Diagnosing tuberculosis among 609 adolescents (aged 12-18 years) with symptoms suggestive of tuberculosis at the Palamaner diagnostic Centre, South India

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ACRONYMS AND ABBREVIATIONS

ARTI	Annual risk of Tuberculosis infection
BCG	Bacillus Calmette-Guérin
BMI	Body Mass Index
DGW	Diagnostic ward
DHEA	Dehydro-epiandrosterone
DOTS	Directly observed treatment short course
HIV	Human immune virus
IGRA	Interferon Gamma Release assays
LJ – Medium	Lowenstein – Jensen Medium
LTBI	Latent tuberculosis infection
MDR TB	Multi drug resistant TB
MGIT	Mycobacterium growth indicator tube
MOTT	Mycobacterium other than tuberculosis
NFHS	National family health survey
NTM	Non Tuberculous Mycobacteria
PCR	Polymerase chain reaction
QFT	QuantiFERON
r RNA	ribosomal Ribo-Nucleic Acid
RNTCP	Revised National Tuberculosis Control Programme
TB	Tuberculosis
TH1 & TH2	T- Helper cells 1 & 2
TST	Tuberculin Skin Test
WHO	World Health Organization
Z – N staining	Zeil – Neelsen staining

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Abstract

Background:

Tuberculosis (TB) accounts for 1.7 million deaths, according to the recent WHO report. India alone accounts for one fifth (21%) of all the TB cases globally. Mycobacterial pathogens are classified into Typical Mycobacteria and Atypical Mycobacteria. Studies have shown that adolescents are a vulnerable age group having higher chance of getting pulmonary TB infection and disease compared to younger children and adults. This age group is a target group for vaccination.

Objectives:

- To estimate the proportion of definite TB and probable TB in 12-18 year old adolescents.
- To estimate the rate of Nontuberculous Mycobacteria (NTM) in the same group.

Methods:

The present study is part of a prospective, observational two year cohort study conducted among school/ college going adolescents, 12-18 years in Palamaner taluk, South India during February 2007 to July 2010. A total of 609 participants attended the diagnostic ward with any of the criteria for referral. The sputum specimens were examined by concentrated AFB smear microscopy and processed for culture. Written informed assent from the subjects and consent from the parents or guardians was obtained at the time of enrollment of the participant before the start of the procedures.

Results:

During the 2 years follow-up of 6643 participants, 609 participants attended the diagnostic ward (DGW) for Tuberculosis testing. Among all these participants, 310 (50.9%) were males and 299 (49.1%) participants were females. 443 (72.9%) participants were referred to DGW based on TST positivity (greater than or equal to 10 mm). A total number of 7 (1.15 %) participants were diagnosed as definite TB, 3 (0.50 %) participants were diagnosed as probable TB. The proportion of 19.05% participants had NTM positive sputum samples. Participants having cough for equal to greater than 2 weeks were, 19 times more likely to become positive for M.tb growth of sputum sample (O.R = 18.60; 95% C.I = 4.02 – 86.05). There was 2 times greater chance of isolating NTM from participants who belongs to Dalit/ Harijan's community.

Discussion:

The proportion of the cases detected in our study was less compared to the estimation by Revised National Tuberculosis Control Programme (RNTCP), in 2007 and what other studies had shown. Most of the participants those referred to diagnostic ward did not show up for TB tests. We consider that, the NTM species isolated in our study are either by chance or by the contamination of sputum samples by environmental Mycobacteria. These isolates therefore had no clinical significance.

Conclusion:

Further studies need to be conducted to ascertain the actual burden of TB disease among all the adolescent age groups including people who don't go to schools. There must be further studies to find out the clinical significance of NTM among the adolescent age groups.

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1. Introduction and literature review:

1.1: Global burden of TB:

Tuberculosis (TB) accounts for 1.7 million deaths, according to recent WHO report (1), this ranks second to HIV as a cause of death globally. There were an estimated 14 million prevalent cases of TB globally, of this India accounts for nearly 1.6 to 2.4 million cases.(1). It was estimated that 9.4 million incident TB cases globally which was about 137 cases per 100 000 population during 2009. Of these cases Asia accounts for 55% and Africa accounts for 30%. It was estimated that out of 9.4 million incident cases in 2009, 1.1 million cases were HIV positive cases. Out of these 1.1 million HIV positive cases 80% were from Africa. There were 1.3 million deaths among HIV negative cases of TB and 0.4 million deaths among HIV infected cases in 2009. In 2008 it was estimated by WHO that 440 000 cases of multi drug resistant TB (MDR TB) were present. China, India, Russian federation and South Africa had the largest number of estimated cases of MDR TB during 2008 (1). The WHO fact sheet dated November, 2010 on tuberculosis, states that persons with TB disease can infect 10 to 15 other persons on an average every year(2). One third of world's population is infected with Tuberculosis. The new smear positive case notification globally was 2.6 million. and in India it was 0.6 million in 2009(1).

1.2: Burden of TB in India:

India alone accounts for one fifth (21%) of all the TB cases globally. The WHO estimates in 2009 states that 2 million incident cases and 3 million prevalent cases of TB were present in India. Out of these 0.13 million incident TB cases were HIV positive during 2009. It was estimated that there were 0.28 million deaths due to Tuberculosis among HIV negative TB

patients during 2009 in India. 17% of the TB patients in India were known HIV positive patients during 2009. WHO estimates in India states that there were 1,660 cases of MDR-TB present during 2009. The new smear positive case notification in India was 0.6 million during 2009(1). There were few studies on adolescent TB giving very little details about pulmonary tuberculosis during the adolescent (12 to 18 years) age group. The nationwide survey was done to know the prevalence of TB by National Tuberculosis Institute; Bangalore had shown the annual risk of infection was 1.5 and the prevalence of infection among 1-9 years age was 8.2 %.(3) These studies have not given much importance to Adolescent age group.

1.3: Non tuberculosis Mycobacteria (NTM):

At present in genus Mycobacteria more than 125 species are identified excluding Mycobacterium *tuberculosis* and Mycobacterium *leprae* (4). Nontuberculous Mycobacteria (NTM) has been classified according to Runyon system of classification(5). This system of classification is becoming less important clinically, after the development of new molecular techniques of isolation of species, which includes 16S rRNA gene sequencing as a standard for isolation of NTM species (4, 6). Based on the guidelines provided by American Thoracic Society/ Infectious disease Society of America, the diagnosis of NTM infection can be done with the combination of clinical, radiological and Microbiological criteria(4).
Mycobacterial pathogens were classified into Typical Mycobacteria and Atypical Mycobacteria (7-8) based on optimal speed of growth and chromogenicity (Runyon system of classification).

1.3.1: Classification of Mycobacteria:

1) Typical: M. tuberculosis, M. bovis, M. avium, M. leprae, M. africanum, M. simiae.

2) Atypical {Mycobacterium other than tuberculosis (MOTT) or Nontuberculous Mycobacteria (NTM)}

Group 1 (Photochromogens)

M.kansasii, M.marinum

Group 2 (Scotochromogens)

M.scrofulaceum (M.marianum), M.szulgai

Group 3 (Non Chromogens)

M.intracellulare, M.xenopi, M.ulcerans

Group 4 (Rapid growers)

M.fortuitum, M.chelonei

The accurate prevalence of the NTM in India is not available. Majority of the studies conducted to know the prevalence of the NTM in India were retrospective. The study conducted by Chakrabarti et al in Chandigarh, North India had shown that from 8.9% of TB suspected patients' Mycobacteria species were isolated from different specimens(9). The detection of the species was done based on tests of Kubica (Biochemical tests used to identify the NTM species and based on these tests NTM were classified by Kubica G.P.) (9-10). The isolation of Nontuberculous Mycobacteria (NTM) in Thiruvallur, South India where the BCG vaccine trail (Chingleput trial) was done was found to be 8.6%. Here also the isolates of species were identified with biochemical tests of Kubica (11). The retrospective study conducted by Christian medical college, Vellore, South India had shown that 3.9% of growth from different clinical samples had shown NTM (12). The study conducted by Chauhan.M.M in rural population of Bangalore had shown that 2.5% of the sputum specimens have yielded NTM species (13).

The rates of infection and disease by Nontuberculous Mycobacteria (NTM) have been reported in the range of 1-15 per 100 000 and 0.1 to 2 per 100 000 in North America (14). The study conducted in South Africa among children had shown that 6% crude yield of Nontuberculous Mycobacteria from the gastric aspirate and induced sputum (15). The prevalence of NTM among Adolescent age group is not known from the previous studies conducted.

1.4: Clinical presentation of Pulmonary TB among adolescents:

The clinical presentation of the TB in adolescent age group is different compared to children and adults. Among adolescents the extra pulmonary tuberculosis is more common compared to adults.(16-19) The study conducted by Sant ´Anna et al. in Mexico among adolescents aged 10 – 18 years had shown that most of the chest radiograph patterns of the pulmonary TB were similar to adult type. Till puberty Pulmonary TB among adolescents is similar to children with Ghon complex or primary complex (Ghon focus, lymphangitis and enlarged regional lymph nodes) and hematogenous dissemination (Miliary TB). After puberty the pathophysiology of pulmonary TB among adolescents is similar to adult type with granulomas on histopathology and cavities and cotton wool lesions on chest radiographs (17). The study conducted by Weber H.C et al, discussed that, the rise in the occurrence of cavitation of lung parenchyma among adolescents with the pulmonary TB starts after puberty(20). The study done by Nelson LJ et al, in USA has also shown that there was more evidence of pulmonary parenchymal cavitation due to TB on chest radiograph among adolescents than adults or children(21). The review paper by Cruz A.T and Starke J.R in Paediatric Respiratory reviews journal have mentioned that fever, cough and productive cough are common symptoms among adolescents and night sweats are uncommon. Haemoptysis and dyspnoea are rare symptoms among adolescents. Respiratory rales are rare

signs among this age group. Other respiratory signs like wheezing, fremitus, dullness to percussion and decreased breath sounds are uncommon among the adolescents(19). The study conducted by Wong K.S et al in northern Taiwan have found that, among all the clinical symptoms of the adolescents with pulmonary TB, cough for more than 4 weeks and haemoptysis symptoms were at an increased risk of acid fast bacilli smear positivity(22).

1.5: Why it is important to study TB among adolescents?

Studies have shown that adolescents are a vulnerable age group having higher chance of getting pulmonary TB infection and disease compared to younger children and adults.(16, 20-21, 23) Studies conducted in United States during 1993 to 2001 had shown that adolescents aged 15 to 18 years were more likely to be smear positive. (21) Studies conducted in South Africa had shown that the adolescents have the high force of infection (24-25). The high force of infection in this age group can be implicated to the increase in the social gathering during this age period, which will lead to increase in the exposure to infectious TB disease (25). During puberty the pulmonary TB disease is mostly characterised by pulmonary parenchymal cavitation and leads to production of sputum with more bacilli which will spread to the community (16-17, 19-20). Because most of this age group have wide social mixing and gathering in schools and colleges, the diseased person has more chance to infect others with TB bacilli.

It is the balance between T cells that is TH1 and TH2 response against TB that causes different cytokine secretion and promotes the non-necrotizing and necrotizing response, respectively. This T cell response is affected by the ratio of dehydro-epiandrosterone (DHEA) and glucocorticoids. The metabolite of vitamin D, 1α 25-dihydroxyvitamin D3 does

play a key role in the immunity against the Tuberculosis.(26-28) The Vitamin D and DHEA levels increase progressively from early adolescence. Hence, the adolescent age group is special because of physiological changes, that occurs during puberty which plays a role in pulmonary TB disease(20). This can explain the higher risk of getting TB disease after recent TB infection and re-infection among adolescent age group compared to children and adults (19, 22).

1.6: Challenges in diagnosing and treatment of the Tuberculosis disease among adolescents:

As the adolescent TB is similar to the TB disease in adults, the diagnostic tests and the diagnostic methods used in adults can also be used in adolescent age group. There are no special diagnostic guidelines for the diagnosis of tuberculosis among adolescents. The delay in the final diagnosis of Tuberculosis disease from the onset of symptoms is a big challenge in adolescents. The study conducted in Toronto, Canada has found that the average time from the onset of symptoms to diagnosis of Tuberculosis disease was 5.25 months with a median of 4 months(18). This delay in diagnosis leads to the delay in the treatment of tuberculosis and thus increased infectivity of the diseased person in the community. It is observed that the compliance to TB treatment in adolescent age group is difficult because of different social issues. It is very important to promote Tuberculosis control programmes in this age group because of their unwillingness to the adherence and acceptance of the anti-tubercular treatment. (16-18).

1.7: Studies in India:

There are less data available about patients having symptoms suggestive of TB in India.

The studies conducted in the tribal population of Madhya Pradesh among symptomatic patients had shown the prevalence of bacillary TB was 387 per 100 000 (29). The tuberculin skin test surveys conducted national wide, between 2000 - 03 among children between 1 to 9 years had shown that the Annual risk of tuberculous infection (ARTI) was 1.5 % (3). This was 1.1 % in south zone, which includes the present study area (30).

The RNTCP status report (TB India 2008) had shown that the annual case detection rate in Chittoor was 159 per 100,000 populations in 2007. The percentage of sputum positive TB cases among TB suspects in Chittoor where the study was conducted was 14% in the same year (31). The annual case detection rate in India was 130 per 100, 000 population and percentage of sputum positive TB cases among TB suspects was 14 in 2007 (31).

1.7.1. Adolescent age group in India:

Census of India 2001, Population projections for India and states 2001-2026, has projected the population aged between 12 to 18 years in Andhra Pradesh state in India is 11.86 millions in 2006 which accounts for 15 percentage of the total population in Andhra Pradesh (32). The same age group in India was projected to be 170 millions in 2006 which also accounted for 15 percentage of the total population in India (33).

1.8: Diagnosis of Pulmonary Tuberculosis:

Clinical spectrum of Tuberculosis (TB) ranges from latent tuberculosis infection (LTBI) to various stages and manifestations of active tuberculosis disease (34-35). In persons having suspicion of Tuberculosis disease, detailed medical history and clinical examination along with laboratory tests and chest X-ray are done to diagnose the disease (34-35). Tuberculin

skin test (TST) and interferon-γ release assays (IGRA) are the tests among many to diagnose the latent TB infection (36-37).

The interferon-γ release assays are T cell assays. The two IGRA tests currently available are T-SPOT.TB test and QuantiFERON-TB Gold test (36, 38). Antibody against Mycobacterium tuberculosis based assays, antigens (Lipoarabinomannan detection assay and MPB64 skin patch test) produced by Mycobacterium tuberculosis assays are other recent immune based diagnostic tests available for diagnosing the probable active Tuberculosis(36). Chest X-ray and clinical assessment of the person have a vital role in differentiating the LTBI from active pulmonary TB disease when TST and IGRAs are used for diagnosis of LTBI.

