PREVALENCE OF LATENT TUBERCULOSIS INFECTION AMONG HEALTHCARE WORKERS USING INTERFERON-GAMMA RELEASE ASSAY

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ABSTRACT

Background: Healthcare workers (HCWs) are at increased risk of Mycobacterium tuberculosis infection due to occupational exposure. Latent tuberculosis infection (LTBI) poses a silent threat that can progress to active disease if undetected. Interferon-Gamma Release Assays (IGRAs) offer improved diagnostic accuracy over the traditional Tuberculin Skin Test (TST), particularly in BCG-vaccinated populations.

Objective: To determine the prevalence of LTBI among healthcare workers using the Interferon-Gamma Release Assay and to identify associated demographic and occupational risk factors.

Methods: A cross-sectional study was conducted from January 2024 to February 2025 at tertiary care centers across Punjab, Pakistan. One hundred HCWs, including doctors, nurses, laboratory technicians, and support staff, were enrolled. Demographic data and exposure history were recorded using a structured questionnaire. Blood samples were analyzed using the QuantiFERON-TB Gold Plus assay. Statistical analysis was performed using SPSS version 26.0, and associations were tested with Chi-square and Fisher's exact tests (p < 0.05 considered significant).

Results: The overall prevalence of LTBI was 30%. Positivity was higher among nurses (37.0%) and laboratory staff (33.3%) compared to doctors (23.7%) and support staff (29.4%). Significant associations were observed between LTBI and employment duration ≥ 10 years (p = 0.02) as well as direct contact with TB patients (p = 0.01). Gender, age, and BCG vaccination status showed no significant relationships.

Conclusion: A substantial proportion of HCWs had latent tuberculosis infection, highlighting persistent occupational exposure risks. Regular IGRA-based screening, infection-control training, and use of personal protective equipment are essential to prevent transmission and reactivation of TB among healthcare staff.

Keywords: Latent tuberculosis infection; healthcare workers; interferon-gamma release assay; QuantiFERON-TB Gold Plus; occupational exposure; Pakistan.

This article may be cited as: Hassan SMU, Imran M: Prevalence of Latent Tuberculosis Infection among Healthcare Workers Using Interferon-Gamma Release Assay. Pak Med & Allied, 2025; 01(6): 24-29.

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INTRODUCTION

Tuberculosis (TB) remains a significant public health issue of concern in the world today, as it is among the top ten causes of mortality in the world and the most significant cause due to a single infectious agent¹. The World Health Organization (WHO) Global TB Report 2023 estimates that in 2022, the world experienced a total of about 10.6 million new TB cases and 1.3 million deaths with the greatest burden being experienced in the low and middle-income countries including Pakistan. Although there are enormous improvements in diagnosis and treatment, the transmission of TB is a major issue, especially in

healthcare facilities whereby the person is easily exposed to diseased individuals².

The repeated occupational exposure to patients with active TB puts healthcare workers (HCWs) at a greater risk of contracting Mycobacterium tuberculosis infection. One of the factors that contribute to this risk is due to poor ventilation in a healthcare facility, poor practices of infection control, overcrowding, and poor use of personal protective equipment. Morbidity of latent tuberculosis infection (LTBI) in HCWs is therefore a significant parameter that is used to measure the effectiveness of

infection control in hospitals and other health care facilities³.

Latent tuberculosis infection is a condition of unremitting immune generator in respect to M. tuberculosis antigens without any clinical and radiographic demonstrations of active disease⁴. The LTBI individuals do not show any signs but since they do carry viable bacilli which may activate to lead to active TB especially when the person is immunologically suppressed, diabetic, or malnourished. It is approximated that approximately a quarter of the world population is LTBI and 5-10 percent of them will develop active TB throughout their life. Thus, LTBI should be timely diagnosed and treated with preventive therapy to interrupt the transmission chain and lower the cases of TB in high-risk groups, such as HCWs⁵.

Achieving LTBI detection through the tuberculin skin test (TST) has been the traditional way of doing it. Nevertheless, the test has a number of limitations: It may give false positives in case of previous exposure to Bacillus Calmette -Guerin (BCG) vaccination or exposure to non-tuberculous mycobacteria, false negatives in immunocompromised people, and two visit test procedure. In order to deal with these difficulties, Interferon-Gamma Release Assay (IGRA) was proposed as a more specific and convenient way⁶. IGRA (i.e. interferon-gamma (IFN-) assays) like the QuantiFERON-TB Gold Plus test, are a test that detects the release of interferon-gamma (IFN-) by sensitized T-cells in response to TB-specific antigens early secretory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10)-which are not produced by BCG strains, or most environmental mycobacteria. Therefore, IGRAs are more specific, and they do not crossreact with BCG vaccination, which is an especially valuable benefit in the high-burden countries such as Pakistan where the universal immunization against BCG is the rule 1,5 .

