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PREVALENCE OF LATENT TUBERCULOSIS INFECTION IN HEALTH CARE WORKERS IN POLAND ASSESSED BY INTERFERON-GAMMA WHOLE BLOOD AND TUBERCULIN SKIN TESTS

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Health care workers (HCWs) are at risk for developing active tuberculosis (TB). The prevalence of latent tuberculosis infection (LTBI) in this group is unknown in Poland, due largely to the problems associated with the interpretation of the tuberculin skin test (TST) in BCG immunized population. The goal of the present study was to assess the prevalence of LTBI in both clinical and non-clinical 155 HCWs (120 females and 35 males) and to compare the groups at different levels of risk. All participants were interviewed using a questionnaire and underwent interferon-gamma whole blood assay (Quantiferon-Tb-Gold) (QTF) and TST. The questionnaire provided information on possible risk factors for LTBI, including demographic and socioeconomic details, the presence of BCG scars, and the degree of occupational exposure. We found that the prevalence of LTBI among HCWs was, on average, 27.1%. A higher risk of acquiring LTBI was associated with certain work locations (TB lab workers - prevalence 50%, TB ward clinicians - 34%, nurses - 30%). The prevalence in analytical lab technicians was 20%, in administration staff was 15%. The HCWs with positive QTF test results were older and worked longer than those who had negative results. There was a significant correlation between the level of IFN- γ and both age ($P < 0.001$) and length of employment ($P < 0.01$). The correlation between the diameter of skin test induration and the magnitude of the INF- γ response also was significant ($P < 0.001$). We conclude that HCWs are at increased risk of infection, suggesting that appropriate preventive strategies should be undertaken. IFN- γ test is a useful tool in detecting LTBI cases in a country where BCG vaccination is a national policy.

Key words: *health care workers, interferon gamma, Quantiferon-TB-Gold, tuberculin skin test, tuberculosis*

INTRODUCTION

Tuberculosis (TB) is a serious public health problem in Poland (1-3). Detection and monitoring of latent *M. tuberculosis* infections are essential to control its spread. Targeted tuberculin skin testing (TST) and drug therapy to prevent latent *M. tuberculosis* infection (LTBI) from progressing to overt disease are important TB elimination strategies in low-incidence countries (4). In Poland, we still focus on the clinical management of patients with active disease, but preventive programs that identify and treat individuals with LTBI will certainly be more widely recommended in the near future. As we approach the elimination phase of TB, due to the steadily declining incidence in the population, the importance of preventive measures will grow. Future TB control strategies will focus on diminishing the prevalence of LTBI to reduce the pool of those infected, from which future cases of TB could arise.

Tuberculin skin test (TST) has been used effectively for detection of latent *M. tuberculosis* infections for more than 100 years, despite many drawbacks such as variability and subjectivity in test application and reading as well as false positive results, especially occurring in BCG-vaccinated populations (5). Nowadays, the advances are provided by a new generation of immunoassays, including Quantiferon-TB-Gold (QTF) based on the detection of IFN- γ secretion by peripheral T cells upon incubation with the specific *M. tuberculosis* antigens ESAT-6 and CFP-10 (6, 7). The ESAT-6 and CFP-10 proteins, secreted by *M. tuberculosis* cells, are potent T-cell antigens which have a role in TB pathogenesis. These antigens are considered to be powerful reagents for the precise detection of LTBI in both BCG-vaccinated and unvaccinated populations (6-8).

As TB in the Polish population has declined, high-risk groups become more visible (1). HCWs are at risk for TB exposure and infection when they care for patients or process clinical samples from TB patients (9-13). The risk of transmission of *M. tuberculosis* from individuals with TB onto other patients and HCWs has been known for many years (12, 13). Therefore, screening of workers for LTBI remains an integral part of tuberculosis control programs in health-care facilities (11). Bacille Calmette-Guérin (BCG) vaccination is a national policy in Poland. The repetitive administration of BCG (and possibly hypersensitization from multiple TST) makes the diagnosis of LTBI, using traditional tuberculin skin tests in Poland, difficult.

The aim of this project was to estimate the prevalence of latent tuberculosis infection in high-risk populations using the TST and QTF, to determine the agreement between both tests, and to compare their relation to risk factors.