In countries like India, detection of acid fast bacilli in sputum smear on microscopy is widely used diagnostic technique for pulmonary tuberculosis disease. This technique is relatively simple, inexpensive, widely applicable and highly specific for Mycobacterium tuberculosis (34-35). There are two procedure for acid fast staining, carbolfuchsin procedure and Flourochrome procedure. Ziel-Neelsen staining (Z-N staining) and Kinyoun methods are used in carbolfuchsin procedure. According to Directly observed treatment short-course (DOTS) strategy and Revised National Tuberculosis Control Programme (RNTCP) in India, conventional direct microscopy of two consecutive days sputum specimen and one of them being a morning sputum specimen is confirmatory diagnostic test for Tuberculosis disease.(39) If at least one sample between two sputum specimen is positive for acid fast bacilli with Z-N staining then the sample is considered as positive for M.*tb* and anti-tuberculosis treatment will be started for that person(39). Studies have shown that sputum processing for centrifugation &chemical process and fluorescent microscopy has higher sensitivity and comparable specificity compared with conventional direct microscopy (40-

42). The limitations for using the Fluorescent microscopy and concentrated sputum examinations at national level TB control programme in countries like India are higher cost of the technique and difficulties in establishing the infrastructure (40-41). Detection of acid fast bacilli in stained smears on microscope may also represent Mycobacterium other than tuberculosis NTM so it is not confirmatory gold standard test for M.*tb* (34-35).

Studies have shown that nucleic acid amplification using Polymerase chain reaction (PCR) has a sensitivity of 75-88% and 100% specificity(35). The PCR confirms the diagnosis of the sputum smear positive cases. The results of the PCR must be interpreted with the clinical evaluation of the patient because the test is positive even in cured and treatment completed cases(35). Culture of the mycobacterium on culture media is the confirmatory gold standard test for definite diagnosis of Tuberculosis (34-36, 43). The culture provides the isolates for identification of species and drug susceptibility testing(43). The culture media available currently are Lowenstein-Jensen media (egg based media), Middlebrook media (agar based media) and a liquid media(35). BACTEC 460 and BACTEC MGIT 960 (Becton Dickinson, USA) are two automated liquid broth culture systems used currently (35, 43). BACTEC 460 system is radiometric system and TK culture media is a new calorimetric system (36, 43-44). The growth in the liquid media is rapid (1-3 weeks) compared to the growth in solid media like Lowenstein-Jensen media and agar media (3-8 weeks). Prolonged time for the growth, contamination and high cost are limitations of the culture. Mycobacteriophage technique is a new rapid, very expensive test for detection of M.*tuberculosis* (35, 43).

The present study is part of a prospective, observational two year cohort study conducted among school/ college going adolescents, 12-18 years in Palamaner taluk with no experimental treatment and with the primary objective of estimating the incidence of

pulmonary tuberculosis disease. In this cohort study the participants were selected from the schools. A stratified randomisation procedure was chosen to assign the selected schools to either active or passive surveillance methodology. After informed consent all the eligible participants were followed during a 2 year follow-up period to assess signs or symptoms of tuberculosis disease according to the type of surveillance i.e. either active or passive. At the start of study (Day 0) blood draw for immunological tests for diagnosis of Tuberculosis and tuberculin skin test were performed among all participants. Parents and adolescents were interviewed with a questionnaire to determine the socio-demographic characteristics of the participants and symptoms suggestive of Tuberculosis. The participants in the active follow-up group were followed up at every third month and asked for any signs or symptoms of Pulmonary TB and blood draw was also performed. At 1 year visit to the participants in active surveillance, Tuberculin skin test was performed. At the final study visit (Day 720) the procedures done at day 0 were repeated among all participants in both active and passive surveillance group. During the follow-up or on the day 0 visits or during the final study visit, if the participant had any referral criteria for diagnostic ward then they were motivated to attend the diagnostic ward (DGW) to test for pulmonary tuberculosis.

2. Rationale:

The data available regarding adolescent TB was very less from the studies conducted globally and in India. As discussed above the adolescent age group is vulnerable and has a higher chance to get Pulmonary TB infection. The risk of getting TB disease after TB infection is high in Adolescent age group compared to adults.(45) This age group is a target group for vaccination. Thus we want see the proportion of definite and probable TB disease among the adolescent age group.

From the study conducted in Chingleput, South India to know the efficacy of BCG vaccine, it was suggested that the exposure of the participants to Nontuberculous Mycobacteria present in the environment might develop immunity and thus the efficacy of BCG could not be elicited properly(11). Keeping this in mind we wanted to determine the frequency of the Nontuberculous Mycobacteria among 12-18 years age adolescents with symptoms suggestive of pulmonary TB, positive TST and recent TB contact in South India.

3. Objectives:

3.1. Primary objective:

- To estimate the proportion of definite TB and probable TB in 12-18 year old adolescents having any referral criteria i.e. symptoms suggestive of TB, recent TB contact and positive Tuberculin skin test (TST) in Palamaner taluk, Andhra Pradesh, south India.

3.2. Secondary objectives:

- To estimate the rate of Nontuberculous Mycobacteria (NTM) in the same group.

4. Methods:

4.1. Study Site:

The study was conducted in Palamaner taluk of Chittoor District of Andhra Pradesh in India. The study area consisted of 397 population units and 384 rural, with an estimated population of approximately 272 000.

4.2. Study population:

Adolescents in the age group of 12-18 years attending the diagnostic ward with referral criteria.

Palamaner, Chittoor district, Andhra Pradesh, South India

4.3. Study design:

The data were collected as part of a prospective, cohort study of school going 12 to 18 years old adolescents, with the primary objective of estimating the two year incidence of tuberculosis conducted during February 2007 to July 2010. The current paper discusses only about the diagnostic ward data, which were collected when the participants were referred to the diagnostic ward if they had any of the criteria for referral.

Number of subjects: A total of 609 participants in 12-18 years age group, who attended the diagnostic ward with any of the criteria for referral.

4.4. Eligibility:

4.4.1. Referral Criteria:

1. Participants having recent history of positive TB contact (contact for more than 8 hours per week).
2. Symptoms suggestive of TB (Unexplained cough, unexplained fever, unexplained weight loss and unexplained night sweats for more than 2 weeks and Haemoptysis)
3. If the participant was diagnosed with pulmonary TB by any other physician
4. Tuberculin skin test (TST) was positive (TST ≥10mm).

4.4.2. Inclusion criteria:

- Male and female adolescent volunteers 12-18 years of age attending high school or junior college in the Palamaner Taluk.
- Informed consent from parent or guardian and assent from the subjects or consent from the subject if he or she is 18 years or older.

4.4.2. Exclusion criteria:

- Plan for the family to move from the study area in two years following enrolment
- Unable to attend follow-up session for reading of the tuberculin skin test.

4.5. Assessments:

4.5.1. Questionnaire:

Once the participant comes to the diagnostic ward the date of diagnostic visit and the way of referral to diagnostic ward i.e. by whom, the participant was referred to the diagnostic ward were noted on the source document from referral form. For self-referral there will be no referral form. Information about current symptoms suggestive of Tuberculosis i.e. any recent illness, recent weight loss, cough, fever, night sweats and haemoptysis were noted down on the questionnaire. The duration of symptoms and history of treatment for those symptoms was noted if it was known by the respondent. The respondent might be parent/ guardian or the participants themselves. The source of information (respondent) was noted on source document. The respondent was also asked about history of current or past tuberculosis disease and history of close contact for more than 8 hours per week, with an adult with proven tuberculosis.

4.5.2. Clinical evaluation:

The height and weight of the participants were recorded. Height was measured to the closest 0.1 cm using a standard measuring tape with the participant standing against a wall in the Frankfurt plane while weight was recorded in clothes but without footwear to the nearest 0.1 kg using a calibrated manual weighing scale (Bhaseen Health Product Pvt. Ltd., Jalandhar, India). Blood pressure, temperature, pulse were noted by the physician or research nurse. Detailed systemic examination of the participant was done by the physician. Body mass index (BMI) for age Z-score, calculated with the Anthroplus software (WHO 2010). Based on the BMI for age Z-score participants were classified into Underweight (BMI – Age Z-score less than or equal to -2), Over weight (BMI – Age Z-score greater than or equal to 1) and Normal (BMI – Age Z-score between -2 to 1; doesn't includes -2 & 1)

A radiograph in posterior anterior view of the chest was taken after the clinical examination by a radiology technician. The radiograph was read by an expert radiologist designated for the study and graded normal, abnormal but not indicating tuberculosis, or abnormal indicating possible tuberculosis. The participant was asked to collect the sputum in a sterile container provided by the research laboratory of the project. The participant was told about the way of collecting the sputum. If the participant was unable to produce the sputum sample naturally then chest physiotherapy was done. Even after chest physiotherapy if he/she were unable to naturally produce the sputum, an induced specimen would be obtained. The participant was asked to collect the second day early morning sputum specimen in a new sterile container. The second day specimen was given to the research field staff by the participant. The field staffs were delegated to bring the specimen to the diagnostic ward. The consistency of the sputum sample i.e. salivary, mucoid, muco-purulent and blood stained was noted. The gender of the participant was not noted in the questionnaire.

4.6. Laboratory Procedure:

The sputum specimens were examined by concentrated AFB smear microscopy and processed for culture. The media used for the culture of Mycobacteria were both Lowenstein-Jensen medium and MGIT medium. If a specimen was culture positive, the species of the isolate were confirmed using a GenoType Mycobacterium CM kit and GenoType MTBC kit.

GenoType Mycobacterium CM kit: It is the test based on the DNA strip technology and identifies the different Mycobacterial species. The Interpretation Chart of this kit identifies following species with conjugate control band, Universal control band, Genus control band and Species specific band:

High GC gram positive Bacterium, Mycobacterium species, M.*avium ssp*, M.*chelonae*, M.*abcessus*, M.*fortuitum*-1, M.*fortuitum*-2, M.*gordonae,* M.*intracellulare*, M.*scrofulaceum*, M.*interjectum*, M.*kansasii*, M.*malmoense*, M.*marinum*, M.*ulcerans*, M.*tuberculosis* complex, M.*peregrinum* (M.*alvei/* M.*septicum*), M.*xenopi*.(46-47)

GenoType MTBC kit: This is also based on DNA strip technology. This kit can identify M.*africanum*, M.*bovis BCG*, M.*bovis ssp bovis*, M.*bovis ssp. caprae*, M.*microti*, and M.*tuberculosis*. This kit is used only after the GenoType Mycobacterium CM kit, identifies M.*tuberculosis complex (47).*

Conjugate Control: "The line must develop in this zone, documenting the efficiency of a conjugate binding and substrate reaction". (Reference)

Universal Control: "This zone detects, as known all Mycobacteria and members of the group, gram positive bacteria with Guanine (G) and Cytosine (C) content. If this zone and conjugate control zone stain positive but the remaining band patterns can't assign to a specific Mycobacteria, additional methods have to be applied to identify the respective bacterial species". (Reference)

Genus Control (MTBC specific band): "This zone hybridizes, as known, with amplicons generated from all members of *Mycobacteria tuberculosis* complex". (Reference)

Reference: Genotype MTBC. Ver1.X Hain Life Sciences. GmbH Nehren, Germany

The reference laboratory is Division of Infectious diseases, St. John's Research institute. The participant was asked to collect a minimum of 5 ml of urine. Urine specimens were frozen and stored for later analysis by a urinary M.*tb* antigen detection assay or molecular diagnostic assay. Results of urine testing were not used in clinical decision-making. Study participants might have blood draw as part of the clinical parameters. All the specimens were sent to the laboratory from diagnostic ward in an ice-pack box at the earliest. The results of these examinations were communicated to the study participant's parent/guardian and medical care provider. If either sputum specimen was smear or culture positive, the results were also reported to the public sector tuberculosis control program (RNTCP-DOTS). If anti tuberculosis treatment was given to the participant, it was recorded in the source documents. If any adverse events occurred at the time of diagnostic visit, those were noted and reported to the institutional review board and sponsors.

4.7. Data management and analysis:

All data collected were entered into customized data acquisition software. A 100% check of the data forms was done to assess missing data and clarity of data prior to data entry. Double data entry was done and disparities reconciled against the data forms. Statistical Package for Social Sciences Version 17.0 (SPSS, 17.0, SPSS Inc, Chicago, IL, USA) has been used for the analysis of the data. The data collected contains the categorical variables, so the proportion, percentage and rates were calculated. The odds ratios and 95% confidence intervals were calculated to know the association between the variables. Binary logistic regression method was used to adjust the confounders. In binary logistic regression the confounding is adjusted for age, gender, cough for more than two weeks, type of cooking fuel used by the household, TB exposure, Body mass index for age Z score and recent TST value in case of Definite TB and definite & probable TB. The same was adjusted for age, gender,

cough for more than two weeks, type of cooking fuel used by the household, TB exposure, Body mass index for age Z score, caste to which the participant belongs and recent TST value in case of NTMs.

4.8. Ethical considerations, ethical review and approval:

Written informed assent from the subjects and consent from the parents or guardians was obtained at the time of enrolment of the participant before the start of the procedure. The study protocol and informed consent forms were reviewed & approved by the Institutional Review Board (IRB) of St John's Medical College, Bangalore and by an Independent Ethics Committee (IEC) of the Aeras Global TB Vaccine Foundation, USA. The studies were also approved by the Ministry of Health Screening Committee of the Government of India (No. 5/8/9/52/2006-ECD-I dt. 10.11.2006).

The study was stopped in September 2008 and restarted in November 2008 because of change in sponsors. When the study was restarted, re-consent was obtained because of change in the protocol.

5. Results:

5.1. Participants:

During the 2 years follow-up of 6643 participants, 609 participants attended the diagnostic ward (DGW) for Tuberculosis testing. Out of 609 participants, 336 (55.2%) were referred from active follow-up and 273 (44.8%) were from passive follow-up. Among 609 participants, 3 participants attended the diagnostic ward 3 times and 16 participants attended

2 times to the diagnostic ward during the period of 2 years. Among all the multiple visits by a single participant, the latest visit to the DGW was taken into the consideration and remaining visits were excluded from the analysis.

