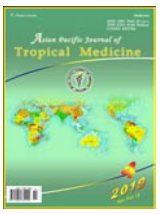

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Latent tuberculosis infection among medical students in Malaysia

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ABSTRACT

Objective: This study aimed to determine prevalence of latent tuberculosis infection among medical students and tuberculosis exposure at the health facilities.

Methods: A cross-section of study year 1 (n=68) and year 5 (n=75) medical students in a local university were recruited for latent tuberculosis infection testing using QuantiFERON-TB Gold Plus and a questionnaire analyzed for multivariate risk.

Results: The majority of the study were vaccinated with BCG. None of year 1 medical students were positive for latent tuberculosis infection, however, six (8.0%) year 5 students were tested positive for latent tuberculosis infection. A higher incidence of year 5 medical students claimed to be exposed to tuberculosis at health facility (65.3% *vs.* 4.4%) and a higher percentage reported contact with tuberculosis case over the preceding year compared to year 1 students (30.7% *vs.* 8.8%).

Conclusion: We observed a higher incidence of latent tuberculosis infection and higher exposure to tuberculosis in health facilities among year 5 medical students. Baseline screening and monitoring for progression to tuberculosis infection may benefit tuberculosis management programs.

1. Introduction

Tuberculosis (TB) is a severe infectious disease caused by *Mycobacterium tuberculosis (M. tuberculosis)*. The World Health Organization (WHO) estimated 10 million incidences of TB with 1.3 million mortality in 2017. Although the rate of TB is generally decreasing 2% per year, it remains an enormous global public health problem[1]. Malaysia is unique in being able to maintain relatively low incidence of TB compared to immediate high burden neighboring countries such as Indonesia, Philippines, Thailand and Vietnam. It is considered a country with intermediate burden with an estimated TB rate of 92/100 000 population per year. However, WHO countries profile on tuberculosis showed an increasing trend in incidence for Malaysia since 2010[2]. A false sense of security may be a reason for the reduced attention given in countries with intermediate burden.

WHO defines latent tuberculosis infection (LTBI) as a state of persistent immune response to stimulation by *M. tuberculosis*

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antigens with no evidence of clinically manifest active TB[3]. At risk groups identified for LTBI testing and treatment include persons living with HIV, HIV-negative household contacts and other HIV-negative at-risk groups. The 2018 WHO guidelines for programmatic management of LTBI recommends that systematic testing and treatment may be considered for health workers in countries with low TB incidence. Positive LTBI individuals are at variable risk for progression to active TB disease. An average of 5%-10% which usually occurs within five years of initial infection has been estimated[4]. Global burden is alarming and under one-fourth of the global population is affected[5] especially in developing countries.

Tuberculin skin test (TST) has been in use for over 100 years to measure hypersensitivity response to *M. tuberculosis* in suspected LTBI. Interferon-gamma release assays (IGRA) were developed to measure T cell-producing interferon gamma (IFN- γ) in whole blood *ex vivo*, thus is more convenient. In low TB incidence settings, IGRAs were shown to be advantageous over TST with higher specificity, better correlation with surrogate measures of *M. tuberculosis* exposure, and less cross-reactivity with the BCG vaccine.

Healthcare workers fall in 'other HIV-negative at-risk groups'. In high burden countries, benefits of treatment may not outweigh harm due to the compromise of reinfection. The decision for systematic testing of LTBI in this group should be made in accordance with the local TB epidemiology and context, health system structure, availability of resources and overall health priorities[1].

Prevalence of LTBI among healthcare workers in Malaysia was determined as 10.6% using the QuantiFERON-TB Gold (QFT) test[6] which is comparatively lower than that tested among healthcare workers in US at 46%[7] and 14.9% in Italy[8]. However, number of immigrants was high in those populations. Screening for LTBI for early diagnosis, determination of baseline levels and prevention to active TB disease are crucial for health care workers including students[8] but is currently not a practice in Malaysia.

The present study is aimed to estimate the prevalence of LTBI among year 1 and year 5 medical students at a local university in Malaysia using IGRA, QuantiFERON-TB Gold Plus.

2. Subjects and methods

2.1. Study design

A descriptive and cross-sectional study was conducted on Year 1 and 5 medical students at the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. Sample size calculation was based on estimating proportionate population using Lemeshow formula, N=(1.96/e) p(1-p), where z=1.96 (95% confidence); e=0.05 (level of precision); p=0.052 (anticipated population proportion). Sample size was determined as N=75 per group. Respondents were selected randomly from name lists[9].