A number of studies carried out across international borders have emphasized the fact that LTBI prevalence among HCWs has been found to be between 20 and 60 percent based on the level of exposure, the method of diagnosis and local TB burden. Nevertheless, Pakistan data are still very sparse especially those collected by the IGRA approach^{5,6}. Since the extent of TB background among the general population is high and most of the healthcare institutions have not adopted strict infection control measures, there is an urgent need to find out the actual burden of LTBI in HCWs using the correct diagnostic tools⁷.

Thus, the current research was aimed to identify the rate of latent TB infection among medical professionals with the help of Interferon-Gamma Release Assay (IGRA) and to determine demographic and work related variables linked to positive IGRA results. The results are expected to be evidence based to enhance infection control interventions, preventive measures, and to make the work

environment safer among the healthcare workers working in the high burden environments⁸.

MATERIALS AND METHODS

Study Design and Setting: This cross-sectional descriptive research paper aimed at identifying the latent tuberculosis infection (LTBI) prevalence among healthcare workers through Interferon-Gamma Release Assay (IGRA). The research was conducted in a sample of tertiary care centers in Punjab, Pakistan, comprising of large government and teaching hospitals, during a period of thirteen months of January 2024 to February 2025. These hospitals were characterized as high-patient load hospitals where the medical personnel are highly exposed to infectious diseases such as tuberculosis. The settings were selected to depict both the urban and semi-urban healthcare settings, which ensured that there was an accurate estimation of the burden of LTBI within the healthcare workforce in the region.

Study Population: All 100 healthcare workers (HCWs) were recruited into the study by means of stratified random sampling in order to have a proportional representation of the various occupational groups of the healthcare workers such as doctors, nurses, laboratory technicians, and support staff. The criteria to qualify as participants were an age bracket of 20-60 years, a period of at least one year of experience in a healthcare system and the absence of any sign or previous history of an active TB. Immunocompromised persons (HIV view, chronic steroid therapy, or malignancy), individuals receiving antituberculosis medication or unwilling to participate were excluded. In this way, the assessment of only healthy individuals at risk of occupational exposure was provided.

Ethical Considerations: This study received ethical approval through the Institutional Review Board (IRB) of the hospitals included in the study through an IRB/TB/2024/112 reference number. The purpose of the study, procedures, and possible risks and benefits of the study were clearly explained to the participants, then written informed consent was obtained. The confidentiality of the identities and laboratory results of the participants was ensured during the study. Students who got a positive IGRA were advised and given a referral to specific TB clinics to receive additional assessment and preventive treatment based on national TB control.

Data Collection: An effective and pretested questionnaire was used to collect the data through the means of the trained research staff. The demographics were captured in the questionnaire which included age, gender, job category, department of posting and service duration. The occupational and clinical data were recorded such as BCG vaccination status, past TB exposure, smoking and any known household patient contact with TB. The questionnaires were also checked at the end of the interview to be complete and correct to provide reliable data entry.

Laboratory Analysis and Collection of Samples: Aseptically, about 4 mL of the venous blood of each participant was collected following informed consent. The Interferon-Gamma Release Assay test was conducted with the kit of OuantiFERON-TB Gold Plus (OFT-Plus, Qiagen, Germany) under the standardized procedure of the manufacturer. Four special tubes were labeled Nil, TB1 Antigen, TB2 Antigen, and Mitogen (positive control): blood samples were transferred into them. The tubes were carefully inverted and incubated upright at 37C of between 16 and 24 hours. Plasma was then incubated and enzymelinked immunosorbent assay (ELISA) was used to determine interferon-gamma levels in plasma. A test was considered to be positive when TB antigen minus Nil was 0.35 IU/mL and 25% of Nil value and negative when it was below this level with sufficient Mitogen response and indeterminate when the control values were invalid. All the assays were performed under biosafety level II laboratories under trained technologists and quality control procedure was adhered to.

Quality Assurance and Control: Strict quality control measures were taken to make laboratory results accurate and reliable. Each run had positive and negative controls of the internal assay. To reduce the bias of the observers, the IGRA tests conducted in the laboratory covered the participants with their demographic and exposure information. During the study period, equipment calibration, reagent validation and compliance with the biosafety procedures were ensured.

