The Serum Level of Tumor Necrosis Factor Alpha and Interleukin-6 in Bipolar Affective Disorder and Schizophrenia *(study in Egyptian sample)*

Hatata H[1] and Attlah MF[2]

[1]Department of Psychiatry, Ain Shams University, Cairo, Egypt.
[2]Department of Microbiology & Immunology, Ain Shams University, Cairo, Egypt.

ABSTRACT

Background: A large body of evidence suggests that immune system dysregulation is associated with bipolar affective disorder (BAD) and schizophrenia**. Objective:** This study is to characterize the immunological variations of patients with BAD in manic phase and patients diagnosed with schizophrenia, by the quantification of the serum levels of tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6). **Subjects and methods:** Thirty four patients with BAD type I diagnosis and 36 physically healthy patients with diagnosis of schizophrenia and 18 matched controls were studied. The inclusion criteria included at least 12 weeks without any kind of psychopharmacological treatment. Exclusion criteria included any infectious diseases, allergies or any other kind of medical illness that required treatment with immunosuppressors, as well as any other diagnosis in axis I. Physical and laboratory examinations was performed to rule out any clinical illness. Enzyme-linked immunosorbent assay (ELISA) was used to analyze the serum cytokines concentration. **Results:** BAD patients showed statistical significant increase in both IL-6 and TNF-α value when compared to controls. On the other hand schizophrenic patients showed statistical significant increase in the serum levels of TNF-α when compared to controls. **Conclusion:** The comparison between both groups suggests that there is a distinctive cytokine pattern for specific disorders. Our results show the existence of alteration in the serum levels of cytokines in both schizophrenia and BAD and this noted activation of the immune system has been demonstrated in both disorders.

Key words: Tumor necrosis factor alpha, Interleukin-6, Bipolar affective disorder, Schizophrenia, Egypt.

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INTRODUCTION

Traditionally, the brain has been considered as an area without contact with immune mediators, protected by the blood brain barrier (BBB)[1]. Current information shows that brain tissue is able to engender immune processes and be influenced by them. The elucidation of the immunology of the central nervous system (CNS) might offer

new insights regarding causes of a number of neuropsychiatric disorders, like Alzheimer's disease, schizophrenia, and mood disorders[1-2]. In the field of mood disorders, the immune system seems to play a particularly important role.

Cytokines are important mediators of the cross-talk between CNS and the immune system, which might have implications for clinical psychiatry[2]. The cytokine production profile of CD4 T helper (Th) lymphocytes allowed the identification of at least two distinct subsets: Th1 and Th2. The Th1 cells produce cells mainly produce interferon gamma (IFN-γ), interleukin-2 (IL-2), tumor necrosis factor-alpha (TNF-α) and interleukin-12 (IL-12), a potent activator of cell-mediated immunity and Th2 cells produce lymphocytes predominantly release interleukin-4 (IL-4), interleukin-13 (IL-13), interleukin-10 (IL-10) and interleukin-6 (IL-6); potent inducers of B-cell immunoglobulin (Ig) type switching to IgE and activators of eosinophil recruitment[3].

Cytokines are proteins or glucoproteins secreted by immune cells in response to noxious stimulus. Acting together, either locally or systemically in the immune response, they function like signals among immune cells. It is now known that cytokines can be secreted not only by immune cells. Cytokines are frequently regulated in cascades, where the induction of early cytokines serves to increase the production of later cytokines[2]. Cytokines interact with the neuroendocrine system, e.g., the hypothalamic-pituitary-adrenocortical (HPA) system, the autonomic system and the neurotransmitter system (dopamine, serotonin and glutamate). Cytokines have high molecular weight but they may cross the BBB either through leaky areas or by active transport[4]. In the brain, they act in specific pathways involved in mood, energy, and activity control[5].