2.2. TB Screening questionnaire

A standardized self-administered questionnaire was distributed to all respondents. Information on demography, BCG vaccination, history of active TB and history of active TB contact were obtained. Assessment for clinical symptoms of TB was also conducted. The respondents were among year 1 and 5 medical students of Malaysian citizenship, any race, both sexes who had consented. Exclusion criteria were respondents with active TB, treated for TB, pregnant, with acute infection, or on immunosuppressive drugs. Sample collection and analysis were conducted in the month of August 2018.

2.3. QuantiFERON-TB Gold Plus (QFT-Plus)

The QFT-Plus (Qiagen) enzyme-linked immunosorbent assay (ELISA) was performed according to manufacturer's instructions. The procedure involved two stages: incubation of blood in TB antigen coated tubes and ELISA detection of IFN- γ in plasma. Briefly, approximately five mL of blood was drawn and one mL was placed into four QFT-Plus blood collection tubes for negative control (lithium heparin alone), positive control (T cell mitogen), TB1 (containing ESAT-6 and CFP-10 peptides) and TB2 (containing ESAT-6 and CFP-10 peptides, as well as shorter peptides of the same antigens designed to specifically stimulate CD8 T-cells). Following 16-24 hour incubation at 37 ℃, plasma was separated by centrifugation and aliquoted into sterile tubes and stored at -30 ℃ until use. Concentration of IFN- γ was determined in 50 μL of plasma by ELISA. Interpretation of the quantitative IFN- γ values was based on the manufacturer's cut-off value where TB1 or TB2 minus Nil must be more or equal to 0.35 IU/mL. All LTBI positive individuals were advised to perform a chest X-ray.

2.4. Ethics approval and consent to participate

Ethical approval was secured from the Institutional Ethics Committee for Research Involving Human Subjects, Universiti Putra Malaysia. Participant consent was obtained and study participants were kept anonymous to maintain their medical confidentiality rights: Personal identifier variables like names were not included in the data collection checklist. The results of the study were reported to the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

2.5. Statistical analysis

Descriptive analysis was performed on Excel worksheet 2013 (Microsoft Office). The statistical analysis was carried out using Fisher's Exact test to determine association between two categorical variables while logistic regression was utilised to estimate odds ratio at 95% confidence interval (*CI*) for multivariate analysis of variables in relation to LTBI infection. $P<0.05$ indicated statistical significance.

3. Results

3. 1. Characteristics of the study subjects

Of the eligible 101 year 1 and 104 year 5 medical students at the

Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 68 (67.3%) year 1 and 75 (72.1%) year 5 students gave consent to the study. The majority were female, reflecting the lower intake of males into medical faculty (27.4% - 33.7%), Universiti Putra Malaysia. The majority were BCG vaccinated following national vaccination requirements. Only one responder, a year 1 student, reported was told had TB. Unlike year 5 students, the majority of year 1 students had low exposure to the health facility or made contact with TB cases in the last one year (Table 1). These are expected as Year 1 students are still at pre-clinical years with minimum exposure to clinical facilities while this group of Year 5 student already had at least two years clinical experience.

3. 2. Latent tuberculosis infection using QFT-Plus

All tested samples achieved in positive controls IFN- γ concentrations of more than 10 IU/mL after mitogen-Nil, fulfilling manufacturer's requirement of more or equal 0.5 IU/mL. These also implied samples collected were healthy and viable. Majority, 98% (140/143) of Nil controls were less than 1.0 IU/mL with highest value of 2.76 IU/mL and still far below the high-nil indeterminate cut point of 8.0 IU/mL. All year one students were negative for the QFT-Plus test. Of the 75 year 5 students, six (8.0%) were positive and 69 (92.0%) were negative. Among the 6 positive cases 2 (2.6%) were male and 4 (5.3%) were female. The overall incidence was 4.2% (6/143).

Table 1. Demographics and exposure of Year 1 and Year 5 medical students in Universiti Putra Malaysia.

Variables	Year 1 (n=68)	Year 5 (n=75)	P
Mean age (year)	20±1	23±1	
Female[n(%)]	47(69.1)	55(73.3)	0.587
BCG vaccination[n(%)]			
Vaccinated	58(85.3)	73(97.3)	
Unvaccinated	2(2.9)	1(1.3)	
Unknown	8(11.8)	1(1.3)	
TB exposure at health facility[n(%)]			
Yes	3(4.4)	49(65.3)	0.000
No	65(95.6)	26(34.7)	
Made contact with TB case last one year[n(%)]			
Yes	6(8.8)	23(30.7)	0.001
No	62(91.2)	52(69.3)	

Of the six positive cases, positive results were detected in 2/6 (33.3%) and 5/6 (83.3%) TB1 and TB2 tubes, respectively. Spearman's rho test showed poor correlation between the two results (R=-0.374, P=0.468).

