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Evaluation of the Performance of QuantiFERON-TB Gold Plus Assay in Human Immunodeficiency Virus Infection

HIV Pozitif Hastalarda QuantiFERON-TB Gold Plus Testinin Performansının Değerlendirilmesi

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Abstract

Introduction: Individuals co-infected with human immunodeficiency virus (HIV) and *Mycobacterium tuberculosis* have an increased risk of the reactivation of latent tuberculosis (TB) infection (LTBI) to active TB. The addition of peptides to stimulate CD8+ T cells is expected to increase the sensitivity of the QuantiFERON®-TB Gold Plus (QFT-Plus) assay in detecting LTBI and TB infection in patients. This study aimed to determine the prevalence of LTBI in patients with HIV infection in our region and to evaluate the possible factors that may interfere with the performance of the QFT-Plus assay in HIV infection.

Materials and Methods: The study included 132 HIV-positive and 133 HIV-negative cases presented to the Ege University Medical Faculty Hospital between January 2016 and December 2019. Demographic and clinical data and laboratory/culture results were obtained from the mycobacteriology laboratory and hospital database.

Results: QFT-Plus positivity rates were 30.1% (40/133) in the HIV-negative and 21.2% (28/132) in HIV-positive groups. The indeterminate results of the HIV-positive and HIV-negative groups were 4.5% and 3%, respectively. The CD4+ T cell count was below 200/mm^3 in five of the six patients in the HIV-positive group with indeterminate results, and the median lymphocyte count was significantly lower. Although no significant difference was found between the median lymphocyte counts of the HIV-positive and HIV-negative group, the positivity rates and secreted interferon (IFN)-ɣ levels were lower, and the indeterminate results were higher in the HIV-positive group than in the HIV-negative group, but the difference was not significant. The rate of QFT-Plus positivity was 32.4% in patients with a viral load below 10,000 copies/ml and 16.1% in patients with a viral load above 10,000 copies/ml (p=0.047).

Conclusion: The positivity rates and secreted IFN-ɣ levels were lower, and the indeterminate results were higher in the HIV-positive group than in the HIV-negative group. The addition of TB2 tube to the QFT-Plus assay could contribute to the sensitivity of the test in both HIV-positive and HIV-negative individuals with LTBI.

Keywords: QuantiFERON-TB Gold Plus, active tuberculosis, human immunodeficiency virus, latent tuberculosis infection, *Mycobacterium tuberculosis*

Öz

Giriş: İnsan immün yetmezlik virüsü (HIV) ve *M. tuberculosis* ile birlikte enfekte olan kişilerde, latent tüberküloz (TB) enfeksiyonunun (LTBI) aktif TB'ye yeniden aktivasyon riski yüksektir. CD8+ T hücrelerini uyarmak için peptitlerin eklenmesinin, hastalarda LTBI ve TB enfeksiyonunu saptamada QuantiFERON®-TB Gold Plus (QFT-Plus) testinin duyarlılığını artırması beklenmektedir. Çalışmanın amacı, bölgemizdeki HIV pozitif hastalarda LTBI prevalansını belirlemek ve HIV enfeksiyonunda QFT-Plus testinin performansını etkileyebilecek olası faktörleri değerlendirmektir.

Gereç Yöntem: Çalışmaya Ocak 2016 ile Aralık 2019 tarihleri arasında Ege Üniversitesi Tıp Fakültesi Hastanesi'ne başvuran 132 HIV pozitif hasta ve 133 HIV negatif olgu dahil edildi. Olguların demografik ve klinik verileri ile laboratuvar/kültür sonuçları mikobakteriyoloji laboratuvarı ve hastane veri tabanından elde edildi.

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Bulgular: QFT-Plus pozitiflik oranları HIV negatif hastalarda %30,1 (40/133) ve HIV pozitif hastalarda %21,2 (28/132) saptandı. İnsan immün yetmezlik virüsü pozitif ve HIV negatif hastaların belirsiz sonuçları sırasıyla %4,5 ve %3 idi. Belirsiz sonuç saptanan altı HIV pozitif hastanın beşinde CD4 T hücre sayısı 200/mm³'ün altındaydı ve medyan lenfosit sayısı anlamlı olarak daha düşüktü (p<0,05). İnsan immün yetmezlik virüsü pozitif ve HIV negatif hastaların medyan lenfosit sayısı arasında anlamlı bir fark olmamasına rağmen, pozitiflik oranları ve salgılanan interferon gamma (IFN-ɣ) seviyeleri HIV pozitif grupta HIV negatif popülasyona göre daha düşüktü ve belirsiz sonuçlar daha yüksekti, ancak iki grup arasındaki fark anlamlı değildi. Viral yükü 10.000 kopya/ml'nin altında olan hastalarda QFT-Plus pozitiflik oranı %32,4, viral yükü 10.000 kopya/ml'nin üzerinde olan hastalarda ise %16,1 olarak bulundu (p=0,047).
Sonuç: Pozitiflik oranları ve salgılanan IFN-ɣ seviyeleri HIV negatif popülasyona kıyasla HIV pozitif grupta daha düşüktü ve belirsiz sonuçlar daha yüksekti. QFT-Plus testine TB2 tüpünün eklenmesi, hem HIV pozitif hem de HIV negatif LTBI bireylerinde testin duyarlılığına katkıda bulunabilir.
Anahtar Kelimeler: QuantiFERON TB Gold Plus, aktif tüberküloz, insan immün yetmezlik virüsü, latent tüberküloz enfeksiyonu, *Mycobacterium tuberculosis*