TNF-α is a 157 amino acid cytokine and is produced in response to injury and inflammatory or infectious stimuli by macrophages, lymphocytes, neutrophils, and structural cells, including fibroblast, smooth muscle cells[6], astrocytes and microglia. It is considered a proinflammatory molecule, augmenting the immune response to help speed-up the elimination of pathogens and resolution of the inflammatory challenge[2]. TNF-α has several effects, including cytotoxicity, antiviral activity, transcription factor activation, and immune response regulation TNF-α is a cytokine that acts synergistically with several other cytokines and in the CNS. Pimarily astrocytes release. TNF-α, but also microglia can release TNF-α after stimulation IL-6 which subsequently stimulate neurons in vitro to secrete dopamine and probably other catecholamines as well[7].

IL-6, a 184 amino acid cytokine, is a multifunctional cytokine produced by lymphocytes, monocytes, microglia, endothelial cells, astrocytes and cortical neurons[8-9]. A significant regulatory role for IL-6 was hypothesized based on their findings that this molecule controls inflammatory responses both at the systemic and local levels in an attempt to re-establish homeostatic conditions[10]. In addition, IL-6 interacts with the HPA axis of the CNS where glucocorticoids have

been shown to suppress the production of IL-6[11-12].

Since the earliest reports on change in cytokines in psychiatric disorders, there has been circumstantial evidence to suggest an association between dysregulation of cytokines in bipolar disorder[13-14] schizophrenia, major depression[15-17].

The role of cytokines in mood disorders was first considered when the cytokine interferon resulted in "sickness behaviour", the symptoms of which are similar to those of major depression. The latter is associated with an increase in pro-inflammatory cytokines such as IL-1, IL-6 and tumor TNF-alpha. These cytokines are potent modulators of corticotrophin-releasing hormone (CRH) which produces heightened HPA activity characterized by increases in ACTH and cortisol, both of which are reported to be elevated in major depression. Antidepressant treatment has immunomodulatory effects with increases in the production of IL-10, which is an anti-inflammatory cytokine[14].

There are some studies describing a potential role of cytokines in the pathogenesis of mood disorder regarding depression. Patients with depression that are otherwise healthy seem to have activated inflammatory pathways, with increased proinflammatory cytokines, acute-phase proteins, and increased expression of chemokines and adhesion molecules. In addition, specific depression symptoms could be associated with increased cytokines levels, like suicidal behavior[18-19].

In the field of BAD the findings are still limited, but there is some data affirming that mania also could be associated with a proinflammatory state. Some studies reported increased proinflammatory cytokines and hyperactivity of Th-1 in bipolar disorder, with significantly higher TNF-α levels in bipolar patients during manic and depressive episodes than normal controls[20]. Kim et al. reported increased production of IL-6 and TNF-α during mania when compared with controls[4].

To date, the most frequently studied cytokines in schizophrenia have been IL-2 and IL-6. Although there is evidence of decreased IL-2 levels in schizophrenia[21-22,] another study did not confirm this trend[23], whereas different study showed the reverse relationship[24]. As for the predicted increase in IL-6 levels in schizophrenia, results reported so far have been more consistent, but not all groups have replicated this observation[15, 17, 25]. As for other cytokines, the lack of consistency of results has been noticed by many authors[2, 26-27].

Our study tries to highlight the immunological alteration in a sample of Egyptian patients suffering from BAD and Schizophrenia by evaluating the levels of TNF-α and IL-6 in both disorders by immune parameters.

SUBJECTS AND METHODS

Subjects:

Among psychiatric patients newly admitted to the institute of psychiatry, Ain Shams University from January 2008 to June 2008, we recruited 36 subjects with schizophrenia (28 males and 8 females) and 34 subjects with bipolar mania (22 males and 12 females). All subjects had active symptoms

at the time of study enrollment who met DSM-IV criteria (American Psychiatric Association, 1994). They were either medication-naive (first onset) or had been free of medication for at least 4 months. 18 healthy age and sex matched Egyptians controls (12 males and 6 females) were recruited from hospital staff.

Each subject was given a diagnostic assessment based on clinical interviews using the Structured Clinical Interview for DSM-IV (SCID-I)[28]. Subjects with any personal or familial history of autoimmune disease, or substance or alcohol abuse were excluded. All subjects were free of chronic and acute physical illness (such as infectious or allergic diseases) associated with abnormal cell-mediated immunity within the 12 weeks before the study. They showed normal laboratory findings in blood chemistry, renal function, thyroid function, and liver function, chest X-ray of the lungs and heart and ECG. All subjects gave oral informed consent to participate in the study after the procedure had been fully explained.

