



Diagnosis and Risk Factors of Latent Tuberculosis Infection among Healthcare Workers Using Whole Blood Human Interferon-gamma Release Assay and Tuberculin Skin Testing

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Authors' contributions

This work was carried out in collaboration among all authors. Author ANU designed the study, wrote the protocol and wrote the first draft of the manuscript. Author OJA wrote part of the manuscript and managed the analyses of the study. Authors NOU and GEU managed the literature searches and performed the statistical analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRID/2020/v3i3330127

Editor(s):

(1) Dr. Bobby Joseph, St. John's Medical College, India.

Reviewers:

(1) Ahmad Salisu Aliyu, Infectious Diseases Hospital, Kano, Nigeria.

(2) Nain Taara bukhari, Agha Khan University Hospital, Pakistan.

(3) Nuhu Sambo, Baze University, Nigeria.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/54935>

Original Research Article

Received 20 December 2019

Accepted 28 February 2020

Published 12 March 2020

ABSTRACT

This study established the diagnosis and risk factors of latent tuberculosis infection (LTBI) among health-care workers in an endemic population using Tuberculin skin test (TST) and Quantiferon TB-gold. A total of 609 Healthcare workers from tuberculosis treatment facilities in Akwa Ibom State, Nigeria were studied. The Interferon-gamma release assay was performed using 3ml of whole blood by ELISA according to the manufacturer's instruction (Cellestis Ltd., Carnegie, Australia) after which 0.1 ml of 5 tuberculin units of Purified Protein Derivative (PPD) was administered intra-

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dermally to each subject. TST results were read after 72 hours by measuring the size of indurations in millimetres. Data were analysed using SPSS version 17 (SPSS Inc., Chicago, Illinois). At the threshold of 10 mm, the prevalence of LTBI by TST was 45.8% and 24.8% at the IGR diagnostic value of ≥ 0.351 IU. Laboratory staff and ward orderlies as well as being in service for >10 years, were more significantly associated with LTBI. A moderate agreement of 76.7%, $k = 0.51$ was obtained between TST at 10 mm, and QFT. Neither previous exposure to TST nor BCG vaccination affected the prevalence of LTBI in the study population. The difference of 54% prevalence of LTBI between TST and QFT may be due to non-tuberculous mycobacterium (NTM) since TST is non-specific. This may have grave implications of drug toxicity and development of resistance to anti-TB drug among individuals harbouring NTM, but receiving anti-TB medication. The 76.7% agreement between the two tests is an indication that the 10 mm cut-off induration for TST is still relevant in the diagnosis of LTBI.

Keywords: *Diagnosis; latent TB infection; QuantiFERON TB gold; Akwa Ibom State.*

1. INTRODUCTION

In most individuals, infection with *M. tuberculosis* can be contained by the host's immune defences thereby making the infection to remain latent [1]. Healthcare workers are particularly important group to study when evaluating latent tuberculosis (TB) infection (LTBI) because their risk of acquiring LTBI is higher than average as a result of their exposure to patients with TB. Currently, it is difficult to predict exactly which of the exposed individuals will develop the disease. Unfortunately, the tuberculin skin testing (TST) which until recently was the only practical way of detecting latent tuberculosis infection (LTBI), does not meet all the diagnostic expectations because of being subjected to considerable variations and other limitations. Some of these variations include false-positive TST responses resulting from either exposure to environmental mycobacteria that share common antigens with *M. tuberculosis* or resulting from prior BCG vaccinations [2]. Technical errors such as placement of measuring apparatus and reading of the TST can also yield false-positive results.

Advances in genomics and molecular biology have led to a promising alternative, the *in vitro* interferon-gamma (IFN- γ) assays [3,4,5] which are based on the concept that T cells of infected individuals released IFN- γ in a significant detectable level. These assays utilise antigens such as the early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10). These proteins are significantly more specific to *M. tuberculosis* than the purified protein derivative (PPD) used in TST, as they are not shared antigens with BCG sub-strains and non-tuberculous mycobacteria species that might cause non-specific sensitization. Also, they are unaffected by previous bacillus Calmette-Guerin

(BCG) vaccination [5]. A limited number of studies evaluating the performance of interferon-gamma release assays (IGRAs) have been conducted in TB endemic settings. Some studies outside Nigeria have examined the use of these tests in healthcare workers (HCWs) [6,7,8,9] but there is dearth of data from Nigerian studies in this regard.

This study evaluated the establishment of diagnosis and risk factors of Latent TB Infection among healthcare workers in Akwa Ibom State, Nigeria using the TST and an enhanced Interferon-gamma release assay (QuantiFERON-TB Gold).

2. MATERIALS AND METHODS

This study was conducted among Healthcare Workers (HCWs) in tuberculosis treatment centers in Akwa Ibom State of Nigeria.

