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QuantiFERON-TB 2G: New Strategy for Diagnosing Latent Tuberculosis

Research article

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Abstract

Introduction: Tuberculosis is one of the most important diseases worldwide, imposing a high financial and emotional burden due to the limitations of its diagnostic tests. The present study aimed to investigate the prevalence of latent tuberculosis using the TST and QuantiFERON TB-2G diagnostic tests among the staff of the Shahid Motahari hospital of Urmia.

Materials and Methods: This was a cross-sectional study conducted on 100 workers of Shahid Motahari hospital of Urmia. The Level of Interferon (IFN) γ production in response to the TB7.7 (Rv2654), CFP-10, and ESAT-6 antigens was investigated by the QuantiFERON (QFT) and ELISA methods. The results were analyzed by QFT software.

Results: According to the results, QFT, compared to PPD, presented a sensitivity of 93.75%, a specificity of 95.23%, a positive predictive value (PPV) of 78.94%, and a negative predictive value (NPV) of 98.76%. Based on the Kappa test, there was a strong direct relationship between the QFT and PPD; however, this relationship was statistically insignificant (P=0.07, Kappa coefficient=0.827).

Conclusion: There was a strong agreement between the QFT and TST tests in identifying patients with latent tuberculosis.

Keywords: Latent Tuberculosis; Diagnostic test; PPD; QuantiFERON TB-2G

Introduction

Tuberculosis (TB) is one of the most important chronic diseases, claiming the seventh rank worldwide regarding prevalence, and it is expected to remain in this rank until 2020 [1]. The evolution of Mycobacterium tuberculosis infection to active TB depends on multifactorial interactions involving various biological dimensions [2]. A small ratio of those infected with M. tuberculosis will develop active TB disease, while more than 90% of them remain asymptomatic. The high rate of latent TB infection is the main obstacle to restraining the disease globally [3,4]. For this reason, the detection and treatment of people with latent TB have a special place in the control program simplemented in different countries, especially those with a high rate of the disease [5,6]. It is estimated that more than one-third of the world's population suffers from latent TB, and there is a high potential in the global population for acquiring this infection. Therefore, it is essential to identify and treat people infected with the latent form of the disease [7] According to the results of different studies, It is estimated that around one-third of the world's population is infected with latent TB (LTBI) with 5-10% life time risk of developing active [8].

Various methods have already been introduced to diagnose latent TB infection, the most important of which include the Tuberculin Skin Test (TST), as well as the tests based on interferon-gamma (IFN-γ) production (such as the T-Spot test, Quanti FERON (QFT) test, and ELISPOT) [9]. The TST is older than other methods and is currently used in many countries as a routine test to evaluate individuals' immune system status in front of M. tuberculosis bacteria [10]. Nevertheless, this method suffers from some limitations, including the non-referral of the people who have undergone the test and should refer for reading the result of the test. Also, this test delivers false negative results in HIV-infected individuals [11,12]. The QuantiFERON-TB Gold in Tube is one of the new methods based on IFN-γ measurement to identify Latent TB Infection (LTBI). The QFT has higher specificity than the TST method; its results are not affected by BCG vaccination, and it can be used in endemic areas. The QFT was approved by the US Food and Drug Administration (FDA) in 2005 as a method for detecting M. tuberculosis infection [13]. Due to immune based reaction of PPD, this test interferes with BCG vaccination but the QuantiFERON gold Mycobacterium tuberculosis derived specific peptides (ESAT-6, CFP-10 and TB7.7, the last one only in QFT-GIT) located in the region of difference (RD-1 and RD-11) of Mycobacterium tuberculosis, IGRAs could elicit a more specific immune reaction

compared to TST, characterized by IFN-γ [14,15]. Since these proteins are not present in the BCG vaccine and are not produced by common environmental mycobacteria (except by M.kansasii, M. rolkai, and M. marinum), this method is more specific than TST in identifying M. tuberculosis. The studies comparing the ISI and QFT methods have reported that the recent test has a higher sensitivity and specificity in identifying patients with either active or latent TB infection [16,17]. the present study aimed to investigate the prevalence of latent TB using the two diagnostic tests of TST and QuantiFERON TB-2G among the staff of the Hospital.