5.2. Description of Participants:

5.2.1: Demographic details:

Table 1 and figure 1, show the age and gender distribution of the participants. Among all these participants, 310 (50.9%) were males and 299 (49.1%) participants were females. 329 (54%) of the total participants attended the DGW, belonging to the age group 14 to 15 years out of which 170 (56.7%) were females and 159 (51.5%) were male participants, followed by 16-17 years age group with 157 (25.9%) participants, out of which 86 (27.8%) were male, and 71 (24%) were female, participants. 70 (11.5%) participants, of which 44 (14.7%) female and 26 (8.4%) male participants belonged to 12-13 years age group and 53 (8.5%) participants were in the age group 18-20 years, with 39 male and 14 female. The mean age of all the participants at the time of DGW visit is 15.06 years (Standard deviation: SD=1.563). The minimum and maximum age of the participants is 12 years and 20 years respectively. The male participants were older than female participants. The mean age of male participants was 15.30 (SD=1.62) and for female participants was 14.81 (SD=1.46).

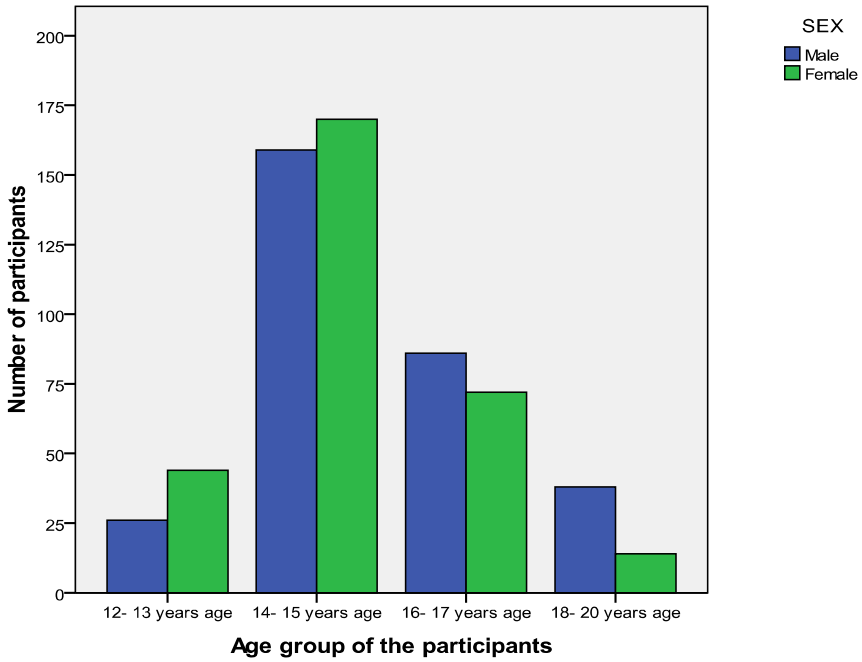

Figure 1: Distribution of the participants according to the age and sex.

5.2.2. Socio-economic characteristics:

Among all the participants majority of the participants' mothers were illiterate (44.5%), 44 % of the participants' fathers had secondary education or more. 82% of the participants were living in houses made of walls with brick and other 18% participants were living in the houses constructed with walls made of mud and others. In 84% of the participants' wood was the household cooking fuel.

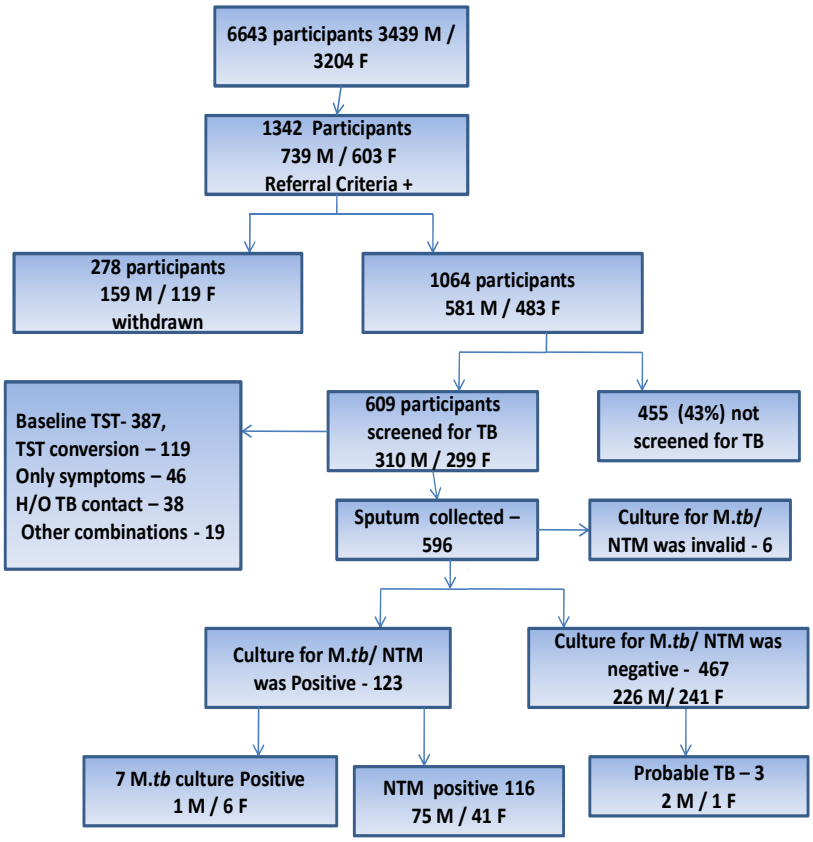

Figure 2: Study Profile

DGW: Diagnostic ward, MTB: Mycobacterium tuberculosis, NTM: Nontuberculous Mycobacteria. TST: Tuberculin skin test, H/O TB: History of TB contact, BTST: Baseline TST, AFB: Acid fast bacilli, Probable TB – Chest X-ray was abnormal for TB

Table 1: Characteristics of participants admitted into the Diagnostic ward for diagnostic evaluation of tuberculosis

Variables	1 Visit	>= 2 Visits

	(n=609)	(n=22)
Age category		
12-13 years	70 (11.5)	0
14 – 15 years	329 (54.0)	8 (36.36)
16 – 17 years	157 (25.8)	8 (36.36)
18 – 20 years	53 (8.7)	6 (27.27)
Gender		
Male	310 (50.9)	14 (63.6)
Female	299 (49.1)	8 (36.4)
Socio-economic characteristics		
Education of Mother		
Illiterate	270 (44.5)	11 (50.0)
Primary	192 (31.6)	5 (22.7)
Secondary or more	145 (23.9)	6 (27.3)
Education of Father		
Illiterate	157 (26.0)	8 (38.1)
Primary	184 (30.4)	2 (9.5)
Secondary or more	264 (43.60	11 (52.4)
Type of wall of house		
Brick	499 (81.9)	19 (86.4)
Others	110 (18.1)	3 (13.6)
Cooking fuel used		
Wood	511 (83.9)	17 (77.3)
Others	98 (16.1)	5 (22.7)

Symptoms [1]		
Cough		
Yes	76 (12.5)	9 (40.9)
No	533 (87.5)	13 (59.1)
Weight Loss		
Yes	29 (4.8)	3 (13.6)
No	579 (95.1)	19 (86.4)
Fever		
Yes	51 (8.4)	3 (13.6)
No	558 (91.6)	19 (86.4)
Haemoptysis		
Yes	6 (1.0)	0 (0)
No	603 (99.0)	22 (100.0)
Night sweats		
Yes	11 (1.8)	0 (.0)
No	597 (98.0)	22 (100.0)
Cough more than 2 weeks		
Yes	46 (7.6)	6 (27.3)
No	563 (92.3)	16 (72.7)
Fever more than 2 weeks		
Yes	25 (4.1)	1 (4.5)
No	583 (95.7)	21 (95.5)
Haemoptysis more than 2 weeks		

Yes	2 (0.3)	0 (.0)
No	607 (99.7)	22 (100.0)
Night sweats more than 2 weeks		
Yes	7 (1.1)	0 (.0)
No	601 (98.7)	22 (100.0)
Participant diagnosed TB prior to diagnostic visit		
Yes	2 (0.3)	0 (.0)
No	607 (99.7)	22 (100.0)
Contact [2]		
Yes	67 (11.0)	6 (27.3)
No	538 (88.3)	16 (72.7)
Recent TST value [3]		
< 10 mm	165 (27.1)	8 (36.4)
≥10 mm	443 (72.9)	14 (63.6)
BAZ [4]		
Underweight	269 (45.3)	10 (47.6)
Normal	312 (52.5)	10 (47.6)
Overweight or Obese	13 (2.2)	1 (4.8)

1. The symptoms were recorded at the time of diagnostic visit in diagnostic ward (DGW). They might not be the referral criteria.
2. The contact with a positive TB case for more than 8 hours a week.

3. Recent Tuberculin skin test (TST) value is the latest test value corresponding to the diagnostic ward (DGW) visit. For one participant TST was administered but was not available for TST reading.
4. BAZ: Body mass index (BMI) for age Z-score. Calculated with the Anthroplus software (WHO 2010). Under weight – Less than or equal to -2; Over weight – Greater than or equal to 1; Normal – Between -2 to 1 (Doesn't includes -2 & 1)

Missing Data: Education of Mother – 1, Education of Father – 3, Weight loss – 1, Night sweats – 1, Contact – 14, Recent TST – 1 and BAZ – 15

5.2.3. Information on exposure according to referral criteria available from Diagnostic ward questionnaire and field questionnaire:

Of the 609 participants attended the DGW, majority (64.6%) of them were referred because of Baseline TST (Day 0 TST) was equal to or greater than 10 mm, 119 (19.5%) had only TST conversion at the time of follow-up, 46 participants (7.6%) had only symptoms suggestive of tuberculosis, 38 (6.2%) had only history of household contact or class room contact with an individual with TB, 2 (0.33%) participants had both symptoms and history of contact as referral criteria, 9 (1.5%) participants had symptoms and TST $\geq$ 10 mm at baseline, 1 (0.16%) participant had symptoms and TST conversion and 2 (0.33%) participants had history of TB contact and TST $\geq$ 10 mm as referral criteria. Table 2 gives the information about the participants' referral criteria to the DGW based on the information available from referral forms and field data.

Table 2: Referral criteria for the participants:

Referral criteria	Number of Participants n = 609 (%)
Only Symptoms suggestive of TB [1]	46 (7.55)
Only History of household contact or class room contact with an individual with TB	38 (6.24)
Only Tuberculin skin test more than or equal to 10mm at baseline	387 (63.55)
Only Tuberculin skin test conversion at the time of follow-up	119 (19.54)
Symptoms and history of TB contact	2 (0.33)
Symptoms and TST ≥ 10 mm	9 (1.48)
Symptoms and TST conversion during follow-up	1 (.16)
History of TB contact and TST ≥ 10 mm at Baseline	2 (0.33)
Self Referral to the DGW [2]	5 (0.82)

1. Symptoms suggestive of TB include: Unexplained cough, unexplained fever, unexplained weight loss and unexplained night sweats for more than 2 weeks and Haemoptysis
2. Among the 5 self referral participants 3 had symptoms suggestive of TB and 2 participants had TB exposure.

The tuberculin skin test results (TST), corresponding to the diagnostic ward visit were imported from the data collected from field questionnaire. The recent TST result using a cut-off value of 10 mm at the time of their diagnostic ward (DGW) visit shows that 443 (72.9%) participants were positive for TST result (greater than or equal to 10 mm) and 165 (27.1%) participants were negative for TST (less than 10 mm). Table 3 shows the frequency of participants according to their recent TST result stratified according to the age and gender.

Table 3: Frequency of participants according to the tuberculin skin test results using a cut-off value of ≥ 10 mm.

		TST < 10 mm	TST ≥ 10 mm	Total[1]
Sex	Male	74 44.8%	235 53.0%	309 50.8%
	Female	91 55.2%	208 47.0%	299 49.2%
	Total	165 100.0%	443 100.0%	608 100.0%
Age group	12- 13 years age	20 12.1%	50 11.3%	70 11.5%
	14-15 years age	85 51.5%	243 54.9%	328 53.9%
	16- 17 years age	42	115	157

		25.5%	26.0%	25.8%
	18-20 years age	18 10.9%	35 7.9%	53 8.7%
	Total	165 100.0%	443 100.0%	608 100.0%

Table 1 describes information regarding the demographic details of the participants, symptoms, Body mass index for age – Z score (BAZ), BCG (Bacillus calmette guarine) scar status of the participant, history of contact and tubeculin skin test (TST) which was recent to the DGW visit.

5.2.4. Anthropometric details:

Among all the participants 52.5% of the participants' Body mass index for age Z (BAZ) score was normal (BAZ was between -2 to 1) and 45.3% of the participants were underweight (Less than or equal to – 2). For 15 participants height and weight were missing so BAZ was not calculated.

5.2.5. Clinical details at the time of DGW visit:

Out of the 609 participants who attended the diagnostic ward, 29 (4.8 %) had experienced recent loss of weight. 76 participants had cough, of these 46 (7.6%) had cough for more than

or equal to 2 weeks. There were 51 (8.4%) participants who had fever at the time of DGW visit and of these, 25 participants had fever equal to or greater than 2 weeks. 6 (1.0 %) participants had haemoptysis and 2 of them had the symptoms for more than 2 weeks. 11 (2.0 %) participants had night sweats at the time of DGW visit and among these participants, 7 (1.1%) participants had the symptom for more than 2 weeks. Two (0.3%) participants were diagnosed as pulmonary TB outside the DGW and prior to their DGW visit, and both of them were on the treatment for tuberculosis. Among all these participants, 67 (11.0 %) of them had experienced a significant exposure to an individual on TB treatment. For 443 (73%) participants, the recent TST corresponding to the DGW visit was positive (equal to or greater than 10mm).

5.2.6. Responders (Informants):

The source of information (respondent) for majority of the participants (65%) was adolescent himself/ herself. It was the mother for 12% of the participants, 15% had father as informants, 3% had grand -parents. For 5% participants it was others.

5.2.7. Diagnostic test results of participants and other details:

All the 609 participants were screened with chest radio graph P-A view for TB disease. For 13 participants neither day 1 or day 2 sputum samples were collected. Of remaining 596 participants 4 participants had only the day 1 sputum sample collected. If only one sample was available, depending on the result this sputum sample, the participant was considered as positive or negative for M.tb or NTM. For 4 participants' sputum samples were contaminated during the culture in either one of the day sample, so the other sample result was considered

for the output (M.tb result/ NTM result). The DNA strip test (GenoType Mycobacterium CM kit and GenoType MTBC kit) results were invalid for 6 participants, so the culture of M.tb/ NTM results for those were considered as missing data. So the result for M.tb or NTM was available for 590 participants.

