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Evaluation of Serum IL-17 and TGF-β Levels in Patients with Brucella Spondylodiscitis before and after Treatment: A Case-Control Study

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Introduction

Brucellosis is the most common bacterial zoonotic disease worldwide and it affects more than half a million people annually, which causes serious and many problems in human health and imposes huge costs on the economies of countries [1,2]

Although this organism is controlled in many countries, it is still endemic to Middle Eastern countries, including Iran [3, 4, 5]. The prevalence of brucellosis in Iran in different provinces has been reported between 0.5 to 10.9% and Brucella is the dominant species of melitensis in Iran [6, 7]

To diagnose brucella spondylodiscitis, various methods such as agglutination, culture, ELISA, etc. are used. Basically, host immunity against Brucella species depends on cellular immunity, which includes active antigen-supplying cells (macrophages and dendritic cells) and CD4 and CD8 T lymphocytes [3, 10]. Increased Th1 cellular immune response due to cytokines such as tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ) and interleukin 12 (Interleukin; IL-12), which are produced at the onset of infection .They clear the Brucella bacteria.

Activation of the Th2 immune response enhances humoral immunity (interleukins 4, 2, 7, 8, and 10) and suppresses macrophage function and increases the risk of infection [8-9].

Th3 cells stimulate the production of transforming growth factor (TGF-β); TGF-β is an anti-inflammatory cytokine that is secreted by active macrophages and T cells and enhances the humoral immune response [12]. It also suppresses cellular immunity at multiple levels and inhibits the function and proliferation of lymphocytes [11]. IL-17 induces the production of IL-12 and IFN-γ in macrophages, thus killing bacteria. It also regulates Th1-induced immunity and the host response to intracellular pathogens. As a result it seems

IL-17 plays an important protective role against Brucella infection [12]. However, the exact role of other cytokines in brucella spondylodiscitis has not been studied. Recently introduced cytokines such as IL-17 and TGF-β have shown new dimensions of the immune response that may address unresolved issues in brucellosis immunology. Given the importance of brucella spondylodiscitis in Iran and considering that, according to our research so far, no study has examined the relationship between serum levels of IL-17 and TGF-β.

The aim of this study was to evaluate the serum levels of IL-17 and TGF-β in patients with brucella spondylodiscitis before and after treatment and to compare it with healthy individuals.

MATERIALS AND METHODS

The type of this study is case-control. Patients in whom the definitive diagnosis of brucella spondylodiscitis was made according to the patients based on clinical and laboratory findings according to brucellosis, was selected as the case group. The control group included healthy patients who had no underlying disease and referred to the rheumatology clinic of Shahid Beheshti Hospital in Kashan only to assess their growth status and were homogeneous in terms of age and sex.

This study was performed on 35 patients with brucella spondylodiscitis and 35 healthy referred to the rheumatology clinic of Shahid Beheshti Hospital in Kashan in 2020. Patients were enrolled in the study who, in addition to the clinical signs consistent with brucella spondylodiscitis, had a Wright or Combs Wright test titer greater than or equal to 1.80 and a 2ME titer greater than or equal to 1.40 [13]. The control group was selected from healthy and asymptomatic patients who referred to the hospital rheumatology clinic only to assess their growth status and were diagnosed with brucella spondylodiscitis due to negative Wright and Combs Wright tests. Patients are the same age and sex as the control group. 5 cc of venous blood was taken from both groups in tubes without anticoagulants and then the samples were transferred to the laboratory for 15 minutes in a centrifuge (Hettich D-78532, Tuttlingen, Germany) at high speed. 3500 rpm was placed to separate the serum. Interleukin-17 and TGF-B were measured by ELISA using a monobind kit (Arizona, USA) before and after treatment. The obtained data will be analyzed using SPSS software version 16 and the significance level is considered less than 0.05.

In order to describe the data, graphs were used and for quantitative variables, mean and standard deviation and for qualitative variables, number and percentage were used. Kolmogorov-Smirnov test was used to test the data for following the normal distribution. Chi-square and t-test were used to analyze the data. P-value less than 0.05 is considered as a significant level.

Result

Table 1: Mean and standard deviation of serum IL-17 level in patients before and after treatment

P-value*	Standard deviation	mean	number	time	variable
<0.001	23.57	83.74	35	Before treatment	serum IL-17 levels
	19.23	24.27	35	After treatment	

The result of paired t-test

According to the table above, the mean serum level of IL-17 in patients before treatment was 83.74 pg / mL and after treatment. pg / ml is 24.27 which is statistically significant (P <0.001).

Table 2: Mean and standard deviation of serum TGF-β level in the case group before and after treatment

P-value*					variable
<0.001	24.44	90.21	35	Before treatment	سطح سرمی TGF-β
	18.3	120.4	35	After treatment	

According to the table above, the mean serum TGF-β level in patients before treatment was 90.21 µg / dl and after treatment. µg / dl is 120.4, which is statistically significant (P <0.001).

DISCUSSION

In this study, serum levels of IL-17 and TGF-β in patients with brucella spondylodiscitis before and after treatment were compared with healthy group and it was found that the mean serum level of IL-17 in the case group was 83.74 pg / mL and in The control group is 25.95 pg / mL, which is significantly higher in the patient group. Also, the mean serum level of TGF-β in the case group was 90.21 pg / mL and in the control group was 125.63 pg / mL that the serum level of this cytokine was significantly reduced in the group of patients. In addition, serum levels of both elements in men compared to women in both case and control groups were not statistically significant. The mean serum level of IL-17 in the case group was 83.74 pg / ml before treatment and 45.27 pg / ml after treatment, but this decrease in IL-17 level was not statistically significant. On the other hand, the mean serum level of TGF-β in the case group was 90.21 pg / ml before treatment and 106.4 pg / ml after treatment, which was a statistically significant increase in serum TGF- β not observed.

