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# Evaluation of Serum Interleukin-17, Transforming Growth Factor-beta Levels in Brucellosis Patients Before and After Treatment

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ABSTRACT

**Introduction:** Brucellosis is a zoonotic disease in humans and animals and is a worldwide public health problem. Changes in inflammatory cytokines levels might be deployed as markers for diagnosing infectious diseases from non-infectious medical conditions. This study aimed to evaluate the relationship between serum levels of interleukin-17 (IL-17) and transforming growth factor-beta (TGF-β) in pediatric brucellosis. **Methods:** The present case-control study included 40 brucellosis patients and 40 matched healthy controls. Serum levels of inflammatory cytokines were measured by ELISA, and the independent student t-test was used to compare the levels in the brucellosis and healthy group. Serum cytokine levels before and after treatment were compared by the paired samples t-test. **Results:** The serum TGF-β level was significantly lower in the patients compared to the control group (90.21 ± 24.44 vs. 125.63 ± 23.28 pg/mL, $P$<0.nv001), and the serum interleukin-17 level was significantly higher in the case group (83.74 ± 23.57 vs. 25.95 ± 17.80 pg/ml, $P$<0.001). After treatment, serum IL-17 levels significantly decreased in the case group. **Conclusion**: In brucellosis patients, the serum IL-17 levels decreased significantly, whereas TGF-β increased significantly in these patients. Hence, the serum levels of these inflammatory cytokines can be indicators for diagnosing pediatric brucellosis.

## INTRODUCTION

Brucellosis is the most common bacterial zoonotic disease worldwide, and it affects more than half a million people annually, which causes severe problems in human health and imposes enormous costs on the international economy [1, 2]. Although this organism is under control in many countries, it is still endemic in the Middle East, including Iran [3, 4, 5]. The prevalence of brucellosis in different provinces of Iran ranges from 0.5% to 10.9%, and *Brucella melitensis* is the dominant species [6, 7].

Various methods, such as agglutination, culture, and ELISA, are used to diagnose brucellosis. Host immunity against *Brucella* species depends on cellular immunity, including active antigen-supplying cells (macrophages and dendritic cells) and CD4 and $CD8^+$ T lymphocytes [3, 9]. 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, clear the *Brucella* bacteria. Activating the Th2 immune response (interleukins 4, 5, and 13) suppresses macrophage function and increases the risk of infection [6-8].

Th3 cells stimulate the production of the transforming growth factor (TGF-β); this cytokine is an anti-inflammatory component secreted by active macrophages and T cells [11]. It also suppresses cellular immunity at multiple levels and inhibits the function and proliferation of lymphocytes [10]. IL-17 induces the production of IL-12 and IFN-γ in macrophages, killing bacteria. It also regulates Th1-induced immunity and the host response to intracellular pathogens.

IL-17 plays an important protective role against *Brucella* infection [11]. However, knowledge of the exact role of other cytokines in brucellosis is limited. 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. No data about serum levels of IL-17 and TGF-β is available.



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This study aimed to evaluate the serum levels of IL-17 and TGF-β in patients with brucellosis before and after treatment and to compare it with healthy individuals.

## MATERIAL AND METHODS

This survey is a case-control study. This study was performed on 40 brucellosis patients and 40 healthy patients referred to the infectious disease clinic of Shahid Beheshti Hospital in Kashan in 2020. Patients had clinical signs, and Wright, Coombs Wright test titer ≥ 1.80 and a 2-Mercaptoetanol titer ≥1.40 [12]. The control group was selected from healthy and asymptomatic patients. Five ml of venous blood was taken from both groups in tubes before and after treatment and 40 healthy children (control group) in Shahid Beheshti Hospital in Kashan.

without anticoagulants. The samples were centrifuged for 15 min at 3500 RPM to separate the serum. Interleukin-17 and TGF-B were measured by the ELISA method using a commercial kit (Arizona, USA) before and after the treatment of patients. The obtained data were analyzed using SPSS software version 16 (IBM® SPSS® Modeler 16.0).

For quantitative variables, mean and standard deviation, and for qualitative variables, number and percentage were used. The Kolmogorov-Smirnov test was used to test the data for following the normal distribution. Chi-square and t-tests were used to analyze the data, and $P$>0.05 0.05 was considered a significant level.

