Psychiatria Danubina, 2012; Vol. 24, Suppl. 1, pp 153–156 *Conference paper*

INCREASED INTERLEUKIN-6 AND TUMOR NECROSIS FACTOR ALPHA IN FIRST EPISODE SCHIZOPHRENIA PATIENTS VERSUS HEALTHY CONTROLS

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SUMMARY

Background: *Inflammatory immune processes have been clearly implicated in the etiopathology of schizophrenia. There are, however, only limited data dealing with immune parameters in the first episode patients with schizophrenia and the course of these parameters during treatment.*

Subjects and Methods: *The presented study compared plasma levels of interleukin (IL)-6, IL-8, IL-10 and TNF-alpha in 25 patients with the first episode of schizophrenia with the minimal exposition of antipsychotics before and after treatment and with age and sex matched group of healthy volunteers. Changes in plasma cytokine levels were investigated after 4 weeks of treatment in relationship with the therapeutic outcome.*

Results: *Our results show significantly increased plasma levels of IL-6 ($p \leq 0.001$) and TNF-alpha ($p \leq 0.001$) in patients at the admission in comparison with healthy volunteers. After 4 weeks of the treatment the PANSS score decreased ($p \leq 0.001$), concurrently the plasma level of IL-6 decreased and TNF-alpha did not show any decrease after treatment. The patients' posttreatment and healthy control group comparison showed higher plasma levels of TNF-alpha ($p=0.008$) and marginally elevated plasma level of IL-6 ($p=0.046$) in the posttreatment group. Plasma levels of IL-8 and IL-10 did not show any significant differences.*

Conclusions: *Our study validated the presence of the proinflammatory state in the first episode of schizophrenia. IL-6 may be considered as a state marker for acute exacerbations and TNF-alpha may be a trait marker of schizophrenia.*

Key words: *schizophrenia – inflammation - interleukin (IL)-6 - TNF-alpha*

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INTRODUCTION

Immune dysfunction represents one of the most important questions in the field of the pathophysiology of schizophrenia because the immunological mechanism would mediate the relationship between genetic vulnerability and environmental factors (Horacek et al. 2011, Watanabe et al. 2010). Immunological changes in disorders with psychotic symptoms have been studied for decades with a focus on the involvement of infectious agents and a disturbed T helper type1 (Th1)/type 2 (Th2) immune response including auto-immune reactions (Hsiao et al. 2011, Wandinger et al. 2011, Watanabe et al. 2010). The cytokine system and cytokine receptors have been described in neurons and glial cells as a major system regulating the cross-talk between the central nervous system (CNS) and the immune system. Circulating cytokines are dysregulated in schizophrenic patients compared to healthy subjects (Reale et al. 2011). Several studies have proved increased plasma cytokine levels or mitogen-stimulated cytokine production in schizophrenia such as interleukin (IL)-1 β, IL-6, IL-10, IL-12, IL-18 and tumor necrosis factor (TNF) - alpha (Chang et al. 2011; Tanaka et al. 2000). A pattern of blunted Th1 immune response, which covers decreased production of IL-2 and interferon gamma (IFN-gamma) and increased Th2 response with elevation of plasma IL-4, IL-10, IL-13, has been described (Muller et al. 2000, Wilke et al. 1996).

In schizophrenia, the positive effect of antipsychotic medication on immune alteration in clinical (Chen et al. 2012) and preclinical studies (Roenker et al. 2011) and the significant therapeutic impact of antipsychotics augmentation with anti-inflammatory medication celecoxib have been described (Akhondzadeh et al. 2007, Muller et al. 2002). Findings in cytokine dysregulation differ in acute and chronic schizophrenia. A recent metaanalysis suggests that cytokines which are elevated in acute exacerbation and decrease with an antipsychotic treatment (IL-1-beta, IL-6 and TGF-beta) may be state markers for acute exacerbations. Permanently elevated cytokines (IL-12, IFN-gamma, TNF-alpha and sIL-2R) may represent trait markers (Miller et al. 2011).

Despite these promising reports the immunological findings in schizophrenia are heterogeneous and often contradictory. The most important confounding variables then comprise length of the disease, applied treatment, comorbidities and additional factors. Although the immunological findings in the first episode of schizophrenia are already described, more evidence of a therapeutic effect on changes in cytokine levels is still needed.

The aims of our study were a) to confirm the Th1/Th2 immune imbalance in the first episodes of schizophrenia, b) to establish the relationship between the intensity of psychopathology and cytokine levels, c) to analyze the influence of the therapeutic outcome on cytokine levels.

