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Comparison of Serum Changes of Interleukin-17A and Interleukin-21 Between Schizophrenic Patients and Healthy Individuals

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Abstract

Background: Immunological alterations in schizophrenic patients have been considered during last decade. There are no remarkable reports on the changes of IL-17A and IL-21 in schizophrenic patients. Therefore, the purpose of this study was to evaluate changes of serum IL-17A and IL-21 in schizophrenic patients in comparison with healthy controls.

Methods: In the present study serum levels of IL-17A and IL-21 in 30 patients with schizophrenia before treatment and three months after treatment were measured by enzyme-linked immunosorbent assay (ELISA) and compare to 30 match healthy control group.

Results: Serum levels of IL-21 in schizophrenic patient was significantly higher than control group (P= 0.001). Serum levels of IL-17A in the schizophrenic patients had no significant changes than the control group (P= 0.4). Serum levels of IL-17A in patients with schizophrenia three months after treatment than before treatment had no significant change (P=0.7) and IL-21 serum levels in schizophrenic patient three month after treatment was not significant changed in comparison with this group before treatment (P= 0.06).

Conclusions: The serum levels of interlukine-21 is elevated in schizophrenic. Results of this study showed that IL-21 might be involved in the pathologic mechanism of schizophrenia.

Keywords: Immunity, Interleukin-17A, Interleukin-21, Schizophrenia.

Introduction

Schizophrenia is a complex brain disorder with characteristic symptoms such as delirium, hallucinations, mental and motor disorders, along with an inability to understand or express reality, and in some cases, suicide attempts. Its clinical signs appear in the late second or third decade of life. The onset of symptoms is about five years earlier than women. Recently, activation of the inflammatory response system and changes in cytokines in schizophrenia have been considered in various studies (1, 2). In addition, there are several reports on the relationship between infection during pregnancy, especially in the second trimester of pregnancy, with prevalence of schizophrenia in children (3, 4). Some studies showed that the increase of IL-8 level in the blood of expected mothers during the second trimester of pregnancy can increases the risk of schizophrenia in children. On the other hand, infection of the central nervous system in childhood has increased the risk of psychotic disorders in older ages (5). Symptoms of

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inflammation have also been observed in the brains of schizophrenic patients (6). Cytokines can partially penetrate the blood-brain barrier and bind to receptors on nerve and glial cells. In addition, cytokines can be produced and released in the central nervous system. Neurons also produce cytokines under certain conditions. Cytokine receptors such as IL-4, IL-6 and IL-10 receptors are present in the central nervous system. The presence of these receptors suggests that cytokines have a direct effect on neuronal function (7, 8). The interleukin-17 family consists of six different interleukins: IL-17A, B, C, D, E, and F. Among these six different interleukins, the biological functions and regulation of IL-17A and IL-17F are well known. These two cytokines have the most structural similarity to each other, and their coding genes are located on a single chromosome close mice and humans. In terms of function, IL-17A induces proinflammatory responses (9, 10). Although IL-17 protects the host against invasion by many pathogens, impaired production can lead to overexpression of proinflammatory cytokines and chronic inflammation, leading to tissue damage and autoimmunity. There are association between IL-17 and many autoimmune diseases such as rheumatoid arthritis and multiple sclerosis (MS). Investigation of IL-17 can be useful for the treatment of autoimmune diseases due to its widespread involvement of in many diseases. Human IL-17A antibodies have now been developed for the treatment of rheumatoid arthritis, psoriasis, and choroiditis (10-12). IL-21 is a cytokine with a quadruple helix. This cytokine is produced by CD4 + activated T cells, natural killer cells, and Th17 cells (13-15). Preliminary studies have shown that IL-21 affects the proliferation and function of natural killer cells, B cells and T cells. Its proven recombinant IL-21 has potent anticancer effects (16). IL-21 inhibitors are currently considered for the treatment of inflammatory diseases and autoimmunity. Recently, the production of IL-21 has been attributed to Th17 cells, which are a subset of CD4 + cells, and the main source of its production is Th-17 cells (17). Because IL-17 and IL-21 are important proinflammatory cytokines produced by the Th17 immune response, and there are few studies on the role of these interleukins in schizophrenia, this study evaluated the serum levels of IL-21 and IL-17 in schizophrenic patients in comparison with normal healthy individuals.

Materials and Methods

In this case-control study, 30 patients with schizophrenia (15 men and 15 women) were selected. Their diagnosis was made by a psychiatrist based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (15, 16). Patients with autoimmune disease or infection were excluded from the study. Thirty healthy individuals (15 males and 15 females) were considered as the control group. Patients were treated with Clozapine (HEXAL company, Germany), Risperidone (JANSSEN company, Germany), and Olanzapine (ELI LILLY company, England). Blood samples were collected from the participants in the morning. After clot formation at room temperature, the samples were centrifuged, and their serum was collected. Immediately after centrifugation, serum samples were transferred to -80 °C freezer. Serum levels of IL-17A and IL-21 were measured by ELISA kits (eBioscience company, BioTec ELx 800, United States, respectively). Statistical analysis was done by SPSS 20. $P < 0.05$ was considered as a significant difference between the experimental groups. The normal distribution of data was determined using the Kolmogorov-Smirnov test. Since the data were not normally distributed, non-parametric Kruscal - Wallis tests were used for statistical analysis of the data for independent groups and Mann - Whitney for dependent groups. Spearman correlation test was used to calculate the correlation coefficient between the studied interleukins.

Ethical Consideration

This study was approved by the ethic committee of Zahedan university of medical sciences (ID: 90-2018). The approved consent was signed by the patients or their guardians.