Methods:

All assays for both patients and controls were carried out in duplicate in the neuropsychiatry laboratory of the institute of psychiatry, Ain Shams University by the same operator.

We collected 6ml venous blood in vaccutainers for both patients groups and control group between 9 am and 11 am, the blood was allowed to clot at room temperature, within 2 hours the serum was obtained by centrifugation at 1500xg for 6 minutes, then serum was kept frozen liquated after separation at -20°C until assayed.

All samples of patients and control were assayed by sandwich ELISA technique (Enzyme linked immunosorbent Assay) commercial kits (Ray Bio Human TNF-α Kit and Ray Bio Human IL-6 Kit) for in Vitro Enzyme linked immunosorbent assay for the quantitative measurement of Human TNF-α and IL-6 in serum. This assay is based on an antibody specific for human TNF-α and IL-6 coated on 96 well plates. Standards and samples were pipetting into the wells so that TNF-α and IL-6 present in samples is bound to the wells by the immobilized antibody. The wells were then washed and biotinylated anti human TNF-α and IL-6 was added. After washing away the unbound biotinylated antibody, HRP conjugated streptavidin is pipette to the wells. The wells were washed again and TMB substrate solution is added to the wells and colour develops in proportion to the concentration of TNF-α and IL-6 bound. The stop solution was added to change the colour from blue to yellow and colour intensity was measured at 450 nm.

The assays were carried out at the same time and in the same run for both TNF-α and IL-6 separately using automated microplate washer DAS and read on DAS analyzer 90 photometer for measuring absorbance (OD) at 450nm. According to the sensitivity of the kit, the minimum detectable colour of TNF-α is typically less than 15pg/ml and of Il-6 was less than 6pg/ml.

Mean absorbance for each set of duplicate standards was calculated. A standard curve OD was plot on Log- Log paper with

standard concentration on the x-axis and absorbance on the Y-axis. The best fit line was drawn through the standard points. Using these curves, the concentrations of both patients groups and control group were calculated.

Statistical analysis:

The data were analyzed statistically using the software statistical package for social science (SPSS) version 14 (SPSS Corp., Chicago, Illinois, USA). The data obtained were expressed as descriptive statistics (mean±standard deviation). Statistics included two tailed unpaired T- test for comparison between two groups and Pearson correlation for correlating categorical parameters. P>0.05 was considered non significant, P<0.05 was considered significant and P<0.001 was considered highly significant. Also A one-way analysis of variance (ANOVA) was performed to compare the different subtypes regarding the same factor.

RESULTS

The present study was carried on 88 subjects; 34 subjects with bipolar mania (22 males and 12 females) with mean age 34.2±10 and 36 subjects with schizophrenia (28 males and 8 females) with mean age 31.4±10 and 18 controls (12 males and 6 females) with mean age 31±5. Table (1) shows the duration of illness of both BAD and Schizophrenia.

By using the modified T test for comparison of the means for TNF-α in both BAD and control groups, there was a statistical significant change in the concentration of TNF-α between the two groups with a p=.045 (table 2).

Table 1: Duration of illness

	N	Mini	Max	Mean	Std. Deviation
BAD	34	1.0	27	10	7,0
Schizo-phrenia	36	1.0	12	5.2	3.4

Table 2: Comparison between BAD and control regarding TNF-α concentration

	Mean	Std. Deviation	T	P- Value
TNF-α BAD	50.7727	85.71937	2.032	.045
-				
TNF-α Control	25.7955	57.44506		

Table (3) shows comparison of the means for IL6 in both BAD and control groups using the modified T test. There was a statistical significant change in the concentration of IL6 between the two groups with a p=.039.

