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A N I N T E R N A T I O N A L J O U R N A L

Higher than Normal Plasma Iinterleukin-6 Concentrations in Brazilian Patients with Mood Disorders

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ABSTRACT

The aim of this work was to study the plasma concentration of IL-6 by ELISA in the patients with mood disorders and normal healthy donors. The plasma concentration of IL-6 was higher in the patients than in the control healthy group. Results suggested that IL-6 could serve as an immunological marker in mood disorder pathogenesis as well.

Key words: IL-6, cytokines, mood disorder

INTRODUCTION

Numerous interactions of the immune system with the central nervous system (CNS) have been described over the the past few years.Therefore, a wide heterogeneity of results can be observed in immunological studies of severe depression (Muller and Ackenheil, 1998). Cytokines play an important role in the different phases of the immune response. The continuous advances in the knowledge of the pathophysioloy of chronic disorders, combined with the progress of radiochemistry, have led to the development of new specific radiolabelled agents for the imaging of chronic diseases (Signore et al, 2002).

The finding that depression often co-exists with some subclinical autoimmune diseases, such as thyroiditis and lupus, suggests that depression may cause alterations in the immune system, or that in fact it is an autoimmune disorder itself. It is known that acute stress may initiate a transient, protective immunological response. Therefore, prolonged or poorly controlled psychosocial stressors may result in changes in different components of the immune system, mainly cytokines, such as INFalfa, INF gamma, IL-1 and IL-6 (Fountoulakis et al., 2004). The IL-6 has been described over the past few years as an important factor involved in a wide range of psychiatric disorders.

Interleukin 6 (IL-6) is a pleiotropic cytokine, which is released from different blood cell types. One function of IL-6 is to activate B cells to synthesize the antibodies (Plata-Salaman, 1991). However, like other cytokines, IL-6 is not only synthesized and released by the immune cells of the peripheral blood, but also produced by the activated astrocytes and microglia cells in the central nervous system (CNS). Several findings

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suggest that IL-6 may mediate the exacerbation of autoimmune disorders in the CNS (Dunn, 1992).
The IL-6 can be involved in the differentiation of B lymphocytes, local IgG synthesis in the CNS, and blood-brain barrier disturbance (Frei et all., 1989). In the hypothalamus, IL-6 can induce the release of growth hormone releasing hormone and TSH, and it stimulates *in vitro* the secretion of prolactin and growth hormone from the pituitary cells (Spangelo et al., 1989).
A strong relationship between the IL-6 and neurotransmitter production has been reported in various studies. IL-6 can stimulate neurons *in vitro* to secrete dopamine and probably other catecholamines (Hama et al., 1991). The peripheral application of IL-6 in the animal experiments was shown to enhance the dopaminergic and serotoninergic turnover in the hippocampus and frontal cortex, without affecting noradrenaline (Zalcman et al., 1994). Conversely, noradrenaline can stimulate astrocytes to release IL-6 (Dunn, 1992). Both observations point to a direct influence of activating cytokines, especially IL-6, on the catecholaminergic neurotransmitter system. The present work aimed to study the effect of the concentration of the IL-6 in mood disorder patients and normal healthy donors.

MATERIALS AND METHODS

Subjects

The subjects included 30 patients with mood disorders, of both the genders, ranging in age from 18 to 68 years and 30 healthy volunteers as controls, also of both the genders ranging in age from 18 to 55 years. The Human Ethics Committee of Universidade Estadual de Londrina approved the present work (CEP 120/01, Resolução 196/96-CNS), and the subjects signed a written informed consent to be included in the present study. Peripheral blood samples were obtained from the patients and control subjects. The patients and control subjects were seen at the Ambulatory Service of Psychiatry, Hospital de Clínicas, Universidade Estadual de Londrina, Londrina, PR, Brazil, during the period of 2001 to 2003. All the subjects, patients and controls were submitted to clinical evaluation according to the Structured Clinical Interview for DSM-IV axis I disorder – clinic version SCID-CV, translated into Portuguese (Del-Ben et al., 2001).

Blood sampling and measurement of plasma levels of IL-6

Venous blood was collected and plasma was obtained by centrifugation at 2000 x g for 10 min and kept frozen at - 20°C until assayed. The IL-6 levels were measured by using the human interleukin-6 (IL-6) ELISA system, RPN 274 (Amersham Pharmacia, Biotech, UK) according to the manufacturer's instructions. All the assays were carried out at the same time and in one run. The plate was read in a microplate spectrophotometer (Reader Sanofi Diagnostic Pasteur PR 2100, USA) at 490nm.

Statistical analysis

Plasma IL-6 levels in the patients and normal healthy donors were compared by Student's t-test using Micronal Origin 4.1 Statistic Program, with the level of significance set at $p<0.05$.

RESULTS AND DISCUSSION

This work compared plasma IL-6 levels of the control group (normal healthy subjects) with patients with depression. The mean plasma IL-6 level of depressive subjects (1.75 pg/ml) was significantly higher than in the normal donor subjects (0.2 pg/ml; $p<0.05$) (Figure 1).
It has been shown that IL-6 can be higher in the depression patients when compared to the healthy subjects (Frommenberger et al., 1997; Lin et al., 1998; Schlatter et al. 2004). The present results were, therefore, compatible with the previous findings. Table 1 shows the concentration of IL-6 reported by many authors.