MATERIAL AND METHODS

Plasma levels of IL-6, IL-8, IL-10 and TNF-alpha were measured in 25 patients with the first episode of schizophrenia with the minimal effect of medication. Demographic data of the studied subjects are summarized in Table 1. We recruited patients from the Psychiatric Hospital Bohnice (Prague) and Prague Psychiatric Centre in the period 2/2010-1/2011 diagnosed by two experienced psychiatrists according to the ICD10 classification system (World Health Organization). We included patients with diagnosis of Acute and transient psychotic disorders. The psychopathology was assessed by the Positive and Negative Syndrome Scale (PANSS) (Kay et al. 1987), firstly during the initial psychiatric interview and secondly on discharge from hospital. We evaluated positive (P1-P7), negative (N1-N7), general (G1-G16) subscales and totals of the PANSS score separately. As a marker of depression symptoms we used the G-6 entry. The diagnosis was reevaluated after 4 weeks with the full clinical examination process (The Mini - International neuropsychiatric interview (M.I.N.I.) (Sheehan et al. 1998); laboratory tests, psychological assessment completed and only patients with a diagnosis of schizophrenia were kept in the group. During the study, 2 patients were excluded because the diagnosis of schizophrenia was not confirmed. The general exclusion criteria were: acute infectious disease, gravidity, lactation, serious endocrine disorder (e.g. diabetes, Cushing, Addison disease), alcohol or drug dependence (except nicotine dependence). The control group was made of 25 age and sex matched group healthy volunteers from the same sociodemographic background. We included men and women who had negative results in M.I.N.I., had no relatives with psychotic illnesses in the first degree and showed no pathology in blood screening tests (blood count, liver and kidney function). The investigation was carried out in accordance with the latest version of the Declaration of Helsinki, written informed consent was obtained from all subjects and the local ethics committees approved the study.

Blood samples were taken after fasting at 6-8 a.m. firstly within 48 hours after admission and then secondly from 18 of the patients after 4 weeks of treatment on discharge. Within the first period patients had taken none or only minimal acute medication. All patients were treated with atypical antipsychotics.

For screening diagnostics (blood count, liver and kidney function) the blood sample was processed in the standard way. For cytokine levels (IL-6, IL-8, IL-10, TNF- alpha) blood samples were processed immediately according to laboratory instructions (the blood clotted for 30 minutes before centrifugation for 10 minutes at 1000Xg). Plasma was immediately removed and frozen in five aliquots and stored at -80°C until analysis. The cytokine levels where quantified in the 36 specimens' collection by the Luminex method (2012) in the Laboratory of immunology and allergology of Charles University in Pilsen. To minimize the risk of possible laboratory inaccuracy the samples were examined in doublets. We used high-sensitivity kits (0.13 pg/ml).

Statistical analyses: Because the data distribution of cytokine plasma levels was not normal (Shapiro-Wilk test), we used non-parametrical statistical tests. To compare groups of patients with healthy controls we used the Mann-Whitney U test, to evaluate the differences between the pre-treatment and post-treatment measurements in cytokines levels we used Wilcoxon matched pairs test. Change in PANSS scores was tested by the paired t-test. To remediate the problem of multiple comparisons we used Bonferroni correction for 12 comparisons. To correlate cytokine levels and PANSS subscores we used the Spearman correlation. All results were assessed at the level p=0.05.

RESULTS

Table 1 shows the descriptive statistics of pretreatment (P1) and post-treatment (P2) subgroups of patients and healthy controls (HC). There were no significant differences in age as well as in the male/female ratio or between the patient (P) and healthy control (HC) groups, thus we assessed males and females together.

Table 1. Basic demographic characteristics for patients' subgroups and healthy control group, PANSS scores for patients' subgroups

Parameter	P1 (N=25)	P2 (N=25)	HC (N=25)	Decrease P1-P2 (%)	p level P1-HC	p level P1-P2
Age	32.32±7.01*	32.32±7.01*	31.12±1.05*		0.5	
Male/Female	16/9	16/9	16/9		1.00	
PANSS P.	24.00±8.49*	9.81±2.94*		14.91±7.40*		<0.0001
PANSS N	19.00±7.75*	11.62±4.36*		7.38±7.07*		0.0001
PANSS T	86.33±22.51*	43.62±9.53*		42.71±19.14*		<0.0001
PANSS G-6	1.95±1.39*	1.43±0.81*		0.52±1.44*		0.11

P1=Patients pretreatment subgroup, P2=Patients posttreatment subgroup, HC=Healthy Control group. PANSS - Positive and Negative Syndrome Scale, P - positive subscale, N - negative subscale, T – total score. Statistically significant results are written in bold text.; *mean ±s.d.