Introduction

Approximately 1/4 of the world population is latently infected with *Mycobacterium tuberculosis*. Individuals with latent tuberculosis infection (LTBI) have a 5-10% lifetime risk of developing active tuberculosis (TB), and most of these cases occur within the first 5 years of infection[1-5]. By contrast, individuals infected with human immunodeficiency virus (HIV) are at a relatively increased risk for active TB development. In HIV infection, even if there is no severe CD4+ T cell decline and patients are under antiretroviral therapy (ART), there is a risk of progression to active disease[6-8]. The World Health Organization (WHO) reported approximately 10 million new TB cases in 2018 and 1.2 million HIV-negative and 251.000 HIV-positive individuals have died from TB[1]. Individuals with LTBI are reservoirs for TB; thus, the treatment of active TB alone is not sufficient for disease elimination. In HIV-positive individuals with LTBI, TB incidence decreases by 62% with isoniazid (INH) prophylaxis[9]. Therefore, WHO recommends the diagnosis and prophylactic treatment of LTBI in HIV-positive individuals who are at risk of developing active TB in high and middle-income countries[1].

There is no gold standard for LTBI diagnosis. However, in recent years, interferon-gamma (IFN-ɣ) release assays (IGRAs) have been widely used in the diagnosis of latent TB[10]. Recently, some modifications were made in the QuantiFERON®-TB Gold in Tube (QFT-GIT) assay, and the QuantiFERON®-TB Gold Plus (QFT-Plus) (Qiagen, Hilden, Germany) test was introduced. In the QFT-Plus test, the levels of IFN-ɣ released from peripheral blood mononuclear cells that are stimulated by early secreted antigen (ESAT-6) and culture filtrate protein (CFP-10) antigens, encoded by the "region of difference-1" of the *M. tuberculosis* genome were measured. In the QFT-Plus assay, two TB antigen tubes (TB1 and TB2) contain ESAT-6 and CFP-10 peptide antigens. The QFT-Plus TB1 tube contains relatively long peptides that stimulate CD4+ T cells, whereas the TB2 tube contains an antigen cocktail consisting of short ESAT-6 and CFP-10 peptides to ensure the release of IFN-ɣ from CD8+ T and CD4+ T cells. The QFT-Plus TB1 tube contains relatively long peptides that stimulate CD4+ T cells. CD8+ T cells are an important component of host immunity and control against *M. tuberculosis*. Significantly higher cytotoxic CD8+ T cell responses are reported in the smear-positive or active pulmonary TB group than in the smear-negative or LTBI patients group, and in HIV-infected active TB group with low CD4+ T cell count, CD8+ T cell antigen response is maintained against *M. tuberculosis*. Based on these findings, the inclusion of peptides to stimulate CD8+ T cells is expected to increase the sensitivity of the test in detecting latent and active TB infection in patients with CD4+ T cell depletion[11-13].

In our country, no study has evaluated the performance of the QFT-Plus assay in HIV-positive cases. Thus, this study aimed to determine the prevalence of LTBI in HIV-positive patients in our region and to evaluate the possible factors that may interfere with the performance of the QFT-Plus assay in HIV infection.

Materials and Methods

HIV-positive and HIV-negative Cases Included in the Study

The study included 132 and 133 patients with and without HIV infection who presented to the Ege University Medical Faculty Hospital between January 2016 and December 2019. The criteria for inclusion in the HIV-negative group were as follows: patients who applied to any outpatient clinic in our hospital, whose lymphocyte values measured simultaneously with the QFT-Plus test were within normal limits, who did not have active TB or LTBI in their pre-diagnosis or diagnosis, and who did not have TB or history of exposure. Patients who received immunosuppressive therapy, which may affect the performance before the QFT-Plus test, or who used drugs such as steroids or tumor necrosis factor inhibitors, were not included in this group. Moreover, patients with malignancy and chronic renal failure were also excluded from this group. Of the 132 patients with HIV infection enrolled in the study, 119 received ART. No information was available about the remaining 13 patients, as they were lost to follow-up or were referred to other hospitals. Demographic and clinical data and laboratory/culture results were obtained from the mycobacteriology laboratory and hospital database.

