases, diabetes mellitus, alcoholism, crowding and indoor air pollution, or the length (delays) of diagnostic and treatment in health-seeking patterns (Dean and Fenton, 2010; Muniyandi and Ramachandran, 2008; WHO, 2008). The importance of geographic and socio-economic location on TB morbidity and mortality rates, for example TB mortality, is an indicator of social health that is affected by cultural beliefs and behaviors toward TB. A Geographic Information System (GIS) is essential analytical tool allowing epidemiologists to work to eliminate TB, to consider and recognize difficult data allowing public health practitioners to recognize geographic dimensions and their incorporation within the new health policy plans. Shetty *et al.* (2006) negate any significant correlation between history of infectious and non-infectious diseases with immigrants SES.

TB-related morbid trends can be caused by interactions with other infections, and observed prevalence and incidence can be due to other non-TB causes (Gomes *et al.*, 2011). The global trends and adverse health impact of HIV and sexual disease infections, viral hepatitis and TB remain among the major and urgent public health

challenges for controlling community morbidity and mortality trends, with devastating fiscal and emotional costs to individuals, families and societies (Dean and Fenton, 2010). Underlying co-morbidities such as immune compromization have been frequently reported as independent predictors of tuberculosis morbidity and mortality (Bradshow *et al.*, 2011). Thesis study results were not associated with infectious and non-infectious diseases as immigrants entered as healthy individuals.

Various inter-related leading risk factors are significant clinical predictors of development of tuberculosis, due to the presence of co-morbidities of other disorders e.g. malnutrition, smoking, alcohol consumption or chronic disorders e.g. cardiovascular diseases and malignancy (Shetty *et al.*, 2006). This can be related to regional, ethnic and cultural causes (Fares, 2011; McKenna *et al.*, 2005; Sia *et al.*, 2010). A significant relationship was established between smoking habits and alcoholism with subsequent development of TB in positive HIV individuals (Dhungana *et al.*, 2008), which, however, was not detected in our research results.

Drinking alcohol and chronic disorders such DM and cardiovascular diseases were not significant factors with LTBI. No effect of those risk factors on TB were reported by Shetty *et al.* (2006) and Feng *et al.* (2011b). Common positive relationships between positive medical history and TB history and higher mortality trends were revealed by Wen *et al.* (2010).

Diabetes mellitus

Diabetes mellitus (DM) is associated with an increased risk of LTBI re-activation and TB disease and should be an important target for intervention and efforts to diagnose, detect, and treat both LTBI and DM that have a beneficial impact on TB control. Jeon and Murray (2008) noted that nearly 60% were unaware of diabetes and related TB risks.

Epidemiologic transitions classically involve shifts from infectious to non-communicable diseases, still one billion individuals are currently living in developing (Asian) countries with important relationships of both TB and diabetes burdens (Goldhaber-Fiebert *et al.*, 2011). Diabetic individuals are at a higher risk of

contracting TB, MTB re-activation and related symptoms than non-diabetics, particularly among males in lower income countries. Rising DM prevalence is related to nutritional status (higher BMI) and higher TB incidence (Dye *et al.*, 2011). Unawareness of immigrants of their diabetes and TB risks should be addressed (Jeon and Murray, 2008). Co-morbidities such as liver disease and cancer associated with old ages increases risks of LTBI/TB (Abuaku *et al.*, 2010; Dye, 2006). An immigrant's denial of smoking or drinking may be due to denial since the majority of subjects were coming from restricted Asian cultures, and TB stigma might be under-estimated (Cramm *et al.*, 2010).

Pharmaceutical and surgical therapy

Pharmaceutical treatments were not significantly associated with TB risks because healthy immigrants were not taking medical treatments such as corticosteroids, immunosuppressive treatment or anti-hypertensive's (Belard *et al.*, 2011; Ladefoged *et al.*, 2011). Self-reporting and related environmental differences are difficult to be globally standardized and the research trials should focus separately according to each country environment and TB morbid trends.

4.9.2.8 Body mass index (BMI) (weight-for-height measure)

Changing body mass index is directly related to TB morbidity and a low BMI is a risk determinant for tuberculosis. Nutritional status is useful for predicting LTBI and prognosis of tuberculosis patients. Falling BMI (defined as less than 18.5 kg/m^2) is associated with higher risk of developing TB (Hang *et al.*, 2011; Shetty *et al.*, 2006). Adverse effects of malnutrition due to MTB infection can cause a rapid rise in TB incidence due to suppression of the systemic immune response and aggravating infection (Dye *et al.*, 2011). Wasting is a prominent feature in developing tuberculosis with incompletely known underlying mechanisms that might be due to immune-endocrine disturbances linked to the consumption wasting status. Ralph and colleagues (2010) revealed an association between proportions of affected lung and CXR cavitary lesion with decreasing BMI category. Dye and colleagues revealed opposite findings and these risk factors positively reduced TB incidence especially in those with increased BMI by gender and rural-urban regions (Dye *et al.*, 2011).

Ladefoged and colleagues recently achieved significant association between malnutrition and TB, but without significance association between lower BMI and TB risk (Ladefoged *et al.*, 2011).

Developmental factors of population growth and changes in demographic factors e.g. rural-urbanizations is related to raising incidence of obesity, and diabetes mellitus and TB. Low BMI patients had nearly a 10-fold increase in mortality, but overweight or obese patients were associated with reduced mortality (Wen *et al.*, 2010). A similar positive association comparing the income of world continental regions and their diabetes prevalence's with overweight (BMI more than 25 kg/m^2) was revealed by Goldhaber-Fiebert *et al.* (2011).

Latent TB carriers are asymptomatic healthy, having normal BMI complicating the issue of MTB carrier suspicion, and necessitates high specificity diagnostic tests. Our findings revealed no direct correlation between BMI and the healthy asymptomatic LTBI immigrants. Even with no significant association, our results revealed that BMI can be a good predictor of LTBI and relative TB risk, but without declaring the mechanism of nutritional status effects. Similar findings were reported by both Lonnroth *et al.* (2010) and Rafiza *et al.* (2011).

4.9.2.9   Smoking

Chest infections (including tuberculosis) and tobacco smoking are the two major causes of respiratory disease and death. Smoking and its chronic cumulative effects are a risk factor for TB which increases susceptibility of the human host and cough-bacillary transmissions from infectious individuals. In the world tobacco smoking ranks fourth among the top ten leading risks for human health and annually causes 5 million deaths. Even though smoking is the single biggest preventable cause of death, there are still an estimated that 1.3 billion smokers (1 billion of them are men) and about 15 billion cigarettes are smoked daily out of 5.5 trillion cigarettes produced globally worldwide.

Smokers are at risk of dying from TB (Kolappan *et al.*, 2002). Ten million deaths by 2020 are estimated, if current smoking continues (WHO, 2009b). Even though

smoking and tuberculosis are preventable killers, both are considered as major public health concerns with geographic overlap between a high prevalence of cigarettes and smoking and societies with high LTBI and TB cases (Pat *et al.*, 2007). Annually, five million deaths are caused by smoking, compared with two to three million deaths from TB (Lin *et al.*, 2007), and globally, one-third of smokers and one-fifth of TB prevalence cases are measured in the East Asian countries, commonly in urban areas (WHO, 2009a). Smoking is determined to be the main risk factor for loss of years of healthy life (disability-adjusted life years or DALYs) in industrialized countries and also among the ten principal risk factors in low-income countries (WHO, 2002b). Smoking has long been associated with low survival in individuals infected with TB and smokers are at increased risk of infections with severe forms of tuberculosis and other respiratory diseases such as pneumonia (Lin *et al.*, 2007). Wen *et al.* (2010) conclude that combination (interaction) effect of smoking and previous TB history reveals increased TB mortality than either smoking or TB history alone. Once a smoker quits smoking, the nine-time higher TB mortality in smokers is reduced to that similar to non-smoker mortality.

Smoking is a rapidly growing global epidemic. Even though still causing up to 9% of all deaths due to adverse effects on local and systemic parts of the immune system, skin and soft tissues and directly on the respiratory tract, an estimate of 10 million adult deaths from all causes in 2030 will be tobacco-related mortality particularly in resource-poor countries of Asia, Africa and South America. Worldwide China is the largest country for production and consumption of tobacco (Liu *et al.*, 1998). A recent modeling study revealed that if China reduced both smoking and solid fuel use added to maintaining 80% of DOTS coverage and TB control, would reduce the annual TB incidence of 14%–52% by 2033 (Hsien-Ho *et al.*, 2008). Adverse effects of smoking are commonly due to inhibition of T cell–mediated immune pathways which produce INF-γ, IL-2, and TNF-α (Pai *et al.*, 2007b).

The prevalence of adults' reported history (current or previous) of tuberculosis was higher among smokers (mainly bidi smokers) than in those who never smoked. Smoking increases the incidence of clinical tuberculosis, predominantly in males and middle-age groups are killed by tobacco from respiratory infections or vascular

related-diseases and losing natural life expectancy (Gajalakshmi *et al.*, 2003). Similar associations were revealed by Kolappan and Gopi (2002). Lin and colleagues, showing a rise of two-fold in the risk of tuberculosis in 17,700 current Taiwanese smokers, and a significant dose-response relationship with number of cigarettes smoked per day and packs per year (Lin *et al.*, 2009).

4.9.2.9.1 Non-beneficial effects of smoking on tuberculosis

The shared clinical picture of symptoms and signs of both smokers and TB patient's makes it difficult to differentiate among both due to deep cough similarities. The presence of tobacco smoke-associated diseases such as chronic obstructive pulmonary disease (COPD) can reduce immunity and masks the physical signs of TB due to similar aggravating complaints and raised disease severity (Awaisu *et al.*, 2010). Cigarette smoking exposure has been shown to reduce IFN-γ production of the lung CD4+ T cells by more than 80% compared with no inhibition on splenic T cells and/or without decreasing the number of CD4+ or CD8+ but causing lower macrophage intracellular killing of MTB, reducing resistance and immunity against tuberculosis with increased bacterial burden (Feng *et al.*, 2011). Also the immunosuppressive effects of tobacco smoke result from a turning off of the production of pro inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and attenuates the systemic inflammatory responses with consequent re-activation of latent tuberculosis, a physiological mechanism termed the 'chronic anti-inflammatory pathway' (Wang *et al.*, 2003).

4.9.2.9.2 Physiological mechanisms of smoking that predispose infection

Cigarette smoking is a known substantial risk factor for important bacterial and viral infections having common physiological mechanisms of smoking predisposal to infection. Firstly, the mechanical and structural changes of the lung physiology of nicotine major toxic compounds turn off the macrophage production of TNF-α, thereby rendering smokers susceptible to the progression of latent TB infection and active TB development (Arcavi *et al.*, 2004; Kopallan *et al.*, 2007; Wang *et al.*, 2003). Secondly, the immunologic mechanisms and smoking habits consistently

increases susceptibility to and aggravate tuberculosis infection and pulmonary tuberculosis, and both have common epidemiological and clinical links (Gajalakshmi *et al.*, 2003; Liu *et al.*, 1998). Both smoking and pulmonary tuberculosis can damage the lungs by reducing the immunological (cellular and immunoglobulins) levels and impairing the phagocytic activity of macrophages and natural killer cells and together with MTB bacilli induces apoptosis of alveolar macrophages (Pat *et al.*, 2007).

In this study the risk of smoking for the development and diagnosis of LTBI and TB suspects was evaluated. Here we have evaluated the risk of smoking to develop latent tuberculosis infection among new immigrants from tuberculosis endemic countries, by using a risk factor questionnaire to assess tobacco smoking and habits.

The negative public health impacts of smoking are increasing risk and morbid rates of burden TB disease among the high- and low-resource countries (Arcavi *et al.*, 2004). A tuberculosis control programme should address the benefits from interventions of reducing tobacco as a potential preventive measure with emphasis on transmission limitation to reduce global burden of TB sustainability due to numerous smoking implications of: 1- active and passive cigarette smoking and risk factor of exposure, 2- development of re-activation dormant TB and 3- progression of primary TB, and 4- increased severity of cavitary disease and death in TB cases (den Boon *et al.*, 2007; Gajalakshmi *et al.*, 2003; Kolappan *et al.*, 2002; Lin *et al.*, 2007; Pat *et al.*, 2007).

Susceptibility to smoking is a known predictor of smoking onset and smoking rates are usually higher among older school students due to increased susceptibility, especially in males from low-income countries (Guindon *et al.*, 2008). Ng *et al.* (2008) revealed that 30% of diagnosed ex-TB patients never been asked about their smoking behaviours. Similar findings were detected in 50% of TB diagnosed patients only received messages about a general advice to quit smoking without explanations or declaration of causes (Pradeepkumar *et al.*, 2008).

4.9.2.9.3   Socio-demographic associations with smoking

Socio-demographic status of TB patients such as gender, overcrowding, nutritional vulnerability and associated chronic non communicable disease is particularly relevant in a country in economic transition (Shetty and Shemko, 2006). Both smoking, obesity and inequalities of TB-related mortality are common among men with a lower level of education (Mackenbach *et al.*, 2008). All forms of tobacco use are an independent risk factor for low body mass index (Pendekar and Gupta, 2007). Similar significant findings were achieved by He and colleagues observed that male smokers had an increased prevalence of TB disease compared with non-smokers (He *et al.*, 2010).

Smoking is sex-linked especially in South East Asia (Guindon *et al.*, 2008). Smokers (especially males) are less adherent to TB treatment and are at higher risks of treatment default, added consequently to persistent TB infectivity and raising their families infectivity due to passive smoking of householders within the households (Lavigne *et al.*, 2006).

Our research findings revealed a significant association between smoking history and LTBI risks. However another multivariate analysis study on an Indian population showed absence of smoking effects on LTBI (Shetty *et al.*, 2006).

4.9.2.9.4   Changes of chest X-ray in smokers with tuberculosis

Risks of TB morbid trends due to tobacco smoking are observed worldwide. Regardless of the specific types of TB outcomes, exposure to tobacco smoke is consistently associated with TB risks. Compared with people who never smoke, smokers have an increased risk of positive latent TB infection, of having active TB, and of dying from TB (Lin *et al.*, 2007).

Smoking affects the clinical manifestations of TB and increases the occurrence of upper zone involvement (cavitary and miliary disease) due to down regulation of TNF-α production from the macrophage and decreased immune response (Altet-Gomez *et al.*, 2005). Pulmonary tuberculosis among smokers has a higher risk of

developing cavitary TB lesions (Altet-Gomez *et al.*, 2005; Kherad *et al.*, 2009). Neither associated abnormal changes were detected in our research CXR findings.

The prevalence of TB among current- (40%) and ex-smokers (14%) in diagnosed TB patients was compared to non-smokers (Awaisu *et al.*, 2010). Tobacco smoking has significant impacts on treatment adherence of LTBI (less compliance), and is also associated with treatment failure, default, and relapse after successful anti-TB treatment (d'Arc Lyra Batista *et al.*, 2008; Kolappan *et al.*, 2002; Ng *et al.*, 2008; Pradeepkumar *et al.*, 2008; Santha *et al.*, 2002). The association between smoking and TB deaths remained significant even after including alcohol use (Pednekar *et al.*, 2007). Except for associated factors of being a female and a non-smoker, socio-economic profile such as age, region of birth, and nicotine dependence among smokers were not associated with adherence to LTBI treatment (Lavigne *et al.*, 2006). Extra supervision should be focused on smokers to ensure compliance with LTBI and anti-TB treatment.

On the contrary Feng *et al.*'s (2011b) results revealed no significant associations between smoking and clinical presentation of PTB. Also Kik *et al.* (2009) revealed no significant association between those had smoked and immigrants having positive TST and both IGRAs.

Cohort studies are valuable and show the smoking effects of increasing TB morbid risks, been examined among ex-smokers through defining related risk factors (Jee *et al.*, 2009), such as tobacco smoking and related LTBI diagnosis (den Boon *et al.*, 2005) and/or with TB disease (Kolappan *et al.*, 2002) and/or TB relapses due to non-adherence to anti-tuberculosis treatment and increased infectiousness (Wang *et al.*, 2009). Doll and colleagues conclusions proved on follow-up study for 40 years on British doctors that death from infectious diseases and PTB was significantly positively associated with cigarette smoking habits (number of cigarettes and current smoking) than non-smokers and ex-smokers stopped at younger ages (less than 35 years) (Doll *et al.*, 1994). Similar to increased TB mortality in active smokers, passive exposure to second hand tobacco smoke in the household also predisposes to the development of TB (Huttunen *et al.*, 2010). For example it is estimated that

among Indian male smokers and non-smokers together, smoking causes half of all deaths from TB (Gajalakshmi *et al.*, 2003).

4.9.2.9.5 Smoking habits

Understanding the tobacco use, habits, attitudes and behaviours of newly diagnosed TB patients is of paramount importance in individualizing smoking cessation intervention via behavioural therapy and in designing effective educational intervention programmes for the prevention of TB through reducing treatment failure and TB recurrence or other poor consequences (Awaisu *et al.*, 2010). Studying the behaviour of smokers and likelihood for TB infection prolongation within communities is difficult due to the socio-economic confounding.

Diagnostic delays of TB are associated with smoking habits, both ex-smoking and current smoking, and consequent complications to advanced poor conditions (Jurcev-Savicevic and Kardum, 2011). Ex-smoking and past history of smoking, in comparison to current smoking, was specifically associated with evidence of LTBI heterogeneous groups (Shetty *et al.*, 2006).

The impact of TB on smokers in the community is also related to hazardous lifestyles and living conditions (Altet-Gomez *et al.*, 2005). Classification of smoking behaviours is difficult; lifetime amount of smoking habits, amount and duration in years, recall bias and lack of objective standards are considered limitation barriers. For example measurement of body biomarkers (e.g. nicotine) in children can determine exposures to environmental tobacco smoke (ETS). Interactions between smoking and adverse effects of other environmental factors was observed due to impairment of T-helper cell immune maturation (Huttunen *et al.*, 2010), and from ETS which are toxic, irritant and directly toxicogenic and contains up to 4,500 free radical compounds (e.g. formaldehyde and acrolein) (Perez-Padilla *et al.*, 2010).

Immigrants who 'ever smoked daily' were having significant positive QFT-GIT results relative to insignificant TST results adjusted for country of origin and recent TB infection should be discriminated (Kik *et al.*, 2009).

Lin *et al.* (2007) meta-analysis concluded that exposure to repairable pollutants from tobacco combustion (dose-response relationship) increases the risk of TB infection even among those exposed to passive smoking e.g. exposure of children to household smoker parents. Pendekar and Gupta's (2007) study revealed that smoking - particularly bidi smoking (the commonest Indian smoking type) - is a known important risk factor with higher probabilities of tuberculosis mortalities than smoking cigarettes (Pendekar and Gupta, 2007).

4.9.2.9.6   Smoking and tuberculosis dose-response relationship

It is very difficult to evaluate the significance of smoking e.g. dose-dependent effects are inconsistently reported, but recent conclusion by Wen *et al.*'s (2010) revealed the presence of association between dose-response relationship of smoking quantity and the intensity of TB infection and mortality (Wen *et al.*, 2010).

Tobacco smoking is positively associated with TB prognosis and health outcomes (active disease and morbid trends) in smokers compared with the non-smoking population. Raised risks of TB deaths among smokers with respiratory TB (PTB) were linked to a dose-response-time relation between the numbers of daily cigarettes consumed (pack-years) and duration of tobacco use e.g. age of starting smoking (Gajalakshmi *et al.*, 2003; Liu *et al.*, 1998; Wang *et al.*, 2009).

Tobacco smoking, which has substantially increased in the developing countries for the last four decades, is associated with positive TST results and a positive pack-years (dose-response) relationship in LTBI cases. den Boon *et al.*'s (2005) results showed that smoking of more than 15 pack-years is associated with positive tuberculin reactivity.

The research study was unable to confirm a strong statistically significant association between smoking and latent tuberculosis infection. Behaviors of tobacco use (addiction) are difficult to change and need to be tackled carefully. Smokers' utilization of tobacco products at a young age increases risks of LTBI suspicion. Smoke-free environments are linked to public community's protection from *Mycobacterium tuberculosis* transmissions and LTBI/TB infections.

4.9.2.9.7   Recommendations for smoking follow-ups

➢ Smokers need extra-supervision to ensure compliance with LTBI treatment and reduce risks of developing active disease.

➢ Tobacco manufacturing should be stopped.

➢ Monitor tobacco use and regular prevention policies. Repeated brief cessation advice is a feasible and inexpensive addition to routine TB case management.

➢ Multifaceted action is required to improve biomass smoke-related illnesses coupled with accurate tuberculosis diagnosis and case management (Hopewell *et al.*, 2006).

4.10 General discussion of LTBI risk factors

Latent tuberculosis and epidemiological risks

To estimate the prevalence of exposure to specific TB risk factors in the population, the relative association between the various predominating factors and LTBI/TB disease should be determined. To understand the reasons for change in TB prevalence over time, it is important to know the change in the exposure of the population to risk factors for TB, such as malnutrition, smoking, diabetes, indoor air pollution, and crowding in relation the low socio-economic status. Repeated LTBI prevalence surveys provide a platform for monitoring the change in prevalence of such risk factors over time. The evidence for a causal link between these and other risk factors is varied and the relative risk of TB associated with these predictive factors cannot be specifically established, and is likely to vary across populations because of effect modification related to a varying mixture of risk factor exposures. Measurement of risk factor exposures necessitates extra questions during data collection (e.g. the questionnaire, clinical history of other disorders). Therefore targeted screening and laboratory diagnostic testing's should follow utilization of assessment tool (e.g. screening questionnaire) able to diagnose 'identifiable' or 'predictive' risk factors to accurately suspect and diagnose LTBI. Random sub-sampling of clustered immigrants can provide a representative sample to determine

the prevalence of exposures to various risk factors in the high-risk groups and population (WHO, 2007b).

Study strengths and limitations

The risk factors studied were associated with MTB exposure and can be generalizable to other situations and cultural settings for large samples. This study is limited in that all the information that was collected was self-reported influenced mainly by recall bias and TB stigma.

4.11 Conclusions

Social determinants of health should be addressed in the design of TB information campaigns and in prioritizing tuberculosis public health interventions. Tuberculosis remains a disease of the socio-economically disadvantaged living in overcrowded environments. The project results proved to be a valuable instrument to assess foreign-born socio-economic situations and LTBI risk factors, which can be a research tool to help to tackle barriers to diagnostics and management for tuberculosis control in Kuwait (Mauch *et al.*, 2011).

4.12 Study recommendations

1- Investigate the causes associated with development of latent tuberculosis among different high-risk groups and recent immigrants to improve the efficacy of screening programmes.

2- Consider new expatriates (mainly adolescents) from deprived communities an important target group for educational interventions and diagnostic testing's by tuberculosis control programmes in low-burden settings (Mahomed *et al.*, 2011).

3- Investigate strictly the contacts of high-risk groups such as targeted active case finding is useful to limit MTB transmission within non-endemic populations.

4- Enhancement of financial resources and decentralization of medical services, especially in low income counties to facilitate the implementation of TB control strategies (Xu *et al.*, 2010).

Using a combination of the significant epidemiological risk factors associated with LTBI and 'TB suspect' diagnosis and the laboratory combination of diagnostic testing's, the outline of evidence-based detection of latent tuberculosis cases in suspected risk groups will be further described in chapter 7.

5 Chapter five

Evaluation of the performance of chest X-ray and tuberculin skin test for diagnosis of latent tuberculosis infection carriers and 'suspect TB' cases

Background

Latent tuberculosis case is defined as normal healthy immigrants with normal CXR and abnormal TST and/or both abnormal IGRAs in the absence of any signs or symptoms of active TB infection. Individual's harboring the dormant *Mycobacterium tuberculosis* bacilli and defined as latent tuberculosis cases are difficult to be clarified as radiological possibility and bacteriological certainty unless post-dormancy bacillary re-activations. Screening of risk groups and immigrants coming from TB-endemic regions is mandatory, worldwide in countries with a TB low prevalence. Weakness of the standard screening policy and loss of MTB carriers are associated with raised morbidity and mortality trends. Absence of a gold standard or accurate laboratory identification for diagnosing the human tuberculosis except isolation of cultured *M. tuberculosis* had unavoidable consequences of delayed diagnosis and uncontrolled spread of TB. For example, even using prescribed LTBI prophylaxis for asymptomatic 'suspect TB' cases without a sound diagnostic result or with laboratory errors and improper management of false positives is associated with a rise of new MTB resistant strains and uncontrolled transmission which disrupts public health. Even with various TB diagnostic tools (pre-arrival and post-arrival) and diagnostic precautions such as testing for TB using CXR and PCR for HIV and hepatitis and availability of free treatment policy, still there is an increase in the number of undiagnosed LTBI and leakage of 'suspect TB' subjects.

Serious TB morbidity/mortality impedes improvements of new diagnostic tests with high accuracy and effective managements. There are still no published data regarding the use of either interferon gamma release assays as a compulsory diagnostic test for immigrant screening coming from high-to-low TB prevalence countries. Even though interferon-γ tests for screening of risk groups is a promising way of detecting LTBI with few false positives, it is limited geographically by the funding resources of the health care system.

Without prompt clinical suspicion and diagnosis, and proper isolation and adequate treatment, not only will TB cause higher mortality, but also outbreaks of health care-

associated TB will afflict other patients and pose a health hazard for health care systems (Freeman *et al.*, 2010; He *et al.*, 2010; Schablon *et al.*, 2009).

The WHO recommends effective TB control, but diagnosis necessitates early detection and management of LTBI individuals at high risk of re-activation and progression to active TB disease forms (Hussein, 2007). For example, treatment of LTBI is a major component of national strategies for elimination, for instance in the United States (Eisenberg and Pollok, 2010). Accurate and early diagnosis of TB infection is crucial but difficult with an inability to recognize the epidemiology of various MTB species and process effective strategies to limit disease spread. A correctly diagnosed case is defined as a culture-positive patient and, worldwide, is used as the gold standard diagnosis, but, results are only achieved after 4-8 weeks, which is critical for both the patient (e.g. disease complications) and community (e.g. increased risks of transmission).

Diagnostic tools can help clinicians to reach a diagnosis or be used to predict the probability of a health event or LTBI and guide decisions to isolate and prophylactically treat suspected MTB carriers. The 'gold standard' diagnostic or reference test should meet a critical criterion supported by a body of evidence and utilization of the reference as a gold standard by the relevant medical specialty community. It should attain both high sensitivity and specificity. Due to the clear limitations of MTB culture, a new schedule needs to be able to diagnose dormant LTBI before active TB flare-ups (commonly pulmonary TB) (Richman, 2008).

In Kuwait case detection and notification rates for all forms of tuberculosis is very high, reaching 89% (77-100%) in 2009 (WHO, 2011b). All new immigrants are accessible at the authorized entry port for implementation of screening procedures; direct interview and the four (two old and two new) diagnostic tests are available.

The aim of chapter five is to assess interventions and evaluate the performance of the two old tuberculosis diagnostic tests ordinarily used for diagnosis of latent tuberculosis infection carriers, and the effect of Bacille Calmette-Guérin (BCG) vaccine on the tuberculin skin test results.

5.1 Chest X-ray

5.1.1 Introduction

Chest radiography is one of the diagnostic methods for differentiating between LTBI and PTB disease and is an indication of medical evaluation for individuals with positive results of other TB diagnostic tests - in particular close contacts to infected TB patients or persons with calcified granulomas, nodular or fibrotic lesions consistent with old TB.

The absence of a gold standard diagnostic for tuberculosis makes chest radiography the cheapest, rapid, most accessible radiological screening test all over the world, as the first line diagnostic approach for chest and lung abnormal findings. Multiple reasons necessities to perform chest radiography for asymptomatic individuals: first to exclude active TB disease; secondly, to assess the evidence of prior TB and other associated relative re-activation risks which can help in guiding treatment decisions; and thirdly, to provide a baseline for future comparisons and further research. Worldwide in developed countries, as part of WHO recommendations, 'post-landing surveillance', ordinary chest X-ray remains the central tool in screening for TB among migrants in most world programmes because radiography offers benefits for screening large numbers of people within high prevalence regions (Rieder *et al.*, 1994).

Advanced TB cases usually reveal marked clinical, radiological improvements and conversion of laboratory-positive test results to negative results. Nevertheless, gross radiological changes, including cavitations, can persist (Douglas *et al.*, 1956) as can the presence of acid fast bacilli in sputum smears (Barnes *et al.*, 1988). Hospital-admitted PTB are often interpreted as a predominant consolidation (the primary lesion) and other pulmonary lung changes such as effusion, reticular fibrosis, excluding cavitations (Wu *et al.*, 2009). In contrast, LTBI suspect carriers are asymptomatic healthy with no exact diagnostic chest X-ray findings.

However sensitivity and specificity of the CXR for diagnosis of active pulmonary TB and LTBI are limited to less than 70% and less than 60%, respectively (Barnes *et al.*,

1988; Cohen *et al.*, 1996). Because of low specificity, if diagnosis among TB suspects (i.e. TB-like symptomatic patient) is based only on CXR, this would lead to a substantial proportion (~ 40%) of over-diagnosis.

LTBI detection as post-active pulmonary disease is regularly revised using WHO guideline, the American College of Radiology and UK National Institute for Health and Clinical Excellence (NICE) to improve diagnostic test accuracy (MacPherson and Gushulak, 2006). LTBI is defined by CXR findings as being an asymptomatic condition with typical evidence of past TB abnormalities having: 1- pleural thickening, 2- upper lobe linear or nodular scarring images, 3- calcified nodules (granulomas), 4- non-calcified nodules, 5- fibrotic scar with/without reduction in volume of upper lobe(s), 6- cranial retraction of pulmonary tissue, 7- calcified mediastinal and hilar lymph nodes, 8- diaphragmatic tenting, and 9- blunt costophrenic angle. Calcified nodules size (commonly between 3-10-mm) and/ or number (between 2 and 5) are typical characteristics for LTBI (Ralph *et al.*, 2010).

5.1.1.1   Merits of chest X-ray

Performance and diagnostic accuracy of chest radiography can be evaluated for TB in humans in the absence of a reference test and of a comparative assessment with other diagnostic tests. The utility of CXR is well established in TB diagnosis and clinical monitoring. Problems in CXR reporting arise from the heterogeneous CXR manifestations of pulmonary TB (e.g. primary versus post-primary disease, adults versus children, immuno-competent versus immuno-compromised) and to inaccuracies inherent in CXR performance and interpretation including limited inter-observer or intra-observer agreement on CXR findings (Ralph *et al.*, 2010).

Although with limitations of CXR sensitivity and specificity reading discrepancies, it remains a pivotal tool for diagnosing chest infections and tuberculosis. Routine admission CXRs are common and applied in hospitals serving populations where TB is common (Barnes *et al.*, 1988).

Annual follow-up screening for active TB cases should be considered seriously to reduce the infectious period and raise the protective effects than only concerned with

screening healthy but high risk immigrants at entry points to non-endemic countries. Radiographic findings play an important role in the clinical diagnosis as a significant predictor of PTB, even in HIV positive cases (Kanaya *et al.*, 2001; Tamhane *et al.*, 2009). A healed calcified lesion in chest radiographs is typical of primary lung exposure to mycobacteria and TB infection (Parrish *et al.*, 1998).

5.1.1.2   Limitations of chest X-ray

Radiography is an important diagnostic procedure in the absence of MTB culture; however, inaccurate interpretation of well-performed CXR can impact case detection and depends on experience and interpretative skills of the reader to exclude LTBI suspects (Bloomfield *et al.*, 1999). Missing PTB and LTBI findings can be due to lack of consistency in reporting and other limiting factors such as clinical training of medical house staff, excessive workloads and the specialty of attending physicians (Wu *et al.*, 2009).

CXR does not play a major role in facilitating PTB diagnosis, with consequent delays in diagnosis and treatment. Missed abnormal radiographic findings play an important role in patient's survival relative to rises in hospital mortalities and exposure of the surrounding community to TB infection with public hazards e.g. medical staff, health care workers, in-house close contacts (Wu *et al.*, 2009). Joshi and others concluded that the majority of asymptomatic HCWs in a high TB-incidence country region showed abnormal radiographic findings, reflecting a combination of recent and past MTB exposures that are not explained by demographic, occupational or immune response factors (Joshi *et al.*, 2007). Limited association was seen between clinical presentation and radiological features of PTB mortalities (Feng *et al.*, 2011b).

Grading of radiological positive findings of LTBI/suspect TB is not standardized due to heterogeneous and inaccurate interpretations of radiographic manifestations of PTB. A standard, simple, numerical risk score, validated against 'suspect TB' radiographic outcome of new immigrants in repeated data sets is still lacking, but case validation is still dependent on medical staff experience. Therefore a simple CXR score can be devised for use for assessment of all adult new immigrants. Ralph

and colleagues (2010) classified abnormalities according to the location (upper, middle or lower lobe) and the type of lesion (cavities, alveolar, reticular, miliary). They constructed a CXR severity grading system in adults with smear-positive PTB which was correlated with clinical and microbiological severity and response to treatment, concluding that cavitary disease show significant relationship between radiological and biological parameters and the 2-month sputum AFB density.

A normal chest radiograph should not exclude latent TB and clinicians are requested to take care of TB past history for individuals coming from endemic regions (Mankia *et al.*, 2011). The proportion of lung affected (cavitations and/or fibrotic infiltrate) is an important measure in many TB CXR grading methods. Creating a CXR score including cavitations and socio-demographics LTBI risk factors such as nationality and smoking also can be considered. Higher scores indicate a greater suspicion of LTBI and assessment of other TB diagnostics is necessitated which are similar to our research study testing's.

5.1.2 Aim

To assess the accuracy of chest radiography (the standard tuberculosis diagnostic test).

5.1.3 Objective and sub-objectives

To assess the diagnostic performance of CXR in diagnosing LTBI in healthy humans with absence of a reference standard test as:

1. Evaluate the performance of chest X-ray in diagnosis of latent tuberculosis infection
2. Introduction of a new chest X-ray scoring system and recording sheet to improve diagnostic performance of latent tuberculosis infection

3. Assess the quality of the reader's chest X-ray radiographic interpretations

5.1.4 Methodology

Excluding one Syrian pregnant housewife, postero-anterior (PA) chest X-ray films for 179 new immigrants were interpreted for evidence of abnormalities indicative for LTBI and TB. CXR hard copies were read independently and blindly by highly-qualified medical staff (three pulmonologists) and re-read by two radiologists. Comments on abnormal findings were recorded in a separate CXR recording sheet (Appendix 6) to interpret the final results and stage scoring (the detailed methodology is discussed in chapter 3).

5.1.4.1 Radiological Criteria for LTBI Diagnosis

Based on a literature review, a frame scoring system was constructed to predict LTBI and TB suspect requiring further diagnostic test(s).

Radiological findings interpreted as an abnormal result consistent with LTBI (probable TB) were further subdivided using a scoring system according to the readers' final interpretation and conclusion of chest X-ray reading (nodular lesion and/or fibrotic lesion and/or cavitatory /granulomatous lesion with/or without calcification and/or other parenchyma lung infiltrates and/or pleural or parenchyma disease). A risk scoring system was introduced to report on the CXR results using a four point scoring system (score I, II, III and IV) to identify predictors of LTBI suspect for re-activations': I: No pathology, II: Pathology not consistent for LTBI, III: Pathology consistent for LTBI, and IV: Pathology highly consistent for LTBI (Table 5.1).

Table 5.1: Chest X-ray scoring system for classification of LTBI categories (Jeong and Lee, 2008; Linh *et al.*, 2007; Ralph *et al.*, 2010; van Cleef *et al.*, 2005; van der Werf *et al.*, 2008; WHO, 2011c; Yang *et al.*, 2007)

CXR result	CXR score	Case definition	Final interpretation
No pathology	I	No TB	Normal
Pathology not consistent with LTBI	II	Suspect LTBI	Abnormal
Pathology consistent with LTBI	III	High suspicion of LTBI	Abnormal
Pathology highly consistent with LTBI	IV	Extremely high suspicion of LTBI	Abnormal

LTBI = latent tuberculosis infection, CXR = chest X-ray, score I = negative and free from abnormalities, score II = at least fibrosis less than 1 cm size and/or nodule size of less 5 mm, score III = at least fibrosis of 1 cm or more size and/or nodule of 5-mm or more size with/without calcification and/or cavitary granulomatous lesion, score IV = score III plus and/or other parenchyma lung infiltrates and/or pleural or parenchyma disease.

A normal CXR was defined as clear lung fields with no abnormality detected. An abnormal CXR was any lung (including pleura) abnormality detected, including opacities, cavitation, fibrosis, pleural effusions, calcification(s) or any unexplained or suspicious shadow (WHO, 2011c).

The score I was labeled as 'normal' ('no LTBI' or 'negative'), whereas, 'abnormal' (or 'positive') CXR results was considered score II 'suspect LTBI' (or 'atypical'), score III as 'high suspicion of LTBI' and score IV were labeled as 'extremely high suspicion of for LTBI'. Scores III and IV are also considered as 'typical' LTBI abnormal lesions and 'definite LTBI diagnosis' for score IV.

5.1.5 Data analysis

Sensitivity and specificity (Knapp and Miller, 1992) were calculated using SPSS version 17.0 Software statistical package. The frequency of positive findings identified separately of the PA radiographs on the basis of the final interpretations

(CXR stage scoring) and positive findings. Agreement between readers was assessed by using the kappa coefficient statistic. A Kappa value of 0.20 or less indicated 'poor' agreement; a kappa value of 0.21–0.40, 'fair' agreement; a kappa value of 0.41–0.60, 'moderate' agreement; a kappa value of 0.61–0.80, 'good' agreement; and a kappa value of 0.81–1.00, 'excellent' agreement (Altman, 1991).

5.1.6 Results

5.1.6.1   Interpretation of chest X-ray radiographs

The distribution of chest radiographic findings according to chest X-ray readers' final comments were as follows (Table 5.2).

Table 5.2: Distribution of chest X-ray findings following a new scoring system of 180 new immigrants to Kuwait during February and May, 2010

CXR score	Final interpretation	TCU n (%)	Pulmonologist (3) n (%)	Radiologist A n (%)	Radiologist B n (%)
I	Normal	179 (100%)	179 (100%)	166 (93%)	168 (94%)
II	Abnormal	0 (0%)	0 (0%)	7 (4%)	2 (1%)
III	Abnormal	0 (0%)	0 (0%)	4 (2%)	9 (5%)
IV	Abnormal	0 (0%)	0 (0%)	2 (1%)	0 (0%)

CXR score **I** = normal, score **II** = suspect LTBI, score **III** = highly suspect LTBI, score **IV** = extremely high (definite) LTBI, TCU = Tuberculosis Control Unit (medical staff), Pulmonologist (3) = three respiratory specialists

All of the 180 new immigrants entered Kuwait after being accepted as healthy. Chest X-rays were performed on 179 of the expatriates. One immigrant - a pregnant Syrian female was excluded, with a temporary certificate issued until childbirth.

The distribution of CXR results of the 180 immigrants that were interpreted by the TCU medical staff opinion and the three pulmonologists according to the defined LTBI case categories did not revealed any statistical significant association. LTBI case frequencies were divided accordingly; 'negligible' in 50.28%, 'low' in

20.112%, 'average' in 0.00%, 'high' in 2.23%, 'extremely high' in 25.7%, and 'cannot be judged' were 1.68% (LLR $\chi^2_{(4)}$ = 7.882, p = 0.096) (Table 5.3, page 287).

Even with detection of abnormal findings in the lung shadows, the three pulmonologists independently interpreted all the 179 CXR films as normal (score I) (LLR $\chi^2_{(4)}$ = 7.882, p = 0.096). Based on the dichotomous classification (TB-related abnormalities or not), pulmonologists and both radiologists were concordant in 89.94% (161/179) cases having normal results. The estimated prevalence of LTBI suspected cases detected by both radiologists was 10.06% (18/179) compared with zero prevalence by both the TCU staff and the three pulmonologists. There were observed statistical significant differences comparing the results of both radiologists' according to the defined LTBI case categories (LLR $\chi^2_{(8)}$ = 52.584, p = < 0.001) (Figure 5.1).
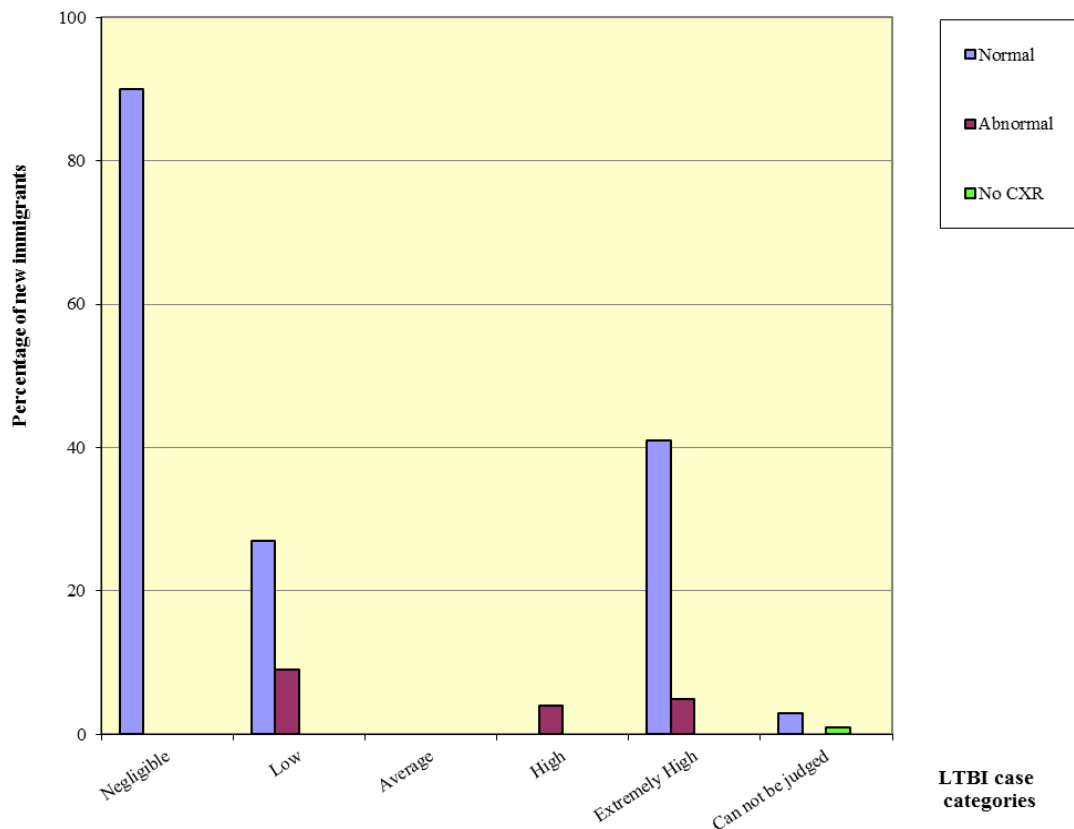
Figure 5.1: Distribution of chest X-ray agreement interpretations by both radiologists' (radiologist A and radiologist B) according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

5.1.6.2   Chest X-ray findings of both radiologists

Excluding the TBU chest X-ray results, fibrotic changes and nodular lesions were commonly detected by all radiography readers (the three pulmonologists and two radiologists). On the contrary chest X-ray films reviewed by the two radiologists revealed different opinions. Table 5.3 reveals the difference of CXR results and CXR score of both radiologists (radiologist A and radiologist B) according to LTBI case categories. The differences of abnormal findings were in scoring of the observed lesions, which were more size-specific when interpreted by each radiologist differently. All findings were statistically significant as shown by the different p-values (Table 5.3).

Table 5.3: Radiologists interpretation following the scoring system of categories for LTBI case definition of 180 new immigrants to Kuwait during February and May, 2010

Chest X-ray	LTBI case definition	Negligible (n=90)		Low (n=36)		Average (n=0)		High (n=4)		Extremely High (n=46)		Can not be judged (n=4)		Total (n=180)		Likelihood ratio	p-value
		n	%	n	%	n	%	n	%	n	%	n	%	n	%		
Radiologist A (CXR result)	**Normal**	90	50.28	28	15.642	0	0	1	0.56	44	24.58	3	1.68	166	92.22	LLR $\chi^2_{(8)}$= 42.006	<0.001
	Abnormal	0	0.00	8	4.469	0	0	3	1.68	2	1.12	0	0.00	13	7.22		
	No CXR	0	0.00	0	0.000	0	0	0	0.00	0	0.00	1	0.56	1	0.56		
Radiologist A (CXR score)	**I**	90	50.28	28	15.642	0	0	1	0.56	44	24.58	3	1.68	166	92.22	LLR $\chi^2_{(16)}$= 46.593	<0.001
	II	0	0.00	5	2.793	0	0	1	0.56	1	0.56	0	0.00	7	3.89		
	III	0	0.00	2	1.117	0	0	2	1.12	0	0.00	0	0.00	4	2.22		
	IV	0	0.00	1	0.559	0	0	0	0.00	1	0.56	0	0.00	2	1.11		
	No CXR	0	0.00	0	0.000	0	0	0	0.00	0	0.00	1	0.56	1	0.56		
Radiologist B (CXR result)	**Normal**	90	50.28	31	17.318	0	0	2	1.12	42	23.46	3	1.68	168	93.33	LLR $\chi^2_{(8)}$= 28.823	<0.001
	Abnormal	0	0.00	5	2.793	0	0	2	1.12	4	2.23	0	0.00	11	6.11		
	No CXR	0	0.00	0	0.000	0	0	0	0.00	0	0.00	1	0.56	1	0.56		
Radiologist B (CXR score)	**I**	90	50.28	31	17.318	0	0	2	1.12	42	23.46	3	1.68	168	93.33	LLR $\chi^2_{(12)}$= 29.751	0.003
	II	0	0.00	1	0.559	0	0	0	0.00	1	0.56	0	0.00	2	1.11		
	III	0	0.00	4	2.235	0	0	2	1.12	3	1.68	0	0.00	9	5.00		
	IV	0	0.00	0	0.00	0	0	0	0.00	0	0.00	0	0.00	0	0.00		
	No CXR	0	0.00	0	0.000	0	0	0	0.00	0	0.00	1	0.56	1	0.56		

Score I = No pathology, Score II = Pathology not consistent with LTBI, Score III = Pathology consistent (significant) for LTBI, Score IV = Pathology highly consistent (highly significant) for LTBI, (-) = undiagnosed LTBI case

Radiologist A's final interpretation reveals 'normal' LTBI finding in 92.22% and 'abnormal' CXR findings of LTBI prevalence in 7.22% (13/180) subjects. The details of these abnormal cases were as follows; 'low' LTBI suspicion in 4.47%, 'high' LTBI suspicion in 1.68%, and 'extremely high' cases in 1.12% (LLR $\chi^2_{(8)}$ = 42.006, p < 0.001) (Table 5.3).