5.2.7.1. Chest X-ray results:

The chest radiograph was abnormal for TB in 7 (1.15%) participants and in 9 (1.5%) participants it was abnormal but not TB. Out of 7 participants, who had chest x-ray abnormal for TB, 4 participants were positive for M.tb on culture of the sputum sample. Remaining 3 participants were negative for the culture. Of the two participants who were diagnosed as Pulmonary TB prior to the DGW visit, chest radiograph was abnormal for TB in one participant and the other's chest radiograph was abnormal, not TB.

5.2.7.2. Sputum sample culture and acid fast bacilli smear results:

Table 4 gives the number of participants with sputum sample positive for smear for AFB and positive for *M.tb* culture. Of 590 participants with sputum culture results available, 7 (1.14 %) participants' sputum samples were culture positive for *Mycobacterium tuberculosis.* Out of these 7 participants, 4 participants' sputum samples' were positive for Acid fast bacilli (AFB) smear on both days. Day 1 and day 2 sputum samples were positive for culture of *M.tb* in 5 participants and in 2 participants only day 1 was positive for *M.tb* culture. In 1 participant only day 2 sputum sample was positive for AFB smear and is negative for culture of *M.tb* and the culture is positive for *Mycobacterium scrofulaceum.* The same participant didn't have any symptoms at the time referral and the recent TST was 20 millimetres

Table 4: *M.tb* culture and sputum smear results:

	Sputum positive for culture of *M.tb* [1]	Sputum negative for culture of *M.tb* [1]	Total
Sputum smear positive for AFB [2]	4 (57.1%)	1 (0.2%)	5 .8%
Sputum smear negative for AFB [2]	3 (42.9%)	582 (99.8)%	585 100%
Total	7 (100%)	583 98.8%	590 100.0%

1. The sputum positive either in day 1 or day 2 or both days sputum
2. The sputum positive for AFB smear either in day 1 or day 2 or both

Table 5 gives the information regarding participants with sputum samples' culture positive for NTM with sputum samples positive for AFB smear. Among all participants who were screened for sputum culture, 116 (19.05%) participants were positive for NTM species on culture in either day 1 or day 2 sputum or both day 1 and day 2. Among 116 participants who were positive for NTM on culture of the sputum sample, 1 participant was positive for AFB smear. *M.scrofulaceum* was isolated from this participants' sputum sample.

Table 5: Participants with sputum positive for culture of MOTT with sputum positive for AFB smear.

	Sputum positive for culture of NTM [1]	Sputum negative for culture of NTM [1]	Total
Sputum smear positive for AFB [2]	1 (0.9%)	0 .0%	1 0.2%
Sputum smear negative for AFB [2]	115 (99.14%)	467 100.0%	582 99.8%
Total	116 (100.0%)	467 100.0%	583 100.0%

1. The sputum positive either in day 1 or day 2 or both days sputum
2. The sputum positive for AFB smear either in day 1 or day 2 or both

5.2.8: Participants with sputum samples positive for culture of Nontuberculous Mycobacteria (NTM) details:

Out of 116 participants positive for NTM, 59 day 1 sputum samples were positive for NTM and 68 day 2 sputum samples were positive for NTM. Both day 1 and day 2 sputum samples were positive for culture of NTM in 11 participants, where as in 105 participants' sputum samples were positive for culture of NTM only either in day 1 or day 2. (Table 6) A total of 127 samples have yielded NTM species from 116 participants. Sputum samples from 6 participants have yielded two different species of NTMs from the day 1 and day 2 sputum samples. From 33 participants a total of 36 sputum samples have shown the growth of

Mycobacterium species, for which the particular species of the Mycobacteria was not identified by the GenoType Mycobacterium CM kit. A total of 19 sputum samples have yielded NTM growth but GenoType Mycobacterium CM kit doesn't show the species for which it belongs, it is then considered as NTM indeterminate. Among all the species of NTM found on culture, *M.fortuitum, M.intracellulare, M.scrofulaceum* have dominated other species i.e. *M.chelonei, M.gordonae, M.interjectum, M.kansasii* on isolation of the species from sputum samples. Table 8 gives the frequency of NTM species. M.*fortuitum* accounts for 24% of the total NTM positive participants followed by M.*intracellulare* in 12.6% of participants and M.*scrofulaceum* in 11% of the participants. In 4 participants *M.kansasii* and in 3 participants *M.gordonae* species were isolated. *M.interjectum* was isolated from 2 participants and *M.chelonei* was isolated in 2 participants.

Table 6: Frequency of NTM species

NTM species	Isolation of NTM by sputum samples (%)	Isolation rates by participants Number (%) [€]
M. *fortitium*	31 (24.41)	31 (25.41)
M. *intracellulare*	16 (12.60)	16 (13.11)
M. *scrofulaceum*	14 (11.02)	13 (10.66)
M. *kansasii*	04 (03.15)	04 (03.28)
M. *gordonae*	03 (02.36)	03 (02.46)
M. *interjectum*	02 (01.57)	02 (01.64)
M. *chelonae*	02 (01.57)	02 (01.64)

Indeterminate [1]	19 (14.96)	18 (14.75)
Mycobacterium species [2]	36 (28.35)	33 (27.05)
Total NTMS	127 (100.0) [3]	122 (100) [4]
MTBC/ NTM Invalid [5]	6	6

€: Participant was counted twice if the two sputum samples yielded two different species. So the number is more than 116 participants.

1. The HAIN kit shows bands for conjugate control and universal control. There was no genus control band. The species of NTM was not mentioned.
2. The kit shows all the three bands (Conjugate control, universal control and genus control). The kit specifies the specimen as Mycobacterium species. The specimen can be looked for further speciation of the bacteria.
3. The number includes both day 1 and day 2 sputum samples isolates, if they are positive in both samples.
4. The number includes both day 1 and day 2 sputum samples isolates, only if there are two different isolates in those two samples of a single participant.
5. The HAIN Genotype was invalid either for MTBC or NTM

MTBC – Mycobacterium tuberculosis complex, NTM – Mycobacterium other than tuberculosis

Conjugate Control: "The line must develop in this zone, documenting the efficiency of a conjugate binding and substrate reaction". (Reference)

Universal Control: "This zone detects, as known all Mycobacteria and members of the group, gram positive bacteria with Guanine (G) and Cytosine (C) content. If this zone and

conjugate control zone stain positive but the remaining band patterns can't assigned to a specific Mycobacteria, additional methods have to be applied to identify the respective bacterial species". (Reference)

Genus Control (MTBC specific band): "This zone hybridizes, as known, with amplicons generated from all members of *Mycobacteria tuberculosis* complex". (Reference)

Reference: Genotype MTBC. Ver1.X Hain Life Sciences. GmbH Nehren, Germany

5.3. Outcome:

The participants with sputum sample positive for culture of *M.tb* either in day 1 or day 2 or both were considered as definite TB. The participant whose chest X-ray showed the changes positive for pulmonary tuberculosis and the sputum sample of the participant was negative for culture of *M.tb* were considered as positive for Probable TB. Figure 1 shows the profile of the study. A total number of 7 (1.15 %) participants were diagnosed as definite TB, 3 (0.50 %) participants were diagnosed as probable TB. The proportion of 19.05% participants had NTM positive sputum samples. Table 7 denotes the characteristics and details of all the 10 participants who were diagnosed as Definite TB or Probable TB. Of the 7 participants who were diagnosed as having definite TB participants, 6 were female. Of those 7 definite TB, 3 participants didn't have any symptoms at the time of DGW visit.

Table 7: Characteristics of the participants with culture positive for M.tb and Chest X-ray abnormal for TB (Definite TB and Probable TB)

SI. No	Participant ID	visit	Gender	Age	BAZ [3]	Smear result	Culture for M.tb	Symptoms[1]	Contact	Chest x-ray	TST values	QFT Value[2]
1	12104	3	Male	17	N	Positive	Positive	Yes	Yes	Abnormal TB	16	+ Ve
2	20116	1	Female	17	U.W	Positive	Positive	Yes	No	Abnormal TB	15	NA
3	23061	1	Female	17	N	Negative	Positive	No	No	Normal	25	+ Ve
4	42107	1	Female	12	U.W	Positive	Positive	Yes	No	Abnormal TB	7	NA
5	52301	1	Female	15	U.W	Negative	Positive	No	No	Normal	17	+ Ve
6	72601	1	Female	15	U.W	Positive	Positive	Yes	No	Abnormal TB	5	NA
7	83013	1	Female	15	U.W	Negative	Positive	No	No	Normal	23	NA
Probable TB												
8	11001	1	Female	15	N	Negative	Negative	Yes	No	Abnormal for TB	4	NA
9	41630	1	Male	16	N	Negative	Negative	No	Yes	Abnormal for TB	23	NA
10	77503	1	Male	14	N	Negative	Negative	No	No	Abnormal for TB	20	NA
Definite in the latest visit and Probable in 2nd Visit												
11	12104	2	Male	16	N	Negative	Negative	Yes	No	Abnormal for TB	10	+ Ve

1: Presence of at least one Symptom suggestive of TB at the time of DGW visit.

2: QFT: QuatiFERON; NA = Not available (QFT was not done for those participants)

3: BAZ: Body mass index (BMI) for Age Z-score. Calculated with the help of Anthroplus software - 2007 (WHO). U.W = under weight – Less than or equal to -2; N = Normal – Between -2 to 1 (Doesn't includes -2 & 1); Over weight – Greater than or equal to 1;

5.4. Association of outcome results with other variables (Characteristics of Participants):

5.4.1: Unadjusted estimates:

Table 8 gives the association between M.tb positive result on culture and symptoms of participants at the time of DGW visit, gender, socio economic status indicators, body mass index for age z-score (BAZ), chest x-ray result and corresponding TST result. The participants who were having history of recent loss of weight at the time of DGW visit had 18 times more chance of sputum being positive for M.tb on culture, compared to who were not having history of recent weight loss (O.R = 18.26; 95% C.I = 3.86 – 86.38). Participants having cough equal to or greater than 2 weeks were, 19 times more likely to become positive for M.tb growth of sputum sample compared to participants without having cough equal to or greater than 2 weeks (O.R = 18.60; 95% C.I = 4.02 – 86.05). The participants with fever for more than or equal to 2 weeks at the time of DGW visit, were having 11 times more chance to be diagnosed as Definite TB (Sputum positive for culture of M.*tb*) and the odds ratio was 11.26 (95% C.I = 2.06 – 61.60). The association of night sweat for more than 2 weeks at DGW visit found significant and the association is weak with odds ratio of 16.02 (95% C.I = 1.67 – 154.31). There were no participants diagnosed as Definite TB, with presence of haemoptysis for more than or equal to 2 weeks at the time of DGW visit. The participants

who had chest x-ray changes for pulmonary tuberculosis had 258 times more chance to become sputum specimen positive for culture of M.tb (O.R = 258; 95% C.I = 39.40 – 1686.46). There was no significant association found with other variable like Socio economic status indicators, BAZ score, TB exposure and corresponding TST result to sputum sample positivity for culture of M.tb.

Table 8: Association of symptoms, age, gender, socio-economic status and other variables with culture results for M.*tb*

Variables	M.tb positive (n=7) [1]	M.tb negative (n=583) [2]	Unadjusted Odds ratio 95% C.I.	Adjusted Odds ratio 95% C.I.
Age category				
12-13 years	1 (14.3)	67 (11.5)	0.77 (0.03 - 8.47)	0.83 (0.07 – 10.37)
14 – 15 years	3 (42.9)	310 (53.2)	0.50 (0.08 - 3.12)	0.29 (0.02 – 4.46)
16 – 17 years®	3 (42.9)	154 (26.4)	---	---
18 – 20 years	0 (0.0)	52 (8.9)		
Gender				
Male	1 (14.3)	301 (51.6)	0.16 (0.02-1.31)	0.06 (0.005 – 0.75)
Female	6 (85.7)	282 (48.4)		
Socio-economic characteristics				
Education of Mother				
Illiterate	2 (28.6)	258 (44.3)	0.27 (0.03-1.72)	

Primary	1 (14.3)	186 (32.0)	0.19 (0.02-1.78)	
Secondary or more®	4 (57.1)	138 (23.7)		
Education of Father				
Illiterate	0 (0.0)	151 (26.0)		
Primary	1 (14.3)	176 (30.3)	0.24 (0.03-2.02)	
Secondary or more®	6 (85.7)	253 (43.6)		
Type of wall of house				
Brick	7 (100.0)	477 (81.8)		
Others	0 (0.0)	106 (18.2)		
Cooking fuel used				
Wood	4 (57.1)	492 (84.4)	0.25 (0.05-1.12)	0.38 (0.05 – 3.22)
Others	3 (42.9)	91 (15.6)		
Caste				
Dalit	3 (42.9)	102 (17.5)	3.54 (0.78 – 16.04)	
Others	4 (57.1)	481 (82.5)		
Symptoms [3]				
Weight Loss				
Yes	3 (42.9)	23 (3.9)	18.26 (3.86 – 86.38)	
No	4 (57.1)	560 (96.1)		
Cough more than 2 weeks				
Yes	4 (57.1)	39 (6.7)	18.60 (4.02 – 86.05)	57.59 (6.06 – 547.64)
No	3 (42.3)	544 (93.3)		
Fever more than 2				

weeks				
Yes	2 (28.6)	20 (3.4)	11.26 (2.06 – 61.60)	
No	5 (71.4)	563 (96.6)		
Haemoptysis more than 2 weeks				
Yes	0 (.0)	2 (0.3)	1.012 (1.003 – 1.02)	
No	7 (100.0)	581 (99.7)		
Night sweats more than 2 weeks				
Yes	1 (14.3)	6 (1.0)	16.02 (1.67 –	
No	6 (85.7)	578 (99.0)	154.31)	
Contact [4]				
Yes	1 (14.3)	63 (10.8)	1.38 (0.16 – 11.61)	3.33 (0.29 – 38.42)
No	6 (85.7)	521 (89.2)		
Recent TST value [5]				
<10	2 (28.6)	163 (28.0)	1.03 (0.20 - 5.35)	0.32 (0.04 – 2.79)
≥10	5 (71.4)	419 (72.0)		
BAZ [6]				
Underweight	5 (71.4)	254 (44.6)	2.97 (0.51-22.29)	6.01 (0.87 – 41.31)
Overweight or Obese	0 (0.0)	13 (2.3)		
Normal®	2 (28.6)	302 (53.1)		
X ray				
Abnormal Tb	4 (57.1)	3 (0.5)	258 (39.40-1686.46)	
Normal	3(42.9)	580 (99.5)		

BCG scar status				
Yes	1 (14.3)	369 (63.3)	0.097 (0.012 –	
No	6 (85.7)	214 (36.7)	0.808)	