Materials and Methods

This cross-sectional study was conducted on the workers of Hospital. Exclusion criteria included: age of <18 years old, pregnancy, recent (less than six weeks) exposure to M. tuberculosis, contact with active TB patients and immunocompromised people. We enrolled all voluntary medical workers in this study. Demographic data such as age and gender were recorded in a checklist. Before performing the TST test, blood samples were obtained for conducting the IFN-γ production assay. The participants were asked about their history of BCG vaccination, and the vaccine scar was checked on all the subjects. The formula developed to estimate a ratio in a population was used to calculate the sample size at the 95% confidence interval (Z1-a/2=1.96) and the error rate of 0.05. According to the results of Vinton et al. (p=0.067) (22), the sample size was calculated as 100. Random stratified sampling was used for recruiting people. Initially, the list of all personnel in different job ranks (faculty members, non-faculty member physicians, nurses, and cleaning staff) was prepared. Then according to the weight of each category, the subjects were randomly chosen from each group.

Interferon-γ production in response to the TB7.7 (Rv2654), CFP-10, and ESAT-6 antigens was assessed by the QFT test and according to the manufacturer's instructions (QuantiFERON-TB Gold, Cellestis Ltd, Carnegie, Victoria, Australia). This test was performed in two steps. In the first step, whole blood was incubated with the mentioned antigens, and in the second step, the serum level of IFN-γ was measured using ELISA. Venous blood was collected into three 1mm heparinized tubes, one of which was used as a negative control and the other tubeas a positive control. Both of these tubes contained phytohemagglutinin, a T-cell mitogen. The third tube, which contained the TB7.7 (Rv2654), CFP-10, and ESAT-6 antigens, was used to assess the status of the patient's immunological memory against M. tuberculosis [18]. After blood samples were added to these tubes, they were incubated at 37°C for 16 to 24 hours. Afterward, sera were obtained by centrifugation and analyzed by the ELISA method to determine IFN-γ concentration according to the kit producer's instructions. Finally, the results were analyzed by QFT software. According to the instructions of the IFN-γ ELISA kit, a level equal to or greater than 0.35 international units/mL was regarded as a latent TB infection [17]. After blood sampling from the right hand, 0.1mL of PPD tuberculin was intradermally injected into the middle part of the left arm. The diameter of induration was measured using a ruler after 48-72 hours, and a diameter above 10mm was considered positive [19]. In this study, the data were analyzed using SPSS 21 statistical software.

Results

In this study, 100 healthcare workers of the hospital were enrolled. The mean age of the participants was 36.26 ±9.54 years, with a minimum age of 21 years and a maximum age of 59 years. In terms of gender, 41(41%) of the staff were male, and 59(59%) were female. Regarding education, 17(17%) workers had diplomas or lower education, 22(22%) had postgraduate degrees, and 61(61%) had bachelor's degrees and higher. Of the participants, 19(19%) showed positive results for the QFT test, and 81(81%) of them had negative results in this test. Regarding the PPD test, 16(16%) had a positive result, and 84(84%) had a negative result. The results of the QFT and PPD tests after 48 hours showed that out of 84 people with negative PPD test results, four (4.8%) individuals rendered positive QFT results, and the other 80(95.2%) people had negative results. Out of 16 individuals who had a positive PPD test result, 15(93.8%) also showed a positive test result for the QFT test, and only one person (6.2%) had a negative. In this study, the agreement percentage between the QFT test and PPD was calculated as 95%. Overall, according to the Kappa test, there was a strong direct but statistically insignificant relationship between the QFT test and PPD (P=0.07; kappa coefficient=0.827) with confidence interval 95% from 0.681 to 0.973. Out of 100 participants in this study, 94(94%) had the scar caused by BCG vaccination, and six (6%) people lacked these scars. All the patients rendering positive results for the PPD and QFT tests were examined for active TB by performing PPD, none of whom showed active disease. Also, no positive history of the infection was identified among the relatives of the participants. Based on the results of this study, the QFT test, in comparison with the PPD test and for detecting LTBI, revealed a sensitivity of 93.75%, a specificity of 95.23%, a Positive Predictive Value (PPV) of 78.94%, and a Negative Predictive Value (NPV) of 98.76% (Table 1).

Table 1: The Comparison of the Results of the QuantiFERON and PPD Tests.