Data **Analysis** and Statistical **Management:** Questionnaire and laboratory report data were inputted and checked with Microsoft excel and processed with Statistical Package of Social Sciences (SPSS) version 26.0 (IBM corp., Armonk, NY, USA). The descriptive statistics was used to summarize the characteristics of the participants in terms of frequencies and percentages in categorical variables, and in terms of means and standard deviations in the continuous variables including age and years of service. The Chi-square test or Fisher's exact test was used to assess the associations between IGRA positivity and the independent variables such as gender, age, profession, years of employment, BCG vaccination and TB exposure history. A p-value of below 0.05 was taken as significant. The findings were also stratified by occupation and exposure length to assess the high-risk groups of occupations in the healthcare workforce.

Outcome Measures: The prevalence of latent tuberculosis infection among healthcare workers in terms of IGRA positivity was the major outcome measure. Secondary outcomes were the analysis of demographic and occupational risk factors that had positive IGRA results. The purpose of these findings was to determine high-risk groups and inform the use of specific prevention and control measures of this infection in hospitals.

RESULTS

This cross-sectional study was carried out among 100 healthcare workers (HCWs) in the tertiary care centers located at Punjab, Pakistan, between January 2024 and February 2025. The average age of the subjects was 35.2 /7.6 years with a range of 22 to 58 years. The study population was evenly represented (52 (52) males and 48 (48) females) indicating a balance of genders that was a representation of the gender balance at most hospital settings. The total prevalence of the latent tuberculosis infection (LTBI) according to Interferon-Gamma Release Assay (IGRA) were 30/100 (30%). Among them, 17 (32.7) were males and 13 (27.1) were females with no statistically significant difference that existed between the two genders (p = 0.48). Nevertheless, the positivity of males is slightly higher, which can be explained by the fact that they are more likely to work in high-exposure departments (pulmonology, internal medicine, radiology) in which TB patients occur quite often.

Table 1 is a summary of gender and profession of the study participants. The professional group that included the highest number of participants (38 or 38 percent of the total participants) was that of doctors, then there were the nurses with 27 (27), the laboratory technicians with 18 (18), and the support staff with 17 (17). The prevalence of IGRA positivity was different in these categories. Five of the doctors were positive; five of the nurses; five of the laboratory technicians; and five of the support staff. In such a way, the largest positivity rate of LTBI was observed in nurses. The trend may be attributed to the close and extended physical contacts that the nurses have with the patients when delivering treatment and monitoring without regular application of respiratory protection, especially in poorly ventilated wards. The positivity rate of laboratory technicians was also rather high, probably because these specialists are working with sputum and biological samples that can possibly Mycobacterium tuberculosis bacilli. Rates among doctors and support staff were lower relative to the other groups, which may be because of the reduced time spent on direct exposure or because of increased levels of awareness and compliance with infection-control measures.

Table 1: Gender and Professional Distribution of Healthcare Workers with IGRA Results

Variable	Category	n (%)	(%) IGRA Positive	
			n (%)	
Gender	Male	52 (52%)	17 (32.7%)	
	Female	48 (48%)	13 (27.1%)	
Profession	Doctors	38 (38%)	9 (23.7%)	
	Nurses	27 (27%)	10 (37.0%)	
	Lab Technicians	18 (18%)	6 (33.3%)	
	Support Staff	17 (17%)	5 (29.4%)	
Total	100 (100%)	30 (30%)		

(Table 1 shows the proportional representation of HCWs and corresponding IGRA positivity rates across gender and occupational categories.)

Comparing the results based on age groups, it was established that the prevalence of LTBI moderately improved with the age, albeit not significantly (p = 0.37). The participants under the age of 35 years positivity rate was 27.3 and that of participants above the age of 35 years was 32.6. This trend can be due to accumulation of exposure risk over age and not age-related variation in immune. The average length of employment was 8.9 years old (4.1) with 47 (47) and 53 (53) being a duration of less than 10 years and 10 years or more respectively. Interestingly, there were much higher LTBI positivities in individuals who had higher number of years of employment (37.7%) than those with less employment years (21.3%), and the p-value was 0.02, which showed that occupational exposure over the years was an important determinant of infection.