## RESULTS

The present study determined the serum levels of IL-17 and TGF-B in 40 children (case group) with brucellosis

**Table 1**. The age range, sex status, number of individuals in each group

| Variable | | Control group No (%) | Case Group No (%) |
|---|---|---|---|
| Sex | female | 21 (52.5%) | 23 (57.5%) |
| | male | 19 (47.5%) | 17 (42.5%) |
| | total | 40 (100%) | 40 (100%) |
| Mean of age | | 2.49± 11.02 | 2.96 ± 12.05 |

In our study, the mean serum level of 83.74 pg/mL for IL-17 in the case group was significantly higher than 25.95 pg/mL in the control group ($P$ <0.001) (Table 1).

Also, the mean serum level of TGF-β was 90.21 pg/mL in the case group and 125.63 pg/mL in the control group, which is statistically significant ($P$ <0.001) (Table 2). The mean serum level for IL-17 in patients before treatment was 83.74 pg/mL, which was reduced significantly to 45.27 pg /ml after treatment ($P$ <0.001) (Table 3).

**Table 2**. The mean and standard deviation of serum IL-17 levels in groups before treatment

| Variable | standard deviation | mean | number | group | *P*-value* |
|---|---|---|---|---|---|
| serum IL-17 levels | 23.57 | 83.74 | 40 | case | <0.001 |
| | 17.80 | 25.95 | 40 | control | |

* The result of the independent t-test

**Table 3**. The mean and standard deviation of serum TGF-β levels in both groups before treatment

| variable | group | number | mean | standard deviation | *P*-value* |
|---|---|---|---|---|---|
| serum TGF-β | case | 40 | 90.21 | 24.44 | <0.001 |
| | control | 40 | 125.63 | 23.28 | |

* The result of the independent t-test

**Table 4**. The mean and standard deviation of serum IL-17 levels in patients before and after treatment

| variable | time | number | mean | Standard deviation | *P*-value* |
|---|---|---|---|---|---|
| serum IL-17 | Before treatment | 40 | 83.74 | 23.57 | <0.001 |
| | After treatment | 40 | 45.27 | 19.23 | |

* The result of the independent t-test

The mean serum TGF-β level in patients before treatment was 90.21 pg/ml, and after treatment increased to 106.4. pg/mL, showing no significant difference ($P$ = 0.24).



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**Table 5**. Mean and standard deviation of serum TGF-β level in the case group before and after treatment

| variable | time | number | mean | Standard deviation | *P*-value* |
|---|---|---|---|---|---|
| serum TGF-β | Before treatment | 40 | 90.21 | 24.44 | 0.24 |
| | After treatment | 40 | 106.4 | 18.3 | |

* The result of the independent t-test

## DISCUSSION

In the present study, comparing the serum levels of IL-17 and TGF-β in patients with brucellosis before and after treatment with healthy individuals revealed a significantly increased IL-17 in the patients (83.74 pg/mL) than the control group (25.95 pg/mL). However, the mean serum TGF-β level in the patient group has significantly reduced (90.21 pg/mL) comparing the control group (125.63 pg/mL). The serum levels for both cytokines in boys and girls among both patients and control groups were not statistically significant. The mean serum IL-17 level in the patient group was 83.74 pg/ml before and 45.27 pg/ml after treatment, showing a statistically significant decrease. 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 not statistically significant.

Cellular immunity is the body's central defense against intracellular organisms such as Brucella. Brucella removal and clearance are mediated by increased macrophage activity through Th1 cellular immunity. Cytokines produced and secreted by various cells in response to proinflammatory mediators and bacteria during this stimulation play a vital role in the pathogenesis of brucellosis [2, 3, 6, 10, 13]. TGF-β is secreted by various cell types, such as macrophages, in response to tissue damage and is a highly potent immunosuppressive cytokine that inhibits the activity of lymphocytes and phagocytes and regulates T cell function [11, 13].