The men's main defense against intracellular organisms such as brucella spondylodiscitis is cellular immunity. Brucella spondylodiscitis removal and clearance is mediated by increased macrophage activity through Th1 cellular immunity. Cytokines that are produced and secreted by various cells in response to proinflammatory mediators and bacteria during this stimulation play a key role in the pathogenesis of brucella spondylodiscitis [3, 2, 11, 14, 9]. TGF-β is secreted by various cell types, such as macrophages, in response to tissue damage. TGF-β is a highly potent immunosuppressive cytokine that inhibits the activity of lymphocytes and phagocytes and regulates T cell function [12, 14].

Rafiei et al. observed that brucella spondylodiscitis is more common in patients with haplotype of mass production of TGF-β gene[15]. Budak et al. also showed that in patients with brucella spondylodiscitis the median TGF-β gene producing the haplotype was significantly increased[16]. In contrast, Bravo et al. reported that the rate of moderate and mass-producing TGF-β genotypes was significantly lower in these patients[12]. In a study by Akbulut et al. there was no significant difference in TGF-β levels between patients and controls[17]. These differences in results may be due to epidemiological, ethnic and geographical differences and study conditions such as the number of patients.

Th17 cells provide host immunity against extracellular bacteria and fungi. These cells differentiate after contact with IL-1, TGF-β and IL-6 [18]. The major cytokines secreted by Th17 include IL-17, IL-21, and IL-22, which act on neutrophils, IgM, and IgA-producing B lymphocytes [18]. IL-17 is a proinflammatory cytokine that has important functions in infectious diseases, autoimmunity and malignancies [19, 20]. IL-17 is also an important link between innate and acquired immunity and is essential for inducing the production of IFN-γ and IL-12 in macrophages and dendritic cells. It appears that IL-17 can activate Th1, which is necessary to control Brucella infection [12].

In this study , the IL-17 level was significantly higher in brucella spondylodiscitis cases than controls and, after brucella spondylodiscitis treatment, the serum levels of IL-17 were decreased. Clapp et al. showed that a live brucellosis vaccine that is able to stimulate the Th17 cell response can protect the mucosa against Abortus Brusella [21]. Rasouli et al. showed that rs8193038 and rs4711998 genotypes and AAGAA interleukin 17 haplotype were the risk factors for brucella spondylodiscitis, while rs3819025AA and rs3819024GG genotypes were protective factors for the disease [22]. These findings suggest that IL-17 may play an important role in protecting against brucella spondylodiscitis infection.

Paula Constanza Arriola Benitez et al. found that *B. abortus* triggers a profibrotic response characterized by inhibition of MMP-9 secretion, inducing concomitant collagen deposition and TGF-β secretion (23)Guillermo Hernán et al said that in brucella spondylodiscitis, increase in TGF-*β* (24).Pasquevich K.A et al showed that IL-17 secreted by Th17 cells plays a core role in protective immunization against brucella spondylodiscitis.(25)

Mariem Kchaou showed that quantitative real-time PCR analysis, preceding and following antibiotic treatment, revealed the association of inflammation in the cerebro-spinal fluid with higher IL-6 and IL-17 expression. These results could improve our understanding of inflammation in a leptomeningial and WM involvement related to Neurobrucellosis for a better diagnosis when clinical, MRI data and hematological routine tests are non-specific(26).

Keramat F et al found the relationship between genetic variants of IL-17 and susceptibility to human brucella spondylodiscitis and found a strong relationship between IL-17 gene SNPs and susceptibility and resistance to this disease. IL-17 has an important role in immunity to Brucella spondylodiscitis and had significantly higher serum IL-17 titers than healthy controls(27)Sofian et al.'s finding that serum IL-17 titers were higher in a brucellosis group than in a control group(28).

In one study found that Outbreak or prevalence of brucellosis in pastoral areas can be prevented upon development of a high-potency vaccine for brucella spondylodiscitis or discovery of a new method for regulating Th1 cell/Th2 cell balance and Treg/Th17 cell balance for brucella spondylodiscitis treatment.(29)

Th17 cells provide the host orga-nism with additional defense against a number of extracellular and intracellular microbial infections and can defend against invasion of Brucella through cooperation with Th1 cells (30). The antibody response to Brucella proteins, mediated by interleukin 17 (IL-17) secreted by Th17 cells, plays a key role in brucellosis vaccine (31).

In one case, noticed an inflammatory process which involves IL-6 and IL-17 cytokines with an increase of IL-6 and IL-17 in PBMCs and CSF. Th17 responses have been shown to contribute to host defence v.s Candida and L. monocytogenes and this Th17 response is favoured when IL-6 is present in high quantities (32)

Our findings indicate that people with these genotypes at the listed positions have a greater risk of developing brucella spondylodiscitis (337, 88, 25, and 103 times, respectively) when exposed to *Brucella*. More assessments of genetic variations and the ability to produce IL-17 in the patients with brucella spondylodiscitis are recommended(33)

Conclusion:

The results showed that serum IL-17 levels decreased significantly whereas serum TGF-β levels increased significantly in brucella spondylodiscitis patients. It is recommended that the serum levels of these inflammatory cytokines can be used as the markers for the diagnosis of brucella spondylodiscitis.

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