Screening for Tuberculosis Infection among Asymptomatic Students of a Nigerian Tertiary Institution: Comparing Three Different Methods

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ABSTRACT

Background: Tuberculosis (TB) screening is not common among asymptomatic individuals despite its global health threat. Exposure of undergraduate medical and allied students to infections, for example TB, during clinical postings and their living conditions underscores the reason for investigating these asymptomatic students living in the hostel of a tertiary institution in a resource poor setting. Further, Interferon Gamma Release Assay (IGRA) is adjudged the best method for screening Latent Tuberculosis Infection (LTBI) but due to its cost, the use of Enzyme Linked Immunosorbent Assay (ELISA) was explored in this study.

Objective: To explore and compare the use of tuberculin skin test (TST), chromatographic rapid blood test and TB ELISA screening technique for detection of TB infection among asymptomatic students in a medical hostel.

Method: One hundred and twenty apparently healthy medical and allied students of the College of Medicine of the University of Lagos, Lagos, Nigeria, were enrolled in this study. Questionnaires were administered to consented participants and TB detection was performed using TST, ELISA and chromatographic rapid blood test methods.

Results: The prevalence of asymptomatic TB using ELISA was 36.7% and 29.2% for TST. These results showed agreement when tested statistically ($X^2 = 1.646; P = 0.199$; concordance agreement of 65.8%; 95% CI) while no TB was detected using the chromatographic rapid blood test kit. The positive and negative predictive values of ELISA were 66.7% and 100% respectively. When tested statistically, there was correlation between TB detection and contact with persons having active TB.

Conclusion: It could be suggested that ELISA and TST method can be used for TB detection among asymptomatic individuals in a resource poor setting where IGRA may be expensive and uncommon.

Keywords: Tuberculosis, tuberculin skin test, ELISA method, blood chromatographic method, asymptomatic students.

INTRODUCTION

Tuberculosis (TB) is a global health threat affecting one-third of the world’s population, especially in poor and developing countries where 95% of global TB cases and 99% of deaths occur (1). It is the leading cause of death due to its association and interaction with HIV infection (2, 3). Tuberculosis currently ranks seventh among the global ranking causes of death and if care is not taken will remain in this position till year 2020, unless intensive efforts are made, despite a projected decline in other infectious diseases (3, 4). Tuberculosis is an infectious disease caused primarily by Mycobacterium tuberculosis which together with M. bovis Calmette Guerin, M. microti, M. africanum and M. carnetti make up the Mycobacterium tuberculosis complex.

Mycobacterium tuberculosis is an Acid Fast Bacteria (AFB) which can form acid stable complexes when certain arcmethylene dyes are used. All species of Mycobacterium have ropelike structures of peptidoglycan that are arranged in such a way to give them properties of acid fast bacteria (5). M. tuberculosis has a tough cell wall that prevents passage of nutrients into and excreted from the cell, therefore giving it the characteristic of slow growth rate. The cell wall of the pathogen looks like a Gram-positive cell wall. Groups at high risk of infection include employees in hospitals, clinics and medical laboratories, and persons who have close contact with someone known or suspected to have active TB (6). The greatest risk of progression from Latent Tuberculosis Infection (LTBI) to active TB occurs in the first 2 years after infection and is very common in overcrowded conditions (7).

The disease usually affects the lungs (pulmonary TB or Koch disease) in most cases and can also spread to other organs with signs such as cough, night sweat, fever, loss of weight, and shortness of breath (8). It is spread through air in form of droplets generated from coughing, sneezing from infected
screening of TB among asymptomatic students