Table 3: Comparison between BAD and controls regarding IL-6 concentration

	Mean	Std. Deviation	T	P-Value
IL6 BAD	12.159	30.613	2.093	.039
-				
IL6 Control	4.44341	12.326		

By using the modified T test for comparison of the means for IL6 in both Schizophrenic and control groups, there was no statistical significant change in the concentration of IL6 between the two groups with a p=0.199 (table 4). Also comparison of the means for TNF-α in both Schizophrenic and control groups showed

statistical significant change in the concentration of TNF-α between the two groups with a p=.014 (table 5).

Table 4: Comparison of IL-6 concentration in Schizophrenic and Controls

	Mean	SD	T	P-
IL6 Schizo	7.6023	17.544	1.293	.199
IL6 Control	4.44341	12.326		

Table 5: Comparison of TNF-α concentration in Schizophrenic and Controls

	Mean	SD	T	P
TNF-α Schiz	75.113	164.769	2.496	.014
TNF-α Control	25.795	57.445		

Table 6: Correlations between Duration of illness and TNF–α, IL-6 in BAD and schizophrenic.

	Duration of Illness	
	r value	p value
TNF–α in BAD	-0.224	0.202
IL-6 in BAD	-0.100	0.525
TNF–α in Schizo	-.130	0.450

By using Pearson's correlation coefficient, the relation between the duration of illness and the TNF-α, IL-6 in schizophrenic and BAD groups was found to be statistically non significant (table 6). Also the relation between the Age and the TNF-α, IL-6 in schizophrenic and BAD groups was found to be statistically non significant (table 7).

By using the one way ANOVA there is no statistical significant difference in the concentration of TNF-α regarding the subtypes of schizophrenia (table 8).

Table 7: Correlations between Age and TNF–α, IL-6 in BAD and schizophrenic.

	Age	
	r value	p value
TNF–α in BAD	-0.246	0.161
IL-6 in BAD	-0.060	0.737
TNF–α in Schizo	-.188	0.273

Table 8: Comparison of TNF-α concentration in different subtypes of schizophrenia

Subtypes of Schizophrenia	N	Mean	SD	P-value
Paranoid	14	174.2857	99.05044	0.952
Hebephrenic	10	177.0000	192.79926	
Undifferentiated	12	200.0000	327.22192	
Total	36	183.6111	216.78037	

DISCUSSION

Among etiological explanations of mood disorders and schizophrenia, several hypotheses concerning immune-related disorders such as infections and autoimmune inflammatory diseases have been proposed[29-30].

Bipolar affective disorder (BAD) is a mental disorder characterized by a phasic course of affective episodes. It produces great suffering to the patients and its prevalence and suicide risk render prophylaxis from it[31].

The principal findings of this study on both serum IL-6 and TNF-α are statistically significantly elevated in bipolar mania.

The result indicates both Th1 and Th2 hyperactivity. This finding is in agreement with those of other studies that demonstrated immunological hyperactivity in bipolar disorder[32-35].

Although the researches on the assessment of the inflammatory response system in

bipolar mania are limited and remain controversial, nevertheless cell-mediated immunity activation in bipolar mania has been suggested.

Further evidence of activation of cell-mediated immunity has been proposed by a number of studies in mania. Maes et al., found that mania was characterized by higher plasma levels of IL-6, soluble interleukin-6-receptor (sIL-6R), soluble interleukin-2-receptor (sIL-2R) and Transferrin receptor (TfR) (32). Kim et al. found that plasma IFN-g and IL-4 were significantly higher in BAD as compared to healthy subjects[36].

In our study we found non-significant correlation between both the duration of illness, age of subjects and elevated IL-6 and TNF-α concentration and in our knowledge this is the first study to correlate duration of illness to these cytokines in biploar mania.

Among manic patients, IL-6 levels returned to the baseline after 6 weeks of treatment with mood stabilizers, but TNF-α level continued to be high. Accordingly, the authors considered that IL-6 is a manic state marker, but TNF-α could be an enduring change[36]. Another recent study, described that both mania and bipolar depression are associated with increased production of proinflammatory cytokines IL-6, IL-8 and TNF-α, even with the use of mood stabilizers or antipsychotic medication. No difference was observed in the concentration of anti-inflammatory cytokine IL-10 or in cortisol concentrations between manic subjects and controls[20].