2.1 Collection of Data

Information on the following variables was collected using a standardized questionnaire: age, gender, educational level, job title, occupational exposure to TB, years of service in health care sector, BCG vaccination, and prior TST. BCG vaccination was verified by confirmation of the presence of scar.

2.2 Test Methods

Tuberculin Skin Testing (TST) was performed using 5TU of the purified protein derivative (PPD). Basically, 0.1 ml volume of the antigen was injected intra-dermally into the dorsal surface of the forearm of each participant with the aid of a disposable tuberculin syringe. The results were read after 48-72 hours and the size of the indurations was recorded in millimetres

with the aid of a transparent meter rule and indurations ≥ 10 mm was considered a positive TST in HCWs [10,11].

For the interferon-gamma assay, the QuantiFERON-TB Gold In-Tube test was used (Cellestis Limited, Carnegie, Australia). This whole blood assay uses overlapping peptides corresponding to ESAT-6, CFP-10, and a portion of tuberculosis antigen TB7.7 (Rv2654). Briefly, 3 ml of blood was drawn from each subject and 1ml delivered into each of the three tubes labelled as nil control, positive control and *M. tuberculosis* specific antigens (ESAT-6, CFP-10 and TB7.7). Tubes were incubated at 37°C overnight before centrifugation, and IFN- γ release was measured by enzyme-linked immunosorbent assay (ELISA) following the protocol of the kit manufacturer. All the assays performed met the manufacturer's quality control standards. Interpretation of test result was done as recommended by the manufacturer and previous study by Pai et al. 2006. A positive QFT-3G was defined as IFN- $\gamma \geq 0.35$ IU/ml if the response to TB antigens minus the negative control was ≥ 0.35 IU/ml and $> 25\%$ of the negative control, negative if these criteria were not met, and indeterminate if either the negative control had a result >8 IU/ml or if the positive control had a result <0.5 IU/ml. Data analysis was performed using SPSS version 17 (SPSS Inc, Chicago, Illinois).

3. RESULTS

Of the 1,110 recruited healthcare workers, 398 (35.86%) later withdrew from participating in the study, leaving 712(64.14%). For those who participated TST results were not available for 98 persons because they did not turn up for a second visit or their readings were invalid. For 5 of the subjects, IGRA results were indeterminate and so valid results for both TST and IGRA were only available for 609 subjects.

Results obtained from this study show higher participation among the Nursing officers (58.1%) and low among the Physicians (5.25%). Adjusted Odds ratios for QFT depending on different putative variables were calculated using Logistic regression.

Table 1 shows the description of the study population and responses from the participants. Among the medical staff, 5.25% were physicians, 58.46% were nurses while laboratory staff and ward orderlies constituted 13.14% and 8.37% respectively. Non-medical staffs were 14.78%.

History of BCG vaccination was recorded for 73.89% of the participants. Tables 2 and 3 show the factors associated with QFT and TST positivity respectively. Adjusted Odds ratios for QFT depending on different putative variables were calculated using logistic regression. A positive QFT and TST results were observed in 151(24.8%) and 279(45.8%) of the participants respectively. In univariate analysis, the prevalence of LTBI assessed by QFT correlated with age, years of service and job category while education was not significant in multivariate analysis.

Table 1. Description of the Study Population

| Variable | N (%) |
|----------------------------------|------------|
| Gender | |
| Female | 410(67.32) |
| Male | 199(32.68) |
| Age(years) | |
| 21 - 30 | 88(14.45) |
| 31-40 | 58(9.52) |
| 41-50 | 307(50.41) |
| 51 – 60 | 156(25.62) |
| Educational Qualification | |
| B. Sc/HND | 58(9.52) |
| College | 337(50.41) |
| GCE/SSC | 156(25.62) |
| FLSC | 58(9.52) |
| Job category | |
| Physician | 32(5.25) |
| Nursing Officer | 356(58.46) |
| Lab Staff | 80(13.14) |
| Ward Orderly | 51(8.37) |
| Admin Staff | 90(14.78) |
| Years of Service | |
| 1 – 10 | 111(18.23) |
| 11 – 20 | 390(64.04) |
| 21 & above | 108(17.73) |
| History of TST | |
| No | 287(47.13) |
| Yes | 322(52.87) |
| BCG Vaccination | |
| No | 13(2.11) |
| Yes | 596(83.89) |

B.Sc = Bachelor of Science, HND = Higher National Diploma, GCE = General Certificate of Education, SSC = Senior School Certificate, FSLC = First School Leaving Certificate. College = Non degree post Secondary Certificate (Registered Nurse)

4. DISCUSSION

The increased risk of healthcare workers for tuberculosis is well established [12,13]. Therefore, screening of HCWs for LTBI and active tuberculosis is fundamental in infection

control programs. To the best of our knowledge, this is the first study that compared the performance of TST and QFT among HCWs in Nigeria.

