

Serum TNF- α , IL-10 and IL-2 in Schizophrenic Patients Before and After Treatment with Risperidone and Clozapine

Abolghasem Ajami¹, Farshideh Abedian^{2*}, Seyyed Hamzeh Hosseini³, Elahe Akbarian⁴, Reza Alizadeh-Navaei¹, Mehrdad Taghipour⁵

¹Molecular and Cell Biology Research Center, ²Department of Immunology, ³Psychiatry Research Center, ⁴School of Medicine, Mazandaran University of Medical Sciences, Sari, ⁵Urology and Nephrology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

ABSTRACT

Background: Schizophrenia is a disorder of the executive function of both sensory and central nervous system. Recent studies suggest that immune mechanisms play a role in the pathophysiology of this disease. The variations in cytokine concentrations have been associated with psychopathology and treatment of schizophrenia. **Objective:** To investigate the changes in serum concentrations of TNF- α , IL-10, and IL-2 in schizophrenic patients before and 40 days after treatment. **Methods:** In a case-control study, 26 schizophrenic patients and 26 healthy individuals were enrolled as the control group. PANSS scale questionnaire was used for diagnosis and assessing the severity of the disease. All patients were then treated with risperidone or clozapine for 40 days. Serum concentrations of TNF- α , IL-10 and IL-2 were measured by ELISA before and after treatment in both groups. Paired *t*-test and Independent *t*-test were used for comparison of data. **Results:** Comparison of TNF- α and IL-10 concentrations in patients before and after treatment revealed a significance decrease of TNF- α and increase of IL-10 concentrations ($p=0.002$, and $p=0.008$, respectively). Serum concentrations of IL-2 were lower than the detection limit of assay and were not detectable. In comparison with healthy controls, serum concentrations of TNF- α in schizophrenic patients were higher, while IL-10 concentrations were lower before treatment although the differences were not significant ($p=0.291$ and $p=0.375$, respectively). There was no correlation between cytokine concentrations and the positive and negative scale (PANSS). Also no significant difference in the admission, relapses, and duration of illness before and after treatment was observed. **Conclusions:** Increase of TNF- α and decrease of IL-10 may have an important role in psychopathology of schizophrenia.

Ajami A, et al. *Iran J Immunol.* 2014; 11(3):200-209

Keywords: Interleukin-2, Interleukin-10, Schizophrenia, Tumor Necrosis Factor alpha

Corresponding author: Farshideh Abedian, Department of Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran, Tel: (+) 98 11 33543081, Fax: (+) 98 11 33543248, e-mail: fabedian1340@gmail.com

INTRODUCTION

Schizophrenia is a disorder associated with sensory system and the administrative function of the central nervous system (CNS) (1). It is one of the most severe mental injuries that begins at the early-old age and affects men and women with the same ratio. The prevalence of this disease is about 1% during the longevity of an adult population and is thought as the most crucial psychiatric disorder (2) with high economical damage. Total therapeutic and indirect costs of the patients reach about 50 billion dollars a year. On the other hand, schizophrenia is a chronic disease and affected patients occupy about 50% of the beds of the mental hospitals.

Antipsychotic drugs are the major treatments for schizophrenia and are divided into two main groups: 1) dopamine receptor antagonists and 2) antagonists associated with serotonin and dopamine, but these medications only eliminate the symptoms of the disorder and do not cure the disease itself (2).

There are various hypotheses about the pathogenesis of this disease, one of which is the immune system interference (1). Documents suggest that the neural, endocrine, and immune systems influence each other using cytokines, hormones, and neurotransmitters (3). Activation of the immune system causes fever and behavioral, neuroendocrine and neuropathologic changes in the CNS (4). These changes efficiently occur by the interaction of cytokines with their receptors on the neurons and the glial cells in the brain (5).

Cytokines are proteins secreted by different cells and perform paracrine, endocrine and even autocrine functions. They can relatively penetrate into the blood-brain barrier and bind to their receptors on the neurons and glial cells (6). Furthermore, cytokines have the capacity to be produced inside the CNS. Although many cytokines are secreted by the glial cells in the brain, some documents reveal that cytokines could also be produced by the neurons under specific conditions (7).