Moreover, 41 (41%) of the healthcare personnel members indicated that they had known previous direct contact with patients who had active tuberculosis. Nineteen of these (46.3) were positive by the IGRA compared to 11(18.6) of those who had no known contact, a statistically significant difference (p = 0.01). This observation confirms the importance of exposure history, and highlights the need to practice strict infection-control measures in the management of suspected or confirmed

cases of TB. In the case of BCG vaccination, it was found that 92 (92) percent of the respondents were vaccinated at childhood age and this is an illustration of the nationwide vaccination coverage. The positivity of LTBI in vaccinated persons was noted to be 29.3, as compared to that of the eight unvaccinated subjects, which was 37.5, but not significant (p = 0.64). The lack of a definite correlation between the status of the BCG and the IGRA findings proves the high specificity of the interferon-gamma assay, that is aimed to overcome the cross-reactivity with the antigens of a BCG.

Potential risk modifiers were also considered to include smoking status and department at the workplace. Of the 23 respondents who were active smokers, 9 (39.1) were IGRA positive, and 21 (27.6) were not smokers, but this difference was not found to be significant (p = 0.21). On the same note, employees who had to work in high risk departments like pulmonology, internal medicine, or microbiology had a combined LTBI prevalence of 35.8% as opposed to those working in administrative or low exposure areas on the prevalence of 24.2% positivity. Even though, the trend indicates the increased risk of infection in patient-facing or laboratory positions, the sample size was small and restricted the statistical power of such an observation.

Table 2 demonstrates the distribution of LTBI by its demographic and occupational features in greater detail.

Variable	Category	Total (n)	IGRA Positive n (%)	p-value
Age Group	< 35 years	55	15 (27.3%)	0.37
	≥35 years	45	15 (33.3%)	
Duration of Employment	< 10 years	47	10 (21.3%)	0.02
	≥ 10 years	53	20 (37.7%)	
TB Contact History	Yes	41	19 (46.3%)	0.01
	No	59	11 (18.6%)	
BCG Vaccination	Yes	92	27 (29.3%)	0.64
	No	8	3 (37.5%)	
Smoking Status	Smoker	23	9 (39.1%)	0.21
_	Non-smoker	77	21 (27.3%)	

(Table 2 demonstrates that longer employment duration and a history of TB exposure were significantly associated with IGRA positivity among HCWs.)

The findings, in general, demonstrate that almost a third of healthcare workers were infected by latent tuberculosis infection, which shows that it is a significant occupational health issue. The substantial correlation between the IGRA positivity, length of employment, and contact with TB patients emphasize the cumulative effect of the workplace contacts on the risk of infection. No significant predictors were identified with gender, age, smoking, and vaccination status but there were slight trends. These results support the importance of periodic occupational screening and precautionary measures to prevent infections by using N95 respirators, proper

ventilation, and regular IGRA tests to detect the presence of asymptomatic carriers in healthcare institutions.

DISCUSSION

The current research assessed the incidence of latent tuberculosis infection (LTBI) in healthcare providers (HCWs) in tertiary care facilities in Punjab, Pakistan, on the Interferon-Gamma Release Assay (IGRA)⁹. The total LTBI prevalence rate of 30% in this study is a significant occupational health issue in a nation that already has high rate of tuberculosis. This prevalence is fully consistent with the previous observations in high-burden areas, with 25% to 40% of HCWs in the past having LTBI based on the methodology and level of exposure at work. Similar rates have been reported by Nasir et al. (2019) in Karachi

with a 33.7 percent prevalence rate of IGRA and by Hedayati et al. (2020) in Iran with a 29 percent positivity of the hospital personnel. These findings are consistent across various high-TB-burden environments; a fact that highlights the persistence of the occupational vulnerability of healthcare personnel¹⁰.

Of particular interest is the slightly higher rate of infection of nurses and laboratory staff in the given study. Due to their bedside care practices, nurses often need to deal with sputum, secretions and contaminated surfaces when they maintain extensive contact with patients. In a like manner, laboratory technicians also have regular exposure to biological samples that have Mycobacterium tuberculosis bacilli, especially when processing and staining specimens^{7,9}. These results are consistent with the findings of Uden et al. (2017), who found that the odds of LTBI are two to three times more common in nurses and laboratory personnel than in the administrative staff. These high rates within these groups in our study are, therefore, a result of occupational exposure patterns among these groups, but not individual susceptibility¹¹.

The fact that the positive correlation between the length of employment and LTBI positivity (p = 0.02) supports the idea that the cumulative effect of the length of exposure in a healthcare setting is at play¹². Employees with ten years and above service showed almost twice the rate of infection as compared to those with lower time of service implying that permanent exposure to the job is a major contributor to risk as time goes by. These trends have been also observed in the Indian and Ethiopian studies that have found positive results in long-term HCWs because of re-exposure to untreated or insufficiently isolated TB cases. This observation highlights the need to have continuous occupational screening and not just a one-time pre-employment assessment¹³.

