Interleukin 18 and interleukin 22 as important immune markers in patients with tuberculosis

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Abstract. Tuberculosis (TB) is a lung infection caused by the bacterium Mycobacterium tuberculosis (MTB). TB is a leading cause of death globally, accounting for approximately 1.5 million deaths per year. The aim of this article was to evaluate the immunological role of Interleukin (IL) 18 and Interleukin 22 in patients infected with MTB and multi-drug resistant tuberculosis MDRTB. A total of 182 individuals suspected with MTB admitted to the tuberculosis center in Al-Najaf City, Iraq. A sputum acid-fast stain was performed for each individual and GeneXpert® heminested real time PCR has been performed for MDRTB detection. The two Interleukins was measurement in serum of infected individual using ELISA technique. Out of 182 individuals, there were 20 infected with MTB and 15 infected with MDRTB. Serum IL18 and IL22 levels were significantly elevated (P<0.05) in patients with MTB and MDRTB groups as compared with control subjects. The MDRTB group showed higher serum IL18 and IL22 levels (P<0.05) than the MTB group. This study showed that IL18 and IL22 had a relationship with MTB and might be used to help diagnose TB; Clinical diagnosis of TB may be aided by these tests. Keywords: IL18, IL22, TB, MTB, MDRMTB, GeneXpert.

1 Introduction

Tuberculosis is a disease caused by the bacterium MTB, it is spread through the air when an infected person coughs or sneezes [1]. In many people, the infection becomes dormant and does not lead to any symptoms [2]. However, in some people the disease progresses to an active infection that causes severe lung damage, fever, night sweats, weight loss, fatigue, and coughing up blood [3]. M. tuberculosis infects the lungs by attaching itself to the tissue lining the lungs [4]. The bacteria produce toxins that disrupt the normal functions of the lungs and cause inflammation, also spread to other parts of the body through bloodstream [5]. As the body fights the infection, it produces inflammatory chemicals called cytokines, these cytokines contribute to the inflammation and damage associated with active TB [6]. One key component of MTB's defense against the host immune system is the production of cytokines, such as IL18, and IL22 which stimulate B cells to produce antibodies that fight infection [7].

IL18 is a powerful pro-inflammatory cytokine involved in the regulation of immune responses [8]. It is secreted by immune cells in response to stimulation by various
pathogens and allergens [9]. Elevated levels of IL18 have been associated with a variety of autoimmune conditions, including arthritis, asthma, and psoriasis [10]. IL18 is also known to induce inflammatory responses in mucosal tissues and contribute to the development of inflammatory bowel disease (IBD) [11]. *M. tuberculosis* has long been recognized as a potent activator of inflammation and IL18 has been identified as a mediator of inflammatory responses induced by MTB infection [12]. IL22 is a pleiotropic proinflammatory cytokine that induces signals in diverse cells of the immune system and plays an important role in the innate immune response, produced by many cell types including dendritic cells, macrophages, mast cells, T cells, B cells, natural killer cells and fibroblasts [13]. It is not found outside the blood/lymphatic system in normal tissues [14]. Therefore, the aim of this study was to evaluate the immunological role of IL18 and IL22 in patients infected with MTB and MDRTB.

2 Methods

2.1 Ethical Consideration

It has been approved by the Institutional Ethics Committees of Kufa University's College of Science and AL-Kufa General Hospital that a study concept for human studies has been approved. Additionally, each participant gave written, informed consent prior to taking part in the study. The World Medical Association's Ethics Code applies to studies involving humans in accordance with the Declaration of Helsinki.

2.2 Individuals

From June to December 2022, 182 males and females suspected of MTB were admitted to the tuberculosis center in AL-Najaf City, Iraq. GeneXpert® heminested real-time PCR was performed for MDRTB detection and ELISA was used to measure two Interleukins in serum samples from infected and healthy individuals [15,16].

2.3 Statistically Analysis

A statistical package (Statistical Package for Social Sciences, version 20, IBM, and Armonk, New York) was used to collect and analyze the data [17,18]. A mean and standard deviation were used to summarize the quantitative data; numbers and percentages were used to summarize qualitative data, correlation was used to determine correlations, and a P-value<0.05 was considered significant [19,20].