Although lithium has been widely used during the last decades and has been proven to be the most effective medicine for this disorder, little is known about its mechanism of action, while its immunomodulatory effects have been found to be conflicting. Additionally, aspects of immune functioning in BAD have been studied not as thoroughly in comparison to other major psychoses as schizophrenia[27] and depression[37-38]. The missing knowledge both in BAD and lithium research areas could be of particular interest, as many studies indicate immune system interference[39].

Regarding Schizophrenic patient group**,** our study shows statistical significant elevation of TNF-α concentration when comparing between schizophrenic group and control group and this is in agreement with the results that concluded patients with an acute exacerbation of schizophrenia had significantly increased production of TNF-α also in their work, they found significant reduction of IL-4 as compared with healthy subjects[40].

No statistical significant difference was observed in serum IL-6 and this is similar with the results of Sinead et al.[40] and differs from the results that have elevation of IL-6 in their Patients[41-42].

Below we explore some of the possible reasons for these discrepancies. Another group found a subgroup of schizophrenic patients with elevated serum IL-6; these patients also had elevated IL-2 production[43]. In addition a group of 128 schizophrenic patients had higher serum IL-6 concentrations than 110 controls[41]. In the latter study, IL-6 was higher in African-American than the Caucasian patients; a

relationship which was not present in our study.

Duration and severity of illness have also been factors in finding IL-6 differences between groups. Also schizophrenic patients in remission had elevated serum IL-6 concentrations[25] and patients who were not comparatively ill had elevated IL-6[44]. Studying a group of patients with a wide duration of illness revealed that those patients who had been ill for the longest time had the highest IL-6 concentrations[41]. Although in our study we found non-significant correlation between the duration of Schizophrenia and the TNF-α concentration.

We compare different subtypes of schizophrenia who had elevation of TNF-α no statistical significance difference was found and to our knowledge this the first study to compare subtypes of schizophrenia regarding elevated TNF-α concentration.

Many studies suggest that serum IL-6 levels are state dependent since acutely ill patients had higher levels than patients in remission[45]. IL-6 levels were also found to be associated with the duration of illness[41, 46] and age[32] in schizophrenic patients. To our knowledge, only two studies have examined CSF levels of IL-6 in schizophrenic patients. Barak et al. found no difference in IL-6 levels between 16 patients and 10 controls using radioactively labeled antibodies whereas other studies were unable to detect IL-6 in the plasma or CSF of 14 patients or nine controls using ELISA[47-48]. This result could further suggest that the immune abnormalities may occur only in a subgroup of patients with schizophrenia.

IL-6 can stimulate neurons in vitro to secrete dopamine and probably other catecholamines as well[49]. The peripheral application of IL-6 in animal experiments enhanced the dopaminergic and serotonergic turnover in the hippocampus and frontal cortex, without affecting noradrenaline[50]. Conversely, noradrenaline can stimulate astrocytes to release IL-6[51]. TNF-α also seems to influence the neurotransmitter balance, probably acting differently in various stages of the disturbance. While the acute release of TNF-α has a stimulatory effect on the catecholaminergic system, chronic release seems to inactivate the catecholamine secretion[52]. This mechanism shows parallels to the pathophysiological mechanisms discussed for schizophrenia, especially since a dopamine hypersecretion has been suggested for acute positive symptoms of schizophrenia and a lack of dopamine release for negative symptoms of schizophrenia[53].

With the introduction of immunological findings in schizophrenia and mood disorder, it is becoming clear that the etiopathology of schizophrenia is related to a range of interacting variables. These include genetic, infectious and immune factors which all function as major predisposing and triggering factors. These multiple factors, like pieces of a mosaic (genetic, infectious and immune), may interplay in different forms, leading to the expression of varying combinations leading to diverse phenotypic expression of diseases[54].

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Addressee of Correspondence:

Hatata H. *Lecturer of Psychiatry, Department of Psychiatry, Ain Shams University, Cairo, Egypt.*

e-mail:*drhatata@yahoo.com*