Concerning LTBI scoring, radiologist A described cases with score II (pathology not consistent with TB) in 3.89%, score III (pathology consistent with TB) in 2.22% and score IV (pathology highly consistent with TB) in 1.11%. Radiologist A's results reveal more score II for the 'low' LTBI cases, and was recorded as fibrosis (fibrotic infiltrates) of less than 1cm and/or nodule size less than 5-mm (LLR $\chi^2_{(16)}$ = 46.593, p < 0.001) (Figure 5.2, Table 5.3).
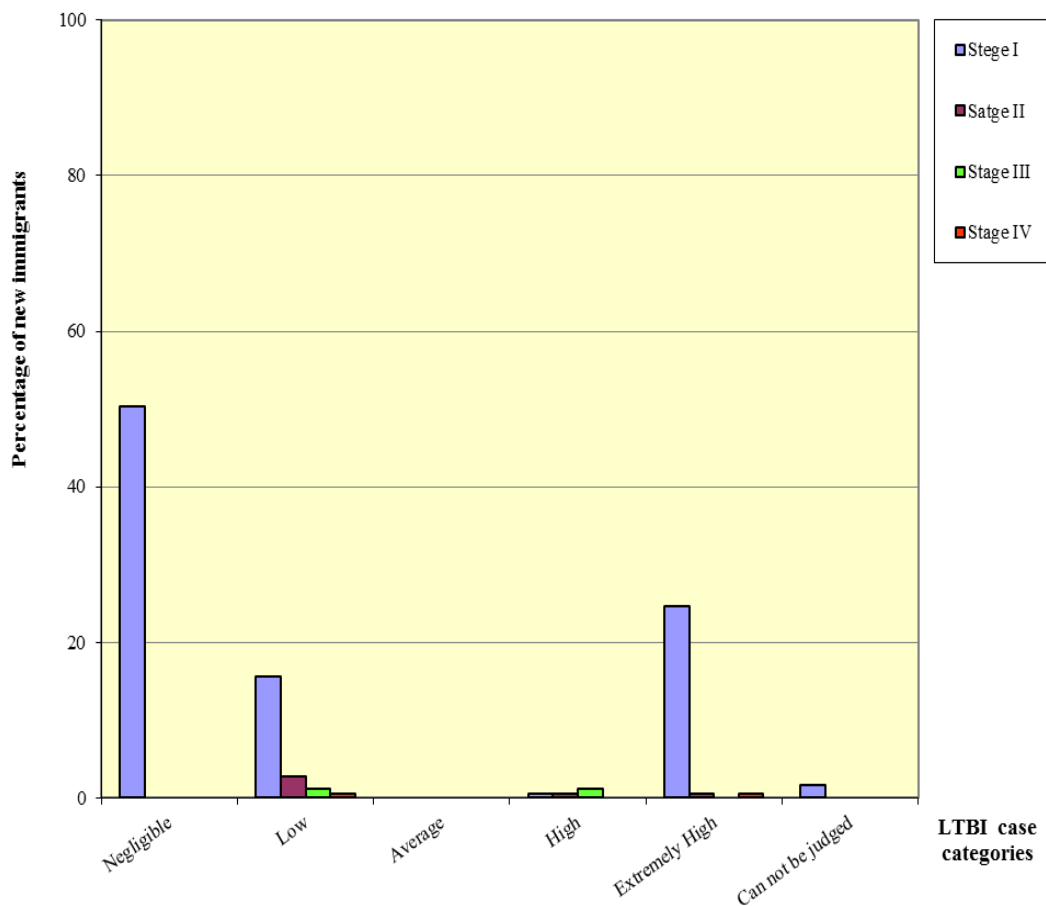
Figure 5.2: Chest X-ray interpretation of radiologist A according to stage scoring system and latent tuberculosis infection (LTBI) categories of 179 new immigrants to Kuwait during February and May 2010

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By contrast radiologist B's final interpretation reveals 'normal' CXR results in 93.33% and 'abnormal' CXR findings were detected in 6.11% (11/180). The details of these abnormal cases were as follows; 'low' LTBI suspicion in 2.79%, 'high' LTBI in 1.12% and 'extremely high' in 2.24% (LLR $\chi^2_{(8)}$ = 28.823, p < 0.001) (Table 5.3).

Concerning LTBI scoring, radiologist B only found cases with score II in 1.11% and score III in 5.00% with no score IV cases. Radiologist B interpreted more score III abnormal findings, and which was in common described as nodule more than 5-mm and/or fibrotic infiltrates more than 1cm (LLR $\chi^2_{(12)}$ = 29.751, p = 0.003) (Figure 5.3, Table 5.3).
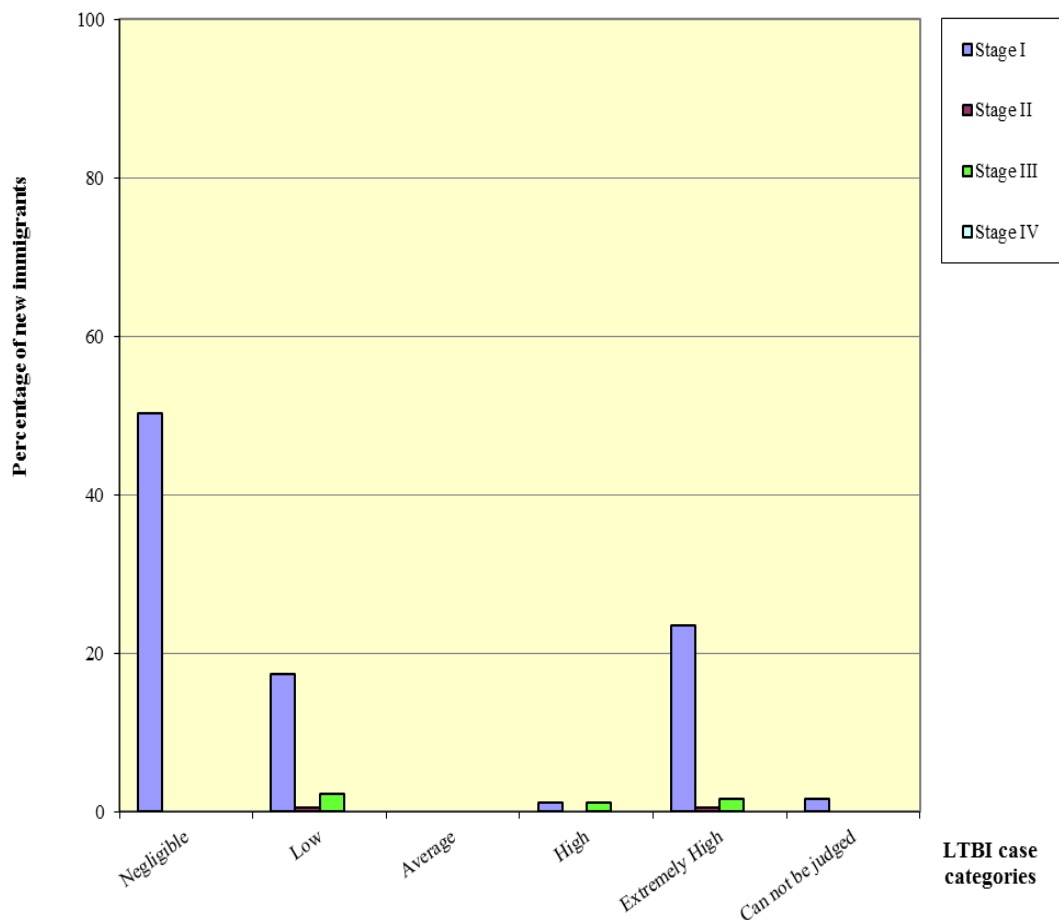
Figure 5.3: Chest X-ray interpretation of radiologist B according to score staging system and latent tuberculosis infection (LTBI) categories of 179 new immigrants to Kuwait during February and May 2010

5.1.6.3 CXR reader agreement

Based on the dichotomous LTBI classification (TB-related result), kappa agreements were calculated as follows (Table 5.4).

Table 5.4: Inter-reader's agreements of chest X-ray interpretation for 179 new immigrants to Kuwait during February and May, 2010

Kappa value	TCU	Pulmonologists (3)	Radiologist A	Radiologist B
Pulmonologists (3)	1.00	---	---	---
Radiologist A	a	a	---	---
Radiologist B	a	a	0.505	---

[a] = cannot be computed by SPSS 17.0 Software, (---) = already present, TCU = Tuberculosis Control Unit staff, Pulmonologists (3) = three respiratory specialists

CXR films, with the exception of one pregnant female, were interpreted as normal results by both TCU staff and the three pulmonologists with 100% concordance (kappa = 1.00). The level of agreement in terms of CXR diagnostic outcome was different. Both radiologists were more likely to give a positive diagnosis in identification of LTBI prevalence having 'moderate' inter-reader agreement (k = 0.505) (Altman, 1991).

Diagnostic lesions of LTBI suspects such as fibrotic infiltrates of more than 5 mm and nodular size of more than 1 cm were the most common forms of parenchymal lesions, and particularly in the lower lobes of the right lung fields were differently detected and interpreted by all the radiographic readers', but with different final scoring of both lung lesions as follows; pulmonologist A (LLR $\chi^2_{(16)}$ = 15.193, p = 0.511), pulmonologist B (LLR $\chi^2_{(24)}$ = 16.655, p = 0.863), pulmonologist C (LLR $\chi^2_{(24)}$ = 19.321, p = 0.735), radiologist A (LLR $\chi^2_{(52)}$ = 58.328, p = 0.254) and radiologist B (LLR $\chi^2_{(40)}$ = 41.091, p = 0.423). No calcified nodules were detected by any of the five film readers'.

5.1.7 Discussion

Chest radiography provides useful information regarding disease extent and progress without an agreed-upon, validated system regarding the severity of CXR abnormalities. The purpose of this research is to introduce a scoring system to improve the radiographic diagnostic performance/detection of LTBI and TB suspects. Clear documentation of adequate diagnosis could potentially reduce the number of unnecessary entries, which would make TB prevention programmes and CXR reading more efficient and cost-effective. The goal of performing CXR in the TB screening programmes prior to employment and after entry of the immigrant's is to search for chest radiographic findings suggestive of active TB or evidence of LTBI lung changes. Abnormal radiographic finding's were fibrotic non-calcified lesions of 2 cm and larger, or large cavitation(s) more associated with MTB transmission (Eisenberg and Pollok, 2010; Lin *et al.*, 2008). Our research finding still revealed nodular sizes more than 1 cm as indicative of prior MTB exposure. Our research finding revealed that more than 1 cm is still indicative of prior MTB exposure.

Leakage of suspected TB cases due to missed diagnosis were detected by our research findings and such leakage is known limiting factor of TB control for decades all over the world. Similar findings were reported by (Barnes *et al.*, 1988; Feingold, 1977).

5.1.7.1   Scoring system

Testing helpful predictors of the presence or absence of LTBI aids construction of a TB predictive model using meaningful CXR LTBI scores, in addition to other diagnostic tests, and epidemiological risk factors related to past TB clinical history (Cohen *et al.*, 1996). The benefit of a combined scoring system is detection of LTBI/TB suspects better than readers' experience. It predicts abnormal changes frequently compared with the biased expected lung changes of one reader and which also affected by inter- and intra-rater differences (usually fair or poor agreement). Severity risk factor measures are needed to reduce the limitation factors such as

nationality and past history of TB infection or smoking. Solari and colleagues' analysis of covariates reveals that patients with previous episodes of PTB are more likely to present hemoptysis and cavities on the CXR even in the absence of MTB (Solari *et al.*, 2008).

The research introduces a new four point scoring system, using scores II, III, and IV, and which represents abnormal CXR results consistently with suspicion of LTBI pathologies. The system was able to show correlation with baseline epidemiologic (socio-demographic) factors and other diagnostic test results. Scoring changes is important to decide before and follow-up after LTBI prophylaxis and active TB management, which improve early diagnosis and prediction of MTB harboring subjects. Similar to our findings, multiple raters' also permit reduction of under- and over-diagnosis of abnormal lung fields (Ralph *et al.*, 2010; van Cleef *et al.*, 2005).

5.1.7.2   Chest X-ray interpretation

Specific abnormal CXR changes for definite diagnosis of LTBI/TB is still absent. Research studies adopt a screening criterion for specific definition of 'TB suggestive CXR findings' had difficult application, similar to the majority of those bacteriologically confirmed cases were belonging to 'non-TB suggestive CXR abnormality' (WHO, 2011c).

Subjective reader interpretation and related miss-diagnosis and miss-leading of LTBI/TB management is restrictive barriers for TB control. The need for a national or universal international standard system for reporting CXR in LTBI and pulmonary TB is acknowledged by the health care systems. Fibrotic lesions of less than 5 mm are defined as well-delineated radiographic lesions compatible with non-endemic regional 'healed' tuberculosis, but not in endemic countries (< 1 cm) (ATS, 2000).

CXR interpretations represent readers' subtlety but still are questionable. Familiarity of TB disease and interpretation experiences of chest radiographic findings are both crucial for LTBI/TB diagnosis which limits the community population with threatens of infection transmission. Our thesis research findings greatly suggest that chest radiographs read by non-radiologists are of extremely low achievement in TB

screening programmes. A recent similar conclusion also has been reached by Eisenberg and Pollock (2010).

'Intentional over-reading' is defined as CXRs read by interpreters who are unable to classify radiographic films as normal (or considered wrongly as abnormal) (WHO, 2011c).

Interpretations for abnormal CXR findings by two independent readers reveal statistical significance in achieving more accurate findings (Gopi *et al.*, 2005). All radiological screening programmes are subject to interpretation error. Experience and the number of interpreters have been shown to affect interpretation. Standardized interpretation should be a goal of all screening programmes using CXRs. Zellweger and colleagues (2006) demonstrated that experience in reading CXRs for possible active TB among asylum seekers can play a significant role in the reproducibility of the reading. Although not done within an immigrant TB screening programme, it has been suggested that TB specialists had better interpretation accuracy in detecting TB related abnormalities. If films are digitized at each site and transmitted electronically to a central reading site, all interpretation can be done by a designated panel of TB radiology experts with an increase in the accuracy of diagnosing active and inactive PTB (Zellweger *et al.*, 2006).

Even though there is confusion among radiologists and clinicians regarding the actual definition of an 'abnormal chest radiograph consistent with prior TB', the American Thoracic Society and the Centers for Disease Control and Prevention still recommend CXR fibrotic infiltrates more than 5 mm (not 10 mm) in non-endemic countries as positive result with high re-activation risks requiring further testing's. For example the ATS and CDC (ATS, 2000) stated that both 'nodules and fibrotic lesions' (with no comments on their size) suggest increased TB re-activation risk compared with opposite findings of other published results (Jasmir *et al.*, 2002).

Inter-rater agreement on cavitations and confluent consolidations are interpreted with higher agreement and are associated with higher AFB density in sputum of pulmonary TB patients and worse lung function than for non-cavitary disease (Palaci *et al.*, 2007; Raplh *et al.*, 2010).

The typical findings of re-activation of LTBI disease characteristically include cavitations and/or infiltrates in the lower parts of upper lobe and/or within the upper parts of lower lobe lung fields, below hilar and parahilar regions (Farman *et al.*, 1986). Cavitary lesions give rise to greater clinical suspicion than alveolar infiltrates and mass lesions in asymptomatic subjects who might suffer of average delays of one month before LTBI/TB diagnosis and treatment (Wu *et al.*, 2009). Size of cavitations' can be used for discriminate accuracy as the best new marker and advantageous without aids for CXR assessment (Ralph *et al.*, 2010). Nevertheless, no single interpreted CXR of the participants revealed any LTBI diagnostic lesion (cavitations') even in those with positive IGRAs result.

The chest X-rays showed high frequencies of fibrotic infiltrates and nodular calcifications characteristic of LTBI radiographic findings for an early compatible primary infection (Lee *et al.*, 2011). Other common radiographic abnormalities of PTB patients on/without treatment are cavity formation, lobar/segmental consolidation and bilateral involvements (Feng *et al.*, 2011b). On the contrary, our research findings were significantly associated with the inability to detect most of these common LTBI abnormal changes by all five film readers'.

Calcified nodular lesions and calcified mediastinal lymph nodes (and enlarged size) represent a common main site of MTB in latent tuberculosis, and is considered as an old, healed tuberculosis lesion with high re-activation risks (Lee *et al.*, 2011). Radiologists could accurately interpret the nodules as an LTBI risk factor. Similar findings were also found by Eisenberg and Pollok (2010) and Horsburgh *et al.*, (2010).

Similar to the research radiologists interpretation, Graham and colleagues (2002) obtained the same inter-reader's agreement (k = 0.53, 'moderate'= 0.40-0.60). Our radiographic findings emphasized chest radiographic evaluation by experienced CXR radiologists and TB specialists. Similar approaches and findings were obtained by Richard *et al.* (2002).

Reducing CXR inter-reader variability is facilitated by interpretation by at least two or three certified radiologists. The contradictory findings of sharing medical

pulmonologists' and radiologists' add little CXR performance to be considered as a diagnostic test in excluding LTBI or prior history of TB affection, which had proved similarly by Eisenberg and colleagues. For example, confusion is common regarding the definition of 'abnormal CXR consistent with prior TB' and the relative significance of non-calcified fibrotic lesions of varying sizes. The findings greatly suggest that single test-strategy using only chest radiograph yield low positive finding's in LTBI/TB immigration screening and/or in pre-employment setting (Eisenberg and Pollok, 2010).

5.1.7.3 Chest X-ray radiography

A CXR result depends on the intensity and presentation of TB disease, which in turn is influenced by a range of other factors such immuno-suppression and atypical findings e.g. HIV/AIDS. Other factors -such as CXR film (quality), functioning CXR machine and the reagents in addition to reader experience and interpretation skills making CXR subject to intra- and inter-reader variation - delay PTB diagnosis. The degree of readers' result concordance, inter-reader variability and LTBI sensitivity are highly dependent on the quality of the films. Radiography and CXR is also unable to distinguish 'smear-positive TB' from 'smear negative TB', whereby all factors can contribute to interpretation and therefore over- and under-diagnosis (van Cleeff *et al.*, 2005; WHO, 2011c).

The useful of CXR in providing information regarding lung shadows and disease extents is still not enough due to lack of a standard and validated grade system for the severity of radiographic abnormalities even in bacteriologically proved PTB.

One postero-anterior radiographic (PA) view is sufficient for TB screening of pre-employment individuals instead of the lateral (LA) view, the latter providing no additional diagnostic value (Eisenberg *et al.*, 2009), but still, chest radiography yield low level of changes in detection of LTBI, and provide no assistance to prioritize a decision for LTBI treatment (Eisenberg and Pollok, 2010). Also elimination of the acquisition of LA for screening of asymptomatic individuals never caused radiologists to change decisions made on the PA view and would therefore reduce radiation exposures (Eisenberg *et al.*, 2009).

5.1.8 Study strengths and limitations

The research study is innovating new suggestions such as:

1. Introduction of a high-quality CXR recording sheet (Appendix 6).
2. Multiple interpretations from various independent chest radiographic readers', of TB-related specialists and radiologists, and simultaneously compared to results issued by the authorized staff of Tuberculosis Control Unit.

The research study faces barriers such as absence of standardized definition to ascertain radiographic appearance of LTBI.

5.1.9 Conclusions

Due to lack of a universal standard and accepted reporting system of chest radiography, then:

1. Review of published guidelines regarding specific radiographic findings of re-activation risks provides no assistance to prioritize LTBI diagnosis/treatment.
2. Chest X-ray is a weak screening tool for detection of abnormal TB-related and latent tuberculosis findings, with consequently,
3. Missing diagnosis of latent tuberculosis and entry to the country (leakage) of LTBI and suspected TB immigrants.
4. The inherent problem of inter-rater differences in radiographic assessment and the low rates of clinician-radiologist agreement between reporters on CXR findings can be solved.
5. Chest radiography provides no assistance in deciding which individuals to prioritize for LTBI chemoprophylaxis unless supported by another specific diagnostic test such as IGRAs which need introduction of a new well-defined scoring system.

5.1.10 Recommendations

Chest radiography diagnostic performance can be improved by the following:

1- Use of the new, simple and well-defined CXR scoring system which is fundamental for higher correct diagnosis of LTBI/TB. Also the scoring system should be revised after implemented on similar study on large sample size of high risk group.

2- Chest radiographic readers need consensus radiographic training to reach maximum inter-rater agreements.

3- Radiologists' should be part of CXR decision-makers for TB reporting. An effort to harmonize interpretation and chest X-ray reporting is needed.

5.2 Tuberculin Skin Test

The second standard diagnostic test for latent tuberculosis infection is the tuberculin skin test (TST).

5.2.1 Introduction

Detection and monitoring of tuberculosis infection is essential to control its spread. Targeted tuberculin skin testing and drug therapy to prevent latent *M. tuberculosis* infection from progressing to overt disease are important TB elimination strategies, particularly in low-incidence countries. The tuberculin skin test has been used effectively for detection of previous MTB infection and LTBI for more than 100 years, despite few drawbacks such as variability and subjectivity in test application and reading, and false positive or false negative results.

5.2.1.1 Tuberculin skin test history

The tuberculin skin test was first described by Robert Koch in 1890, but developed by Charles Mantoux, a French physician in 1907 who gave the test its eponymous name, or PPD for Purified Protein Derivative. Tuberculin is a glycerol extract of the bacillus, with PPD introduced in 1934 in its standardized version. Later, in 1939, PPD-S was produced by Seibert and Glenn, and remains as the standard dose of 5 tuberculin units (0.1ml) is injected intradermally. In the UK, Mantoux test used different type of 2TU (tuberculin RT23) of SSI {Staten Serum Institute} dissolved in 0.1ml solution (CDC, 2011; MoH, 2005).

5.2.1.2　Immunological reaction

The TST (skin test reagent) reaction for diagnosis of mycobacterial infection is caused by a localized delayed-type hypersensitivity (DTH) versus the injected PPD at the site of injection, which are tuberculin's composed of a crude mixture of heat-denatured proteins derived from cultures of *M. tuberculosis*. The relationship of PPD responses to the immune T-lymphocytes' release interferon gamma (INF-γ) in peripheral blood after presentation to MTB antigens remains unclear. BCG vaccination alters the interpretation of a positive PPD, and therefore TST interpretation depends on the presence or absence of previous BCG.

5.2.1.3　Tuberculin skin test interpretation

Manifestation of a positive TST represents reactive type-IV delayed hypersensitivity reaction. TST induration between 5- and 10-mm can persist for up to 25 years after BCG vaccination (Burl *et al.*, 2010). The interpretation of the test is based on an individual's epidemiological risk factors for TB infection and disease progression. There is an inherent relationship between PPD and local prevalence of LTBI. Geographic and racial differences can determine exposures to mycobacteria other than *Mycobacterium tuberculosis* (NTM) and the host immune status. TST conversions can be false positives in a low-risk travel population, even in absence of exposure to MTB (Freeman *et al.*, 2010). A common confounder, which affects TST reactivity, is prior vaccination with the compulsory Bacille Calmette-Guérin vaccine, the strain and dose of BCG used, and method of vaccine administration or age of vaccination. TST cannot be positive in certain biological factors due to suppression of type-IV delayed hypersensitivity and T-lymphocytes such as malignancies and viral infections (e.g. HIV) (LoBue *et al.*, 2010). Chan and colleagues (2008) concluded in their longitudinal study of 783 children in Taiwan vaccinated with BCG at birth that 10-mm induration should be an optimal 'cut-off' for children younger than 7 years of age, with 21-mm being more appropriate for the first year of life compared with only 5-mm 'cut-off's in non-endemic countries.

5.2.1.4　Merits of the tuberculin skin test

In addition to the routine diagnostic and screening methods, the tuberculin skin test is still used for the last 100 years worldwide (NICE, 2006). TST is still the most common technique for diagnosing LTBI infection, even among BCG-vaccinated populations, because of low cost, easy application and interpretation, and is still used to screen contacts of active TB patients and other populations at high TB risk (ATS, 2000). TST use is based on purified protein derivative (PPD), is inexpensive and easy to perform for identification of MTB-infected subjects.

TB skin testing at immigrant entry centres that serve large foreign-born populations can be effective. Li and colleagues (2010) detected LTBI prevalence of positive TST in 24.4% (higher among foreigners) in BCG-vaccinated which help to target TST testing before immigrant's chemoprophylaxis.

TST is currently a standard and reliable test for immune-diagnosis of TB infection (Lin *et al.*, 2008). For persons with LTBI having normal immune responsiveness, TST sensitivity can approach 100%. The specificity is approximately 99% in populations with less antigenic cross reaction in those without history of BCG vaccination or exposures to other mycobacterial organisms (ATS and CDC, 1999). Positive TST in contacts of a TB patient is indicative of LTBI and was detected (2.4 times higher) than in contacts without history of exposure to TB patient was observed by Lin and colleagues (2008). Controlling TB in the US follows reports of the Institute of Medicine which recommends compulsory TST for all immigrants and treating LTBI prophylactically before US entry (legal green card). Hill and colleagues concluded their study on 2,348 household Gambian contacts to sputum smear TB diagnosed patients that either positive TST or T-SPOT *.TB* test was sufficient indication for preventive treatment without replacement of the Mantoux test by an ELISPOT test (Hill *et al.*, 2008).

5.2.1.5 Disadvantages of the tuberculin skin test

The TST is inadequate for a confident diagnosis or exclusion of LTBI/TB infection (low sensitivity and specificity), but is currently used as the standard second choice after abnormal CXR to detect LTBI even without differentiating past and present TB infection. TST sensitivity (which is poor in children) is affected by several factors

such as the interval post-infection, desensitization and observer variation, whereas, the specificity (which is poor in adults) is influenced by previous sensitization (result of exposure to *M. avium*, *M. paratuberculosis* and environmental mycobacteria) and by skin tuberculosis (Monaghan *et al.*, 1994). Independent risk factors for a positive TST reaction in household contacts were detected more in those having longer affection of TB index case and suffer from treatment delays (Lin *et al.*, 2008).

Uncertainty of past history of previous BCG vaccination might affect the interpretation of TST reactions and the likelihood options of the effective treatments. Factors affecting TST reaction are commonly related to two factors; vaccine and dose (some contain bacillary virulence and produce different sensitivities) and time of TST reading (Horwitz *et al.*, 1972). A meta-analytical study proved that previous BCG administration increased the likelihood of TST false-positive results up to 15 years post-vaccination (Richeldi, 2006) while interferon gamma release assay (IGRAs) results are independent of BCG vaccination (discussed in chapter 1 section 1.4.2; IGRAs). TST is not helpful in identifying (if positive reaction) or excluding (if negative result) latent TB infection in patients taking immune-suppressive treatments (Mankia *et al.*, 2011).

5.2.1.6   Contradictory responses
5.2.1.6.1   False-positive reactions

False-positive reaction can be defined as positive TST induration with no history of infection with *M. tuberculosis*. The causes of false-positive results can include, but not be limited to, infection with non-*Mycobacterium tuberculosis* (especially in low prevalence non-endemic countries) or previous BCG vaccination or prior TST testing (Farhat *et al.*, 2006). Two-step TST testing measures conversion reading (negative-to-positive reaction) of more than 72 hours but is less reliable in BCG vaccinated individuals (Lalvani *et al.*, 2001b; Tat *et al.*, 2005). Another confounding factor is tobacco smoking, associated with positive TST reaction in LTBI cases and need to be further studied (den Boon *et al.*, 2005).

5.2.1.6.2 False-negative reactions

Absence of a TST reaction in persons infected with *M. tuberculosis* can result from; cutaneous anergy, recent TB infection (within 8-10 weeks of exposure), very old TB infection, very young age (less than 6 months old), recent live-virus vaccination (such as measles) or recent viral illnesses (such as hepatitis and chickenpox), or overwhelming TB disease. Also incorrect TST administration and reaction interpretation can also reduce a negative result (Bergamini *et al.*, 2009; Jong Lee *et al.*, 2010). For example the Heaf test was discontinued in UK after observed of small test head fragments were found at the injection sites (Zellweger *et al.*, 2006). Low rates of positive TST might be associated with under-estimation and subjective interpretation, which should rely on the readers' experience (Chang *et al.*, 2010). Hardy *et al.* (2010) showed that a higher cut-off point for a positive TST (> 15-mm) lead to missing cases of LTBI

5.2.1.6.3 Booster phenomenon

Skin test conversion from primarily negative TST reactions to positive result due to loss of early immunologic reactivity to MTB. This can represents an immunologic boosting phenomenon of previous MTB infection after subsequent repeated TST within short period of time (2-24 weeks). The initial test stimulates the immune system of a previously infected with MTB due to the ability of their immune system to react against the second test, known as 'booster effect'. The 'two-step method' to be repeated in one to three weeks is recommended (Al Mazrou, 2004; CDC, 2010b). Boosting can even occur two years after the first TST.

5.2.2 Aim

To assess the accuracy of tuberculin skin test (the second standard tuberculosis diagnostic test).

5.2.3 Objectives

To assess the diagnostic performance of TST in diagnosing LTBI in healthy humans with absence of a reference standard test as:

1. Evaluate the performance of tuberculin skin test in diagnosis of LTBI and 'suspect TB' cases
2. Introduce a new recording sheet for tuberculin skin test evaluation

5.2.4 Methodology

The skin was injected with 0.1 mL (5 tuberculin units [TU]) of Tubersol® (Connaught Laboratories, Willowdale, Ontario, Canada) using the Mantoux method to the 180 participants' volar forearm. The diameters of the resulting wheal reactions were measured within 48 and 72 hours after puncture (not more than 5 days) after application. Induration size on the volar forearm was measured in millimetres (mm) using a flexible reading ruler. All the results obtained were double-interpreted (nurse and researcher) and recorded as either positive (if ≥ 10-mm) and negative result (< 10-mm). Both body weight and body height were measured to calculate body mass index (BMI). Arms were checked for the presence of a BCG scar.

5.2.4.1 Scoring criteria of tuberculin skin test reaction

Following the Ministry of Health (Kuwait) and the American Thoracic Society and Centers for Disease Control and Prevention guidelines (ATS and CDC, 2000), a TST response was defined as a skin test reaction to antigens (PPD) derived from *Mycobacterium tuberculosis*. A positive TST was defined as an induration reaction to PPD of more than or equal 10-mm cut-off point for LTBI diagnosis. The TST result was confirmed after double interpretation by both the nurse and the researcher. Any adverse reactions at the injection site during TST manipulation and result interpretation were recorded. All results were been documented in the TST recording sheet by the nurse (Appendix 7). The LTBI scoring system was graded according to Mantoux reaction size induration (mm) (Table 5.5).

Table 5.5: Tuberculin skin test response and scoring system of latent tuberculosis infection reaction (ATS and CDC, 2000; CDC, 2005c; CDC, 2010b, MoH, 2010)

Mantoux response at 48-72 hours (until 120 hours)	Score Grade	LTBI Reaction
0	0	Negative
<5	1	Reactive
5 - <10	2	Borderline
10 - <15	3	Positive
15 - <20	4	Highly positive
> 20	5	Definite

A positive TST reaction is defined as any reaction of more than or equal 10-mm ($\geq$ 10-mm) of induration in recent immigrants (within last 5 years) from high-prevalence countries. A reaction of more than 5-mm (> 5-mm) of induration was considered positive in those coming from non-endemic countries and/or with a history of recent contacts of infectious TB cases and/or persons with fibrotic changes on chest radiographs consistent with prior TB. Also a reaction of more than 15-mm (> 15-mm) of induration was accepted as positive in immigrants with no known risk factors for TB (ATS and CDC, 2000; CDC, 2005c; CDC, 2010b)

5.2.5 Results

98.33% (177/180) fulfilled the inclusion criteria for TST results. The TST results of three excluded immigrants were as follows: two participants came on day 7 (Indian electrician male and Nepali housemaid female) and one participant did not return for TST reading (Ethiopian housemaid female) (Table 5.6).

Table 5.6: Results of tuberculin skin test performed to 180 new immigrants to Kuwait during February and May, 2010

TST result	Number of immigrants (%)
Included TST within 24-72 hours (until 120 hours)	177 (98.33%)
Excluded TST (follow-up after 5 days)	2 (1.11%)
Excluded TST (lost follow-up)	1 (0.55%)

A modified 'cut-off' point value of TST induration was proposed for classifying TST results into cutaneous grading and scoring system of LTBI diagnosis in healthy adults. The frequency of LTBI reactions in the new immigrants including the three missed follow-up participants are shown in Table 5.7.

Table 5.7: Skin test response and score frequency of latent tuberculosis infection of the 180 new immigrants to Kuwait during February and May, 2010

Mantoux response (mm)	Score grade	LTBI diagnosis	Final result	n (%)
0	I	Negative (no reaction)	Normal	170 (96.04%)
1 - < 5	II	Normal (reactive)	Normal	6 (3.38%)
5 - < 10	III	Borderline	Normal	1 (0.05%)
10 - < 15	IV	Positive	Abnormal	0 (0.00%)
15 - < 20	V	Highly positive	Abnormal	0 (0.00%)
> 20	VI	Definite	Abnormal	0 (0.00%)
Excluded	-	Cannot be judged	-	3 (1.66%)

Modified 'cut-off' point value of TST induration was more than 10-mm, score (-) = no score for excluded case (due to missed follow-up or result reading after 5 days), mm = millimetre induration (diameter size swelling)

Applying the tuberculin skin test responses' according to the LTBI case category classification showed the significant statistical differences as shown by the different p-values in the following results (see Table 5.8).

Table 5.8: Distribution of tuberculin skin test results according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

Tuberculin Skin Test		Negligible (n=90)		Low (n=36)		Average (n=0)		High (n=4)		Extremely High (n=46)		Can not be judged (n=4)		Total (n=180)		Likelihood ratio	p-value
		n	%	n	%	n	%	n	%	n	%	n	%	n	%		
TST follow-up	Normal	90	100.00	36	100.00	0	0.00	4	100.00	46	100.00	1	25.00	177	98.33	LLR $\chi^2_{(4)}=$ 26.017	<0.001
	Missed	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	3	75.00	3	1.67		
TST score	Score I (<5)	90	100.00	36	100.00	0	0.00	3	75.00	46	100.00	1	25.00	176	97.78	LLR $\chi^2_{(8)}=$ 33.865	<0.001
	Score II (5-9)	0	0.00	0	0.00	0	0.00	1	25.00	0	0.00	0	0.00	1	0.56		
	Missed	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	3	75.00	3	1.67		
TST cutaneous induration size (mm)	0 (Negative-No reaction)	85	94.44	36	100.00	0	0.00	3	75.00	45	97.83	1	25.00	170	94.44	LLR $\chi^2_{(12)}=$ 37.947	<0.001
	<5 (Reactive-Normal)	5	5.56	0	0.00	0	0.00	0	0.00	1	2.17	0	0.00	6	3.33		
	5-9 (Reactive-Borderline)	0	0.00	0	0.00	0	0.00	1	25.00	0	0.00	0	0.00	1	0.56		
	Missed	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	3	75.00	3	1.67		
TST adverse reaction	No	90	100.00	36	100.00	0	0.00	4	100.00	46	100.00	1	25.00	177	98.33	LLR $\chi^2_{(4)}=$ 26.017	<0.001
	Missed	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	3	75.00	3	1.67		

Average LTBI category is zero (means no detected cases), Missed = excluded case due to missed follow-up or came after 5 days for TST result reading

The comparison between TST follow-up results (normal versus missed cases) according to LTBI case categories showed a statistically significant difference (LLR $\chi^2_{(4)} = 26.017$, p < 0.001) (Table 5.8). Distribution of the TST stratified cutaneous induration size according to the defined LTBI case categories revealed the following: healthy participants having normal results with no skin reaction (zero mm) were 94.44% (170/180), in comparison to participants who had small reactive reaction (between 1 and < 5-mm) in 3.33% (6/180) or those having normal response with borderline reaction (between 5 and < 10-mm) in 0.56% (1/180) (one Sri Lankan housemaid female) (LLR $\chi^2_{(12)} = 37.947$, p < 0.001) (Figure 5.4, Table 5.7 and Table 5.8).
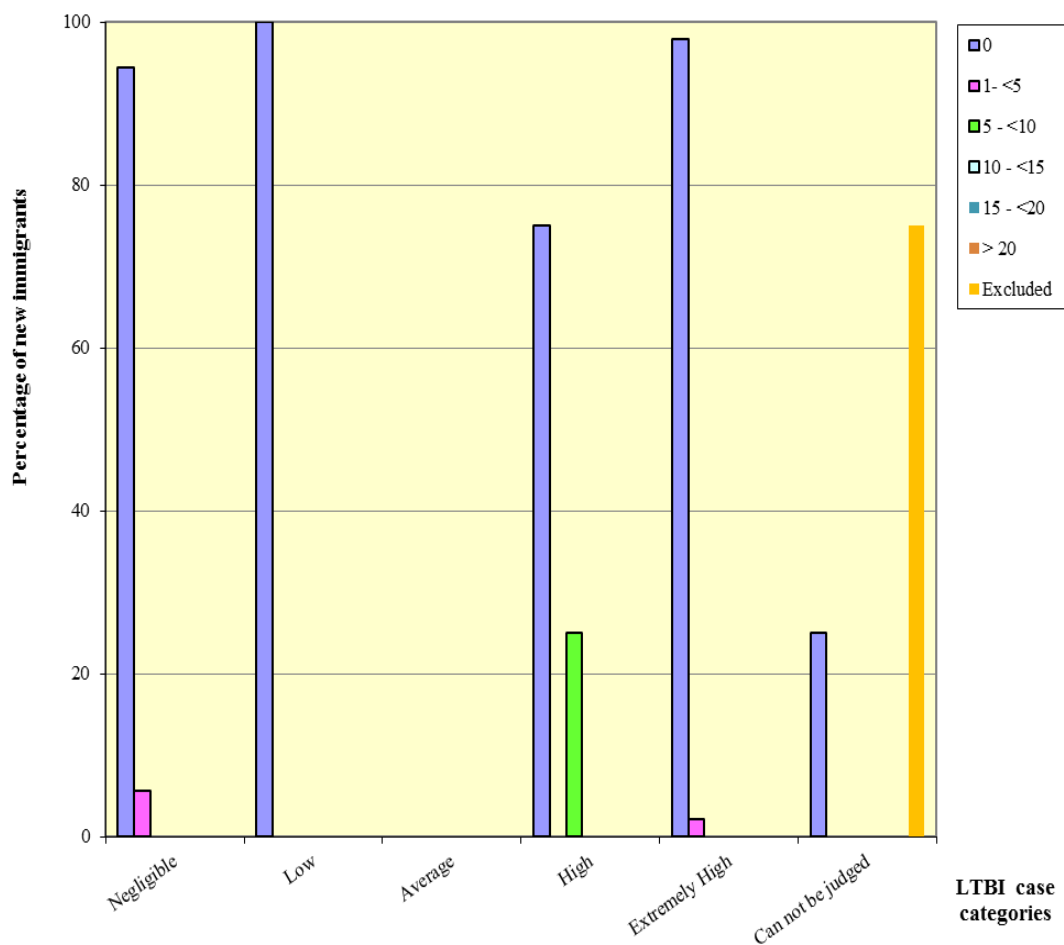
Figure 5.4: Distribution of tuberculin skin test reaction and cutaneous induration (millimetre) according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

According to the Mantoux reaction, the prevalence of LTBI in all the included new immigrants was zero% (0/177) using the ordinary TST method. Applying the classification criteria of LTBI, all participants were belonging to the normal results of score I (zero = negative reaction) and score II (= < 5-mm induration) in 97.78% (176/180). Immigrants had score III (5-9-mm induration) were 0.56% (1/180) with no observed positive abnormal reaction above the modified 'cut-off' value (> 10-mm) (LLR $\chi^2_{(8)}$ = 33.865, p < 0.001) (Figure 5.5, Table 5.7 and Table 5.8).
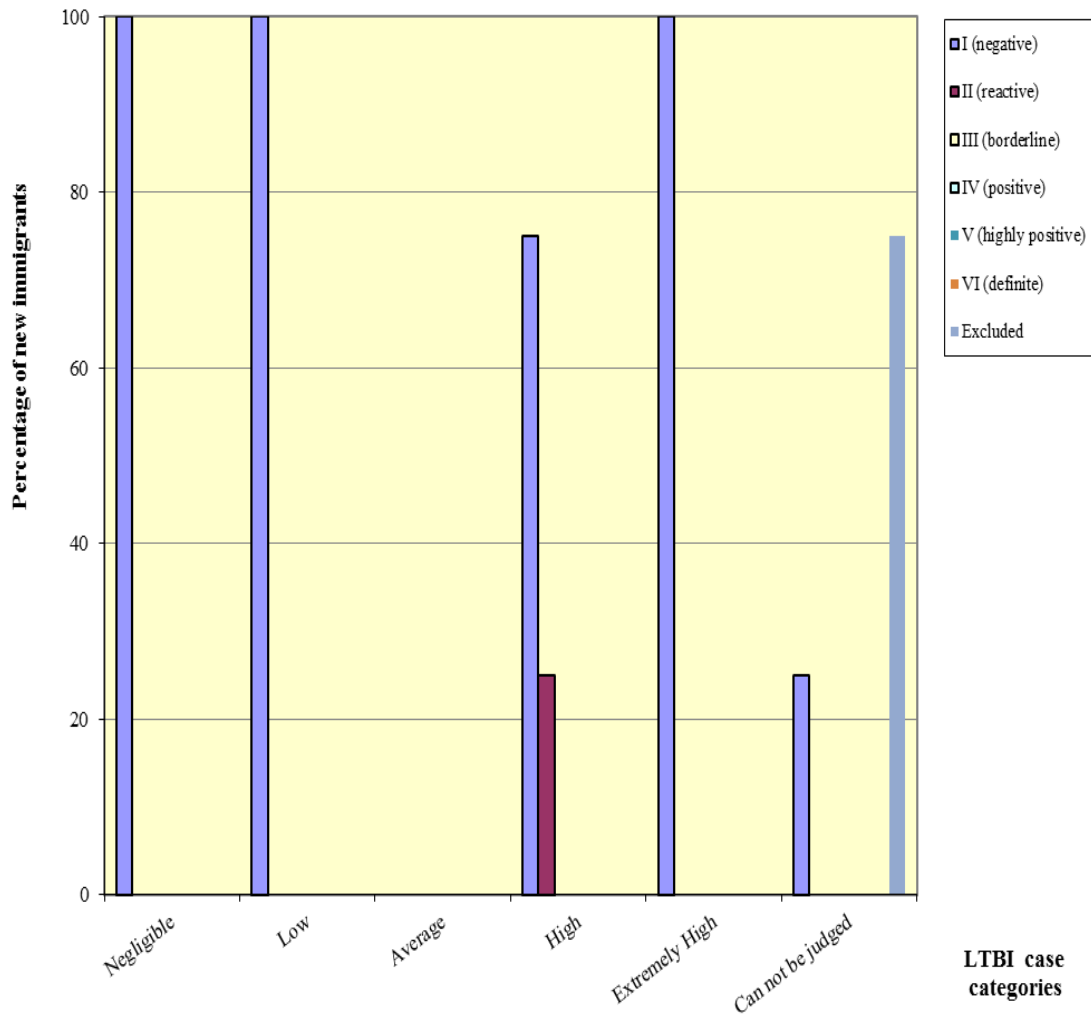
Figure 5.5: Distribution of tuberculin skin test score according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

Absence of adverse reactions was noticed in all participants due to proper administration of TST by the head nurse and the research strictly addressed notification of any future complications or swelling reactions and enforce of further follow-ups (LLR $\chi^2_{(4)} = 26.017$, p < 0.001) (Table 5.8).

5.2.6 Discussion

The TST has been the only established screening method for tuberculosis for over a century. Although the TST is inexpensive, easily available and accessible and is the preferred test in most TB-prevalent settings, it has recognized limitations, including subjective interpretation, false positivity, cross reactivity with non-tuberculous mycobacteria, administration errors and the requirement for two visits. In addition to these limitations, low diagnostic accuracy and the unavailability of better screening tests in resource-limited settings makes MTB transmission difficult to control. Still the diagnosis of latent TB infection is currently based on the century-old tuberculin skin test even though a positive reaction can result from previous infection by MTB, BCG vaccination or cross-reactions with nontuberculous mycobacteria. Used as the second choice standard test after CXR, TST results help clinicians to estimate accurately the risk of TB re-activation for patients and reinforce treatment adherence for LTBI (Horsburgh *et al.*, 2004).

The TST cannot distinguish between previous BCG vaccination and TB infection due to poor specificity in BCG-negative people and poor sensitivity in children. A meta-analysis proves that previous BCG administration increases the likelihood of TST false-positive results up to 15 years post-vaccination (Richeldi, 2006) while the interferon gamma release assay results are independent of BCG vaccination (will be further discussed in chapter 6). Similar to these results Chang *et al.* (2010) concluded that an increased TST reaction was associated with previous BCG vaccination. Meta-analysis of 56 studies reveals similar findings of a higher reaction risk versus 5 TU PPD (Wang *et al.*, 2002). With the exception to those who have never been vaccinated TST reactions of up to 18-mm in diameter are likely to be the result of prior vaccination, rather than old TB infection and no need for preventive chemotherapy (Tissot *et al.*, 2005). BCG vaccinated individuals above the age of one

year produce more frequent, more persistent and larger TST reactions (Farhat *et al.*, 2006).

The effect of BCG on the TST reaction is observed mainly in the first years after BCG administration. Previous BCG vaccination and the boosting phenomenon were significantly associated with positive TST and reactions miss-interpretations (Chang *et al.*, 2010; Wang *et al.*, 2002). The proportion of prior BCG-vaccinated individuals having positive TST results reaches 90% (Horwitz *et al.*, 1972). TST induration between 5- and 10-mm persists for up to 25 years after BCG vaccination, depending on several factors; 1- strain and dose of BCG used, 2- method of vaccine administration, 3- the timing of vaccination, 4- number of BCG vaccinations administered, 5- cross-reactivity with other environmental mycobacteria, 6- age of vaccination and other associated factors such as nutritional status and genetic factors (Burl *et al.*, 2010). A positive TST result in children below 5 years of age, especially if they are not BCG-vaccinated, must be taken as evidence of TB infection (Mendez-Echevarria *et al.*, 2011). The presence of a false negative TST because of poor T cell function can be detected in small children.

In contrast to previous findings, my results of tuberculin skin tests showed no statistically significant association between previous BCG vaccination and presence of a BCG scar in relation to LTBI categories. A history of BCG vaccine is not a contraindication for tuberculin skin testing or treatment for LTBI in persons with positive TST results. TST reactions should be interpreted regardless of BCG vaccination history (CDC, 2010b). Absence of positive TST reactions in our study strengthens the inference that BCG is not interfering with TST interpretation even though 86.11% (155/180) of participants had already been vaccinated against TB with BCG around pre-school ages (discussed further in the next section 5.3; BCG). Similar related finding we rerecorded by Minodiier and colleagues (2010), namely that a positive TST is more likely to be related to an increased duration of TB exposure in the TB-endemic country of birth rather than to previous BCG vaccination.

