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Interlukine-17 and TGF-β levels in patients with acute brucellosis before and after treatment

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Background/aim: T-helper cell type 1 (Th1)/Th2 cytokine balance is involved in the resistance or susceptibility to *Brucella* infection. The analysis of cytokine levels is valuable to determine the role of the immune system in *Brucella* prognosis. The aim of this study was to investigate the levels of serum interleukin-17 (IL-17) and transforming growth factor beta (TGF-β) and their alterations with treatment in patients with acute brucellosis.

Materials and methods: TGF-β was tested in 33 acute brucellosis patients and 19 controls and IL-17 was analyzed in 40 patients and 12 controls. Cytokine levels were tested in controls and patients before and after treatment by ELISA.

Results: TGF-β levels were significantly lower in brucellosis cases compared to controls. At the end of the treatment, the serum levels of this cytokine had increased, but there was no significant difference between this cytokine level before and after treatment. The IL-17 level was significantly higher in the brucellosis group compared to controls and its value decreased in patients at the end of treatment without any significant difference.

Conclusion: This study indicated that TGF-β was lower and IL-17 was higher in brucellosis cases and, after treatment, the serum level of TGF-β increased and that of IL-17 decreased in these patients.

Key words: Brucellosis, transforming growth factor beta, TGF-β, interleukin-17, IL-17

1. Introduction

Brucella species are gram-negative, nonmotile, nonencapsulated, and facultative intracellular bacteria that can cause chronic zoonotic disease in humans (1,2). Domestic and wild animals are reservoirs of the bacteria, which are usually transmitted to humans through the consumption of contaminated unpasteurized dairy products, direct contact with infected animals, and inhalation of infected aerosols (3). Brucellosis is the most common bacterial zoonotic disease worldwide, over half a million people being infected annually, and it causes serious health problems and economic losses (4,5).

Despite control of this organism in many countries, it remains endemic in the Mediterranean and Middle Eastern regions, including Iran (2,6,7). The prevalence of brucellosis in Iran has been reported from 0.5% to 10.9% in different provinces. It has been reported that the majority of isolates were identified as *Brucella melitensis* and it is highly endemic in certain parts of Iran, such as Markazi Province, with a 5-year incidence of about 40/100,000 (8,9).

Brucella spp. survive within a variety of cells, including macrophages, and spread in mononuclear phagocytes to reticuloendothelial sites (10,11). Both cell-mediated and humoral immunity are responsible for the clearance of *Brucella* infection (11,12). Host protection against *Brucella* spp. primarily depends on cell-mediated immunity, involving mainly activated antigen-presenting cells (macrophages, dendritic cells) and CD4+ and CD8+ T-lymphocytes (2,10).

Cytokine profiles can be considered as T-helper cell type 1 (Th1), Th2, or Th3 responses. The Th1/Th2 balance

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may be involved in the susceptibility or resistance to brucellosis (1). Promoting Th1 cell-mediated immune response causes clearance of *Brucella* and is under the control of major cytokines like interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and interleukin-12 (IL-12), which are produced at the onset of infection. However, activation of Th2 responses promotes humoral immunity (IL-4, IL-5, IL-9, IL-13, IL-10), suppresses the macrophage function, and increases the susceptibility to infection (13–16).

The Th3 subset induces transforming growth factor beta (TGF-β), a multifunctional cytokine that regulates several pathways in the growth and differentiation of many cell types (1). It is an antiinflammatory cytokine secreted by activated T cells and macrophages, and it promotes the humoral response (17). This cytokine suppresses cellular immunity at multiple levels and inhibits lymphocyte proliferation and function (18).

The IL-17 family is a group of newly described cytokines. The IL-17 cytokine family consists of six members (IL-17A to IL-17F) and five receptors (IL-17RA to IL-17RD and SEF) (10). IL-17A (hereafter referred to as IL-17)-producing T cells are distinct from the classic Th1 and Th2 cells (19,20). IL-17 induces IL-12 and IFN-γ production in macrophages and mediates bacterial killing. It also regulates IL-12/Th1 immunity and the host responses to intracellular pathogens. It thus seems that IL-17 may have a main role in protection against *Brucella* infection (10).