®: Reference variable

1. The number denotes the number of definite TB; the participants' sputum samples were positive for culture of *Mycobacterium tuberculosis (M.tb)* either in day 1 or day 2 or both sputum samples
2. The participants' sputum sample was negative for culture of *Mycobacterium tuberculosis (M.tb)* in both sputum samples. If there was only one sputum sample available and negative for M.tb, it is also considered as negative for M.tb. For 13 participants sputum samples were not collected as per old protocol. For 5 participants the HAIN Genotype was invalid for M.tb.
3. The symptoms were recorded at the time of diagnostic visit in diagnostic ward (DGW). They might not be the referral criteria.
4. The contact with a positive TB case for more than 8 hours a week.
5. Recent Tuberculin skin test (TST) value is the latest test value corresponding to the diagnostic ward (DGW) visit.
6. BAZ: Body mass index (BMI) for age Z-score. Calculated with the help of WHO provided software. Under weight – Less than or equal to -2; Over weight – Greater than or equal to 1; Normal – Between -2 to 1 (Doesn't includes -2 & 1)

Missing Data: Education of Mother – 1, Education of Father – 3, Participants diagnosed as TB prior to diagnostic ward visit – 1, recent tuberculin skin test (TST) value - 1 and BAZ – 15

Table 9 gives the information regarding the association between being positive for Definite TB or Probable TB and not TB with symptoms of participants at the time of DGW visit, gender, Socio economic status indicators, Body mass index for age z-score (BAZ) and corresponding TST result. The univariate analysis done among participants with definite TB or probable TB had shown that, the participants with history of recent loss of weight at the time DGW visit had 17 times more chance to be diagnosed as definite TB or probable TB. Presence of cough for more than or equal to 2 weeks among participants at the time of DGW visit had 9 times more likely to be diagnosed as either definite TB or probable TB with an odds of 9.25 (95% C.I = 2.51 – 34.15). The participants with history of fever for more than or equal to 2 weeks at the time of DGW visit had 7 times more chance to be diagnosed as either definite TB or probable TB, with an odds of 7.00 (95% C.I = 1.40 – 35.1). The presence of night sweats for more than 2 weeks at the time of DGW visit were found significant but the lower limit of the 95% confidence interval was near 1 with odds ratio of 10.6 (95% C.I = 1.16 – 97.58). The significant association of fever and night sweats were weak because of quite less number of the probable TB or definite TB among symptomatic. There was no probable TB or definite TB participants with history of haemoptysis for more than 2 weeks. The association with other variables including age, gender, socio economic status indicators, night sweats, history significant exposure to an individual on TB treatment and TST results on a cut-off value of 10 mm (latest TST which was corresponding to the DGW visit) and BAZ score found insignificant.

Table 9: Association of symptoms, age, gender, socio-economic status and other variables with Definite or Probable TB.

Variables	Definite/ Probable TB (n=10) [1]	Not TB (n=580) [2]	Unadjusted Odds ratio 95% C.I.	Adjusted Odds ratio (95% C.I.)
Age category				
12-13 years	1 (10.0)	67 (11.6)	0.57 (0.02 - 5.60)	0.51 (0.05 – 4.91)
14 – 15 years	5 (50.0)	308 (53.1)	0.62 (0.14 - 2.80)	0.30 (0.03 – 3.34)
16 – 17 years®	4 (40.0)	153 (26.4)	-----	-----
18 – 20 years	0 (0.0)	52 (9.0)		
Gender				
Male	3 (30.0)	299 (51.6)	0.40 (0.10 - 1.57)	0.27 (0.06 – 1.21)
Female	7 (70.0)	281 (48.4)		
Socio-economic characteristics				
Education of Mother				
Illiterate	5 (50.0)	255 (44.0)	0.68 (0.15 - 3.05)	
Primary	1 (10.0)	186 (32.1)	0.19 (0.01 -1.78)	
Secondary or more®	4 (40.0)	138 (23.8)		
Education of Father				
Illiterate	0 (0.0)	151 (26.2)		
Primary	3 (30.0)	174 (30.2)	0.62 (0.13 - 2.70)	
Secondary or more®	7 (70.0)	252 (43.7)		

Type of wall of house				
Brick	9 (90.0)	475 (81.9)	1.99 (0.25 – 15.87)	
Others	1 (10.0)	105 (18.1)		
Cooking fuel used				
Wood	7 (70.0)	489 (84.3)	0.43 (0.11 - 1.71)	0.61 (0.12 – 3.1)
Others	3 (30.0)	91 (15.7)		
Caste				
Dalit	4 (40.0)	101 (17.4)	3.16 (0.88 – 11.41)	
Others	6 (60.0)	479 (82.6)		
Symptoms [3]				
Weight Loss				
Yes	4 (40.0)	22 (3.8)	16.90 (4.45 – 64.25)	
No	6 (60.0)	558 (96.2)		
Cough more than 2 weeks				
Yes	4 (40.0)	39 (6.7)	9.25 (2.51 – 34.15)	18.62 (3.66 – 94.77)
No	6 (60.0)	541 (93.3)		
Fever more than 2 weeks				
Yes	2 (20.0)	20 (3.4)	7.00 (1.4 – 35.1)	
No	8 (80.0)	560 (96.6)		
Haemoptysis more than 2 weeks				

Yes	0 (.0)	2 (0.3)	1.02 (1.01 – 1.03)	
No	10 (100)	578 (99.7)		
Night sweats more than 2 weeks				
Yes	1 (10.0)	6 (1.0)	10.63 (1.16 – 97.58)	
No	9 (90.0)	574 (99.0)		
Contact [4]				
Yes	2 (20.0)	62 (10.7)	2.09 (0.43 – 10.06)	3.07 (0.55 – 17.13)
No	8 (80.0)	518 (89.3)		
Recent TST value [5]				
<10	3 (30.0)	162 (28.0)	1.10 (0.28 – 4.31)	0.48 (0.10 – 2.35)
≥10	7 (70.0)	417 (72.0)		
BAZ [6]				
Underweight	5 (50.0)	254 (44.9)	1.18 (0.29 – 4.74)	1.62 (0.42 – 6.22)
Overweight or Obese	0 (0.0)	13 (2.3)	------	------
Normal®	5 (50.0)	299 (52.8)		
BCG scar status				
Yes	3 (30.0)	367 (63.3)	0.25 (0.064 – 0.97)	
No	7 (70.0)	213 (36.7)		

®: Reference variable

1. The number denotes the number of definite TB; the participants' sputum samples were positive for culture of *Mycobacterium tuberculosis (M.tb)* either in day 1 or day 2 or both sputum samples

2. The participants' sputum sample was negative for culture of *Mycobacterium tuberculosis (M.tb)* in both sputum samples. If there was only one sputum sample available and negative for M.tb, it is also considered as negative for M.tb. For 13 participants sputum samples were not collected as per old protocol. For 5 participants the HAIN Genotype was invalid for M.tb.
3. The symptoms were recorded at the time of diagnostic visit in diagnostic ward (DGW). They might not be the referral criteria.
4. The contact with a positive TB case for more than 8 hours a week.
5. Recent Tuberculin skin test (TST) value is the latest test value corresponding to the diagnostic ward (DGW) visit.
6. BAZ: Body mass index (BMI) for age Z-score. Calculated with the help of WHO provided software. Under weight – Less than or equal to -2; Over weight – Greater than or equal to 1; Normal – Between -2 to 1 (Doesn't includes -2 & 1)

Table 10 represents the association between sputum culture results for NTM (sputum sample having NTM species on culture and no NTM growth) with symptoms, history of TB contact of participant at the time of DGW visit, gender, socio economic status indicators, BAZ score and corresponding TST result. Results for NTM among participants positive for *M.tb* were considered as missing data in the analysis. Male participants had 200% more chance of becoming NTM positive on culture of the sputum samples compared to female participants with an odds of 1.90 (95% C.I = 1.25 – 2.90). There was 2 times more chance to isolate NTM from the participants who belongs to Dalit/ Harijan's community, the odds ratio was 1.91 with 95% C.I of 1.18 – 3.11.

The 2 participants with presence of haemoptysis for more than 2 weeks at the time of DGW visit had isolation of NTM on culture. The association found significant with odds of 5.06 (95% C.I = 4.30 – 5.96). The GenoType Mycobacterium CM kit had isolated M.*fortuitum* and M.*intracellulare* from these two participants. There was no significant association found with other symptoms suggestive of Tuberculosis and presence of NTM in sputum samples. The other socio economic status indicators of the participants and tuberculin skin test (TST) also didn't show any significant association with presence of NTM species.

Table 10: Association of symptoms, age, gender, socio-economic status and other variables with MOTT result of the sputum culture

Variables	MOTT positive (n=116) [1]	MOTT negative (n=467) [2]	Unadjusted Odds ratio 95% C.I.	Adjusted Odds ratio
Age category				
12-13 years	11 (9.5)	56 (12.0)	0.94 (0.32 – 2.74)	0.90 (0.44 – 1.88)
14 – 15 years	60 (51.7)	250 (53.5)	1.15 (0.50 - 2.68)	0.69 (0.32 – 1.50)
16 – 17 years	36 (31.0)	118 (25.3)	1.46 (0.70 - 2.61)	1.14 (0.39 – 3.35)
18 – 20 years®	9 (7.8)	43 (9.2)		
Gender				
Male	75 (64.7)	226 (48.4)	1.95 (1.28 – 2.97)	1.94 (1.23 – 3.05)
Female	41 (35.3)	241 (51.6)		
Socio-economic characteristics				
Education of Mother	54 (47.0)			

Illiterate	37 (32.2)	204 (43.7)	1.26 (0.72 - 2.22)	
Primary	24 (20.9)	149 (31.9)	1.18 (0.64 – 2.17)	
Secondary or more®		114 (24.4)		
Education of Father				
Illiterate	29 (25.0)	122 (26.3)	0.99 (0.57 –1.70)	
Primary	38 (32.8)	138 (29.7)	1.15 (0.69 –1.89)	
Secondary or more®	49 (42.2)	204 (44.0)		
Type of wall				
Brick	91 (78.4)	386 (82.7)	0.76 (0.46 –1.26)	
others	25 (21.6)	81 (17.3)		
Cooking fuel used				
Wood	101 (87.1)	391 (83.7)	1.31 (0.72 – 2.37)	1.12 (0.59 – 2.12)
Others	15 (12.9)	76 (16.3)		
Caste				
Dalit	30 (25.9)	72 (15.4)	1.91 (1.18 – 3.11)	2.0 (1.2 – 3.32)
Others	86 (74.1)	395 (84.6)		
Symptoms [3]				
Weight Loss				
Yes	5 (4.3)	18 (3.9)	1.12 (0.41 – 3.09)	
No	111 (95.7)	449 (96.1)		
Cough more than 2 weeks				
Yes	9 (7.8)	30 (6.4)	1.23 (0.57 – 2.66)	1.11 (0.47 – 2.61)

No	107 (92.2)	437 (93.6)		
Fever more than 2 weeks				
Yes	3 (2.6)	17 (3.6)	0.70 (0.20 – 2.44)	
No	113 (97.4)	450 (96.4)		
Haemoptysis more than 2 weeks				
Yes	2 (1.7)	0 (.0)	5.1 (4.32 – 6.01)	
No	114 (98.3)	467 (100.0)		
Night sweats more than 2 weeks				
Yes	0 (.0)	6 (1.3)	1.25 (1.20 – 1.30)	
No	116 (100.0)	461 (98.7)		
Contact [4]				
Yes	16 (13.8)	47 (10.1)	1.43 (0.78 – 2.63)	1.16 (0.59 – 2.26)
No	100 (86.2)	420 (89.9)		
Recent TST value [5]				
< 10 mm	33 (28.7)	130 (27.8)	1.04 (0.66 – 1.64)	1.01 (0.61 – 1.67)
≥10 mm	82 (71.3)	337 (72.2)		
BAZ [6]				
Underweight	58 (50.9)	196 (43.1)	1.36 (0.88 – 2.10)	1.27 (0.81 – 1.98)
Overweight or Obese	2 (1.8)	11 (2.4)	0.84 (0.12 – 4.16)	1.35 (0.28 – 6.55)
Normal®	54 (47.4)	248 (54.5)		
X ray				

Abnormal TB	0 (0.0)	3 (0.6)	1.250 (1.20 – 1.30)	
Not TB	116 (100.0)	464 (99.4)		
BCG scar status				
Yes	71 (61.2)	298 (63.8)	0.90 (0.59 – 1.36)	
No	45 (38.8)	169 (36.2)		

®: Reference variable

1. The number denotes the number of participants with NTM positive sputum samples; the participants' sputum samples were positive for culture of Non tuberculosis bacteria (NTM) either in day 1 or day 2 or both sputum samples
2. The participants' sputum sample was negative for culture of Non tuberculosis bacteria (NTM) in both sputum samples. If there was only one sputum sample available and negative for NTM, it is also considered as negative for NTM. For 13 participants' sputum samples were not collected as per old protocol, for 1 participant the HAIN Genotype was invalid for NTM, 7 participants were positive for M.tb and 5 participants were showing HAIN genotype as invalid for M.tb.
3. The symptoms were recorded at the time of diagnostic visit in diagnostic ward (DGW). They might not be the referral criteria.
4. The contact with a positive TB case for more than 8 hours a week.
5. Recent Tuberculin skin test (TST) value is the latest test value corresponding to the diagnostic ward (DGW) visit.
6. BAZ: Body mass index (BMI) for age Z-score. Calculated with the help of WHO provided software. Under weight – Less than or equal to -2; Over weight – Greater than or equal to 1; Normal – Between -2 to 1 (Doesn't includes -2 & 1)

Missing Data: Education of Father – 3 and BAZ – 12

5.4.2. Confounder-adjusted estimates:

The Binary logistic regression had shown that the Female participants had 94% less chance to be diagnosed as definite TB compared to Male participants. Other associations were not changed after logistic regression. The adjusted odds have not changed with definite TB/ Probable TB after regression analysis.

The association between presence of NTM species with symptoms, TST result, gender, BAZ score, BCG scar status, socio economic status indicators and history of TB contact were adjusted with binary logistic regression method. The covariates included in the regression method were gender of the participants and age of the participants, cough, type of cooking fuel, history of TB contact, TST result and BAZ (Body mass index for age Z – score) score of the participant and caste to which the participant belongs. This analysis did not change the significant association between the gender and presence of NTM species in sputum and also the significant association between the communities to which the participants belong and NTM isolation from the sputum sample. The logistic regression also had shown that male participants were having 2 times more chance to be positive for NTM species on culture of the sputum specimen using GenoType Mycobacterium CM kit compared to the female participants. And the sputum from the participants belonging to Dalit community had 2 times more chance to isolate NTM species using the GenoType Mycobacterium CM kit. Table 8, 9 and 10 gives the adjusted estimates.