This study was approved by the Medical Research Ethics Committee of the Ege University Faculty of Medicine (approval no: 04/03/2020-20-3T/22, date: 04.03.2020). This study was conducted retrospectively using the information obtained from the records of the patients who presented to the outpatient clinic. Patients who visited the outpatient clinic are informed about the tests requested, but written consent is not routinely obtained.

QFT-Plus Assay

The QFT-Plus assay was performed according to the recommendations of the manufacturer. Moreover, 1 ml of venous blood was collected from the patients into the nil control, mitogen control, TB1 antigen, and TB2 antigen tubes supplied in the kit. Tubes were incubated at 37 °C for 16-24 h within 16 h after collection. After incubation, tubes were centrifuged at 3000 × g for 15 min to separate the plasma and were stored at 4 °C for 0-4 days. Then, 50 µL of the freshly prepared working conjugate was added to all enzyme-linked immunosorbent assay (ELISA) wells. Thereafter, 50 µL of plasma samples were added to the nil, TB1 antigen, TB2 antigen, and mitogen wells and incubated for 2 h at room temperature. After washing, 100 µL of the enzyme-substrate solution was added to the wells and incubated for 30 min at room temperature. Then, 50 µL of the stop solution was added to all wells, and the optical density of each well was measured in the ELISA reader. Optical density values were analyzed using the QuantiFERON-TB Gold Analysis Software. The results of the analysis were evaluated as positive, negative, or indeterminate. A positive test was defined as antigen-nil ≥0.35 IU/ml and ≥25% of the nil sample, whereas a negative test was defined as antigen-nil <0.35 IU/ml or ≥0.35 and <25% of nil when mitogen-nil was ≥0.5 IU/ml. The results were considered indeterminate if 1) nil >8 IU/ml or 2) antigen-nil ≥0.35 IU/ml and <25% of nil when the nil was ≤8.0 IU/ml and the mitogen response was <0.5 IU/ml[14].

Statistical Analysis

Statistical analyses were performed using the IBM Statistical Package for the Social Sciences Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA). Cross-tables were created for categorical variables, and chi-square analysis was performed. The Shapiro-Wilk test was used to check whether numeric variables had normal distribution. For variables without normal distribution, the comparison of two groups was made by the Mann-Whitney U test. Categorical variables were shown as numbers and %, whereas numerical variables as median (minimum, maximum). A p value <0.05 was considered significant.

Results

The QFT-Plus results were evaluated in the HIV-positive group (n=132) with a median age of 32 and HIV-negative group with a median age of 39 (n=133) (Table 1). The median lymphocyte count was 1825/mm^3 in the HIV-positive group and 1865/mm^3 in HIV-negative group. In the HIV-positive group, the median CD4+ T and 133 cell counts were 381 and 882/mm^3, respectively.

Table 1. Characteristics of the HIV-positive and HIV-negative group and evaluation of positive QFT-Plus results in these groups

Characteristics of individuals	**HIV-positive**	**HIV-negative**	**p value**
Age median (min-max)	32.5 (19-67 years)	39 (19-55 years)	**0.013**
Sex, % (n) Female % (n) Male	 11.36 (15) 88.63 (117)	 48.87 (65) 51.12 (68)	**p<0.001**
Median lymphocyte count	1825/mm^3 (160-8010)	1865/mm^3 (620-3220)	0.598
CD4 cell count Median (min-max) <200/mm^3, %(n) 200-349/mm^3, %(n) 350-499/mm^3, %(n) >500/mm^3, %(n)	 381/mm^3 (12-1601/mm^3) 20.45 (27) 24.24 (32) 24.24 (32) 31.06 (41)		
QFT-Plus results in the HIV-positive group and HIV-negative group			
	HIV-positive (n, %)	HIV-negative (n, %)	p value
TB 1 and TB 2 Tube positive	16 (57.1%)	25 (62.5%)	0.705
TB 1 Tube positive	4 (14.3%)	7 (17.5%)	
TB 2 Tube positive	8 (28.6%)	8 (20%)	
Total	28 (100%)	40 (100%)	

Min-max: Minimum-maximum, HIV: Human immunodeficiency virus, QFT-Plus: QuantiFERON®-TB Gold Plus, TB: Tuberculosis

While QFT-Plus was positive in 21.2% (28/132) and indeterminate in 4.5% (6/132) of the patients in the HIV-positive group, it was positive in 30.1% (40/133) and indeterminate in 3% (4/133) of the patients in the HIV-negative group. After excluding indeterminate results, the median CD4+ T cell count was 394/mm^3 in the HIV-positive group. The lymphocyte count was below 200/mm^3 in five of six patients with indeterminate results, and the median lymphocyte count was 43/mm^3. The difference between the two groups was significant (Mann-Whitney U test, p=0.008). The results of the QFT-Plus in the HIV-positive and HIV-negative groups are summarized in Table 2. The relationship between the QFT-Plus results of the HIV-positive group and the median lymphocyte counts is summarized in Table 3.