Previously, some cytokines such as IFN-γ, IL-2, IL-3, IL-4, IL-12, and IL-6 have been studied in human brucellosis (21–24). However, the precise role of other cytokines in brucellosis has not been studied. Newly described cytokines such as IL-17 and TGF-β introduce new aspects of the immune response that might clarify the unsolved problems in the immunology of brucellosis. The aim of this study was to investigate the levels of serum IL-17 and TGF-β and their alterations with treatment in patients with acute brucellosis.

2. Material and methods

This study was carried out with patients with acute brucellosis and aged-matched controls living in Markazi Province of Iran (Arak city) from January 2013 to January 2014. Arak is the capital of Markazi Province with an estimated population of over 500,000 and is situated in a brucellosis-endemic area. The project was approved by the Arak University of Medical Sciences ethics committee. Informed consent was obtained from all subjects before participation.

Patients were selected from among individuals attending a private clinic in Arak. A clinical diagnosis of brucellosis was made on the basis of the symptoms (such as fever, night sweating, malaise, weakness, anorexia, and weight loss), compatible clinical findings (such as splenomegaly, arthralgia, lymphadenopathy, and myalgia), and standard tube agglutination (STA = Wright) test dilution of ≥1:160 in the presence of 2-mercaptoethanol (2ME) agglutination, ≥1:40. The control group was selected from among healthy individuals residing in the same geographical area.

Serum samples were collected from brucellosis patients before receiving any treatment and from controls. All samples were tested for IL-17 and TGF-β levels by enzyme-linked immunosorbent assay (ELISA). The commercial enzyme immunoassay kits used were from Affymetrix eBioscience, Vienna, Austria. All assay protocols, cutoffs, and result interpretations were carried out according to the manufacturer's instructions.

All patients then received a regimen of doxycycline (100 mg twice daily) plus rifampicin (600 mg daily) for 6–8 weeks. After treatment, serum samples were collected again from patients and IL-17 and TGF-β levels were tested with the same ELISA kits.

2.1. Statistical analysis

Data were entered into SPSS 16.0 (SPSS Inc., Chicago, IL, USA). For data analysis, the Mann–Whitney U test and Wilcoxon signed-rank test were used. $P < 0.05$ was considered statistically significant. Data are presented as the mean ± standard deviation or absolute number with percentage, as indicated.

3. Results

TGF-β levels were tested in 33 acute brucellosis patients and 19 age-matched controls. The mean age of patients was 23.2 ± 15.2 years; 54.5% of them were male and 45.5% were female. IL-17 levels were analyzed in 40 patients (with mean age of 23.9 ± 15.6) and 12 controls. Among those patients, 57.5% were male and 42.5% were female.

IL-17 and TGF-β levels of the study group (before and after the treatment) and the control group are shown in Table 1.

TGF-β levels was significantly lower in acute brucellosis cases compared to the control group ($P<0.001$). At the end of treatment, the serum levels of this cytokine had increased, but there was no significant difference between the mean level of this cytokine before and after treatment (Wilcoxon signed-rank test) (Table 2).

The IL-17 level was significantly higher in the acute brucellosis group compared to controls ($P < 0.01$) and its value decreased in patients at the end of treatment without any significant difference (Table 2).

4. Discussion

In this study, we analyzed serum TGF-β and IL-17 levels in a control group and acute brucellosis patients before

Table 1. Serum levels of TGF-β and IL-17 in the healthy control group and acute brucellosis patients before and after treatment.

Cytokines	Acute brucellosis group		Control group
	Before treatment	After treatment	
TGF-β (pg/mL)	84.48 ± 47.46	91.2 ± 63.2	120.11 ± 40.61
IL-17 (pg/mL)	80.2 ± 125.9	67.9 ± 131.6	25.03 ± 32

Values are presented as mean ± standard deviation. TGF-β: Transforming growth factor beta, IL-17: interleukin-17.