QuantiFERON/PDD	Negative n (%)	Positive n (%)	Total n (%)
Negative	80(95.2)	1(6.2)	81(81)
Positive	4(4.8)	15 (93.8)	19(19)
Total	84(84)	16(16)	100(100)

Discussion

Tuberculosis is one of the most important diseases worldwide Kappa=, imposing a high financial and emotional burden due to the limitations of its diagnostic tests [20]. The results of different studies show that the IFN-γ production assay using the CFP-10, ESAT-6, and Rv2654 antigens has considerably higher specificity than PPD [21]. In addition, other advantages of the IFN-γ production assay compared to PPD include no need for revisiting after 48 hours and the availability of its results within 24 hours [22]. The aim of the present study was to determine the prevalence of latent TB among the workers of hospital in 2017 using two diagnostic tests, the TST and QuantiFERON TB-2G. In this study, a total of 100 people were enrolled. The mean age of the participants was calculated as 36.26±9.54 years, with a minimum age of 21 years and maximum age of 59 years. Our results demonstrated that based on the Kappa coefficient, there was a strong direct relationship between the QFT and PPD tests; however, this relationship was not statistically significant. According to our results and compared to PPD, the QFT test delivered a sensitivity of 93.75%, specificity of 95.23%, the PPV of 78.94%, and the NPV of 98.76% for detecting latent TB. The efficiency of these two methods (i.e., QFT and TST) and their agreement in identifying patients with LTBI have been assessed in different countries. In this vein, the findings of the present study were consistent with the reports of Brook, et al. [23] in Denmark, Ourr, et al. in the UK [24], and Alvarez, et al. [25] in Spain but contradicted the findings of the studies conducted in Korea by Kang, et al. [16] and in Iran by Vaziri, et al. [26]. In 2012 study, Onur et al. compared the specificity of the QFT test with that of the tuberculin skin test in detecting TB infection in children and reported that there was no correlation between positive results in the skin test and the positivity of the QFT test in children with latent TB. In the recent study, 80% and 66.7% of the children showed positive results for the skin test and QFT test, respectively [27].

Overall, the results of the above-mentioned study were consistent with the findings of the present study [27]. In another similar study conducted by Balkhy, et al. [28], the efficiency of the QFT-II QaurEFON-E and TST tests in the diagnosis of latent TB infection was investigated in the general population of Saudi Arabia. The recent study showed that 124(9.1%) and 127(9.3%) participants had positive results in the QFT-GIT and TST tests, respectively, indicating a considerable agreement between the two tests (88.8%) according to the Kappa coefficient. The results of the above study were consistent with our observations. In another 2015 study conducted by Masoumi Asl, et al. [29], the TST and QuantiFERON TB Gold In-Tube (QFT-GIT) tests were used to determine the prevalence of LTBI in healthy children vaccinated for BCG, and the correlation between the two tests was also investigated. The results showed that the prevalence of LIBI was 3.8% based on the TST test and 2.2% based on the QFT-GIT test. Only one child tested positive for both the TST and QFT-GIT tests. Twenty cases showed positive results for the QFT-GIT test but negative results for the TST test, and in 36 cases, the TST test was positive, while the QFT-GIT rendered negative results. Finally, 910 cases showed double negative results for the TST and QFT-GIT tests. Overall, the results of the recent study indicated a poor correlation between the TST and QFT-IT tests based on the Kappa correlation coefficient, which was inconsistent with our findings in the present study. The findings of Karimian et al. showed that the agreement percentage between the QFT and PPD tests was 89.3% (Kappa coefficient=0.052), which was close to the value obtained in the present study. However, regarding the Kappa coefficient, we witnessed a strong correlation between the QFT and PPD tests, which was not in line with the above-mentioned study [30].

Although multiple studies have indicated the higher specificity and sensitivity of the QFT test compared to the TST, there are inconsistencies between studies in terms of the agreement rate between these two tests. The reasons for such differences can root in geographical variabilities in the prevalence of TB, various lifestyles and health attitudes of the people of different countries, people's knowledge in dealing with TB patients, different treatment and preventive strategies of countries, the level of contact with TB patients, routine vaccination programs, the prevalence of non-tuberculosis mycobacterial infections, genetic differences, and finally, the impact of HLA haplotypes on the extent and quality of immune responses.

Conclusion

In this study, we investigated 100 medical workers, to determine the prevalence of LTBI using the QFT and TST tests. Our findings showed a strong agreement between the two tests in identifying people with latent TB.

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