The TST should not be used to overlook CXR findings. Data suggest that cases of LTBI can potentially be missed due to the high 'cut-off' point (> 10-mm) for a

positive TST in non-endemic countries such as Kuwait, with consequent missing of LTBI immigrant cases. The size of TST reactions might remains the same without increase in most immigrants, could be due to medium or high level of allergic responses against low virulent organisms and able to 'burns out' or kill the invading bacilli. The situation recommends TST re-testing to follow the natural history of BCG vaccination (Horwitz *et al.*, 1972). However risk factors for positive TST is increased due to longer hours of MTB exposures in endemic countries.

5.2.6.1 Is TST no more a 'second choice' for LTBI detection?

Although TST is an important tool for detecting TB infection and has been well-studied all over the world to detect LTBI, it is not a 'gold standard'. TST positive results can be considered as clinical predictors only for smear-negative TB patients (Kanaya *et al.*, 2001), and the TST is an imperfect tool for detecting latent or active tuberculosis infected with viable MTB organisms.

Others have also found that the TST is imperfect as a routine screening test for BCG-vaccinated or other persons at low risk for TB infection such as low prevalence regions and Kuwait (CDC, 1995). Variability of TST reactions and interpretation ambiguity do not allow dependence on TST result for LTBI/TB management. An updated report of CDC proved that neither the presence of induration reaction nor the TST size will provide exact protection against TB disease or to determine whether the reaction is due to TST or previous BCG (CDC, 2006).

Because the majority of immigrants received BCG at older pre-school ages, our negative TST findings support the theory that minimal TST indurations in vaccinated individuals if BCG was administered in infancy during the post-vaccination period (especially more than 10 years) versus more persistent and larger TST reactions in those who received BCG after infancy (Crampin *et al.*, 2009; Farhat *et al.*, 2006).

The study results suggested that the diagnostic use of TST should be improved to detect MTB infection by modification of the 'cut-off' point values for adults and in particular healthy immigrants coming from TB-endemic regions. On the contrary

Zhang *et al.* (2010) concluded that 10-mm induration should be used as the 'cut-off' point in endemic countries.

The possibility of cross-reaction of the TST with BCG vaccination in BCG-vaccinated populations attenuates TST specificity in diagnosing tuberculosis. The TST reveals a significant association with individual's BCG status (which is compulsory in all TB endemic and Asian countries) in having significantly lower specificity and diagnostic accuracy than IGRAs blood tests in identification of LTBI and MTB infection (Richeldi *et al.*, 2009, Zhang *et al.*, 2010). PPD cross-reactivity causes TST poor specificity response to MTB infection due to shared antigens with *M. bovis* and other environmental NTM having false positive TST results more frequently in older age groups (> 40 years), and with bigger TST induration more than 10-mm (> 10-mm) or other non-significant induration (> 20-mm), while compared to false negative in anergic immuonocompromised people (Lalvani *et al.*, 2001b; Menzies *et al.*, 2008). Accordingly, the study negative TST results did not usually contribute to the risks of re-activation of TB so, unless TST re-testing and having recent conversion to positive reaction then still cannot be considered as diagnosed LTBI.

Despite cross-reactivity with other non-mycobacteria present in the BCG, Wang *et al.* (2002) in a meta-analysis of 26 studies from 1966-1999 concluded a positive reaction of 15-mm in diameter was more likely to be caused by *M. tuberculosis* infection than by previous BCG vaccination, (consistent with our proposed new scoring system for suspicion for carriers of LTBI and MTB).

Diagnosed LTBI immigrants are miss-diagnosed using the TST alone. Low socio-economic status of TB endemic countries is correlated with false-positive TST (LTBI diagnostic test) which results from routine BCG vaccination and exposure to environmental bacteria (Baker *et al.*, 2008; Boccia *et al.*, 2009; Elender *et al.*, 1998; Lienhardt *et al.*, 2005; Mangtani *et al.*, 1995). Also socio-economic characteristics such as adulthood ages above 19 years, cultural and psychological factors, high family income and received BCG vaccination were significant risk factors for non-compliance with tuberculin skin test follow-ups, the management and prevention of

non-compliance to the test (da Rocha *et al.*, 2011). The results described here were not influenced by the immigrant's surrounding environment in Kuwait.

There was no statistically significant association between the Mantoux test result and presence of a BCG scar. Our results for all new immigrants coming from high TB incidence regions were also not significant. The effect of BCG vaccine on TST induration response will be further discussed in chapter 5, section 5.3).

TST conversion to positive reactivity can be due to accurately diagnosed LTBI (Mancuso *et al.*, 2008) or waning of a positive tuberculin reaction in specific delayed type hypersensitivity, indicating that mycobacterial antigen might be degraded and disappear from the body (Wiker *et al.*, 2010). The two-step strategy is currently recommended for LTBI screening, but with the disadvantage of clinician counsel overloads and false-positive cases e.g. hypersensitivity reaction with BCG vaccination or missing false-negative cases e.g. immune-compromised individuals. Otherwise, result re-reading after one-week and/or re-testing after six weeks might facilitate correct decisions (Topic *et al.*, 2009). Both conversion types did not meet the research criteria because the TST were not repeated but can be considered as a confirmatory test for either positive IGRAs in vaccinated new immigrants, with or without CXR testing.

The usefulness of TST in diagnosing LTBI remains debated, especially since the introduction of more sensitive and specific IGRAs. INF-γ release assays provide a new tool for LTBI diagnosis and surveillance for new TB infections, and allow highly specific ex-vivo testing, with no boosting phenomenon. 'RD-1'-based INF-γ release assay is more specific than the conventional TST using PPD for diagnosing latent TB in high BCG coverage population such as Asian countries (Zhang *et al.*, 2010). Blood INF-γ correlates with TST reactivity but IL-10 is inversely correlated with the size of TST induration suggesting separate factors involved in controlling T-lymphocytes and the TST erythematous reaction (Burl *et al.*, 2010). For a high suspicion of LTBI, at least both TSTs and either IGRAs should be performed, especially those with prior BCG vaccination (Smith *et al.*, 2011). TST is not a reliable test in school children less than 15 years or in those BCG-vaccinated in infancy, and in those at low risk of TB infection. TST shows high specificity and

confirmatory to the new QNF-GIT screening test for LTBI diagnosis and is one of the recommendations of this study (Chun *et al.*, 2008; Jacobs *et al.*, 2011).

PPD tuberculin has low specificity, especially in BCG positively vaccinated individuals, and necessitates discovery of new protein(s) specific to MTB like IGRAs antigens, ESAT-6 and CFP-10 and TB7.7 to improve sensitivity and specificity of immunologic response and to replace TST in diagnosing LTBI/TB (Ferrara *et al.*, 2006; Franken *et al.*, 2007; Lalvani *et al.*, 2001b; Pai *et al.*, 2005; Zhang *et al.*, 2010). A new alternative to ordinary PPD reagent, DPPD (recombinant 6kDa, ESAT-6 and CFP-10 antigens), is recognized only by the T cells of TB patienta and not by other healthy or BCG-stimulated cells. In the Philippines, a new patch-based TB test employed the MPH 64 (a new specific MTB antigen) and test continues for 3-4 days post-application with higher sensitivities (98%) and specificities (100%) (Tiwari *et al.*, 2007). Similar diagnostic trials for new MTB antigens are mandatory to improve TST specificity.

5.2.7 Study strength and TST reliability

1. Even with exclusion of three immigrants (one expatriate drop-out and two other cases returned after 5 days), still participant's follow-up was 98.33% at the time addressed for the inclusion criteria. High rates of non-compliance can be observed in other studies among different population groups, still non-compliance was not a limitation barrier in our research study efficacy.

2. TST limitation: False-positive and false-negative results due to inter-observer variability were solved through double checking by the administrative nurse and the researcher. A false-negative result due to cutaneous anergy was not reported while the recent immigrants supposed to be healthy for employment in the country; similar finding was achieved by Richeldi *et al.* (2009).

3. Negative history of previous diseases in addition to perfect intradermal administration of PPD reveals absence of 'boosting effect ' or any TST adverse reactions except for small subcutaneous redness in a few immigrants (LLR $\chi^2_{(4)}$ = 26.017, p < 0.001).

5.2.8 Conclusions

1. Identification of latent *Mycobacterium tuberculosis* infection in new immigrants is that need to be solved:

2. Diagnosis can be summarized in that LTBI cases are missed on the basis of a PPD test result hampered by reduced sensitivity of the tuberculin skin test which has critical problems as the second choice for diagnostic criteria of MTB infection.

3. Treatment can be summarized in that inaccurate treatment of LTBI on the basis of tuberculin skin test results due to false-positives occurring after BCG vaccination and exposure to *Mycobacterium bovis* antigens or exposure to nontuberculous mycobacteria and/or the non-reactive false negatives.

5.2.9 Recommendation

1- Introduction of a new recording sheet for tuberculin skin test involving some major TB-associated risk factors (TST adverse reaction, BCG scar and body mass index) added to each immigrant identity number and date of TST performance plus follow-up notes.

2- Even for the accepted TST use for new immigrant screening, old TST guidelines should be revised and replaced by higher specificity diagnostic tests such as IGRAs.

3- The diagnostic use of the TST may be improved by modifying the 'cut-off' point values used to detect MTB infection and to diagnose LTBI according to the geographic region and epidemiological burden of tuberculosis. Targeted TST testing plays a role only as a confirmatory test in reducing unnecessary testing and treatment.

The rule effect of BCG vaccination on the reaction of tuberculin skin test and chest X-ray will be discussed in the next section 5.3.

5.3 Bacille Calmette-Guérin (BCG)

5.3.1 Introduction

The Bacillus Calmette-Guérin (or Bacelle Calmette-Guérin) (BCG) vaccine has existed for 90 years (first used in 1921) and is the most widely used of all current national childhood immunization programmes. Protection is insufficient, and does not prevent primary infection and, more importantly, does not prevent re-activation of latent pulmonary infection, the principal source of bacillary spread in the community. The impact of BCG vaccination on transmission of *Mycobacterium tuberculosis* is not clear, it being unclear whether BCG confers lifelong immunity. TB incidence rose again after abandoning routine BCG in the mid-1980s even with the introduction of other preventive measures (Ladefoged *et al.*, 2011). Latent TB infections are common among immigrant children from high TB incidence countries and require screening for TB development. For example BCG immunization is offered in the UK to previously unvaccinated, TST-negative new entrants below the age of 16 years who were born in, or lived for at least 3 months in, endemic countries (RCP-London, 2010). BCG has a role in aiding the clearance of a large bacillary load during chemotherapeutic treatment, with possibility of in-vivo cooperation between anti-TB antibiotics and the host immune response to reduce the mycobacterial cell burden (Lalvani *et al.*, 2010).

5.3.1.1 BCG vaccine history

Following Robert Koch's discovery of *Mycobacterium tuberculosis* in 1882, vaccine trials against TB were set out and in 1908 Leon Calmette, a French bacteriologist, and Camille Guérin, a French veterinarian at the Pasteur Institute, Lille, were able to devise a vaccine by attenuating a *Mycobacterium bovis* strain to lose its virulence. After thirteen years and 230 passages the new attenuated strain was protective in animal models due primarily to the loss of the genes in the 'Region of Difference'-1 (RD-1) region of the *M. bovis* genome. In 1921 the first dose of BCG was given as a disease-modifying agent before the era of antibiotics to an infant suffering from infectious tuberculosis and cared for by his grandmother. The BCG vaccine is

injected intra-dermally or sub-cutaneously as a 0.1 ml single injection into the upper arm (left deltoid muscle) and injection is preceded by tuberculin skin testing.

5.3.1.2 BCG efficacy

BCG itself still forms part of the strategies to improve TB vaccine solutions (Lalvani *et al.*, 2010). The efficacy of BCG vaccine is globally variable (from zero to 80% protection) and maximally effective when administered to the immature immune system to enhance maturation of cell-mediated immunity during the neonatal and infancy periods (Grange *et al.*, 2011). The induction of both antigen specific Th1 (long term) and Th2 (short term) cytokine responses, with BCG scar formation have a strong association with high INF-γ responses to PPD indicating high exposure to environmental mycobacteria (Djuardi *et al.*, 2010). The immune system is configured and maintained differently in different countries for both induction and maintenance of vaccine-induced immunity. Prior environmental exposures to NTM are responsible for blocking of BCG's action rather than BCG masking (Crampin *et al.*, 2009). Causes of low BCG efficacy against PTB are attributed to: 1)- continuous exposure to environmental mycobacteria and/or co-infections, 2)- genetic variation of the target population or of vaccine strains (lead to MTB virulence), and 3)- nutritional differences among vaccinated subjects. BCG-produced antioxidants suppress host immunity and vaccine efficacy to prevent pulmonary TB (Frantz *et al.*, 2010; Sadagopal *et al.*, 2009).

5.3.1.3 BCG advantages/benefits

Worldwide populations are obliged to continue using BCG due to low cost and high safety. TB control is largely dependent on BCG and early detection of active and latent tuberculosis (Figure 5.6).
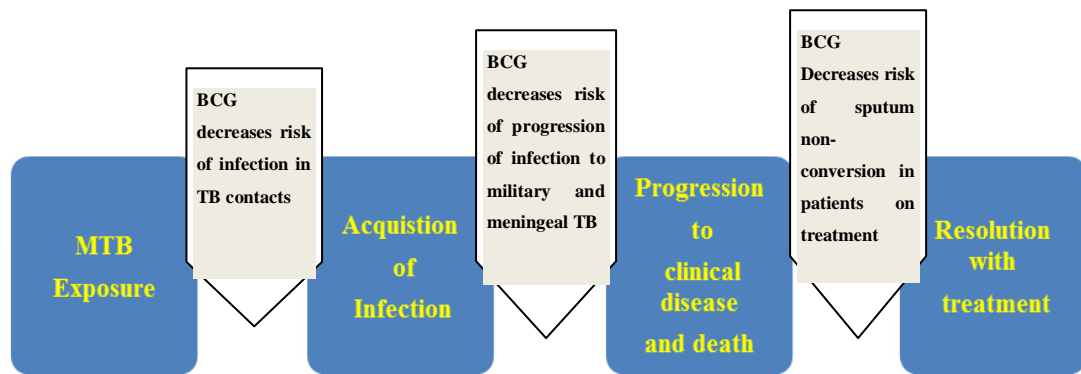
Figure 5.6: Schematic representation of the protective effects of BCG at different steps in the natural history of tuberculosis. Grey arrows represent effects of BCG discovered in the last five years while the blue boxes represent effects historically known (Lalvani *et al.*, 2010) (26/01/2012)

5.3.1.4   BCG disadvantage/adverse effects

TB prevention requires elimination of latent MTB infection, which the BCG vaccines are ineffective against. Lack of full protection against TB was not overcome by repeating BCG vaccination in adults, although protection against leprosy was improved (Crampin *et al.*, 2009). Repeated BCG vaccination after MTB exposure has detrimental effects ('Koch phenomenon') promoting IL-17-dependent immunopathologies of the lung (Sadagopal *et al.*, 2009; Thaiss and Kaufmann, 2010). Exposure contacts to environmental mycobacteria prior to BCG vaccination can block the protection against subsequent *M. tuberculosis* and/or mask the protective effects of BCG vaccine efficacy (Frantz *et al.*, 2010). Local hypersensitivity and cutaneous reactions and, less commonly, BCG-oma (abscess) or ugly large and raised ulceration scars (keloid) (Langenskiold *et al.*, 2008) or BCG-osis (fatal dessiminated BCG disease) (Grange, 1998) are significant adverse effects.

5.3.2 Objective

Assess the effect of BCG vaccine on the results of the tuberculin skin test according to the defined case categories of latent tuberculosis infection.

5.3.3 Methodology

BCG vaccination status was ascertained from the participants through reliable past history of vaccination and/or presence of a characteristic BCG scar.

5.3.4 Results

Analysis of the effects of Bacillus Calmette-Guérin vaccination status in relation to the defined cases of latent tuberculosis infection categories is shown in Table 5.9.

Table 5.9: Distribution of Bacillus Calmette-Guérin (BCG) vaccination status against tuberculosis according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

BCG vaccination against TB		Negligible (n=90)		Low (n=36)		Average (n=0)		High (n=4)		Extremely High (n=46)		Can not be judged (n=4)		Total (n=180)		Likelihood ratio	p-value
		n	%	n	%	n	%	n	%	n	%	n	%	n	%		
History of BCG vaccination	Yes	78	87	28	77.78	0	0	4	100.00	41	89	3	75.00	154	85.56	LLR $\chi^2_{(8)}$= 5.470	0.706
	No	10	11	7	19.44	0	0	0	0.00	5	11	1	25.00	23	12.78		
	Unknown	2	2	1	2.78	0	0	0	0.00	0	0	0	0.00	3	1.67		
Age of BCG vaccination (years)	Median (IQR)	5.00 (2)		6.00 (3)		-	-	7.50 (6)		5.00 (2)		4.00 (-)		5 (2)		KW $\chi^2_{(4)}$= 3.330	0.504
Presence of BCG scar	Positive	79	87.78	27	75.00	0	0	4	100.00	41	89.13	4	100.00	155	86.11	LLR $\chi^2_{(4)}$= 6.104	0.192
	Negative	11	12.22	9	25.00	0	0	0	0.00	5	10.87	0	0.00	25	13.89		
Animal exposure in mother country	Yes	51	56.67	18	50	0	0	1	25.00	24	52.17	0	0.00	94	52.22	LLR $\chi^2_{(4)}$= 7.928	0.094
	No	39	43.33	18	50	0	0	3	75.00	22	47.83	4	100.00	86	47.78		
Having cow?	Yes	16	17.78	5	14	0	0	0	0.00	7	15.22	0	0.00	28	15.56	LLR $\chi^2_{(4)}$= 3.114	0.539
	No	74	82.22	31	86	0	0	4	100.00	39	84.78	4	100.00	152	84.44		

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Total immigrants answered 'yes' for history of previous BCG vaccination were 85.56% (154/180) and only 1.67% (3/180) who answered 'unknown' and did not know about their BCG past history. These findings were not statistically significant when compared with LTBI categories (LLR $\chi2_{(8)}$ = 5.470, p = 0.706) (Figure 5.7, Table 5.9).
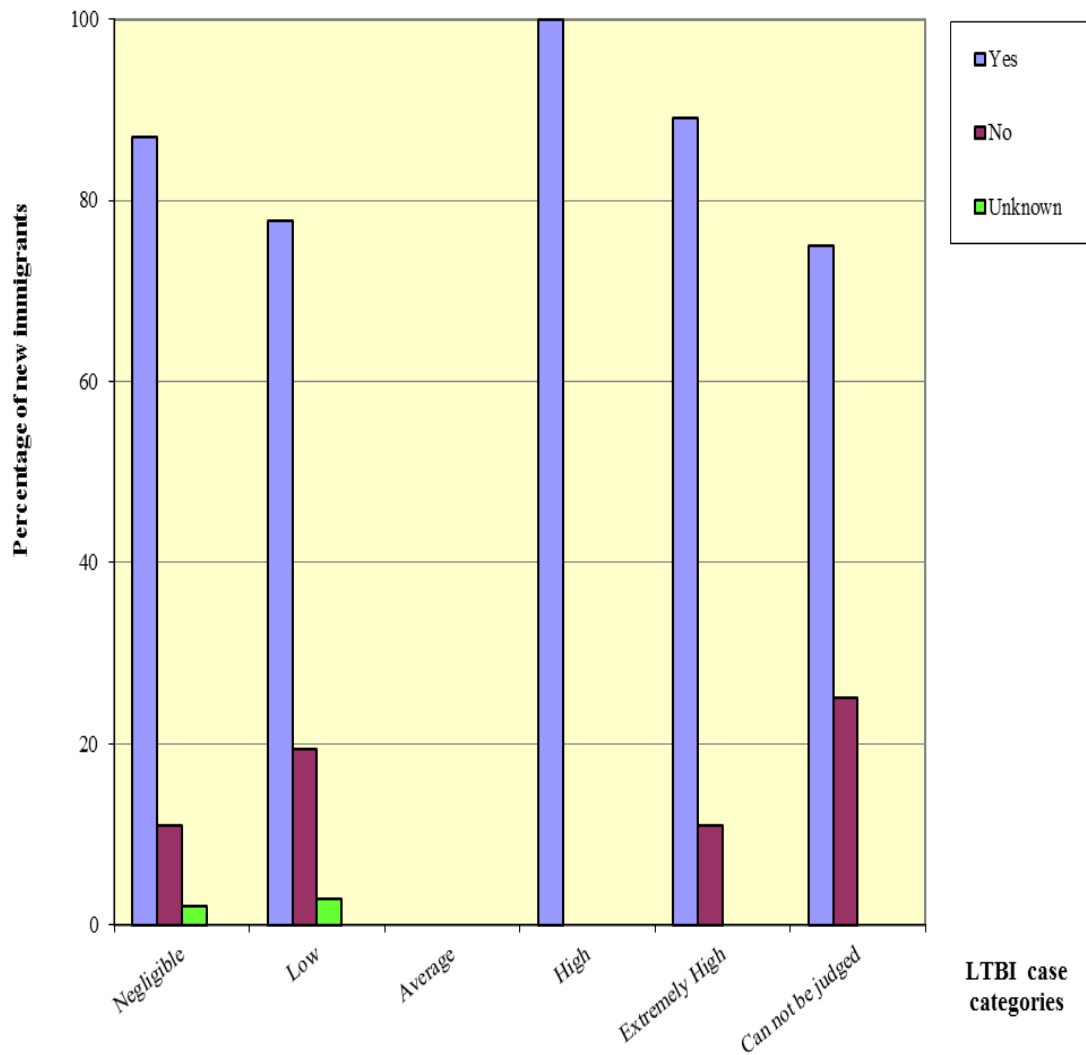
Figure 5.7: Distribution of BCG vaccination history according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

Presence of BCG vaccination scar was not statistically significant to be a risk factor for LTBI development or to cause positive TST reaction even in those classified as 'high' and 'extremely high' LTBI cases were having forearm scar. A positive scar was detected in 86.11% (155/180) (LLR $\chi^2_{(4)} = 6.104$, p = 0.192) (Figure 5.8, Table 5.9).
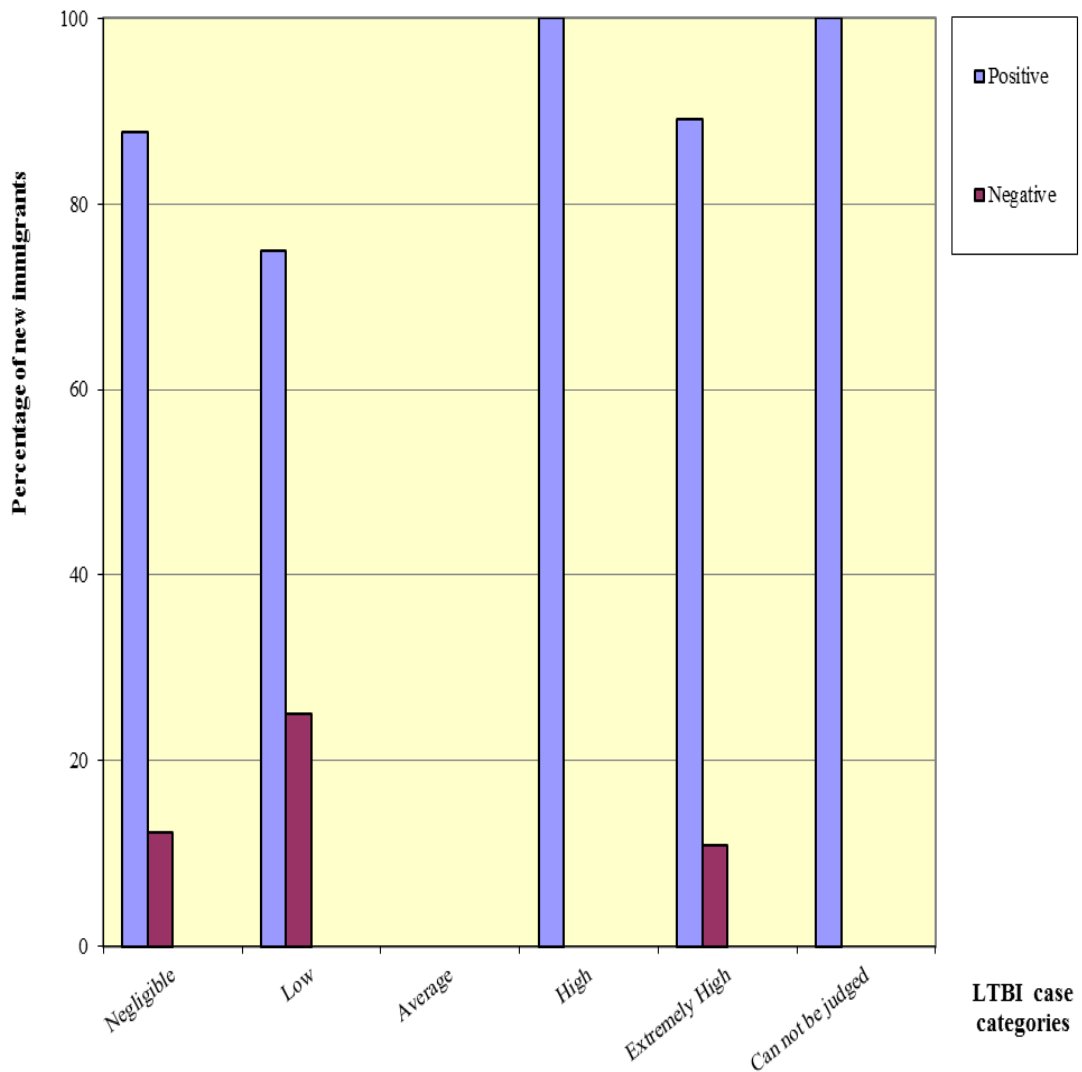
Figure 5.8: Distribution of presence of BCG vaccination scar according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

The overall median age of BCG vaccination for the 180 participants was 5 years (IQR = 2 years). Both 'negligible' and 'extremely high' LTBI groups were having similar median ages to the sample median age. The 'high' LTBI group had been vaccinated at older ages (median = 7.5 years, IQR = 6 years) but the 'low' LTBI

group been vaccinated at median of 6 years (IQR = 3 years). The minimum (youngest) age of vaccination was one year versus the oldest maximum age of 13 years was answered. However these findings did not show a statistically significant difference between normal vaccination ages for LTBI development (KW $\chi^2_{(4)}$ = 3.330, p = 0.504) (Figure 5.9, Table 5.9 and Table 5.10).

Table 5.10: Distribution of BCG vaccination ages according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

Age of BCG vaccination (year)	Negligible (n=90)	Low (n=36)	Average (n=0)	High (n=4)	Extremely High (n=46)	Cannot be judged (n=4)
Minimum	1	2	0	4	1	1
Q1	4	4.25	0	4.25	4.5	2.5
Median (IQR)	5 (2)	6 (3)	-- (--)	7.5 (6)	5 (2)	4 (--)
Q3	6	6.75	0	10	6	5
Maximum	13	11	0	10	7	6

(--) means cannot computed by SPSS Software, IQR = interquartile range, Q = quartile

Different BCG vaccination status at different median ages of LTBI defined categories is shown in Figure 5.9.
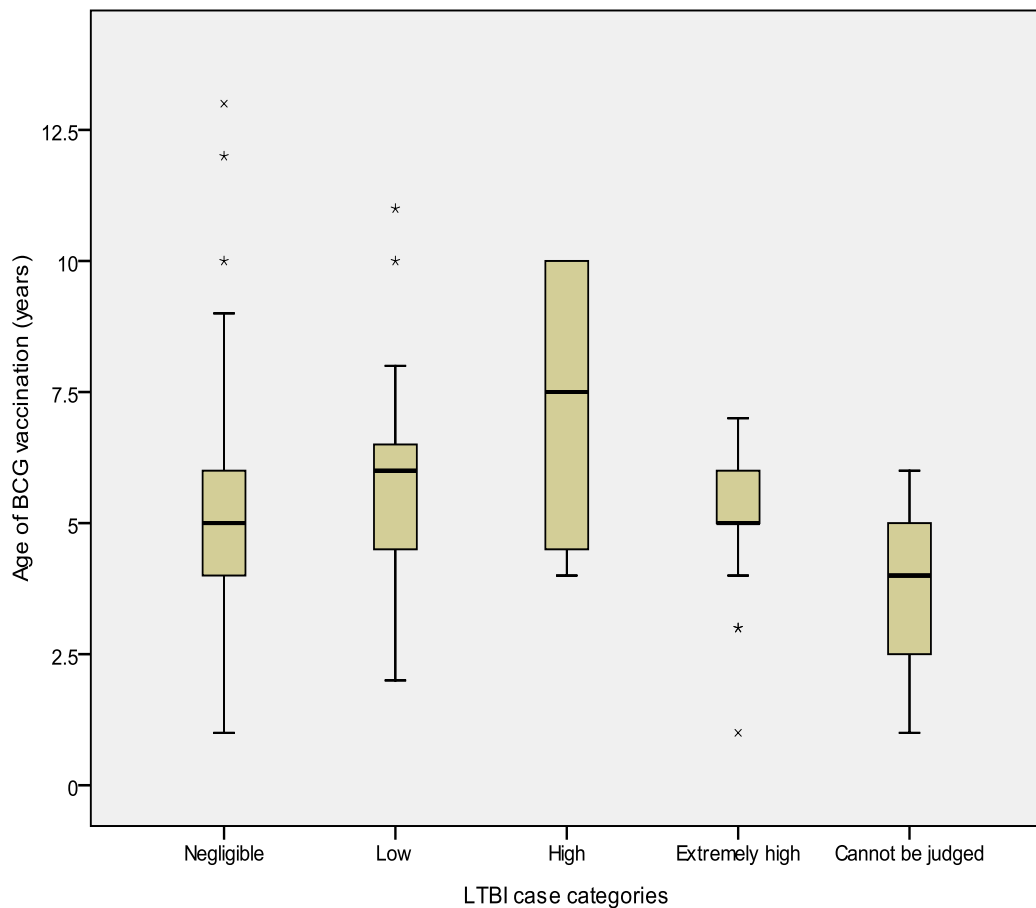
Figure 5.9: Box-and-whisker plots of distribution of BCG vaccination age (years) according to the defined case categories of latent tuberculosis infection for 180 new immigrants to Kuwait during February and May, 2010 *Outlier values, [x] Maximum and minimum outlier values

Immigrants having a history of exposure to different animal and poultries in their mother country were 52.22% (94/180), and had a significant statistical difference at the 10% level of significance according to the defined LTBI categories. Table 5.9 shows the distribution of the majority for those considered as 'negligible' LTBI cases in 56.67% and 'extremely high' LTBI individuals in 52.17% (LLR $\chi 2_{(4)} = 7.928$, p = 0.094) (Figure 5.10). On the other hand, a correlation between owning and exposure to cattle in mother countries and LTBI development was detected only in 15.56% (28/180) of total immigrant cases, but was not statistically significant (LLR $\chi 2_{(4)} = 3.114$, p = 0.539) (Table 5.9).
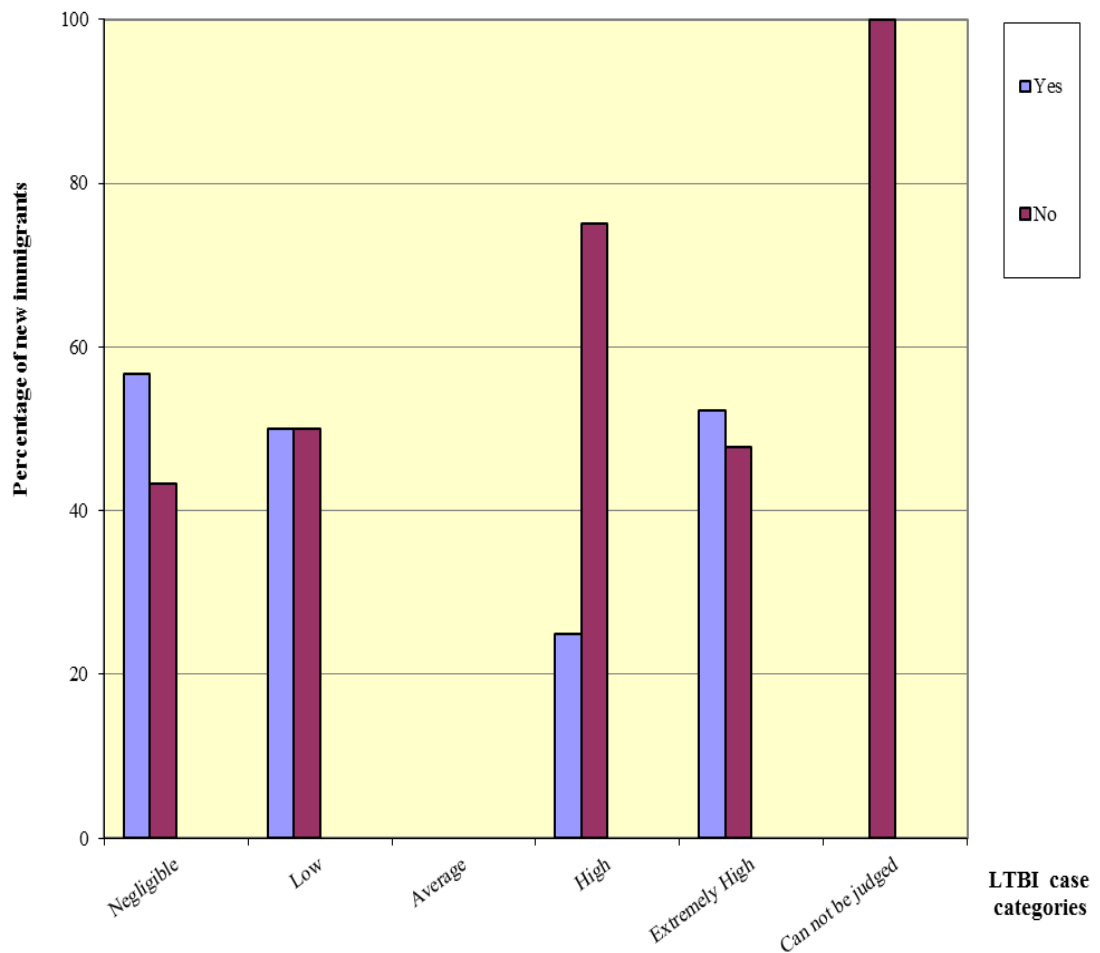
Figure 5.10: Distribution of animal exposure in mother country according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

5.3.5 Discussion

A history of BCG vaccine is not a contraindication for tuberculin testing. TST is extremely limited as a diagnostic tool to prove whether a positive result is caused by MTB infection or cross-reactivity resulting from BCG vaccination, especially in TB endemic countries such as India or China, with a 98% inoculation rate of BCG. Since BCG-TST positive reactivity wanes with time (if more than five years have elapsed since administration of BCG vaccine) - a positive TST reaction is most likely a result of exposure to *M. tuberculosis* infection (CDC, 2005c).

5.3.5.1 BCG administration

The vaccine is ineffective against latent MTB. Administration routes and BCG dose and age of vaccination can affect its efficacy and safety and scar formation (Jeremiah *et al.*, 2010). The dependence on scar formation as evidence of vaccine intake is affected by high prevalence (up to 50%) of vaccine recipients unable to form scar, which is not correlated with BCG-induced development of adaptive cellular immune responses (Lalvani *et al.*, 2010). On the other hand Burl and colleagues (2010) concluded in a longitudinal study that a lack of BCG scar was unlikely to be due to incorrect administration of BCG vaccine

5.3.5.2   Timing of BCG vaccination

The age of BCG vaccination can be related to TST positivity of vaccination during infancy or after childbirth than older childhood period (Wang *et al.*, 2002). In China, BCG vaccination is highly recommended and has been compulsory since the late 1970s and is usually given to all newborns within the first 3 months of life and without booster vaccinations proved that no influences or interferences of BCG on TST reactive responses (He *et al.*, 2010). Gomes and colleagues (2011) have recently concluded that waning of the BCG-induced protection was associated with raised risks of TB morbid rates in children aged between three and five years. A global meta-analysis suggested that minimal effects on TST for BCG vaccination received in infancy (especially more than 10 years after vaccination), and BCG received after infancy produces frequently more persistent and larger TST reactions (Farhat *et al.*, 2006).

5.3.5.3   Testing for the BCG scar

Successful vaccination can be assessed by the presence or absence of a BCG scar (Djuardi *et al.*, 2010). Emphasis on skin testing in BCG-vaccinated individuals should be considered in the appropriate clinical setting (Wang *et al.*, 2002). A BCG scar is associated with a three-fold increased risk of sputum culture conversion (Jeremiah *et al.*, 2010). Similar to our research results Kik *et al.* (2009) shown no association between the presence of BCG scar with recent exposure to TB in immigrants having positive IGRAs and TST results. Similarly there was no

association between BCG scar and household contacts to diagnosed TB patients and progression to active TB (Hill *et al.*, 2008).

On the other hand, absence of an immunization scar was significantly associated with the severe disease form related to the amount of exposure and can be a determinant factor for TB infection. The occurrence of vaccinated TB infected children without scar production underpins the importance of reviewing vaccination health records (Soysal *et al.*, 2005).

This study proved that the presence of a BCG scar and related TST negative results of all immigrants were not significantly associated with a higher prevalence of LTBI. Similar findings were noted by Demkow *et al.*, (2008) and by Kik *et al.*, (2009). Other statistical significance also detected the absence of risk difference in TB patient contacts having positive BCG scar compared to other normal control contacts without scars (ATS and CDC, 1999; Lin *et al.*, 2008).

A statistically significant relation between the number of BCG scars and the diameter of TST, which was significantly higher in HCWs with two BCG scars than HCWs with one scar and HCWs without a BCG scar (Cagalayan *et al.*, 2011).

Similar to the majority of BCG-related publications, the size of BCG scar was not measured in our research because did not correlate with protection against TB, and also is not an indication for diagnosis (or presence) of LTBI. A similar result was also achieved by Crampin *et al.* (2009).

5.3.5.4   Waning of BCG efficacy

TST reactivity caused by BCG vaccine generally wanes with the passage of time, but periodic skin testing may prolong reactivity in vaccinated persons, the phenomenon of 'boosting reactivity' (CDC, 2010b). On the contrary, TST reaction size and TST results were not affected by the time since the last dose of BCG vaccination, the number of BCG scars or BCG vaccination schedule in children, and significantly affected the decision for LTBI prophylaxis (Babayigit Hocaoglu *et al.*, 2011). Exposure to previous BCG vaccination did not significantly reduce the risk of being

diagnosed as active or latent TB was also revealed by Caley *et al.* (2010), in common with our findings.

5.3.5.5   Animal exposure

Grafein *et al.* (2011) concluded that there was an absence of a significant association between the presence of LTBI and a BCG scar, but presence of a significant association with consumption of unpasteurized (cows) milk in the past 6 months, which can be related to *Mycobacterium bovis.* Bradshaw *et al.* (2011) suggested that advancing age increases the likelihood of exposure to unpasteurized dairy products and cross-reactions of environmental mycobacterial antigens using IGRA tests. A similar significance in our research results of those participants having past history of exposures to various animals and positive BCG scar without LTBI diagnosis.

5.3.5.6   New vaccine challenges

A new TB vaccine designed and evaluated to raise the protective efficacy and with less boosting phenomenon of the immune responses, then was primed by the vaccinated BCG, which means can prevent initial infection but not eradicate an established pathogen or to prevent latent infection (Grange *et al.*, 2011). Current TB vaccine development is focused on a phenomenon called 'heterologous prime-boost strategy'; 'priming' the infant with BCG or recombinant/genetically modified BCG (rBCG) followed by 'boosting', using some MTB antigens that are delivered in a different way. Boosting includes viral vectored vaccines and fusion protein vaccines which even can be given to children and adolescents or adults (Hawkridge *et al.*, 2011; Lalvani *et al.*, 2010). RUTI (fragmented MTB cells) is a new efficacious vaccine that slows LTBI progression in experimental mice by inducing a strong immune response of mixed nature (Th1/Th2/Th3 cells) and promoting latent bacilli antigen recognition through inducing Th1 response then producing specific antibodies able to control MTB re-activation (Cardona, 2006).

5.3.5.7   Is IGRAs assay saving the problem of BCG-TST positive cross-reaction?

BCG acts as a surrogate marker in epidemiological screening trials estimating LTBI measured by either IGRAs, and can be used as the basis for the development of new vaccines (Soysal *et al.*, 2005). On the other hand, IGRAs tests can detect latent tuberculosis infection by measuring interferon gamma releases in response to antigens present in *M. tuberculosis*, and not confounded by previous BCG vaccination and most non-tuberculous mycobacterial antigens. IGRAs by evidence-based are more sensitive and specific than TST and CXR for LTBI diagnosis in BCG vaccinated subjects, saving costs and reducing health system overburden (Al-Orainey, 2009). This is done through enumeration of INF-γ-secreting T cells (T-SPOT *.TB* test) or by measurement of INF-γ concentration (QuantiFERON Gold In-Tube test or ELISA test) (Eisenberg and Pollock, 2010).

IGRAs test are less confounded by BCG vaccination than the association between BCG and TST positive results (CDC, 2011b; Pai *et al.*, 2006). IGRAs use *M. tuberculosis* specific antigens and do not cross react with BCG and therefore do not cause false-positive reactions in BCG recipients (CDC, 2010b). BCG was not found to be as a risk factor associated with IGRA positivity in immigrants (Pareek *et al.*, 2011). There was a lack of consistency between TST and QFT-GIT results in subjects with previous BCG vaccination (Huang *et al.*, 2010).

As a new tool, the IFN-γ release assay has been used in counties with a low incidence of pediatric TB and low rate of BCG vaccination for detecting outbreaks in schools, monitoring therapy or diagnosis of tuberculosis and NTM disease (Sun *et al.*, 2010). BCG vaccination of school children was significantly associated with negative IGRAs (Bradshow *et al.*, 2011). The finding by Weir and colleagues (2006) in a study on older school children prior to vaccination revealed higher INF-γ production to PPD in Malawi than in the UK where exposure to environmental mycobacteria is lower. History of BCG vaccination was found to be predictive of positive TST and negative QNF-GIT in healthy individuals but not among HIV sero-positive subjects due to HIV-associated immunosuppression (Santin *et al.*, 2010).

5.3.6 Study limitation

Absence of BCG scar in 25 participants forearm might present on other body sites such as thigh, which was ethically difficult for researcher to check out, and therefore immigrant was considered as unvaccinated.

5.3.7 Conclusions

Despite the promising research for new vaccine against tuberculosis, still BCG vaccine administration, with its deficiencies, should be continued as the only important tool (option) in controlling the harmful effects of tuberculosis, especially in endemic countries. Whether TST-positive reactions should be interpreted regardless of BCG vaccination history is still unclear which necessitate massive research trials comparing between the national resident and the various foreign-born residents and recent expatriates.

5.3.8 Recommendations

1- The relationship between immunogenicity (ability to induce delayed hypersensitivity reaction-type 4 against TST in the response to BCG vaccination need to be elucidated.

2- Considering the confounding effect of risk factors, BCG efficacy and protection need further evaluation using IGRAs in both vaccinated and non-vaccinated population.

3- Developments of new vaccines occur to raise efficacy of protection against LTBI/TB risk and replace BCG in the future. New vaccine candidates from preclinical to clinical testing might be accelerated by development of biomarkers able to predict the pre-clinical outcome of MTB such as adjuvant or viral-vectored antigens in recombinant BCG (rBCG) (Kaufmann, 2011).

Assessment and comparison of the two new tuberculosis (IGRAs) diagnostic tests will be detailed in chapter six before adherence to the newly defined evidence-based epidemiological-laboratory criteria used for early detection of latent tuberculosis cases, which will has a positive impact on future reduction of tuberculosis morbid

infection and laboratory costs, and overburdens of the health care services as described in chapters 7, 8, and chapter 9.

6 Chapter six

Evaluation of the performance of two new diagnostic tests used for diagnosis of latent tuberculosis infection carriers and 'suspect TB' cases

Background

During the last two decades, molecular biological techniques to identify mycobacterial bacilli in pulmonary secretions have become more sensitive, with rapid MTB detection and accreditation for international use. Early detection of inactive bacillary carriers can prevent the spread of TB and reduce the high prevalence of latent tuberculosis infection, thus helping prevention of re-activation risks. In the absence of a true reference test for TB standard diagnosis, it is difficult to establish the actual diagnostic sensitivity and diagnostic specificity of TB diagnostic tests, consequently adding limitations to TB control. Chest X-ray and the tuberculin skin tests are of extremely low performance to detect LTBI in the immigrant screening programs, but IGRAs add accuracy for LTBI diagnosis and can be considered as a potential replacement test on the in vitro quantification of the immune cell response and increase prevention of MTB transmission.

Compared with PPD (the choice for latent tuberculosis diagnosis), IGRAs biomarker tests detect the release of INF-γ response to specific tuberculous antigens. Two antigens, the early-secreted antigenic target 6-kDa protein (ESAT-6) and culture filtrate protein 10 (CFP-10), are products of a genomic region (RD1) that is present in all MTB and pathogenic *M. bovis* strains, but is absent from all BCG vaccine and NTM stains. Thus, the diagnosis of latent tuberculosis infection has increased in sensitivity, specificity and positive predictive value through the use of IGRAs, which are superior to replace both TST and CXR. The ability of IGRAs to distinguish LTBI from active TB in most circumstances allows new strategic guidelines for screening of LTBI and 'suspect TB' carriers in high-risk groups from TB endemic countries. IGRAs are predicted to become routine diagnostic tools in clinical practice in non-endemic countries.

The aim of this work is assessment of interferon gamma release assays performance in detection of dormant mycobacterium bacilli in high-risk groups, in the absence of a gold standard test.

6.1 QuantiFERON Gold In-Tube test (QFT-GIT)

6.1.1 Introduction

New diagnostic tests being developed and evaluated to optimize the use of current diagnostic tests to validate accuracy, predictive values, cost-effectiveness and impacts in prediction of future clinical managements. In vitro biomarker diagnostics are regularly validated against the standard tests. The QuantiFERON test and the specific QuantiFERON Gold In-Tube test (by means of the added specific proteins of MTB) are widely used for the identification of tuberculosis infection. The culture of clinical samples alone for intermittent excretion of MTB is an insensitive indicator of human infectiousness, which necessitates serologically-based assays without conversion or cross-reactivity to other mycobacterias. The in vitro biochemical test is based on quantification of interferon gamma levels from the stimulated peripheral blood lymphocytes, by which able to detect dormant bacilli at early stages of MTB infection before re-activation. Previous findings greatly suggest that both CXR and TST are not enough to diagnose latent tuberculosis cases in the immigrant screening programmes (Eisenberg and Pollock, 2010). In comparison with TST, both IGRAs more accurately discriminate and diagnose LTBI, based on the risk of TB exposure (Jong Lee *et al.*, 2010).

