

SERUM LEVELS OF INTERLEUKIN-6 AND TUMOR NECROSIS FACTOR- ALPHA IN EXACERBATION AND REMISSION PHASE OF SCHIZOPHRENIA

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SUMMARY

Background: The variations in proinflammatory cytokine levels have been associated with schizophrenia (SCH), duration of illness, psychopathology and treatment. The aim of the study was to investigate serum levels of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α) in schizophrenic patients during exacerbation and remission, and its association with course of illness and therapy.

Subjects and methods: We measured serum levels of IL-6 and TNF- α in 43 schizophrenic patients in exacerbation and remission and compared them to 29 healthy controls, matched by sex, age, body mass index (BMI) and smoking habits. The severity of psychopathology was assessed using the Positive and Negative Syndrome Scale (PANSS).

Results: There was no difference in levels of IL-6 and TNF- α in exacerbation compared to remission in schizophrenic patients. IL-6 was higher and TNF- α was lower in schizophrenic patients in both exacerbation and remission in comparison with healthy controls. TNF- α in exacerbation was in negative correlation with IL-6 in remission. No statistical significance was found between levels of cytokines and sex, age, BMI, smoking habits, antipsychotic medication, duration of treatment and duration of illness. IL-6 levels were in positive correlation with the age of onset and the duration of untreated psychosis. In schizophrenic patients on adjunctive treatment with mood stabilizers, TNF- α levels increased in remission.

Conclusion: Our results suggest that the connection between schizophrenia, cytokines and medication is multifaceted, and not necessarily linear. Adjunct mood stabilizers not only ameliorate psychopathology, but might convey immunomodulatory effects as well. Further longitudinal studies could elucidate potential beneficial effect of combined therapy in treatment of SCH.

Key words: schizophrenia - tumor necrosis factor-alpha - interleukin-6 - antipsychotics - mood stabilizers

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INTRODUCTION

The interconnection between immune abnormalities and schizophrenia (SCH) has been studied for decades and still represents a topic for debate. Growing evidence suggests that specific cytokines play a pivotal role in signaling the brain to produce neurochemical, neuro-endocrine, neuroimmune and behavioral changes (Reale et al. 2011). Cytokines are signaling proteins of the immune system which are involved in important functions of the brain such as the regulation of mood (McNamara & Lotrich 2012), the sleep-wake cycle (Weschenfelder et al. 2012) and food consumption (Ramos et al. 2004). So far, SCH has been associated with altered levels of various cytokines and their soluble receptors, such as interleukin (IL)-6, soluble interleukin-6 receptor (sIL-6R), interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-4 (IL-4), tumor necrosis factor-alpha (TNF- α) (O'Brien et al. 2008, Frommberger et al. 1997, Maes et al. 2002, Müller et al. 1997). Increased concentrations of IL-6 are connected to negative phenomenology, duration of illness (Akiyama 1999, Ganguli et

al. 1994), resistance to therapy (Lin et al. 1998), and unfavorable course of illness (Müller et al. 2000). Elevated serum concentration of IL-6 was also observed in acute phase of illness (Frommberger et al. 1997), as well as in patients with chronic form of SCH (Akiyama 1999, Zhang et al. 2002). Several studies found increased concentrations of TNF- α in patients in exacerbation of SCH (Naudin et al. 1997, O'Brien et al. 2008, Theodoropoulou et al. 2001), while others proved differently (Xu et al. 1994). Particular "confounding" factors such as age, smoking, body mass index (BMI), sex, and infection also have a bearing on levels of IL-6 and TNF- α (Haack et al. 1999, Himmerich et al. 2009). Moreover, numerous studies demonstrated that treatment with antipsychotics may affect levels of cytokines (Pollmächer et al. 1996), and that antipsychotics could have antiinflammatory effects on microglial activity, which is elevated in SCH (Monji et al. 2009). On the other hand, the influence of other psychotropic drugs such as mood stabilizers, anxiolytics and anticholinergic, also used in treatment of SCH as adjunct therapy and proven to be beneficial, have not been studied in

detail (Afshar et al. 2009, Leucht et al. 2007, Schwarz et al. 2008, Tiihonen et al. 2009, Volz et al. 2007).