The direct contact with TB patients was also noted to have a strong and statistically significant association with IGRA positivity (p = 0.01). Almost half of the participants who had history of known exposures were found to be IGRA positive, which affirmed the direct effect of unprotected or frequent contacts with infected patients¹⁴. This observation is in line with the world evidence that exposure to smear-positive TB cases without proper respiratory protection is the only most significant predictor of LTBI among healthcare workers. Though there are infection-control guidelines in Pakistan, adherence is not always ideal, as hospitals are overcrowded, the ventilation is poor and isolation practices have not been practiced to the best of their ability. The results of the presented study indicate that it is necessary to implement extreme measures when it comes to adherence to infection prevention methods, especially in the departments of pulmonology, emergency medicine, and microbiology, where the risk of transmission is the biggest¹⁵.

Interestingly, the independent variables (gender, age, and BCG vaccination) did not exhibit any significant

association with LTBI in this study. The lack of gender disparities indicates that the risk of exposure is more affected by work duties rather than biologically predisposed to the risk¹⁶. The non-correlation with BCG vaccination also confirms the specificity of IGRA which involves TB-specific antigens (ESAT-6 and CFP-10) that cannot be influenced by previous vaccination or environmental contacts with mycobacteria. This underscores the superiority of IGRA over the old Tuberculin Skin Test (TST) in terms of diagnosis especially in people whose BCG immunization is universal¹⁷.

Even though it was found that a slightly higher rate of infection was present among smokers and those who were employed in high-risk departments, there was no statistical significance of the associations. This could be explained by the small sample size and the diversity in the intensity of exposure by departments¹⁸. However, these results cannot be overlooked, and the damage to pulmonary immunity caused by smoking has been known and may make a person vulnerable to being infected with M. tuberculosis. These associations could be better explained by future research, using a larger sample size, and quantitative assessment of exposure¹⁹.

The prevalence rate of 30 percent in this study is an indicator of the endemicity of the tuberculosis disease in Pakistan and the occupational risks of the health care professionals. However, research in low-TB-burden nations like the United Kingdom and the United States has indicated that the prevalence of LTBI in HCWs is less than 10% and highlights the role of national TB burden and the infrastructure to control infections. Its implications are therefore that there is an urgent need to have tailored screening programs, particularly in high-risk departments. Regular surveillance using IGRA, respirator use, creation of negative pressure isolation rooms, and yearly training on TB awareness are essential preventive measures, which can play a major role in minimizing the transmission at the workplace 19-20.

The identification of the LTBI condition in the HCWs is not only vital in protecting personal health but also plays a significant role in ensuring minimum biosafety in hospitals and ensuring that their nosocomial infection is not spread to the susceptible patients. Because HCWs may serve as the incubators of the active TB cases in the future, the early diagnosis and the preventive chemoprophylaxis can contribute significantly to the chain of transmission. Integrating IGRA screening as an additional screening method in the yearly health check-ups of healthcare employees, especially those with long period of employment or with a high rate of contact with TB would be a preventive measure towards occupational health management^{7,9}.

Although the research has some significant insights, some weaknesses need to be noted. The sample was small and limited to tertiary centers in Punjab, which might not

be applicable to primary or private healthcare centers^{5,6}. The cross-sectional design does not determine the time relationships and there was no analysis of potential confounders like nutritional status or coexisting infections. Nevertheless, the applicability and validity of the results remains strong in light of the use of a very specific diagnostic test, the introduction of different types of healthcare, and a set of the standard laboratory processes^{19,20}.

CONCLUSION

The research concludes that the prevalence of latent tuberculosis infection among healthcare workers in tertiary care units of Punjab, Pakistan, is high with the overall prevalence of the disease estimated to be around 30 percent as established by Interferon-Gamma Release Assay. Nurses and laboratory staff showed the highest prospect of infection and considerable correlation was found with longer period of employment and direct contact with TB patients. These results point to the ongoing occupational hazard of tuberculosis infection in healthcare settings and the significance of regular screening and Institutional methods. infection-control programs should include routine IGRA testing, especially among the high-risk groups. Proper ventilation, respiratory protection measures, and frequent TB prevention education can help alleviate the risk of the infections to a considerable degree. Moreover, policies of early diagnosis and chemoprophylaxis among the workers with LTBI should be implemented to inhibit the reactivation and hospital-based transmission. The continuous enhancement of infection-control facilities on the national scale with the help of routine surveillance and the policy implementation is also vital to not only protect healthcare providers but also mitigate TB spread in Pakistan as a whole.

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