MATERIAL AND METHODS

This study was approved by a local Ethical Committee of the Institute of Tuberculosis and Lung Diseases in Warsaw, Poland. All study participants provided written informed consent. A hundred and

fifty five subjects (120 women and 35 men); aged 24-69 (mean 42.1 \pm 9.8SD years) were enrolled, including both clinical and non-clinical health care workers. Clinical health-care workers included those with direct patient contact or contact with TB patient's sputum samples: doctors (n=31), nurses (n=19), and staff of TB labs (n=46). Nonclinical health-care workers included those who did not have direct patient contact, such as administration (n=19) and analytical laboratory employees (n=30). All TB and non-TB staff underwent chest radiological screening biannually. Neither immunocompromised nor pregnant individuals participated in this study. Patients with a history of TB were excluded. All subjects were BCG vaccinated. The study population underwent screening examination including face-to-face interview by means of a questionnaire and physical examination. Interviewers were medical doctors experienced in TB. The questionnaire contained information about age, gender, history of risk factors, degree of occupational exposure (year of training and active clinical/nursing practice in the health care service, job category), non-medical tuberculosis contact, smoking and alcohol consumption, any coexisting illness, medication(s) received, known radiographic changes and prior knowledge of sensitization to TST. Demographic and clinical data were correlated with TST and the QFT-Gold results. Everyone identified as LTBI positive within the medical service was offered information on the published sensitivity and specificity of the tests, the potential value of these tests, and counseling on the risks and benefits of chemoprophylaxis.

Tuberculin skin test (TST)

Trained health care workers performed and interpreted the results 48 h after application according to the American Thoracic Society (ATS) and Centers for Disease Control and Prevention guidelines (14). The positive interpretation of a TST is an area of induration \geq 10 mm.

QFT assay

Venous blood was collected into heparinized tubes provided by the manufacturer of the test. Blood was transported to the lab in the upright position with the use of portable blood incubator. The QFT assay was performed in accordance with the manufacturer's instructions. The testing was conducted in two parts, an overnight culture of blood with stimulation antigens and the subsequent quantification of IFN- γ production. Following an overnight incubation at 37°C in humidified atmosphere, the supernatant plasma was harvested and then stored at -80°C until the analysis. All samples were assayed for IFN- γ in a single ELISA run. Results were calculated and interpreted according to the manufacturer's instructions. Calculations were performed using software provided by the manufacturer's kit (Analysis Software v1.51 Cellestis).

Statistical analysis

Differences between the examined groups were compared using the Mann-Whitney U test. Correlation between TST and INF- γ level was assessed with Spearman's correlation test. Statistical significance was accepted at $P < 0.05$. All analyses were performed with a Statistica software program.

RESULTS

The prevalence of LTBI among HCWs was, on average, 27.1%. A higher risk of acquiring LTBI disease was associated with certain work locations (TB lab workers - prevalence 50%, TB ward clinicians - 34%, nurses - 30%). The

Table 1. Age, length of employment, and magnitude of TST induration in the HCW groups with positive and negative QTF test results.

	QTF positive	QTF negative	P<
Mean age (yr)	49.5 ±12.5	37.8 ±1.8	0.01
Mean time of employment (yr)	25.3 ±11.8	13.1 ±1.2	0.01
Mean TST induration (mm)	16 ±1.9	11.4 ±1.2	0.05

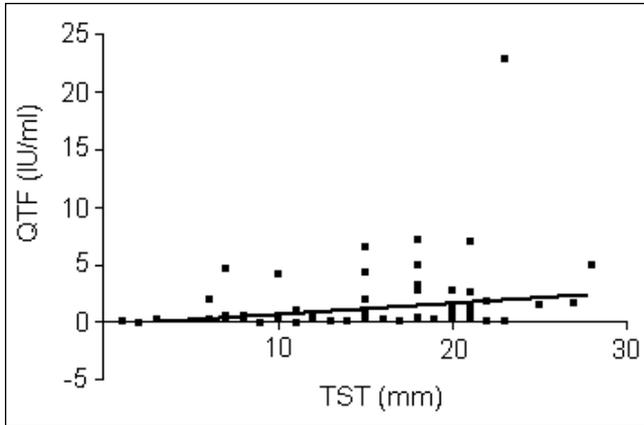


Fig. 1. Correlation between INF-γ production and the magnitude of TST induration in health care workers.

