Journal of Bacteriology and Parasitology
ISSN: 2155-9597

Verso et al., J Bacteriol Parasitol 2017, 8:5
DOI: 10.4172/2155-9597.1000324

Research Article
OMICS International

Screening for Latent Tuberculosis Infection among Students of Healthcare Professions and Postgraduates of the Faculty of Medicine of the University of Palermo

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Received date: September 27, 2017; **Accepted date:** October 19, 2017; **Published date:** October 26, 2017

Abstract

Introduction and objective: Italy is a country with a low incidence of tuberculosis and in the last fifty years the annual number of TB cases decreased from 12,247 to 4,418, showing a reduction of approximately 64% in the number of cases and 71% in incidence. Despite of this encouraging trend, in the last years the epidemiology of tuberculosis changed and today it is a re-emerging infectious. The aim of this study is to measure the prevalence of positivity to tuberculosis infection (latent TB) in students, without any obvious manifestation of disease, attending degree courses of the health care professions and postgraduate medical courses of the School of Medicine of the University of Palermo, Italy.

Materials and methods: A cross-sectional observational study in students of nursing, midwifery, dentistry degree courses and in resident physicians of postgraduate medical schools was carried out from January 2012 to July 2016. Mantoux test was performed and all positive cases were tested with Interferon-Gamma Release Assay (IGRA).

Results: Of the 1,351 subjects evaluated, 25 (1.8%) resulted positive to Mantoux test; in 17 students (1.2%) the diagnosis was confirmed with IGRA. Positive cases were significantly more frequent among students attending Postgraduated Medical School Courses ($p<0.001$) and were older than negative cases ($p<0.001$).

Conclusion: This study suggests that in our geographic area, latent TB shows a relatively low prevalence among students of medical schools. Despite of this evidence, and considering that several students have been found to be positive for TB, this infectious disease has to be considered a re-emerging biohazard that requires preventive strategies for the containment of the risk in exposed workers as well as in the general population.

Keywords: Medical and nursing students; Occupational biohazard; Quantiferon; Latent tuberculosis; Mantoux skin test

Introduction

Tuberculosis is a re-emerging infectious disease in several high-income countries. In Italy there is a paucity of updated data and the latest official data have been published by the Ministry of Health and include notifications recorded in 2008 [1,2]. In the last fifty years (1955-2008), the national reporting system observed that the annual number of TB cases in Italy decreased from 12,247 to 4,418; similarly the annual crude rate has lowered from 25.26 cases per 100,000 inhabitants to 7.41, showing a reduction of approximately 64% in the number of cases and 71% in incidence. The crude mortality rate decreased from 22.5 to 100,000 inhabitants in 1955 to 0.7/100,000 in 2006. Although these data could induce optimism, it is well known that TB is changing its trend and is currently re-emerging in our country.

In Italy, country with low incidence, TB control is performed through the prevention of the transmission of the bacterium from infectious persons, but also preventing the progression from latent TB to active disease in subjects with this condition.

In 2014, there were estimated 9.6 million new TB cases in the world: 5.4 millions among men, 3.2 millions among women and 1.0 million among children. There were also 1.5 million TB deaths (1.1 million among HIV-negative people and 0.4 million among HIV-positive people), of which approximately 890,000 were men, 480,000 were women and 140,000 were children [3]. World Health Organization (WHO) estimates that a third of the world's population guests Mycobacterium tuberculosis without any disease manifestation (latent TB) [4].

In immunocompetent subjects the risk of developing the active disease during the entire life is 10%, more probably during the first 2/5 years after exposure. A person with latent tuberculosis infection (LTBI) is a subject who came in contact with bacillus but, thanks to an

efficient immune response, did not develop the disease: is asymptomatic and not contagious.

TB is a professional risk for many workers, first of all health care workers.

The main tool for health surveillance of workers exposed to the TB contagious is the monitoring of latent tuberculosis infection through:

- The identification of infected persons to prevent the evolution from infection to disease.

- The review of the adequacy of the protocols.

- The reclassification of the risk levels.