individuals and less commonly via skin wounds (9). People nearby may become infected by inhaling the bacteria. Mycobacterium tuberculosis can remain viable in air for a long time or in dust particles at home for weeks. However, transmission occurs after a substantial amount has been inhaled (8, 10). Exposure to individuals having active TB can lead to one of the following three outcomes (11): the innate immune system can destroy the tubercle bacilli leaving no evidence of TB exposure, the tubercle bacilli can escape the immune system and disseminate causing primary tuberculosis when bacterial growth is controlled, while some survive and persist leading to LTBI (11). In the later, such individuals are infected with M. tuberculosis for many years without showing signs or symptoms or spreading it to others. However, in a situation when the immune system is weakened, like in diseases such as diabetes, Human Immunodeficiency Virus (HIV) infection, malignancy, extremes of ages, chronic kidney disease etc. latent TB can develop into active TB (8, 10). A person with active TB will infect 10 to 15 persons on the average if untreated (12). The World Health Organization (WHO) has estimated that one-third of the total world population is latently infected with M. tuberculosis and 5-10% of the infected individuals will develop active TB disease during their life time (3, 13). However, the risk of developing active disease is 5-15% every year and lifetime risk is approximately 50% in HIV co-infected individuals (10, 14). Most of the active disease cases in low TB incidence countries arise from pool of latently infected or asymptomatic individuals. This process is poorly understood and only a few risk factors have been defined. Therefore, early detection and management of LTBI is important in the eradication of TB disease. In countries with high incidence of TB, active transmission occurs in crowded places and small households (15, 16).

Tuberculosis is a serious public health problem in Nigeria, with an estimated prevalence of over 900,000 TB cases. Nigeria ranks 5th among the 22 high TB burden countries of the world. In Nigeria, Lagos (8200 per annum) and Oyo (6000 per annum) are some of the cities having high TB notification rate (17). Furthermore, Nigeria is a very populous nation where the public rarely knows the TB burden because of lack of appropriate national survey and this may be contributing to the prevailing inappropriate care seeking behaviour and poor awareness of the disease in the country (18). The outcome of TB control is determined by clinical and social factors like delayed presentation and health care utilization which depend on the knowledge and awareness of TB symptoms among the population (19, 20), especially the asymptomatic group.

For over a century, the only method of diagnosing asymptomatic TB has been the Tuberculin Skin Test (TST) or Mantoux test (21). Its major flaw is its inability to differentiate individuals infected with M. tuberculosis from individuals immunized with other Mycobacterium such as Bacille Calmette Guerin (BCG) (22). Interferon Gamma Release Assay (IGRA) was developed to address the short comings of the TST. This new in-vitro test assesses the immunologic reactions of cytokines to specific antigens to M. tuberculosis (23). This test is very costly and uncommon in this environment, hence this study attempts to explore and compare ELISA TB screening, TST and blood chromatographic test for TB as a possible way of screening TB among asymptomatic individuals in a resource poor setting like Nigeria where IGRA is still uncommon and costly.

MATERIALS AND METHODS

Study Population

It was an exploratory study designed to compare three methods of TB detection using a blood chromatographic rapid kit (Diaspot), TST and Immunoglobulin M (IgM) ELISA kit. The total number of study participants recruited was 120 (38 females and 82 males) healthy asymptomatic undergraduate medical and allied students who volunteered to participate after proper informed consent. All the recruited participants were living in the medical hostel of a tertiary institution in Nigeria and they all fulfilled the inclusion criteria for the study participation. A formal power calculation was not performed since the study was exploratory (24). The study spanned a period of three months and the health status of each individual was assessed based on their medical history disclosed through questionnaire interview. Volunteers with the following conditions were excluded from the study: current or past history of chronic illness such as diabetes, cancer, kidney disease, leukaemia, and clinical evidence of active TB or previous treatment of TB.

Data Collection

Questionnaires were administered to consented students in their hostels in order to collect information on their general health status and sociocultural/ possible predisposing factors. The questionnaire was in English, which is the official language of communication in Nigeria. It was also used to obtain socio-demographic information, awareness of the warning signs of tuberculosis, the predisposing risk factors, and TB medication, cure and treatment duration. The question on awareness of the disease was based mostly on non-personal experience; however, it was able to assess the individual’s level of enlightenment and education on public health.

Blood samples were collected from the participants before the TST was done on their skin. The blood samples were collected into plain bottles and sera were separated from the whole blood samples and stored in cryovial containers before the serological tests were performed.