The involvement of cytokines produced by the microglia, astrocytes, neurons, and endothelial cells in the pathogenesis of different psychiatric disorders, like schizophrenia, acute depression, panic disorders and autism, have been widely surveyed (8). On the other hand, the expression of the cytokines in the CNS refers to the role of this intercellular messenger in different physiologic processes such as CNS differentiation (9), synaptic transmission (10), cognition (11), regulation of the hypothalamus-hypophysis axis (HPA) (12), dream (13), and appetite (14).

There is some evidence that supports the cytokine changes in the schizophrenia and acknowledges the effect of antipsychotic drugs in the regulation of these changes (15,16); however, there are many controversies in different studies. Theodoropoulou *et al.* reported the increased concentrations of TNF- α in schizophreniac patients (17) compared to the control group, while others reported a decrease in TNF- α concentrations (18) or even normal concentrations of TNF- α (19). There are studies showing increased concentrations of TNF- α in treated cases compared to the control group (20,21), whereas others reported the decreased concentrations of TNF- α in treated patients (22,23). These controversial results have also been observed in studies measuring other cytokines such as IL-10 and IL-2. Regarding IL-10, Kaminska *et al.* (24) and O'Brien *et al.* (25) demonstrated decrease of IL-10, whereas Chang *et al.* (26) and Kunz *et al.* (27) reported increase of IL-10, and Kubistova *et al.* demonstrated the normal amount of IL-10 in schizophrenic patients (28). Also, Zhang *et al.*, revealed

increase of IL-2 while Manderan *et al.* (16) and Theodoropoulou *et al.* (17) reported decrease of IL-2 in this diseases.

In this study, the serum concentrations of TNF- α , IL-2, and IL-10 as innate, adaptive, and regulatory cytokines were measured in patients with schizophrenia before and after treatment and compared with the control group to reveal the probable role of these cytokines in the pathogenesis of schizophrenia.

MATERIALS AND METHODS

Inclusion Criteria. A case-control study was conducted in patients who referred to the Zare Hospital, Sari, Iran, with a diagnosis of schizophrenia during July 2008 to January 2011. The diagnosis was confirmed by administration of interviews such as SCID (Structured Clinical Interview for DSM-IV). All patients were asked to complete a written consent form, before entering the study. Also the project was approved by the Ethics Committee of Mazandaran Medical University. Patients who were diagnosed for the first time or individuals whose disease relapsed due to the lack of compliance to therapy and the physician had used second-generation antipsychotic drugs for their treatment, enrolled in the study.

Subjects. Thirty patients who had the eligibility criteria were selected for this study. Due to the lack of enough serum samples of the second sampling stage, four patients were excluded and finally the results of 26 patients were analyzed. Those patients who had used drugs in addition to second-generation medications or suffered from other psychiatric disorders were also excluded. 26 individuals were also selected as controls. Individuals in the control group were blood donors who referred to the blood transfusion organization Sari, Iran and had no history of antipsychotic drug taking and no past history of any psychiatric disorders. These individuals were age and sex matched with the cases and had normal CRP and ESR levels.

Data Collection Sampling and Treatment. Data were collected by using two questionnaires for evaluating the schizophrenic symptoms, demographic features, and also the medication history. An International PANSS questionnaire (29) was used to assess positive and negative symptoms in patients. After confirming the diagnosis of schizophrenia in patients, the treatment with risperidone and clozapine was initiated based on the patient's condition. Clozapine was used when their previous treatments had not been successful or when the treatments had caused adverse side effects. Moreover, risperidone was administered once or twice daily in other cases. Before the treatment 5 ml of venous blood was taken and serum was isolated. All samplings were conducted during the morning hours. The sera were immediately sent to a central laboratory and stored in -70°C degrees. Sampling was repeated 40 days after the start of treatment. Five milliliters of venous blood was also taken from each individual of the control group and the serum was separated in the same conditions as the case group.

ELISA Assay. Serum concentrations of TNF- α , IL-10, and IL-2 were measured using commercial ELISA kits (Avibion, Finland) according to the manufacturer's instructions. Laboratory experiments were done in a blind format where the information about the samples was not revealed until the time of analysis.