This study has shown the prevalence of latent TB infection by TST to be 45.8%. On the other hand, the prevalence as assessed by IGRA is 24.8%, about 2-fold lower than TST's result. However, the high prevalence of LTBI in healthcare workers as demonstrated in this study is not surprising as Nigeria is among the high TB burden nations. Currently, Nigeria occupies a significant position among the high burden nations and the results obtained here is consistent with estimates from other developing countries [14,15,16].

From similar studies, higher differences in infection rates of LTBI among HCWS as assayed by these two methods have been documented. Nienhaus et al. [17] investigated 261 healthcare workers from different hospitals for pulmonary

infections in Germany and found a prevalence of 9.6% with IGRA compared to 24.1% with TST. Schblon et al. [18] also tested 270 healthcare workers in a hospital for pulmonary diseases but in the northern part of Germany and had a prevalence rate of 7.2% with IGRA and 30.7% with TST. The relatively low positivity rates of IGRA compared with TST in this study further confirms the low specificity of TST especially in BCG vaccinated individuals [16,19] and those infected with non-tuberculous mycobacteria (NTM) as earlier reported [16,20]. It therefore suggests the extent of over-diagnosis of LBTI in studies that solely rely on TST results. Asuquo et al. [21] reported a 45.8% prevalence among smear positive patients and healthy individuals using TST in Calabar, Nigeria. In IGRA, the level of IFN- γ produced by lymphocyte cells sensitized with antigens such as ESAT-6 and CFP-10, so measured, are significantly more specific to *M. tuberculosis* infection than PPD, and are not shared with BCG sub-strains or several non-tubercular mycobacteria species that might

Table 2. Frequency, Adjusted Odd Ratios (OR) and 95% Confidence Interval (CI) for Covariates Associated with QFT Results

| Covariates | No. positive/Total tested (%) | Adjusted Odd Ratio | 95%CI |
|-------------------------------|-------------------------------|--------------------|-------------|
| Age | | | |
| 21-30 | 8/88 (9.1) | 1.00 | |
| 31-40 | 16/58 (27.6) | 2.10 | 0.80-5.56 |
| 41-50 | 47/307 (15.3) | 0.76 | 0.31-1.88 |
| 51 & above | 80/156 (51.3) | 2.67 | 0.99-7.20 |
| Gender | | | |
| Male | 53/199 (26.6) | 1.00 | |
| Female | 98/410 (23.9) | 0.75 | 0.49-1.14 |
| Educational Attainment | | | |
| University | 8/58 (13.8) | 1.00 | |
| College | 93/337 (27.6) | 1.89 | 0.85-4.19 |
| Secondary | 20/156 (12.8) | 1.70 | 0.67-4.34 |
| Primary | 30/58 (51.7) | 3.47 | 1.33-9.00 |
| Years of Service | | | |
| 1-10 | 3/111(2.7) | 1.00 | |
| 11-20 | 93/390 (23.8) | 10.92 | 3.06-39.05 |
| 21 & above | 55/108 (50.9) | 26.23 | 6.47-106.28 |
| Job Category | | | |
| Physician | 3/32 (9.4) | 1.00 | |
| Nurses | 82/356 (23.8) | 3.32 | 0.90-12.31 |
| Lab. staff | 27/80 (83.8) | 16.53 | 3.41-80.20 |
| Ward orderly | 16/51 (31.4) | 13.07 | 2.00-85.24 |
| Admin. Staff | 23/90 (25.6) | 6.75 | 1.23-36.94 |
| History of TST | | | |
| No | 66/287 (23.0) | 1.00 | |
| Yes | 85/322 (26.4) | 0.88 | 0.53-1.49 |
| BCG Scar | | | |
| No | 3/13(23.1) | 1.00 | |
| Yes | 146/596(24.5) | 0.42 | 0.12-1.54 |

Table 3. Frequency, adjusted Odd Ratios (OR) and 95% confidence interval for covariates associated with TST results at 10 mm indurations