After excluding indeterminate results, the positivity rates were 22.2% (28/126) in the HIV-positive group and 31% (40/129) in the HIV-negative group (chi-square test, p=0.113). The QFT-Plus positivity rates were 23% (26/113) in male patients of the HIV-positive group, and 36.5% (23/63) in male patients of the HIV-negative group (chi-square test, p=0.055), 15.4% (2/13) in female patients of the HIV-positive group and 25.8% (17/66) in female patients in the HIV-negative group (chi-square test, p=0.424). The QFT-Plus positivity rates were 17.5% in patients aged <30 years in the HIV-positive group, 14.3% in those aged <30 years in the HIV-negative group (chi-square test, p=0.681), 26.1% in those aged >30 years in the HIV-positive group, and 37.2% in those aged >30 years in the HIV-negative group (chi-square test, p=0.133). In the HIV-positive group, the QFT-Plus positivity rates in patients with CD4 lymphocyte count <200/mm^3, 200-349/mm^3, 350-499/mm^3, and >500/mm^3 were 13.6%, 34.4%, 15.6%, and 22.5%, respectively (chi-square test, p=0.214). Moreover, the QFT-Plus positivity rate was 32.4% in patients with a viral load <10,000 copies/ml and 16.1% in patients with a viral load >10,000 copies/ml (chi-square test, p=0.047).

Both TB1 and TB2 tubes were positive in 16 (57.1%) patients in the HIV-positive group and 25 (62.5%) patients in the HIV-negative group. The details of the positive QFT-Plus results of these patients are summarized in Table 1. The median IFN-ɣ concentration secreted from TB1 tube was 2.2 IU/ml in 32 HIV-negative cases and 0.94 IU/ml in 20 HIV-positive cases (Mann-Whitney U test, p=0.094). The median IFN-ɣ value released from the TB2 tube was 1.6 IU/ml in 33 HIV-negative cases and 0.93

Table 2. QFT-Plus results of TB1 and TB2 tubes in the HIV-positive group (n=132) and HIV-negative group (n=133)

HIV-positive					
Tube		**TB2**			
		Positive	**Negative**	**Indeterminate**	**Total**
	Positive	16	4	0	20
TB1	Negative	8	98	0	106
	Indeterminate	0	0	6	6
	Total	24	102	6	132
HIV-negative					
Tube		**TB2**			
		Positive	Negative	Indeterminate	Total
	Positive	25	7	0	32
TB1	Negative	8	89	0	97
	Indeterminate	0	0	4	4
	Total	33	96	4	133

HIV: Human immunodeficiency virus, QFT-Plus: QuantiFERON®-TB Gold Plus, TB: Tuberculosis

Table 3. Relationship between median lymphocyte counts and QFT-Plus results of the HIV-positive group

Feature	**QFT-Plus result**				
	Positive			**Negative**	**Indeterminate**
	TB1 + TB2 positive	**TB1 positive**	**TB2 positive**		
Median lymphocyte count (cells/mm^3)	1700	1700	1950	1835	720
Median CD4+ T cell count (cells/mm^3)	409	273	397	401	43
Median CD8+ T cell count (cells/mm^3)	1006	1112	1149	880	244

HIV: Human immunodeficiency virus, QFT-Plus: QuantiFERON®-TB Gold Plus, TB: Tuberculosis

IU/ml in 24 HIV-positive cases (Mann-Whitney U test, p=0.077). Six patients in the HIV-negative group had >10 IU/ml of IFN-ɣ levels in TB1 and TB2 tubes, whereas no patients in the HIV-positive group had >10 IU/ml. The levels of IFN-ɣ secreted from the TB1 tube in HIV-positive and HIV-negative groups are summarized in Figures 1A and 1B. IFN-ɣ levels secreted from the TB2 tube are summarized in Figures 2A and 2B.

Discussion

In parallel with the incidence of TB, LTBI rates differ significantly among countries, and IGRA positivity rates increase proportionally with age[15]. When indeterminate results were excluded, the QFT-Plus positivity rate was 31% (40/129) in the HIV-negative and 22.2% (28/126) in the HIV-positive group. Although the positivity rate was lower in the HIV-positive group than in the HIV-negative group, the difference was not significant. Petruccioli et al.[4] reported that the QFT-Plus positivity rate was higher (80-68.7%) in HIV-negative TB group than in the HIV-positive TB group. Moreover, in previous studies conducted with QFT-GIT, the sensitivity of the test decreased, especially in HIV/TB co-infected group with CD4+ T cell count <200 cells/mm^3[15-18]. By contrast, König Walles et al.[19] reported LTBI rate of 33% in both HIV-positive and HIV-negative pregnant group using the QFT-Plus test in an Ethiopian study. Our results