3 Results

3.1 Total Patients and Age Groups

Thirty-five of 182 individuals were infected with MTB, 20 were male (57.1%) and 15 were female (42.9%). The most infected age group was 41-50 with 18 cases (51.5%) (10 male and 8 female) followed by age group 20-30 with 8 cases (22.8%) and 31-40 with 7 cases (20%), the age group 51-60 was the lowest infected with 2 cases (5.7%) (Table 1), the statistical analysis proved (P-Value 0.3674) non-significant differences between ages between; male (36.06 ± 2.608) and female (37.33 ± 2.353) (Figure 1).
Table 1. Total patients infected with *mycobacterium tuberculosis*.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Male No.(100%)</th>
<th>Female No.(100%)</th>
<th>Total No.(100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-30</td>
<td>5</td>
<td>3</td>
<td>8(22.8)</td>
</tr>
<tr>
<td>31-40</td>
<td>4</td>
<td>3</td>
<td>7(20)</td>
</tr>
<tr>
<td>41-50</td>
<td>10</td>
<td>8</td>
<td>18(51.5)</td>
</tr>
<tr>
<td>51-60</td>
<td>1</td>
<td>1</td>
<td>2(5.7)</td>
</tr>
<tr>
<td>Total No.(100%)</td>
<td>20(57.1)</td>
<td>15(42.9)</td>
<td>35(100)</td>
</tr>
</tbody>
</table>

Fig. 1. Statistical analysis in ages between male and female infected with mycobacterium tuberculosis. MTB: *Mycobacterium tuberculosis*.

On the other hand, Out of 35 patients infected with *mycobacterium tuberculosis* there were 15 infected with MDRTB, 9 male (60%) and 6 female (40%). Age group 51-60 was the most infected with 8 cases (53.4%) (5 male and 3 female) followed by age group 41-50 with 5 cases (33.4%) while, the age groups 20-30 and 31-40 were the lowest in infected with one case for each age group (6.6%) (Table 2), the statistical analysis (Figure 2) proved there was no significant differences (P-Value 0.2173) in ages between male (45.64 ± 2.632) and female (49.17 ± 2.638). Figure 3 showed MDRTB strain isolated from sputum of male with 45 years old stained with acid fast stain.

Table 2. Total patients infected with multi-drug resistant *mycobacterium tuberculosis*.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Male No.(100%)</th>
<th>Female No.(100%)</th>
<th>Total No.(100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-30</td>
<td>1</td>
<td>0</td>
<td>1(6.6)</td>
</tr>
<tr>
<td>31-40</td>
<td>1</td>
<td>0</td>
<td>1(6.6)</td>
</tr>
<tr>
<td>41-50</td>
<td>2</td>
<td>3</td>
<td>5(33.4)</td>
</tr>
<tr>
<td>51-60</td>
<td>5</td>
<td>3</td>
<td>8(53.4)</td>
</tr>
<tr>
<td>Total No.(100%)</td>
<td>9(60)</td>
<td>6(40)</td>
<td>15(100)</td>
</tr>
</tbody>
</table>
Fig. 2. Statistical analysis in ages between male and female infected with Multi-drug resistant *mycobacterium tuberculosis*. MDRTB: Multi-drug resistant *Mycobacterium tuberculosis*.

Fig. 3. Multi-drug resistant *Mycobacterium tuberculosis* cells isolated from the sputum of a 45-year-old male stained with acid-fast stain.

3.2 Interleukin 18

Figure 4 showed the results of statistical analysis of interleukin 18 levels in patient's serum and control. A significant increase has been showed (P-Value 0.0247) of serum interleukin 18 level (10.76±1.061) in patients infected with MTB as compared with control (8.554 ± 0.4850). Also, a significant increase has been indicated (P-Value<0.0001) in serum interleukin 18 level (15.17±0.5436) in patients infected with MDRTB as compared to control (Figure 5). Serum interleukin 18 level was higher (P-Value 0.0003) in MDRTB patients as compared to MTB (Figure 6).
Fig. 4. Statistical analysis of interleukin 18 levels in patient's serum infected with *Mycobacterium tuberculosis* and control. MTB: *Mycobacterium tuberculosis*.

Fig. 5. Statistical analysis of interleukin 18 levels in patient's serum infected with Multi-drug resistant *Mycobacterium tuberculosis* and control. MDRTB: Multi-drug resistant *Mycobacterium tuberculosis*.