Making the best estimate of incident LTBI high risk groups such as new expatriates will provide data to guide and support policy recommendations. IGRAs are an accurate indicator of LTBI, providing more sensitive and specific results when diagnosing actively infected subjects to be treated. Until recently, the only tool for detection of LTBI was the tuberculin skin test, but recently the IGRAs, as well as QNF-GIT, have offered themselves as alternative or adjunct tests to the PPD test (Delgado Naranjo *et al.*, 2011). QNF-GIT is a valuable public health tool with potential advantages over the standard TB diagnostics. Systemic reviews show evidence that IGRAs, especially QFT-GIT, have excellent specificity that is unaffected by BCG vaccination. Development of new national guidelines incorporating IGRAs in screening is occurring. Some high-income with TB low-

incidence countries, such as the United States have, recommended IGRAs for repeated screening of health care workers (CDC, 2005; Zwerling *et al.*, 2011).

6.1.1.1 QNF-GIT merits

These are rapid ex vivo blood-based tests, requiring a single screening visit, and, in contrast to the TST, can be repeated without sensitization or boosting. They require only one visit and do not require a baseline two-step protocol. In addition to ESAT-6 and CFP-10 antigens which are used in the QNF test group, QuantiFERON-TB Gold In-Tube (QNF-GIT) test is the latest new version approved internationally and commercially licensed which includes addition of a third MTB specific antigen; TB7.7. All three antigens are not present in BCG or in most NTMs therefore increasing the specificity for diagnosing the presence of MTB and LTBI than PPD of Mantoux test (LoBue *et al.*, 2010; Sun *et al.*, 2010).

QNF-GIT assays show higher sensitivity as well as being more specific than traditionally used PPD tests, and the results of ESAT-6, CFP-10 and TB7.7 IFN-γ-based tests do not interact with NTM and are not affected by prior BCG vaccination (Drobniewski *et al.*, 2007; Hardy *et al.*, 2010; Lalvani *et al.*, 2001b; Pai *et al.*, 2005; Sauzullo *et al.*, 2008). Interestingly, a positive Quantiferon tests in TB contacts is more predictive of subsequent active TB than a positive skin test (Diel *et al.*, 2008), and does not produce false positive results to the extent that the skin test does. QNF-GIT avoids useless, expensive treatments and unwanted effects of anti-tuberculosis medicines of miss-diagnosed individual from having LTBI/TB (Cagalayan *et al.*, 2011)

Conflicted estimates of LTBI risks and uncertainty testing's among expatriates and travelers such as military in Germany, Canada and USA had lead to variable screening policies and guidelines (Freeman *et al.*, 2010). In developed countries, LTBI screening using QNF-GIT including the two-step preceded by positive TST is recommended (NICE, 2011; Pooran *et al.*, 2010).

Despite difficulties in isolation of MTB, still QNF-GIT test provided correct diagnosis and confirmation of LTBI/TB in BCG vaccinated school children having

positive TST and which is extremely challenging to be recommended as a gold standard test (Delgado Naranjo *et al.*, 2011; Jacobs *et al.*, 2011). Also, progression from LTBI to active tuberculosis forms was more reliably identified using QNF-GIT than TST in high-risk populations, especially in children (CDC, 2005; Chun *et al.*, 2008; Diel *et al.*, 2011).

6.1.1.2   QNF-GIT demerits

IGRA's performance varies in different settings. QNF-GIT results in high-TB burden regions are influenced by low immune responses such as HIV co-infection. Under-estimation of LTBI prevalence using QNF-GIT might be attributed to differences in methods of BCG vaccination (Jong Lee *et al.*, 2010). Even though QFT assays provide more accurate and quicker results, they are more expensive (due to high reagents cost) and produce indeterminate or nonreactive results in immuno-compromised conditions such as malnutrition and tropical infections common in low income and TB endemic countries (Baboolal *et al.*, 2010; Balcells *et al.*, 2008; Thomas *et al.*, 2010). QNF-GIT gave indeterminate results more frequently in children less than 4 years of age than in older children above 14 years of age, because of low levels of test mitogen responses (Bergamini *et al.*, 2009). Other risk factors for false negative and indeterminate results of QNF test were old age, low BMI and wasting status, HIV co-infection (suppression of mitogenic response) and HLA-genotype (demonstration of new MHC class II allele) suppressing INF-γ response (Hang *et al.*, 2011). Mahomed *et al.* (2011) concluded that the LTBI prevalence of large sample adolescents was nearly the same as detected by positivity of TST or QNF-GIT.

6.1.2 Aim

To assess the accuracy of QuantiFERON-TB Gold In-Tube test (new biomarker TB test).

6.1.3 Objective

To evaluate the diagnostic performance of QuantiFERON-TB Gold In-Tube test in diagnosing latent tuberculosis infection in healthy humans with absence of a reference standard test.

6.1.4 Methodology

Quantiferon-TB Gold In-Tube tests were performed according to the manufacturer's (Cellestis Limited, Carnegie, Victoria, Australia) specifications and CDC approval (CDC, 2011b). Control materials and *M. tuberculosis* antigens (ESAT-6, CFP-10, TB7.7) for QFT-GIT are contained in special tubes used to collect blood for the test, thus allowing more direct testing of fresh blood. One tube contains test MTB antigens that consist of a single mixture of 14 peptides representing the entire amino acid sequences of ESAT-6 and CFP-10 and part of the sequence of TB7.7. The two accompanying tubes serve as negative and positive controls: the negative-control tube contains heparin alone, and the positive-control tube contains heparin, dextrose, and phytohemaglutinin.

Blood (1 ml) was collected into each of the three tubes, mixed with the reagents already in the tubes, and incubated for 16–24 hours. Plasma was separated, and the IFN-γ concentration in the plasma was determined using a sensitive single ELISA run machine. To interpret the calculated results of QNF-GIT was in accordance with the Cellestis manufacturere's instructions and as approved by the FDA. The TB response was calculated as the difference in IFN-γ concentration in plasma which is released by the sensitized CD4+ T-cells stimulated with MTB antigen (i.e., the single cocktail of peptides representing ESAT-6, CFP-10, and TB7.7) minus the IFN-γ concentration in plasma from blood incubated without antigen (i.e. Nil).

The results were defined 'positive' if the INF-γ value after stimulation with TB-antigen minus the value in the Nil control was more than 0.35 IU/ml and more than 25% of Nil; 'negative' if value of TB-antigen minus Nil was less than 0.35 IU/ml or if that difference was more than 0.35 IU/ml and less than 25% of Nil, with Mitogen minus Nil more than 0.5 IU/ml; 'indeterminate' for TB antigen minus Nil less than

0.35 IU/ml or more than 0.35 IU/ml and less than 25% of Nil, with Mitogen minus Nil less than 0.5 IU/ml, or every time Nil was equal 0.8 IU/ml. Results are calculated by the Cellestis manufacturer's Software programme.

6.1.5 Results

Interferon gamma levels were measured using QNF-GIT ELISA and results of the 180 participants along with the LTBI case categories were represented accordingly (see Table 6.1).

Table 6.1: The distribution of QuantiFERON Gold In-Tube test results according to latent tuberculosis infection case categories of 180 new immigrants to Kuwait during February and May, 2010

QuantiFERON GOLD In-Tube test		Negligible (n=90)		Low (n=36)		Average (n=0)		High (n=4)		Extremely High (n=46)		Can not be judged (n=4)		Total (n=180)		Likelihood ratio	p-value
		n	%	n	%	n	%	n	%	n	%	n	%	n	%		
QNF-GIT result	Abnormal	0	0.00	4	11.11	0	0	0	0.00	46	100.00	1	25.00	51	28.33	LLR $\chi^2_{(4)}$= 184.972	<0.001
	Normal	90	100.00	32	88.89	0	0	4	100.00	0	0.00	3	75.00	129	71.67		
Interferon gamma level score	<0.35	90	100.00	32	88.89	0	0	4	100.00	0	0.00	3	75.00	129	71.67	LLR $\chi^2_{(4)}$= 195.007	<0.001
	0.35-<1.00	0	0.00	3	8.33	0	0	0	0.00	17	36.96	0	0.00	20	11.11		
	1.00-	0	0.00	0	0.00	0	0	0	0.00	7	15.22	0	0.00	7	3.89		
	1.50-	0	0.00	0	0.00	0	0	0	0.00	1	2.17	0	0.00	1	0.56		
	2.00-	0	0.00	0	0.00	0	0	0	0.00	3	6.52	0	0.00	3	1.67		
	3.00-	0	0.00	1	2.78	0	0	0	0.00	3	6.52	0	0.00	4	2.22		
	5.00-	0	0.00	0	0.00	0	0	0	0.00	2	4.35	0	0.00	2	1.11		
	10.00-	0	0.00	0	0.00	0	0	0	0.00	2	4.35	0	0.00	2	1.11		
	15.00-	0	0.00	0	0.00	0	0	0	0.00	3	6.52	0	0.00	3	1.67		
	>20.00	0	0.00	0	0.00	0	0	0	0.00	8	17.39	1	25.00	9	5.00		
Interferon gamma level (IU/ml)	Median (IQR)	0.02 (0.06)		0.13 (0.25)		-	-	0.06 (0.29)		1.46 (12.23)		0.04 (18.71)		0.07 (0.46)		KW $\chi^2_{(4)}$= 105.220	<0.001

Average LTBI category is zero (means no detected cases), (-) means cannot be computed by SPSS Software programme

There were no missing results for the 180 participants. The prevalence of LTBI having positive results was 28.33% (51/180). Negative QFT-GIT results were detected in 71.67% of participants (129/180) and no indeterminate results were reported (Table 6.1).

The distribution of QNF-GIT results according to LTBI case categories. New immigrants having abnormal 'positive' results were detected in the entire 'extremely high' LTBI group as 100% (46/46) and the rest were in the 'high' LTBI group as 11.11% (4/36). These differences were statistically significant (LLR $\chi^2_{(4)} = 184.972$, p < 0.001) (Figure 6.1, Table 6.1).
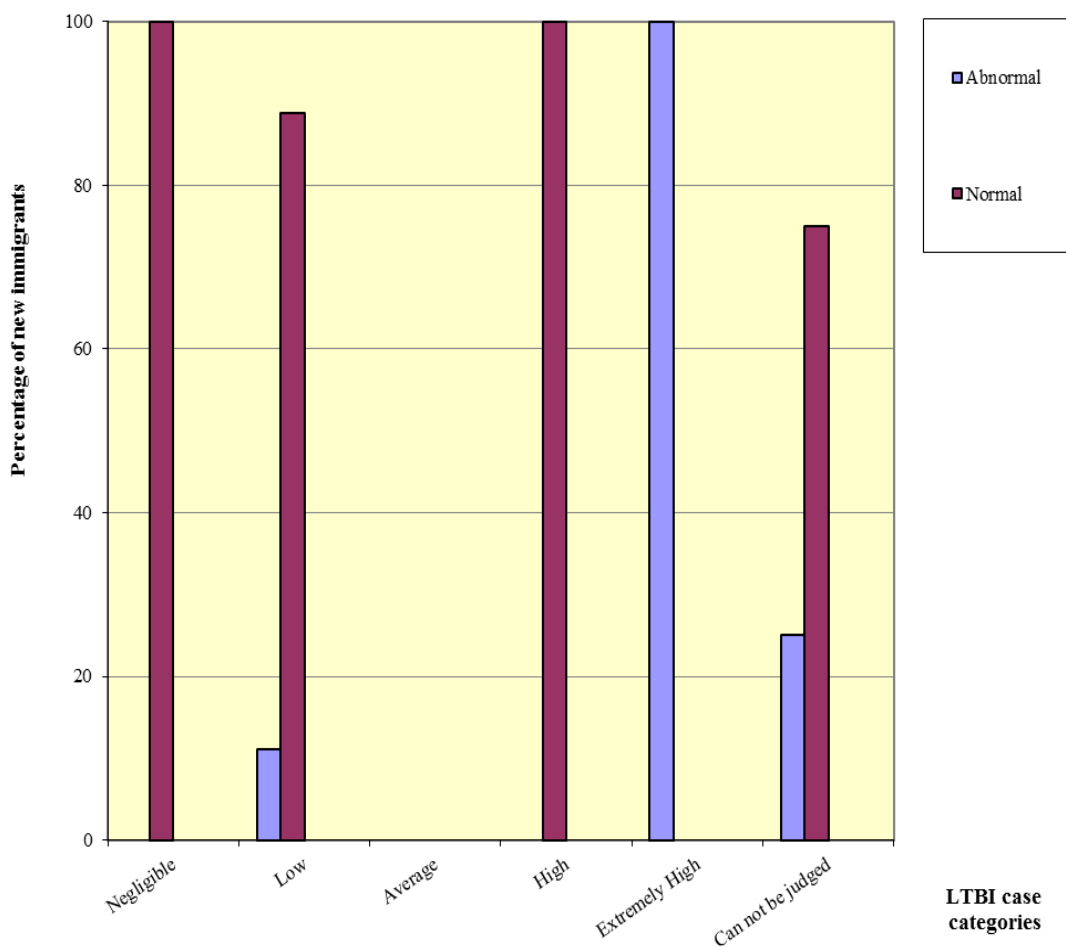
Figure 6.1: Distribution of QuantiFERON Gold In-Tube test results according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010 (Normal interferon gamma levels = < 0.35 IU/ml, Abnormal INF-γ = ≥ 0.35 IU/ml)

The scores of our invented quantitative of interferon gamma levels were found statistically significant with the LTBI defined categories (LLR $\chi^2_{(4)}$ = 195.007, p < 0.001) (Figure 6.2, Table 6.1). Those LTBI defined cases having 'abnormal' scores of high INF-γ levels lay between 0.35 and less than one IU/ml in 11.11% (20/180) followed by INF-γ levels ranges of more than 20.00 IU/ml in 5% (9/180) and INF-γ levels ranges between 1 and 1.50 IU/ml in 3.89% (7/180). All 'negligible' LTBI categories had normal results below 0.35 IU/ml. In contrast, all 'extremely high' LTBI defined cases had high INF-γ levels above 0.35 IU/ml and high score results.
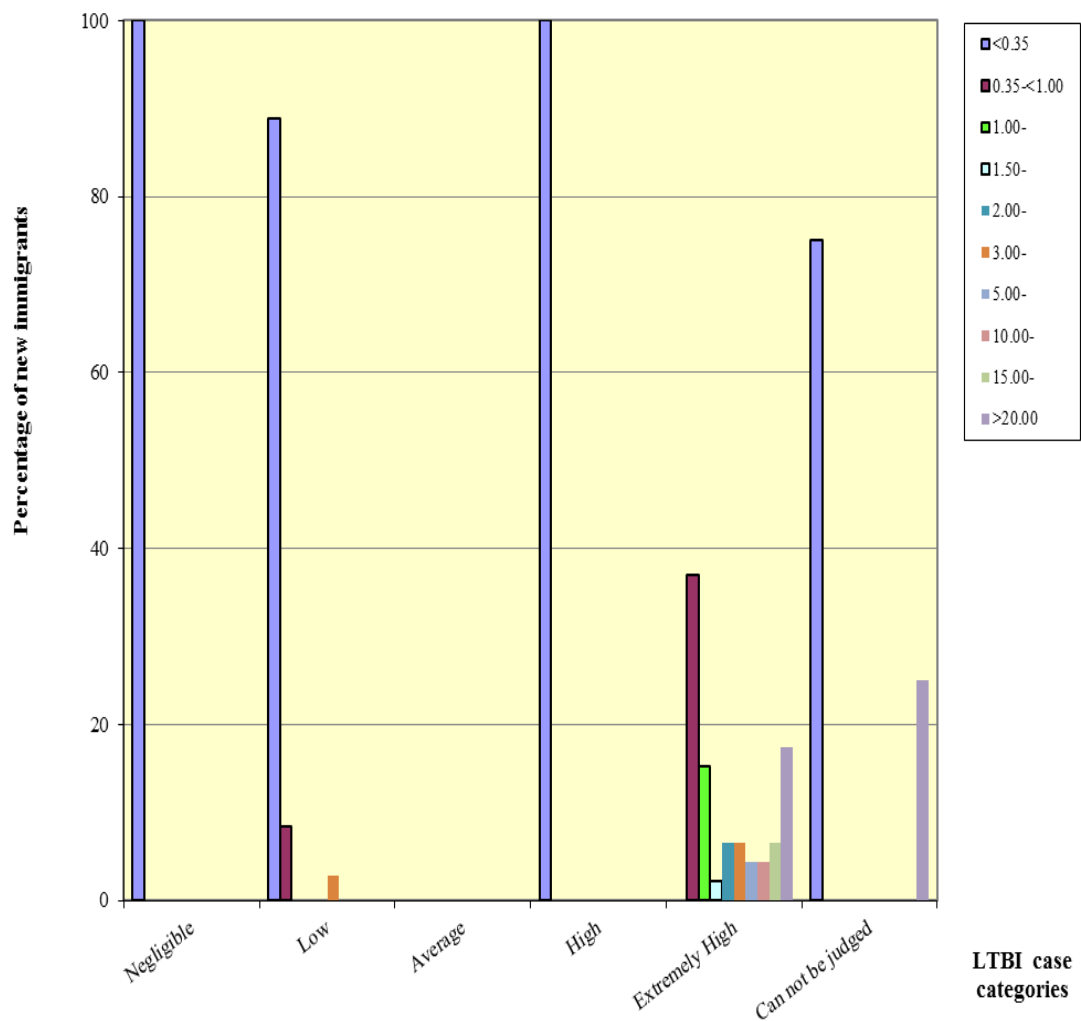
Figure 6.2: Distribution of interferon gamma levels of QuantiFERON Gold In-Tube test results according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

The median interferon gamma level of the total immigrants was 0.07 IU/ml (IQR = 0.46 IU/ml, SIQR = 0.23). Similarly, the other LTBI categories also had normal median INF-γ levels less than 0.35 IU/ml. On the other hand abnormally high median levels of INF-γ were detected in the 'extremely high' LTBI group reaching 1.46 IU/ml (IQR = 12.23 IU/ml), in addition to the highest calculated of both the minimum- (0.36 IU/ml) and the maximum- (30.52 IU/ml) INF-γ levels for the same group (KW $\chi^2_{(4)}$ = 105.220, p < 0.001) (Table 6.1, Table 6.2). The quantitative interferon gamma levels (IU/ml) that had been measured by ELISA for all involved participants are represented accordingly in Table 6.2 and Figure 6.3.

Table 6.2: Distribution of interferon gamma levels (IU/ml) measured by QuantiFERON Gold In-Tube test according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

Interferon gamma levels (IU/ml)	Negligible (n=90)	Low (n=36)	Average (n=0)	High (n=4)	Extremely High (n=46)	Can not be judged (n=4)
Minimum	-0.26	-0.78	--	-0.08	0.36	-0.02
Q1	-0.01	0.0150	--	-0.060	0.6450	-0.02
Q2 (Median)	0.0150	0.1300	--	0.0600	1.4600	0.0400
Q3	0.0525	0.2600	--	0.2325	12.8775	18.6925
Maximum	0.3100	3.070	--	0.27	30.52	24.89

(--) = means cannot computed by SPSS Software prgramme, (-) = negative result means immigrant antigens is less than the positive control (mitogen) antigens

The distribution of different interferon gamma levels measured by the ELISA of QuantiFERON Gold In-Tube test according to the defined cases of latent tuberculosis infection for 180 new immigrants to Kuwait is shown in Figure 6.3.
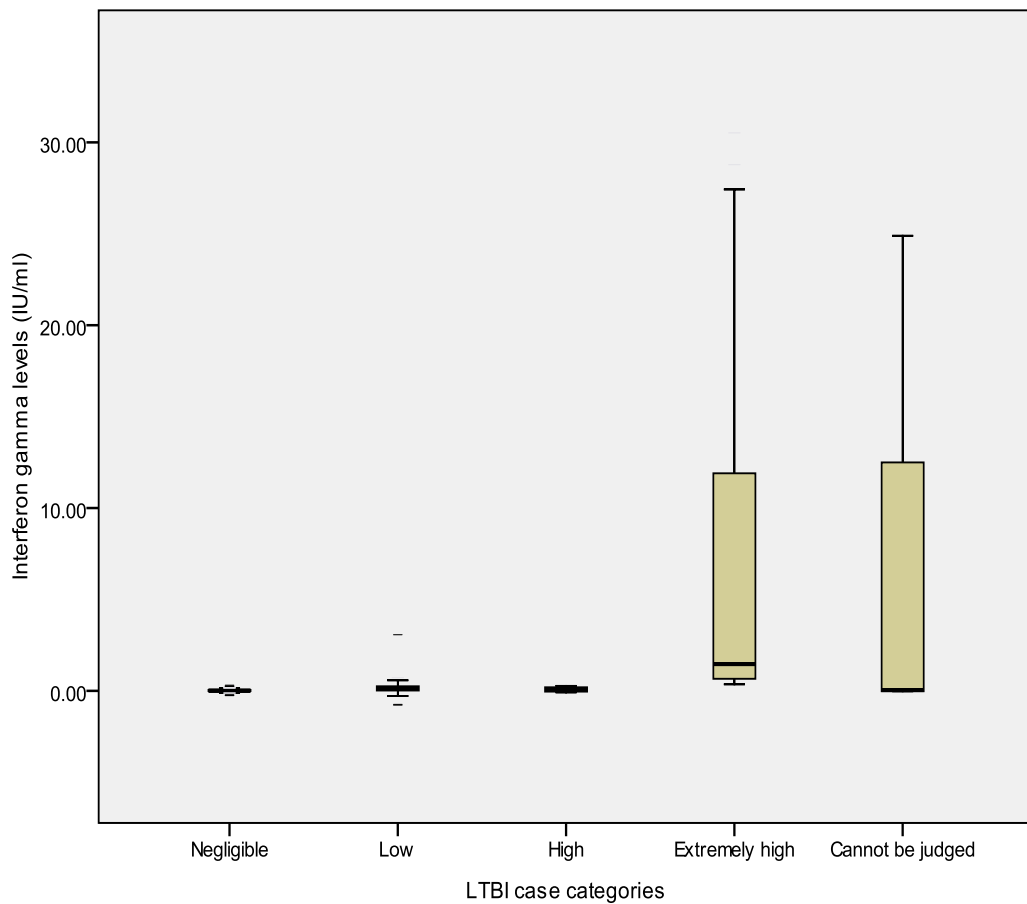
Figure 6.3: Box-and-whisker plots of distribution of interferon gamma levels (INF-γ) measured using QuantiFERON Gold In-tube test according to latent tuberculosis infection categories in 180 new immigrants to Kuwait during February and May, 2010 (INF-γ = interferon gamma level)

6.1.6 Discussion

Notifications of incident cases of tuberculosis increased over the last one decade in non-endemic countries such as Kuwait (HVS, 1984-2009) and the UK (Coker *et al.*, 2006) due to morbid increments of TB in foreign-born residents. Effective control of TB requires identification of latent TB in populations (travelers' and immigrants) arriving from high-risk countries. The weakness of the traditional screening strategy in recent immigrants from highly endemic countries is the low sensitivity/specificity of chest radiography. The higher QFT-GIT specificity prevents unnecessary overload on health care systems even though more expensive, still represents a cost-effective alternative tool to TST in targeted screening programmes. QFT-GIT able to identify

more LTBI cases than the NICE guideline even with low sensitivity of both TST and QNF-GIT tests in HIV-infected persons (Weinfurter *et al.*, 2011). A QFT-first protocol was proven by the study of Hardy and colleagues (2010) on UK foreign-born population to be cheaper and less costly than a CXR-first protocol, due to the reduced number of CXRs required (Hardy *et al.*, 2010). Diel *et al.* (2007) reached the same conclusions.

QuantiFERON-TB Gold In-Tube test appears to be a valuable public health tool with potential advantages over the PPD. The high specificity and sensitivity of QNF-GIT suggest that it can replace the traditional (CXR and TST) screening tests, especially for immigrants within less than two years from entry to low incidence countries due to higher risks of LTBI re-activations. For immigrants from high risk countries, QFT blood testing followed by CXR is feasible and cheaper for TB screening than the traditional screening using the ordinary CXR followed by TST for radiographically suspicious positives (Hardy *et al.*, 2010). Similar aims of targeted screening of immigrants of LTBI diagnosis were recently tested by Legesse *et al.* (2011) and Nenadic *et al.* (2011). A two-step strategy for the diagnosis of LTBI, using QNF-GIT to confirm positive TST cases was significantly cost-effective (NICE, 2011). Both IGRAs and QFT-GIT were cost-effective in reducing screening costs in TB contacts (Hardy *et al.*, 2010).

6.1.6.1   QNF-GIT results

The increase in the prevalence of diagnosed cases of LTBI and 'suspect TB' measured by the QNF-GIT positive results 28.33% supported by a negative CXR and TST, and who had been missed by the ordinary screening programme applied within the Tuberculosis Control Unit in Kuwait. Hardy and colleagues were able to diagnose missed immigrant cases by the NICE protocol implemented in UK (Hardy *et al.*, 2010; Nice, 2006).

Our research results suggested that QFT-GIT is more reliable than the ordinary diagnostic tests for identifying population at risk of harbouring MTB before progression to active TB. Other evidence-based results of positive QNF-GIT were similar to other achieved published results (Coker *et al.*, 2006; Hardy *et al.*, 2010;

Kik *et al.*, 2009). For example positive QFT-GIT is associated with the expatriate's country of origin.

Excluding active TB cases, the LTBI prevalence in our sample of recent immigrants ranged from zero% with CXR and TST to 28.33% with positive QFT-GIT. When considering any subject positive to at least one diagnostic test, the prevalence will increase. An Italian study on the new immigrants reached the same conclusion (Saracino *et al.*, 2009).

6.1.6.2 Interferon gamma levels of QNF-GIT

Interpretation of QNF-GIT results and IFN-γ levels did not correlate with positive TST, and is not limited by indeterminate results our sample participants where all having no history of any health disorders such as anegry or malnutrition. These results concur with Thomas *et al.* (2010). Measurement of IFN-γ production is helpful to diagnose active tuberculosis in clinically diagnosed and confirmed TB cases. The estimated prevalence of LTBI was increased using the lower QFT-GIT 'cut-off' point of IFN-γ levels, which are recommended by the manufacturer (Legesse *et al.*, 2011). Immigrants with negative TST and positive QFT-GIT results usually showed INF-γ levels close to the 'cut-off' value of 0.35 IU/ml (Jong Lee *et al.*, 2010). Therefore using an uncertainty zone around a new 'cut-off' point (e.g. 0.2 to 0.7 IU/ml) can improve the discrimination between non-specific variations for LTBI diagnostic 'cut-off' point and true conversion to positive result or reversion negative results (Schablon *et al.*, 2010a). Harada *et al.* (2006) used a grey zone of 0.10 to 0.35 IU/ml as a standard for the conversion results. Follow-up for positive baseline QNF-GIT and to detect of converters (positive- or negative-) should be sited before the diagnosed LTBI individuals developing into active TB, which was also recommended by Schablon *et al.* (2010a).

The research described here observed high median levels of the released IFN-γ levels of 1.46 IU/ml and were detected for all 'extremely high' LTBI immigrants, which can be supported by other research study results to be diagnostic level for LTBI without the need for other diagnostic testing's ($KW\chi^2_{(4)} = 105.220$, $p < 0.001$) (Table 6.1). In addition, the observed IFN-γ levels of more than one (> 1.00) IU/ml can be

accepted as the positive standard level value was statistically significant in those 'extremely high' LTBI at risk of MTB re-activation (LLR $\chi^2_{(4)} = 195.007$, $p < 0.001$) (Table 6.1).

The effect of changes in 'cut-off' points of TST induration and interferon gamma levels of QNF-GIT should be considered for the 'extremely high' LTBI immigrants coming from high incidence TB regions. Degrees of concordance are significantly correlated between the level of IFN-gamma production and diameter of TST skin induration (Saracino *et al.*, 2009). Co-incident changes between both tests were achieved in a similar target study implemented by Saracino and colleagues (2009). Other studies relate higher concentrations of IFN-γ levels to epidemiological risk groups s such as children and the elderly, using positive QNF-GIT (Bergamini *et al.*, 2009), and country of origin (Kik *et al.*, 2009), and other different TB risk groups; in TB patients was higher (INF-γ = 15.5 +/- 4.5 IU/ml) than nurses (high TB risk group) (INF-γ = 11.7 +/- 5.5 IU/ml) and medical students (low TB risk group) (INF-γ = 1.3 +/- 0.9 IU/ml) (Eum et al., 2008).

However other researchers related the high IFN-γ levels with the magnitude of TST induration (Demkow *et al.*, 2008). Negative TST results in our research were difficult to correlate with IFN-γ levels. Limits of the negative and positive results of QNF-GIT are difficult to be defined because of the high number of immigrant's spontaneous conversions and reversions, therefore, serial testing's are recommended in other immigrant studies. Further research is required to detect the lowest 'uncertainty' score ranges of IFN-γ levels in LTBI cases.

Nenadic *et al.* (2011) have recently revealed that IFN-γ level concentrations in children were similar in LTBI and TB disease, and either before or at the end of anti-TB treatment. Therefore concluded that IGRAs is not a useful screening tool to monitor the treatment of infected children diagnosed with LTBI or active TB. Also, Drobniewski and colleagues (2007) detected no correlation between the mean IFN-γ levels and age or number of years of medical practice.

Even though IGRAs showed good correlation with occupational risk factors for TB exposure in low-incidence settings (Zwerling *et al.*, 2011), still the number of

medical experience (practice per years) were not significantly associated with interferon gamma levels using QNF-GIT in endemic countries, such as Russia (Drobniewski *et al.*, 2007).

6.1.6.3 QNF-GIT accuracy

IGRAs are known to be an accurate indicator of LTBI, providing higher specificity of diagnosis and reducing the number of treated subjects, and with lower indeterminate results, similar to other study findings (Diel *et al.*, 2010; Ferrara *et al.*, 2006). QFT-GIT increased the percentage of possibly diagnosed LTBI. In common with our research findings for identifying TB infection, there were no false negative or false positive results from a combination of both QNF-GIT and TST in relation to BCG (Jong Lee *et al.*, 2010). In BCG-vaccinated immigrants, our research findings approved significantly that QFT-GIT can be considered as a useful confirmatory test for TST-positive individuals and as a replacement test for TST-negative individuals in cases of LTBI diagnosis versus the unreliable negative TST. Jacobs *et al.* (2011) came to the same conclusion. QNF-GIT diagnostic accuracy will be further discussed in chapter 7.

6.1.6.4 BCG vaccine and QNF-GIT results

Both IGRAs are the most promising tests for LTBI diagnosis because of the improvement of specificity and convenience, especially in BCG-vaccinated populations (Eriksen *et al.*, 2010; Sun *et al.*, 2010; Zhang *et al.*, 2010). Estimation of the probability of having a positive QNF-GIT test can be as a function of other associated LTBI risk factors such as BCG vaccination. The recent study by Mendez-Echevarria and colleagues (2011) revealed a significant association between absence of BCG scar and positive QNF-GIT.

QNF-GIT results were significantly not affected by prior BCG vaccinations (Cagalayan *et al.*, 2011; Hardy *et al.*, 2010), and is known as excellent diagnostic tool for screening of nosocomial transmission, especially in BCG vaccinated people at high risk of exposure to MTB (Cagalayan *et al.*, 2011; Choi *et al.*, 2008; Jong Lee *et al.*, 2010). The evaluation of occupational risk such as health care workers factors

done by Pai *et al.* (2005) revealed a stronger but non-significant association between positive IGRAs and the TST results, explained by differences in the prevalence of LTBI due to TB exposure.

QNF-GIT and TST agreement was associated with a significant rate of prior BCG vaccination (Vinton *et al.*, 2009). IFN-γ assays have a useful role in screening high-risk groups of having LTBI and who are BCG vaccinated, such as TB health care workers. Similar associations, including higher risk staff, have been reported by others (Eum, *et al.*, 2008; Harada *et al.*, 2006; Mirtskhulava *et al.*, 2008; Schablon *et al.*, 2009).

6.1.6.5   Is QNF-GIT preferred over tuberculin skin test?

The potential benefits of QFT-GIT over the traditional TST were recognized in the study performed by Hardy and colleagues (2010). The comparative results revealed that immigrants with LTBI and positive results of QNF-GIT were not associated with negative TST results, even though the majority of participants (86.11%) had a past history of BCG vaccination. QNG-GIT and TST disagreement was more frequently observed in LTBI cases than in normal non-infected individuals or tuberculosis disease cases (Mendez-Echevarria *et al.*, 2011). Similar to the research conclusion from this study on healthy adults, IGRAs results should be seriously considered in those young children having TST-negative/QTF-positive discordance because these false negative of LTBI diagnosed cases cannot be ruled out for future MTB re-activations (Mendez-Echevarria *et al.*, 2011; NICE, 2011). Higher TST indurations in LTBI diagnosed patients that have positive QNF-GIT were statistically significant and comparable to the results of those with smaller TST size (Cagalayan *et al.*, 2011; Mendez-Echevarria *et al.*, 2011). In this study all TST indurations below 10-mm were read as normal.

QFT-GIT permitted the identification of more LTBI cases than TST. Normally, the greater specificity of QNF-GIT can be emphasized to the discordance against the positive TST results, which are usually justified by previous BCG vaccinations or non-mycobacterial infections (Pai *et al.*, 2007; Saracino *et al.*, 2009). QNF-GIT is

considered an alternative to TST for detection of LTBI in populations with compulsory BCG vaccination (Cagalayan *et al.*, 2011).

Both IGRAs respond to MTB exposure revealed positive association with increasing TST induration (Adetifa *et al.*, 2007). Legesse and colleagues (2011) concluded that positive induration of TST more than 10-mm size was significantly associated with higher QNF-GIT positivity than the zero mm TST swelling. However our research findings were statically significant not associated with TST results and/or the size of indurations.

Low rate of positive QNF-GIT results with a positive TST result support exposures to NTM. Reversion of the initially low-positive QNF-GIT to normal INF-γ levels indicates that IGRAs might be useful for the diagnosis of later re-infections (van Brummelen *et al.*, 2010). The conclusion was that the mean TST indurations were of no difference in the QNF positive than the negative group. Similar findings were also revealed by Demkow and colleagues' (2008) study of health care workers (a high risk group). Other studies also revealed lack of strong associations of either TST or QNF-GIT positivity to risk factors related to household infectivity and MTB exposure transmission (Shanaube *et al.*, 2011).

6.1.7 QNF-GIT strengths

1. Based on a whole-blood ELISA, QNF-GIT overcomes TST weaknesses such as the need for return visits, reader variability, variable specificity, cross-reactivity with BCG vaccine and non-tuberculous mycobacterial infection (Cagalayan *et al.*, 2011). Indeterminate QNF-IT results were observed in non immunosuppressed healthy adult immigrant's of all included age groups, to be very low (zero%), indicating laboratory technician familiarity of samples manipulations and the diagnostic performance of QNF-GIT assays after blood samples were withdrawn. Similar results were also noted by Ferrara *et al.* (2006) and Hardy *et al.* (2010).

2. Indeterminate results occur due to a lack of lymphocyte activation after stimulation with the mitogen when the time between blood sampling and blood incubation is less than 2 hours, the percentage of indeterminate results

consequently decreasing (Mendez-Echevarria *et al.*, 2011). All of our samples were incubated in the first hour following venepuncture, which may explain the zero percentage of indeterminate results. In the future, the cost of testing should be reduced to avoid repetition of QNF-GIT testing.

6.1.8 QNF-GIT weakness

The QuantiFERON-TB Gold In-Tube and the TST cannot discriminate between active and latent TB infection in the current screening on immigrants, and in particular in those coming from TB-endemic areas which limit the free use of chemoprophylaxis. The uncertainty zone of QNF-GIT and interferon gamma levels leads to missing suspicious cases in high-risk groups. The positive QNF-GIT used to determine latent TB in foreign-born immigrants reflects prior TB exposure in the country of origin, but also can be related to recent TB infection. This late reactivity limits QNF-GIT usefulness unless supported with other diagnostic tests such as the highly specific T-SPOT *.TB* test. The same conclusion was also reached by Kik *et al.* (2009).

6.1.9 Conclusions

Limitation in implementation of the new diagnostic tests restricts TB prevention. The QuatiFERON Gold In-Tube test is a true innovation in clinical practice for TB control and elimination. QNF-GIT increases the identification of LTBI cases among recent immigrants to Kuwait. According to the evidence-based superior sensitivity and specificity, the use of QNF-GIT in the screening of latent TB infection is comparable to and predominates over the conventional screening with the chest X-ray and tuberculin skin test, particularly in BCG vaccinated immigrants. Even though the use of single cut-off point criteria for QuantiFERON Gold In-Tube test can lead to over-diagnosis of TB infections, still interferon gamma levels of more than one ($\geq$ 1.00) IU/ml can be considered as the lower limit for LTBI definite diagnosis. Clinical evaluations then help prevent unnecessary diagnosis and therapy of miss-diagnosed healthy individuals.

6.1.10 Recommendations

Based on the interferon gamma levels of the QuantiFERON Gold In-Tube test results, we recommend:

1- QNF-GIT can be considered as complementary rather than mutually exclusive test in immigrant screening and follow-up using the 'two-step' strategy approaches in conjunction with chest X-ray.

2- Periodic QNF-GIT testing for the detection of latent TB as an alternative to CXR and TST in high-risk populations such as health care workers (e.g. laboratory technician) with routine BCG vaccination programmes. A new criterion to define the 'cut-off' point diagnostic of LTBI need to be further established, especially in high-risk groups.

3- The classification of LTBI case categories should be strengthened and supported by testing QNG-GIT on larger cohort studies performed on high-risk immigrants newly entering the country from TB endemic areas to evaluate the ONF-GIT positivity and the diagnostic levels of the released interferon gamma suggestive of LTBI.

6.2 T-SPOT .*TB* test

6.2.1 Introduction

Early diagnosis of *Mycobacterium tuberculosis* infection is an essential and difficult step in tuberculosis control and elimination. LTBI incidence can be over- or under-estimated using the standard TB diagnostic tests such as chest X-ray or tuberculin skin test, which have a low sensitivity and specificity, especially in TB endemic countries with a predominantly BCG-vaccinated population (Sun *et al.*, 2010; Zhang *et al.*, 2010).

The T-SPOT .*TB* test is a variant of the in-vitro enzyme-linked assay technique for enumerating effector T-lymphocytic cells that secrete IFN-γ upon overnight stimulation with peptide pools of two specific antigens from *Mycobacterium tuberculosis*; ESAT-6 and CFP-10, which is more specific and sensitive than the

standard LTBI test. The ELISPOT (enzyme-linked immunospot assay) has the highest sensitivity and is the most promising test to diagnose both LTBI and active TB due to improvement of its specificity and convenience, especially in *M. bovis* BCG-vaccinated populations. Sensitivity of T-SPOT .*TB* assay to detect active TB infection was not influenced by the TST results that were reliably low in healthy individuals (Dilektasli *et al.*, 2010; Zhang *et al.*, 2010), and is consistently higher than that of the QFT-GIT (Diel *et al.*, 2010).

T-SPOT .*TB* assay measures T-lymphocytic white cells, which play a major role in measuring the health of the immune system, in particular, against bacterial infections. 'CD positive', or CD4+ (or T-'helper' cells) account for 30% and 70% of the total lymphocytes (between 500 and 1,000 cells per microlitre (µL). T-lymphocytes which had CD4+ molecules on its surface can lead the attack against intruders by sending signals, releasing cytokines and growth factors to activate and regulate the immunity response against *Mycobacterium tuberculosis*. Mean CD4+ counts of tuberculosis infection are significantly lower than in healthy subjects.

The aim of TB control programmes is to reduce TB disease by rapid diagnosis. The T-SPOT .*TB* assay is more accurate for the diagnosis of TB and correlates better with the existence of risk factors for TB infection. The T-SPOT .*TB* assay is helpful for LTBI diagnosis in high-risk groups if carried out either with TST and/or to confirm the TST result (Ozekinci *et al.*, 2007).

Rapid determination of MTB infection will accelerate TB diagnosis and enable early treatment/control of TB. Detection of LTBI will prevent re-activation progression to active TB (Simsek *et al.*, 2010). In addition to rapid diagnosis of active TB, T-SPOT .*TB* test allows rapid exclusion of people suspected of having active TB (Meier *et al.*, 2005). Similar conclusions were reached by Zhang *et al.* (2010), who demonstrated that T-SPOT .*TB* assay was more sensitive than conventional TST for detection of active TB infection in BCG-vaccinated populations.

6.2.1.1   T-SPOT .*TB* assay merits

T-SPOT *.TB* assay describes both qualitative (type of immune protein) and quantitative (number of responding CD4+ cells) variables (Sun *et al.*, 2010). T-SPOT *.TB* test, having a higher specificity than QNF-GIT or the standard old tests, can identify TB cases in relation to population risks, and is not confounded by BCG vaccination. For example, TSPOT *.TB* test is more specific than TST among BCG-vaccinated people, compared to equal specificity in patients without BCG history (Sun *et al.*, 2010). Close contacts having BCG negative and TST positive (BCG-/TST+) results are less likely to have positive T-SPOT *.TB* test than BCG vaccinated healthy people (Brodie *et al.*, 2008).

The in-vitro IGRA is a useful tool to detect LTBI individuals without been affected or cross-reacted by BCG vaccination and to exclude reliably the active infected cases in low endemic settings, rather than intermediate and high TB-burden countries (Dilektasli *et al.*, 2010).

T-SPOT *.TB* test can serve as a potential predictor of therapeutic efficacy. INF-γ producing T-cell responses against *Mycobacterium tuberculosis*-specific antigens were significantly decreased during treatment with anti-TB regimens which can be a reliable reference for therapeutic success and improvement of active TB patients (Zhang *et al.*, 2010).

T-SPOT *.TB* test is significantly cost-effective and cost-saving, with superior specificity compared with both TST and QNF-GIT tests. Even with repeated TST over two years, still no false positive results of the of T-SPOT *.TB* test due to absent induction of T-cell responses (Richeldi *et al.*, 2006). A large cohort study revealed that the costs of a LTBI screening and treatment programme of 1,000 patients using TST alone was more expensive (U.S.$695,820) than using TST followed by T-SPOT *.TB* test (U.S.$342,563) or T-SPOT *.TB* test alone (U.S.$387,136) (Wrighton-Smith *et al.*, 2006). Using T-SPOT *.TB* alone, or in combination with TST test, for screening of close contacts before isoniazid preventive treatment of LTBI was highly cost-effective in reducing the burden of TB and effective in non-endemic countries (Diel *et al.*, 2007).

6.2.1.2   T-SPOT *.TB* deficiencies

Although it has relatively high sensitivity and specificity, the higher costs and laboratory complexity of test should be considered. The use of IGRAs to diagnose active TB is still debatable and further large studies are needed. Even though they have greater diagnostic accuracy than the standard diagnostics, still the IGRAs and T-SPOT .*TB* test are affected by cellular immunity that result in more indeterminate results even in immuno-competent individuals. These invalid results occur because of false negative results (i.e., T-SPOT .*TB* test fails to give a reportable result or there is insufficient response in positive-control wells) (Bergamini *et al.*, 2009; Brodie *et al.*, 2008; Diel *et al.*, 2010; Passalent *et al.*, 2007). Also subjective variation is reported in the interpretation of T-SPOT .*TB* test results between different observers compared to the newly automated reader (still not implemented in Kuwait), and which can lead to LTBI miss-/over- (wrong) diagnosis.(Dilektasli *et al.*, 2010).

6.2.2 Aim

To assess the accuracy of T-SPOT .*TB* test (the new biomarker tuberculosis test).

6.2.3 Objective

To evaluate the diagnostic performance of T-SPOT .*TB* test in diagnosing latent tuberculosis infection in healthy humans with absence of a reference standard test.

6.2.4 Methodology

The ex-vivo ELISPOT assay was performed on fresh blood samples, which was previously detailed in chapter 3 (Materials and Methods; T-SPOT .*TB* test – section 3.12.2.1.1). The isolated peripheral blood mononuclear cells are incubated for 16–20 hours in the presence of ESAT-6 and CFP-10 protein antigens (ABC, Imperial College, London, UK). Phytohaemaglutinin (PHA; Sigma-Aldrich, UK) was the positive control. Sensitized T-cells will secret IFN-γ in the blood of persons infected with *Mycobacterium tuberculosis*. Next, enzyme-labeled secondary antibodies are added, which bind to other epitopes of the examined cytokine. T-lymphocytic counts were separated and detected by the T-SPOT .*TB* manual and eyeglass reading along with the LTBI defined cases. Interpretation and assay criteria were applied

accordingly: T-SPOT .*TB* results are interpreted by subtracting the spot count in the Nil Control well from the spot count in each of the Panels, according to the following algorithm: 1- The test result is 'positive' if (Panel A minus Nil Control) and/or (Panel B minus Nil Control) is more than, or equals, six colony forming unit (> 6 spots), 2- The test result is 'negative' if both (Panel A minus Nil Control) and (Panel B minus Nil Control) is less than, or equal to, five spots ($\leq$ 5 spots). This includes values less than zero. An abnormal or 'positive' result indicates that the sample contains effector T-lymphocytic cells reactive to MTB, whereas, a normal or 'negative' result indicates that the sample probably does not contain effector T-cells reactive to MTB. Indeterminate results are represented by an inability to read the number of spots.

6.2.5 Results

T-SPOT .*TB* test results were measured for all 180 participants. Table 6.3 shows the distribution of T-SPOT .*TB* assay results along with the LTBI defined categories.