Therefore, in the current study our aim was to investigate: i) whether the levels of IL-6 and TNF- α in schizophrenic patients alter during exacerbation and remission; ii) if there is a difference between levels of interleukins in patients suffering from SCH in exacerbation and remission in comparison to healthy controls; iii) if there is association between sex, age, BMI and smoking habits and cytokine levels iv) if there is a connection between interleukins level and age of onset, duration of untreated psychosis, duration of illness and duration of treatment; v) if there is a difference in levels of interleukins in patients treated with different medication.

SUBJECTS AND METHODS

Forthy-three inpatients hospitalized at the Clinic for Psychiatry, Clinical Center of Serbia, Belgrade provided informed written consent to participate in the study. The study was approved by the Ethics' Committee of School of Medicine in Belgrade and the Board of the Clinic of Psychiatry. The study protocol was in accordance with the Declaration of Helsinki. All patients fulfilled the DSM-IV (American Psychiatric Association 1994) criteria for schizophrenia. All patients experienced an acute exacerbation of illness and all had history of at least one previous psychotic episode. Positive and Negative Syndrome Scale (PANSS) (Kay et al. 1987) was used to assess the severity of illness. Diagnosis and PANSS evaluation were carried out by two experienced research psychiatrists through complete semi-structured interviews in combination with all other available medical records. Patients who had been examined and given the same diagnosis by two experienced psychiatrists were recruited into the study. All clinical data concerning the course of illness and previous antipsychotic treatment were obtained by reviewing complete medical records and charts. The age of onset was defined as the age when symptoms first appeared as reported by the patients, family members or medical staff. Duration of untreated psychosis was defined as period in-between the appearance of the first symptoms of the illness and patient's age at the beginning of psychiatric treatment. Total duration of antipsychotic treatment was defined as time in years on any antipsychotic during lifetime. At the time of hospital admission, all subjects were free of major psychotropic drugs for at least 6 weeks. Patients with a history of any other psychiatric illness, substance or alcohol abuse, organic mental disorder were excluded. The exclusion criteria were also acute or chronic physical diseases, pregnancy or breast feeding, history of systemic, endocrine, immune disorders, infections, allergies, obesity or under-nutrition with recent weight loss, smoking more than 20 cigarettes per day. All subjects were free of anti-inflammatory and immunosuppressive therapy. Patients

were found to have normal blood and urine tests, normal liver and renal profile and normal electrocardiogram (EKG) and electroencephalogram (EEG) on admission as well as in a remission phase. The blood samples from the patients were collected before application of antipsychotic therapy in current psychotic episode – in exacerbation phase (T1) and in remission (on average after 6 weeks - T2). Decrease of $\geq 50\%$ on PANSS scale was assumed as remission. All biochemical measurements were conducted blind to diagnostic status. All patients included in the study were treated with the antipsychotic medication during their hospital treatment. In addition to antipsychotics, the treatment of certain patients included mood stabilizers, anxiolytics and anticholinergics.

Twenty-nine healthy individuals matched by sex, age, BMI and smoking habits to the SCH patients, were recruited from the staff of Clinic for Psychiatry and Institute for Biochemistry. They were screened for basic sociodemographic parameters, personal and family history of mental disorders (first degree relatives). None of the healthy controls had a history of psychiatric disorder, drug and alcohol abuse, serious acute or chronic somatic illness. All were free of psychotropic medication, anti-inflammatory or immunosuppressive therapy. Healthy volunteers' blood samples were collected only once.