Fig. 6. Statistical analysis of interleukin 18 levels in patient's serum infected with *Mycobacterium tuberculosis* and Multi-drug resistant *Mycobacterium tuberculosis*. MTB: *Mycobacterium tuberculosis*, MDRTB: Multi-drug resistant *Mycobacterium tuberculosis*. 
3.3 Interleukin 22

Figure 7 of the current study revealed that interleukin 22 levels were significantly (P-Value 0.0400) higher in patients infected with MTB (17.71±2.782) than those in control groups (12.78±1.057), while interleukin 22 levels were significantly (P-Value < 0.0001) higher in patients infected with MDRTB (23.01±0.7953) than in control groups (Figure 8). Compared to patients infected with MTB, MDRTB patients had significantly higher levels of interleukin 22 (P-Value 0.0373) (Figure 9).

**Fig. 7.** Statistical analysis of interleukin 22 levels in patient's serum infected with *Mycobacterium tuberculosis* and control. MTB: *Mycobacterium tuberculosis*.

**Fig. 8.** Statistical analysis of interleukin 22 levels in patient's serum infected with Multi-drug resistant *Mycobacterium tuberculosis* and control. MDRTB: Multi-drug resistant *Mycobacterium tuberculosis*.
Patients with MTB Patients with MDRTB

Serum concentration of IL-22 pg/ml

Fig. 9. Statistical analysis of interleukin 22 levels in patient's serum infected with *Mycobacterium tuberculosis* and Multi-drug resistant *Mycobacterium tuberculosis*. MTB: *Mycobacterium tuberculosis*, MDRTB: Multi-drug resistant *Mycobacterium tuberculosis*.

4 Discussion

Tuberculosis was most prevalent in the 41-50 age groups according to our results. Tuberculosis is a bacterial infection that can infect people of all ages, TB can affect the lungs, but it is most commonly a lung disease in people over 50 years old. There are three types of TB: pulmonary TB, multi-drug resistant TB and abdominal TB [21]. Tuberculosis is usually spread from person to person through the air via coughing or sneezing by someone who has active TB. The bacteria responsible for causing tuberculosis are killed in the air by the normal immune system of people without the disease. However, if a person with active TB coughs or sneezes, the germs can be inhaled by others and become trapped in the small airways of the lungs where they begin to multiply. People with latent TB may have inactive TB bacteria in their lungs but without symptoms [22]. They do not have any symptoms and are not infectious to other people so they do not have to be treated. Those who are infected with TB, but have no symptoms are called latent TB. They are at risk of developing the disease or spreading it to others. Latent TB is common and is estimated to affect up to one third of the population around the world [23].

In this study, IL-18 and 22 were higher levels in patient's serum with MTB than control, while there was significant increase in two interleukins levels in patient's serum with MDRTB as compare with MTB. Multi-drug resistant tuberculosis is a serious problem, especially in regions with poor health care systems. MDRTB is caused by a strain of MTB that is resistant to at least isoniazid and rifampicin, the two most effective drugs for treating TB [24]. Extensively drug-resistant tuberculosis (XDRTB) is MDRTB that is resistant to fluoroquinolones and second-line injectable drugs. The spread of resistant TB is primarily caused by weak medical systems, amplification of resistance patterns through movement of people and products, and lack of public awareness of the disease. Because of this, global programs to prevent the spread of MDRTB have been largely unsuccessful [25]. New medications are in development that target the IL-18/TB axis and may provide better therapeutic benefits with fewer side effects. A potential treatment option for these diseases is a molecule known as an antagonist of the IL-18 receptor, which binds to the receptor and prevents it from activating downstream. Interleukin-22 is a cytokine that belongs to a family of cytokines called lymphokines is a proinflammatory cytokine that has been shown...
to play an important role in the innate immune response [26]. This protein is a member of the interleukin family, which are proteins that mediate cellular immune responses by binding to specific cell surface receptors and then triggering a specific biological response [27, 28]. IL-22 has been shown to be important in the inflammatory response because it is produced by a variety of cells in the immune system that respond to infection or injury by recruiting other immune cells to the site of injury or infection [29,30].

5 Conclusion

This study showed that IL18 and IL22 had a relationship with MDRTB and might be used to help diagnose TB; Clinical diagnosis of TB may be aided by these tests.

References