5.5. Other analysis:

Among all the participants attended the diagnostic ward, 19 participants had DGW visits more than once. Of these 19, one participant attended the DGW, 3 times was positive for sputum AFB smear only during the 3rd visit. The participant was also positive for culture of *M.tb* only during the 3rd visit. The same participant was showing chest x-ray abnormal for TB during 2nd and 3rd visit to the DGW. One participant had chest x-ray abnormal, not TB during the 1st visit to the DGW whose X-ray was normal in following visit. All other participants had normal chest x-ray. 1 participant was positive for NTM (M.*intracellulare*) on culture of day 1 sputum sample during the 1st visit. Three participants were positive for NTM on culture of day 2 sputum samples during their 1st visit. One participant was positive for NTM on culture of day 2 sputum sample during latest visit (2nd visit).

Table 11 explains the characteristics between the participants with single visits to the DGW and the participants with multiple visits. Among all the participants, the participants who attended the DGW more than once were more likely to be older age group, belonging to Muslim religion, part of active surveillance of the study and had a significant exposure to TB. Except these differences, the participants who visited the DGW more than once are characteristically similar to the participants who attended only once to the DGW during the 2 year follow-up.

Table 11: Comparison of characteristics between the participants with single visit to DGW and multiple visits (2 or more visits) to DGW.

	Single Visit (N = 590)	Multiple visits (N=19)	
Age			

12 - 13 years	70 (11.9 %)	0 (0.0 %)	P = 0.01
14 – 15 years	322 (54.6%)	7 (36.8%)	
16 – 17 years	150 (25.4%)	7 (36.8%)	
18 – 120 years	48 (8.1%)	5 (26.3%)	
Gender			
Male	299 (50.7%)	11 (57.9%)	P = 0.536
Female	291 (49.3%)	8 (42.1%)	
Religion			
Hindu	530 (89.8%)	12 (63.2%)	P < 0.001
Muslim	52 (8.8%)	7 (36.8%)	
Christian	8 (1.4%)	0 (0.0%)	
Caste			
Dalit/ Harijan	105 (17.8%)	5 (26.3%)	P = 0.342
Others	485 (82.2%)	14 (73.7%)	
Walls			
Brick	483 (81.9%)	16 (84.2%)	P = 0.808
Others	107 (18.1%)	3 (15.8%)	
Surveillance group			
Active	320 (95.2%)	16 (84.2%)	P = 0.01

Passive	270 (98.9%)	3 (15.8%)	
Cooking Fuel			
Wood	495 (83.9%)	16 (84.2%)	P = 0.971
Others	95 (16.1%)	3 (15.8%)	
Exposure to TB (≥ 8 hours per week)			
Yes	62 (10.6%)	5 (26.3%)	P = 0.03
No	524 (89.4%)	5 (73.7%)	
Education of Mother			
Illiterate	260 (44.2%)	10 (52.6%)	P = 0.768
Primary	187 (31.8%)	5 (26.3%)	
Secondary or more	141 (24.0%)	4 (21.1%)	
Education of Father			
Illiterate	150 (25.6%)	7 (38.9%)	P = 0.162
Primary	182 (31.0%)	2 (11.1%)	
Secondary or more	255 (43.4%)	9 (50.0%)	

The participants who attended the DGW were characteristically similar to the participants who didn't attend the DGW during the follow-up even though they had been referred. Except that, the male participants were more likely to deny for attending the DGW for diagnosis of TB compared to female. Table 12 gives the details of the same.

Table 12: Comparison of characteristics between the participants screened for TB disease and not screened for TB disease.

	Screened for TB disease (Responders) (N = 609)	Not Screened for Tb disease [1] (Non Responders) (N = 733)	
Age[2]			
12 years	176 (28.9 %)	176 (24.0%)	P = 0.217
13 – 14 years	312 (51.2%)	392 (53.5%)	
15 – 16 years	96 (15.8%)	130 (17.7%)	
17 – 18 years	25 (4.1%)	35 (4.8%)	
Gender			
Male	310 (50.9%)	429 (58.5%)	P = 0.005
Female	299 (49.1%)	304 (41.5%)	
Religion			
Hindu	542 (89.0%)	650 (88.7%)	P = 0.149
Muslim	59 (9.7%)	80 (10.9%)	
Christian	8 (1.3%)	3 (0.4%)	
Caste			

Dalit/ Harijan	110 (18.1%)	141 (19.2%)	P = 0.583
Others	499 (81.9%)	592 (80.8%)	
Walls			
Brick	499 (81.9%)	582 (79.4%)	P = 0.242
Others	110 (18.1%)	151 (20.6%)	
Surveillance group			
Active	336 (55.2%)	373 (50.9%)	P = 0.117
Passive	273 (44.8%)	360 (49.1%)	
Cooking Fuel			
Wood	511 (83.9%)	636 (86.8%)	P = 0.139
Others	98 (16.1%)	97 (13.2%)	
Education of Mother			
Illiterate	270 (44.3%)	351 (47.9%)	P = 0.194
Primary or more	339 (55.7%)	382 (52.1%)	
Education of Father[3]			
Illiterate	157 (26.0%)	217 (21.7%)	P = 0.119
Primary or more	448 (74.0%)	511 (70.2%)	

1: Includes withdrawn participants;

2: Age of the participant at the time of enrolment i.e. at Day 0.

3: For 9 participants the education of father is missing

Table 13 gives the information regarding the demographic details of the participants who didn't attend the diagnostic ward even though they had referral criteria. The participants were categorized into group of participants withdrawn from the study and group of participants who were active during the entire 2 year follow-up. Participants who were withdrawn were most likely to be older age group and were in active surveillance of the study. Otherwise the two groups were distributed equally in gender wise and other socioeconomic characteristics wise.

Table 13: Comparison of the demographic details of participants withdrawn from the study and active during the follow-up among participants who didn't attend the DGW even after referral from the field

	Active[1] (%) (N = 454)	Withdrawn [2](%) (N = 279)	
Age[3]			
12 years	131 (28.9)	45 (16.1)	P < 0.001
13 – 14 years	239 (52.3)	153 (54.8)	
15 – 16 years	66 (14.5)	64 (22.9)	
17 – 18 years	18 (4.0)	17 (6.1)	
Gender			
Male	270 (59.5)	159 (57.0)	P = 0.508
Female	184 (40.5)	120 (43.0)	

Religion			
Hindu	401 (88.3)	249 (89.2)	P = 0.925
Muslim	51 (11.2)	29 (10.4)	
Christian	2 (0.4)	1 (0.4)	
Caste			
Dalit/ Harijan	91 (20.0)	50 (17.9)	P = 0.479
Others	363 (80.0)	229 (82.1)	
Walls			
Brick	356 (78.4)	226 (81.0)	P = 0.4
Others	98 (21.6)	53 (19.0)	
Surveillance group			
Active	217 (47.8)	156 (55.9)	P = 0.03
Passive	237 (52.2)	123 (44.1)	
Cooking Fuel			
Wood	401 (88.3)	235 (84.2)	P = 0.11
Others	53 (11.7)	44 (15.6)	
Education of Mother			
Illiterate	225 (49.6)	126 (45.2)	P = 0.25

Primary or more	229 (50.4)	153 (54.8)	
Education of Father[4]			
Illiterate	129 (28.6)	88 (31.8)	P = 0.37
Primary or more	322 (71.4)	189 (68.2)	

1: Active group participants were those who continued in the study during two years follow-up.

2: Participants withdrew from the study during the two years follow-up.

3: Age of the participant at the time of enrolment i.e. at Day 0.

4: For 5 participants the education of father was missing data

6. Discussion:

6.1. Validity:

6.1.1. Internal Validity:

6.1.1.1 *Selection Bias:* All the study participants were school going children and were distributed evenly with respect to age and gender. The data regarding for some variables mentioned below were missing; education of mother in 1 participant, education of father in 3 participants, information regarding history of weight loss in one participant, night sweats in 1 participant, history of significant TB contact in 14 participants, recent TST result was not available for 1 participant and BMI for age Z – score was not available for 15 participants. We considered the missing data of these will not change the output in the analysis. All the participants had referral criteria, at the time of DGW visit. 5 participants attended the DGW directly, without referral letter from the field. These participants were considered as "self-referral" participants. Of these 5 participants, 3 had symptoms and 2 participants had history

of TB exposure which met our referral criteria. Among all the visits to DGW by a participant, we considered only latest visit in order to maintain even distribution and uniformity of the data.

6.2.1.2 *Information Bias:* The information regarding the clinical history was collected from participants themselves in 65%. For 27% of participants, it was the parents and remaining 8% it was grandparents or other relatives who provided the information.

Among 13 participants who attended the DGW before the restart of the study, as per old protocol the sputum was not collected because the chest x-ray was normal and the physician who examined the participant decided not to process the sputum sample for Acid fast bacilli smear or culture. So for these participants sputum samples were not collected. The data for these participants was considered as missing data. Among 609 participants, for 6 participants the Genotype Mycobacterium CM report for Mycobacterium tuberculosis complex was invalid. So for these 6 participants the data was considered as missing data. We have ignored the potential yield of these 19 participants (13 and 6 participants, total 19 participants) and considered the inclusion of these in the analysis will not change the output results.

For 4 participants whose sputum samples were contaminated (either day 1 or day 2), the uncontaminated sputum sample culture result was considered in the analysis.

The confounding for outcome measures was controlled with Binary logistic regression. The effect measures were adjusted for Socio economic status indicator (Walls type of the houses, cooking fuel used for cooking), gender, age, cough for more than or equal to 2 weeks, recent TST result, Body mass index Z – scores and recent contact with TB.

6.1.2. External Validity (Generalizability):

The study participants were all school going children residing in semi urban and rural areas. The results were internally valid among the participants, so the results can be applied to school going adolescent in India and other high TB prevalent countries.

The National family health survey (NFHS – 3), India (2005 – 06) had shown that only 41% of adolescents between age 15 – 17 years were attending the school in year 2005 – 06 (48). This study was conducted only in school going children, which restricts the generalizability of the results. We cannot apply the results for all the adolescent age group in India.

6.2: Sampling:

The participants were referred to the diagnostic ward (DGW) as part of the prospective cohort study. The schools in the study area were selected in a random sampling procedure to include in the study. The selected schools were assigned to either active or passive surveillance methodology after a stratified randomization procedure. After the randomization, eligible participants from schools were invited to participate in the study. A total of 6643 participants were enrolled and of this 609 participants attended the DGW.

The nonparticipation of participants to DGW for TB diagnostic evaluation, who had referral criteria, might have affected the output results. As the study is the part of vaccine trials, it was started with the main objective of looking at the incidence of definite TB in the study group. In the course of study the number of incident cases was less than the number that was anticipated before the start of study. So the referral criterion has been revised and instead of

TST more than 15 mm as per the old protocol, TST value of more than 10 mm was considered as referral criteria. Some of the participants have completed their two years follow-up by the time decision of this revision was taken. Due to this reason even though there were 1343 participants who had referral criteria, only 609 (45.4%) participants attended the DGW. The remaining 733 (54.6%) did not attended the DGW for the TB evaluation. The male participants were more likely to non-participate the DGW for TB evaluation compared to female participants. In other characteristics both the groups (Participants who attended the DGW and who didn't attend the DGW even though they were referred to DGW) were similar.

6.3. Main Results:

6.3.1: Definite and Probable Tuberculosis:

The present study had shown that 1.15 percentages of adolescent participants were diagnosed as definite TB**.** The proportion of probable tuberculosis among the participants was 0.5 %. Both probable and definite Tuberculosis together, accounts for 1.64 percentages. This proportion of the cases detected in our study was less compared to RNTCP estimation, in 2007 and what other studies had shown. 14% of TB suspects (Includes all age groups) in Chittoor district were sputum positive TB cases in 2007 where the study was conducted (31). The figure we got in the study might be the under estimation of the true proportion of TB in the community. This may be because we have taken only the school going children in the study, but the burden is more among the school dropout children. There might be one more reason for this under estimation of the true result, that is, most of the participants who were referred to the DGW did not show up for the TB diagnostic tests.

The study conducted among tribal population of Madhya Pradesh, in 2007 - 08 had shown that 4.4 percentage of the participants between 15 – 24 years of age group, who had symptoms suggestive of TB, were sputum positive(29). The wide gap in the above results with our results can also be explained by following reasons:

1. In RNTCP there are only symptomatic patients and there were no Tuberculin skin test positive patients. We have considered culture positive for M.*tb* as definite TB unlike only smear positive in RNTCP.
2. In RNTCP, the results apply to all age groups, not only in school going adolescent children.
3. The study conducted in Madhya Pradesh shows the results among tribal population which is demographically different from our study population.
4. The less proportion of the definite and probable Tuberculosis cases can be implicated to the uniqueness among the school going adolescent children.

In our study, the univariate analysis had shown that participants with presence of cough for more than or equal to 2 weeks had 19 times more chance to be diagnosed as definite TB and 9 times more chance to be diagnosed as either definite TB or probable TB. The retrospective study conducted by Wong et al, had shown that the adolescents who had cough for more than 4 weeks had an increased risk of acid fast bacilli smear positivity. The odds ratio was 13.8 with 95% C.I = 2.3 – 83.1 (49). Unlike the earlier study, in our study we used culture and molecular DNA test to diagnosis pulmonary TB. Even then our findings are similar to the previous studies. The review article by Cruz AT and Starke JR also states that among adolescents cough is more common symptom(19). This supports that among adolescents cough more than two weeks is characteristic symptom.

In our study the univariate analysis has shown that presence of recent weight loss at the time of diagnostic ward visit among participants is one of the characteristic symptoms for diagnosis of pulmonary TB among adolescents. They are 18 times more likely to be diagnosed as definite TB and have 17 times more chance to be diagnosed as either definite TB or probable TB. This finding in our study is similar to the previous papers (19).

Fever for more than 2 weeks also found to be an important symptom for tuberculosis among our study population. The baseline disease survey conducted in South India has shown that, fever for more than 1 month doesn't have significant role in diagnosing pulmonary tuberculosis(50). The study group in this survey was aged more than 15 years which is different from our study. This paper didn't discuss the presence of recent loss of weight and night sweats among the pulmonary TB cases. In our study the participants with night sweats for more than two weeks were found to be more likely to be diagnosed as definite TB or both definite and probable TB. But the lower limit of 95% confidence interval is nearer to 1, which denotes that association is weak.