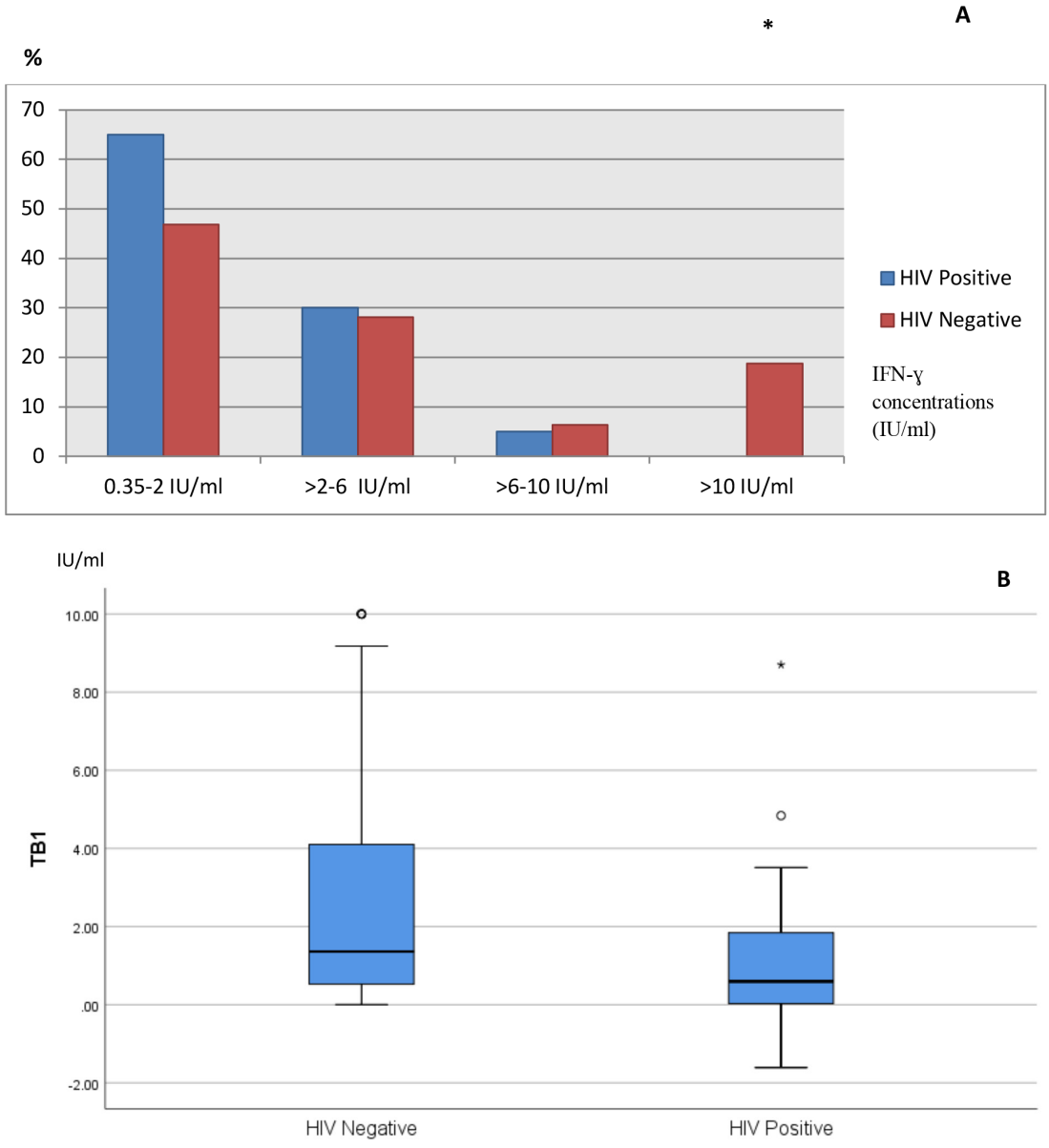

Figure 1. Median IFN-ɣ values (A) and IFN-ɣ concentrations (B) released in TB1 tubes of the HIV-positive group (n=20) and HIV-negative group (n=32) *(Mann-Whitney U test, p=0.094)