According to the guidelines of the Italian Ministry of Health, published in 2009, the recommended procedure is based on the tuberculin test (TST) [5]. The procedure can be integrated using Interferon Gamma (IGRA) as confirmation of a positive TST. Exclusive use of TIG, if available, can be evaluated if the group in question has high rates of vaccination with BCG or are expected high rates of positive TST (Mantoux test). A positive TST was defined as an induration measuring ≥ 10 mm in healthy subjects. It is necessary to perform test IGRA (interferon-gamma release assay) in subjects with positive Mantoux test or in cases of lesser reliability testing (eg. vaccinated with BCG).

Finally, subjects with Mantoux test and IGRA positive are visited by the specialist (infectious disease specialist, pulmonologist).

The aim of this study is to measure the prevalence of positivity to tuberculosis infection in students without any obvious manifestation of disease, attending schools of the health care professions, or postgraduate medical courses of the School of Medicine of the University of Palermo, Italy.

According to the Italian laws, these students, performing the practical training in the various hospitals of the territorial training network, are considered workers exposed to biohazards, as well as physicians and nurses regularly employed and working in various departments, and therefore they regularly undergo health surveillance.

Materials and Methods

This retrospective observational study analyzed data collected as a consequence of the health surveillance carried out from January 2012-July 2016 in students of medicine, nursing, midwifery, dentistry degree courses and in resident physicians of postgraduate medical schools of the University of Palermo (specialization schools).

For each student, we compiled a clinical record, including information such as relatives' diseases and personal remote and proximate pathologies (anamnesis). Then, we conducted an objective exam and Mantoux test. Always, we asked the subjects the informed consent to the processing of data; for this study, we obtained the Local Ethic Committee the approval, despite it's not required in Italy for observational retrospective studies [6].

Information on the following variables was collected using anamnesis' data: age, sex, nationality, incidence of the disease in the country of origin (low/medium/high), parental history of TBC, degree or specialization and year (with caution if the student had already carried out training courses in hospital or not), any previous vaccination for TB (BCG vaccination). Tuberculin Skin Test (TST) was performed by trained personnel following standard procedures: 0.1 ml of purified protein derivative (Tubertest®; Sanofi Pasteur, Lyon, France) was injected to each participant subject [5]. The TST was administered to the volar side of the forearm of the participants and read 48-72 h after the application by experienced personnel. All TST positive cases were also tested with an Interferon-Gamma Release Assay (IGRA; QuantiFERON® TB-Gold Cellestis, Victoria, Australia) to confirm the diagnosis of LTBI, because of its major specificity compared with conventional TST.

The QFT (Quantiferon TB-Gold) is performed on peripheral blood collected directly in the 3 vacuum tubes, 1 ml per vacuum tube. The tubes are: Nil (negative control in which there is no stimulus), TB antigen (in which there are peptides from ESAT-6, CFP10 of RD1 region and TB7.7 antigen from RD11 region), Mitogen (positive control in where is the phytohemagglutinin, as T lymphocyte stimulator, to verify that the patient is not immunodepressed). The tubes containing the blood are then taken to the laboratory and incubated at 37°C and processed after 16-20 hours. The measurement of IFN-γ production occurs through an immuno-enzymatic system (ELISA).

The null control must be less than or equal to 8.0 IU/ml and the mitogenic value should be equal to or greater than 0.5 IU/ml, or the value of the TB-null antigen must be equal to or greater than 0.35 IU/mL to obtain a valid result for Quantiferon TB gold in test tubes. The data analysis is performed by reporting the optical density values of the standards and the samples used.

The cut-off value for the Quantiferon TB gold test is 0.35 IU/ml greater than the null control for the plasma sample stimulated with the TB antigen: an IFN-γ ≥ 0.35 IU/ml (TB antigens minus negative control) was considered a positive test. Subjects who have an antibody TB response above the cut-off rate are probably infected [7].

The measured IFN-γ concentration level is not correlated with the stage or degree of infection, the immunological response level, or the probability of progression of the disease. A positive test does not necessarily indicate the presence of active tuberculosis.

The students IGRA positive were examined by an infectious diseases specialist and underwent chest radiography, to determinate a possible active infection.

Finally, the data were statistically analyzed, in order to identify possible relations with conditions who may become real risk factors (such as age, gender and country of origin) on the basis of statistical significance (p-value <0.05). Absolute and relative frequencies were calculated for qualitative variables, while quantitative variables were summarized as mean (standard deviation; SD) when normally distributed or otherwise as median (interquartile range; IQR). Data normality was verified by the Shapiro–Wilk test for normality. Categorical variables were analysed using chi-squared test (Mantel-Haenszel) or Fisher's exact test. Medians and means were compared by using the Mann–Whitney or Student t test. Statistical analysis was performed with R software version 3.3.2 (October 2016).