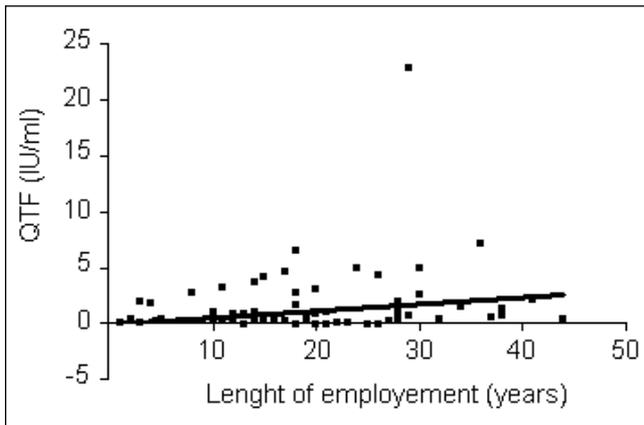


Fig. 2. Correlation between INF-γ production and the length of employment in health care workers.

prevalence in analytical lab workers group was of 20% and in administration staff group of 15%. All microbiologists processing sputum from TB patients without modern safety facilities were infected. Gender was not associated with a positive QTF test result. The HCWs with positive QTF test results were older and worked longer than individuals with negative results (Table 1). The mean TST induration was bigger in the QTF positive than negative group (Table 1). The prevalence of

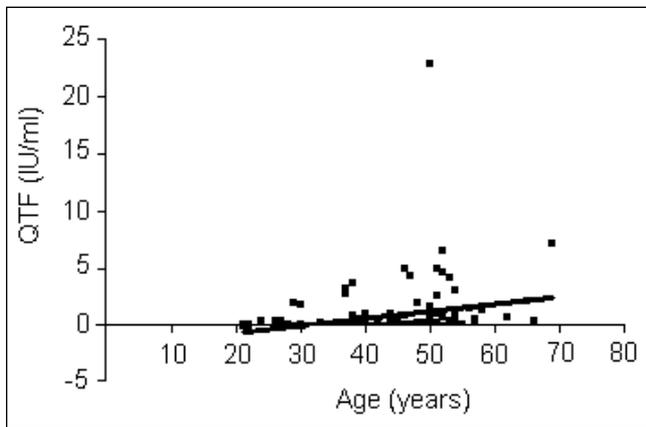


Fig. 3. Correlation between INF- γ production and the age of health care workers.

LTBI in HCWs older than 40 years was 62%, and in the group younger than 40 years, it was 22%. Among HCWs working less than 10 years, infection rate was 33% and in those working longer than 10 years, it was 56%. All employees of TB lab equipped with modern safety facilities were free from infection. The presence of a BCG scar was not significantly associated with a higher prevalence of LTBI.

The correlation between the diameter of the skin test induration and the magnitude of the INF- γ response was highly significant ($P < 0.001$) (Fig. 1). Consistency of both tests was 82%. There also were correlations between the level of INF- γ , on one side, and age ($P < 0.001$) (Fig. 2) or length of employment ($P < 0.01$) (Fig. 3), on the other.

DISCUSSION

The rate of TB in the general population in Poland was 24.3/100000 in 2004 (1), but TB incidence among HCWs staff in contact with TB was 204-721/100000 in 2001 (2). There is little information available on the rate of LTBI infection in HCWs, due largely to the methodological problems associated with interpreting TST in a population that is universally immunized for BCG and have had multiple TST examinations. TST has been used for many years to screen contacts of patients with tuberculosis and other populations of high TB infection risk (5, 11). However, TST is based on a crude mixture of poorly defined mycobacterial antigens, some of which are shared with the *M. tuberculosis* complex, environmental non-tuberculous mycobacterial strains, and the vaccine substrain *M. bovis* BCG (5).

In the present study, we attempted to establish the rate of LTBI infection in HCWs using a novel *in vitro* INF- γ assay. The QuantiFERON-TB-Gold test has been recently developed to overcome some of the limitations of the TST (6, 7). This provides an opportunity for targeted intervention. An effective program for

high-risk groups in low-incidence countries includes chemoprophylactic treatment (11). The common policy in Poland is to apply chemoprophylaxis only in children and in dual HIV and TB infected patients. However, if we have a more accurate tool to detect latent TB in BCG vaccinated subjects, a national policy of preventive therapy may also include other risk groups, including HCWs. The goal of the present study was to compare an *in vitro* response, as measured by QFT test results, with the size of the TST induration in HCWs. Both tests were simultaneously performed in 155 subjects divided into the different subgroups to estimate the prevalence of latently infected clinical staff within the TB medical services (high-exposure group), in comparison with other medical staff, not primarily working with TB patients (low exposure). The study supports the hypothesis that there is a higher prevalence of LTBI in HCWs within the TB services, including those working in TB labs. There was considerable heterogeneity in the risk of LTBI between different occupations: doctors and nurses in TB wards and TB laboratory personnel had a high incidence of TB disease, while the incidence of LTBI was lowest in administrative staff. The analysis demonstrates that work in TB services was the most important risk factor for LTBI, reflecting extensive exposure to *M. tuberculosis*. Our results are consistent with those of other studies in HCWs from a high (India, Russia, and Georgia) and middle (Japan) incidence countries (13, 15-18). In our study, the prevalence of LTBI in senior years was two times greater than that in junior years. A study from India reported a 4-fold higher prevalence in medical students who were more than 23 years of age than in those aged 18-20 years (13).