Brucellosis is common in patients with a haplotype of the TGF-β gene mass production [14]. In patients with brucellosis, the TGF-β gene producing the haplotype was significantly increased [15]. In contrast, the rate of moderate and mass-producing TGF-β genotypes was significantly lower in these patients [11]. There was no significant difference in TGF-β levels between patients and controls [16]. These variations may be due to epidemiological, ethnic, and geographical differences and the 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 primary cytokines secreted by Th17 include IL-17, IL-21, and IL-22, which act on neutrophils, IgM, and IgA-producing B lymphocytes [17]. IL-17 is a proinflammatory cytokine with essential functions in infectious diseases, autoimmunity, and malignancies [18, 19]. This cytokine 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. IL-17 activates Th1, which is necessary to control *Brucella* infection [11]. In this study, the IL-17 level was significantly higher in brucellosis cases than in controls, and after brucellosis treatment, the IL-17 serum levels were decreased. A live brucellosis vaccine stimulating the Th17 cell response could protect the mucosa against *Brucella abortus* [19]. It showed that rs8193038 and rs4711998 genotypes and AAGAA interleukin 17 haplotypes were the risk factors for brucellosis, while rs3819025AA and rs3819024GG genotypes were protective factors against the disease [20]. These findings suggest that IL-17 may be essential in protecting against *Brucella* infection.

*B. abortus* triggers a profibrotic response characterized by inhibiting MMP-9 secretion, inducing concomitant collagen deposition and TGF-β secretion [21]. TGF-β increases in brucellosis [22]. IL-17 secreted by Th17 cells plays a critical role in protective immunization against brucellosis [23].

Quantitative real-time PCR analysis, preceding and following antibiotic treatment, revealed the inflammation association in the cerebrospinal fluid with higher IL-6 and IL-17 expression. These results could improve our understanding of inflammation in a leptomeningeal and WM involvement related to neurobrucellosis for a better diagnosis when clinical, MRI data and routine hematological tests are nonspecific [24]. A survey on the relationship between genetic variants of IL-17 and susceptibility to human brucellosis found a strong relationship between IL-17 gene SNPs and susceptibility and resistance to this disease. IL-17 has a role in immunity [25]. Serum IL-17 titers were higher in the brucellosis group than in the control group [5].

One study found that outbreaks or prevalence of brucellosis in pastoral areas can be prevented upon developing a high-potency vaccine for brucellosis or discovering a new method for regulating Th1/Th2 cells balance and Treg/Th17 cell balance for brucellosis treatment [26].

Th17 cells provide the host organisms with additional defense against several extracellular and intracellular microbial infections and can defend against the invasion of *Brucella* through cooperation with Th1 cells [19]. The antibody response to *Brucella* proteins, mediated by interleukin 17 (IL-17) secreted by Th17 cells, plays a crucial role in the brucellosis vaccine [27]. One case noticed an inflammatory process involving IL-6 and IL-17 cytokines with an increase of IL-6 and IL-17 in



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PBMCs and CSF. Th17 responses have been shown to contribute to host defense vs. *Candida* and *L. monocytogenes*, and this Th17 response is favored when IL-6 is present in high quantities [28].

Our findings indicated that people with these genotypes at the listed positions have a greater risk of developing brucellosis (337, 88, 25, and 103 times, respectively) when exposed to *Brucella*. More assessments of genetic variations and the ability to produce IL-17 in patients with brucellosis are recommended [25]. In this study, we concluded that the serum level of IL-17 in patients with brucellosis increased significantly compared to healthy adults, while the serum level of TGF-β decreased not significantly. TGF-β is an anti-inflammatory cytokine that is secreted by macrophages and T cells and enhances the humoral immune response. TGF-β is a cytokine with different functions and regulates several pathways in the growth and differentiation of many types of cells. It also suppresses cellular immunity at multiple levels and inhibits the function and proliferation of lymphocytes [29]. Therefore, the level of these two cytokines, especially interleukin 17, can be used to diagnose brucellosis. After treatment, the serum level of IL-17 in the patients' group was significantly reduced, but the level of TGF-β did not change significantly. Therefore, serum levels of these two cytokines may be used as diagnostic markers in brucellosis patients.

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## CONFLICT OF INTEREST

The authors declare that no conflicts of interest are associated with this manuscript.

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