Table 6.3: The distribution of T-SPOT .*TB* test results according to latent tuberculosis infection case categories of 180 new immigrants to Kuwait during February and May, 2010

T-SPOT. *TB* test		Negligible (n=90)		Low (n=36)		Average (n=0)		High (n=4)		Extremely High (n=46)		Can not be judged (n=4)		Total (n=180)		Likelihood ratio	p-value
		n	%	n	%	n	%	n	%	n	%	n	%	n	%		
T-SPOT. *TB* test response	**Reactive**	0	0.00	23	63.89	0	0	4	100.00	46	100.00	1	25.00	74	41.11	LLR $\chi^2_{(8)}$= 192.884	<0.001
	Non-reactive	88	97.78	13	36.11	0	0	0	0.00	0	0.00	3	75.00	104	57.78		
	Indeterminate	2	2.22	0	0.00	0	0	0	0.00	0	0.00	0	0.00	2	1.11		
T-SPOT. *TB* test result	**Abnormal**	0	0.00	23	63.89	0	0	4	100.00	46	100.00	1	25.00	74	41.11	LLR $\chi^2_{(8)}$= 192.884	<0.001
	Normal	88	97.78	13	36.11	0	0	0	0.00	0	0.00	3	75.00	104	57.78		
	Indeterminate	2	2.22	0	0.00	0	0	0	0.00	0	0.00	0	0.00	2	1.11		
T-lymphocyte count score	**<50**	3	3.33	0	0.00	0	0	0	0.00	1	2.17	0	0.00	4	2.22	LLR $\chi^2_{(20)}$= 21.218	0.384
	50-	15	16.67	4	11.11	0	0	1	25.00	6	13.04	1	25.00	27	15.00		
	100-	40	44.44	20	55.56	0	0	3	75.00	17	36.96	3	75.00	83	46.11		
	200-	25	27.78	12	33.33	0	0	0	0.00	20	43.48	0	0.00	57	31.67		
	300-	4	4.44	0	0.00	0	0	0	0.00	2	4.35	0	0.00	6	3.33		
	>400	3	3.33	0	0.00	0	0	0	0.00	0	0.00	0	0.00	3	1.67		
T-lymphocyte cell count	**Median (IQR)**	177.00 (99)		180.00 (74)		-	-	146.00 (105)		196.00 (80)		145.00 (109)		180 (89)		KW $\chi^2_{(4)}$= 4.409	0.353

Average category = no detected cases, (-) means cannot be computed by SPSS Software programme

The predicted prevalence of LTBI and 'positive' results of T-SPOT .*TB* test in the group of 180 immigrants subjected to the test were detected in 41.11% (74/180), and was divided accordingly; both 'high' LTBI and 'extremely high' LTBI had abnormal results in 100% and 'low' LTBI in 63.89% (23/180). The 'indeterminate' results were for two cases and both were belonging to the 'negligible' LTBI cases 1.11% (2/180) cases. 'Negative' results were obtained in 57.78% (104/180) of immigrants, 97.78% (88/90) of which in the 'negligible' LTBI group and 36.11% (23/36) in 'low' LTBI immigrants group (LLR $\chi^2_{(8)}$ = 192.884, p < 0.001) (Figure 6.4, Table 6.3).
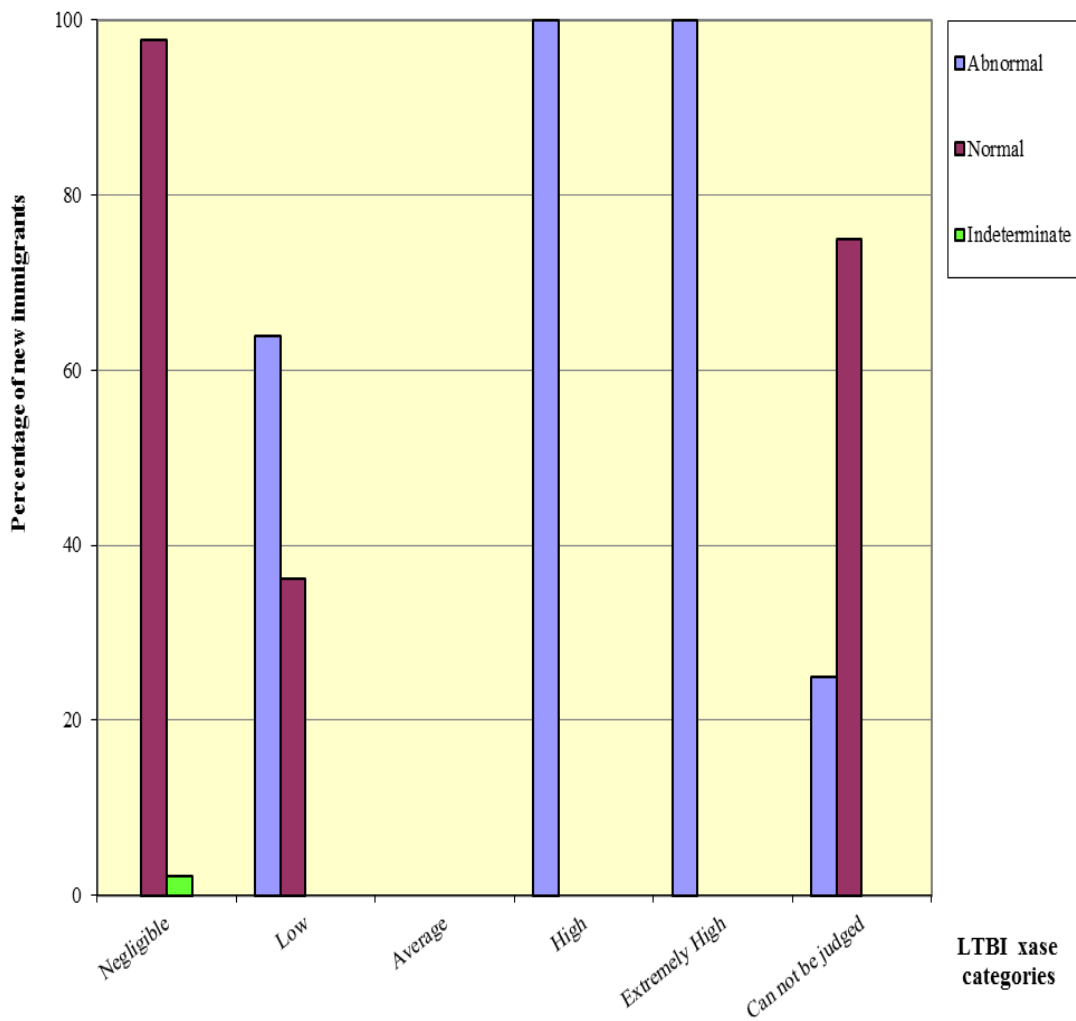
Figure 6.4: Distribution of T-SPOT .*TB* test results according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

Distributions of the score number of T-lymphocytes were detected invariably in the blood samples by T-SPOT *.TB* test according to the different LTBI defined categories of 180 new immigrants. The majority of participants had CD4+ T-lymphocytes per microlitre (µL) of between 100 and 200 cells in 46.11%, between 200 and 300 cells in 31.67% and between 50 and 100 cells in 15% of the total blood samples. However, these findings were not revealed a statistically significant difference of median scale number of T-lymphocytes (LLR $\chi^2_{(20)}$ = 21.218, p = 0.384) (Figure 6.5, Table 6.3).
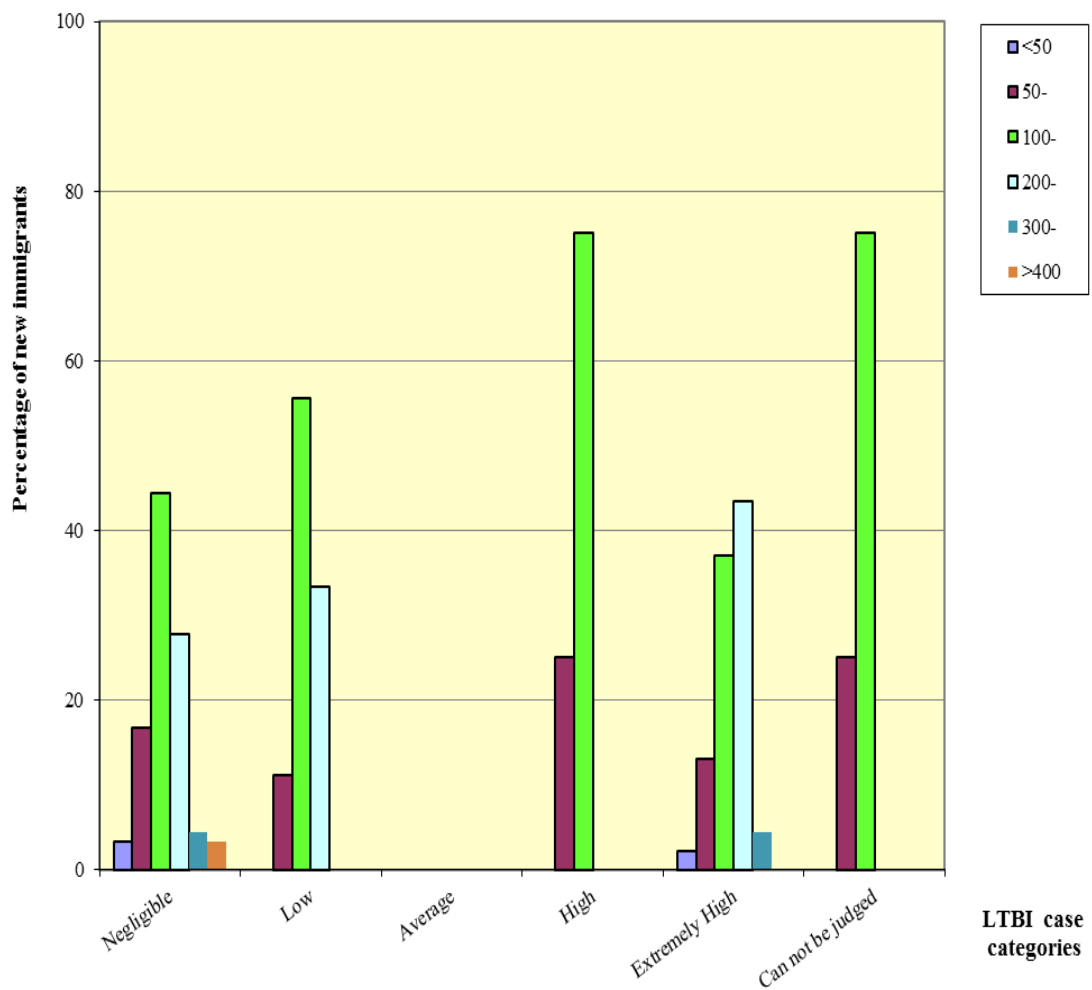
Figure 6.5: Distribution of number of T-SPOT *.TB* test lymphocytes per microlitre according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February and May, 2010

The overall median CD4+ T-lymphocytic cell count of the total immigrants was 180 cells per microlitre (cells/µL) (IQR = 89 cells/µL); similar to the normal accepted human cell levels. The highest median T-lymphocytic cell counts was measured in the 'extremely high' LTBI, equaling 196 cells/µL (IQR = 80 cells/µL), whereas, the lowest median CD4+ cell level was calculated in the 'high' LTBI group as 146 cells/µL (IQR = 105 cells/µL). The lowest calculated CD4+ was 20 cells/µL and the highest CD4+ was 419 cells/µL, and both belonged to the 'negligible' LTBI group. However, these differences were not statistically significant (KW $\chi^2_{(4)}$ = 4.409, p = 0.353) (Figure 6.6, Table 6.3).

The numbers of enumerated T-lymphocytes separated by T-SPOT .*TB* assays for all the 180 involved participants are represented accordingly in Table 6.3, Table 6.4 and Figure 6.6.

Table 6.4: Distribution of number of T-lymphocyte cells count per microlitre separated by the T-SPOT .*TB* test according to latent tuberculosis infection categories of 180 new immigrants to Kuwait during February- May, 2010

T-lymphocyte cells count per microlitre	Negligible (n=90)	Low (n=36)	Average (n=0)	High (n=4)	Extremely High (n=46)	Can not be judged (n=4)
Minimum	20	52	--	55	48	83
Q1	110.75	130.50	--	72	131.50	87.25
Q2 (Median)	177	180	--	146	196	145
Q3	209.25	204.25	--	176.50	211.75	196
Maximum	419	271	--	179	321	198

(--) means cannot computed by SPSS Software

The distribution of total T-lymphocyte counts measured by the T-SPOT .*TB* test according to the defined cases of latent tuberculosis infection for 180 new immigrants is shown in Figure 6.6.
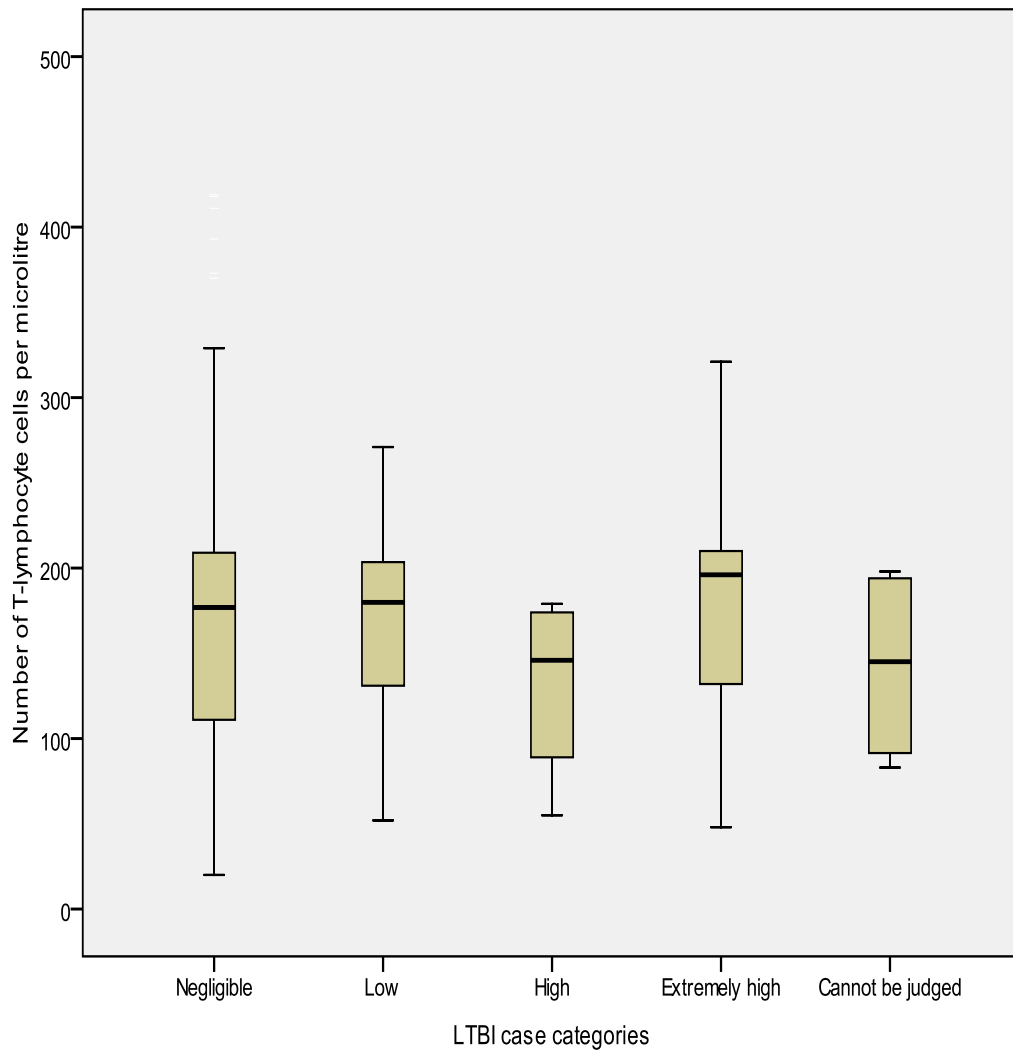
Figure 6.6: Box-and-whisker plots of median T-lymphocytes (CD4+) per microlitre (μL) separated by T-SPOT.*TB* assay according to the defined cases of latent tuberculosis infection (LTBI) of 180 new immigrants to Kuwait during February and May, 2010

6.2.6 Discussion

Screening for latent tuberculosis infection in new immigrants is mandatory before entry to non-endemic countries and Kuwait, but its diagnosis still poses a challenge. Diagnostics of latent TBI have been based for the last century on the old tuberculin skin test. However, a positive reaction can result not only from infection with *Mycobacterium tuberculosis* but also from BCG vaccination or cross-reaction with non-tuberculous mycobacteria. Several studies have shown that IGRAs and the T-

SPOT *.TB* test are superior diagnostic tools in the identification of individuals infected with TB (Anderson *et al.*, 2000; Borkowska *et al.*, 2011; de Andrade Lima *et al.*, 2011; Dilektasli *et al.*, 2010; Ewer *et al.*, 2003; Ferrara *et al.*, 2006, Pai *et al.*, 2005).

6.2.6.1   T-SPOT *.TB* results

The two IGRA tests perform similarly with superior diagnosis of LTBI and suspect TB new immigrants. The ELISPOT test was more sensitive than the QFT-GIT and the two old diagnostic tests for diagnosing latent TB immigrant carriers (41.11% versus 28.33% for QNF-GIT, 10.06% for chest X-ray and zero% for tuberculin skin test). Significant discordance between the two IGRAs and between each and the TST remain largely unexplained (Adetifa *et al.*, 2007).

6.2.6.2   T-lymphocyte cell counts

The cellular immunity of T-lymphocytes plays a role in the immune system and is an important factor in the outcome of tuberculosis infection. These cells have no phagocytic activity and cannot kill infected host cells or pathogens. Rather, they activate and direct other immune cells such as activation and growth of cytotoxic T cells, and in maximizing bactericidal activity of phagocytes such as macrophages.

The present study shows that 77.78% of total immigrants had median T-lymphocytes levels between 100 and 300 cells/µL, which is lower than normal cell counts (between 500-1,000 cells/µL) (Davoudi *et al.*, 2008). This proved that suppression of cellular immunity occurs even in the asymptomatic immigrants who were diagnosed with latent TB and had abnormal low CD4+ cell counts similar to any tuberculosis infection such as pulmonary and military TB. Comparative studies depend on standard CD4+ cell counts more than 100 cells/µL as the lowest diagnostic level for cellular quantitative comparison in a whole blood assay against positive TST (Lawn *et al.*, 2007). Both HIV infection (such as TB/HIV patients) and the lower immunological CD4+ cells are associated with a high risk of a normal CXR, even though symptoms of cough and dyspnea and sputum smear positivity are associated with a higher TB risk (Pepper *et al.*, 2008). INF-γ-producing T-cell responses against

Mycobacterium tuberculosis-specific antigens decrease during the treatment with anti-TB regimens (Chee *et al.*, 2008; Zhang *et al.*, 2010).

CD4+ lymphocyte cell counts are known to be associated with the clinical and radiographic presentation of LTBI and PTB (Asimos *et al.*, 1996; Davoudi *et al.*, 2008; Greenberg *et al.*, 1994), and lead to TB suspicion in immigrants (Martin *et al.*, 2011), which can be related to various CXR findings. The extent of CXR cavitation (which was an absent finding in this research) can be directly correlated with CD4+ cell counts, and the radiographic changes may reflect immune-suppression at the time of MTB infection (Sacks *et al.*, 1999). Suspects with more than 200 CD4+ lymphocytes/μL demonstrate atypical CXR with more apical infiltrates and cavitations, whereas, those with less than 200 CD4+ lymphocytes/μL often have lower lobe infiltration, opacities, interstitial nodules and hilar or mediastinal lymphadenopathy. Similar findings were observed by both Keiper *et al.* (1995) and Geng *et al.* (2005). Quantitative T-cell response was higher and revealed high median spot forming cells of monomorphonuclear T-cells in active TB cases compared to their LTBI contacts (Chee *et al.*, 2009).

Martin and colleagues (2011), in contrast to our findings, recently revealed a statistically significant association between tuberculosis and high CD4+ lymphocytes. Also Zhao and colleagues (2011) revealed a significant increase in the mean value of peripheral blood mononuclear cells using T-SPOT *.TB* test and the induration size of positive TST.

6.2.6.3 BCG vaccine and T-SPOT *.TB* results

Ewer *et al.* (2003) studied latent TB among school pupils in a British school and achieved 89% concordance between both T-SPOT *.TB* and TST and concluded that BCG vaccination did not influence the results of T-SPOT *.TB* test, compared with higher TST indurations in BCG-vaccinated children. Dilektasli *et al.* (2010) concluded that diagnostic accuracy of T-SPOT *.TB* assay was markedly higher for LTBI diagnosis in BCG-vaccinated individuals than TST performance. According to Borkowska *et al.* (2011), discordance of positive TST was due to BCG vaccination and discordance of positive T-SPOT *.TB* occurred due to lack of skin reaction to tuberculin in those participant's with poor health status. T-SPOT *.TB* positivity

among BCG-scar students was significantly lower than the TST positive rate, but not for 899 'no-scar' Chinese students, denoting no BCG vaccine effect of the high specific T-SPOT *.TB* results (Zhao *et al.*, 2011).

6.2.6.4   Is T-SPOT *.TB* assay to be preferred over TST?

The T-SPOT *.TB* test was superior to the tuberculin skin test in diagnosing latent TBI and offers a more sensitive and accurate approach than TST in the identification of tuberculosis infection (de Andrade Lima *et al.*, 2011). Variability of T-SPOT *.TB* positive results were not consistent with TST indurations in the healthy immigrants, which indicates that 10-mm diameter size cannot be used as a diagnostic 'cut-off' induration for LTBI diagnosis in BCG-vaccinated TB endemic populations and which need to be increased. A good correlation between positive T-SPOT *.TB* test results and TST diameter of induration of more than 15-mm was recently achieved by Borkowska and colleagues (2011). TST sensitivity increases and specificity decreases if induration size is accepted as more than 15-mm (> 15-mm), in comparison to a higher specificity of T-SPOT *.TB* assay was achieved by Dilektasli *et al.* (2010). In contrast to our study, 10-mm size was accepted by Zhang *et al.* (2010) as the 'cut-off' size for immigrants.

Our research findings concluded that T-SPOT *.TB* assay can be used in active TB patients during anti-TB chemotherapy which can serve as potential predictor of treatment accuracy and also representing LTBI prevalence better than undetected cases by the TST. The same conclusion was reached by Zhang *et al.* (2010). Other studies, with the similar conclusion that T-SPOT *.TB* assay improved the sensitivity for detection of active TB over both TST and QFT-GIT in non-endemic countries, are by Zhang *et al.* (2010) and Zwerling *et al.* (2011)..

Wrighton-Smith and colleagues (2006) have suggested that a TST followed by a T-SPOT *.TB* test protocol is cost-effective for contact screening for latent TB infection. However, the negative results of well-performed TST's described here did not support such follow-up criteria of LTBI and TB contacts.

Borkowska *et al.* (2011) obtained general concordance of 79% in a sample of healthy volunteers at risk of harbouring MTB in three subgroups; health care employees, suspected tuberculosis cases and suspected individuals to have other than

tuberculosis. According to Brodie *et al.* (2008), the general concordance between T-SPOT *.TB* test and TST results among BCG-vaccinated immigrants was 64%, compared with 82% in unvaccinated individuals.

In Gambia, a recent study on 2,348 household contacts showed excellent TST and T-SPOT *.TB* test performance for the diagnosis of LTBI from recent exposure, and concluded that positivity by either test can be an indication for preventive chemoprophylaxis without favouring TST replacement by the ELISPOT assay (Hill *et al.*, 2008).

For the T-SPOT *.TB* test, none of the independent variables and included risk factor predictors for LTBI such as age, nationality and place of birth, vaccination history or close contact with a patient with active TB disease was significantly associated with positive IGRAs results, compared with TST-BCG vaccination positivity (Sun *et al.*, 2010).

6.2.7 T-SPOT *.TB* strengths

1. The observed indeterminate (two samples) results were interpreted to have low number of T-lymphocytic cells, and which might occur because of insufficient INF-γ production in PHA mitogen well and also can be associated with malnourished (under-nutrition) immigrants coming from low income and food resource countries. But the assay nevertheless shows superior specificity in detection of INF γ-producing sensitized CD4+ cells (Dilektasli *et al.* 2010; Kobashi *et al.*, 2009). The same finding were noted even at younger ages (Bergamini *et al.*, 2009), compared with indeterminate results of QNF-GIT or false negative TST.

2. Sample transportation within 15 minutes and technical sample preparation in the central laboratory did not influence the occurrence of indeterminate results.

3. Performance of IGRAs blood withdrawal was conducted before tuberculin administration of TST that reduce unreliable results of IGRAs due to the release of INF-γ from the sensitised T-cells for up to 6 months following TST (Borkowska *et al.*, 2011).

6.2.8 T-SPOT .*TB* limitations

1. IGRA diagnostic tools are limited in underestimating the true number of LTBI cases. The high cost of T-SPOT .*TB* test and the test implementation by the central laboratory of tuberculosis in the Tuberculosis Control Unit for only four days per week (except Thursday) making it difficult to be a compulsory screening diagnostic for high risk groups. The results of Hill and colleagues (2008) do not support the appropriateness of a two-stage TB approach suggested by the NICE guidelines (NICE, 2006) and only seven of the 21 latent TB progressors to active TB might benefit from the preventive treatment. Cost-effectiveness of ELISPOT test approach as diagnostic or confirmatory test requires further study.

2. Even though indeterminate results are known to be less frequent in T-SPOT .*TB* test than QFT-GIT (result of choice of 'cut-off' point read by Cellestis Software) but still our research observed two indeterminate results of T-SPOT .TB test and not for QNF-GIT (Kobashi *et al.*, 2009).

6.2.9 Conclusions

The study revealed that the T-SPOT .*TB* assay is a good diagnostic tool in identifying latent tuberculosis carriers. The use of T-SPOT .*TB* assay in high-risk groups and new immigrants who are miss-diagnosed or missed by the negative ordinary CXR and TST is helpful and can enhance LTBI diagnosis even in BCG-vaccinated community. At public health policy level in Kuwait, the T-SPOT .*TB* test results support the addition and/or replacement of the CXR or Mantoux test with a T-cell based test for the diagnosis of LTBI in any suspected new immigrant.

6.2.10 Recommendations

1- T-SPOT .*TB* assay should be used as an alternative diagnostic approach or as a complementary tool for suspected CXR or negative TST cases, and also to exclude positive TST caused by BCG vaccination.

2- Large cohort study is warranted to study the correlation between T-SPOT .*TB* test results and lower TST indurations (such as 5-mm) in high resource countries.

3- To evaluate the clinical utility of T-SPOT .*TB* assay in accurate diagnosis of LTBI in high risk groups such as health care workers and laboratory employees.

4- Assessment of levels of sencitized T-lymphocytes using T-SPOT .*TB* assay should be repeated on diagnosed TB patients before, during and after anti-TB therapy to find diagnostic correlation with active tuberculosis convertors.

A combination of the evidence-based radiographic and laboratory diagnostic tests (chapters 5 and 6) and evidence-based epidemiological risk factors (chapter 4) which would facilitate prediction and early diagnosis of latent tuberculosis infection in MTB harbouring and suspected cases will be described in chapter seven.

7 Chapter seven

Evidence-based laboratory diagnostic criteria for detection of latent tuberculosis infection and 'suspect tuberculosis' cases – Classification

''Absence of a gold standard test to correctly identify the sensitivity and specificity of each of the four diagnostic tests poses a challenge for evaluation and comparative assessment (Kunst, 2006)''

7.1 Introduction

Despite management advances worldwide, tuberculosis still remains a serious uncontrolled disease with threatening consequences, such as nosocomial outbreaks of TB/HIV co-infections and the rise of drug-resistant strains (Sacks *et al.*, 1999; He *et al.*, 2010). Accurate and timely detection of LTBI hiding bacilli is crucial but difficult due to inability to recognize the epidemiology of TB species and the implemented (whether effective) strategies to limit its spread. Therefore an important factor in successful elimination of TB is accurate and timely detection of LTBI to enhance early effective prophylaxis and help to reduce progression from dormancy to overt disease and consequently prevent further MTB transmissions. Delayed diagnosis may worsen TB into a more severe illness and increase the risk of morbidity and mortality. Therefore worldwide concerns are always targeted toward screening the high risk groups and foreign-born immigrants by encouraging detection of infectious transmission and allowing health care systems to operate effectively. Active case findings are influenced by the degree of diagnostic indication (clinical suspicion) and the quality of laboratory facilities. The performance and accuracy of diagnostic tests for LTBI/TB varies with the stage and consequent disease severity. For example, IRGAs are currently recommended for those with positive TST and normal CXR to confirm the diagnosis of LTBI (CDC, 2011b; Hardy *et al.*, 2010).

A conflict in risk estimation of TB disease and limitations of LTBI diagnostic tests plays a role in MTB infectiousness and uncontrolled epidemiological spread (Freeman *et al.*, 2010). The diagnosis of tuberculosis in patients with a negative radiographic or bacteriological examination is still problematic in clinical settings. The newly developed IGRAs, including both T-SPOT *.TB* and QFT-GIT, show advances in TB diagnosis, which require research to discriminate the accuracy of all diagnostic tests (Ferrara *et al.*, 2006; Jong Lee *et al.*, 2010; Kobashi *et al.*, 2009; Zwerling *et al.*, 2011). Tuberculosis threats rise in those having a positive IGRAs (QuantiFERON and/or T-SPOT *.TB* tests) and negative TST with free radiographs leaving many high-risk immigrants (mainly from endemic countries) unidentified, if

not tested with IGRAs. To overcome the TST shortcomings, use of IGRAs is currently recommended as two-step strategy (CDC, 2011b; Jong Lee *et al.*, 2010).

Choice of LTBI screening test

Indications for tuberculosis infection diagnostics should depend on the followings 1- the purpose of the test (whether monitoring, follow-up or research), 2- the target population for screening, 3- sample turnover (number of samples tested per day), 4- availability of technically skilled personnel and equipments, 5- the time within assay performed (e.g. IGRAs within 6 hours), and 6- the costs, including instruments, reagents, shipping, laboratory infrastructure and labour cost.

Pai *et al.* (2006) compared the performance and operational characteristics of the three laboratory TB diagnostic tests, recently supported by CDC (2011b) (Table 7.1).

Table 7.1: Comparison of the operational characteristics of the old tuberculin skin test and the two new interferon- gamma release assays used for latent tuberculosis infection diagnosis (Pai *et al.***, 2006)**

Performance and operational characteristics	Tuberculosis diagnostic test		
	TST	**QFT-GIT**	**T-SPOT .*TB***
Initial Process	Intradermal injection	Process whole blood within 16 hours	Process peripheral blood mononuclear cells within 8 hours
Mycobacterium tuberculosis **antigen used**	Single antigen (PPD RT23)	Single mixture of synthetic peptides representing ESAT-6, CFP-10 & TB7.7	Separate mixtures of synthetic peptides (ESAT-6 & CFP-10)
Cross-reactivity by prior BCG or with NTM	Yes	Less likely (with exceptions)	
Correlation with MTB exposure intensity	Partial	Yes (better correlation with exposure than TST)	
Test	In vivo skin test	In vitro blood test	
Reliable (reproducibility)	Moderate and variable	High (no inter-subject variability)	
Boosting phenomenon	Yes (if repeated)	No	
Result conversions and reversions	Yes	Not always	
Adverse reactions	Rare (discomfort skin or anaphylactic reaction)	Rare	
Material costs	Low (Euro 23, $ 9.79, £8)	Moderate-high (Euro 129, $37.5, £20-40)	
Patient visit to complete testing	Yes (once at least)	No	
Laboratory infrastructure (e.g. instruments)	No	Yes	
Time to obtain a result	2-3 days	< 1 day (16-24 hour)	
Trained personnel	Yes (intradermal)	Yes (Phlebotomy)	
Measurement (result given in)	Induration swelling (millimetre)	INF-γ concentration units	Number of INF-γ producing cells (spots number)
Possible results	Positive, negative	Positive, negative, indeterminate	Positive, negative, indeterminate, borderline
Inter- or intra-reader variability	Yes (subjective)	No (objective)	

Regardless of the tuberculosis diagnostics used, targeted testing is critical in reducing unnecessary testing and treatment (Mancuso *et al.*, 2011). CDC guidelines recommend targeted testing of only persons with known risk factors for TB, specifically stating that ''targeted tuberculin testing programs should be conducted only among high risk groups and discouraged in those at low risk'' (CDC, 2000; CDC, 2011a). But a known limitation is the limited data on the use of both IGRAs tests to predict who will progress to TB disease in the future (CDC, 2011c). In addition, using ordinary chest radiography is the most critical step of missing cases with high index of clinical suspicion for LTBI re-activations in TB non-endemic countries and pulmonary tuberculosis in TB-endemic areas, particularly in foreigners/immigrants with past history of contracted TB disease or contact with pulmonary TB infection (Wu *et al.*, 2009). False-positive TST results due to prior BCG vaccination lead to unnecessary treatment of presumed wrongly diagnosed latent tuberculosis infection where IGRAs recently approved as more sensitive and specific in this suspicious individuals. NICE guidelines currently further recommended IGRAs for those with positive TST and normal CXR to confirm LTBI diagnosis, while the old criteria recommended only CXR for screening immigrants coming from countries with TB incidence more than 40 per 100,000 populations (NICE, 2006). A two-step strategy is still recommended for LTBI diagnosis using the Mantoux skin test as a screening tool and QNF-GIT as a confirmatory test (NICE, 2006), or using either IGRAs (CDC, 2011b), but requires further research.

The previous findings in chapter five suggested that LTBI prevalence in the new immigrants screening measured using both CXR and TST are of extremely low to yield LTBI prevalence in addition to no bacteriological confirmation, compared with a higher LTBI prevalence's detected by the new biomarker IGRAs assays in chapter six, which were also similar to other publications and research programme findings (ATS, 2000; Brodie *et al.*, 2008; CDC, 2011b; Demkow *et al.*, 2008; Diel *et al.*, 2010; Diel *et al.*, 2011a; Eisenberg and Pollock, 2010; Ferrara *et al.*, 2006; Hardy *et al.*, 2010; Mahan *et al.*, 2011; Mazurek *et al.*, 2007; Mazurek *et al.*, 2010; Menzies *et al.*, 2007; Nienhaus *et al.*, 2011; Rangaka *et al.*, 2011; Soysal and Bakir, 2011; Sun *et al.*, 2011; Wrighton-Smith *et al.*, 2006).

7.1.1 Aim

The main aim is to assess the evidence-based laboratory diagnostic testing for detection of latent tuberculosis infection among new immigrants to non-endemic Kuwait.

7.1.2 Objectives

To estimate the accuracies of both the old and new diagnostic tests for detection of latent tuberculosis with absence of a gold standard diagnostic test able to rule out *Mycobacterium tuberculosis* exposures.

7.1.2.1   Sub-objective 1:

The first sub-objective is to introduce a new classification tree and scoring system of evidence-based radiographic-laboratory tuberculosis diagnostics combinations for detection of latent tuberculosis infection and 'suspect TB' carriers.

7.1.2.2   Results of sub-objective 1

According to the evidence-based laboratory-related criteria for LTBI diagnosis which were previously described in chapter three; section 3.16 (Table 3.2) the study subjective one facilitates the use of the estimated accuracies of the four tuberculosis diagnostics help to determine the frequency and percentage distribution of LTBI defined categories. Similarly, using the optimal accuracy after implementation of the two old and two new diagnostic tests either singly or in combination permitted estimation of suspicious tuberculosis cases or facilitate TB control.

Following the use of risk assessment definitions for defining LTBI case categories according to Thrusfield M. (2007), the study results permitted the introduction of new screening criteria and tuberculosis testing guidelines for diagnosis of latent tuberculosis in the high risk groups and general population (Table 7.2).

Table 7.2: New evidence-based radiographic-laboratory classification criteria for the diagnosis of latent tuberculosis infection case categories and suspect tuberculosis cases implemented on the 180 new immigrants to Kuwait during February and May, 2010

Test score	CXR	TST	QNF-GIT	T-SPOT *.TB*	LTBI case diagnosis	Frequency (%)
0	x	x	x	x	Cannot be judged	4 (2.2)
1	-	-	-	-	Negligible	90 (50.0)
2	+	-	-	-	Low	9 (5.0)
3	-	+	-	-	Low	0 (0.0)
4	-	-	+	-	Low	4 (2.2)
5	-	-	-	+	Low	23 (12.8)
6	+	+	-	-	Average	0 (0.0)
7	+	-	+	-	High	0 (0.0)
8	-	+	+	-	High	0 (0.0)
9	+	-	-	+	High	4 (2.2)
10	-	+	-	+	High	0 (0.0)
11	-	-	+	+	Extremely High	41 (22.8)
12	+	-	+	+	Extremely High	5 (2.8)
13	-	+	+	+	Extremely High	0 (0.0)
14	+	+	+	+	Extremely High	0 (0.0)

CXR = chest X-ray, TST = tuberculin skin test, QNF-GIT = QuantiFERON Gold In-Tube test, T-SPOT *.TB* = T-SPOT *.TB* test, LTBI = latent tuberculosis infection, (+) = positive result, (-) = negative result, (x) = no result and case excluded

Continue of Table 7.2: *Risk assessment definitions according to Thrusfield M. (2007): Negligible (of 'likelihood') = That need not to be considered, Insignificant (c.f. 'significant'), Insignificant (of 'likelihood') = Unimportant, trifling (c.f. 'significant'), Low (of 'likelihood') = Less than average; coming below the normal level, Average (of 'likelihood') = The usual amount or extent or rate, High (of 'likelihood') = Extending above the normal or average level, Extremely (of 'likelihood') = Outermost; furthest from the centre; situated at either end; the highest or most extreme degree of anything.*

The percentage distribution of the defined LTBI case categories implemented on the 180 new immigrants that was described in Table 7.2 is represented accordingly in Figure 7.1.
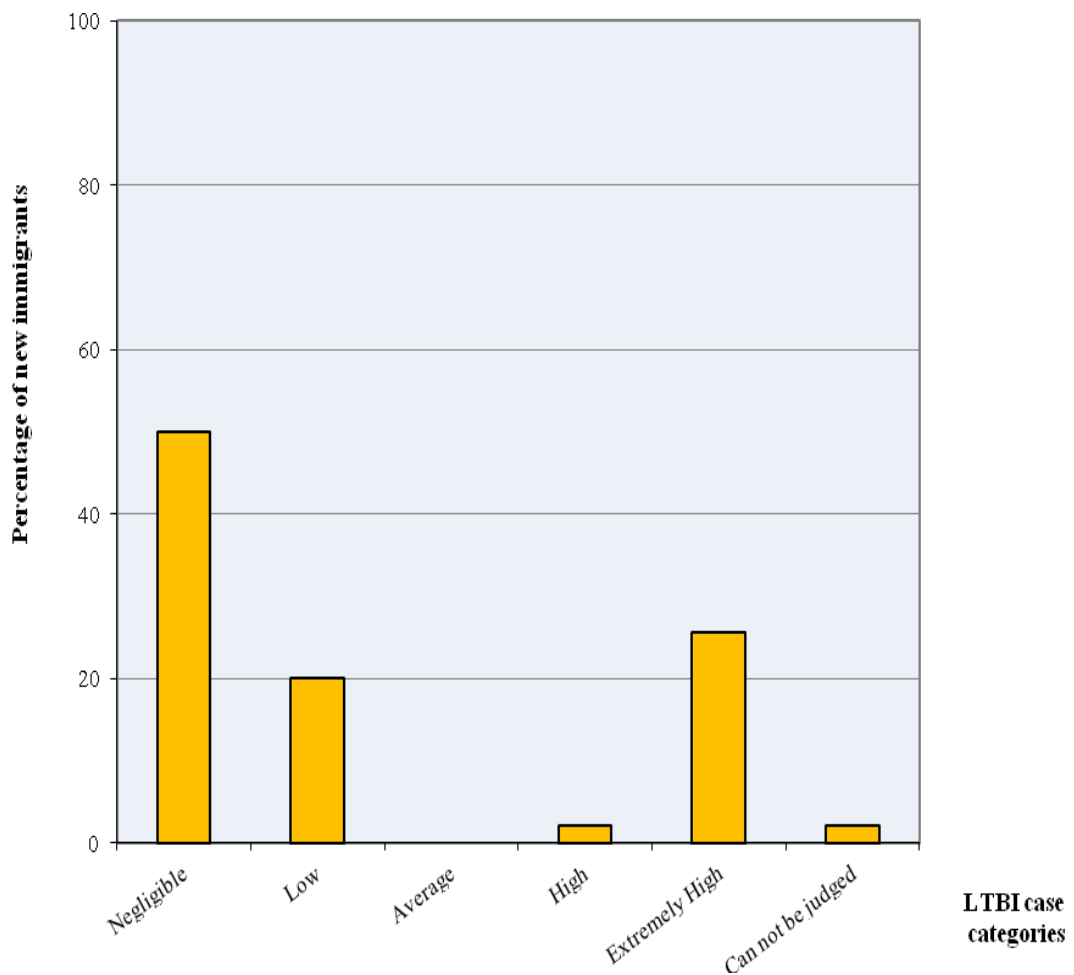
Figure 7.1: Percentage distribution of the defined latent tuberculosis infection case categories representing the sample 180 new immigrants to Kuwait during February and May, 2010

The study results permitted introduction of new screening guidelines for latent tuberculosis in high risk groups and general population (Table 7.2). These new testing's guidelines can be accepted as 'the paradox of gold standard' for development of an interventional strategies involving the use of various diagnostics for latent tuberculosis diagnosis and will have useful applications to field research projects toward preventing tuberculosis worldwide.

Diagnostic test accuracy cannot be accurately determined because of absence of the gold standard for latent and active tuberculosis for comparison. Therefore it is impossible to definitely rule out MTB exposure which can be a significant limitation in IGRAs specificity (Dilektasli *et al.*, 2010).

7.1.2.3   Sub-objective 2:

The second sub-objective is to assess the performance characteristics, through comparative evaluation, of the accuracy of the two old and the two new laboratory diagnostic tests in the clinical diagnosis of latent tuberculosis infection.

7.1.2.3.1   Methodology of sub-objective 2

Data on the diagnostic test outcomes in question were extracted by the same reviewers from the full text of studies that satisfied the research inclusion criteria as explained in chapter 3 (Materials and Methods; section 3.18):

1. Sensitivity (SN), defined as the ability of a test to identify latent tuberculosis infection among new immigrants as abnormal or 'positive' result by any result of the four combination tests.
2. Specificity (SP), defined as the ability of the test to identify subjects without suspicion or evidence of LTBI as normal or 'negative' test results of all four diagnostic tests.
3. Positive predictive value (PPV), defined as the probability that the positive result is a true positive, whereas, the negative predictive value (NPV) of the test is the probability that the negative result is a true negative.

4. Diagnostic accuracy (DA) of the testing's combinations, calculated according to the following equation; SN+SP/SN+SP+PPV+NPV (Knapp and Miller, 1992; Simundic, 2008; Thrusfield, 2007).

'Indeterminate' result of IGRAs is defined as the number of a test failed to give a reportable finding result. Our research study accepted any defined indeterminate result as normal or 'negative' result.

Implementation of various tuberculosis diagnostics, either singly or in combination, to 'maximize' the accuracy of latent tuberculosis infection diagnosis and high risk group carriers of MTB was undertaken. Diagnostic-test parameters outcomes (SN, SP, PPV, NPV, and DA) and their 95% confidence intervals, for the four tuberculosis diagnostic test statistics, were calculated using the SPSS 17.0 Software.

7.1.2.3.2 Results of sub-objective 2

Table 7.3 shows the diagnostic-test parameters outcomes and related 95% confidence intervals and kappa values (detailed in chapter 3: Materials and Methods) for various combinations of the four tuberculosis diagnostic tests. The parameter outputs involved only test results for the included immigrant cases (defined as the total sample number for each combined diagnostic test) for each two combinations of the three implemented tuberculosis diagnostics, with exclusion of the TST (due to negativity of all included 180 participants).

Table 7.3: Diagnostic accuracy of various combinations of the four screening diagnostic tests used for diagnosis of latent tuberculosis infection suspected cases implemented on the 180 new immigrants to Kuwait during February and May, 2010 (LTBI = latent tuberculosis infection, CXR = chest X-ray, TST = tuberculin skin test, QNF-GIT = QuantiFERON Gold In-Tube test, T-SPOT .TB = T-SPOT .*TB* test, SN = sensitivity, SP = specificity, PPV = positive predictive value, NPV = negative predicitive value, DA = diagnostic accuracy, TCU = medical staff of the Tuberculosis Control Unit, N/A = cannot be computed by SPSS Software programme)

TB diagnostic test combinations	Total included sample	SN (95% CI)	SP (95% CI)	PPV (95% CI)	NPV (95% CI)	DA	Kappa (limits)
CXR (two radiologists) & CXR (TCU/three pulmonologists)	179	N/A	94.32	0.00	100.00	94.32	N/A
TST & CXR (TCU/three pulmonologists)	176	N/A	100.00	N/A	100.00	100.00	N/A
QNF-GIT & CXR (TCU/three pulmonologists)	179	N/A	71.59	0.00	100.00	71.59	N/A
T-SPOT .*TB* & CXR (TCU/three pulmonologists)	179	N/A	58.52	0.00	100.00	58.52	N/A
TST & CXR (two radiologists)	176	N/A	94.32	0.00	100.00	94.32	N/A
QNF-GIT & CXR (two radiologists)	179	30 (10.8-60.3)	71.60 (64.4-77.9)	5.88 (2-15.9)	94.53 (89.1-97.3)	69.27	0.002 (-0.100-0.105)
T-SPOT .*TB* & CXR (two radiologists)	179	60 (31.3-83.2)	59.76 (52.2-66.9)	8.11 (3.8-16.6)	96.19 (90.6-98.5)	59.78	-0.031 (-0.157-0.096)
QNF-GIT & TST	177	N/A	71.75	0.00	100.00	71.75	N/A
T-SPOT .*TB* & TST	177	N/A	58.76	0.00	100.00	58.76	N/A
QNF-GIT & T-SPOT .*TB*	180	92.16 (81.5-96.9)	79.07 (71.3-85.2)	63.51 (52.1-73.6)	96.23 (90.7-98.5)	82.78	0.627 (0.507-0.746)

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Values that could not be calculated by SPSS 17.0 were presented as N/A (not available values) due to absent of positive diagnostic test results, which lead to zero positive predictive values.

The highest calculated sensitivity was calculated between the two IGRAs 92.16% (95% CI: 81.5-96.9), whereas, the lowest sensitivity was calculated between both the QNF-GIT and chest radiography read by the two radiologists 30% (95% CI: 10.8-60.3). The highest specificity was measured in comparison between the two IGRAs 79.07% (95% CI: 71.3-85.2), in comparison to the lowest specificity was calculated between the T-SPOT *.TB* test in compared with chest X-ray read by radiologists 59.75% (95% CI: 52.2-66.9).

Both the highest PPV 63.51% (95% CI: 52.1-73.6) and the highest NPV 96.23% (95% CI: 90.7-98.5) were calculated in comparison between both IGRAs assays. On the other hand, the lowest PPV was 5.88% (95% CI: 2-15.9) and the lowest NPV was 94.53% (95% CI: 89.1-97.3) and were both belonging to the comparison between both the QNF-GIT and chest radiography read by the two radiologists. The highest diagnostic accuracy was calculated for comparison between both IGRAs as 82.78%, whereas, the lowest diagnostic accuracy was calculated in comparison between both T-SPOT *.TB* test versus chest X-ray read by the two radiologists as 59.78%.