HIV: Human immunodeficiency virus, IFN: Interferon

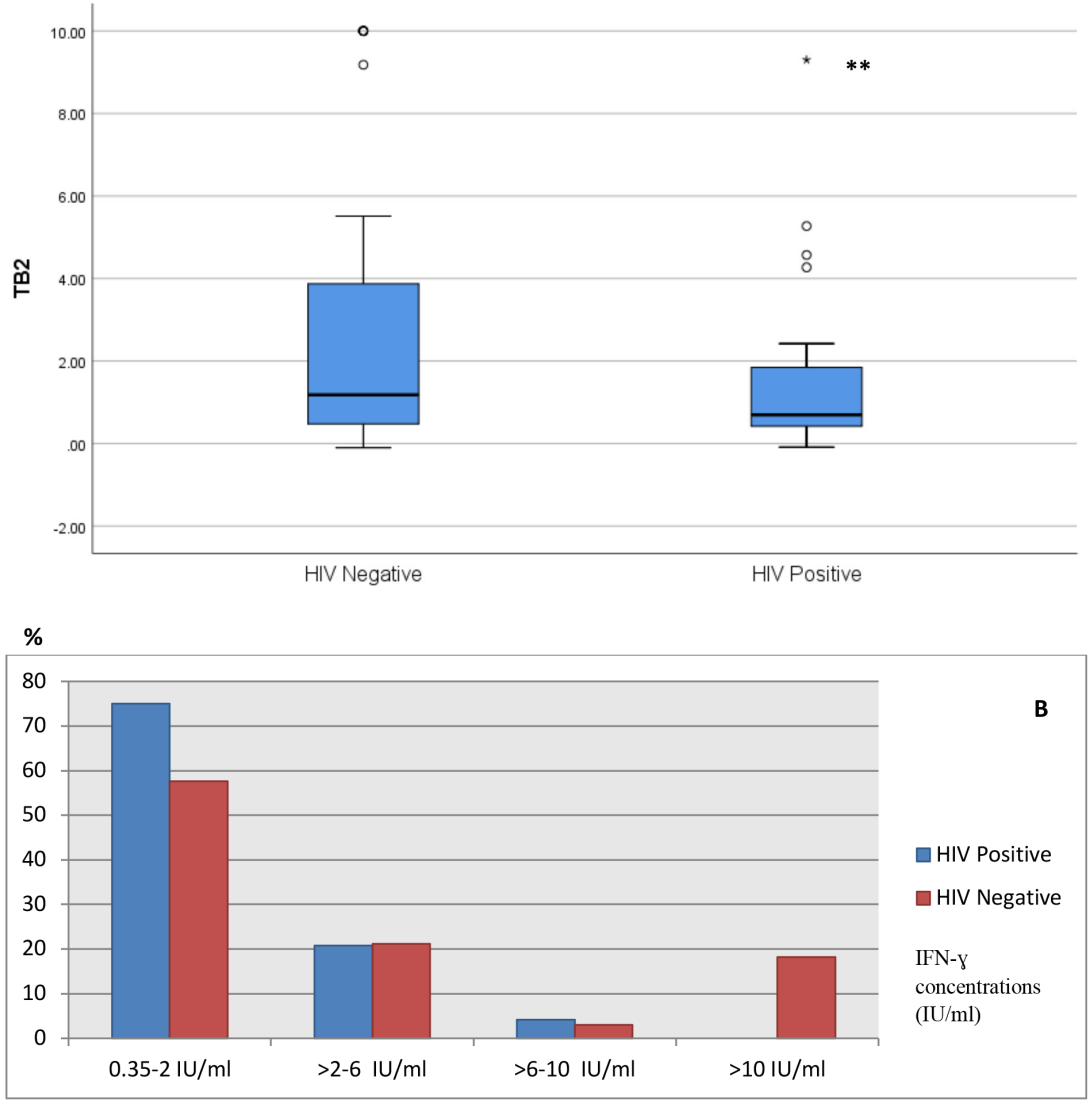

Figure 2. Median IFN-ɣ values (A) and IFN-ɣ concentrations (B) released in TB2 tubes of HIV-positive group (n=24) and HIV-negative group (n=33) **Mann-Whitney U test, p=0.077

HIV: Human immunodeficiency virus, IFN: Interferon

also support the finding that the positivity rate of the QFT-Plus test may be lower in the HIV-positive group.

In our study, indeterminate results were 4.5% in the HIV-positive and 3% in the HIV-negative group. Although the rate of indeterminate results was higher in the HIV-positive group, no significant difference was found between the two groups (p>0.05). Petruccioli et al.[4] reported 0% and 6.2% of indeterminate results in the QFT-Plus test in the HIV-negative TB group and HIV-positive TB group, respectively. The HIV-positive group with CD4+ T cell count <200 cells/mm^3 are known to have higher rates of indeterminate results because of the inadequate mitogen response[16]. In our study, the CD4+ T cell count was below 200/mm^3 in five of the six patients in the HIV-positive group with indeterminate results, and the median lymphocyte count was significantly lower (p<0.05).

Among HIV-positive and HIV-negative cases, the QFT-Plus positivity rate was higher in patients aged >30 years and in men. Moreover, a higher QFT-Plus positivity rate was found in both male and female patients in the HIV-negative group, although not significant. In a study conducted in Turkey, the QFT positivity rate increased in proportion to age and was also higher in men than in women (27.7-26.6%)[15].

No association was found between CD4 lymphocyte counts and QFT-Plus positivity rates. However, QFT-Plus positivity rates were twice as high in patients with a viral load <10,000 copies/ml than in patients with a viral load >10,000 copies/ml (p<0.05). Therefore, this might be due to the high viral load negatively affecting the CD4 lymphocyte response.