Collection of blood samples and biochemical analysis

Blood samples were taken from antecubital vein between 7 a.m. and 8 a.m. into a vacutainer and they were left at room temperature for 1h to leave time for blood to clot. Serum and blood cells were separated by centrifugation (15 min, 3000 rpm). Serum samples were stored at -80°C until TNF- α and IL-6 measurements were made. TNF- α and IL-6 levels were measured by Bio Legend ELISA MAX Deluxe Sets (Bio Legend, San Diego, CA). Each sample was run in duplicate and the average was obtained.

Statistical analysis

In the study, the differences in means between patients and healthy controls were tested with independent samples t-test. To explore the difference in means between acute phase and remission in patients with schizophrenia paired t-test was used as appropriate. One-way analysis of variance (ANOVA) was used to compare the differences in means between different subgroups of patients with schizophrenia. For discrete variables, study groups were compared by a chi-square test. Pearson's correlation coefficients were calculated to examine the relationships between TNF- α and IL-6 levels and clinical variables. Results are presented as mean \pm standard error of mean (SEM) and $p < 0.05$ was regarded as significant for all comparisons.

RESULTS

Demographic and clinical characteristics of the study participants are presented in Table 1. Schizophrenic patients and healthy controls were matched by age, sex, smoking habits and BMI.

Immunological findings

Interleukins' serum levels in patients with SCH in exacerbation phase (T1), in remission phase (T2) and healthy controls are shown in Figure 1. There was no differences between levels of TNF- α in exacerbation of illness and remission ($t=-0.019$, $p=0.985$). Similarly, no statistical significance was found between serum concentrations of IL-6 in exacerbation and in remission ($t=-0.564$, $p=0.576$). TNF- α in exacerbation was negatively correlated to IL-6 in remission ($r=-0.301$, $p=0.050$), while in patients during the remission IL-6 did not correlate to TNF- α in remission ($r=-0.243$, $p=0.117$). Serum concentrations of TNF- α in SCH group were significantly lower in acute exacerbation of illness ($t=-2.115$, $p=0.041$) as well as in remission phase ($t=2.139$, $p=0.039$) in comparison to healthy controls. Sera concentrations of IL-6 were higher in the group of patients in exacerbation ($t=2.001$, $p=0.050$), as well as, in remission ($t=-2.594$, $p=0.012$) when compared with the group of healthy controls.

Immunological parameters and course of illness, age, gender, BMI and smoking habits

Sera concentrations of IL-6 in SCH group in exacerbation of illness (T1) correlate to duration of untreated psychosis ($r=0.462$, $p=0.003$), as well as to the age of onset ($r=0.459$, $p=0.004$). No connection was found between immune parameters and duration of treatment, nor with duration of illness. No statistical significance was found between levels of cytokines and age, gender, BMI and smoking habits.

Levels of interleukins and pharmacotherapy

TNF- α levels in patients in exacerbation ($F=0.897$, $p=0.416$) and in remission ($F=0.431$, $p=0.653$), as well as IL-6 levels in exacerbation phase ($F=0.677$, $p=0.514$) and in remission ($F=0.078$, $p=0.925$) did not statistically differ depending on applied antipsychotic therapy (FGA, SGA, clozapine). Higher frequency of increased TNF- α was observed in remission in those who received antipsychotic and mood stabilizer ($\chi^2=8.378$, $p=0.004$, $\Phi=0.488$). No alternations were indicated regarding IL-6 in patients who were taking mood stabilizer along with antipsychotic. The difference in cytokine levels related to different types of mood stabilizers were not accessed due to insufficient number of patients on mood stabilizers, which unabled further statistical analyses. There was no statistical significance between levels of cytokines and anxiolytics and anticholinergics.