Considering the two indeterminate results of T-SPOT *.TB* test as 'normal', then both IGRAs assays revealed concordant 'abnormal' results in 26.11% (47/180) and concordant 'normal' results in 56.66% (102/180). Discordance of IGRAs were detected as the following; T-SPOT *.TB*+/QNF-GIT- in 15% (27/180), whereas, QNF-GIT+/T-SPOT *.TB*- in 2.22% (4/180) (Figure 7.2). Similarity of IGRAs results was observed with 'good' kappa agreement of 0.627 (0.507-0.746) for LTBI detection (Figure 7.2, Table 7.3).

Agreement between both IGRAs (T-SPOT *.TB*+/QNF-GIT+) was the highest 83.2% (k = 0.66) compared with lower T-SPOT *.TB*+/TST+ agreement of 64.5% (k = 0.34) or QNF-GIT+/TST+ agreement of 58% (k = 0.26 (Dominguez *et al.*, 2008).
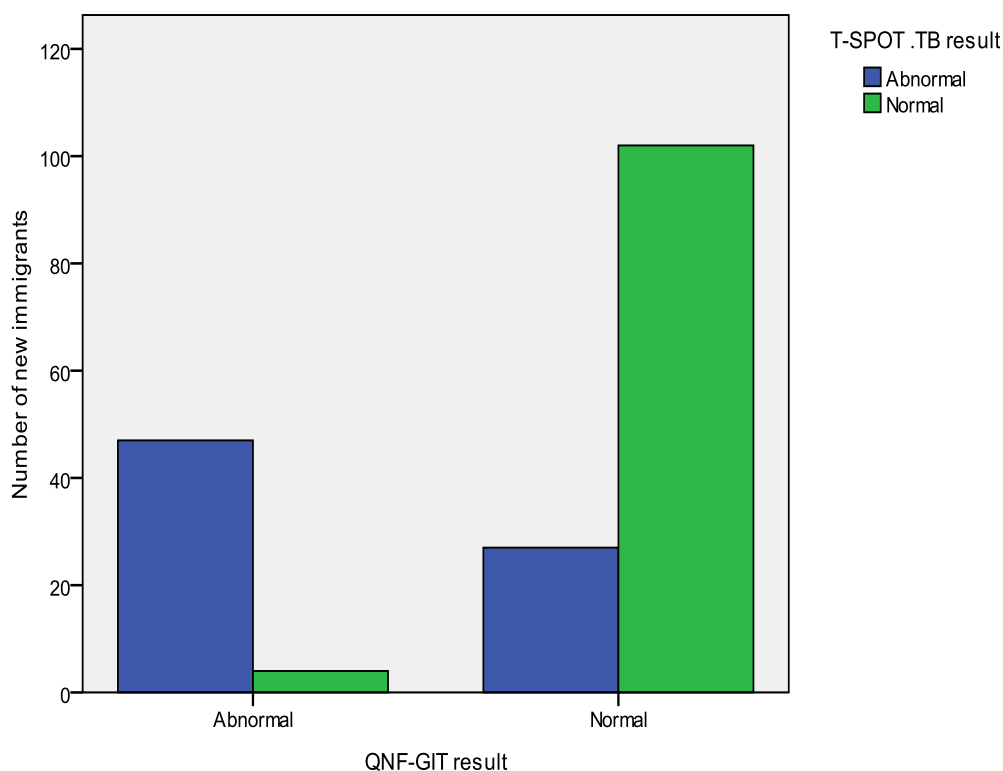
Figure 7.2: Percentage distribution of the defined latent tuberculosis infection case categories representing the sample 180 new immigrants to Kuwait during February and May, 2010

7.1.3 Discussion

Early diagnosis of *Mycobacterium tuberculosis* infection is an essential, but difficult, step in tuberculosis control and elimination. Screening and treating individuals harbouring LTBI will minimize the risk of subsequent development to active TB, especially in immigrants from TB endemic regions. LTBI incidence can be over- or under-estimated using the standard TB diagnostic tests such as chest X-ray or the tuberculin skin test, which have a low sensitivity and specificity, especially in TB endemic countries with predominantly BCG-vaccinated populations (Sun *et al.*, 2010). Current evidence supports use of IGRAs. In comparison with the TST, the newer commercial IGRAs were superior and more specific for detecting confirmed

active TB disease, especially when performed in developed countries where economic resources are of little concern (Diel *et al.*, 2010).

To evaluate the implications of screening on arrival, this research explored the changes the prevalence of latent tuberculosis in new immigrants coming from high-incidence TB areas. Identifying the role of new TB screening strategy in early and accurate detection of LTBI and indirectly reducing the consequent TB outcomes. IGRA results related to measures of the level of exposure better than the PPD test did (Arend *et al.*, 2007). For example, molecular epidemiology suggests that the majority of resistant TB cases are due to ongoing transmission of drug-resistant MTB strains, which is likely to be the result of diagnostic delay, thereby emphasizing the need for rapid accurate diagnostics and comprehensive contact tracing of high risk groups. Current diagnosis of LTBI in the low-income and high-burden regions relies on chest X-ray and TST following suspected abnormal CXR findings of any healthy asymptomatic expatriate. The high non-compliance rates reduce the efficacy of TB disease control measures. Socio-economic characteristics such as age and behavioural attitudes of the studied groups towards monitoring are common cultural or psychological factors influencing participants' opinions. For example, higher family income predisposes to non-compliance with test administration among medical students, versus compliance among physiotherapy students (da Rocha *et al.*, 2011).

7.1.3.1 Absence of a gold standard test

While positive smear microscopy and bacteriology culture can still be accepted as the gold standard in laboratory diagnostics, remarkable technical progress has been made recently with advance in MTB detection through the implementation of ex vivo interferon-gamma release assays for the detection of latent TBI. The IGRAs were developed to overcome the difficulties and limitations of the standard tuberculin skin test in accurately diagnosing latent tuberculosis. Still fewer publication of literature reviews are available on using IGRAs to screen immigrants within TB control programmes (Carvalho *et al.*, 2005; Winje *et al.*, 2008).

It is impossible to establish the actual sensitivity and specificity of any of the four TB diagnostics because of the lack of a gold standard for the diagnosis of LTBI (Borkowska *et al.*, 2011), and surrogate measures, such as gradient of recent exposure, have been used to evaluate IGRAs (Brodie *et al.*, 2008, Ewer *et al.*, 2003; Lalvani *et al.*, 2001b),

To improve LTBI/TB diagnostic specificity, new IGRAs have been developed, but updated IGRA guidelines are always needed worldwide. The accumulating evidence suggests that the commercial IGRAs have higher sensitivity to detect infection after known *M .tuberculosis* exposure in regions with low tuberculosis incidence and specificity than the TST in detecting LTBI, particularly in BCG-vaccinated patient's who comprise the majority of the developing world. For example, IGRAs are suggested to be superior in detecting recent rather than old infection (Mazurek *et al.*, 2010).

Diagnostics accuracy estimates can be used to determine guidelines for the optimal implementation of the two old and two new diagnostic tests; either singly or in combination, and to maximize diagnostics accuracy. These guidelines may have useful applications to field research projects and the development of intervention strategies involving the use of laboratory tests to control and prevent TB worldwide.

The results show considerable diversity in the recommendations on IGRAs, with four approaches commonly proposed: 1- a two-step approach of TST, followed by IGRA either when the TST is negative (to increase sensitivity, mainly in immune-compromised individuals), or when the TST is positive (to increase specificity, mainly in BCG-vaccinated individuals); 2- either TST or IGRA (but not both), 3- IGRA and TST together (to increase sensitivity even with decreased TST specificity), and 4- IGRA only to replace the TST. All these IGRAs testing guidelines are supported with evidence-based results and periodical updates should be monitored (CDC, 2011b).

Interferon-gamma release assays can be used at the community level for epidemiological studies and LTBI detection to control MTB transmission due to

immigration, and is not suggested for serial testing of active tuberculosis diagnosis (Legesse *et al.*, 2011; Nenadic *et al.*, 2011). IGRAs have been reported to be better predictors of the risk of developing tuberculosis disease than the TST, and correlate better with the intensity and duration of TB exposure than the TST (Mendez-Echevarria *et al.*, 2011; NICE, 2011). Old studies performed in the developed countries have shown superior sensitivity and specificity of the currently available two IGRA kits for the diagnosis of latent TBI, as compared with TST (Ewer *et al.*, 2003). New guidelines of some developed countries are starting to recommend a two-step strategy for the diagnosis of LTBI, using IGRA to confirm positive TST cases, and has significant cost-benefit reductions in TB chemoprophylaxis administration (CDC, 2011b; NICE, 2011).

Considering the confounding effect of risk factors, there is a need for further evaluation using IGRAs in both vaccinated and non-vaccinated new immigrant populations, which can estimate (measure) the protective effect of BCG vaccination against latent TB infection. Evidence of protection (assessed by positive IGRAs) and reducing risk of severe TB disease forms (66% reduction), and cost effectiveness of BCG immunization was highlighted for revision in the next-step of universal BCG vaccination and careful monitoring and follow-up for TB incidence associated with high travel and MTB exposures of international groups (Eriksen *et al.*, 2010).

Similar to our comparable results, both IGRAs were superior in sensitivity and specificity, and were acceptable substitutes for chest radiography and the tuberculin skin tests, without complications such as exaggerated delayed type hypersensitivity reactions (Tavast *et al.*, 2009). However, the accuracy of IGRAs for diagnosing latent tuberculosis remains unknown because of the absence of a reference/gold standard diagnostic test. Additional evidence-based data are needed before deciding that positive IGRAs result should receive tuberculosis preventive chemoprophylaxis with caution. Ultimately, the gold standard for evaluating the significance of diagnostic tests is the consequent progression to active TB disease therefore long-term longitudinal follow-up will be required to clarify the predictive values of all diagnostics (Mahan *et al.*, 2011).

7.1.3.2   Accuracy of tuberculosis diagnostic tests

Diagnostic accuracy is a measure of proportions of the correct test results and is measured by assessment of specificity (proportion of true negatives that are actually negative test results) and sensitivity (proportion of true positives that are actually positive test results). Assessments of accuracy of *Mycobacterium tuberculosis* diagnostic tests are difficult because there is no 'gold standard' to confirm a diagnosis of LTBI, but, 'approximations' of accuracy can be made by testing populations with known characteristics (Mazurek *et al.*, 2010).

Estimation of diagnostic accuracies permits defining LTBI prevalence more precisely. Due to the lack of a gold standard in LTBI diagnostics, it is impossible to establish the sensitivity or specificity of any of the four TB diagnostic tests. This correlation was not specifically defined in any of the previous studies of TB diagnostics. No single diagnostic test is able to exclude MTB exposure which can be related to significant reduction in IGRA's specificity and accuracies (Dilektasli *et al.*, 2010). Therefore, our study analyzed the diagnostic accuracies between the combination results of two diagnostics which possibly can be considered as a substitute criteria used for diagnosis of LTBI and estimation of TB risks (Borkowska *et al.*, 2011).

All diagnostic tools (old and new tests) are being developed and evaluated after careful evaluation of accuracy, society impacts and cost-effectiveness. Estimation of diagnostic test accuracy for maximum TB diagnosis would help to determine guidelines for the optimal implementation of these tests, either singly or in combination (Diel *et al.*, 2011a). The greater attainable specificity and sensitivity in diagnosing and detecting LTBI cases of both IGRAs and the requirement of a 'one-visit-result' is a compelling advantage over the old less specific diagnostic test and' two-visit-result' TST test (Table 7.1). IGRA tests did not show false positive results in BCG-vaccinated individuals similar to the observed boosting phenomenon leading to false positive TST (Borkowska *et al.*, 2011). Erikson *et al.* (2010) and Zhang *et al.* (2010) concluded that a significantly lower proportion of IGRA positivity was seen in BCG vaccinated people than in non-vaccinated individuals.

Sun *et al.* (2011) undertook a meta-analysis from 2000 to 2011; the sensitivities of both IGRAs and the TST in active tuberculosis were similar. The pooled specificity was 100% for ELISA and 90% for ELISPOT, but was lower for the TST (56%), and even lower (49%) in children with positive BCG vaccination.

LTBI and are a more specific way of diagnostic and reducing the number of treated patients IGRAs represent advances in tuberculosis immunology and evolutionary microbial biology and are designed to replace TST for LTBI diagnosis because of their logistical advantages and enhanced specificity over TST. For example, the boosting effect of IGRAs results by the TST was absent in the findings of Bradshaw *et al.* (2011). Both diagnostic tests still lack the expected sensitivity and reproducibility. IGRAs are more accurate indicators, and are a valuable public health tool with diagnostic advantages over the PPD test, which require improvements in governmental feasibility resources.

The present study attempted to establish the rate of LTBI infection in new immigrants using the novel T-cell based in vitro IGRAs recently developed to detect LTBI and overcome the limitations of the CXR and TST, which provides for targeted intervention through a new screening policy. A similar attempt was performed by the American Thoracic Society (ATS, 2000). A combination of both QNF-GIT and TST add higher sensitivity, even in LTBI endemic areas such as Indonesia, but with lowered specificity for active TB disease (Rutherford *et al.*, 2010). Our findings revealed QNF-GIT specificity was consistently high same as T-SPOT *.TB* test specificity, and with lower indeterminate estimates in the new expatriates. Similar results on adult participants were also detected by other screening findings (Diel *et al.*, 2010; Ferrara *et al.*, 2006). The T-SPOT *.TB* test can be comparatively better over the TST in routine clinical use in identifying the BCG-vaccinated high risk groups and foreign-born populations from endemic regions (Brodie *et al.*, 2008).

Even the achieved high correlation and agreement between both IGRAs assays than between the TST and either IGRAs assay, still little discrepancy is present between both IGRAs which needs future study evaluations at different IGRAs 'cut-off' levels

and re-test the predictive values of diagnostics result before development of active TB disease (Arend *et al.*, 2007).

T-SPOT .*TB* test had significantly higher specificity, with less indeterminate results, even in immunosuppressed hospital patients with LTBI or suspected active TB disease than QNF-G and TST (Ferrara *et al.*, 2006). On the other hand, our research T-SPOT .*TB* test results were more specific, but had two indeterminate results, compared with no indeterminate results in QNF-GIT.

Regardless of any test used, targeted testing is critical in reducing unnecessary testing and treatment for accurately diagnosed conditions. As with the TST, testing with IGRAs can give false-positive results if used in low-prevalence populations. Current guidelines and evidence available on the use of IGRAs do not address the importance of serial testing or provide the guidance to properly interpret IGRA results, which need to be updated with specific recommendations (Zwerling *et al.*, 2011).

Future directions in IGRA development are always reviewed for new targeted indications. As a new promising tool, both interferon gamma release assays were used in countries with a low incidence of pediatric TB and a low rate of BCG vaccination to detect outbreaks in schools (Ewer *et al.*, 2003), to monitor anti-TB therapy (Sun *et al.*, 2010; Zhang *et al.*, 2010) or to diagnose of tuberculosis and NTM disease.

Nowadays, the use of IGRAs is increasingly recommended, but still their role in the diagnosis of active tuberculosis remains unclear. For example, diagnostic sensitivities of both IGRAs are higher than that of the TST but are still not high enough to be used to rule out TB. Sound evidence for the use of IGRAs requires independent and carefully designed prospective studies that the thesis aim and methodology were focused. Even with restrictions of low PPV, IGRAs diagnostic results to diagnose active TB in the last years are promising (Lalvani *et al.*, 2001a; Lalvani *et al.*, 2001b; Meier *et al.*, 2005; Simsek *et al.*, 2010).

Correlations with or confirmatory to other TB diagnostic tests in predicting the clinical outcomes and stage markers of disease severity, IGRAs were also used for

monitoring treatment response in the context of TB clinical trials. For example, diagnostic scores can be utilized in a separate and comparable patient population (Ralph *et al.*, 2010). IGRAs showed good correlation with occupational risk factors for TB exposure in low-incidence settings (Zwerling *et al.*, 2011). Still persistent positive IGRA responses following old TB infection among immigrants limit their usefulness, and by which cannot be considered as a discriminative test to reflect 'recent' TB infection in contact investigation (Kik *et al.*, 2009). Also serial IGRAs results should be interpreted cautiously because of their high variability associated with both conversion and reversion phenomena.

Extensive studies evaluating the cost, feasibility, and effectiveness of new screening policies, such as using IGRAs, is a national policy pre-requisite. Internationally, new intervention guidelines will impact on clinical practice differently. IGRA positivity is not influenced by BCG-vaccination, and is associated with exposure to contagious tuberculosis cases and remote TB exposures (Diel *et al.*, 2011a). IGRAs high specificity will stop unnecessary preventive prophylaxis in BCG-vaccinated individuals with a false-positive TSTs, whereas improved sensitivity for LTBI detection in individuals with weakened cellular immunity at highest risk and vulnerable groups (for example malnutrition or HIV-positive individuals) will enable reliable targeted testing and treatment before progressing to active tuberculosis. The role of IGRAs in the diagnosis of active TB is still not clear, but either these tests can be useful as adjunct tests in diagnostic work-ups of suspected TB cases. Irrespective of the economic resources, Diel *et al.* (2010) concluded that IGRAs are superior to the TST for detecting confirmed active TB disease, and also for LTBI diagnosis by Dilektasli and colleagues which was more specific if applied in non-endemic and developed countries (Dilektasli *et al.*, 2010).

Fifteen studies assessed the prediction ability of the studied population before developing active TB concluded that neither IGRAs nor TST were not highly accurate or specific. Until accurate predictive biomarkers are identified, existing IGRAs for latent tuberculosis infection should be preferred over the TST and chosen, in addition, on the basis of higher specificity in different populations, cost and feasibility, and individuals' preferences (Rangaka *et al.*, 2011).

The clinical performance of the T-SPOT .*TB* assay, for TB (active and latent infection) diagnosis, in adults and children, in immune-compromised and immuno-competent individuals, in BCG-vaccinated subjects, and in pulmonary and extrapulmonary TB diseases, has better sensitivity and specificity than the TST (Soysal and Bakir., 2011).

Prevalence of LTBI was 77% in Georgian HCWs, detected by using combination of QNF test and TST, and was significantly higher than single use test (QNF +ve detect LTBI prevalence 66 % and 60 % for TST +ve result). Length of employment > 5 years was associated with TST +ve result (OR 5) against QNF +ve test (OR 2.26) with moderate agreement of 73% with positive TST $\geq$ 10-mm (k = 0.43) (Mirtskhulava *et al.*, 2008). A study done on 119 crack cocaine-smoking drug users in Houston, USA showed LTBI prevalence of 28% using TST and 34% using both IGRAs. Among TST negative participants, 18% were detected by QNF-G test and 21% by T-SPOT .*TB* test (Grimes *et al.*, 2007).

On the other hand Mandalakas and colleagues (2011), assessing the value of IGRAs and TST, revealed similar accuracy for the detection of TB infection or the diagnosis of disease in children which is target evaluation for further research.

New guidelines in some developed countries are starting to recommend a two-step strategy for the diagnosis of LTBI, using IGRA to confirm positive TST cases, and has significant cost-benefit reductions in TB chemoprophylaxis administration (CDC, 2011b; NICE, 2011). IGRAs can be used also for epidemiological studies for LTBI detection at the community level to control immigration MTB transmission and can be used for serial testing of active tuberculosis (Legesse *et al.*, 2011; Nenadic *et al.*, 2011).

7.1.3.3   Tuberculosis diagnostics concordance/discordance

Multiple studies have confirmed concordance between IGRA and TST tests, but the reported concordance is variable between the four diagnostic tests for diagnosis of LTBI in new immigrants and high risk groups. For example, in people with discordant test results (i.e., one positive and the other negative), decisions about

medical or public health management will require individual judgment to assess the quality and magnitude of each test result, such as the size of TST indurations or the IGRAs TB response (Mazurek *et al.*, 2010). The finding of TST +/QTF − discordant results in presumed LTBI suspected cases was recently revealed as a well-described phenomenon (Mendez-Echevarria *et al.*, 2011).

Among 759 BCG-unvaccinated individuals with no history of exposure to occupational TB, there were concordant negatives of both IGRAs in 80.1% compared with concordant positives in 9.5% versus discordant results in 10.4%. (Arend *et al.*, 2007).

The poor correlation between the TST and QFT-GIT assays in the present study is consistent with previous studies in Japan (Harada *et al.*, 2006), South Korea (Choi *et al.*, 2008) and Australia (Vinton *et al.*, 2009). Immigrants screened for TB revealed more TST+/QTF− discordance in the BCG-vaccinated population than in the unvaccinated group (Mendez-Echevarria *et al.*, 2011). Another example of similar discordance between a TST at 'cut-off' point more than 5-mm of skin indurations and QFT-GIT at a 'cut-off' point more than 0.35 IU/ml level of IFN-γ is described by Legesse and colleagues (2011). The low level of concordance between the TST and IGRAs tests was mainly because the two assays evaluate different mechanisms of immunological response against MTB elicited by different antigens (Ferrara *et al.*, 2006; Harada *et al.*, 2006; Mazurek *et al.*, 2007; Menzies *et al.*, 2007; Pai *et al.*, 2005; Saracino *et al.*, 2009; Winje *et al.*, 2008).

Discordance of TST and IGRAs results have been noted in other study results (Ferrara *et al.*, 2006; Harada *et al.*, 2006; Mazurek *et al.*, 2007; Pai *et al.*, 2005; Winje *et al.*, 2008), but the disagreement factors are still unclear whether adding IGRAs alone or as an alternative to TST is currently questionable. For example TST negative/QFT-GIT positive discordance was significantly associated with foreign birth and age more than 50 years (Weinfurter *et al.*, 2011), and similar to our TST-/QNF-GIT+ discordance findings of while conversely, TST-/QFT-GIT+ discordance was associated with foreign birth and being black, and aged between 30 and 49 years.

Discordance between TST induration and INF-γ levels indicates that different factors, such as interleukin-10 (IL-10), might contribute to regulating the size of the TST reaction (Burl *et al.*, 2010). In mice, IL-10 is found to be an important chronic inflammatory regulator and has been shown to decrease DTH-IV and expression of cutaneous lymphocyte-associated antigen (CLA) on T cells (Sigmundsdottir *et al.*, 2004). Other studies observed that the majority of discordant results were as TST positive/IGRA negative, with concerns that IGRAs may not be as sensitive as the TST for diagnosis of LTBI in very young and immune-compromised subjects such as children, and also whether the primary infection was very old with recent diagnosis or complications (Bakir *et al.*, 2008; Farhat *et al.*, 2006).

A study by Cagalayan and colleagues (2011) revealed intermediate concordance between both the TST and QNF-GIT, and QNF-GIT diagnostic accuracy was only 65.38%. Rutherford and co-workers (2010) observed no differences between patients with discordant results of the TST and QFT-GIT in Indonesia (TB endemic country), and similar test discordances was associated with foreign-born and old persons (Weinfurter *et al.*, 2011), and recent immigrants (Saracino *et al.*, 2009).
Other studies (Arend *et al.*, 2007; Igari *et al.*, 2007; Luetkemeyer *et al.*, 2007; Pai and Menzies, 2007) found poor agreement between TST induration of more than 15-mm and QFT-GIT.

Concordance was unachieved if TST 'cut-off' was raised to more than 15-mm which might miss LTBI due to MTB infection than due to NTM or BCG (Menzies *et al.*, 2007; Mazurek *et al.*, 2007; Saracino *et al.*, 2009; Wang *et al.*, 2002). In contrast, Brodie and colleagues (2008) found general concordance between T-SPOT .*TB* test and TST results among BCG vaccinated immigrants was 64% compared to 82% in unvaccinated individuals. Poor concordance, due to BCG vaccination and/or NTM infection, has been described in other studies (Carvalho *et al.*, 2005; Ferrara *et al.* 2006; Mazurek *et al.*, 2007; Menzies *et al.*, 2007; Saracino *et al.*, 2009).

7.1.3.4 Tuberculosis diagnostics agreements

Agreement between tests for *Mycobacterium tuberculosis* infection varies widely in different studies (Chee *et al.*, 2008; Choi *et al.*, 2008; Leung *et al.*, 2008). Agreement

is known to be affected by various factors such as the prevalence of LTBI infection, estimates of recent and remote exposure history, socio-demographic characteristics (e.g., age, race, prior BCG vaccination and coexisting diseases), and technical errors such as test interpretation. Similar to our findings, baseline epidemiological and demographic characteristics such those aging more than 28 years, male and birth in TB endemic regions and recorded diagnostics positive results of both IGRAs are associated with increased likelihood of LTBI diagnosis more than positive TST (Mahan *et al.*, 2011).

Positive IGRA results with negative TST and CXR results strongly pointed to a higher probability of LTBI/TB infection in BCG vaccinated subjects, in comparison to positive TST and negative IGRAs result. In our study, optimum agreement can be reached by lowering the 'cut-off' value for both IGRAs and TST with CXR suspicious findings for latent TB. Arend and colleagues (2007) differed, in concluding that maximum agreement (more than 89.5% achieved or k = 0.59) can be reached through increasing sensitivity by lowering the 'cut-off' value of the QNF-GIT but improving specificity by raising the 'cut-off' value for the T-SPOT .*TB* test. As expected, lowering the 'cut-off' value of the QNF-GIT to more than 0.1 IU/ml instead of $\geq$ 0.35 IU/ml increases the total number of positive tests (Mahan *et al.*, 2011).

Agreement of both the QNF-GIT and TST tests was 'excellent' in tuberculosis disease cases and non-vaccinated children, but on the other hand, disagreement between the tests was frequently observed among latent tuberculosis infection cases than in non-infected normal children or TB disease cases (Mendez-Echevarria *et al.*, 2011), in comparison to lower observed or 'moderate' agreement between both tests (TST and QNF-GIT) detected by Weinfurter and colleagues' (2011) study of 1,653 foreign-born and homeless individuals.

Even though both T-SPOT .*TB* and TST tests show 'good' agreement in the control normal group compared with psoriasis patients in a highly tuberculosis endemic setting, T-SPOT .*TB* test was still superior to TST in diagnosing latent tuberculosis infection (de Andrade Lima *et al.*, 2011).

Pai and colleagues' (2005) findings on healthy adults from a rural community in South Africa (TB endemic country) demonstrated 'poor' agreement between the TST and IGRAs, with a high proportion of individuals (between 30% and 56%) with TST indurations more than 15-mm who tested negative on the IGRAs The poorer agreement between TST indurations more than 15-mm and QNF-GIT positive results was also observed elsewhere (Luetkemeyer *et al.*, 2007; Pai and Menzies, 2007; Saracino *et al.*, 2009). Zhao and colleagues (2011) relate low agreement between the T-SPOT *.TB* test and the TST due to BCG vaccination.

The agreement between the TST and IGRAs in non-BCG-vaccinated individuals was higher than that in BCG-vaccinated children. IGRAs specificity was far greater than the TST, particularly in BCG-vaccinated children (Sun *et al.*, 2011). Consistent with our research's 'good' agreement findings, the two commercial IGRAs (QNF and T-SPOT *.TB* tests) also revealed good agreement and were cost-effective in screening contacts of tuberculosis patients (Diel *et al.*, 2007; Dominguez *et al.*, 2008). Using different TST and QNF-GIT 'cut-off's, the highest concordance was obtained for QNF-GIT at units more than 2.64 IU/ml (more than manufacturer's 'cut-off' up 0.35 IU/mL) and TST at more than 10-mm (Saracino *et al.*, 2009).

7.1.4 Study strength

1. This is the first study to compare the diagnostic performance effectiveness of targeted testing by using 'simultaneously': 1- the two old TB diagnostic tests (chest X-ray and tuberculin skin test), and 2- the two new biomarker IGRA tests on a high risk group (new immigrants) for latent tuberculosis infection diagnosis.
2. Both IGRA assays were highly reliable and reproducible, and the findings substantially lying within-subject variability during the study period which can be considered when interpreting serial testing's in comparable populations and similar settings.

7.1.5 Study limitations

A major limitation in the study is lack of a standard diagnosis of LTBI. Reproducibility is low and small sample size limits generalization of the new

diagnostic strategy which needs further studies on large number of participants to determine the diagnostic performance precisely. Repeatability might be difficult for other researchers to perform similar methodology of implementing the four TB diagnostic tests simultaneously. The two old diagnostics weaken the performance and diagnostic accuracy of the new IGRAs assays.

7.1.6 Conclusions

Because of higher sensitivity and specificity of both IGRAs, it should be no longer justified to perform lung X-ray or TST alone to define (diagnose) LTBI and 'TB suspect' cases. Advantages of both IGRAs overlapped over the performance of chest X-ray and tuberculin skin test in detecting latent tuberculosis and allowing the exclusion of *Mycobacterium tuberculosis* infection. Both IGRAs are useful for screening high-risk groups and recent immigrants, even without other diagnostic results. The study findings suggested the targeted screening of recent and foreign-born immigrants using quantiFERON Gold In-Tube test, in addition to the ordinary chest X-ray, would enhance the diagnosis of latent tuberculosis and *Mycobacterium tuberculosis* carriers, especially in situations where the old diagnostics are unreliable such as in young children and immunocompromised individuals.

IGRAs are reported to be better predictors of the risk of developing tuberculosis disease than the TST, and correlate better with the intensity and duration of TB exposure than the TST (Mendez-Echevarria *et al.*, 2011; NICE, 2011). The study results permitted introduction of new screening guidelines for latent tuberculosis high risk groups and the general population (Table 7.3). In view of the high risk of *Mycobacterium tuberculosis* carriers, a combination of either IGRAs or chest X-ray interpreted by a radiologist will be appropriate for diagnosis of latent tuberculosis cases and exclusion of active tuberculosis. After IGRA evaluation, an alternative strategy can be followed before starting preventive prophylaxis to suspected LTBI immigrants. Compulsory QNF-GIT followed by complementary CXR can be cost-effective and available diagnostic tools for immigrant screening (Hardy *et al.*, 2010).

Even with difficulty to differentiate between active and latent tuberculosis using the 'cut-off' validity values recommended by the manufacturers, still both IGRAs can add scientific knowledge about the global epidemiology of LTBI/TB.

7.1.7 Recommendations

Even with the substantial progress in documenting the utility of IGRAs compared with chest radiography and the tuberculin skin test, further additional research is needed, and future studies should focus on determining the values and limitations of the IGRA assays and their related importance to medical care, such as tuberculosis control and elimination. From the previous significant findings, future research recommendations can be targeted accordingly;

1- Are IGRAs (the new diagnostics) better at predicting subsequent active tuberculosis than CXR and TST (the old diagnostics)?.

2- Are high-risk persons with discordant results of negative TST or CXR diagnostic results and positive of either IGRAs test are truly at higher risks to develop active tuberculosis compared with immigrants with concordant negative results of both the old and new diagnostics ?.

3- Can sensitivity and specificity of IGRAs be improved by modification in testing methods, application of different interpretation criteria, or inclusion of additional new specific antigens?.

8 Chapter eight

Evidence-based detection of latent tuberculosis infection

Abstract

Diagnosis and treatment of latent tuberculosis infection is a cornerstone of tuberculosis control all over the world. Since the last century, the tuberculin skin test has been the only means of diagnosing the disease, but missed dormant inactive tuberculosis carriers, in contrast to new promising diagnostic tools - namely, interferon-gamma release assays - for diagnosis and tuberculosis surveillance. This study focuses on the use of both 'evidence-based' epidemiological characteristics and appropriate laboratory diagnostic tests for screening high-risk cases.

8.1 Introduction

Awareness of the global burden and threat of communicable diseases and the opportunities for disease control has permitted the development of various international partnerships and alliances to coordinate efforts to control diseases globally, an example being the global campaign to eradicate smallpox in the 1960s and 1970s. Similarly, the high burden of TB disease, with almost four deaths each minute worldwide, has stimulated of the World Health Organization Stop TB Strategy (WHO-STOP TB) and the Global Plan to Stop TB plan, the goal being eradication of tuberculosis by 2050. Additionally, the United Nations Millennium Development Goals (UNMDGs) aim to halve TB incidence, prevalence and deaths by 2015, compared with their levels in 1990, and has already saved 14 million lives (WHO, 2010c). Even with having a similar aims and targets with the Stop TB Partnership programme to globally accelerate social and political action against tuberculosis, still MDGs of eliminating TB will not be achieved by 2050 (less than one case per 1 million population). Goals unachieved due to: 1- incident cases are in major occurring within population-dense TB endemic regions of fast growing populations; Africa and Asia, 2- TB persistence in communities with poor health infrastructure, which lead to 3- increase in drug-resistant cases and TB/HIV co-infection cases. Therefore these unidentified gaps in both national- and international-directed TB control efforts in which political governments should address the preventive challenges on regular pattern (Jassal and Bishai, 2010). Most countries in sub-Saharan Africa (an HIV pandemic region) reported that more than 30% of active TB are TB/HIV co-infected (WHO, 2009a; WHO, 2011a).

The global cost of the partnership's activities until 2015 is estimated at U.S.$56 billion, of which U.S.$47 billion for implementation of available diagnostic interventions and U.S.$9 billion for research and development. However, an overall estimated funding gap for the Global Plan was U.S.$30 billion due to several countries reduced spending on overseas developmental programs. These developing countries are instead trying to compensate for the annual 100 million persons entering poverty and increased household overcrowding and poor nutrition, which

are known risk factors to enhance the incidence and transmission of TB. Shortage in governmental contributions and budgeted plans lead to scientific obstacles toward failure to end the burden of human-TB suffers and prevalence's (Figure 8.1).

2005

At least 70% of people with sputum smear-positive TB will receive a diagnosis and at least 85% successfully treated.

2015

The global burden of TB will be reduced by 50% relative to 1990 levels.

2050

The global incidence of active TB will be less than 1 case per million population per year.
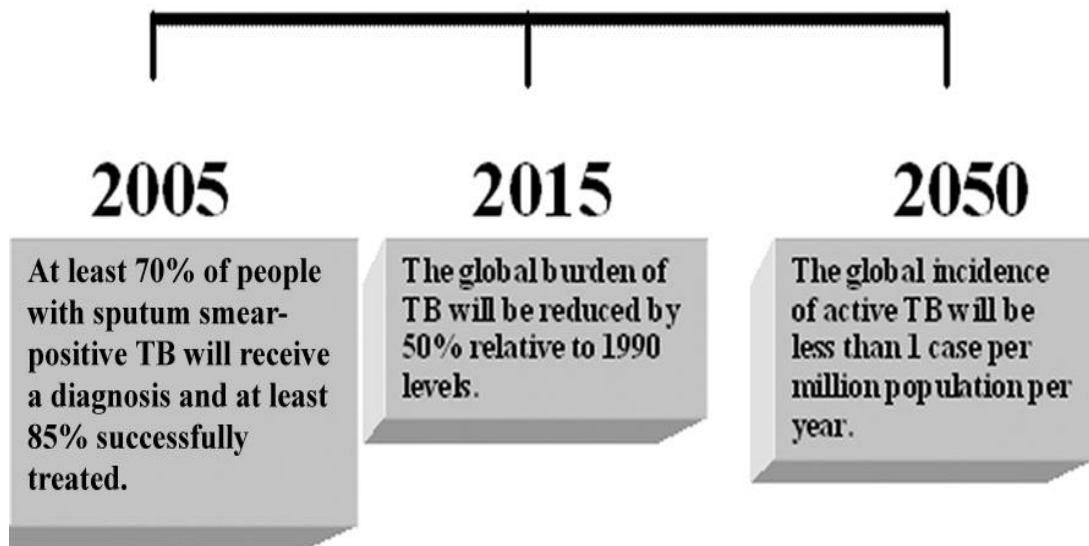
Figure 8.1: Three key time points in achieving tuberculosis elimination by 2050, if all criteria established by the Stop TB Partnership are effectively obtained (Jassal and Bishai, 2010)

Tuberculosis elimination criteria for any society can be met only if new infections are effectively prevented. Delay in tuberculosis diagnosis is an important factor in the spread of TB and a poor outcome for patients due to inadequate health-seeking behavior at patient level or inadequate diagnosis at the level of the health care system. Research studies have investigated the causes of delay among diagnosed cases, but, only few have investigated causes of delays among undiagnosed individuals detected in the community. Therefore, case-finding in the community and investigating the risk factors among suspected cases of latent tuberculosis are crucial to the development of optimum interventions that will improve early case detection through determining the prevalence of tuberculosis suspects. Preventive measures for future cases of TB in non-endemic areas is to identify and provide chemoprophylaxis to individuals with latent tuberculosis infection, in addition to tracing suspected TB cases with minimal diagnostic delay. Understanding the overlapping socio-economic nature of TB epidemics and their structural determinants is key to the design and

implementation of effective prevention programmes. Epidemic control of chronic communicable diseases should be preceded by long-term planning that is able to change specific dynamics of disease transmission such as promoting research and development of improved diagnostics, drugs and vaccines that are provided in community implementation programmes (Jassal and Bishai, 2010).

Research- and health-related data sources among new immigrants can identify more precise population- and individual-level risk factors associated with both differential risks for LTBI and risk of progression to active TB, which can be generalized and applied on a regional basis (Freeman *et al.*, 2010). Individual risk behaviours influence the probability of contact with other infected or infectious individuals and can delay TB diagnosis with further MTB transmission from LTBI cases that are generally defined as positive TST and/or positive IGRAs with no evidence of active TB disease form. Therefore a new evidence-based LTBI classification can be used to predict TB suspicious cases that require isolation before re-activation active TB disease or producing cross infections. History about risk factors using questionnaire-guided evidence-based results can help health care workers to recognize TB suspicious cases and to confirm diagnosed cases (Solari *et al.*, 2008).

8.2 Aim

To define latent tuberculosis infection index case by which able to raise the quality of health care services.

8.3 Objectives

1. To apply prediction criteria based on evidence-based epidemiological and laboratory diagnostic criteria on high-risk group which permit rapid identification of latent tuberculosis immigrants.
2. To introduce a new evidence-based laboratory criterion using diagnostic measures for LTBI detection.

8.4 Discussion

Tuberculosis remains a major global health threat. The latest reportable cases by WHO in 2009 observed more diagnosed and loss of lives than in the previous years. For example 9.4 million incident cases of active TB been diagnosed in 2008 was increased to 10 million in 2010 around the world. The majority of incidences were due to MTB re-activation from reservoir of LTBI (WHO, 2010c).

Mycobacterium tuberculosis causing tuberculosis remains a formidable health problem and disease control is largely based on early detection and treatment of infected persons with active or latent TB disease (WHO, 2011a).

The target of studies worldwide is to reach a timely and accurate diagnosis of latent tuberculosis infection which enables reduction of the risk of progression to severe tuberculosis. Individual risk behaviours influence the probability of contact with other infected or infectious individuals and can produce delays in TB diagnosis with consequent uncontrolled MTB transmission. Several trials of clinical prediction rules have been developed to recognize TB suspicious cases by using a scoring system composed of TB risk (questionnaire-related) variables and diagnostic tests (laboratory-related) similar to Solari and colleagues (2008) trial.

Tuberculosis was firmly put on the global health agenda because of: first TB identified by the Global Burden of Disease Study in 1990 (GBD 1990) as one of the top 10 causes of mortality and morbidity worldwide, second the case fatality rate for untreated TB disease reached 50% and costing loss of millions of young adult healthy, and third anti-TB management with DOTS follow-ups was supervened by the Stop TB strategy in 2006 revealed social and economical barriers were the most effective targets for saving costs of all health interventions. Among the follow-up successes of the WHO strategies worldwide, 36 million patients were treated between 1995 and 2008 and 8 million deaths were averted (WHO, 2009a), but still both DOTS and the Stop TB Strategy are only prioritized/restricted to sputum smear positive for TB infectious cases in low-resource countries such as Africa.

Tuberculosis has become an international public health priority due to the migratory flow of high-risk groups from high incidence countries, leadings to micro-epidemics in closed environments (e.g. hospitals, jails), and the recent AIDS pandemic (TB/HIV syndemic) (Dye *et al.*, 1999).

The CDC recommendation for LTBI screening of foreign-born individuals is still not commonly practiced. Challenges include low health seeking behaviours for screening for latent infection and active disease, as well as suboptimal treatment compliance. The reasons for these barriers vary by country of emigration and duration of acculturation. For example, the majority (half) of the active TB disease in the USA immigrants is due to re-activation of LTBI dormant bacilli (Geng *et al.*, 2005).

Variable pulmonary impairments (from mild to severe dysfunctions) after tuberculosis such as obstructive and/or restrictive lung abnormalities. These deformity patterns resulting in common from caseating granuloma and cavity formation followed by anatomic changes, which are occurring more in PTB compared to LTBI. Worldwide, human function-associated disabilities require aggressive case prevention strategies and post-treatment follow-up evaluations (Pasipanodya *et al.*, 2007).

TB is a preventable and treatable condition, but if left untreated can be life-threatening. Worldwide, inconsistent policies are due to the uncertainty regarding risk for LTBI among long-term travelers from low- to high-risk countries, with difficulties in estimating the risk for LTBI, as measured by TST conversion. From a clinical perspective, identification/diagnosis and treatment of LTBI is important in reducing the risk of progression and associated complications. Making the best estimation of LTBI incidence can provide scientific data to support policy recommendations, and estimation of LTBI prevalence (e.g. using IGRAs) can help to evaluate the performance of health policies and interventions. LTBI is an important occupational problem among health care service and staff (Demkow *et al.*, 2008).

The two billion people estimated to harbor latent MTB carry 2-23% lifetime risk of developing re-activation TB and constitute a major impediment to TB control efforts. For all who became infected, the course and duration of progression to active disease

are highly variable. Lack of uniformity in disease manifestations among infected individuals reflects complex interaction between genetic and environmental factors and both influence susceptibility to infection and disease pathogenesis. For example nested IGRA-TST maximizes the sensitivity for LTBI diagnosis and has the potential to identify MTB mode and can be applied to the high-risk populations (Dodd *et al.*, 2010).

The challenges of TB control remain unachieved, and scientific and financial investments are both required for further substantial progress, aiming to create a strategy that allows prevention and/or sterile eradication of *Mycobacterium tuberculosis* infection. The study by Berry and colleagues (2010) reported identification of a 393 gene set which enables a gene expression signature to differentiate between latent and active TB transcriptomes compared with healthy individuals. Duarte and colleagues (2011) revealed the presence of HLA-DRB*14 allele frequency higher in a small sample of TB patients compared with a control group of healthy staff on daily exposure to TB. This could be a susceptibility allele toward evolution of TB disease.

8.4.1 Definition of latent tuberculosis infection case

Latency is a dynamic event where the active immune response keeps controlling the persistence of mycobacteria, and re-activation of 5 to 10% of LTBI will lead to active TB. Because tuberculosis itself is a stigmatizing disease, latent tuberculosis definition through reporting of the predictive risk factors such as history of contact with a tuberculosis patient case and related results of the diagnostic tests might be able to rule out a diagnosis of developing active tuberculosis in high-risk group immigrants.

Latent tuberculosis infection can be defined as the presence of any tuberculous lesion which fails to produce any symptoms of its presence, and is also considered as a clinical syndrome with absence of clinical symptoms and signs. The immune responses surround foreign pathogens into inactive (quiescent) state, which control bacillary spread and inhibit disease transmission and lowering public health risks.

Within two to four weeks, MTB bacilli can proliferate intracellularly within alveolar macrophages of the lungs and disrupt macrophage acidification to prevent phagosome–lysosome fusion. Inadequate host immunity permits tubercule bacilli to escape causing transient bacteraemia and dissemination throughout the body. Recruitment of both T-cells and macrophages can control the infection and immunological memory persist for decades (for up to 25 years) in the form of a positive delayed hypersensitivity reaction-type IV to the PPD of *Mycobacterium tuberculosis* and calcified scar lesion in chest radiographs (Parrish *et al.*, 1998).

8.4.1.1 Latency timing

Tuberculosis latency is characteristic of TB disease, and is a life-long variable period (sometimes decades) following MTB bacillary infection. Other results suggest no relationship between re-activation latency period and raised TB incidence diagnosed in the young population due to hyperendemicity (den Boon *et al.*, 2005). It is difficult to attribute observed disease rates to risk factors or current environmental exposures particularly in immigrants from high-incidence regions. Active TB disease in low-endemic countries arises from a growing proportion of latently-infected individuals facing risks of re-activation. Rates of increments of the latent infection continued to be high in endemic regions with high infection pressure such as in India and South Africa, because of maintained exogenous re-infection phenomenon during latency period (Wiker *et al.*, 2010). Cases of latent TB infection convert to active TB within the first two years of infection. Diagnostic delay of TB among pastoral communities in Africa revealed the longest patients' delay from developing countries with a mean of 130 days (range: 10-1800 days), and 50% had pulmonary TB with positive sputum, and each patient can dispense up to 3,500 bacilli per cough, infecting 10 to 15 people each year, raising the public suffer (Gele *et al.*, 2009).

8.4.1.2 Human latent tuberculosis

Individuals with latent tuberculosis infection should be identified as sources of persistence and multiplication of viable *Mycobacterium tuberculosis* bacilli localized within macrophages, and also defined as asymptomatic subject without clinical manifestation of disease. Such individuals are also benefit from subsequent

chemoprophylaxis as important preventive measure before development of active disease and consequently reducing TB incidence. Tuberculosis acid-fast bacilli, the characteristic bacterial forms, usually failed to be revealed in microscopic examination of infected tissues of latent dormant TB suspects. Initial absence or low predominance of MTB bacillary emerging antigens for sensitizing immunity added to the gradual degeneration and necrosis of antigen-specific T-cells in the infected lungs, and would physically prevent cellular penetration and promote MTB bacillary resistance (Henao-Tamayo *et al.*, 2009). Paradox observation is opposite to the expects that the latent bacilli might be altered to non-acid fast status or may remain acid fast but in a number low enough to defy microscopic detection. Location of the latent bacilli within the old pulmonary granulomatous lesions (e.g. lungs) and dissemination permits bacilli seeding to their early bactermic phase, post-exposure.

Latent bacilli are expected to be in a metabolically active state of stationary (slow growth within granuloma) evidenced by response of LTBI cases to chemoprophylaxis treatment and reduced risk of developing reactivated TB. Mycobacterium bacilli are spore-like and metabolically inactive, with evidence of sporulation regulatory genes, such as sigma factor gene (sigF gene), which permit MTB 'invisibility' to the host immune system for long periods (Parish *et al.*, 1998).

8.4.2 Identification of latent tuberculosis index case

Tuberculosis is the only disease clarified as clinical probability, radiological possibility and bacteriological certainty. Matching of laboratory diagnostic tests with epidemiological risk factors can predict and help pick up latent tuberculosis cases, preventing bacterial flare-up risks and facilitating elimination/control of tuberculosis. The mechanisms by which *Mycobacterium tuberculosis* establishes a latent metabolic state is still unknown, but theoretically accepted that reduced oxygen tension might trigger the bacillus to enter a state of latency.