| Covariates | No. positive/Total tested (%) | Adjusted Odd Ratio (95%CI) |
|----------------------------------|--------------------------------------|-----------------------------------|
| Age | | |
| 21-30 | 19/88 (21.6) | 1.00 |
| 31-40 | 25/58 (43.1) | 1.75(0.79-3.85) |
| 41-50 | 116/307 (37.8) | 0.68 (0.32-1.43) |
| ≥51 | 119/156 (76.3) | 2.47 (1.02-5.97) |
| Gender | | |
| Male | 75/199 (37.7) | 1.00 |
| Female | 204/410 (49.8) | 1.51 (1.04-2.20) |
| Educational Qualification | | |
| B.Sc/HND | 20/58 (34.5) | 1.00 |
| College | 191/337 (56.7) | 2.38 (1.27-4.46) |
| SSCE | 36/156 (23.1) | 1.12 (0.54-2.32) |
| FSLC | 32/58 (45.8) | 1.19 (0.52-2.73) |
| Years of Service | | |
| 1-10 | 18/111 (16.2) | 1.00 |
| 11-20 | 179/390 (45.9) | 2.20 (1.07-4.53) |
| >21 | 82/108 (75.9) | 6.80 (2.70-17.11) |
| Job Category | | |
| Med Officer | 8/32 (25.0) | 1.00 |
| Nurses | 178/356 (50.0) | 2.01 (0.78-5.18) |
| Lab. staff | 41/80 (51.3) | 31 (4.06-31.46) |
| Ward orderly | 19/51 (37.3) | 2.92 (0.98-8.70) |
| Admin. Staff | 33/90 (36.7) | 3.00 (1.14-7.89) |
| History of TST | | |
| No | 99/287 (34.5) | 1.00 |
| Yes | 180/322 (55.9) | 0.000 |
| BCG Scar | | |
| No | 6/13(46.15) | 1.00 |
| Yes | 266/596 (44.6) | 0.000 |

cause non-specific sensitization [5]. The over-diagnosis of LTBI as observed with TST in this and other studies may be due to infection with the Non-Tuberculous Mycobacterium (NTM) since TST is non-specific [16]. Where routine diagnosis and treatment of TB relies only on TST, it may implicitly have grave implications of drug toxicity and development of resistance to anti-TB drug in individuals harbouring NTM, but receiving anti-TB medication. Studies conducted on disease progression and probability of developing TB disease have shown higher rates of individuals with LTBI progressing to TB disease among those with positive IGRA (14.6%) compared to TST (2.3%) [13]. This implies that the 5-10% progression rate estimated by WHO (Stop TB partnership) using TST is lower than the actual rate if analysed by IGRA [18]. This further indicates the need for treatment of LTBI among healthcare workers in developing countries. If HCWs develop active TB, they are at risk of transmitting the infection to their patients, including those who are immuno-compromised [22].

The risk factors identified in this study are length of time of service and age. Both univariate and multivariate analysis showed that positivity of latent TB infection was significantly higher among older participants employed for 11 years or more years in the healthcare sector. Age and length of service have earlier been shown to be important in several other studies [14,15,16]. In the healthcare sector, it is a reflection of variation in exposure frequency and intensity as corroborated in previous studies [16]. Pai et al. [9] also found an association between prevalence of LTBI with longer duration of employment. They found that those who were employed for 10 years or more had a threefold increase of prevalence of LTBI compared to those who worked less than a year. Longer duration of employment was associated with longer cumulative exposure to patients with TB, hence cumulative probability of having LTBI. Although direct contact with TB patients has always been shown to be risk factor in many previous studies [14,15,16], this study did not find a strong effect across all the categories of

healthcare workers. This is not surprising however, as more than 85% of the study participants reported direct contact with TB patients thereby reducing the predictive value of this exposure factor. Data obtained from this study show that working as a laboratory staff and as a Ward orderly was significantly associated with LTBI.

This study has demonstrated no impact of previous BCG vaccination on TST and IGRA. Earlier studies have also shown BCG vaccine to cause a negative impact on TST/IGRA results [23,4]. This could be because the participants of this study largely comprised of adult above the age of 20 years, who had received BCG vaccine at birth according to government policy on expanded programme on Immunization (EPI). In individuals vaccinated at infancy, TST response due to BCG vaccine wanes rapidly and becomes insignificant after 10 years [24,25]. Another explanation could be attributed to miscalculation of BCG vaccination status of the individuals owing to the use of scar as a proxy [24]. Recent tuberculin surveys from India involving more than 100,000 participants have also shown that BCG vaccination does not influence the estimation of annual risk of infection [24]. However, BCG vaccine might have an effect on TST in other populations, depending on vaccine strain, frequency and time since vaccination was administered [16,25].

Data obtained in this study could not be compared to the general population since there are no base-line epidemiologic data available for LTBI in the general population.

5. CONCLUSION

In conclusion, the results obtained in this study have been validated by other reports claiming superiority of IGRA to TST methods in LBTI diagnosis. It has provided a base-line information regarding diagnosis of LTBI and infection risk among healthcare workers involved in TB control in Akwa Ibom State. Obtaining base-line and valid information on LTBI among healthcare worker population is important given that one of the priorities of TB control is the implementation of effective TB infection control measures.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely

no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

The study protocol was approved by the ethics review committee of the Akwa Ibom State Ministry of Health. All the participants gave written informed consent prior to their inclusion in the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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