Statistical Analysis. Data was analyzed using SPSS 18.0 software (SPSS, Chicago, IL, USA). Serum concentrations of cytokines in patients before treatment and forty days after onset, and also changes in PANSS scale data were compared using paired-*t*-test.

Pearson test was used to determine the correlation between serum concentrations of cytokines before and after treatment. Spearman test was used for evaluating the relationship between cytokines concentrations and PANSS scale. The independent *t-test* was used for examining the relationship between disease relapse, times of hospitalization, disease duration with cytokines concentrations and also for differentiating the efficacy of the clozapine and risperidone on cytokine concentrations. P values less than 0.05 were considered statistically significant.

RESULTS

Of 52 subjects who participated in the study, 26 patients belonged to the case group and 26 belonged to the control group. Patients and controls were matched for age and sex. The mean ages of the case and control groups were 33.62 ± 9.49 yrs and 33.92 ± 8.86 yrs, respectively. In total, 40 (76.92%) participants were male and 12 (23.08%) were female. An international PANSS questionnaire was used for detecting positive and negative symptoms of schizophrenic patients and to assess the response to treatment in this study. The mean scores of the evaluated variables in this questionnaire are mentioned in Table 1.

Table 1. Mean scores of positive and negative symptoms before and after the treatment.

PANSS Questionnaire Scores	Before the Treatment	After the Treatment	P Value
Mean total score	87.62 ± 22.99	52.92 ± 12.63	0.00
Mean of positive symptom scores	21.46 ± 8.44	11.46 ± 4.07	0.00
Mean of negative symptom scores	25.46 ± 7.77	15.92 ± 6.76	0.00

Serum concentrations of the TNF- α , IL-10, and IL-2 were measured in schizophrenia patients before and after treatment and compared by paired *t-test* (Table 2). Concentration of these cytokines were also measured in sera of healthy control group and compared with patients by *t-test* (Table 2).

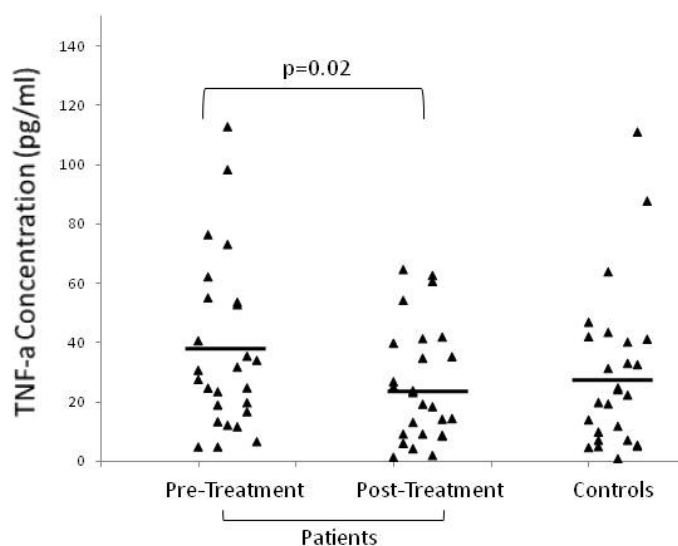
Results showed statistical significant changes between concentrations of TNF- α and IL-10 in the case group before and after treatment. However, serum concentrations of IL-2 in all case and control groups were less than the sensitivity of the assay, and therefore, IL-2 was not detectable in sera of schizophrenia patients as well as the control group. In other words, we could say that the concentration of the IL-2 in the schizophrenia patients were not increased and were the same as the control group.

Table 2. Serum concentrations of cytokines in patients (before and after treatment) and in control group.

Variant	Concentration before treatment (pg/ml)		Concentration after treatment (pg/ml)		P Value*
	Case	Control	Case	Control	
TNF-α	37.168 \pm 5.604	29.007 \pm 5.210	25.471 \pm 3.818	29.007 \pm 5.210	0.002
IL-2	0.092 \pm 0.011	0.071 \pm 0.003	0.070 \pm 0.011	0.071 \pm 0.003	Lesser than kite sensitivity
IL-10	3.496 \pm 1.234	10.716 \pm 7.207	4.161 \pm 1.057	10.716 \pm 7.207	0.008

*The provided P-value shows the differences between serum concentrations of cytokine in case group before and after treatment, not between case and control group.