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Results

Comparison of serum IL-17A concentrations in different study groups

Results showed that there was no significant difference between pre-treatment schizophrenic patients and control group in term of serum levels of IL-17A (P= 0.4). There was no significant difference between serum concentrations of IL-17A before and three months after treatment in schizophrenic patients' group (P=0.7). Also, there was no significant in serum concentrations of IL-17A between patients with schizophrenia three months after treatment and healthy control group (P=0.1) (Table 1).

Table 1. Comparison of serum levels of IL-17A and IL-21 in two groups.

IL type	Control (A)	Case		Comparison A and B	Comparison A and C	Comparison C and B
		before treatment (B)	Three months after treatment (C)			
IL-17A pg/ml	6.8±4.6	8.9±6.96	8.31±4.2	0.4	0.1	0.7
IL-21 pg/ml	18.4±7.56	33.82±9.45	31.92±2.12	0.001*	0.001*	0.06

*Significant data is indicated by an asterisk.

Comparison of serum IL-21 concentrations in different study groups

Results showed that there is a significant difference in serum levels of IL-21 between pre-treatment schizophrenic patients and control group (P= 0.001). There was no significant difference between serum concentrations of IL-21 before and three months after treatment in schizophrenic patients' group (P=0.06). Also, there was significant difference in serum concentrations of IL-21 between patients with schizophrenia three months after treatment and healthy control group (P=0.001) (Table 1).

Evaluation of correlation coefficient between cytokines IL-17A and IL-21 in schizophrenic patients

The correlation coefficient between IL-17A and IL-21 in schizophrenic patients was determined by Spearman's correlation test and P <0.01 was considered as the correlation criterion. The results of this test showed that there is not any correlation between IL-17A and IL-21 and in this disease (Table 2).

Table 2. Evaluation of the correlation between IL-17A and IL-21 in schizophrenic patients

Variable	R	P value
Correlation between IL-17A and IL-21	0.02	0.9

Calculation of blood factors in two groups

There was no significant difference between groups in term of blood factors including white blood cell (WBC), liver enzymes ALT, AST and hemoglobin (Hb) (Table 3).

Table 3. Comparison of biochemical factors in patients with schizophrenia and controls.

Variable	Control	Case	P value
WBC*1000/µl	7.9 ± 0.3	8.02 ± 0.3	0.8
ALT (IU/L)	25.3 ± 1.9	28.54 ± 1.3	0.2
AST (IU/L)	20.4 ± 2.07	25.75 ± 2.3	0.1
Hb (mg/dl)	13.7 ± 0.4	14.5 ± 0.4	0.2

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Discussion

Cytokines are a large family of low molecular weight proteins. These agents have a wide range of functions in innate immunity and inflammatory responses. Cytokines are able to cross the blood-brain barrier and interact between the central nervous system and the immune system, and regulate activities such as neuronal migration, synapse maturation, and differentiation of neurons. However, imbalance between these inflammatory mediators can lead to neuritis, nerve damage, and nerve degeneration. Thus, cytokines can be involved in a large number of psychological disorders (17, 18). This study was designed for comparison of serum levels of IL-17A and IL-21 between schizophrenic patients and healthy persons.

Our results showed that the serum levels of IL-17A in schizophrenic patients were not significantly different with the control group. IL-17A is a proinflammatory cytokine that is primarily produced by Th17 cells and stimulates macrophages and the secretion of other proinflammatory cytokines such as IL-1, IL-6, TNF-α, chemokines, and metalloproteins. Therefore, IL-17A causes inflammation by induction of these factors. IL-17A, as the major cytokine in central nervous system disorders, is produced in the central nervous system mainly by microglia cells. Regulation of feedback of cytokines of Th17 family leads to stability of IL-17A level. So, in our study, the observed non-significant difference between schizophrenic patients and control group may be due to this feedback regulation. Borovcanin et al. showed that serum levels of IL-17 in schizophrenic patients were significantly lower than in controls (19). The exact mechanism of this reduction is not yet known, but IL-4 may induce an increase in TGF-β production and a decrease in IL-17A production. Previous studies have been showed that IL-4 can reduce IL-17A production by reducing receptor/ ligand expression or reducing their function (20, 21).

The results of this study showed that the serum levels of IL-21 in patients with schizophrenia were significantly increased compared to the control group. IL-21, as an inflammatory cytokine, is produced by Th17 cells. IL-21 causes the production and maturation of natural killer cells, increases IFN-γ synthesis, and cytolytic activity. By increasing IFN-γ, microglia are activated in the brain. Activated microglia secrete proinflammatory cytokines, resulting in complications of schizophrenia (22, 23). One of the reasons for the significant increase in IL-21 in schizophrenia is induction of its expression through the activation of the transcription factor STAT-3. Activation of STAT-3 stimulates the development and differentiation of Th17 cells, which in turn increases IL-21 production. Another reason is the effect of IL-21 on regulatory T cells. In this way, IL-21 inhibits the STAT5a and STAT5b phosphorylation. This situation leads to inactivation of these transcription factors. Th17 cell differentiation increases followed by an increase in IL-21 (23-26). In addition, in our study, the level of blood factors including white blood cell (WBC), liver enzymes ALT, AST, and hemoglobin (Hb) in patients with schizophrenia and healthy controls were measured and compared. Data showed that there is no significant difference in term of these factors. This indicates that these factors do not play an active role in the development of schizophrenia.

IL-21 is significantly increased in patients with schizophrenia compared to healthy individuals. So, this interleukin may be involved in the pathogenesis of schizophrenia. Further studies are needed to identify more details about the role of IL-21 in the disease as well as identification of related pathways in the diagnosis and treatment of the disease.

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Conflict of interest

The authors declare no conflict of interest.

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