Positive QFT-Plus results were separated into three groups by their ability to respond to "both TB1 and TB2," "TB1 only," and

"TB2 only" to evaluate the effect of HIV infection on the assay. Although no significant difference was found between the two groups, the positivity rates to "both TB1 and TB2" (62.5-57.1%) and "TB1 only" (17.5-4.3%) were higher in the HIV-negative LTBI group and the positivity rate to "TB2 only" (28.6% to 20) was higher in the HIV-positive LTBI group. Petruccioli et al.[4] reported positivity rates to "both TB1 and TB2," "TB1only," and "T2 only" of 80%, 11%, and 8.9% in the HIV-positive LTBI group, 94.7%, 3.9%, and 1.3% in individuals with recent infection, and 85.3%, 2.9%, 11.8% in individuals with remote infection, respectively. Compared with HIV-positive LTBI, the positivity rate to "both TB1 and TB2" was significantly higher in the HIV-negative group with recent infection, whereas no difference was observed in the HIV-negative group with remote infection. König Walles et al.[19] found reported positivity rates of 89.7%, 5.7% and 6.3% to both TB1 and TB2, TB1 only, and T2 only in the HIV-negative LTBI group, whereas 87.4%, 6.3%, and 6.3% in the HIV-positive LTBI group, respectively. In contrast to other studies, both TB1 and TB2 positivity rates were significantly lower in both HIV-positive and HIV-negative groups in our study. On the contrary, in concordance with the results of other studies, the HIV-positive LTBI and HIV-negative LTBI groups had similar TB1 only and TB2 only responses, and the addition of the TB2 tube contributed to the positivity of the assay in both the HIV-positive and HIV-negative groups.

Although no significant difference was found between the mean lymphocyte count of the HIV-positive and HIV-negative groups, the IFN-ɣ levels secreted in both TB1 and TB2 tubes were higher in the HIV-negative LTBI group than in the HIV-positive LTBI group. Similarly, Petruccioli et al.[4] showed that CD4+ T cell count did not affect IFN-ɣ values in the HIV-positive LTBI group, the CD8+ T cell response was comparable in the HIV-positive and HIV-negative groups, but the CD4+ T cell response was impaired in the HIV-positive group. Patients with HIV infection are at risk of progressing to active TB disease, even without a severe CD4+ T cell depletion and despite ART[6-8]. These findings suggest a possible decrease in immune function in patients with HIV infection even if there is no quantitative decrease in CD4+ T cells.

Study Limitations

The small number of cases was the major limitation of the study. In addition, age and gender were compared in terms of the similarity of the groups, and a difference was found between the groups.

Conclusion

This study showed that the positivity rates and secreted IFN-ɣ levels were lower and the indeterminate results were higher in the HIV-positive group than in the HIV-negative group. However, these findings were not significant. Although the average CD4+ T cell count was within normal limits in the HIV-positive group, the lower positivity rate and lower IFN-ɣ concentrations may be due to the decrease in response to antigen in CD4+ T cells. Therefore, the TB2 tube added to the QFT-Plus assay could contribute to the sensitivity of the test in both HIV-positive and HIV-negative LTBI groups.

Ethics

Ethics Committee Approval: This study was approved by the Medical Research Ethics Committee of the Ege University Faculty of Medicine (approval no: 04/03/2020-20-3T/22, date: 04.03.2020).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.D.G., Concept: C.Ç., Design: C.Ç., Data Collection or Processing: M.Y.D., T.G.A., Analysis or Interpretation: M.Y.D., İ.A., A.Z., M.N.O., Literature Search: M.Y.D., C.Ç., Writing: M.Y.D., C.Ç., İ.A., A.Z., A.D.G., T.G.A., M.N.O.

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References

1. World Health Organization (WHO). Global tuberculosis report 2019. Available from: https://www.who.int/health-topics/tuberculosis#tab=tab_1
2. Houben RM, Dodd PJ. The global burden of latent tuberculosis infection: a re-estimation using mathematical modeling. PLoS Med. 2016;13:e1002152.
3. Fitzgerald DW, Sterling TR, David W. *Mycobacterium tuberculosis*. In: Bennett JE, Dolin R, Blaser MJ, editors. Mandell, Douglas and Bennet's Principles and Practice of Infectious Diseases, 8th ed. Elsevier Saunders; Philadelphia; 2015. p. 2787-823.
4. Petruccioli E, Chiacchio T, Navarra A, Vanini V, Cuzzi G, Cimaglia C, Codecasa LR, Pinnetti C, Riccardi N, Palmieri F, Antinori A, Goletti D. Effect of HIV-infection on QuantiFERON-plus accuracy in patients with active tuberculosis and latent infection. J Infect. 2020;80:536-46.
5. Mazurek GH, Jereb J, Vernon A, LoBue P, Goldberg S, Castro K; IGRA Expert Committee; Centers for Disease Control and Prevention (CDC). Updated guidelines for using Interferon Gamma Release Assays to detect *Mycobacterium tuberculosis* infection-United States, 2010. MMWR Recomm Rep. 2010;59:1-25.
6. Girardi E, Sabin CA, d'Arminio Monforte A, Hogg B, Phillips AN, Gill MJ, Dabis F, Reiss P, Kirk O, Bernasconi E, Grabar S, Justice A, Staszewski S, Fätkenheuer G, Sterne JA; Antiretroviral Therapy Cohort Collaboration. Incidence of tuberculosis among HIV-infected patients receiving highly active antiretroviral therapy in Europe and North America. Clin Infect Dis. 2005;41:1772-82.
7. Lawn SD, Myer L, Edwards D, Bekker LG, Wood R. Short-term and long-term risk of tuberculosis associated with CD4 cell recovery during antiretroviral therapy in South Africa. AIDS. 2009;23:1717-25.

