Serum level of IL-2, IgG and IgM in Treated and Untreated TB Patients

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Abstract

Background: In tuberculosis (Tb) infection, a majority of individuals exposed to its etiological agent Mycobacterium tuberculosis (MTB) become infected and receive protection from developing into an active disease. This resistance to mycobacterial infection is because of the interaction between macrophages and T-cells, dependent on interplay of cytokines made by each cell. Objective: The present study aims to detect IL-2, IgG and IgM in treated and untreated TB patients in Babylon Governorate.

Results: The results of this study are showed that patients with untreated tuberculosis have mean of IL-2 is significantly higher than patients with treated or control groups, but there were slight differences between treated and the control group. Level of IL-2 for untreated, treated, and control group are (1.584± 0.559 g/L), (1.143± 0.492 g/L) and (1.614± 0.692 g/L), respectively. The mean of IgG in untreated patients was higher than in the treated patients, there are (22.094± 1.299g/L) and (17.376± 2.494 g/L), respectively and the normal value of IgG is (6.3-16 g/L). The IgM mean value in whole groups of the study had located in the normal value, the mean of IgM in the untreated patients is (1.584± 0.559 g/L), treated patients is (1.143± 0.492 g/L), and normal value is (0.3-2.5 g/L). Conclusion: The results of our study were concluded that IL-2 level in untreated patients is higher than in treated patients and control. IgG antibody level is in TB patients (untreated and treated patients) much higher than the normal value, and its level in untreated is higher than treated patients. IgM was not showing significant changes in both treated and untreated patients and all them in the normal value of IgM level.

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Introduction

About one third of the world population is infected with Mycobacterium tuberculosis, the causative organism of tuberculosis [25]. After presentation of M. tuberculosis, produce inflammatory cytokines and chemokines that serve as a signal for infection. The monocytes, neutrophils, and lymphocytes migrate to the focal site of infection, but they are unable to wipe away the bacteria efficiently. During this time, the bacilli resist the bactericidal mechanisms of the macrophage (phagolysosome) by preventing phagosome-lyosome fusion, multiply in the phagosome, and cause macrophage necrosis [5]. In tuberculosis (Tb) infection, a majority of individuals exposed to its etiological agent Mycobacterium tuberculosis (MTb) become infected and receive protection from developing into an active disease. This resistance to mycobacterial infection is because of the interaction between macrophages and T-cells, dependent on interplay of cytokines produced by each cell [16], studies on understanding of how cytokine production would contribute to host defense and to immune-pathogenesis of Tb still remain to be understood [8]. Interleukin-2 (IL2), put out by activated T-cells, is significant in the activation and proliferation of these cells [10]. IL2 together with interferon-γ, is released by T-helper (Th-1) cells, and these two cytokines have roles in the protective immune response [17]. sIL-2R could play a regulatory role in the immune response. Although the exact immunological role of sIL-2R is not well-made, it may function as a marker of disease activity in patients with systemic lupus erythematosus, rheumatoid arthritis [18], and pulmonary disorders, such as tuberculosis (TB). In patients with old inactive TB, the serum sIL-2R levels were not significantly dissimilar from those of normal control subjects. In contrast, active pulmonary TB is linked up with markedly elevated sIL-2R levels [6].

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The immunology of tuberculosis and the significance of delayed hypersensitivity as protective immunity have been extensively studied in the light of modern sophisticated immunological techniques [11,26]. The clinical usefulness of detection of serum immunoglobulin IgG and IgM antibodies have been reported in tuberculosis and other pulmonary diseases [19]. Host humoral response to mycobacterial antigens depends upon the production of different types of antibodies (immunoglobulins) and is the expression of the functional capacity of immunoglobulin producing cells [26].

MATERIALS AND METHODS

Patients:
The samples of blood are collected from sixty patients suspected which has surfing from tuberculosis infection, in the Specialized Chest and Respiratory Center, Hilla City during the period from February to June 2015. The clinical signs were recorded for each patient, including the night sweating, fever, loss of weight, and history of dry cough. Checklist sheets were drawn out for each patient including age, gender, radiological finding.