8.4.2.1   Immunological definition of latently infected cases

The immune system is the single and most important factor for the control of LTBI and halting MTB spread. Improving discrimination between LTBI and the active

symptomatic form of TB disease is a priority, mainly in areas where treating all infected cases is not possible. But it is still not understood how the immune system controls LTBI except for the major role of CD4+ T-cells and antagonizing effects of tumour necrosis factor- alpha (TNF-α) (produced by CD4+ cells), which result in re-activation of LTBI (Mankia *et al.*, 2011). Number of tuberculin-specific CD4+ T-cells in peripheral blood carrying either of activation markers such as IFN-γ (with dominance; is a marker for LTBI) and/or IL-2 in LTBI cases, were higher in LTBI than active pulmonary TB patients due to migration of active CD4+ T-cells to infected tissues (Streitz *et al.*, 2010). Mantoux test and IGRAs can identify a memory of a previous adaptive immune response against *Mycobacterium tuberculosis* bacilli. Worldwide, the diagnosis of LTBI is made with positivity of TST and recently on the T-cell-based IGRAs before initiation of chemo-preventive therapy (LoBue *et al.*, 2010).

8.4.2.1.1 The immune response and evasion strategies of *Mycobacterium tuberculosis*

After the infection of two to four weeks, specific CD4+ and CD8+ responses are evident, and cells produce INF-γ and TNK-α to enhance the effectors' mechanisms of innate immunity. The adaptive immune response against MTB encompasses CD4+ and CD8+ effecter cells' expansion and generation of long-lived memory T-cells. CD4+ cells are considered the main source for INF-γ, a critical cytokine for an anti-mycobacterial protective immune response. Diminished INF-γ production correlates with disease severity and development of extra-pulmonary TB or low immunity, whereas increased production levels are indicative of actively replicating mycobacteria in those recently exposed to active TB cases and are biomarkers of high risk susceptibility from previous MTB infection (Rueda *et al.*, 2010).

Adaptive immune responses through the immune cells and the fibrotic wall surrounding the granulomas will prevent bacterial and outbreak spreading for long periods. After being inhaled, MTB are phagocytosed by antigen-presenting cells (APCs) in the lung, such as alveolar macrophages (AM), lung parenchyma

macrophages (LPM) and dendritic cells (DC), which elicit local inflammatory responses, for recruitment of mononuclear cells (MNC) to target the infection.

Within the granuloma MTB will be enclosed and prevent phagosome acidification to survive and escape within the cytosol of the granuloma (defined as aggregated of macrophages, neutophils, and monocytes). Caseation of granulomas denotes massive cell death and difficulties in enclosing MTB bacilli even though apoptotic cell death would impede MTB spread. Another strategy of MTB evasion is blocking apoptosis through inhibiting prostaglandin E2 (PGE2) production, which is crucial for T-cells in containment of MTB. CD4+ T-cells, predominantly T-helper 1 (TH 1) exerting protection by producing IFN-γ after activation by antigen presenting cells from MHC class II molecules. Other strategic evasion of MTB is through inhibiting MHC-II expression and antigen presentation via Toll-like receptor 2 (TLR2). IFN-γ as a key cytokine activates macrophages (protective immunity) to kill intracellular *Mycobacterium tuberculosis* through the induction of nitric oxide (NO). Also, CD8+ T-cells contribute to host defences by cytokine production and also by perforin- and granzyme-mediated cytotoxic activity, but require APCs exogenous presentation with MHC class II molecules, which is also inhibited by the MTB bacilli. Post-infection, memory response cells are able to elicit a secondary response after re-encountering the pathogens (Feng Y. *et al.*, 2011; Thaiss and Kaufmann, 2010).

Acting as a co-factor for induction of antimycobacterial activity, vitamin D is a potent immuno-modulator of the innate immune responses and its deficiency is associated with LTBI. Serum levels less than 20 ng/mL impair the macrophage-initiated innate immune response against *Mycobacterium tuberculosis* (Talat *et al.*, 2010).

8.4.2.1.2   Predictors of latent tuberculosis infection

Millions of people are living with latent tuberculosis infection, representing a reservoir of potentially active tuberculosis disease, and are associated with re-activation consequences such as intense suffering, permanent disability, and high economic costs for populations, especially as the MTB bacillus spreads.

This section summarizes the current knowledge of epidemiological and laboratory diagnosis aspects of LTBI discussed in chapters 4, 5 and 6. We have developed a study proposal for LTBI definition and a classification tree using common predictor factors from the history of epidemiological characteristics (questionnaire-related) and radiographic-laboratory (four diagnostic tests-related) investigational data from a representative sample of high-risk groups (the 180 new immigrants) which has been validated during February and May 2010.

The study proposal for LTBI definition might facilitate early prediction of LTBI and help staff of the health care system through clinical prediction rules (CPRs) in identification of suspected pulmonary and extra pulmonary tuberculosis. CPRs are tools to help clinicians to reach a diagnosis or predict/guide decisions towards the probability of a health event (active TB) before it occur especially in high-risk group populations (Solari *et al.*, 2008). Also combining the results of TB laboratory diagnostics increases the sensitivity of detection of LBTI, which has confirmed and supported our study recommendations. For example, our results approved that negative diagnostic results of the TST should not be enough alone to exclude LTBI/TB, but can be interpreted in conjunction with other clinical and diagnostic findings. Baboolal and colleagues (2010) also described similar significant findings (Baboolal *et al.*, 2010).

The relationship between the four tuberculosis diagnostic tests results and the major socio-demographic variables and common risk factors of tuberculosis can be assessed. The predictor risk variables and laboratory results facilitating definition of latent tuberculosis infected cases are listed in Tables 8.1, 8.2, and 8.3, respectively.

8.4.2.2 Epidemiological definition of latent tuberculosis index cases

The epidemiological risk factors (which can be accepted as predictors) for suspected TB cases and *Mycobacterium tuberculosis* carriers can be direct or indirect, and are outlined in Table 8.1.

Table 8.1: Classification of evidence-based epidemiological risk factors and related diagnostic levels of both IGRAs for development of latent tuberculosis infection and tuberculosis suspicious carriers of 180 new immigrants to Kuwait during February and May, 2010

Variable	Likelihood ratio	p-value	%
Socio-demographic characteristics			
Age group	LLR $\chi^2_{(24)}$ = 38.244	0.033	92.22
Gender (Male)	LLR $\chi^2_{(4)}$ = 14.008	0.007	76.1
Nationality (Endemic countries)	LLR $\chi^2_{(52)}$ = 72.522	0.032	82.9
Ethnicity (Asian-East Asian)	LLR $\chi^2_{(16)}$ = 26.478	0.028	75
Total number of sleeping rooms (1-4)	KW $\chi^2_{(4)}$ = 8.977	0.062	95
Risk factors for *Mycobacterium tuberculosis* infection			
Microbus/bus/train	LLR $\chi^2_{(4)}$ = 14.884	0.005	75.6
Tuberculosis knowledge			
General knowledge	LLR $\chi^2_{(4)}$ = 16.103	0.003	82.2
Cough	LLR $\chi2_{(8)}$ = 24.553	0.002	81.1
Hemoptysis	LLR $\chi2_{(8)}$ = 22.590	0.004	68.3
Fever	LLR $\chi2_{(8)}$ = 22.032	0.005	71.1
Night sweating	LLR $\chi2_{(8)}$ = 15.726	0.046	38.9
Generalized weakness	LLR $\chi2_{(8)}$ = 24.435	0.002	68.3
Weight loss/wasted	LLR $\chi2_{(8)}$ = 24.824	0.002	68.3
Vomiting/diarrhea	LLR $\chi2_{(8)}$ = 17.087	0.029	43.9
Sources of tuberculosis knowledge			
Study area (e.g. school/friend)	LLR $\chi2_{(4)}$ = 11.512	0.021	41.1
Public shared talks	LLR $\chi2_{(4)}$ = 11.236	0.024	81.1
TV/Newspaper/magazine advertisement	LLR $\chi2_{(4)}$ = 15.275	0.004	76.7
Medical campaign/Poster	LLR $\chi2_{(4)}$ = 16.627	0.002	58.9
History of environmental contacts (inside- and outside- households)			
Duration of contact to outside non-households (> 6 hours)	LLR $\chi^2_{(12)}$ = 20.285	0.062	88.9
Have been infected or contacted with TB case	LLR $\chi^2_{(8)}$ = 13.799	0.087	6.1
TB stigma (in-door close contacts)	LLR $\chi^2_{(4)}$ = 11.047	0.026	7.8
Risk factors for progression of infection to active tuberculosis: History of smoking			
Positive history of smoking (current and ex-smokers)	LLR $\chi^2_{(4)}$ = 9.135	0.058	51.1
Laboratory diagnostic result			
Interferon gamma level (IU/ml) = 0.07 (0.46)	KW $\chi^2_{(4)}$ = 105.220	< 0.001	
T-lymphocytes number (cells/µl) (IQR) = 180 (89)	KW $\chi^2_{(4)}$ = 4.409	0.353	

Analysis of epidemiological risk factors such as socio-demographic characteristics associated with tuberculosis infection/exposure can be utilized for defining latent tuberculosis cases to achieve variable uniformity for comparison with other LTBI studies. Increased TB awareness is needed, in particular in low TB incidence communities and in immigrants originating from high-endemic areas. Health care professionals should be aware that immigrants from LTBI/TB endemic countries remain at risk of re-activation after they immigrate. Also TB controllers need to focus on particular immigrants, location sites, cultural behaviours, and risk groups such as family/children contacts (Cowie and Sharpe, 1998).

Knowledge of different socio-demographic factors relating to tuberculosis permits the development of appropriate integrated social and health policy responses, and quantitatively can determine the disease-specific cost benefit. Pai and colleagues (2005) reported a four-fold higher prevalence of LTBI in Indian medical students aged more than 23 years than in those aged between 18 and 20 years, whereas, older age ranges between 15 and 34 years were significantly detected by Langlois-Klassen and colleagues (2011) as targets for preventive management, compared to the average age of 30 years in our non-endemic country.

Epidemiological risk factors can be supported with positive diagnostics result(s), and vice versa, by which can increase diagnosis of suspected latent tuberculosis. Chest radiographic findings must be interpreted in the context of the individual's exposure and medical history which immigrant's should sign for information responsibility. This facilitates assessment of the challenges of re-activation prediction (Eisenberg and Pollok, 2010).

Communities are still focusing only on the clinical management of patients with active disease, but preventive programmes can identify and treat prophylactically individuals with LTBI and are recommended for the future. Cost-effectiveness of immigrant screening then follow addressing the characteristics of the population's health in addition to assessing the impacts of enhanced screening policies. Cost-effectiveness of immigrant screening guidelines is further explained in chapter 9.

8.4.2.3   Laboratory definition of latent tuberculosis index cases

A 'definite' case of tuberculosis is normally defined as any condition with positive-culture-confirmed disease due to *Mycobacterium tuberculosis* complex. Any new immigrant can be defined by an evidence-based definition in those healthy, without any clinical manifestations of active TB disease, and who were diagnosed by positive radiological changes of MTB in chest radiographs, or by having positive results of either or both IGRAs (QNF-GIT more than 0.35 IU/ml or T-SPOT *.TB* tests, interpreted having more than or equal six spots ($\geq$ 6 spots)) and without radiological abnormalities. Laboratory reports also define latent tuberculosis infection alternatively as any case 'other than definite' (Rieder *et al.*, 1994).

The introduction of diagnostic interventional and targeted measures can improve LTBI detection and raise the quality of health care. The results of four different tuberculosis tests (the two old; CXR and TST, and the two new immunological IGRAs) were included for comparison of new evidence-based combinations. Evidence-based laboratory criteria of diagnostic tests for diagnosing latent tuberculosis and *Mycobacterium tuberculosis* carriers can be integrated, as outlined in Table 8.2 (described in detail in chapter 5) and Table 8.3 (as detailed in chapter 6).

Table 8.2: Diagnostic result frequencies of evidence-based laboratory diagnostic tests in predicting the development of latent tuberculosis infection and *Mycobacterium tuberculosis* suspicious carriers in 180 new immigrants to Kuwait during February and May, 2010

Variable	Statistical correlation	p-value	Frequency (%)
CXR (score II, III, or IV)	$LLR\chi^2_{(16)} = 46.593$	< 0.001	18 (10.06)
QNF-GIT (positive result > 0.35 IU/ml)	$LLR\chi^2_{(4)} = 184.972$	< 0.001	51 (28.33)
T-SPOT.*TB* (positive result > 6 spots)	$LLR\chi^2_{(8)} = 192.884$	< 0.001	74 (41.11)

CXR = chest radiography, QNF-GIT = QuantiFERON Gold In-Tube test, T-SPOT.*TB* = T-SPOT .*TB* test

Including those excluded cases and added as having negative test results (each according to its relevant diagnostic test), then combinations of the laboratory diagnostic test results to achieve more evidence-based of the highest diagnostic accuracy for detection of LTBI are shown in Table 8.3.

Table 8.3: Evidence-based results of combinations from three tuberculosis diagnostic tests and diagnostic performances in 180 new immigrants to Kuwait during February and May, 2010

TB diagnostic test combinations	Normal	Abnormal	Diagnostic accuracy
	Frequency (%)	Frequency (%)	Percentage %
Chest X-ray (radiologist) plus QNF-GIT	175 (97.2)	5 (2.8)	69.27
Chest X-ray (radiologist) plus T-SPOT *.TB*	171 (95)	9 (5)	59.78
Both IGRAs (QNF-GIT plus T-SPOT *.TB*)	133 (73.9)	47 (26.1)	82.78

Radiologist = means chest radiography interpreted by at least one radiologist

The highest accuracy of LTBI diagnostics was a combination of IGRAs (82.78%), then CXR (with radiologist) plus QNF-GIT and CXR (with radiologist) plus T-SPOT *.TB* test respectively (69.27%, 59.78%). The cost-effectiveness of dual-testing screening guidelines for LTBI detection in immigrant's is further discussed in chapter 9.

8.4.3 New laboratory classification against a 'proposed' gold standard test'

Another aim was testing a new current recommended criterion for the diagnosis of LTBI in asymptomatic and apparently normal recent immigrants. Accepting the results of only one type of TB diagnostic test can limit the value of evaluation of high-risk immigrants and under-estimate LTBI prevalence, in which our study recommended LTBI assessment by at least two TB diagnostic methods. Another new guideline (a two-test strategy) for the evidence-based diagnostic accuracy of TB diagnostic test combinations previously shown in Table 8.3 was re-tested as a combination of two diagnostic tests against a 'proposed' gold standard test for latent tuberculosis infection, shown in Table 8.4.

Table 8.4: Estimation of diagnostic test accuracy for combinations of tuberculosis diagnostic test results versus a 'proposed' gold standard test implemented in 180 new immigrants to Kuwait during February and May, 2010 (GST = proposed as theoretical-laboratory 'gold standard test')

TB diagnostic test combinations versus 'proposed gold standard test' (GST)	SN (95% CI)	SP (95% CI)	PPV (95% CI)	NPV (95% CI)	DA	Kappa (p-value)	Likelihood ratio (LLR χ^2)	p-value
CXR (radiologist) plus QNF-GIT versus T-SPOT *.TB* (GST)	93.24 (85.1-97.1)	100 (96.5-100)	100 (94.7-100)	95.50 (89.9-98.1)	97.22	-0.056 (0.007)	9.094	0.003
CXR (radiologist) plus T-SPOT *.TB* versus QNF-GIT (GST)	90.2 (79-95.7)	96.9 (92.3- 98.8)	92 (81.2-96.8)	96.15 (91.3-98.3)	95.00	-0.039 (0.063)	3.086	0.079
Both IGRAs (QNF-GIT plus T-SPOT *.TB*) versus CXR (radiologist) (GST)	74.07 (66.8-80.2)	72.22 (49.1-87.5)	96 (91-98.3)	23.64 (14.4-36.3)	73.89	0.011 (0.865)	0.028	0.866

CXR = chest radiography, QNF-GIT = QuantiFERON Gold In-Tube test, T-SPOT.*TB* = T-SPOT *.TB* test, SN = sensitivity, SP = specificity, PPV = positive predictive value, NPV = negative predictive value, DA = diagnostic accuracy

Table 8.4 shows that the results of a combination of chest radiography (interpreted by a radiologist) and QNF-GIT test against T-SPOT .*TB* test as a 'proposed' gold standard test revealed the highest diagnostic accuracy of 97.22% with statistical significance (LLR $\chi^2_{(1)}$ = 9.094, p = 0.003). Another combination of CXR plus T-SPOT .*TB* test against QNF-GIT as theoretical gold standard revealed diagnostic accuracy of 95% but with no significant statistical relationship (LLR $\chi^2_{(1)}$ = 3.086, p = 0.079). A combination of both IGRAs versus chest radiography as 'a proposed' TB gold standard also revealed the lowest diagnostic accuracy of 73.89, without statistical significance (LLR $\chi^2_{(1)}$ = 0.029, p = 0.866).

8.4.4 Research evidence-based supports

Our findings support those of Moon and others (2011); the first combination of CXR and QNF-GIT diagnostics can be recommended for all new immigrants carrying *Mycobacterium tuberculosis*, and can be started as ordinary testing in high-income and low endemic countries instead of applying only CXR, by which latent tuberculosis and foreign-born suspicious cases can be detected (Moon *et al.*, 2011).

Latent TB infection can be miss-diagnosed because of over-reliance on positive TST, due to antigen interactions from prior BCG vaccination and environmental non-mycobacterial infection, versus lower screening costs using QNF-GIT for TB contacts as shown by Hardy *et al.* (2010). On the contrary our research findings revealed under-estimation of LTBI if relied only on TST results. Analysis of prospective cohort studies using TST indurations of more than 10-mm and/or radiographic evidence of old and healed TB calcifications has demonstrated that the lifetime risk of TB re-activation reached more than 20% of the population (in particular, adults older than 35 years or children less than five years) (Horsburgh, 2004).

The new IGRAs diagnostic techniques have a higher specificity and sensitivity than the Mantoux test (standard LTBI diagnostic) for LTBI diagnosis in recent immigrants and high suspicious TB cases, and whether BCG vaccinated or not (Al-Orainey, 2009; Brodie *et al.*, 2008; Diel *et al.*, 2010; Grimes *et al.*, 2007; Menzies *et*

al., 2007; Orlando *et al.*, 2010; Pai *et al.*, 2005; Pai *et al.*, 2006; Tavast *et al.*, 2009). Regular IGRAs screening instead of only chest radiography in the high-income countries is recommended same as our study recommended indicators. Using IGRAs achieved majority of all health care workers to be latently infected with tuberculosis in the high TB incidence such as India, attributable to increased occupational exposure to *Mycobacterium tuberculosis* plus repeated exposures in the community (Joshi *et al.*, 2007). Lower IGRAs positive results than TST are related to a lower QNF-GIT sensitivity but higher specificity (Orlando *et al.*, 2010).

8.5 Conclusions

Detection of latent tuberculosis cases in certain foreigners and nationalities can facilitate future tuberculosis control and prevention. Susceptibility to MTB infection, the pathogenesis and the asymptomatic/symptomatic manifestations of LTBI/TB will depend on the immunological status of the exposed hosts, which can be indirectly determined by combinations of both evidence-based epidemiological co-morbid risk factors and/or laboratory diagnostic test results. The challenges of LTBI control and TB elimination then can be rapidly met.

8.6 Study recommendations

1- New similar study trials should be tested on large sample of recent expatriates of implementing both evidence-based epidemiological and laboratory diagnostic criteria using both QNF-GIT plus chest X-ray for defining a latent tuberculosis index case. Then,

2- Compare the research findings to other samples of high-risk groups; first from recent immigrants (from TB endemic countries) and second from foreign-born residents of similar immigrant nationalities living in Kuwait.

9 Chapter nine

Cost effectiveness of chemo-preventive screening for immigrant's

9.1 Introduction

Latent tuberculosis cases are undetermined/under-estimated in the high-incidence settings, in which 9.4 million active TB disease cases recently correspond to an estimated incidence of 140 per 100, 000 population occurring throughout the world in 2009. On the other hand, out of these total active TB cases only 5.7 million (new cases and relapsed cases) were actually notified to the various countries' national tuberculosis programmes (NTP) and the rest were based only on estimated assessments of effectiveness of surveillance systems with an estimated 1.4 million (15%) incident TB/HIV co-infections in 2008 (WHO, 2010c; WHO, 2010g).

During infection with *Mycobacterium tuberculosis*, it is still unknown whether the organism inside the body is in a persistent slow replicating state or is settled in a dormant non-replicating state, with the latter ultimately possibly causing a latent infection with the potential to reactivate to active disease forms. International TB epidemiology and endemic veracity of world countries limit TB diagnosis and management even the outlines recommended by the WHO. The majority of new TB cases arise from re-activation of dormant foci of infection. Thus, management of LTBI is a major recommended national strategy for TB disease prevention (Horsburgh *et al.*, 2004).

The aim, worldwide, is to manage/control and prevent tuberculosis, but, there is only limited evidence to support the current TB screening guidelines, which are still unable to identify individuals with latent tuberculosis in particular from TB endemic regions. TB control can be supported through chemoprophylaxis and prevention of individual cross-infections such as those occurring in newly hired health care workers, or prevention of secondary transmission using preventive medication when immigrants enter from highly endemic countries (He *et al.*, 2010; Schwartzman and Menzies, 2000).

Successful control of LTBI/TB in low-incidence countries will depends on early detection of patient with the acute clinical form of the disease, in addition to effective screening and treatment programmes for the identification and preventive prophylaxis of latent tuberculosis cases at high risk of MTB re-activation/progression (Orlando *et al.*, 2010).

Evidence-based approaches and tuberculosis control policy should enable clearer determination of the risk factors influencing tuberculosis incidence that are tailored to local needs. Effective estimation of health impacts of both the broad social and economic policy interventions then can focus on the disease- or population-specific public health programmes.

9.2 Aim

To describe the cost-effectiveness of preventing latent tuberculosis infection in a screened high-risk group of new immigrants.

9.3 Discussion

The challenges for ease access to the primary health care system and follow-up control of treatment adhesion added to contacts tracing, all can be facilitated by 'effective' screening and treatment programmes for recent immigrants (Orlando *et al.*, 2010).

Improvement of the effectiveness of tuberculosis control programmes in Kuwait can be achieved by identifying cost-effective priorities for screening for latent tuberculosis in new immigrants entering the country each day. New 'two-step' LTBI screening guidelines can facilitate progress towards TB elimination by prioritizing screening for new immigrants arriving from TB high-incidence and endemic countries, in particular for specified epidemiological variables.

9.3.1 Screening of high-risk groups (new immigrants)

Screening is a systematic search for LTBI/TB, often performed by radiological investigation, and/or followed by the Mantoux skin test for positive radiographic findings, and newly recommended to use the biomarker (immunological) IGRAs tests. Increased knowledge of LTBI progression rates in those individuals having positive IGRAs (one-step or two-step strategy) would help to improve the hypothetical cost-effectiveness outcomes were statistically achieved in the systemic review analysis performed by Nienhaus and colleagues (Nienhaus *et al.*, 2011).

A major concern of public health is the screening of high-risk populations such as foreign-born and recent immigrants or contacts to actively infected TB patients. In a recent survey of 26 European countries, 13 reported and strictly implemented national TB screening programmes for immigrants and/or refugees, while only a few of them recommended treatment for LTBI detected by the screening (Coker, 2004). The observed results of Losi and colleagues (2011) were similar to our study findings, and highlighted the potential advantages and concerns of using a blood test for diagnosing LTBI in a 'two-step' strategy in foreign-born children. Uncontrolled intensification of immigrant screening and TB surveillance were unable to reflect the challenges of LTBI prevention. Then the issue had been solved by careful planning, pilot testing, enhanced information sharing, health care services availability/training, and continuous programme evaluations (Richard *et al.*, 2005).

The study findings are relevant to public-health interventions, the ultimate aim of LTBI screening being the prevention of progression to active TB using chemoprevention. Whether the introduction of IGRAs in the TB screening of new immigrants is cost-effective need always to be studied according to each the country health system. Recent immigrants from the TB endemics (less than two years since immigration) can re-activate and develop active TB disease which should be targeted. In countries with an incidence of active TB less than 10/100,000 habitants (such as Kuwait) the screening strategies for the identification of those high risk subjects are cost effective for TB control and reduce epidemic risk (Orlando *et al.*, 2010).

9.3.2 Is screening immigrants for latent tuberculosis a cost-effective preventive procedure?

Routine screening of new immigrants and foreign-born high-risk groups, although costly, would help to detect latent tuberculosis cases early, to improve public health. Screening and identification of latent tuberculosis cases was found cost-effective before development of new active TB cases, thus preventing future cases of active tuberculosis in UK (Pareek *et al.*, 2011).

Identify 'suspect TB' carriers was tested through building a classification tree using epidemiological-radiographic-laboratory test results was described in chapter 8, section 8.4.2.

The prevalence and predictive factors associated with tuberculosis in adolescents were found to be latently infected with MTB in TB high-burden settings, in endemic predictable regions with socio-demographic and economic poverty-related factors and by which able to predict the risk of TB infection. Such deprived adolescents should be the target group for educational interventions by TB control programmes (Mahomed *et al.*, 2011).

The guidelines of LTBI screening can raise the community health toward TB elimination by prioritizing screening for close contacts of TB infected patients and the foreign-born residents and/or new immigrants regardless of their time living or even if coming to non-endemic countries was observed to be more cost-effective than only TST screening (Linas *et al.*, 2011), which was consistent with our study's recommendations. The lower sensitivity/specificity of TST can be associated with drop-out follow-ups of participants which can reduce the effectiveness of the CXR-TST dual strategy of LTBI detection in suspected tuberculosis cases (Orlando *et al.*, 2010). Systematic review studies of cost and cost-effectiveness for different TB-screening strategies revealed with evidence-based that either IGRA single-screening strategy was cost-effective than the IGRA two-step strategy or other used guidelines (Nienhaus *et al.*, 2011).

Systematic screening is known to have a positive impact on community health. Raising the level of public awareness of LBTI/TB, early detection and effective treatment, early contact recognition, adoption of protective measures, and effective screening of LTBI cases, are all necessary for controlling the risk of TB in a country (Horsburgh, 2004).

Regular reliable surveillance is essential part for any TB control programme and under-registration is rare in Kuwait due to high-notification system. In Kuwait, post-entry registration of immigrants denotes a strong and effective immigration

registration system and within short time, which coincide with strict TB case notification and 100% detection rates. This denotes strict legislative laws and implemented interventions to increase the registration completeness and legal notification of both active TB cases and latent TB cases requiring chemoprophylaxis (WHO, 2010i).

The screening policy of new immigrants recommended by WHO, implemented all over the world and compulsory in Kuwait, is primarily using chest X-ray and secondly by TST or other diagnostic test for suspected CXR positive findings (Al Jahdali *et al.*, 2010; CDC, 2011a; Hardy *et al.*, 2010). Educational programmes should re-enforce the consequences of treatment delay among tuberculosis cases which contributes to the greatest number of infectious days. Also clarifications are needed of the loss of follow-ups and the related threatened negative expectations of MTB transmission within the family and society (Gust *et al.*, 2011). Positive diagnostic results can be approved as a reference standard for LTBI diagnosis and reflects exposures to MTB among high-risk groups and foreign-born residents from endemic countries (O'Donnell *et al.*, 2010).

With the absence of a gold standard TB test, evidence-based future guidelines for diagnosis of LTBI case must be re-organized, periodically updated, and without financial conflicts or health care overloads.

The six Gulf state countries are one of the largest markets for immigrant Arab and Asian job seekers. After the discovery of oil, the absence of a workforce necessitated employment of an expatriate labour force, which brought negative socio-economic, cultural and health consequences, such as changes in infectious disease epidemiology. Planned migration screening programmes for TB can be improved using evidenced-based identification criteria of suspect MTB carriers, through cooperative research between countries. Direct costs in the developed/in-migrant countries include expenditures on hospitalization and transportation, consultation fees, diagnostic tests and medicines/treatments fees. Indirect costs on the health care system can be accessed through the days lost from work due to symptomatic

complaints or health-seeking behaviours such as recurrent visits for uncontrolled relapse of active TB disease (Pantoja *et al.*, 2009).

Post-arrival immigrant screening decreases the infectiousness period and identifies cases of latent TB infection. For example our research findings if implemented can facilitate improvements in the morbid rates of Kuwait residents through the immigrant's future screening for latent TBI without missing cases.

Pareek and colleagues (2011) advanced the evidence-base by using the more accurate and empirical IGRA screening, which can be objectively applied and achieved cost-effective screening of immigrants at different regions. Screening of all immigrants aged less than 35 years from any country would cost more than £1.5 million and could prevent 44.5 cases of tuberculosis, whereas application of NICE guidelines would cost about £850,000 and only prevent 13.2 cases of active disease in UK.
Even with overall discordances between TST and IGRAs, various studies proved that the performance of interferon gamma release assays for LTBI diagnosis was higher and more efficient than the gold standard TST (Ferrara *et al.*, 2006; Harada *et al.*, 2006; Mazurek *et al.*, 2007; Pai *et al.*, 2005; Pai *et al.*, 2006; Richeldi, 2006; Sauzullo *et al.*, 2008; Winje *et al.*, 2008).

Screening for LTBI with positive TST followed by an IGRA was a cost-effective approach for the high-income and low-risk countries. The UK-NICE guidelines protocol estimated about £47.67 per screened immigrant and about £160.81 per LTBI case identified (NICE, 2006). Pooran and colleagues (2010) showed that, per 1,000 screened contacts, the TST/IGRA dual screening strategies using TST/T-SPOT *.TB* was costed at £162,387 versus TST/QFT-GIT costs of £157,048, which costed less than the single strategy counterparts (T-SPOT *.TB* and QFT-GIT; £203,983 and £202,921 respectively). Single IGRAs strategies should be requested accurately by the health care staff and only to those truly suspected/infected TB cases and harboring MTB, with subsequent correct treatment/follow-up of LTBI cases in the high-risk groups. A dual strategy is more cost effective than a single strategy, but this conclusion is sensitive to the used screening test assumptions and LTBI prevalence.

The implementation of LTBI screening programmes is strictly indicated only in settings where LTBI prophylaxis and follow-up can be offered and monitored. The use of IGRAs to screen immigrants in TB control programmes have been recommended by Carvalho and others (2005) and Winje and others (2008). The average cost of identifying one latent tuberculosis case following UK-NICE guidelines was £160.81, whereas, using the first-line QFT followed by CXR protocol, it was only £93.16 (Hardy *et al.*, 2010). Recent analysis of the available 13 studies on cost-effectiveness provides strong evidence in support of the use of IGRAs in screening the high-risk groups such as new immigrants and their close contacts from high-incidence countries (Nienhaus *et al.*, 2011).

9.3.3 Preventive therapy (chemoprophylaxis) of latent tuberculosis infection

Tuberculosis is still a global public health problem and a major leading cause of infectious disease morbidity and mortality all over the world, particularly in low-income and middle-income countries, especially where tuberculosis is hyper-endemic because of increased exposure and transmission.

The American Thoracic Society and Center for Disease Control and Prevention recommended using the terminology 'Treatment of Latent Tuberculosis Infection' instead of 'Preventive Therapy' or 'Chemoprophylaxis', which mean prevention of development of active TB disease in individuals that are currently well and asymptomatic, but infected and carrying *Mycobacterium tuberculosis* (ATS and CDC, 2000). For example, immigrants born in high-incidence countries that had arrived within less than two years averaged 25 years of age on arrival are major targets for preventive therapy (Langlois-Klassen *et al.*, 2011). Preventive efforts are noticed to begin LTBI prophylaxis and ensure treatment follow-ups. Even after initial screening, treated cases can drop-out and miss the medication schedule follow-ups. Therefore, LTBI will not be prevented because of non-adherence to LTBI treatments. Our findings were generally similar to WHO guidelines and recommended strict follow-up to ensure LTBI/TB control.

Before LTBI prophylaxis, active TB disease should be excluded, and then the full prophylactic regimen should be followed carefully. Screening and preventive prophylaxis of LTBI are key tools for reduction in the risk of developing active TB among immigrants from endemic and non-endemic countries by more than 50%, and is targeted for TB control programmes which depend on screening biomarkers (CDC, 2000; Rieder *et al.*, 1994). For example, a positive TST can be confirmed by positive IGRAs in BCG-vaccinated individuals, and then LTBI chemoprophylaxis can be initiated after exclusion of active TB (Al Jahdali *et al.*, 2010).

Treatment of latent tuberculosis infection is an essential component of tuberculosis control and elimination. The goal of preventive treatment is to minimize the transmission of *Mycobacterium tuberculosis* by carrying out a public health action to prescribe and follow-up completion of treatments. Therefore, treatment of latent TB reduces the risk of TB infection progressing to TB disease (CDC, 2011d). LTBI is associated with a lower burden (or number) of MTB organisms, therefore requiring fewer drugs compared with the four-drug therapy of active TB without creating drug-resistance (Al Jahdali *et al.*, 2010).

Isoniazid preventive therapy (IPT) is cheap and highly efficacious (up to 90%) in stopping progression to active TB among TST-positive people, but, despite having an effective drug, LTBI progression to active TB still largely unchecked. Efficacy and compliance results were considerably higher and acceptable alternative using a new regimen of short-course regimen with isoniazid for six months and/or rifampicin for three or four months than a nine-month of isoniazid treatment, which may also was more effective against infection with isoniazid-resistant or rifampin-susceptible mycobacteria with uncommon drug-related adverse effects in children and is a recommendation for adult investigation (Al Jahdali *et al.*, 2010; Spyridis *et al.*, 2007). Table 9.1 shows the latest CDC recommendations and the future preventive therapy currently recommended for worldwide countries.

Table 9.1: Treatment regimens for latent tuberculosis infection in adults (CDC, 2011d)

Drugs	Duration	Interval	Minimum dose (mg)	Maximum dose (mg)
Isoniazid	9 months	Daily	270	300
		Twice weekly*	76	900
Isoniazid	6 months	Daily	180	300
		Twice weekly*	52	900
Isoniazid and Rifapentine	3 months	Once weekly*	12	INH (900)/Rif (900)
Rifampin	4 months	Daily	120	450-600

*means use Directly Observed Therapy, short course (DOTS)

Note: CDC recommends that the combination of rifampin (RIF) and pyrazinamide (PZA) should generally not be offered for LTBI treatment due to reports of severe liver injury and death.

Chemoprophylaxis of LTBI using a short-course regimen is indicated to ensure that infection does not progress to active TB. Studying the socio-cultural believes and behavioural attitudes can demonstrate the treatment acceptance/adherence in the foreign-born individuals and in-migrants (Minodier *et al.*, 2010). Out of 1,414 close contacts having similar exposures revealed, 903 refused chemoprevention and 19 developed active TB within four years and all of the latter previously had positive QFT-GIT results and the progression rate was higher in children than for adults (Diel *et al.*, 2011b).

LTBI therapy consists of nine-month course of daily isoniazid (INH) or combination of isoniazid-rifampicin (INH-R) for three months, and monthly clinic visits for monitoring medication side effects. The majority of contacts showing evidence of harbouring dormant LTBI were reduced by seventy percent of the risk of progressive developments to active disease by completing a six-to-nine month's course of INH (Salazar-Vergara *et al.*, 2003; Spyridis *et al.*, 2007). A recent cost-effective analysis, which considered INH-related hepatoxicity and post-exposure TB contacts, concluded that a dual strategy of TST followed by an IGRA was more cost-effective (Pooran *et al.*, 2010). Diel and colleagues (2007) described a previously similar cost-effective study of the outcomes of INH treatment for close contacts in non-endemic countries such as Switzerland.

The use of rifapentine (900 mg) plus isoniazid (900 mg) (combination-therapy group) for 3 months was as effective as the standard regimen 9 months of isoniazid (100 mg) (isoniazid-only group) alone in preventing tuberculosis at high risk groups, and had a higher treatment completion rate for the shorter therapeutic duration (82.1% versus 69% respectively) (Sterling *et al.*, 2011). Patients with LTBI treated with rifampin for 4 months show higher completion rate than those given INH for nine (91.3%) or even four months (77.2%), respectively (Young *et al.*, 2009).

The shorter regimen and application of directly observed preventive therapy (DOPT) increases therapeutic completion rates (Sterling *et al.*, 2011). Rifamycin is a new medication that has strong sterilizing activity against both dormant and semi-dormant TB bacilli and more potent than INH regimen alone. Rifapentine (long acting rifamycin/isoniazid combination) is better tolerated with less hepatotoxicity compared with rifampicin or pyrazinamide (Yew *et al.*, 2007). In addition to higher therapeutic compliance with short-course regimens, rifampicin can be considered as replacement therapy for children with LTBI in countries with more than 11% of isoniazid resistance (Finnell *et al.*, 2009).

9.3.4 Obstacles to latent tuberculosis infection prevention

The chronic slow growth of the mycobacterium tubercle bacillus is the greatest obstacle to rapid diagnosis of LTBI/TB disease, but unfortunately, there are still no accurate guidelines to indicate how LTBI can be prevented. Early detection and treatment of active TB cases by DOTS cannot reduce MTB transmission among immigrants because most active TB occurs due to re-activation of infections acquired in their country of origin (El-Hamad *et al.*, 2001). Reduction of exposure risks should be a high priority, especially for occupational and environmental TB risk factors. Loss of infected skilled labourers and health care workers such as nurses and doctors who are essential in the fight against TB disease (Bradshaw *et al.*, 2011; He *et al.*, 2010; Jong Lee *et al.*, 2010; Rafiza *et al.*, 2011). In the Poland IGRAs are not licensed added to the absence of utilization guidance complicated subsequently with higher risks of nosocomial exposures and MTB transmission between HCW's and to the general population (Demkow *et al.*, 2008).

The introduction of a free TB treatment policy in the high income countries with no financial complaints still barriers to tuberculosis care are detected among migrants occurs due to weak health care system. Limited access to medical services was reduced by solving the financial stress/constraints, however, these limitations cannot be accepted by those immigrants being laid off from work (cause of in-migration) or facing the community avoidance after diagnosed to have LTBI/TB (Wei *et al.*, 2009). Inadequacies in the screening process and increased TB prevalence in the general population can occur due to increased TB frequencies (Mankia *et al.*, 2011). When IGRAs are used to determine latent TB infection in foreign-born individuals, positive findings relate to recent TB infection and also reflect prior TB exposure in the country of origin (Kik *et al.*, 2009).

Diagnostic test cost-effectiveness can be enhanced by increased adherence to chemoprophylaxis recommendations (Schwartzman and Menzies, 2000). Risk factors of treatment non-compliance due to the long preventive treatment duration of INH for nine months or INH-R combination for three months, and adverse effects in addition to the needs for regular monitoring during treatment, results in suboptimal treatment completion rates, which are closely related to demographic factors e.g. age, gender, smoking habit, different education level and recent immigrants less than five years of residence and/or with social maladaptations e.g. unemployment (Anibarro *et al.*, 2010; El-Hamad *et al.*, 2001; LoBue and Moser, 2003; Young *et al.*, 2009). High medication completion levels are directly but marginally related to short treatment regimens (Spyridis *et al.*, 2007). Non-adherence to preventive measures during screening is under-estimating LTBI prevalence post-treatment. Multi-generational households and unstable living cultures are socio-demographic factors associated with poor adherence to LTBI prophylaxis (Minodier *et al.*, 2010). Language barriers between immigrants and medical providers are a major obstacle to effective surveillance of health care (CDC, 2007). The appearance of drug-resistant tuberculosis is another urgent pre-request for LTBI detection through screening of high risk groups. In 2008, 440,000 multidrug resistant cases emerged globally, with an estimated 50% in India and China (WHO, 2010h). In Kuwait, nine cases out of reported 428 TB confirmed cases were recently confirmed and started treatment, and all were tested for MDR-TB (WHO, 2010i). LTBI conversion (after lifetime

dormancy) and risks of progression to active TB disease ranges from 10% to 20% also impedes TB control (Horsburgh, 2004).

9.3.5 Cost-effectiveness of evidence-based new recommendations for using IGRAs

Tuberculosis has enormous public health and economic implications in high-burden countries, therefore providing targeted interventions is recommended, particularly for those high-risk groups (WHO, 2008a). Wiker *et al.* (2010) concluded that in several populations, latent tuberculosis appears to be more rapidly cleared than previously thought. The early detection and treatment of LTBI constitutes an essential component of the tuberculosis control strategy in resource-rich and low-prevalence countries and helps to interrupt MTB transmission. The risk of nosocomial transmission and incidence of TB in the majority of high-income countries has dramatically declined after implementation of a recommended set of infection-control practices to reduce TB burden (Drobniewski *et al.*, 2007; Seidler *et al.*, 2005).

Radiographic CXR screening of immigrants from TB endemic countries can save money, but remains less diagnostic than the new biomarker IGRAs or PCR. Compared with no screening, radiographic screening (cost U.S.$3,943 per prevented active TB case) prevented 4.3% of expected active TB cases in the highest risk group, and 8% in the lowest risk people (U.S.$236,496 per case prevented). Also tuberculin skin testing further reduces the expected incidence to 8% and 4% respectively, but still is less cost-effective than adding either IGRAs (Schwartzman and Menzies, 2000).

Routine careful scheduling and case counseling followed by monitoring of LTBI diagnosed cases and utilizing with completion of the low-cost chemo-preventive medication especially for contacts of diagnosed active TB patient are recommended (Anibarro *et al.*, 2010; Young *et al.*, 2009). A school-based TB-screening programme was effective when targeted towards children of recent immigrants detected early with LTBI (Minodier *et al.*, 2010). IGRAs screening for LTBI treatment was found highly cost-effective in reducing the burden of active TB disease (Diel *et al.*, 2007).

Gray and colleagues (2011) recently detected that out of 94 HIV patients with QNF-GIT-positive results, 45 patients were not re-tested and all were offered LTBI INH-preventive treatment , and none developed active tuberculosis during follow-up.

LTBI prevalence through nosocomial transmission of MTB is an important occupational problem among health care workers, and risk reductions should be a priority (He *et al.*, 2010; Seidler *et al.*, 2005), and need strict infection control (Sacks *et al.*, 1999), which necessities accurate LTBI diagnosis through serial screening using IGRAs instead of the annual chest X-ray similar to our research recommendations.

Several studies on cost-effectiveness provide strong evidence supporting the use of IGRAs in screening risk groups such as HCWs and immigrants from high-incidence countries and close contacts of patients developing active TB (Nienhaus *et al.*, 2011). Moon and colleagues' (2011) results showed a high prevalence of positive TST and QFT-Gold in 173 samples of laboratory personnel which recommended the use of IGRAs to screen for LTBI and as helpful confirmatory follow-up tests for MTB infection. Similarly, Hardy and colleagues (2010) revealed that QNF-GIT followed by chest X-ray can be a complementary and available cost-effective diagnostic tool for immigrant screening because of the reduced number of CXR's following the positive QNF-GIT results. Screening for latent TB using blood biomarker tests (IGRAs) is obligatory before starting anti-tumour necrosis factor treatment for rheumatologic disorders, and instead of tuberculin skin test especially in the BCG vaccinated populations (Anderson *et al.*, 2000; de Andrade Lima *et al.*, 2011). The use of IGRAs for identifying subjects requiring anti-tuberculosis chemoprophylaxis also might be considered a better cost-effective long-term curative strategy (Diel *et al.*, 2007).

The choice of diagnostic test should be based on cost-effectiveness. Diel *et al.* (2008) evaluation of 601 close TB contacts detected higher QNF-GIT accuracy and specificity than TST for the presence (or diagnosis) of LTBI who will progress to active TB. Similarly, QFT-GIT was found more reliable than the TST in identifying those individuals who soon might progress to active TB, especially in children (Diel *et al.*, 2011b). The higher QFT-GIT specificity over TST prevents unnecessary overloads on the health care system and, even though IGRAs are more expensive,

can represent a cost-effective alternative to TST in high-risk and targeted screening programmes (Orlando *et al.*, 2010).

Kowada and colleagues (2008) evaluated different screening strategies against a hypothetical cohort of 1,000 immunocompetent 20-year-old individuals had contact with sputum-smear-positive PTB patients. The study findings of a base-case analysis, the QFT-alone strategy was dominant (U.S.$S471.54; 28.1099 quality-adjusted life-years [QALYs]) and the most cost-effective for TB screening in close contacts within a medium-incidence country with a completely BCG-vaccinated society like Japan, compared with the combined TST/QNF strategy (U.S.$500.55; 28.1087 QALYs) and the TST-alone strategy (U.S.$573.98; 28.1079 QALYs).

Pooran and colleagues' (2010) analysis revealed that the cost-effectiveness of five different screening criteria for contact tracing over two years post contact in UK was the two-step strategy using both TST and T-SPOT *.TB* test (£37,206 per case of active TB prevented), followed by the near similar costs as the TST and QFT strategy (£37,699 per case of active TB prevented) as the most cost-effective strategies, in comparison to the use of TST alone was the least cost-effective (£47,840 per active TB case prevented).

In contrast, Mancuso and colleagues' (2011) findings detected that 'targeted' strategies' costs were over U.S.$250,000 per case prevented, whereas, 'universal' testing strategies' costs were over U.S.$700,000 per case prevented in rising-incidence countries. Even though targeted testing offered the best solution, it is still expensive compared with no testing, and 'sequential' testing using both TST and IGRAs. However 'targeted' and 'universal' testing strategies using the TST alone was the slightly more cost effective strategy than targeted testing using either QuantiFERON-TB Gold In-Tube or T-SPOT *.TB* test for LTBI diagnosis.

9.4 Conclusions

Screening for latent tuberculosis infection can be justified among populations at a higher risk of carrying *Mycobacterium tuberculosis* infection and developing

tuberculosis disease. Awareness of the health care staff about early detection and chemoprophylaxis and aggressive follow-ups proved reduction of TB prevalence risks. Targeting of the most vulnerable high-risk groups and new immigrants with diagnostic interventions is essential for the progress of preventive control measures. Either IGRAs can be implemented cost effectively with or without positive radiographic interpretation. A global dual-strategy approach for LTBI diagnosis was possibly more cost-effective strategy than a single strategy for the healthcare contacts in UK (NICE, 2011; Pooran *et al.*, 2010), which can be tested in Kuwait to estimate LTBI prevalence before diagnostic guideline generalization.

9.5 Study recommendations

Screening and resources for planning, training and supervision are required to safeguard health quality. All these can directly and indirectly assess the country national health system;

1- There is a requirement for funds for the development of new technologies, with better use and scale-up of the existing tuberculosis diagnostic tools, and adequate treatment capacity to care for the increasing numbers of diagnosed latent tuberculosis cases. This should greatly reduce the costs and work time in the future for the high-risk group comprising recent immigrants entering from tuberculosis-endemic countries.