The comparison of the serum concentrations of the TNF- α and IL-10 before and after treatment declare the significant decrease in TNF- α in the treated schizophrenia patients, ($p=0.002$; Figure 1) while IL-10 increases after treatment ($p=0.008$; Figure 2). The comparison of the serum concentrations of the cytokines before treatment with the control group demonstrated that the serum concentrations of TNF- α in the schizophrenia patients is more than healthy control ($p=0.291$) but serum concentrations of the IL-10 is less in the control group ($p=0.375$).

**Figure 1.** Serum concentrations of TNF- α in patients (before and after treatment) and in control group.

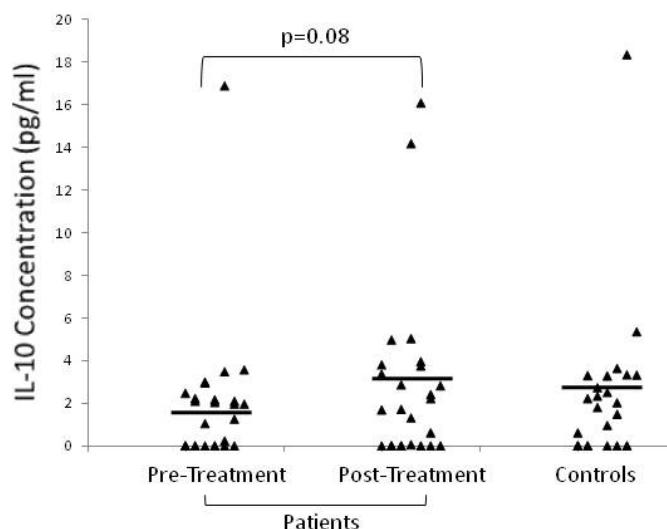


Figure 2. Serum concentrations of IL-10 in patients (before and after treatment) and in control group.

Of 26 Patients treated with second generation of antipsychotic drugs, 16 patients treated with the risperidone and 10 individuals treated with the clozapine. To investigate the comparative effects of the above drugs on the level of TNF- α and IL-10, the independent samples *t-test* was used. Although the treatment caused a significant decrease in the serum concentrations of TNF- α and an increase in IL-10 concentration, the comparison of the effect of these two drugs did not demonstrate any significant difference. In other words, although risperidone has more decreasing effect on TNF- α level and more increasing effect on IL-10 than clozapine, the differences did not reach the significant level.

To investigate the relation between the frequency of hospital admissions, frequency of relapses and the duration of the illness with the concentration of the TNF- α and IL-10 Pearson Correlation Test was used. Results showed that there was no relation between the frequency of hospital admissions, frequency of relapses and the duration of the illness with the concentrations of the TNF- α and IL-10.

PANSS score in schizophrenia patients were analyzed by Spearman test to evaluate the relationship between cytokines concentrations and symptom scores (total, positive, and negative scores). There was no significant relation between the scores of PANSS questionnaire and the serum concentrations of the TNF- α and IL-10, before and after the treatment.

DISCUSSION

In this study, the serum concentration of TNF- α in the patients with schizophrenia was higher than the control group. After treatment, however, it was decreased to normal concentrations similar to that of the control group. This was in agreement with the data

given by Monteleone *et al.* following ten weeks (22) and Kim *et al.* following six weeks of treatment (23). Also, Dunjic-Kostic *et al.* indicated decreased concentrations of TNF- α during acute and remission phase (18). Other studies showed increased serum concentrations of TNF- α after four to six weeks of treatment (17,20,21,28,30) or no change in its concentrations (19,24,27). TNF- α is a pro-inflammatory cytokine, which is increased in innate immune responses and also during Th1 and Th17 activation. It may also take part in the pathogenesis of schizophrenia by activating the Hypothalamo-Pituitary-Adrenocortical (HPA) axis, activating secretion of serotonin as a neurotransmitter and stimulating the indoleamine 2-3-dioxygenase which leads to elimination of tryptophan and activation of kynurenine metabolites, or releasing of the neurotoxic glutamic acid (31). In this study, serum concentrations of IL-10 in the schizophrenic patients showed decline in comparison with the control group and increase in the patients under treatment. Studies of Kaminska *et al.* (24) and Obrien *et al.* (25) showed decline of IL-10 in the schizophrenic patients, while data given by Chang *et al.* (26) and Kunz *et al.* (27) indicated increased serum concentrations of IL-10. However, Kubistova *et al.* (28) reported no change of this cytokine in schizophrenia. Considering the anti-inflammatory properties of IL-10, which could prevent the release of pro-inflammatory cytokines such as TNF- α , decreased serum concentrations of IL-10 in the schizophrenic patients indicates the lack of regulatory activities of immune system that could help in increased TNF- α levels and its effects.