Multivariate analysis determined a 22.1 increased risk of LTBI among Year 5 students, and LTBI was also increased with increased age (Table 2). Female had a lower risk (OR=0.62) of LTBI. Students were also at increased risk if exposed to TB facilities (OR=1.63) or had contact with TB case (OR= 3.28). However, none of these were statistically significant.

Table 2. Multivariate analysis of demographics and tuberculosis (TB) exposure stratified for latent tuberculosis infection among the study population.

Variables	OR	95% CI	P
Year (Year 1 *vs.* Year 5)	22.1	0.00-0.00	0.99
Age	6.46	0.43-96.46	0.18
Sex (male *vs.* female)	0.62	0.90-4.26	0.63
Exposed to TB facility (unexposed *vs.* exposed)	1.63	0.14-18.56	0.69
Contact with TB case (no contact *vs.* with contact)	3.28	0.41-26.29	0.26

4. Discussion

The overall incidence of LTBI among medical students detected in this study (4.2%) was slightly lower than reported LTBI among medical students in high burden countries *i.e.* 5.2% in Korea[10] and 15.2% in South Africa[11]. These studies used older versions of QFT in-Tube kit. As expected, TST testing in these previous studies identified higher numbers of positive cases which were 23% in Korean and 26.6% in South African medical students as this method is not specific to latent TB infection. Using TST method, Brazil estimated 6.9%[12] while Italy, a low burden country recorded only 0.8%[8] among medical students. However, discordant results with negative TST and positive QFT are also observed (15.7%), requiring more detailed analysis[13].

In this study, two samples were concordant *i.e.* positive for both TB1 and TB2 tubes. The four others had discordant results *i.e.* three positive for only TB2 while another positive for only TB1. A study assessing performance of the new QFT-Plus tubes for LTBI in pregnant women[14] also observed discordant results but only in 29 (10.5%) of positive cases (n=277) of which 16 (5.8%) were TB1+/TB2- and 13 (4.7%) TB1-/TB2+. Responses of peripheral blood monoclonal cells from individuals with active TB and LTBI incubated in TB1 and TB2 tubes, evaluated by flow cytometry showed while CD4 responses were induced in both tubes, CD8 T-cell response was mainly in TB2 tubes[15]. This corresponded with the format of the kit. Thus, observation of single TB1 tube positivity was unexpected. Nevertheless, the IFN- γ concentration was low at 0.44 IU/mL. Of the remaining cases, majority (4/5) were TB1-/TB2+. The results here suggested that activation were mainly of CD8 T cells. These results are expected to be true positives as these cases were, in general, exposed to TB facilities/made contact with TB cases.

The increased risk of LTBI among Year 5 medical students who, as expected are also older, is likely due to the higher risk of being exposed to TB facilities and TB cases associated with LTBI. While there is minimal early exposure during pre-clinical years, in clinical years students are expected to spend more time at hospital facilities

and experience close contact while clerking patients. Healthcare workers who do not work in healthcare facilities with a TB control program, are less likely to have LTBI[16]. In TB-designated hospitals, regular infection control training for healthcare workers, regular maintenance of ultraviolet disinfectant equipment were associated with lower LTBI[17]. While Malaysia is only moderate burden for TB, increased risk are expected where TB patients are warded and special facilities are inadequate. Urgency and vigilance in this matter is most important. A meta-analysis across 16 countries showed the overall risk of both LTBI and TB among healthcare workers continues to be significantly higher than the general population[18].

Among positive QFT subjects, the majority (4/6) were female. Multivariate analysis, however, showed an increased risk among males. These results were due to the lower number of male medical students in our study. Higher incidence among males, are concordant with other studies, as discussed by Rafiza *et al.*[6]. None of the multivariate analysis were statistically significant which is likely due to the overall low number of positive cases (4.2%). This also indicated that though there was increased incidence of TB, the number infected was still low. A reason for this is likely due to the fact that the majority of medical students here (as well as the rest of the nation) are vaccinated with BCG. Research using the QFT test observed risk of LTBI is increased with absence of a BCG scar[16].

The risk of progression from LTBI to active disease among this group of at-risk population is still unclear. Thus, research priorities should include need for identification of risk factors to determine potential benefits from public health interventions and LTBI treatment[3].

Limitations of the study included the small sample size and dependence on accurate responses from participants.

We observed significantly higher incidence of LTBI and higher exposure to TB in health facilities among year 5 medical students. A direct relationship cannot be concluded as baseline results were not available. Thus, baseline screening should be part of TB management procedures among medical students. Follow-up of this cohort of positive cases may aid to further understand performance of the kit and reveal risk factors in progression to active TB.

Conflict of interest statement

We declare that we have no conflict of interest.

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