Table 1. Demographic and clinical characteristics of schizophrenic patients and healthy controls

	Schizophrenia (n=43)		Control (n=29)
	T1	T2	
Gender (M/F)	19/24		12/17
Age (X \pm SEM)	36.63 \pm 1.50		34.00 \pm 1.01
BMI(kg/m ²) (X \pm SEM)	22.70 \pm 0.24		22.21 \pm 0.36
Smokers (yes, %)	72.1		62.1
PANSS (X \pm SEM)			
Total score	112.42 \pm 4.25	49.63 \pm 3.58	
Course of illness (years, X \pm SEM)			
Age of onset	24.89 \pm 0.71		
Duration of untrated psychosis	1.53 \pm 0.47		
Duration of treatment	7.86 \pm 1.10		
Duration of illness	10.42 \pm 1.29		
Medication			
Antipsychotics (CPE mg, X \pm SEM)			
First generation	403.64 \pm 55.84		
Second generation	193.75 \pm 33.81		
Clozapine	601.66 \pm 78.03		
Additional therapy (yes, %)			
Mood stabilizers	46.5		
Anxiolytics	83.7		
Anticholinergics	18.6		

Abbreviations: T1, exacerbation phase; T2, remission phase; BMI, body mass index; PANSS, Positive and Negative Syndrome Scale; CPE, chlorpromazine equivalents

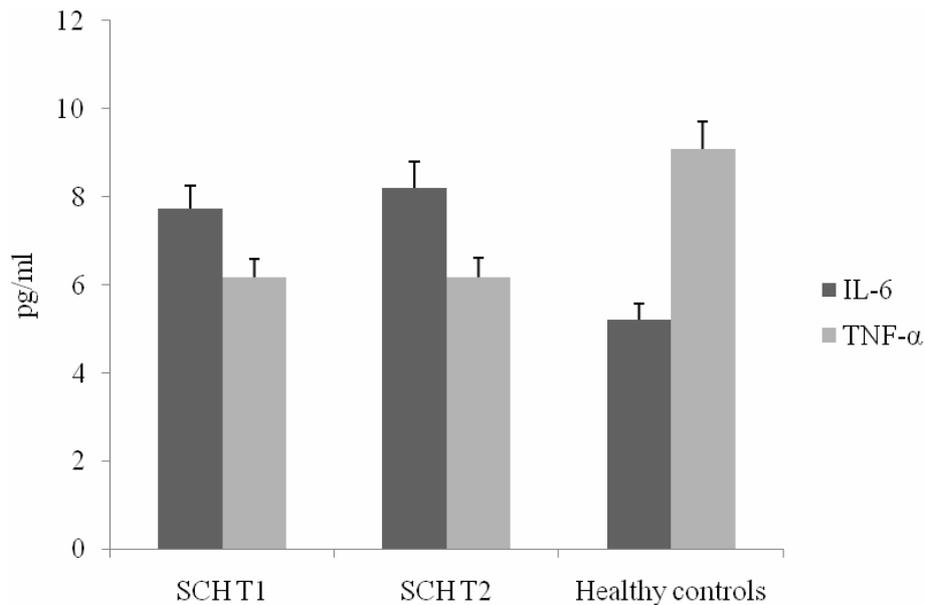


Figure 1. Serum levels of IL-6 and TNF- α in healthy controls and patients with SCH in acute phase (T1) and remission (T2). Data presented as mean \pm SEM. Significantly lower serum levels of TNF- α were found in SCH T1 (6.16 ± 0.52) and SCH T2 (6.17 ± 0.47) compared to healthy controls (9.08 ± 1.27). Significantly higher serum levels of IL-6 were found in SCH T1 (7.72 ± 1.15) and SCH T2 (8.21 ± 1.04) compared to healthy controls (5.21 ± 0.50).