IL-2 Assay:
Add Sample: one hundred µL of Standard, Blank, or Sample per well. The blank well had added with References Standard & Sample Diluent. Solutions had added to the tail end of the micro ELISA plate well, avoid inside wall touching and foaming as possible. Mix it gently. Pass over the plate with sealer we provided. Incubate for 90 minutes at 37˚C. Biotinylated Detection Ab: The liquid of each well had removed, do not wash. Immediately 100µL of Biotinylated Detection Ab working solution was added to each well. Cover with the Plate sealer. The plate was gently tap to ensure thorough mixing. Incubate for 1 hour at 37˚C. Wash: Each well was aspirated and wash, the operation was replicated three times. Each well was washed by filling with Wash Buffer (approximately 350µL) (a squirt bottle, multi-channel pipette, manifold dispenser or automated wash are needed). Removal of liquid complete at each pace is essential. After that the final wash, remaining Wash Buffer had removed by aspirating or decanting. Invert the plate and pat it against thick clean absorbent paper. HRP Conjugate: One hundred microliter of HRP Conjugate working solution had added to each well. Cover with the Plate sealer. Incubate for 30 minutes at 37˚C. Wash: Repeat the wash process for five times as conducted in step3. Substrate: Ninety microliter of Substrate Solution had added to each well. Cover with a new Plate sealer. Incubate for about 15 minutes at 37˚C. The plate had protected from light. The response time can be reduced or expanded according to the actual color change, but not more than 30minutes. When apparent gradient appeared in standard wells, the user should terminate the response. Stop: Fifty µL of Stop Solution had added to each well. Then, the color turns to yellow immediately. The order to add stop solution be the same as the substrate solution.

OD Measurement: The visual density (OD value) had determined by each well at one time, using a micro-plate reader set to 450 nm. The user should open the micro-plate reader in advance, preheat the instrument, and set the testing parameters.

IgG and IgM procedure:
• Reagents
  Package
  Reagent-1 1× 35ml
  Reagent-2 1×7ml
  Buffer solution (1-Reagent) stored at 2-25˚C and antiserum solution (2-Reagent) stored at 2-8˚C are stable until expiry date printed on the package.

• Concentration in the test:
  Tricine buffer (pH 80); PEG; sodium chloride; antihuman IgG or IgM antiserum; HEPES buffer (pH 7.4); stabilizers.

• Specimen:
  Serum
  Specimen without lipemia or hemolysis is recommended.
  Specimen can be stored up to 3 days at 2-8˚X or up to 6 months at -20˚C.

Procedure:
These reagents may be used in automatic analysers ACCENT-200 and ACCENT-200 II GEN. 1-Reagent and 2-Reagent are ready to use.
For reagent blank 0.9% NaCl is recommended.
Results analysis:
IgM normal value (0.3-2.5 g/L).
IgG normal value (6.3-16 g/L).

Results:
**IL-2, IgG and IgM test results:**
Mean of IL-2, IgG and IgM level shown in table (1). In patients with untreated tuberculosis the mean of IL-2 is significantly higher than patients with treated or control groups, but there are slight differences between the treated and the control group. Level of IL-2 for untreated, treated, and control group are (169.429±69.139 pg/ml, 96.997±27.113 pg/ml) respectively, the untreated vs. treated P.value (0.003) significantly. untreated vs. control P. value is (0.005) significantly, and treated vs. control P. value is (0.521) non significantly. IgG mean in untreated patients is higher than in treated patients, there are (22.094±1.299 g/L, and 17.376±2.494 g/L, respectively), significantly P.value is (P<0.01) and the normal value of IgG is (6.3-16 g/L). The IgM mean value in all groups of the study are located in the normal value, the mean of IgM in the untreated patients is (1.584±0.559 g/L), treated patients is (1.143±0.492 g/L), nonsignificant P. value is (P>0.05), and normal value is (0.3-2.5 g/l).