We couldn't elicit any other association among participants who were diagnosed as definite TB or probable TB with characteristics like gender, age, TB exposure, socio economic status indicators, tuberculin skin test result and body mass index for age Z – score. The study conducted by Kolappan C et al among individuals aged more than 15 years, in South India had shown that males have 2.5 times more chance to be diagnosed with pulmonary TB compared to females(51). The same finding was not observed in our study. This can be implicated to the peculiarity of the characteristics of school going adolescent age group. The matched case – control study conducted in St. John's Medical college hospital, Bangalore have found that lack of separate cooking facilities was a risk factor for tuberculosis and

higher education level was significantly protective against tuberculosis(52). Because of this we looked at the education levels of both parents of the participants and the association with definite TB and either definite or probable TB and we didn't find any association. The study conducted by Mishra VK et al. in 1992 - 93 found that biomass cooking fuel increases the risk of tuberculosis (53). In our study we didn't find any association between the biomass cooking fuel and Tuberculosis.

We found that there were three participants who didn't have any symptoms at the time of DGW visit but were diagnosed as definite TB. This can be attributed to the primary infection of tuberculosis which may have progressed to disease without symptoms. This can be implicated to shortest time interval between primary tuberculosis infections to the development of disease which is more common among adolescents with minimal or no symptoms at starting of the disease as described by B.J Marias, R.P. Gie et al (IJTLD – 2004) (54).

6.3.2. Nontuberculous Mycobacteria (NTM) isolates:

In our study we found that 19.2 % of the participants' sputum samples were positive for Nontuberculous Mycobacteria (NTM). This is much higher than the previous studies, which had shown the isolation rates of NTM in south India and North India (9, 11, 13). The study conducted by Karak et al in Calcutta, India has reported that 17.4% of sputum specimens from fibrocavitary lung diseases have yielded NTM (55). The high ambient temperature present in major parts of South India encourages the growth of Mycobacteria in the environment (9, 11). Because of this, confirmation of the NTM infection must be done with positive culture from at least two separate sputum samples (4). But in our study the NTMs were isolated from single occasion. None of the participants whose culture results positive for

NTM had chest X- ray abnormalities. So as with other studies we consider that these NTM are either by chance or by the contamination of sputum samples by environmental Mycobacteria (11). These isolates therefore had no clinical significance.

In our study we found that male had 2 times more risk of getting infected with NTM as compared to females. The same finding is also found in previous studies (14). This gender difference can be attributed to the wide exposure of the male to the environment compared to females in South India because of the social and economic disparities. The studies have also shown that increasing age is a risk factor for the NTM infection (14). But in our study we couldn't find any association with increased age from 11 years to 20 years among the adolescent participants.

We found that the participants belonging to Dalit community have a 2 times higher chance of getting infected with NTM compared to participants belonging to other communities. This is because of low levels of hygiene in the same population. This can be attributed to the low socio-economic status of the Dalit community population in South India (NFHS -3, 2005 – 06).

The high proportion of particular species like M.*fortuitum, M.intracellulare* and M.*scrofulaceum* in our studies is similar to the rate of the isolates in other earlier studies (11-12, 55). This finding in our study signifies that the NTM prevalence among adolescent age group is similar to the general population in South India.

The current study has found that the participants who had haemoptysis for more than 2 weeks have 5 times higher chance of producing NTM isolates from the sputum samples. These two participants yielded M.*fortuitum* and M.*intracellulare* from the sputum samples. These

Mycobacteria might be responsible for producing haemoptysis in those participants. It is known that M.*intracellulare* and M.*fortuitum* are pathogenic Mycobacteria and produces pulmonary disease (4). But in our study we didn't repeat the test to isolate the same species from another sputum sample, so the significance of these Mycobacteria can't be ascertained.

6.4. Conclusion:

6.4.1. For Research:

6.4.1.1: Mycobacterium *tuberculosis*:

Even though the current study has shown a lower proportion of TB disease among the school going adolescents in South India, there must be further studies to ascertain the actual burden among all the adolescent age groups which includes the adolescents who do not go to school.

6.4.1.2. Nontuberculous Mycobacteria:

The NTM detected in our study had no clinical significance. There must be further studies to find out the clinical significance of NTM among the adolescent age groups with confirmation of NTM infection according to the clinical, microbiological and radiological criteria.

6.4.2. For practice/ policy:

6.4.2.1: Mycobacterium *tuberculosis*:

The National Tuberculosis control programmes have to be strengthened among the school going adolescent age groups because of the vulnerability of the adolescents towards Tuberculosis disease.

6.4.2.2. Nontuberculous Mycobacteria:

The NTM is more common among the Dalit community; this might be because of their living conditions, which have to be improved. The findings in our study suggest that the NTM

situation among adolescent is not different from the general population in South India. The exposure to the NTM must be considered during the conduction of new vaccine trails among the adolescents, because it may affect the vaccine efficacy.

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Appendix A - DAY 0 Questionnaires:

Enter # recorded on page 3 only if child is enrolled in study.
PID # ___ ___ -- ___ ___ ___

INFORMED CONSENT PROCESS

Date of consent discussion: __ __ / __ __ / __ __ __ __
(d d / m m / y y y y)

Village name	**Parent's name**	**Address and Contact details**
_____ **School/College name**	**Adolescent's Details** **Reported Age:_____ _____Yrs** **Initials:_____ _____ _____**	Land line:_____ Mobile:_____ E-mail:_____

Was ADEQUATE TIME given for parent/ 18 year old adolescent's consideration, questions, and answers?	☐ YES		☐ NO
Does parent/ 18 year old adolescent express understanding of the PURPOSE of the study?	☐ YES		☐ NO
Does parent/18 year old adolescent express understanding that participation is VOLUNTARY?	☐ YES		☐ NO
Does parent/ 18 year old adolescent express understanding WHAT PARTICIPATION INVOLVES?	☐ YES		☐ NO
Does parent/18 year old adolescent express understanding of the difference between ACTIVE and PASSIVE follow-up?	☐ YES		☐ NO
Does Parent know that their child will also be discussed **separately** about the study and their participation? (Check NA in the case of 18 old adolescent)	☐ NA	☐ YES	☐ NO
Does parent/18 year old adolescent give permission to review the school and or other records for an official date of birth?	☐ YES		☐ NO
Was Informed Consent signed? If YES, date consent signed: __ __ / __ __ / __ __ __ __ (d d / m m / y y y y)	☐ YES		☐ NO
If NO, record the reason			
Record approximate length of time taken to complete Informed Consent Process.			

_____
Name of study team member who conducted consent process

_____
Signature of study team member who conducted consent process *Date*

INFORMED ASSENT PROCESS

Study Team Member Code
☐☐☐

(Only if adolescent is less than 18years of age)

Date of assent discussion: __ __ / __ __ / __ __ __ __ (d d / m m / y y y y) Or ☐ NA

Version of the consent used:

☐ Original/ same language as consent form *(for the age group between 15-17)*

☐ Simplified version of the consent *(for the age group between 12-14)*

Was ADEQUATE TIME given for adolescent's consideration, questions, and answers?	☐ YES	☐ NO
Does adolescent express understanding of the PURPOSE of the study?	☐ YES	☐ NO
Does adolescent express understanding that participation is VOLUNTARY?	☐ YES	☐ NO
Does adolescent express understanding WHAT PARTICIPATION INVOLVES?	☐ YES	☐ NO
Does adolescent express understanding of the difference between ACTIVE and PASSIVE follow-up?	☐ YES	☐ NO
Is adolescent interested to participate?	☐ YES	☐ NO
Was Informed Assent signed? If YES, date assent signed: __ __ / __ __ / __ __ __ __ (d d / m m / y y y y)	☐ YES	☐ NO
If NO, record the reason		
Record approximate length of time taken to complete Informed assent Process.		

_____

Name of study team member who conducted assent process

_____

Signature of study team member who conducted assent process *Date*

Study Team Member Code

☐☐☐

SCREENING and ENROLLMENT

Date of screening/enrollment (Study Day 0): __ __ / __ __ / __ __ __ __ (d d / m m / y y y y)		
Adolescent's date of birth __ __ / __ __ / __ __ __ __ (d d / m m / y y y y)	**Adolescent's Gender** **M or F** _____	**Name of the School/College** **Grade(7 to 12)**:_____ _____

Was copy of signed consent given to parent or 18 year old adolescent?	☐ YES	☐ NO
Was copy of signed assent given to adolescent?	☐ NA ☐ YES	☐ NO
Does family plan to live within study area for next 2 years?	☐ YES	☐ NO
Is adolescent able to attend follow-up visit for reading Mantoux test?	☐ YES	☐ NO
Does adolescent meet all eligibility criteria?	☐ YES	☐ NO

If **YES**, record sequentially assigned **PID** # ____ ____ - ____ ____ ____

Surveillance group assigned to: ❒ ACTIVE

❒ PASSIVE

If NO, do not assign a PID #; Record reason not enrolled:

Give parent completed Study Participant ID Card. Review the contact information noted on the card with them. Tell parent and or participant to bring the PID card and show when ever they contact the physician or the study staff.

The adolescent is considered enrolled in the study when the PID # is assigned. From this point forward, use the initials and the PID # as identifiers of the adolescent.

_____

Name of study team member who conducted screening and enrollment process

_____

Signature of study team member who conducted screening and enrollment process *Date*

STUDY DAY 0

Socio-economic factors:

Religion:

- ❑ Hindu
- ❑ Muslim
- ❑ Christian
- ❑ Sikh
- ❑ Jain
- ❑ Buddhist
- ❑ Other; specify: _____
- ❑ Not answered

Caste:

- ❑ Dalit/Harijan
- ❑ Others
- ❑ Not answered

Highest level of education attained:

Mother:

- ❑ Illiterate
- ❑ Primary
- ❑ Secondary
- ❑ High school
- ❑ Higher secondary
- ❑ College
- ❑ Not answered

Father:

- ❑ Illiterate
- ❑ Primary
- ❑ Secondary
- ❑ High school
- ❑ Higher secondary
- ❑ College
- ❑ Not answered

Occupation:

Mother: _____ Or, ❑ Not answered

Father: _____ Or, ❑ Not answered

Completed by Headquarters

☐☐

☐☐

What is the total monthly household income? _____ Rupees Or, ❑ Not answered

What are the walls of the house made of?

- ❑ Packed mud
- ❑ Stone
- ❑ Bamboo
- ❑ Thatch
- ❑ Wood
- ❒ Brick
- ❑ Other; specify: _____
- ❑ Not answered

Does the house have an electricity connection?

- ❑ Yes
- ❑ No
- ❑ Not answered

Which type of fuel is used as major cooking fuel?

- ❑ LPG
- ❑ Electricity
- ❑ Wood
- ❑ Kerosene
- ❑ Coal
- ❑ Agricultural residue
- ❑ Others, Specify_____

Study Team Member Code

□□□

STUDY DAY 0(continued)

Details of BCG Immunization:
Was participant immunized with **BCG**?

- ❑ Yes
- ❑ No
- ❑ Not known
- ❑ Not answered

History of TB disease *Any YES answer either from the parent or from the participant has to be considered as positive*	Parent interview Study Team Member Code ☐☐☐	Participant interview Study Team Member Code ☐☐☐
Did your child/you have TB disease in the past	❑ Yes, indicate the age of the child____ ❑ No ❑ Not known	❑ Yes ❑ No ❑ Not known
Is your child/ Are you currently taking TB medications ***If* YES *Mantoux should not be applied***	❑ Yes ❑ No ❑ Not known	❑ Yes ❑ No ❑ Not known
History of **positive TB contact** (for more than 8 hrs/week)	❑ Yes ❑ No ❑ Not known	❑ Yes ❑ No ❑ Not known
Symptoms <u>for more than two weeks</u> Unexplained Cough	❑ Yes ❑ No	❑ Yes ❑ No
Unexplained Weight loss	❑ Yes ❑ No	❑ Yes ❑ No
Unexplained Fever	❑ Yes ❑ No	❑ Yes ❑ No
Unexplained Night sweats	❑ Yes ❑ No	❑ Yes ❑ No
Haemoptysis(Blood seen in mucous when cough)	❑ Yes ❑ No	❑ Yes ❑ No
Hospital admission(in the past six months for any illness)	❑ Yes ❑ No	❑ Yes ❑ No

STUDY DAY 0 (continued)

Diagnosis of any acute or chronic diseases in the past	Parent interview	Participant interview
Gastro enteritis	❑ Yes ❑ No ❑ Not known	❑ Yes ❑ No ❑ Not known
Pneumonia	❑ Yes ❑ No ❑ Not known	❑ Yes ❑ No ❑ Not known
Meningitis	❑ Yes ❑ No ❑ Not known	❑ Yes ❑ No ❑ Not known
Asthma	❑ Yes ❑ No ❑ Not known	❑ Yes ❑ No ❑ Not known
Diabetes	❑ Yes ❑ No ❑ Not known	❑ Yes ❑ No ❑ Not known
Anemia	❑ Yes ❑ No ❑ Not known	❑ Yes ❑ No ❑ Not known
Others	❑ Yes ❑ No If **YES** specify: _____	❑ Yes ❑ No If **YES** specify: _____
If any of the above answers is YES then check YES otherwise check NO	❑ Yes ❑ No	❑ Yes ❑ No

Name of family doctor: _____ Or, ❑ None

Location: _____

STUDY DAY 0 (continued)

Physical measurements

Weight: ___ ___ ___. ___ Kg

Height: ___ ___ ___. ___ cm

Presence of BCG scar

Whether **BCG** scar is present?

- ❑ Yes
- ❑ No

Blood Draw for Research of Immunology of TB

Draw the blood before applying the Mantoux. Blood should be collected and labeled as per instruction. ***Not more than two trails*** *should be made for the blood draw.*

Date of blood draw:___ ___/ ___ ___/ ___ ___ ___ ___
dd mm yyyy

Time of blood draw *(use 24 hour clock)*: ___ ___ : ___ ___
hh mm

Number of CPT tubes used for blood collection:

- ❑ 1
- ❑ 2
- ❑ 3

If the number of tubes is less than 3 fill the Protocol Deviation Form. CPT tubes should be kept in the cool box to maintain temperature at 2-8°C and transport to the Lab within 4 hours from the time of collection.

Blood collected for Serum preparation:

- ❑ Yes
- ❑ No

Study Team Member Code

☐☐☐

Hemoglobin concentration:

____ ____ - ____ g/dL

Study Team Member Code

☐☐☐

STUDY DAY 0(Continued)

Tuberculin skin test: *(If the adolescent is taking TB medication then check NA. Test will be read within 2 – 4 days after application. Test should be applied after the blood draw.)*

Date applied: __ __ / __ __ / __ __ __ __ (d d / m m / y y y y) Or ❑ NA

Study Team Member Code ❑❑❑

Time applied *(use 24 hour clock)*:__ __ : __ __ (h h : m m)

Date read: __ __ / __ __ / __ __ __ __ (d d / m m / y y y y)

Study Team Member Code ❑❑❑

Reading time *(use 24 hour clock)*:__ __ : __ __ (h h : m m)

Result *(Record widest transverse measurement in whole number)*: ___ ___ mm

*If the Tuberculin skin test reaction is less than 5mm apply the second test 1-4 weeks a*fter the first test was applied to the opposite arm. The larger
will be used as baseline value.