DISCUSSION

Our results showed some difference in cytokine levels between patients suffering from SCH and healthy controls. We observed higher concentrations of IL-6 and lower levels of TNF- α in both exacerbation and remission in SCH patients in comparison to healthy control group. Elevated levels of IL-6 in SCH patients were reported in numerous previous studies (Frommberger et al. 1997, Gangluli et al. 1994, Naudin et al. 1997, Zhang et al. 2002). However, results regarding levels of TNF- α have been less conclusive, reported to be increased, decreased or unchanged (Baker et al. 1996, Naudin et al. 1997, Xu et al. 1994, Stefanis et al. 1994, Theodoropoulou et al. 2001). Observed discrepancy with some of the previous research results may occur due to different biological material that was analyzed (serum vs. plasma vs. CSF), different assay method, small sample size, various phases of disease (acute vs. chronic, active phase vs. remission) and confounding factors such as BMI, smoking, age, and gender. Furthermore, previous therapy was often not taken systematically into account (Singh et al. 2009, Haack et al. 1999). Our study included subjects with normal BMI, similar number of smokers and non-smokers, free of any other medical condition or any medication. Such inclusion criteria could be useful to control the effect of confounding factors, but might pose the boundary in terms of real clinical situation. Since many patients with SCH suffer from some co-morbid condition (Kozumplik et al. 2009, Rubeša et al. 2011, Oreški et al. 2012), our findings could apply to a limited group of individuals. Furthermore, our research showed that increased concentrations of IL-6 in SCH, as well as

decreased concentrations of TNF- α in patients were manifested both in exacerbation of illness and remission. In contrast, most prevailing results speak in support of higher concentrations of IL-6 in the exacerbation of illness and its reduction during remission (Frommberger et al. 1997, Pae et al. 2006), indicating elevated IL-6 to be a state marker of exacerbation (Naudin et al. 1997). Moreover, a meta-analysis performed by Miller et al. (2011) which took into consideration 40 studies, showed that TNF- α as trait marker for SCH is heightened during the acute deterioration of illness, but it remains the same after treatment. Our results do not support either notion. We found elevated IL-6 and decreased TNF- α to remain unchanged in SCH patients, irrespectively to the phase of illness. This might be due to timeline of blood draws, which was before and approximately after 6 weeks of treatment. Some of the previous investigations had their samples taken after a longer period of time (8-12 weeks) (Monteleone et al. 1997, Erbağci et al. 2001, Zhang et al. 2005). Nevertheless, the criteria for remission were fulfilled in all subjects, and we performed the blood draw at first time-point upon reaching remission. Another interesting finding in our study is associated to an interconnection between IL-6 and TNF- α levels. We found that TNF- α in exacerbation was in negative correlation with IL-6 in remission. This is contrary to Naudin's et al. (1997) study, who showed these two interleukins to be in positive correlation. Our finding could be artifact, but could also reflect complex and systematic immunological interplay. Furthermore, gender, age, smoking habits and BMI are commonly reported to affect cytokine levels. Thus, Haack et al. (1999) indicate that age induces increased activity of the

TNF- α and IL-6. Still, Maes et al. (1994) found that heightened concentrations of IL-6 were manifested only in younger patients, but not in those aged over 35. Baker et al. (1996) found higher levels of TNF- α in women in comparison to men, while Ganguli et al. (1994) had results proving to the contrary. Demirijan et al. (2006) observed that smoking habits can induce increase of TNF- α and IL-6, while Zhang et al. (2008) noticed that serum levels of cytokines were lower among smokers when compared to non-smokers. Our results are not in the line with these findings, since none of these variables were associated neither with IL-6 nor TNF- α levels across the subject groups. Our findings correspond to Schmitt et al. (2005) research, who found no correlations between age, sex and cytokine levels in SCH patients or healthy controls.

The second part of our study included analysis of a relationship between clinical features and pro-inflammatory cytokines. We found that IL-6 solely, positively correlated with the age of onset and duration of untreated psychosis, while no association was established between cytokines levels and duration of illness. Namely, it has been shown that increased concentrations of IL-6 are brought into relation with duration of illness (Akyma 1999) and unfavorable course of illness (Müller et al. 2000). On the other hand, Pae et al. (2006) did not find a correlation between baseline levels of cytokines and the duration of illness and the age of onset. Analogous data may speak in support that the differences in immune status may alter in different stages of illness. Moreover, they could be different in early onset and late onset psychosis. Narayan et al. (2008) speaks favorably of the change of the nature of SCH with its progression. Consequently, short-term illness is related to alternations in gene transcription, RNA expression, metal binding and vesicle mediated transport, while long-term illness is linked to inflammation, stimulus response and immune functional status. Naudin et al. (1997) found that plasma levels of TNF- α and IL-6 were increased in patients with chronic form of SCH (average duration of illness 14 yrs). However, further in their research, the same authors stated that the value of TNF- α did not alter in relation to the duration of illness. Furthermore, IL-6 level varied and was higher in patients who were receiving treatment for more than 15 yrs. We did not establish any association between the duration of illness and cytokine levels. Moreover, our sample consisted of relatively younger subjects (aged around 36) who have been receiving treatment for about 10 yrs, which adds to the one of the reasons for not altering immune parameters related to the duration of illness.

