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## **Screening of Latent Pulmonary Tuberculosis infection in Kurdistan regional-Iraq using QuantiFERON-TB gold plus technique**

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**Abstract**---Background and Objective: Pulmonary tuberculosis (PTB) is a serious disease that mainly affects the lungs. The aim of this study was a screening of latent pulmonary tuberculosis (LTBI) in Kurdistan Regional-Iraq, using QuantiFERON-TB Gold Plus technique (QFT-Plus). Methodology: The study analyzed 24 close contact persons (11 male and 13 female) their ages ranged from 16 to 66 years. Interferon-gamma had been measured by sandwich ELISA. Results: Our result showed diagnosis of (3\24) contacts Infected with LTBI, (1\24) contact intermediate and (20\24) normal individual persons by QFT-Plus technique. Conclusion: Our study recommends screening for LTBI in populations at increased risk (close contacts persons with active pulmonary tuberculosis patients) and QFT-Plus is important test for diagnosis of PTBI.

**Keywords**---pulmonary tuberculosis, LTBI, QFT-Plus, Kurdistan-Iraq.

**Introduction**

Tuberculosis (TB) remains the single largest infectious disease, a total of 1.5 million people died from TB in 2018 (including 251 000 people with HIV). This disease occurs as a result of infection by a group of bacteria such as *M.tuberculosis* complex(MTBC) and non-mycobacterium tuberculosis(NMT).<sup>1</sup> In

most individuals, *Mycobacterium tuberculosis* bacteria is cleared initially by host defenses, in some cases remain latent, and the individual is asymptomatic and noninfectious. However, LTBI has the potential to develop into active tuberculosis at any time. Identification and treatment of LTBI can reduce the risk of development of the disease by as much as 90 percent and so has the potential to protect the health of the individuals as well as the public by reducing the number of potential future sources of infection.<sup>2,3</sup>

IGRA test detects PTBI by measuring T-cells immuno-response, which release IFN- $\gamma$  following stimulation by specific antigens (ESAT-6, CFP-10, and TB7.7). Unlike other tests, IGRA avoids the cross-reaction of BCG vaccination.<sup>4</sup> The course of tuberculosis is dependent on the interactions of the *M.tuberculosis* and the immune system of the host. When person inhalants air droplets containing mycobacteria and arrive at its target(epithelial cells of the lung), innate immunity such as macrophages are rapidly activated and the adaptive immune system such as T-lymphocytes synergistically cooperates for preventing of growth and spreading of the bacteria.<sup>5,6</sup>

### Material and Methods

We conducted a cross-sectional study from September 2019 to April 2020 in the International Center for Chest and Respiratory Diseases in the Kurdistan provinces of Iraq ( Erbil, Dohuk, and Sulaymaniyha cities ). A ten milliliters(10 ml) of blood were drawn and collected from 24 participants, then centrifuged (3000 rpm for 10 minutes), and the serum was pipetted into Epp. tubes and storage at -20C until analyzed later. We measured serum IFN- $\gamma$  levels by the quantitative sandwich ELISA method depending on the procedure according to the working method which recommended by QFT-Plus, QIAGEN, Germany.<sup>7</sup> We used IBM SPSS 22.0 to analyze the study data. To compute the statistical difference between concentration of IFN- $\gamma$  in TB1 and TB2 tubes, we used the Chi-square, T-value and mean $\pm$ standard deviation (SD) used to represent IFN- $\gamma$  levels in TB1 and TB2 tubes. A p-value less than 5% was considered statistically significant.

### Results

Results of this article showed identification of three patients (12.5%) infected with PTBI( 2 male,1 female), one participant male was Indeterminate(4.2%) and 20(83.3%) healthy individual ,(8 male, 12 female) P<0.05, Table 1.

Table 1. QFT-Plus results of contact persons

Final result	male(%) n	female(%) n	Total(%)	Mean of age(year)
positive	2(8.3)	(4.2)1	3(12.5)	38.7 $\pm$ 12.6
negative	8(33.3)	(50)12	20(83.3)20	35.8 $\pm$ 6.1
Indeterminate	1(4.2)	( 0 )0	1(4.2)	44.0
Total	11(45.8)	13(54.2)	24 (100)	-----
Chi Square = 27.250 P <0.05				

On the other hand, our results showed that mean levels of IFN- $\gamma$  in TB2 tubes were higher than in TB1 tubes in all participates ( $P=0.579$ , T-Value =  $-0.603$ ), Table 2.