Tuberculin Skin Testing

The tuberculin skin test was performed by intradermally injecting 0.1 ml of Tuberculin Purified Protein Derivative (PPD) with tuberculin syringe. The preferred site for this test was either the flexor or dorsal surface of the forearm about 4 inches below the elbow joint. This site was cleansed with methylated spirit and allowed to dry. Conversely, the stopper of the PPD vial was cleaned with spirit and 0.1 ml of Tuberculin PPD solution was drawn into the sterile insulin syringe of 29G gauze needle. Tuberculin PPD was then injected intradermally by inserting the tip of the needle into the most superficial layers of the skin with the needle bevel pointing upwards. As the solution
was injected, a pale white bleb 6 to 10 mm in diameter was formed at the needle point which was quickly reabsorbed thereafter.

Results of the tuberculin skin test were read 72 h after injection. Induration only was considered while interpreting the test. The diameter of induration was measured transversely to the long axis of the forearm and recorded in millimetres. Reactions were classified as positive when induration measures 10 mm or more (25). This indicates hypersensitivity to tuberculoprotein and indicates past or present infection with *M. tuberculosis*. It was determined as doubtful when induration was between 5 and 9 mm (25). In cases like this, the test was repeated at another site on the skin. This was done to rule out cross reactions from other Mycobacteria infections. The results were recorded as negative when induration was less than 5 mm (25). This indicates lack of hypersensitivity to tuberculoprotein and the fact that tuberculosis infection is highly unlikely.

**Chromatographic Rapid Blood Test**

The separated and stored sera in the cryovials were used for this rapid test and the ELISA IgM test. The test device was removed from the sealed foil pouch and used immediately. Thereafter, the dropper was used to dispense 3 drops of serum to the specimen well of the test device and 1 drop of buffer was added and the time was set for 10 min. The results were interpreted as positive when 2 distinct coloured lines appear, one line should be in the control region (C) and another line should be in the test region (T); negative when one coloured line appears in the control region (C) and no apparent coloured line appears in the test region (T); and as invalid when control line fails to appear. The relative sensitivity of the kit as stated is 83.0%, the relative specificity is 98.9% and the relative accuracy 95.6%.

**Mycobacterium tuberculosis IgM Enzyme Immunosorbent Assay (ELISA) Test**

All reagents and samples were brought to room temperature before use and all standards and samples were assayed in duplicates. The procedure for the test was as prescribed by the manufacturer (26). The optical density (OD) for the participants sera were compared with the value for the cut-off standard (0.4855). Therefore, if the value of the sample is ≥ 0.4855, it is POSITIVE while ≤ 0.4855 is NEGATIVE. The performance characteristics of the kit are intra-assay precision = 7.6% and inter-assay precision = 9.4% (26). The clinical specificity and clinical sensitivity are 99% and 100% respectively.

**Statistical Analysis**

The results obtained were analysed using EPI informatics software (EPI- INFO), Chi square and SPSS statistical packages.

**RESULTS**

**Prevalence of Asymptomatic TB among Studied Participants**

The prevalence of asymptomatic TB among studied participants was 44/120 (36.7%) for the ELISA test and 35/120 (29.2%) for the Tuberculin Skin Test (Mantoux test). These results were tested statistically and there was no significant difference ($\chi^2 = 1.646; P$-value = 0.199). Meanwhile, the blood chromatographic rapid test kit (Diaspot) was 0/120 (0.0%) (Table1).

**Table1: Prevalence of Asymptomatic TB among Studied Participants**

<table>
<thead>
<tr>
<th>No. of Participants</th>
<th>No. Positive (%)</th>
<th>No. Negative (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 120</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA Test</td>
<td>44 (36.7)</td>
<td>75 (62.5)</td>
<td>120 (100.0)</td>
</tr>
<tr>
<td>Tuberculin Skin Test</td>
<td>35 (29.2)</td>
<td>85 (70.8)</td>
<td>120 (100.0)</td>
</tr>
<tr>
<td>Blood Chromatographic Rapid Test (Diaspot)</td>
<td>0 (0.0%)</td>
<td>120 (100)</td>
<td>120 (100.0)</td>
</tr>
</tbody>
</table>

ELISA and TST ($\chi^2 = 1.646; P$-value = 0.199).

**Demographic Data of Studied Participants**

Out of the 120 students recruited, there were 39 females and 81 males (Female: Male; 1:2). The age range of the recruited participants was between 18-39 years with mean and median age of 21.9±2.95 years and 21.9 years respectively. Twenty four of the participants (20%) were between 18–19 years age range. 15/120 (12.5%) had contact with active TB patients, 54/120 (45%) had received BCG vaccine, while 66% were not vaccinated before (Table 2). Sixteen (29.6%) of these participants that had taken BCG were Mantoux positive by TST while 44.4% were TB positive using ELISA.