Table 2. Mean levels of TGF-β and IL-17 in brucellosis patients before and after treatment (Wilcoxon signed-rank test).

Cytokines	Mean before brucellosis treatment	Mean after brucellosis treatment	P-value
TGF-β	15.6	16.2	0.13
IL-17	21.8	18.1	0.52

TGF-β: Transforming growth factor beta, IL-17: interleukin-17.

and after treatment. TGF-β was significantly lower and IL-17 was significantly higher in acute brucellosis cases compared to the control group. After treatment, the serum levels of TGF-β increased and IL-17 decreased in brucellosis patients without any significant difference.

The main defense against intracellular organisms like *Brucella* is cellular immunity. Elimination of *Brucella* is mediated by enhanced macrophage activation by Th1 cell-mediated immunity. Cytokines released during this stimulation and produced by various cells in response to proinflammatory mediators and bacterial products play a critical role in the pathogenesis of brucellosis (1,2,11,14,15).

TGF-β is secreted by many cell types, including macrophages, in response to damaged tissues. TGF-β is a very powerful immunosuppressive cytokine that blocks the activation of lymphocytes and phagocytes and modulates T-cell function (17,25).

Rafiei et al. (26) found that a high-producing haplotype of the TGF-β gene was more common in brucellosis patients than controls. Budak et al. (27) showed that the frequency of the intermediate producer haplotype of TGF-β was significantly higher in brucellosis patients. In contrast, Bravo et al. (17) reported that the frequency of high and intermediate producer genotypes of TGF-β were significantly lower in patients compared with controls. In a study by Akbulut et al. (28), no significant differences were found between patient and control groups regarding TGF-β levels. We found that TGF-β was significantly lower in brucellosis cases than controls and, after brucellosis treatment, the serum levels of TGF-β were increased. These discrepancies in results may be explained by epidemiological, ethnic, and geographic variations and study conditions such as the number of the patients.

Th17 cells mediate host immunity against extracellular bacteria and fungi. These cells differentiate after exposure to IL-1, TGF-β, and IL-6 (29). The main secreted cytokines from Th17 cells are IL-17, IL-21, and IL-22 and their main effector cells are neutrophils, IgM/IgA B cells, and IL-17 CD4 T cells (19). IL-17 is a proinflammatory cytokine that has important functions in infectious diseases, autoimmune disorders, and malignancies (30,31). IL-17 is an important link between the adaptive and innate immune responses and it is required for the induction of IFN-γ and IL-12 in macrophages and dendritic cells. It seems that IL-17 can affect the activation of Th1 immunity, which is necessary for controlling *Brucella* infection (10).

We found that the IL-17 level was significantly higher in brucellosis cases than controls and, after brucellosis treatment, the serum levels of IL-17 were decreased. We could not find any study on IL-17 levels in brucellosis patients so it was impossible to compare our results to others studies, but there are a few surveys on the importance of IL-17 in brucellosis immunopathogenesis, as we showed in our investigation. Clapp et al. (32) showed that a live brucellosis vaccine that is capable of stimulating potent Th17 cell responses can contribute to the protection against mucosal challenge with *B. abortus*. Rasouli et al. (10) reported that the IL-17 rs4711998, rs8193038, and rs3748067 AA genotypes and AAGAA haplotype could be considered as susceptibility factors for brucellosis while the IL-17 rs3819024GG and rs3819025AA genotypes might

be resistance factors against the disease. These findings suggest that IL-17 may have a main role in the protection against *Brucella* infection.

In conclusion, our results indicated that TGF-β and IL-17 are involved in the pathogenesis of brucellosis and the levels of these cytokines can influence the outcome of brucellosis infection. Due to the low number of patients in our study, these results need to be confirmed in larger patient cohorts and with subjects from different ethnic groups.

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