_____

Name ***Signature***

Repeat Tuberculin skin test *(If the first test result is less than 5mm repeat the test 1-4 weeks after the first test was applied to the opposite arm)*

Date applied: __ __ / __ __ / __ __ __ __ (d d / m m / y y y y) Or ❑ NA

Study Team Member Code ❑❑❑

Time applied(*(use 24 hour clock)*:__ __ : __ __ (h h : m m)

Date read: __ __ / __ __ / __ __ __ __ (d d / m m / y y y y)

Study Team Member Code ❑❑❑

Reading time *(use 24 hour clock)*:__ __ : __ __ (h h : m m)

Result *(Record widest transverse measurement in whole number)*: ___ ___ mm

The larger result will be used as baseline value.

_____

Name ***Signature***

Baseline *(write the first tuberculin skin test result as the baseline value if it is more than 5mm otherwise compare first and second test results; use the larger result as baseline value.)*

Baseline value:___ ___ mm

STUDY DAY 0 (continued)

Sputum Collection:

Is the participant able to provide spot sputum sample?

❑ YES
❑ NO, provide the sputum container with appropriate label, PID# and participant initials; and (including instructions for sputum collection)

Date of Early morning Sputum Collection: __ __ /__ __/ __ __ __ __ Or ❑ NA

***The date on which the baseline evaluations such as Blood collection, TST and or the sputum are made is considered as* Study Day 0.**

If the adolescent has been enrolled in the ACTIVE group, remind adolescent of the date for the next scheduled study visit, Study Day 90.

Adolescents enrolled in the PASSIVE group will not have a scheduled study visit until Study Day 720.

STUDY DAY 0 (continued)

Referral for TB evaluation

Was participant referred to TB evaluation?

❑ Yes, check the reason for Referral

❑ Positive symptoms and Positive TST

❑ Positive AFB smear

❑ No

If **YES**, has the zone supervisor been informed? ❑ Yes ❑ No

Date of referral: __ __ / __ __ / __ __ __ __
dd mm yyyy

Has referral slip been sent for TB evaluation? ❑ Yes ❑ No ❑ Not known

Education

Was a list of TB symptoms given to Adolescent and explained about the need to seek treatment?

❒ Yes
❒ No

Reminder to adolescent

Adolescent will be reminded to inform their parents and contact either study staff or their family doctor or a TB clinic or study office (contact number printed on Study ID Card) if they have any of these symptoms in future as:

(Check to indicate adolescent was reminded)

❒ Unexplained cough for two weeks or more
❒ Unexplained weight loss for two weeks or more
❒ Unexplained fever for two weeks or more
❒ Unexplained night sweats for two weeks or more
❒ Haemoptysis

STUDY DAY 0 NOTES: _____

_____

_____

_____

_____

_____

_____

Name ***Signature*** ***Date***

Study Team Member Code

❑❑❑

Appendix B – DGW Questionnaires:

DIAGNOSTIC VISIT

Date of Diagnostic visit: __ __ / __ __ / __ __ __ __
d d m m y y y y

Referral from *(check one)*: ❒Self-referral

❒ Active follow-up visit

❒ Passive surveillance *(check one)*:

❒ Hospital/primary health centre/private practitioner

❒ Microscopy centre; ❒ Emmaus Swiss Hospital
❒ Other

❒ Field study team *(between scheduled visits for Active group, or passive surveillance for Passive group)*

❒ Study Day 0
❒ Positive AFB smear
❒ Positive symptoms and Positive TST

❒ TST conversion

Study Team Member Code
❒❒❒

Physical assessment *(Place leading zero if necessary.)* **Date of assessment** __ __ / __ __ / __ __ __ __
d d m m y y y y

Weight ___ ___ ___ . ___ kg

Height ___ ___ ___ . ___ cm

Temperature ___ ___ . ___ °C

Heart rate ___ ___ ___ bpm

Blood pressure ___ ___ ___ mm of Hg

Mid-arm circumference ___ ___ . ___ cm

DIAGNOSTIC VISIT (continued)

Participant interview

Date of interview __ __ / __ __ / __ __ __ __
d d m m y y y y

Is participant experiencing:

Recent illness
❒ Yes; specify_____ How many days _____
Treated: ❒ Yes; specify treatment _____
❒ No
❒ No

Recent loss of weight ❒ Yes
❒ No

Cough ❒ Yes; how many days _____
Treated: ❒ Yes; specify treatment _____
❒ No
❒ No

Fever ❒ Yes; how many days _____
Treated: ❒ Yes; specify treatment _____
❒ No
❒ No

Haemoptysis ❒ Yes; how many days _____
Treated: ❒ Yes; specify treatment _____
❒ No
❒ No

Night sweats ❒ Yes; how many days _____
Treated: ❒ Yes; specify treatment _____
❒ No
❒ No

Was the child diagnosed with TB prior to diagnostic visit?
❒ Yes, if Yes is the participant currently taking any TB medication?,
❒ Yes; number of days already on treatment: _____ days
(Record on TB Treatment Record case report form.)
❒ No
❒ No

DIAGNOSTIC VISIT (continued)

Significant exposure (e.g. approximately 8 hours) to an individual on TB treatment

❒ Yes
❒ No

Respondent/source of information *(check all that apply)*:
❒ Mother
❒ Father
❒ Grand parent
❒ Adolescent
❒ Other; specify:

_____

Study Team Member Code
□□□

Brief review of systems: physical exam

HEENT ❒ Normal ❒ Abnormal, specify:
_____

Dermatologic ❒ Normal ❒ Abnormal; specify:
_____

Neurologic ❒ Normal ❒ Abnormal; specify:
_____

Respiratory ❒ Normal ❒ Abnormal; specify:
_____

Cardiovascular ❒ Normal ❒ Abnormal; specify:
_____

Gastrointestinal ❒ Normal ❒ Abnormal; specify:
_____

Urogenital ❒ Normal ❒ Abnormal; specify:
_____

Musculoskeletal ❒ Normal ❒ Abnormal; specify:
_____

Failure to thrive (FTT)? ❒ Yes ❒ No

Study Team Member Code
□□□

_____

Name ***Signature***

DIAGNOSTIC VISIT (continued)

Chest radiograph *(A-P)* **Date:** __ __ / __ __ / __ __ __ __
d d m m y y y y

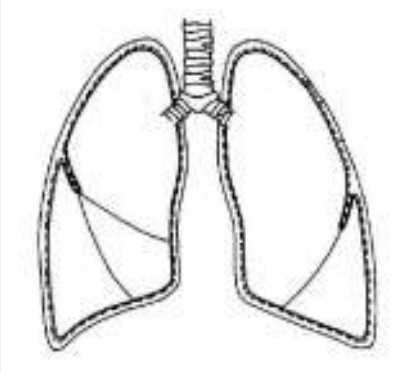
- ❒ Normal
- ❒ Abnormal, TB
- ❒ Abnormal, not TB

Impression

Apex:

Hilar:

Mid-zone:



Right Left

Lower zone:

NOTES:

_____
_____
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_____
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_____
__

Name ***Signature*** ***Date***

Study Team Member Code

❒❒❒

DIAGNOSTIC VISIT (continued)

*If the chest X-ray result is '**Abnormal, TB'**, then fill the following section otherwise, check **'Not applicable'** and fill the diagnostic visit summary form at the end.*

Sputum Smear ❒ Not applicable

1 of 2: Sputum

Date obtained: __ __ / __ __ / __ __ __ __
d d m m y y y y

__ ❒ Natural
❒ Induced

Consistency:
❒ Salivary
❒ Mucoid
❒ Muco purulent
❒ Blood stained

Result:
❒ Positive; specify:
❒ Scanty
❒ 1+
❒ 2+
❒ 3+
❒ Negative

NOTES:
_____
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Name ***Signature*** ***Date***

DIAGNOSTIC VISIT (continued)

2 of 2: Sputum

Date obtained: __ __ / __ __ / __ __ __ __
d d m m y y y y

❒ Natural
❒ Induced

Consistency:

❒ Salivary
❒ Mucoid
❒ Muco purulent
❒ Blood stained

Result:

❒ Positive; specify:
- ❒ Scanty
- ❒ 1+
- ❒ 2+
- ❒ 3+

❒ Negative

NOTES:

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__

Name ***Signature*** ***Date***

DIAGNOSTIC VISIT (continued)

CULTURE

1 of 2: Sputum

Date obtained: __ __ / __ __ / __ __ __ __
dd mm yyyy

- ❒ Positive
- ❒ Negative
- ❒ Contaminated

If culture result is positive, record speciation. Otherwise, check Not applicable.

❒ Not applicable *(Check if culture was negative; Do not complete below.)*

HAIN:

MTB	❒ Positive	❒ Negative
MOTT	❒ Positive	❒ Negative
BCG disease	❒ Positive	❒ Negative

If culture result is MTB positive, record drug sensitivities. Otherwise, check Not applicable.

❒ Not applicable *(Check if culture was MTB negative or contaminated; Do not complete below.)*

Drug sensitivities:

Streptomycin	❒ Sensitive	❒ Resistant
Isoniazide	❒ Sensitive	❒ Resistant
Rifampicin	❒ Sensitive	❒ Resistant
Ethambutol	❒ Sensitive	❒ Resistant

NOTES:

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Name ***Signature*** ***Date***

DIAGNOSTIC VISIT (continued)

CULTURE

2 of 2: Sputum

Date obtained: __ __ / __ __ / __ __ __ __
d d m m y y y y

- ❒ Positive
- ❒ Negative
- ❒ Contaminated

If culture result is positive, record speciation. Otherwise, check Not applicable.

❒ Not applicable *(Check if culture was negative; Do not complete below.)*

HAIN:

MTB	❒ Positive	❒ Negative
MOTT	❒ Positive	❒ Negative
BCG disease	❒ Positive	❒ Negative

If culture result isMTB positive, record drug sensitivities. Otherwise, check Not applicable.

❒ Not applicable *(Check if culture was MTB negative or contaminated; Do not complete below.)*

Drug sensitivities:

Streptomycin	❒ Sensitive	❒ Resistant
Isoniazide	❒ Sensitive	❒ Resistant
Rifampicin	❒ Sensitive	❒ Resistant
Ethambutol	❒ Sensitive	❒ Resistant

NOTES:

_____
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Name ***Signature*** ***Date***

DIAGNOSTIC VISIT (continued)

Instructions for blood draw:

- A blood draw of 29 ml would occur at the time of TB diagnosis, for **those diagnosed with TB,** provided that it was at least three months since the previous blood draw.
- Participants may have additional 10 ml blood drawn as part of the tuberculosis diagnostic process to assess clinical parameters prior to starting treatment (e.g., CBC or serum chemistry).

Is a blood draw required for participant?

❑ Yes, check if ❑ 29 ml
❑ 10 ml
❑ both 29 ml and 10 ml
❑ No, Do not complete the following section

(To be filled by Staff Nurse only)

Date of blood draw:___ ___/ ___ ___/ ___ ___ ___ ___ (dd / mm / yyyy) Or ❑ Not applicable

Time of blood draw *(use 24 hour clock)*: ___ ___ : ___ ___ (hh : mm)

Blood Draw for Research of Immunology of TB

Blood should be collected and labeled as per instruction. ***Not more than two trails*** *should be made for the blood draw.*

Number of CPT tubes used for blood collection:

Check 'Not applicable' if only 10 ml blood has to be collected

❑ 1
❑ 2
❑ 3
❑ Not applicable

If the number of tubes is less than 3 fill the Protocol Deviation Form. CPT tubes should be kept in the cool box to maintain temperature at 2-8°C and transport to the Lab within 4 hours from the time of collection.

Blood collected for clinical parameters assessment:

❑ Yes
❑ No
❑ Not applicable

Study Team Member Code

☐☐☐

Summary of Diagnostic Process Date: __ __ / __ __ / __ __ __ __
d d m m y y y y

TB medication

One-week supply given ❒ Yes ❒ No ❑ Not applicable

Treatment started prior to discharge ❒ Yes ❒ No ❑ Not applicable

If medication supply is given, or if treatment has been started prior to discharge, inform zone supervisor in order to record on TB Treatment Record.

Follow-up Letters

Parent(s) or guardian will be provided a letter with all known test results (e.g., CXR, initial smear result) and an explanation of their meaning, and instructions to contact the family doctor or local health clinic for follow-up if child was diagnosed with TB. The letter will indicate that a follow-up letter will then be sent if any results are pending; a follow-up letter will then be sent to the parent(s) with the remaining results (e.g., culture) and diagnosis.

If the parent has specified a primary care doctor, letter(s) will also be sent to the doctor at the same time of the letter(s) sent to the parent(s).

Only if the child is diagnosed with TB, the local TB officer will receive a letter describing the diagnostic results, the diagnosis, and treatment recommendation.

Letter sent / provided to:

Parent: Initial ❒ Yes ❒ No; reason _____
Follow-up ❑ Pending ❑ Not applicable

Primary care doctor: Initial ❒ Yes ❑ Not applicable
Follow-up ❑ Pending ❑ Not applicable

Local TB officer (upon diagnosis) ❒Yes ❑ Pending ❑ Not applicable

DIAGNOSTIC PROCESS SUMMARY:_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Name ***Signature*** ***Date***

Study Team Member Code

❒❒❒

TB TREATMENT RECORD

☐ **Check if TB treatment is NEVER GIVEN to the adolescent during the entire 2-year study period. If treatment is given, complete the information below. Use an additional TB Rx form if necessary.**

	Treatment Record one drug per line using full generic name.	Start date dd mm yyyy	Stop date dd mm yyyy	Treatment prescribed by Record role and affiliation, and name if known
Record treatment started PRIOR to admission to Diagnostic visit in this section.				
1				
2				
3				
4				
5				
6				
Record treatment started AFTER Diagnostic visit in this section.				
1				
2				
3				
4				
5				
6				

Treatment Outcome *(Check one.)*

- ❒ Cured
- ❒ Treatment completed
- ❒ Died
- ❒ Failure
- ❒ Defaulted
- ❒ Transferred out

NOTES:

_____

_____

__

_____

__

_____

__

Name ***Signature*** ***Date***

Study Team Member Code

❒❒❒