2- There is a need for analysis of cost-effectiveness of latent tuberculosis infection screening using the new dual-step diagnostic criteria.

3- Future studies are suggested to evaluate chemo-prophylactic efficacy using short-term one-drug regimens, in addition to effective diagnostic tools delivered through the authorized or specialized health care centres and medical care services to all suspect immigrants from tuberculosis endemic and Asian (particularly East Asian) countries at point of entry/registration.

10 Chapter ten

General discussion

It is important for any researcher to think about the bigger picture/context/issues that frame and inform TB infection and policy in non-endemic countries. Innovating and testing for evidence-based thesis structure (included in the nine chapters headings and described the contents/arguments of each) can be useful for other innovative frameworks through different data analysis and result interpretations toward raising the community health.

10.1 Introduction

The aims and objectives of this thesis have concentrated on 'accurate' and 'early' diagnosis of latent tuberculosis infection, which serves three major purposes; first, enabling an evidence-based diagnosis of latent TB cases and allowing future timely interventions; secondly, allowing epidemiological characterization of high-risk groups and 'suspect TB' cases where better TB control can be facilitated; and thirdly, indirectly assessing the health care system and related medical services of the country's residents.

Public health authorities should ensure they are;

➢ offering appropriate health care and treatment services,
➢ carrying out contact tracing of LTBI diagnosed cases, in addition to
➢ early recognition of local outbreaks and monitoring of tuberculosis epidemiology represented by the recommendations of the WHO.

As tuberculosis is the only disease clarified as clinical possibility, as radiological probability and as bacteriological certainty, therefore, *Mycobacterium tuberculosis* carriers and 'suspect TB' cases have to be early diagnosed and managed before bacillary re-activations

10.2 Discussion

The problem of latent tuberculosis infection is:

Nearly two billion of the world's population are infected at least once with *Mycobacterium tuberculosis*, and are considered to have latent tuberculosis infection. The high-risk groups at risk of re-activation do not secrete the harbored and dormant bacilli, which are hard to diagnose by conventional laboratory tests such as sputum smear and bacterial culture. With the absence of a gold standard test for LTBI diagnosis, the results of various diagnostics for asymptomatic individual are still without clinical significance. To date, there are no evidence-based data to assess the performance for IGRAs in comparison with the TST, and the latter test remains the recommended test for LTBI diagnosis all over the world. Practical, cost-effective strategies to identify immigrants with latent tuberculosis infection and to deliver treatment for accurately diagnosed latent TB infection are still unavailable, but are needed in the future.

Chapter 1 presented the general view of international pandemic latent tuberculosis infection and active tuberculosis disease and its related consequences, with the need for global epidemic control. Immigration of latent tuberculosis infected cases from tuberculosis-endemic regions is considered the main risk factor for bacillary transmission to low incidence countries. The problem with tuberculosis in the low prevalence countries in particular is the continuous unresolved leakage of latent tuberculosis immigrant cases, where increased tuberculosis morbidity and resurgence of *Mycobacterium tuberculosis* drug-resistance strains are internationally observed problems. Early identification and successful treatment of latent tuberculosis cases remains the most effective step against MTB transmission and towards its prevention (Orlanso *et al.*, 2010).

Chapter 2 explained the consequences of dominance of foreign-born residents due to continuous in-migration, even with controlled case notification of all infected tuberculosis cases in non-endemic countries such as Kuwait, and consequent increments of all tuberculosis morbid rates since the last three decades (HVS, 1984-2009). Therefore continuous supervision of the control measures of all infectious diseases and tuberculosis is necessitated. The higher fatality rates in Kuwaitis, compared with the majority of diagnosed active tuberculosis cases in the foreign born residents requires further genotypic polymorphism analysis studies, especially within

the first one and two years post-entry due to high risks of MTB exposure-transmissions. Without screening and treatment for latent tuberculosis, case notification rates of active tuberculosis disease among high-risk groups and recent immigrants remain high (El-Hamad *et al.*, 2001).

Chapter 3 described the material and methods for screening a sample of 180 new immigrants during obligatory registration at entry points using an epidemiological questionnaire and three tuberculosis diagnostic tests, in addition to the ordinary chest X-ray. Our findings show a significant difference between recent immigrant individuals for various variables and diagnostic test results, similar to Losi and colleagues' (2011) recent findings in foreign-born children's of settled immigrants to Italy.

Chapter 4 demonstrated the commonest causes and evidence-based related risk factors for latent tuberculosis infection and active tuberculosis disease, which was one of the main objectives of this research work. The results of the questionnaire showed that social determinants of health should be addressed in the design of tuberculosis information campaigns. Barriers to diagnostics and management for TB control in Kuwait can be tackled by prioritizing tuberculosis public health interventions, as also recently noted by Mauch *et al.*, (2011). Worldwide, LTBI diagnosis is still made using the tuberculin skin test, but this has disadvantages, such as shared interaction with the Bacille Calmette-Guérin vaccine antigens (applied in 99% of countries) and false positive TST outcomes. Chest X-ray and the tuberculin skin test should not be continued as the ordinary diagnostic tests for abnormal chest findings and latent tuberculosis infection, but can help as confirmatory tests.

Universal chest radiography and tuberculin skin test in a large pre- or post-employment TB screening program was of low yield in the detection of active TB or the increased LTBI re-activation risks, and provided no assistance to decide which individuals to prioritize for LTBI chemoprophylaxis.

The second objective was to demonstrate the potential utility and performance of both the two traditional tests used for diagnosis of latent tuberculosis infection; namely, chest X-ray and tuberculin skin test. This was addressed in **chapter 5**. A

new radiological criterion for LTBI diagnosis (chapter 5; section 5.1.5, Table 5.1) was developed from combination of previous evidence-based findings. The presence of radiologist opinions during interpretation is strictly recommended to raise the weakness of radiographic diagnosis from other non-specialized authorities. Both radiologists also were able to detect the abnormal CXR findings (10.8%) which were similar to the average worldwide LTBI prevalence (5-10%). Additionally, following the diagnostic criterion and scoring system of tuberculin skin test response of latent tuberculosis infection reaction (chapter 5; section 5.2.5, Table 5.5), which is followed by the Ministry of Health in Kuwait as recommended by ATS and CDC, 2000; CDC, 2005c; CDC, 2010b, had still revealed a low diagnostic performance, with a need to present a new TST recording system for the non-endemic countries.

Recently, interferon-gamma release assays were demonstrated to be excellent tests for detection of latent tuberculosis, and replacement alternative tests characterized with superior sensitivity and specificity to the CXR and TST and/or CXR alone in recent adult immigrants. Moreover, there was no interference from BCG vaccine, with consequent better prediction of latent tuberculosis cases (Al-Orainey, 2009; Brodie *et al.*, 2008; CDC, 2011b; Diel *et al.*, 2010; Gray *et al.*, 2011; Grimes *et al.*, 2007; Mazurek *et al.*, 2007; Menzies *et al.*, 2007; Moon *et al.*, 2011, Orlando *et al.*, 2010; Pai *et al.*, 2005; Pai *et al.*, 2006; Tavast *et al.*, 2009).

Chapter 6 described the comparative performance of the two-biomarker IGRAs diagnostics (QuantiFERON Gold In-Tube test and T-SPOT *.TB* test) for detection of LTBI in high-risk groups. Kik and colleagues (2009) observed that positive IGRAs results might be associated with previous remote infection without evidence of clear re-activation. Interferon gamma detective tests, both IGRAs, are reported to be better predictors and to correlate more the MTB exposure-re-activation risks toward developing tuberculosis disease than the standard Mantoux test (Mendez-Echevarria *et al.*, 2011; NICE, 2011).

Chapter 7 noted that absence of a gold standard test to correctly identify the sensitivity and specificity of each of the four tuberculosis diagnostic tests (Kunst, 2006), thus challenging evaluation of test performance and comparative assessment

of different tests. A proposed new classification for detection of latent tuberculosis infection and 'suspect tuberculosis' cases using evidence-based laboratory diagnostic criteria was statistically tested and elucidated (chapter 7; section 7.3.3, Table 7.2).

Chapter 8 re-capitulated the significant results of previous chapters 4-7 for the identification of latent tuberculosis infection index cases diagnosed from the evidence-based immunological (section 8.4.2.1), epidemiological (section 8.4.2.2.1, Table 8.1) and laboratory diagnostics (section 8.4.2.2.2, Table 8.2). A new two-step diagnostic criterion was tested versus a theoretical 'proposed' gold standard test and estimated for test combination accuracy (chapter 8; section 8.4.2.3, Table 8.5).

Any clinical, radiological or bacteriological evidence of tuberculosis disease contra-indicates chemo-preventive therapy, which should be prescribed only for definitely diagnosed LTBI cases after exclusion of active TB disease (Al Jahdali *et al.*, 2010). The literature does not yet provide sufficient data on the definite accuracy of TST, CXR or IGRAs in detecting latent tuberculosis cases - even in high-risk groups. An urgent preventive measure through a new and more accurate diagnostic strategy requires further studies to determine the optimal use of new developed diagnostic algorithms.

Chapter 9 described the cost-effectiveness of high risk group screening able to structure an evidence-based new recommendation for using a two-step diagnostic criteria procedure, using either IGRAs as compulsory test for high-risk groups and complementary CXR read by radiologists (Hardy *et al.*, 2010; Losi *et al.*, 2011; Nienhaus *et al.*, 2011). This would raises the infection control measures and reduce the consequent rises in tuberculosis morbid rates previously explained in chapter 2. Chapters 7 and 9 demonstrated that immigrants should be screened at access points using the two-step combination criterion of both chest radiography (read by at least one radiologist) plus QuantiFERON Gold In-Tube test, before being identified as candidates for LTBI prophylaxis (Orlando *et al.*, 2010).

10.3 Final conclusions

Neither interferon gamma release assays nor chest radiography and the tuberculin skin test have high accuracy for the prediction of latent tuberculosis infection or active tuberculosis cases, although the use of interferon gamma release assays in certain high-risk groups such as immigrants from TB endemic regions can reduce the number of people considered for chemo-preventive therapy. Regardless of the diagnostic test(s) used, targeted testing for high-risk groups within low prevalence populations is critical in reducing unnecessary testing(s), which is similar to the recent conclusion of Mancuso *et al.* (2010). The recommended two-step strategy is cost effective than a single strategy for immigrant screening, depending on the country economic funds for IGRAs implementation and LTBI prevalence in relation to rising incidence of active TB and/or drug-resistance cases (Pooran *et al.*, 2010). Until more specific and predictive biomarkers are identified, the existing interferon gamma release assays - QuantiFERON Gold In-Tube test in particular - can be chosen for detection of latent tuberculosis infection on the basis of calculated relative specificity in different populations, logistics, test cost, and also patients' preferences, rather than on predictive suspicions alone. A similar conclusion was reached by Rangaka *et al.* (2011).

10.4 Final recommendations

1- Latent tuberculosis infection can be detected on the basis of history taking of epidemiologically-related risk factors, physical examination and chest X-ray plus either IGRAs.

2- Screening should be a component of a wider intervention approach, even with overloads of false positive results on the health care system, thus exceeding the barrier of higher costs of a single test (Orlando *et al.*, 2010). Data improvements to assess the frequency and duration of cost-effective screening and access of healthcare services would ensure a continuum of preventive care for high-risk groups.

3- We introduce the new classification (or 'Edinburgh classification') for detection of latent tuberculosis cases (Table 10.1). The research results can be supported

through implementation of our screening methodology on large sample size of high-risk groups, and by which can evaluate the cost-effectiveness of the recommended dual-step diagnostic testing of QNF-GIT and chest radiography. Also subsequent periodic monitoring for suspect tuberculosis cases is recommended to compare the rates of sensitivity and specificity between different methods (Losi *et al.*, 2011; Nienhaus *et al.*, 2011). This is crucial, before generalization of the new diagnostic guidelines.

4- Health system problems caused the biggest barrier to migrant patients' access to TB care. Enhancement of financial resources and decentralization of medical services, especially in low income counties, is crucial and is recommended for the implementation of the TB control strategies (Xu *et al.*, 2010). It is also essential to provide social welfare, including living subsidies, for poor migrant TB patients because the free treatment policy alone has little effect in reducing migrant patients' financial stress (Wei *et al.*, 2009). Therefore tuberculosis screening programmes considering two-step diagnostics for LTBI should ensure medically evaluation and therapeutic completion for positive results individuals (Oxlade, 2007).

5- Application of a whole-genome sequencing (WGS) for all *Mycobacterium tuberculosis* isolates and testing for gene polymorphism which might be associated with susceptibility to tuberculosis. Analysis should be performed for different high-risk group nationalities particularly for East Asian regions associated with multi-drug resistance strains of *Mycobacterium tuberculosis* bacilli newly discovered in Kuwait (Feng J.Y. *et al.*, 2011; Ladefoged *et al.*, 2011; WHO, 2010i). DNA sequencing can identify drug susceptibility and genetic characterization of *Mycobacterium tuberculosis*, which was a recent finding, observed in Thuong *et al.*, 2011, and is recommended as the secondary laboratory diagnostic tool for the future, worldwide.

Table 10.1: New classification representing categorization criteria for diagnosis of latent tuberculosis infection cases using a combination of four-tuberculosis diagnostic tests and score for latent tuberculosis infection case diagnosis

Tuberculosis diagnostic test results'				Test score	LTBI case definition
Old (Standard)		New			
CXR	TST	QFT-GIT	T-SPOT .*TB*		
-	-	-	-	1	**Negligible**
+	-	-	-	2	**Low**
-	+	-	-	3	**Low**
-	-	+	-	4	**Low**
-	-	-	+	5	**Low**
+	+	-	-	6	**Average**
+	-	+	-	7	**High**
-	+	+	-	8	**High**
+	-	-	+	9	**High**
-	+	-	+	10	**High**
-	-	+	+	11	**Extremely High**
+	-	+	+	12	**Extremely High**
-	+	+	+	13	**Extremely High**
+	+	+	+	14	**Extremely High**

CXR = chest X-ray, TST = tuberculin skin test, IGRAs = interferon gamma release assays, QFT-GIT = QuantiFERON Gold In-Tube test, T-SPOT .*TB* = T-SPOT .*TB* test, LTBI = latent tuberculosis infection

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* Note that all website citations as accessed on 29/03/2012 unless otherwise stated.

Appendexes

Appendix 1: Table A represents tuberculosis hospital discharges for every 1,000 population according to gender and nationality in Kuwait during a 26-year-period (1984-2009) (HVS, 1984-2009

Year	MK	FK	TK	MNK	FNK	TNK	MT	FT	GT
1984	0.30	0.22	0.26	0.70	0.47	0.62	0.58	0.35	0.49
1985	0.23	0.24	0.23	0.75	0.50	0.65	0.57	0.38	0.48
1986	0.21	0.20	0.20	0.61	0.39	0.52	0.47	0.30	0.40
1987	0.23	0.18	0.20	0.49	0.37	0.45	0.40	0.28	0.35
1988	0.26	0.27	0.27	0.41	0.22	0.34	0.38	0.23	0.32
1989	0.21	0.23	0.22	0.35	0.26	0.31	0.32	0.25	0.29
1990	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
1991	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
1992	0.19	0.14	0.17	0.33	0.22	0.29	0.27	0.18	0.23
1993	0.20	0.18	0.19	0.37	0.36	0.37	0.31	0.26	0.29
1994	0.15	0.08	0.12	0.36	0.34	0.36	0.29	0.20	0.26
1995	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
1996	0.21	0.20	0.20	0.35	0.32	0.34	0.31	0.26	0.29
1997	0.26	0.16	0.21	0.34	0.34	0.34	0.32	0.26	0.29
1998	0.19	0.18	0.18	0.31	0.30	0.30	0.27	0.24	0.26
1999	0.21	0.11	0.16	0.28	0.30	0.29	0.26	0.21	0.24
2000	0.09	0.08	0.09	0.06	0.11	0.08	0.07	0.10	0.08
2001	0.08	0.08	0.08	0.06	0.09	0.07	0.07	0.08	0.07
2002	0.08	0.07	0.08	0.06	0.12	0.08	0.06	0.10	0.08
2003	0.08	0.07	0.07	0.06	0.09	0.07	0.06	0.08	0.07
2004	0.07	0.06	0.06	0.07	0.11	0.08	0.07	0.08	0.07
2005	0.21	0.14	0.18	0.21	0.29	0.24	0.21	0.22	0.22
2006	0.18	0.11	0.14	0.22	0.30	0.25	0.21	0.21	0.21
2007	0.14	0.11	0.12	0.19	0.26	0.21	0.17	0.20	0.18
2008	0.13	0.08	0.10	0.18	0.26	0.21	0.17	0.19	0.17
2009	0.12	0.09	0.11	0.24	0.35	0.28	0.21	0.24	0.22

MK = Kuwaiti males, FK = Kuwaiti females, TK = total Kuwaiti's, MNK =non-Kuwaiti males, FNK = non-Kuwaiti females, TNK = total non-Kuwaiti's, MT = total males, FT = total females, GT = grand total (**Source**: 'HEALTH, KUWAIT' annual publications; 1984-2009 (N/A = data not available due to Iraqi invasion on 1990-1991, N/S = data not available due to implementation of incomplete new statistical system for data collectionl)

Appendix 2: Table B represents tuberculosis hospital discharges for every 1,000 hospital discharges according to gender and nationality in Kuwait during a 26-year-period (1984-2009) (HVS, 1984-2009)

Year	MK	FK	TK	MNK	FNK	TNK	MT	FT	GT
1984	4.32	1.96	2.86	10.27	2.56	5.77	8.42	2.36	4.81
1985	3.13	2.05	2.47	8.90	2.72	5.35	7.05	2.48	4.36
1986	2.85	1.64	2.11	7.69	2.28	4.55	6.07	2.04	3.68
1987	2.98	1.53	2.10	6.30	2.19	3.93	5.17	1.94	3.27
1988	2.47	1.56	1.92	6.41	1.66	3.71	5.07	1.62	3.06
1989	2.12	1.39	1.68	5.61	2.09	3.61	4.46	1.83	2.92
1990	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
1991	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
1992	2.29	1.03	1.52	6.50	2.57	4.48	4.35	1.63	2.80
1993	2.11	1.24	1.58	6.63	2.88	4.70	4.40	1.91	3.00
1994	1.59	0.57	0.97	6.86	2.67	4.69	4.31	1.47	2.71
1995	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
1996	N/S	N/S	1.58	N/S	N/S	4.12	N/S	N/S	2.81
1997	3.89	1.46	2.36	11.32	3.94	6.98	7.59	2.59	4.54
1998	2.21	1.38	1.71	7.57	2.92	5.03	4.98	2.08	3.30
1999	2.24	0.84	1.41	6.51	2.94	4.61	4.39	1.77	2.92
2000	0.95	0.61	0.74	1.51	1.08	1.28	1.22	0.81	0.99
2001	0.77	0.55	0.64	1.54	0.91	1.20	1.14	0.70	0.89
2002	0.84	0.53	0.66	1.43	1.33	1.38	1.13	0.88	0.99
2003	0.99	0.59	0.75	1.81	1.14	1.44	1.40	0.83	1.07
2004	0.85	0.49	0.64	1.88	1.26	1.56	1.39	0.84	1.08
2005	2.58	1.27	1.82	6.21	3.41	4.74	4.51	2.29	3.28
2006	2.23	1.05	1.55	6.77	3.48	5.04	4.68	2.24	3.34
2007	1.77	1.07	1.37	6.20	3.51	4.77	4.18	2.29	3.14
2008	1.68	0.78	1.16	6.41	4.02	5.15	4.27	2.42	3.26
2009	1.50	1.03	1.24	7.59	4.61	6.00	4.81	2.91	3.78

MK = Kuwaiti males, FK = Kuwaiti females, TK = total Kuwaiti's, MNK =non-Kuwaiti males, FNK = non-Kuwaiti females, TNK = total non-Kuwaiti's, MT = total males, FT = total females, GT = grand total (**Source**: 'HEALTH, KUWAIT' annual publications; 1984-2009   (N/A = data not available due to Iraqi invasion on 1990-1991, N/S = data not available due to implementation of incomplete new statistical system for data collectionl)

Appendix 3: Table C represents tuberculosis cause-specific mortality rates for every 100,000 mid-year populations according to gender and nationality in Kuwait during a 26-year-period (1984-2009) (HVS, 1984-2009)

Year	MK	FK	TK	MNK	FNK	TNK	MT	FT	GT
1984	6.48	4.19	5.32	1.21	0.78	1.06	2.82	2.36	2.63
1985	3.52	5.51	4.52	1.74	1.02	1.46	2.36	3.11	2.69
1986	1.69	0.28	0.98	0.30	0.72	0.46	0.79	0.52	0.67
1987	1.36	1.62	1.49	1.01	0.46	0.79	1.13	0.99	1.07
1988	2.97	1.55	2.28	0.35	0.88	0.56	0.97	1.09	1.02
1989	2.86	0.75	1.83	0.11	0.34	0.20	0.76	0.47	0.64
1990	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
1991	0.33	0.00	0.17	N/A	N/A	N/A	N/A	N/A	N/A
1992	0.64	0.34	0.50	0.41	0.32	0.38	0.50	0.33	0.43
1993	0.94	1.23	1.09	1.39	0.00	0.95	1.23	0.68	1.01
1994	1.18	0.60	0.89	0.31	1.02	0.53	0.60	0.80	0.68
1995	2.03	0.57	1.30	0.93	1.41	1.08	1.28	0.99	1.17
1996	1.65	0.00	0.84	0.42	0.62	0.48	0.83	0.30	0.63
1997	1.90	1.06	1.47	0.73	0.49	0.65	1.09	0.76	0.96
1998	0.78	0.25	0.52	0.12	0.74	0.32	0.33	0.50	0.39
1999	0.50	0.74	0.62	0.68	2.38	1.23	0.63	1.57	1.00
2000	1.95	0.24	1.08	0.43	0.23	0.37	0.90	0.23	0.64
2001	0.95	0.46	0.70	0.43	1.54	0.79	0.59	1.01	0.76
2002	1.38	0.22	0.79	0.81	1.63	1.08	0.98	0.96	0.97
2003	0.89	0.65	0.77	0.38	0.98	0.57	0.53	0.82	0.64
2004	1.29	0.63	0.95	0.43	1.11	0.65	0.68	0.88	0.76
2005	0.84	1.01	0.92	0.38	1.38	0.69	0.50	1.21	0.77
2006	0.61	0.39	0.50	0.42	1.30	0.69	0.47	0.88	0.62
2007	0.79	0.57	0.67	0.44	1.82	0.87	0.53	1.29	0.81
2008	0.57	0.55	0.56	0.35	0.96	0.54	0.40	0.80	0.55
2009	1.28	1.05	1.16	0.75	1.03	0.85	0.89	1.04	0.95

MK = Kuwaiti males, FK = Kuwaiti females, TK = total Kuwaiti's, MNK =non-Kuwaiti males, FNK = non-Kuwaiti females, TNK = total non-Kuwaiti's, MT = total males, FT = total females, GT = grand total (**Source**: 'HEALTH, KUWAIT' annual publications; 1984-2009   (N/A = data not available due to Iraqi invasion on 1990-1991, N/S = data not available due to implementation of incomplete new statistical system for data collectionl)

Appendix 4: Table D represents tuberculosis case fatality rate (TB deaths for every 100 TB hospital discharges) according to gender and nationality in Kuwait during a 26-year-period (1984-2009) (HVS, 1984-2009)

Year	MK	FK	TK	MNK	FNK	TNK	MT	FT	GT
1984	21.43	19.44	20.59	1.74	1.66	1.72	4.87	6.72	5.41
1985	15.19	23.17	19.25	2.33	2.05	2.25	4.17	8.30	5.55
1986	8.00	1.43	4.83	0.50	1.83	0.88	1.68	1.71	1.69
1987	6.02	8.96	7.33	2.04	1.23	1.78	2.82	3.49	3.05
1988	11.27	5.63	8.45	0.84	4.10	1.66	2.56	4.66	3.21
1989	13.56	3.28	8.33	0.32	1.30	0.64	2.40	1.86	2.20
1990	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
1991	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
1992	3.39	2.38	2.97	1.25	1.49	1.32	1.83	1.83	1.83
1993	4.62	6.78	5.65	3.79	0.00	2.60	3.99	2.56	3.47
1994	7.69	7.14	7.50	0.84	3.03	1.48	2.07	3.94	2.64
1995	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
1996	7.79	0.00	4.08	1.19	1.92	1.40	2.73	1.15	2.18
1997	7.22	6.45	6.92	2.14	1.44	1.91	3.45	2.99	3.29
1998	4.23	1.45	2.86	0.39	2.50	1.06	1.21	2.12	1.54
1999	2.35	6.52	3.82	2.40	7.81	4.23	2.39	7.47	4.13
2000	21.62	2.86	12.50	7.14	2.13	4.85	12.90	2.44	8.00
2001	12.50	6.06	9.23	6.67	17.50	11.00	8.70	12.33	10.30
2002	17.14	3.13	10.45	14.04	13.33	13.68	15.22	9.78	12.50
2003	11.76	9.68	10.77	6.45	10.64	8.26	8.33	10.26	9.20
2004	18.18	11.11	15.00	6.25	10.34	7.97	9.73	10.59	10.10
2005	3.92	7.14	5.23	1.79	4.71	2.89	2.36	5.42	3.54
2006	3.37	3.51	3.42	1.88	4.40	2.79	2.21	4.18	2.94
2007	5.71	5.26	5.51	2.40	6.95	4.18	3.04	6.56	4.46
2008	4.48	7.32	5.56	1.94	3.65	2.65	2.39	4.23	3.14
2009	10.94	11.11	11.02	3.12	2.99	3.06	4.23	4.35	4.28

MK = Kuwaiti males, FK = Kuwaiti females, TK = total Kuwaiti's, MNK =non-Kuwaiti males, FNK = non-Kuwaiti females, TNK = total non-Kuwaiti's, MT = total males, FT = total females, GT = grand total (**Source**: 'HEALTH, KUWAIT' annual publications; 1984-2009   (N/A = data not available due to Iraqi invasion on 1990-1991, N/S = data not available due to implementation of incomplete new statistical system for data collectionl)

Appendix 5: Questionnaire of immigrant participants

Data Collection Sheet (Questionnaire)

<u>PhD project title</u>: '**The challenges of Tuberculosis Prevention through Early Detection of Latent Tuberculosis Infection in New Immigrants to the State of Kuwait'**

Q1	Serial number			Participant name			Date	…/…/…
A)- Socio-economic/Demographic Characteristics:								
Q2	Age (years) :	[……….]						
Q3	Gender :	Male [1]		Female [2]				
Q4	Nationality :	[……………........................]						
Q5	Place of birth :	Country [……………..………]			City [………………….…..…………]				
Q7	Marital status :	Single [1]		Married [2]		Widowed [3]		Divorced/Separated [4]
Q8	Ethnic origin :	Asian [1]		Black [2]		White [3]		Caucasian [4] Other [5]
Q9	Education :	Illiterate [1]	Primary [2]		Intermediate [3]		Secondary [4]	
		Diploma [5]	University & above [6]					
Q10	Occupation (in mother country):				[…………………………………..]			
Q11	Duration of employment experience (years) :				[…………]			
Q12	Total individual income (country currency) (U.S.$):				[…….……]			
Q13	Total individuals living in the same place (family size):				[…….……]			
Q14	Total number of general rooms in the living place :				[…………..]			
Q15	Total number of sleeping rooms in the living place :				[…….……..]			
B)- Entry to the State of Kuwait:								
Q16	Date of entry to Kuwait :				[…..../……./…….]			

Q17	Proposed occupation in Kuwait :	[………………….]	
Q18	Did you live previously as a resident in Kuwait?	Yes [1]	No [2]
Q19	If Yes, since when from last visit to Kuwait (year):	[……………...…..]	
Q20	Diagnostic test(s) performed before entry to Kuwait?		

			Yes [1]	No [2]
	A	Chest X-ray	Yes [1]	No [2]
	B	Sputum microscopy		
	C	Tuberculin skin test		
	D	Forced cough test		

C)- Tuberculosis Disease History

Q21	Any history of travel or working outside mother country of origin :	Yes [1]	if Yes, go to **Q22**
		No [2]	if No, go to **Q 24**
Q22	Which country been worked in and total duration (years)?		

	Country (worked in)	Duration (year)
A		
B		
C		
D		
E		

Q23	Which country been visited/traveled to and total duration (weeks)?

	Country (travelled to)	Duration (week)
A		
B		
C		
D		

D)- Tuberculosis disease knowledge:

Q24	Do you have access to medical/health care service :	Yes [1]	No [2]	Unknown [3]
Q25	What is the average duration to reach the nearest medical/health care service(minutes):	[…………..…]		
Q26	What is your general knowledge about tuberculosis?	Correct [1]	Incorrect [2]	

Q27		What is your knowledge about common symptoms/signs of TB disease:			
Respiratory complaint			Yes [1]	No [2]	Unknown [3]
	A	Continuous productive cough (+/- thick colored sputum)			
	B	Hemoptysis (bloody cough)			
Constitutional complaint			Yes [1]	No [2]	Unknown [3]
	C	Fever (+/- shivering)			
	D	Night sweating (drenching fever)			
	E	Generalized weakness			
	F	Weight loss (wasted)			
	G	Vomiting/Diarrhoea			
	H	Body pains (joint/bone)			
	I	Other			
Example(s) of other TB complaint(s): [……………………………………………..……..]					

Q28		From which of the following sources was knowledge obtained?		
		Knowledge source	Yes [1]	No [2]
	A	Family member/Relative		
	B	Study area (school, subject)		
	C	Work (peer, close contact)		
	D	Public shared talks		
	E	TV/newspaper advertisement		
	F	Medical campaign/health-related poster		
	G	Other		
Examples(s) of other knowledge source(s): [………………………………………………….]				

Q29	Do you have history of BCG vaccination against TB disease:	Yes [1]	No [2]	Unknown [3]
Q30	If Yes, at which age of vaccination: […………………………………...]			
Q31	Have you been infected or contacted with TB diagnosed patient? if No or Unknown, go to **Q40**	Yes [1]	No [2]	Unknown [3]
Q32	If Yes, TB disease was diagnosed by:	Physician [1]	Other (e.g. lay person) [2]	

Q33	What was the duration of complaints before TB diagnosis (years)	[………………………………..…]		
Q34	Has anti-TB therapy been prescribed? if No, or Unknown, go to **Q40**	Yes [1]	No [2]	Unknown [3]

Q35	Compliance to prescribed anti-TB therapy?			
	Anti-TB therapy course and outcome	Yes [1]	No [2]	Unknown/Not remember [3]
A	Complete			
B	Cured			
C	Default			
D	Failure			
E	Relapse			
F	Resistance (MDR/XDR)			
G	DOTS follow-up			

Q36	What is the type of anti-TB therapy prescribed?			
	Prescribed anti-TB therapy	Yes [1]	No [2]	Unknown/Not remember [3]
A	Rifampicin			
B	Perizanamide			
C	INH			
D	Ethambutol			
E	Other (e.g. streptomycin)			

Q37	Have anti-TB therapeutic adverse effect(s) been noticed? if No or Unknown, go to **Q40**	Yes [1]	No [2]	Unknown [3]

Q38	What type of anti-TB therapy adverse effects?		
	Adverse effect(s)	Yes [1]	No [2]
A	Fever		
B	Vomiting/Diarrhea		
C	Orange-red urine		
D	Yellowish skin discoloration		
E	Other		
Example(s) of other adverse effect(s) :	[……………………..……………..…]		

Q39	If Yes, then against which type of anti-TB therapy?	[……………………………………………]			

E)- Tuberculosis-Associated Risk Factors: TB exposure

Q40	What is the average number of daily contacts to? :				
	Inside household (HH) area :	[……………………………...]			
	Outside non-household (NHH) area :	[……………..…………..…]			

Q41	What is the average duration (hour) of contact to? :				
	Household (HH) area :	<1 [1]	2-5 [2]	6-10 [3]	>10 [4]
	Non-household (NHH) area :	<1 [1]	2-5 [2]	6-10 [3]	>10 [4]

Q42	Any previous history of contact with TB diagnosed/infected patient: if Yes, go to **Q43** if No or Unknown, go to **Q46**	Yes[1]	No [2]	Unknown [3]	

Q43	What is the type of relationship to TB diagnosed patient? :				
	Relationship		Yes [1]	No [2]	
	A	Close contact/indoor family contact (e.g. parent/family member)			
	B	Non-close/outdoor contact (e.g. relative/neighbor/friend/work peer)			
	C	Other			

Examples of relationship(s) :	[……….…………..]

Q44	What is the average number of daily contacts to TB patient in? :				
	Inside household (HH) area :	[…….…………..…]			
	Outside non-household (NHH) area :	[………...…………]			

Q45	What is the average duration (hours) of contact TB patient in?				
	Household (HH) area :	<1 [1]	2-5 [2]	6-10 [3]	>10 [4]
	Nonhousehold (NHH) area :	<1 [1]	2-5 [2]	6-10 [3]	>10 [4]

Q46	What is the common route of outdoor transport(s)? :				

Transport route		Yes [1]	No [2]
A	Walking		
B	Bicycle/Tricycle/Motorcycle		
C	Microbus/Bus/Train		
D	Car		
E	Other		

Examples(s) of other route(s) (e.g. airplane, ship) :	[……………………………………….………]

G)- TB exposure place/living area in Kuwait:

Q51	What is the total number of individual(s) living in the same room: [………………………......]
Q52	What is the general condition of sleeping area/place? :
	Presence of sleeping closed-room with good ventilation [1]
	Presence of sleeping closed-room without good ventilation [2]
	Presence of outdoor exposure area [3]

H)- History of Smoking & Smoking Behaviors:

Q53	Are you a :	Current smoker [1]	Ex-smoker [2]	Never smoke [3] if Never smoke, go to **Q60**	
Q54	What is the starting age of smoking (years) ? :	[……………………………………….……...]			
Q55	If ex-smoker: What was the age of stop smoking (years)?:	[…………………………………………….]			
Q56	What is the total duration of smoking (years)?:	<1 [1] 1-2 [2]	3-5 [3] 6-10 [4]	>10 [5]	
Q57	What type of smoking habit preferred? :				

		Smoking type	Yes [1]	No [2]	
	A	Cigarette (manufactured)			
	B	Pedis (manual)			
	C	Tambaco			
	D	Birri			
	E	Hukka (hubble bubble)			
	F	Shisha/Qedo			
	G	Cigar			
	H	Pipe			
	I	Other			

Example(s) of other smoking habit(s) :	[……………………………………………………….]

Q58	What is the frequency consumption of cigarette (number of cigarettes/day) :	<5 [1]	5-10 [2]	10-20 [3]	>20 [4]
Q59	What is the frequency consumption of other preferred smoke(s)/day:	<5 [1]	5-10 [2]	10-20 [3]	>20 [4]

I)- History of infectious/non-infectious(chronic) disorder(s):

Q60	History of catching the following disorder(s) and total duration of complaint(s) since diagnosis (years)?:				
	Disorder	Yes [1]	No [2}	Unknown [3]	Duration (year)
A	Skin allergy				
B	Malnutrition				
C	Malaria				
D	Asthma				
E	Hepatitis				
F	HIV/AIDS				
G	Silicosis				
H	Alcoholism				
I	Diabetes mellitus				
J	Cardiovascular disease				
K	Blood disorder				

	L	Other				
Example of other disorder(s) :			[………………………………………….……...]			
Q61	Any previous history of surgical procedure?:		Yes [1]		No [2]	
Q62	If Yes, what type of surgical procedure? :		[………………………………………….]			
Q63	Any history of taking medical/pharmaceutical drugs:		Yes [1]		No [2]	
Q64	If Yes, what type of medical/pharmaceutical drugs?:		Corticosteroids [1]			
			Anti-allergic steroids [2]			
			Immunosuppressive(s) [3]			
			Herbal medicine [4]			
			Other (5) [………………………………….]			
Researcher: Dr. Adel Mohanna AL-Harbie PhD (Resrarch-Infectious Diseases) University of Edinburgh-UK						

Chest X-Ray Recording Sheet

PhD project title: '**The Challenges of Tuberculosis Prevention through Early Detection of Latent Tuberculosis Infection in New Immigrants to the State of Kuwait'**

A) - Parenchyma abnormality

A.1 Abnormal changes

Finding	RL	Size (mm)	Number	LL	Size (mm)	Number
Cavity						
Fibrosis						
Infiltration						
Opacity/ consolidation						

A.2 Nodule abnormality: Nodule number: RL ( )   LL ( )

Size (mm):   <5 ( )   >5 ( )

Shape: Round ( )   Irregular ( )   Homogeneous ( )   Heterogeneous ( )

A.3 Cavity Pathology

Finding	RL	LL
Cavity (granuloma)		
Calcified		
Non-calcified		
Number		

A.4 Other CXR changes

Finding	RL	LL
Bronchectasis		
Honeycomb fibrosis		
Collapse		
Lobar volume loss		

B) - Pleural abnormality

Finding	RL	LL
Thickening/ scar		
Apical caps		
Effusion		
Obscured costo-phrenic angle		

C) - Central structure abnormality

Finding	RL	LL
Tracheal deviation		
Hilar elevation		
Mediastinal shift		
Pericardial effusion (calcified)		
Bone abnormality (vertebral column)		

D) - Lymphadenopathy abnormality

Finding	Hilum	Mediastinum	Calcified	Non-calcified	Number
RL					
LL					

Scoring system (chest X-ray score reading)		
CXR result	Score	Final score assessment
Normal (no pathology)	I	
Pathology not consistent with TB	II	
Pathology consistent (significant) for TB	III	
Pathology highly consistent (highly significant) for TB	IV	

RL = right lung, LL = left lung

Appendix 7: Tuberculin skin test sheet of immigrant participant

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Tuberculin Skin Test Recording Sheet

PhD project title: '**The Challenges of Tuberculosis Prevention through Early Detection of Latent Tuberculosis Infection in New Immigrants to the State of Kuwait'**

Date of TST performance: [..../..../....] Date of TST result reading: [..../..../....]

TST cutaneous induration size (mm) = [.....................]

 Grading system according to Mantoux reaction:

Induration transverse diameter size (mm)	Participant TST result	Grade	Score level
< 5		1	Negative
5-<10		2	Borderline
10-15		3	Positive
>15		4	highly Positive

TST adverse reaction(s): Negative [.....] -   Positive [.....]:

[Hematoma ( ) Arm swelling ( )   Fever ( )   Bleeding ( )   Abscess/discharge ( ) Allergic itching ( ) Vesicular rash ( )   Bullae ( )   Skin ulcer ( )   Other ( )]

Body weight (Kg): [....]   Body height (M): [....]   Body mass index: [....]

BCG vaccination scar: Positive [......]   Negative [......]

Nurse: Signature:

 Researcher: Dr. Adel Mohanna AL-Harbie   Signature:

PhD-Research-Infectious Diseases; University of Edinburgh-UK

</div>

Appendix 8: QuantiFERON Gold In-Tube test result sheet of immigrant participant

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<u>QuantiFERON Gold In-Tube test Result Sheet</u>

<u>PhD project title</u>: '**The Challenges of Tuberculosis Prevention through Early Detection of Latent Tuberculosis Infection in New Immigrants to the State of Kuwait'**

Subject ID. number:

Date of performance:

Interferon gamma level (INF-γ) (IU/ml): […………….]

QNF- GIT result: Positive [….]   Negative [….]   Indeterminate [….]

Reader's comment:

Laboratory technician: Signature:

Researcher: Dr. Adel AL-Harbie   Signature:

       PhD (Research-Infectious Diseases)

       University of Edinburgh-UK

</div>

Appendix 9: T-SPOT .*TB* test result sheet of immigrant participant

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T-SPOT .*TB* Test Result Sheet

PhD project title: '**The Challenges of Tuberculosis Prevention through Early Detection of Latent Tuberculosis Infection in New Immigrants to the State of Kuwait**'

Subject ID. name/serial number:

Date of T-SPOT .*TB* test performance:

Panel A (ESAT-6) Response: Spot number [……..]

Panel B (CFP-10) Response: Spot number [……...]

Positive Control Panel, 'Mitogen': Spot Number [………]

Negative Control Panel, 'Nil': Spot Number [………]

Test Response: Reactive [……]   Nonreactive […...]   Indeterminate [……]

T-SPOT .*TB* test Result: Positive […..]   Negative […..]   Indeterminate […..]

Lymphocyte cell count: […………..]

Laboratory technician: Signature:

Researcher: Dr. Adel Mohanna AL-Harbie   Signature:

              PhD (Research-Infectious Diseases)

              University of Edinburgh- UK

Appendix 10: Immigrant informed consent (English)

<div style="border:1px solid">

Adoption agreement to participate in the research study
<u>Informed Consent</u>

<u>PhD project title</u>**: 'The Challenges of Tuberculosis Prevention through Early Detection of Latent Tuberculosis Infection in New Immigrants to the State of Kuwait'**

This study aims to examine accuracy of new diagnostic tests in detecting latent tuberculosis infection which is part of PHD research study in medicine (Infectious Diseases) for assessment in diagnosing inactive form of tuberculosis and you are free to participate in the study. If you agree, please give accurate answers to questions of the annexed questionnaire under supervision of the researcher Dr. Adel Mohanna AL-Harbie (department of Public Health).

The research also aims to measure the body weight, body height and taking blood sample only 11 ml for tests, performing tuberculin skin test and returning within 2-3 days checking for skin test result provided not having any previous skin allergy in addition to shoulder skin examination for previous BCG vaccination scar.

Researcher undertakes to preserve the privacy of information and deal with it with utmost secrecy, declined to name or personal use only participant and within the framework of research and in accordance with the ethics of medical research.

You are free to agree or not agree to participate in this study with also complete freedom to refuse to answer any question from the questionnaire in the event of approval.The health services will still be fully provided for you even in case of disagreements to participate in the study.

For extra information you are most welcome to call Dr. Adel Mohanna AL-Harbie mobile: (00965) 66011406

Are you OK to participate in the study?   Agree: Signature:

We thank your cooperation with us.

<u>Researcher</u>: Dr. Adel Mohanna AL-Harbie

       PhD (Research–Infectious Diseases)

       University of Edinburg-UK

</div>

Appendix 11: Immigrant informed consent (Arabic)

<div dir="rtl">

<u>اقرار الموافقة على المشاركة فى دراسة البحث</u>

(Informed consent)

<u>عنوان الدراسة</u> **"الاكتشاف المبكر لعدوى مرض الدرن الكامن ومنع انتشاره باستخدام الطرق التشخيصية وتقييمها على الوافدين الجدد فى دولة الكويت"**

وهى جزء من رسالة الدكتوراة في الطب للباحث الدكتور عادل مهنا الحربى من إدارة الصحة العامة وتهدف الدراسة الى الوقاية من مرض الدرن عن طريق تقييم الفحوصات الجديدة واستخدامها لتشخيص مرض الدرن الكامن

ولك كامل الحرية في أن تقررا لمشاركة ضمن هذه الدراسة والمساهمة بحرية تامة وبإجابات دقيقة على أسئلة الاستبيان المرفق مع الباحث كما سيتم عمل الفحوصات والمتضمنة على قياس الطول والوزن واخذ عينة دم بحجم 11ملم اجمالى لكلا الفحصين المطلوب دراستهما وسيتم عمل فحص الجلد الدرنى فحص الجلد فى مدة 2-3 فى منطقة العضد وضرورة الرجوع للتاكد من نتيجة(skin test) (tuberculin ايام وايضا فحص وجود اثر سابق لتطعيم الدرن فى منطقة الكتف أو الفخذ للتعرف على مدى وجود ندبات بسبب اخذ تطعيم سابق ضد مرض الدرن.

وفى حالة موافقتك فان نتائج فحوصات البحث لن تعرضك لأى مخاطر ولن تعيق من دخولك للبلاد ومن حقك الانسحاب فى اى مرحلة من المراحل ومن حقك التحفظ على الاجابة على بعض الاسئلة.

وفى حالة عدم الموافقة فان ذلك لن يؤثر على الرعاية المقدمة لك.

ويتعهد الباحث بالمحافظة على خصوصية المعلومات والتعامل معها بسرية تامة مع عدم الإفصاح عن اسم او شخصية المشارك واستخدامها فقط ضمن إطار البحث وفقا لأخلاقيات البحوث الطبية.

وأيضا الحرية التامة لرفض ولكم كامل الحرية في الموافقة او عدم الموافقة على المشاركة فى هذه الدراسة الإجابة على أي سؤال من أسئلة الاستبيان.

بالباحث واذا رغبت فى الحصول على اى معلومات فلا تتردد فى الاتصال

نقال : 66011406 (00965)

فهل انت موافق على المشاركة فى الدراسة؟

التوقيع: موافق:

نشكر لك تعاونك معنا،،،،،

<u>الباحث</u> : **د/ عادل مهنا الحربي**

(رسالة دكتوراة- الامراض المعدية)

جامعة ادنبرة/ اسكتلندا

</div>

Appendix 12: Researcher adoption pledge (English)

<div style="border:1px solid black; padding:1em;">

<u>**Researcher adoption pledge**</u>

The title of research study for a doctorate in the Epidemiology of Infectious Diseases – Department of Public Health (Ministry of Health) is;

<u>PhD project title</u>**: 'The Challenges of Tuberculosis Prevention through Early Detection of Latent Tuberculosis Infection in new immigrants to the State of Kuwait'**

 and to assess tuberculosis epidemiology.

I promise to maintain complete confidentiality of information and not circulated outside the framework of scientific research in accordance with the ethics of medical research and trust of the human rights.

<u>Researcher</u>:   Dr. Adel Mohanna AL-Harbie   <u>Signature</u>:

PhD (Research-Infectious diseases)

University of Edinburgh-UK

</div>

Appendix 13: Researcher adoption pledge (Arabic)

<div dir="rtl">

اقرار تعهد

يهدف عنوان بحث دراسة الدكتوراة فى الامراض الوبائية والمعدية ـ قسم الصحة

العامة الى فائدة منع انتشار عدوى مرض الدرن عن طريق الاكتشاف المبكر'

لمرض الدرن الكامن باستخدام الطرق التشخيصية وتقييمها على الوافدين الجدد

فى دولة الكويت

</div>

PhD project title: 'The Challenges of Tuberculosis Prevention through Early Detection of Latent Tuberculosis Infection in New Immigrants to the State of Kuwait'

<div dir="rtl">

ودراسة حالة مرض الدرن فى دولة الكويت كما اتعهد بالمحافظة التامة على

سرية المعلومات وعدم تداولها خارج اطار البحث العلمى وذلك لضمان حقوق

الافراد المشاركين فى الدراسة وفقا لاخلاقيات البحوث الطبية

اسم الباحث: د/ عادل مهنا الحربى

موضوع البحث: رسالة دكتوراة ـ الامراض المعدية

التوقيع:

</div>