Results

The main characteristics of the 1,351 subjects included in the study are shown in (Table 1).

As shown in (Table 1), there was a higher prevalence of women in the observed sample and of students of Postgraduate Medical Courses; almost all enrolled subjects were born in Italy, except for two students

of the Nursing Degree Course: a 18 years old girl coming from Madagascar, negative to Mantoux test, and a 21 year old boy, born in Poland, positive to Mantoux test, but negative to IGRA Assay TB Gold. This latter subject referred to have been vaccinated in his country of origin. Twenty-five subjects resulted positive to Mantoux test; in 8 cases IGRA test was negative, probably due to previous vaccination with BCG, whose efficacy is not optimal in all vaccinated subjects. Overall, 1.8% subjects resulted positive in Mantoux test, but only 1.2% were confirmed by IGRA test. Students who resulted positive in both tests were examined by an infectious diseases specialist and underwent chest radiography, to determinate a possible active infection. Each of them was diagnosed with latent TBC and given an indication to undergo chemoprophylaxis. Only 2 subjects adhered to the drug treatment and follow-up, probably scared by the hepatotoxicity of antituberculous drugs.

	Total of students
Total, n (%)	1,351 (100)
Sex, n (%)	
- Males	494 (36.6)
- Females	856 (63.4)
Age in years, mean ± SD	25.7 ± 5.6
Country of birth, n (%)	
- Italy	1,349 (99.9)
- Other	2 (0.1)
University course, n (%)	
- Medical School courses	862 (63.8)
- Postgraduate Medical School courses	489 (36.2)
University training	
- Clinical	974 (72.1)
- Preclinical	377 (27.9)
Tb vaccination	
- Yes	69 (5.1)
- No	1,282 (94.9)
Mantoux tuberculin skin test positivity	
- Yes, with negative IGRA test	8 (0.6)
- Yes, with positive IGRA test	17 (1.2)
- No	1,326 (98.2)

Table 1: General characteristics of subjects (N=1,351) included in the study.

In table 2 are shown factors potentially associated to skin positivity in our sample.

Analyzing the characteristics of positive subjects to Mantoux skin test, although the low percentage found in enrolled students, we can affirm that subjects with positive tuberculin reaction were statistically significantly older (31.0 vs. 25.6 years, p<0.001) and attended postgraduate medical school courses (p<0.001). Of course, among vaccinated students there was a statistically significant higher percentage of positive to skin test (p <0.001). No statistically significant differences were observed between males and females and between those who attended clinical or preclinical university training. The same statistically significant differences have been confirmed in subjects found positive both to skin test and IGRA, as shown in table 3. It is important to observe that among vaccinated subject 12 students resulted to be infected by *Mycobacterium*.

	Tuberculin reactivity (by Mantoux test)		**p-value**
	Positive	**Negative**	
Sex, n (%)			
- Males	10 (2.0)	484 (98.0)	0.89[a]
- Females	15 (1.8)	841 (98.2)	
Age in years, mean ± SD	31.0 ± 4.6	25.6 ± 5.5	<0.001[b]
University course, n (%)			
- Medical School courses	4 (0.5)	858 (99.5)	<0.001[c]
- Postgraduate Medical School courses	21 (4.3)	468 (95.7)	
University training			
- Clinical	21 (2.2)	953 (97.8)	0.26[c]
- Preclinical	4 (1.1)	373 (98.9)	
Tb vaccination			
- Yes	14 (20.3)	55 (79.7)	<0.001[a]
- No	11 (0.9)	1,271 (99.1)	

Table 2: Factors associated with tuberculin reactivity among students and postgraduates at the University of Palermo (Sicily). [a]Chi-squared test; [b]Student t test; [c]Fisher's exact test.

After finding skin test positive, we met again these students and asked if they remembered having had contact with patients with TB in the workplace or in other contexts: only a student of nursing degree course referred that his grandmother was affected by TB when he was young; all other students did not know how and when they were infected.