<table>
<thead>
<tr>
<th>Test</th>
<th>Untreated PT</th>
<th>Treated PT</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>169.429±69.139 pg/ml</td>
<td>96.997±27.113 pg/ml</td>
<td>78.997±0.909 pg/ml</td>
</tr>
<tr>
<td>IgG</td>
<td>22.094±1.299 g/L</td>
<td>17.376±2.494 g/L</td>
<td>(6.3-16 g/L) normal value</td>
</tr>
<tr>
<td>IgM</td>
<td>1.584±0.559 g/L</td>
<td>1.143±0.492 g/L</td>
<td>(0.3-2.5 g/L) normal value</td>
</tr>
</tbody>
</table>

Discussions:
IL-2 is an important immunomodulatory cytokine that is produced by multiple cell types including activated T-cells, dendritic cells, and NK cells and is crucial both for the immune responses against many infectious diseases and for the maintenance of tolerance [14]. IL-2 is extensively investigated, especially in T-cell based studies and has shown potential as a diagnostic marker for TB disease [2]. The classical cytokines such as IL-2, TNF-α, IL-6 and IFN-γ, have been reported to correlate with disease activity during active pulmonary TB [13]. The soluble interleukin-2 receptor (sIL-2R) is released along with interleukin-2 from activated T lymphocytes. The main function of sIL-2R is regulation of the immune response by binding IL-2, which results in blocking the biological functions of this cytokine [20]. Shiratsuchiet al., [21] suggested that IL-2 is important during regulation of immune responses to intracellular organisms like M. tuberculosis. Berktaaset al., [1] suggested that IL-2 is important cytokines in Th1-mediated cellular immunity and T-cell-mediated bactericide. In conclusion, IL-2 concentration was decreased significantly after 2 months of therapy. These matching with our study results.

Turgut et al., [23] who reported that mean serum IL-2 levels are higher in patients with active tuberculosis than in patients with inactive tuberculosis or in healthy controls, but these values decline with therapy to the levels similar to those displayed by the inactive tuberculosis and control groups after treatment of six months. These studies were in agreement with our study results. The emergence IL-2-secreting cells in patients after antibiotic treatment could be considered to be a consequence the expansion of central memory T-cells, caused by the reduced of M. tuberculosis antigen load [15].

In the untreated TB patients there was a high level of M. tuberculosis cells that leads to activated immune cells, such as (T-cells, dendritic cells, and NK cells) and these activated immune cells produce IL-2 that leads to elevation in IL-2 level in serum, while in patients who taking therapy the M. tuberculosis cells level has declined that leads to decline of activated immune cells and IL-2 level respectively.

The protective immune response in TB is cell mediated, but an antibody response is common and may be correlated with the lack of an effective cell-mediated response [7]. The knowledge of the humoral immune responses at various stages of TB infection and disease could help to elucidate the complex interaction between host and pathogen [3]. Feng et al., [9] suggested that the IgG antibody level is higher in the most active TB patients with or without the anti-TB chemotherapy than that in health control, and the positive rate of IgG was highest among the three isotypes, indicating that the IgG antibody was the most extensive antibody isotype. Welch et al., [24] suggested that the IgG antibody level is higher in most advanced and extensive forms of the disease. Patients with active TB usually exhibited strong IgG responses, but poor IgM. All these studies are matched with our study that illustrating that all patients have an AFB smear positive (treated and untreated), IgG recording high level in both treated and untreated patients in compared with the control group, while IgM level there is no significant change in the mean value in all groups of patients. The IgG mean value in untreated patients higher than in treated, significantly.

IgG measurement alone, cannot differentiate patients with active disease from those who had TB in the preceding few years. However, the IgG level is valuable in differentiating patients with positive TB tests [12]. Charpinet al.,[4] previously showed this relationship between IgG levels and the previous TB disease.
minority of the patients showed slight raised IgM. These are the group that had no history of TB in the past. Shin et al., [22] suggested that the IgM antibodies appear first and are produced in large quantities in response to any antigen and decline in the more advanced phases. Several authors have suggested that IgM antibodies are produced mainly during the early phase of primary TB infection.

In our study all samples taken from both treated and untreated patients, and have a positive AFB. The mean level of IgG in untreated patients significantly higher than in treating patients, and both were higher significantly than the normal value (6.3-16 g/L), and IgM was not showing significant changes in both treated, and untreated patients and all them in the normal value of IgM level (0.3-2.5g/L). Maybe to say that all the patients were in the chronic stage of infection with TB and skip the acute stage of infection.

REFERENCE