Table 2. Mean of IFN- $\gamma$  levels in TB1 and TB2 of QFT-Plus tubes

QFT-Plus results	Mean of IFN- $\gamma$ in TB1 IU/ml (Mean $\pm$ SD)	Mean of IFN- $\gamma$ in TB2 IU/ml (Mean $\pm$ SD)
Positive tubes	1.07 $\pm$ 1.22	1.6 $\pm$ 1.59
Negative tubes	0.2 $\pm$ 0.169	0.222 $\pm$ 0.173
Indeterminate tubes	1.22	1.71
T-Value = $-0.603$ , P-Value = $0.579$		

Also, this presented study showed, that a significant rate of concordance of IFN- $\gamma$  levels in TB1 and TB2 tubes for positive QFT-Plus patients (double- positive), As there was an immuno-response in TB1 and TB2 tubes in positive QFT-Plus patients (3\3) that exceeded the cut-off value ( $\geq 0.35$  and  $\geq 25\%$  of Nil) IU/ml, as well as there was an immuno-response in the mitogen tube and the level of IFN- $\gamma$  in the Nil tube was more than 8 IU/ml, Also the immuno-response in one Indeterminate tube was double-positive and exceeded cut-off ( $\geq 0.35$ ) IU/ml, but the value of IFN- $\gamma$  level in Nil tube was less than 8 IU/ml, on the other hand, there were a twenty negative QFT-Plus persons, Seventeen of them did not have an immuno-response in both tubes (double-negative), while three of them had an immuno-response that exceeded the cut-off value (double-positive), but the level of IFN- $\gamma$  in mitogen was less than 0.5 IU/ml and did not exceed 0.25% of Nil tube value, ( $P = 0.0471$ ). Table 3

Table 3. immuno-response state results for QFT-Plus test

QFT-Plus results	No.of double-positive immuno-response persons n(%)	No.of double-negative immuno-response persons n(%)	Total n(%)
Positive	12.5)3 (	0)0(	3(12.5)
negative	(12.5)3	(70.8)17	20(83.4)
Indeterminate	(4.2)1	(0)0	1(4.2)
Total n(%)	(29.2)7	(70.8)17	24(100)
* Chi-Square = 11.657 P-Value = 0.0471			

## Discussion

Diagnostic methods for detection PTB are constantly evolving to find fast and accurate techniques to directly detect MTB and antitubercular resistance Because both controls of PTB infection and management of patient are dependent on early and accurate diagnosis of PTB.<sup>3,8</sup> This study presented important information on the spreading of pulmonary tuberculosis in Kurdistan Regional-Iraq. It is worth noting that this presented study has one limitation, represented by the small number of participates (24 subjects). The current study was consistent with the results of Almufly, et al,<sup>9</sup> that showed (12)% and (2)% of Healthcare workers (HCWs) were positive and Indeterminate for QFT-Plus in Dohuk city respectively.

But Al-Lami, et al,<sup>10</sup> showed (27.8)% of HCWs were positive for LTBI, The inconsistent of findings between two studies because the different geographic locations in the same country, and the small number of participates in this presented study.

It is worth noting that the prevalence of LTBI in the Asia countries are varied, such as the prevalence of LTBI in this study was higher than that reported from Malaysia <sup>11</sup>, Japan<sup>12</sup> , which showed rates of (10.6)%, and (9.9)%, respectively, whereas it was lower than that reported from Taiwan <sup>13</sup>, South Korea <sup>14</sup>, India <sup>15</sup>, and China <sup>16</sup>, which showed (14.5)%, (17.2)%, (31)%, and (33.6)%, respectively. On the other hand, this presented results were partial consistent with the results of Kim,et al<sup>17</sup> who found that the mean of IFN- $\gamma$  levels in TB2 tube for positive QFT-Plus were higher than TB1 (2.83) and (2.39) IU/ml (  $p=0.422$ ), respectively. while they found the mean of IFN- $\gamma$  levels of TB1 and TB2 for indeterminate person were (0.01) IU/ml, also they found an asymmetry of immuno-response in the four participates tubes (single-positive and single-negative).the cause of this partial consistent of two studies are the different of geographic locations in two countries, Additionally there are unique specific antigens (ESAT-6, CFP-10 and TB7.7) in TB2 tubes rather than TB1 that stimulated Immuno-response of CD8 and CD8-Tcells to produce IFN- $\gamma$  in TB2.<sup>18</sup>

Also our data disagreed with Italian study done by Coppeta et al<sup>19</sup> whose showed a rate of concordance of IFN- $\gamma$  levels in TB1 and TB2 tubes for positive QFT-Plus patients (double- positive) was (88.0)% , while (12)% of them were single-negative. The main reason for the difference between the results of the two studies is due to the difference in the habits and health system, in addition to the different geographical locations in both countries.

### **Conclusion**

Although Iraq is a relatively high TB burden country, the prevalence of LTBI among Kurdistan Regional-Iraq is high to some extent comparing with other studies in same countries. It is important to educate people about the disease, especially those who have been in contact with the patient within the family. QFT-Plus is one of the most important test for diagnosis PTBI because it don't have cross-reactively with BCG-vaccine.

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### **Ethical Clearance**

The study Ethical Committee at scientific research by ethical approval of both health and higher education and scientific research ministries in Iraq.

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**Conflicts of interest:** There are no conflicts of interest.

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