The third part of our study included analysis of a relationship between medication and cytokine levels. We did not observe differences in cytokine levels depending on applied antipsychotic therapy (FGA, SGA, clozapine). Surprisingly, in schizophrenic individuals on both mood stabilizers and antipsychotics,

TNF- α levels increased in remission. Namely, it is acknowledged that antipsychotics may influence cytokines levels, but results are controversial (Zhang et al. 2004, Song et al. 2000, Maes et al. 2002, Pae et al. 2006, Lu et al. 2004). Moreover, elevated serum levels of IL-6 are associated with teraporesistance, while in treatment responsive individuals they are unchanged (Lin et al. 1998). In our study there were no treatment-resistant individuals, which could be one of the explanations why there were no changes in IL-6 and TNF- α concentrations neither in acute nor in remission phase. On the other hand, little consideration has been devoted to investigation on how mood stabilizers manifest their immunomodulatory effect. It has been found that lithium reduces plasma concentrations of acute phase proteins, which are markers of inflammatory response (Maes et al. 1997, Sluzewska et al. 1997). Valproate also suppresses TNF- α production and performs inhibitory effect on nuclear factor kB (NF-kB) production *in vivo* in human glioma cells (Ichiyama et al. 2000). It was also noted that lithium leads to an increase in some of T helper-2 (Th-2) cytokines and a decrease in some T helper-1 (Th-1) cytokines (Rapaport & Manji 2001). As for our results, we can assume that they support favourable effect of augmentation by mood stabilizers (considering that total PANSS scores were reduced by around 50%) still, it could be argued about potential dual effect of TNF- α in terms of neuro-protection, considering that TNF- α features several effects such as cytotoxicity, antiviral activity, transcription factor activation and immune response regulation (Bhardway & Aggarwal 2003). Activation of tumor necrosis factor receptor (TNF-R1) could trigger dual cascade in various types of cells and lead to apoptosis on one hand and to proliferation, differentiation and survival on the other (Brietzke & Kapezinski 2008). Further investigations could elucidate whether TNF- α might be more vulnerable parameter, especially in the context of different medication.

Limitations

Our study has several limitations. Most importantly, the patients who were admitted to the ward had an acute deterioration due to a reduced compliance. Therefore, the amount of therapy they received, it could not be estimated with certainty. Considering the fact that mood stabilizers proved to be beneficial when augmented to antipsychotics in treatment of SCH, as well as for the clinical status itself (agitation, aggressive behavior), they were used despite making the sample pharmacologically more "dirty". Drug-naïve, first-time hospitalized patients, were not included in the study since they did not fulfill inclusion criteria. Some studies were conducted on drug-naïve or medication-free patients. However, this was not the case with our study. In addition to that, our study provides a realistic clinical picture in a tertiary health care center.

CONCLUSION

In current study we observed differences in pro-inflammatory cytokine serum levels in schizophrenic patients compared to healthy subjects. Clinical parameters such as age of onset and duration of untreated psychosis were related to IL-6 levels, while TNF- α serum levels correlated to combined therapy in some SCH patients in remission. Augmentation with mood stabilizers could exert potential immunomodulatory effects in SCH patients. Further prospective studies are needed to investigate deeper into biological mechanisms that lie behind these complex interconnections.

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