8. Tiberi S, Carvalho AC, Sulis G, Vaghela D, Rendon A, Mello FC, Rahman A, Matin N, Zumla A, Pontali E. The cursed duet today: tuberculosis and HIV-coinfection. Presse Med. 2017;46:23-39.

9. World Health Organization. Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resource-constrained settings, Geneva, Switzerland, 2011. Available from: http://whqlibdoc.who.int/publications/2011/9789241500708_eng.pdf

10. Metcalfe JZ, Everett CK, Steingart KR, Cattamanchi A, Huang L, Hopewell PC, Pai M. Interferon-γ release assays for active pulmonary tuberculosis diagnosis in adults in low- and middle-income countries: systematic review and meta-analysis. J Infect Dis. 2011;204(Suppl 4):S1120-9.

11. Rozot V, Vigano S, Mazza-Stalder J, Idrizi E, Day CL, Perreau M, Lazor-Blanchet C, Petruccioli E, Hanekom W, Goletti D, Bart PA, Nicod L, Pantaleo G, Harari A. *Mycobacterium tuberculosis*-specific CD8+ T cells are functionally and phenotypically different between latent infection and active disease. Eur J Immunol. 2013;43:1568-77.

12. Day CL, Abrahams DA, Lerumo L, Janse van Rensburg E, Stone L, O'Rie T, et a Day CL, Abrahams DA, Lerumo L, Janse van Rensburg E, Stone L, O'rie T, Pienaar B, de Kock M, Kaplan G, Mahomed H, Dheda K, Hanekom WA. Functional capacity of *Mycobacterium tuberculosis*-specific T cell responses in humans is associated with mycobacterial load. J Immunol. 2011;187:2222-32.

13. Sutherland JS, Young JM, Peterson KL, Sanneh B, Whittle HC, Rowland-Jones SL, Adegbola RA, Jaye A, Ota MO. Polyfunctional CD4+ and CD8+ T cell responses to tuberculosis antigens in HIV-1-infected patients before and after anti-retroviral treatment. J Immunol. 2010;184:6537-44.

14. QuantiFERON®-TB Gold Plus, ELISA package inserts, quiagen. Available from: http://www.quantiferon.com/wp-content/uploads/2017/04/English_QFT_ELISA_R04_082016.pdf

15. Çavuşoğlu C, Durusoy R, Yaşar M, Kayın M. Evaluation of QuantiFERON®-TB Gold in Tube Test and Tuberculin Skin Test in the Diagnosis of *Mycobacterium tuberculosis* Infection. Mikrobiyol Bul. 2017;51:115-26.

16. Cheallaigh CN, Fitzgerald I, Grace J, Singh GJ, El-Eraki N, Gibbons N, Keane J, Rogers TR, Clarke S, Bergin C. Interferon gamma release assays for the diagnosis of latent TB infection in HIV-infected individuals in a low TB burden country. PloS One. 2013;8:e53330.

17. Weinfurter P, Blumberg HM, Goldbaum G, Royce R, Pang J, Tapia J, Bethel J, Mazurek GH, Toney S, Albalak R; Tuberculosis Epidemiological Studies Consortium. Predictors of discordant tuberculin skin test and QuantiFERON®-TB Gold In-Tube results in various high-risk groups. Int J Tuberc Lung Dis. 2011;15:1056-61.

18. Oni T, Gideon HP, Bangani N, Tsekela R, Seldon R, Wood K, Wilkinson KA, Goliath RT, Ottenhoff TH, Wilkinson RJ. Risk factors associated with in determinate gamma interferon responses in the assessment of latent tuberculosis infection in a high-incidence environment. Clin Vaccine Immunol. 2012;19:1243-7.

19. König Walles J, Tesfaye F, Jansson M, Tolera Balcha T, Winqvist N, Kefeni M, Garoma Abeya S, Belachew F, Sturegård E, Björkman P. Performance of QuantiFERON-TB Gold Plus for detection of latent tuberculosis infection in pregnant women living in a tuberculosis- and HIV-endemic setting. PLoS ONE. 2018;13:e0193589.