The IFN- γ based assays appear to offer a rapid *in vitro* testing requiring a single visit, which is less subjective than TST and can be repeated as an annual occupational screening. Nevertheless, larger cohort studies are necessary to assess the value of these tests, risks of active TB development in positive individuals, and effectiveness of preventive therapy based on IFN- γ test results. The results may modify institutional infection control and occupational health policies. Negative-pressure facilities are available in Polish hospitals, including those centers dealing exclusively with TB (2). In Poland, unlike in Western Europe, a common feature of TB control is the use of ultraviolet germicidal radiation and daily surface disinfection (2). However, continuous effort leading to larger implementation of laminar-flow chambers (present nowadays in 41% of TB labs) may reduce the number of nosocomial transmissions in TB laboratories in Poland.

Preventive therapy given to those with a positive tuberculin test substantially decreases the risk of development of active TB (9). Chemoprophylaxis following a positive TST in Poland is limited largely to younger age groups. All HCW staff is radiologically screened for TB annually with referral to the TB services for full treatment if appropriate. Within the TB services, isoniazid prophylaxis is not widely used, as there is an argument that it would be of limited value due to continued exposure. On the other hand, a reduction of exposure risk should be a high priority, because occupational TB leads to the loss of skilled HCWs, who are

essential in the fight against TB. Unfortunately, there are no guidelines to indicate how this should be done. In the United States and other high-income countries, the risk of nosocomial transmission of TB declined with the reduction in incidence of TB disease in the population (9, 19). This has led to recommendations for a comprehensive set of infection-control practices to protect HCWs and reduce nosocomial transmission (20). In the years following the publication of those recommendations, there was a dramatic decline in the burden of TB among HCWs (9, 20-22). In a recent review of TB among HCWs in high-income countries, the overall incidence of TB disease in the general population and native-born HCWs was less than 10 and 25 per 100,000 per year, respectively (9, 23). The situation is different in Poland, as we still focus largely on case detection and treatment of active cases. At present, IFN- γ tests are not licensed in the Poland, and there are no guidelines on the use of these tests. HCWs in Poland have a higher risk of TB infection than the estimates of risk in the general population (1). In our study, more years of clinical training and greater exposure to TB patients were risk factors for LTBI, and this provides additional prove for nosocomial transmission. Our study provides evidence that a reduction in the risk of LTBI in TB lab can be achieved with implementation of modern safety facilities, but this needs to be evaluated in larger, better-controlled studies.

In summary, there is consistent epidemiologic evidence that TB is an important occupational disease in HCWs. In our study, the infection rate correlated with indicators of exposure, including longer time of employment and workplace that has been identified as high risk. Finally, we noticed that the incidence of infection is lower in TB laboratories which implemented infection-control measures. With the recent emergence of extensively drug-resistant tuberculosis, the need to implement infection-control measures has been reemphasized by global agencies such as the WHO and the Stop TB Partnership (24). Currently available evidence is limited, but it suggests that relatively simple interventions, such as early diagnosis of TB, segregation of infectious TB patients, or education and training of HCWs, might be effective. High rates of LTBI would support the need for the review and reinforcement of institutional cross-infection measures. Additional low-cost measures could include engineering controls, such as exhaust ventilation, improved natural ventilation, or sunlight (24). In high-income countries, there are guidelines to minimize the transmission of TB in health-care facilities (9, 16, 25). Administrative controls help reduce the exposure of HCWs to people with TB. Environmental controls (isolation rooms - present in 50% of Polish hospitals dealing with TB patients) aim to prevent the spread and to reduce the concentration of infectious droplets in the air. Respiratory-protection controls (personal devices with HEPA filters - present only in 12.5% of TB centers in Poland) aim to reduce the risk of infection when exposure to *M. tuberculosis* is unavoidably high (9, 16).

We conclude that latent TB is an important occupational problem among HCWs, in particular those working in the TB services in Poland, as a consequence

of lack of isolation facilities in many TB centers and weak infection control measures. These observations suggest that appropriate preventive strategies and staff health monitoring should be improved. Moreover, extensive studies evaluating the cost, feasibility, and effectiveness of these interventions are urgently needed. IFN- γ tests are useful tools in detecting LTBI cases in a country where BCG vaccination is a national policy.

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