Table 2. Plasma levels of cytokines for patients´ subgroups and healthy control group, p – levels before Bonferroni correction

Cytokine	P1 (N=25) 1 (pg/ml)	P2 (N=25) 1 (pg/ml)	HC (N=25) 1 (pg/ml)	p level P1-HC	p level P1-P2	p level P2-HC
IL-6	3.17 (1.35-11.94)	1.97 (1.26-3.01)	0.82 (0.57-1.99)	0.0004	0.0028	0.0038
IL-8	4.74 (3.37-6.06)	4.28 (2.55-5.41)	3.63 (3.03-6.27)	0.62	0.09	0.6415
IL-10	8.86 (5.08-17.99)	9.50 (5.00-14.91)	6.26 (4.72-10.15)	0.0715	0.3195	0.1653
TNF-alpha	4.98 (4.16-7.00)	5.64 (4.17-7.25)	2.81 (2.47-5.09)	0.0013	0.5905	0.0007

P1=Patients pretreatment subgroup, P2=Patients posttreatment subgroup, HC=Healthy Control group
1Data are presented as a median and (interquartile range). Results that are statistically significant after Bonferroni correction for 12 comparisons are written in bold text.

The patients' pretreatment and healthy control group comparison after Bonferroni correction showed significantly higher plasma levels IL-6 (p=0.0048), TNF-alpha (p=0.0156) in the P1 group. The patients' pretreatment and posttreatment comparison revealed a decrease after acute treatment in plasma levels of IL-6 (p=0.0336). There was no decrease in TNF-alpha. The patients' posttreatment and healthy control group comparison showed higher plasma levels of TNF-alpha (p=0.0084) and marginally elevated plasma level of IL-6 (p=0.0456) in posttreatment group. Plasma levels of IL-8 and IL-10 did not show any significant differences (Table 2).

The posttreatment patients' subgroup showed a decrease in the total PANSS score as well as in all three subscales scores (positive, negative symptoms and general psychopathology) ($p \leq 0.001$). We found no significant correlation between PANSS subscales for positive and negative subscales and the total PANSS score or between the differences in psychopathology and differences in cytokine levels before and after treatment.

DISCUSSION

The main finding of our study is the increased plasma levels of IL-6 and TNF-alpha in schizophrenia sample. Elevation of these cytokines is congruent with the well-described pro-inflammatory state in schizophrenia (Miller et al. 2011, Potvin et al. 2008). Increased IL-6 and TNF-alpha are both pro-inflammatory mediators produced predominantly by macrophages. Increased plasma levels of IL-6 associated with schizophrenia are confirmed by several meta-analyses (Miller et al. 2011, Potvin et al. 2008, Watanabe et al. 2010). In the central nervous system, IL-6 stimulates the release of acetylcholine, serotonin and corticotropin releasing hormone (CRH). IL-6 has been found to regulate brain development, synaptic plasticity, and various behaviours related to feeding, sleep and stress (Bauer et al. 2007). The IL-6 elevation is also described in mood disorders and it is possible that it deals with the affective psychopathology in psychotic disorders. In our data there was no correlation between IL-6 plasma levels and depressive symptoms. A significant increase in IL-6 production is also seen in acute stress (Segerstrom et al. 2004). The change in IL-6 plasma level could reflect the unspecific factors related to the fact the onset of schizophrenia is often preceded by the stress triggers (social stress, travelling, infection etc.).

TNF-alpha is a ubiquitous pro-inflammatory cytokine elevated in Th1 and Th17 immune response. TNF-alpha might contribute to the pathogenesis of schizophrenia by activation of the hypothalamo-pituitary-adrenocortical (HPA) axis, activation of neuronal serotonin transporters, stimulation of the indoleamine 2,3-dioxygenase which leads to tryptophan depletion and activation of kynurenine metabolites, or by neurotoxic release of glutamate (Himmerich et al. 2009).

The antipsychotic treatment had a clear influence on the decrease of PANSS scores, which was most apparent in the positive subscale. After 4 weeks of treatment there was a decrease of IL-6. Meanwhile, the plasma level of TNF-alpha did not change. In comparison with the healthy control group, TNF-alpha remained significantly higher whereas IL-6 showed marginal difference at discharge. Our findings are fully congruent with the recent metaanalysis which documented that IL-6 is a possible state marker for acute exacerbations and TNF-alpha may be a trait marker of acute psychotic condition (Miller et al. 2011).

The alternative explanation for the elevation of IL-6 and TNF-alpha is the effect of environmental factors such as latent toxoplasmosis. This infection is 2-3 times more prevalent in schizophrenia and pro-inflammatory cytokines would represent the causal mediators of this risk factor (Beaman et al. 1994, Horacek et al. 2011).

A limitation of the study is the size of the group. A larger group would make it possible to analyze more characteristics of the group.

CONCLUSIONS

Our results confirm the pro-inflammatory state in the first episode of schizophrenia and document the decrease of IL-6 plasma level after acute treatment. TNF-alpha did not show any change. IL-6 may be considered as a state marker for acute exacerbations and TNF-alpha may be a trait marker of schizophrenia.

Acknowledgements

This work was supported by grant NT/13843 from the Ministry of Education, Youth and Sports of the Czech Republic.

Conflict of interest: None to declare.

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