In this study, serum concentrations of IL-2 in the case and control groups was very low and was not measurable by our assay, suggesting no change in IL-2 concentration in sera from schizophrenic patients. Hence, we can assume that endocrine IL-2 has no effect on schizophrenic pathogenesis. Data given by Manderan *et al.* (16) and Theodoropoulou *et al.* (17) showed decrease of serum concentrations IL-2 while that of Zhang *et al.* (23) revealed increase of this cytokine.

Various data given by different authors regarding the serum concentrations of cytokines in the schizophrenic patients are controversial which could be due to the differences in the cytokine measuring techniques or in the type of samples being tested (serum, plasma, or CSF), small sample sizes, sampling during different phases of the disease (acute, chronic, active phase, or remission), types of antipsychotic drugs and treatment responsiveness (32).

Two studies by Haack *et al.* (32) and Singh *et al.* (33) showed that patients' characteristics such as age, sex, smoking, body mass index (BMI), infectious diseases, and the previous non-systematic treatment could affect the serum concentrations of cytokines. One study indicated the increase of TNF- α in the schizophrenic patients even after treatment and they related this increase to silent toxoplasmosis, which is two to three times more prevalent in schizophrenic patients (34,35). In this study, we tried to control the confounding variables to obtain more accurate results. Therefore, we included patients with normal concentrations of CRP and RF to exclude any infection and inflammation. Also, we only included patients under treatment with the second generation of antipsychotic drugs, and the severity and response to treatment in schizophrenic patients was evaluated through measuring of PANSS.

Our data shows no significant relationship between serum concentrations of IL-10 and TNF- α prior to and after treatment, using the PANSS questionnaire (total, positive, and negative symptoms). Kubistova *et al.* (33) indicated no significant association between serum concentrations of IL-10 and TNF- α using the PANSS questionnaire which is in agreement with our findings.

In this study, after 40 days of treatment with risperidone and clozapine the serum concentrations of IL-10 and TNF- α were determined. It was found that, both types of the drugs used, had effect on treatment of diseases (PANSS score) and increase or decrease of these cytokines. Although risperidone has more effects on the mean concentrations of these cytokines compared to the clozapine, there was no significant difference between these two drugs in treatment effect and cytokine concentrations.

In the present study, no significant relationship was observed between frequency of hospital admission, relapse and duration times of illness with the serum concentrations of TNF- α and IL-10 prior to and after the treatment. Few studies have been conducted to determine the relationship between these factors and serum concentrations of cytokine.

One limitation of the present study was measuring of cytokines in the serum. Since most biological activities of cytokines is exerted in the paracrine and autocrine modes, it would have been more accurate to measure them locally in the CNS of age/sex matching groups and include patients treated with one type of antipsychotic drugs which then could give more credible results.

In conclusion, our data showed that increase of TNF- α and decrease of IL-10 might have an important role in the psychopathology of schizophrenia, as we showed an increase in serum levels of TNF- α in contrast to a low level of IL-10 in patients with schizophrenia. Therefore, decrease and increase in cytokine serum levels in health and disease may be attributed to host and environmental factors. A further investigation is needed to explore if the increase and decrease in serum levels of TNF- α , IL-10 are directly related to the etiology of schizophrenia.

ACKNOWLEDGEMENTS

This work was financially supported by Mazandaran University of Medical Sciences (MAZUMS). Also thanks to the laboratory staff of Zare Hospital for blood sampling and Dr. Fazlali and Dr. Nazemi (Residents of psychiatrics) for helping in data collection. Special thanks to Ms. Mina Rostami, Dr. Mehran Taghipour and Dr. Sovaïd Taghipour for their critical reading of the manuscript.

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