**Table 2: Demographic Data of Studied Participants**

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Variable (N=120)</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>81 (67.5)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>39 (32.5)</td>
</tr>
<tr>
<td>2.</td>
<td>Age</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18 – 19</td>
<td>24 (20.0)</td>
</tr>
<tr>
<td></td>
<td>20 – 21</td>
<td>37 (30.8)</td>
</tr>
<tr>
<td></td>
<td>22 – 23</td>
<td>30 (25.0)</td>
</tr>
<tr>
<td></td>
<td>&gt;23</td>
<td>29 (24.2)</td>
</tr>
<tr>
<td>3.</td>
<td>Mean age</td>
<td>21.9±2.95</td>
</tr>
<tr>
<td>4.</td>
<td>Lived/ Been in contact with Persons with TB Disease</td>
<td>15 (12.5)</td>
</tr>
<tr>
<td>5.</td>
<td>Have taken BCG Vaccine</td>
<td>54 (45.0)</td>
</tr>
<tr>
<td>6.</td>
<td>Not Vaccinated</td>
<td>66 (55.0)</td>
</tr>
<tr>
<td>7.</td>
<td>Number of Persons per Room</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2–4</td>
<td>52 (43.3)</td>
</tr>
<tr>
<td></td>
<td>5–8</td>
<td>52 (43.3)</td>
</tr>
<tr>
<td></td>
<td>9–12</td>
<td>16 (13.3)</td>
</tr>
</tbody>
</table>

The percentage of Mantoux positive among participants in relation to number of persons per room is as shown on Figure 1.
Screening of TB among Asymptomatic Students

Detection Results of Studied Participants that had Contact with TB Persons

Nine of the fifteen participants who had contacts with TB persons, 9/15 (60%) were positive for TB while 40% (6/15) of these were positive to Mantoux by the tuberculin skin test (Table 3). This was tested statistically and found to be strongly correlated (correlation coefficient (r) = 0.88).

Table 3: Detection Results of Studied Participants that had Contact with TB Persons

<table>
<thead>
<tr>
<th>Number of Participant (N=15)</th>
<th>ELISA (%)</th>
<th>Mantoux (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number positive</td>
<td>9 (60.0)</td>
<td>6 (40.0)</td>
</tr>
<tr>
<td>Number negative</td>
<td>6 (40.0)</td>
<td>9 (60.0)</td>
</tr>
<tr>
<td>Total</td>
<td>15 (100.0)</td>
<td>15 (100.0)</td>
</tr>
</tbody>
</table>

There was a correlation between TB detection and contact with persons with active TB (correlation coefficient (r) = 0.88). ELISA: % Sensitivity = 100%; % Specificity = 66.7%; Positive Predictive Value (PPV) = 66.7%; Negative Predictive Value = 100%.

Age and Sex Distribution of TB Positive Participants Using ELISA and TST Methods

Twenty-four participants were between the ages of 18 and 19 years; 11 (45.2%) of these were positive using ELISA (5 males and 6 females), while 9 (37.5%) were positive by the TST (8 males and 1 female) in this same age group (Tables 4 and 5). The others were negative. A total of 29 students were above 23 years in which 9 were positive by ELISA and 8 positive by TST (Tables 4 and 5). The results were tested statistically and there was no association between TB detection and age or sex of the participants using either ELISA or TST (P (Fisher exact) = 0.227 and P (Fisher exact) = 0.062 respectively).

DISCUSSION

In this study, we evaluated the use of tuberculin skin test, blood chromatographic rapid test and TB ELISA IgM method to detect asymptomatic TB among students in the medical hostel of the College of Medicine of the University of Lagos, Ili-Araba, Lagos, Nigeria. There was no significant difference ($\chi^2 = 1.646$, P = 0.199) in the prevalence of asymptomatic TB among these medical students using ELISA (36.7%) and TST (29.2%). This result agrees with the finding on TST in Ethiopia, although IGRA was used instead of ELISA used in this study (24). In the study, an agreement was observed between TST and IGRA among young apparently healthy adults. This shows the adeptness of TST in the diagnosis of asymptomatic TB. Since TST is adjudged the standard method of determining whether a person is possibly infected with M. tuberculosis, this could suggest that ELISA method can be used in place of IGRA in a setting like ours where IGRA is uncommon or still costly. This is in view of the fact that the TST results agree with those obtained with ELISA. The prevalence of 29.2% detected in this study using TST is also comparable to the result obtained in a study by Dagnew et al. and Legesse et al. in Ethiopia (24, 27).