	Tuberculin reactivity (by Mantoux test) associated to IGRA positive		**p-value**
	Positive	**Negative**	
Sex, n (%)			
- Males	6 (1.2)	490 (98.8)	0.89[a]
- Females	11 (1.3)	845 (98.7)	
Age in years, mean ± SD	32.0 ± 3.9	25.6 ± 5.5	<0.001[b]
University course, n (%)			
- Medical School courses	2 (0.2)	860 (99.8)	<0.001[c]

- Postgraduate Medical School courses	15 (3.1)	474 (96.9)	
University training			
- Clinical	15 (1.5)	959 (98.5)	0.22[c]
- Preclinical	2 (0.5)	375 (99.5)	
Tb vaccination			
- Yes	12 (17.4)	57 (82.6)	<0.001[c]
- No	5 (0.4)	1,277 (99.6)	

Table 3: Characteristics of subjects found positive both Mantoux test and IGRA. [a]Chi-squared test; [b]Student t test; [c]Fisher's exact test.

Discussion and Conclusion

The analysis of the results shows an extremely low prevalence of latent TBC in the examined sample. This finding is consistent with other similar studies in our area and can be explained, at least in part, by considering that Sicily is one of the Italian areas with the lowest prevalence of active infection and with decreasing tendencies, according to the latest available data [1,2].

In fact, similar studies performed in recent years at the Universities of Parma, Naples (L.Vanvitelli) and Genoa found prevalence of suspected latent tuberculosis in students of the Schools of Medicine respectively: in Parma of 3% at TST (almost all foreigners: we have not data on IGRA); at Naples of 1.2% at TST, confirmed at IGRA test only in 0.1% of the sample; at Genoa of 1.4% at TST, confirmed only in 0.5% at IGRA test: almost all those subjects were born in a country with a high TB incidence [8-10]. Also in a similar German study, the prevalence of positivity in nursing students was 0.6% [11].

In our study there was a higher probability of positivity in older students, and in those who attended postgraduate courses (schools of specialization); it could be assumed that the contagion could have occurred in the attended hospital departments. In fact, it is frequent that patients with undiagnosed active TB go to other hospital departments (orthopaedics, gynaecology, cardiology, etc.) to be cured of other illnesses, representing a biohazard for these workers, generally unready to implement prevention strategies for this type of disease [12].

In fact, we found positive tests (skin and serological) in students of specialization schools in all areas, both in Medicine and in Surgery. It is therefore necessary to train and inform all health practitioners of the potential specific risk of contracting this disease, even if students are not involved in as specialist in infectious diseases or pneumology. It cannot be excluded that in the future, in Sicilian hospital workers and medical students, TB will reverse its decreasing trend as well as hospital infectivologists point out, noting the increase in hospital admissions of patients with active TB.

However, it should be highlighted that Sicily, economically depressed and in serious difficulty due the economic crisis, usually is not considered as possible residency for immigrants coming from poor and high incidence of tuberculosis infections Countries [13]. On the other hand, subjects coming from these nations are already present in our territory and often live in degraded urban and residential environments, and this increases the possibility of contagion and the evolution of the infection.

In any case, even with low incidence of infection in this geographic area, it is indispensable to work for limiting the evolution from latent to active TBC in all population, including exposed workers, encouraging the adoption of recommended therapeutic strategies that, unfortunately, are not suggested and applied uniformly by all the specialists and in all the geographical areas of our country [14-17]. In addition, research into new active prophylactic means should be stepped up, promoting the production of new vaccines more efficient than those currently in use for immunizing the general population and all at high risk of exposure workers [18]. Also in this study, we can see how vaccine protection is not always effective, and according to this consideration, we found 12 previously vaccinated subjects who were infected.

It is necessary to respect primary prevention procedures, ensuring infected patients in appropriate environments, teaching workers in respecting the use of personal protective equipment (facial masks) also for assisting suspected cases, always trying to protect health safety [19-20].

From the diagnostic point of view, the use of the IGRA test in our study, as in many others analogues carried out in various countries, showed to be an extremely useful tool in the diagnosis of latent TB, also to discriminate positivities due to previous vaccinations that, in our study, have been not related in the anamnesis by the enrolled subjects.

In conclusion, our findings suggest that, both from a Public Health and occupational medicine view, it is necessary to be vigilant in this aggressive pathology, by implementing all the prevention strategies for every worker exposed to the risk of contagion, first of all by using good practices of primary and secondary prevention and increasing workers' knowledge about the problem [21-24].

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