However, the TST result should be interpreted with caution because of some limitations that may result in TST false-
negative and/or positive reactions such as malnutrition and parasitic infections, possibly overwhelming TB disease or difficulties with the method of TST administration and interpretation of the reaction (28). The prevalence observed in this study population is worrisome as it is known that 5% of people exposed to *M. tuberculosis* will progress to active TB within 2 years and another 5–10% will develop the disease sometimes later in life (29, 30).

A higher prevalence of TB was reported among the males (30.9%) than the females (25.6%) using TST which also correlates with the work done in Ethiopia (25, 28), although they recorded a bit higher prevalence. However, this difference was not statistically significant, therefore does not suggest that males are more prone to asymptomatic TB than females. It is important to mention that all the samples tested with the blood chromatographic rapid test method in this study were negative. This may suggest the unreliability of possibly the particular batch of the rapid test kit used and the detection limit of the kit when it pertains to LTBI.

It was discovered that some risk factors are associated with the detection of TB among individuals recruited for this study. One of such risk factors considered was contact with TB persons. This was found to be a risk factor, which is similar to the results obtained in Malaysia, Zambia and China (31–34). Although their studies were carried out among healthcare workers, it is comparable to our study population (students in medical hostel) who sometimes do clinical postings which may expose them.

There was no association between asymptomatic TB detection and the age and sex of the study population. This was important in determining whether BCG vaccination could have effect on the method used for the asymptomatic TB detection. This is because it has been observed that BCG vaccination lasts for about 15 years, depending on the age of the vaccination of the individual (35). This may not have had any influence on the detection methods employed in this study as there was no association between BCG vaccination and TB detection when tested statistically using the different methods. This may be due to the fact that most of the study participants were between 18–39 years which is probably the undergraduate age in Nigeria. Although studies have shown that BCG vaccinated individuals are more likely to have positive TST result (36, 37), this was not the case in this study. Not all the samples of the individuals that have taken BCG were positive for TST and/or ELISA. This tally with a meta-analysis of several studies that showed that the effect of BCG is minimal after 10 years (38) and elsewhere it was documented that it wears out after 15 years of vaccination (39–41). On the other hand, the BCG vaccine may not have an effect on the ELISA method since the antigens contained in the assay are not present in any BCG strain (42). This is in consonance with our findings as there was no significant association between BCG vaccination and TB detection using ELISA. This may suggest that BCG vaccination at infancy may provide less protection as the individual ages.

The prevalence of asymptomatic TB in this study is seemingly high. This is not desirable because it may serve as a reservoir for future TB epidemic in this environment. However, the possible sources of this infection among the participants should be investigated to forestall rising prevalence, considering the fact that these medical and allied students are trained to be future healthcare practitioners.

For the three different test methods evaluated to detect asymptomatic TB, it could be adjudged that TST and ELISA are the most reliable while TST proved to be a reliable standard method for asymptomatic TB detection. It is therefore suggested that ELISA IgM can be used in resource poor settings like Nigeria where IGRA is not common and/or costly. However, it should be used in combination with TST to detect asymptomatic TB.

**CONCLUSION**

This study has shown that ELISA and TST method can be suggested for TB detection among asymptomatic individuals in a resource poor setting where IGRA may be expensive and uncommon. The study recorded a seemingly high prevalence; this signals the need for periodic surveillance of asymptomatic TB among populations prone to overcrowding such as in students’ hostels. This will help contain the incidence of the disease from such circumstances in the future, especially among medical students routinely exposed to hospital environment with possible air droplets of this infective organism.

**CONFLICT OF INTEREST**

There is no conflict of interest in this study.

**REFERENCES**

Screening of TB among Asymptomatic Students