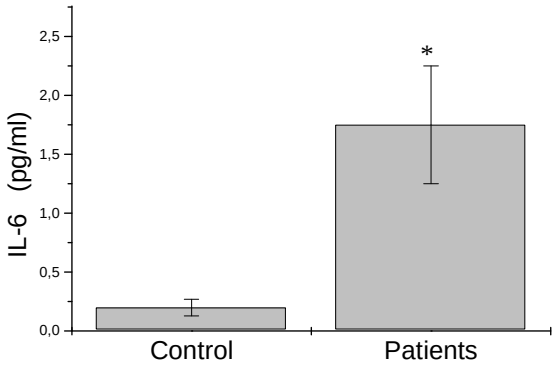

Figure 1 - Quantification of plasma IL-6 in patients and healthy donors. The cytokine was quantified using the *human interleukin-6 (IL-6)* ELISA *system*, RPN 2754 (*Amersham Pharmacia, Biotech* UK). * The plasma concentration of IL-6 was significantly different higher in patients than in the control group ($p<0.05$). (*Origin 4.1)*

Table 1 - Comparison of concentration of IL-6 in different types of disorders.

Year	Author	IL-6	Situation or Approach
1995	Maes et al.	Lower	Anti-psychotic treatment
1997	Frommberger et al.	Higher	Depression and schizophrenic patients
1998	Lin et al.	Higher	Schizophrenic patients
2001	Musselman et al.	Higher	Major depression in cancer patients
2002	Ushiroyama, et al.	Higher	Menopausal women with and without depression
2004	Schlatter et al.	Higher	Major depression
2004	Kubera et al.	Higher	Antidepressant treatment
2009	Gabbaya et al.	Higher	Major depression
2010	Forti et al.	Higher	Major depression
2010	Podlipný et al.	Lower	Self-reported depression
2010	O'Donovan et al.	Higher	Anxious individuals

Controversy remains over the influence of psychotic drugs, where many investigations have reported a decrease in the plasma IL-6 levels as a result of anti-psychotic treatment (for example, Maes et al., 1995). However, Kubera et al. (2004) found increased plasma levels of IL-6 in the patients undergoing antidepressant treatment (imipramine, venlafaxine, 5-HTP and fluoxetine). The results showed that even the patients treated with different types of drugs had an increase in the plasma IL-6.

A large body of evidence suggests that immune system dysregulation is associated with major depressive disorder in adults. Findings from Gabbaya et al (2009) suggest that immune system dysregulation may be associated with the adolescent major depressive disorder, with an imbalance of Th1/Th2 shifted toward Th1, as documented in adult major depressive disorder. There was a trend towards the increased IL-6 plasma levels in the adolescents with major depressive disorder compared to controls. Forti et al (2010) found that the baseline IL-6 and ACT (1–antichymotrypsin) were increased in prevalent major depression and relevant depressive symptoms.

Some findings suggest that serum IL-6 levels may be especially high in the patients with an unfavorable course of disease (Lin et al., 1998; Frommberger et al., 1997). The increased plasma levels of IL-6 in those psychiatric patients seemed to be related to the clinical course, especially changes to psychotic symptoms such as delusions or disorganized thinking.

It is also believed (although not well documented) that the production of proinflammatory cytokines (such as IL-1, IL-6, INFalfa, INFgamma), whether in the context of therapeutic administration or medical illness, could induce the symptoms that closely resemble major depression (Raison et al., 2002). These observations allowed to infer that the increased production of proinflammatory

cytokines might play a crucial role in the immune and acute phase response in the depression (Fountoulakis, et al. 2004).

Higher than normal plasma IL-6 concentrations were associated with a diagnosis of major depression in cancer patients. IL-6 may contribute to symptoms that overlap with those seen in major depression (Musselman et al., 2001).

The association between the depressed mood and serum level of IL-6 has already been described as significantly stronger in men than in women (Penninx et al., 2003). Ushiroyama, et al. (2002) observed elevated plasma interleukin-6 (IL-6) and soluble IL-6 receptor concentrations in menopausal women with and without depression.

A typical finding, which has been replicated several times and supports the inflammatory hypothesis of depression, is the elevation of blood pro-inflammatory cytokines such as interleukin (IL)-1, IL-6 and tumor necrosis factor-α (TNF-α) in the patients suffering from depression. Study from Podlipný et al (2010) compared the levels of interleukin (IL)-6, IL-8, IL-10 and tumor necrosis factor-α (TNF-α) in the population samples characterized by a high or low level of self-reported depression. The authors found that the IL-6 serum level in the people with symptoms of depression was lower. However, the difference was on the border of statistical significance. Depressive symptoms, poor sleep quality, and systemic markers of inflammation (IL-6) are frequently associated (Prather et al., 2009)

It is known that the influence of neuroleptic medications cannot be ignored because the doses of neuroleptics are not fixed after the admission. There are several observations showing that the antipsychotic therapy with neuroleptics is accompanied by a functional decrease in the IL-6 system. Wichers and Maes (2002) reported on the psychoneuroimmuno-pathophysiology of cytokine-induced depression in humans. A significant decrease of IL-6 during therapy with neuroleptics was described by Maes and coworkers (1995). On the other hand, Kubera et al. (2004) reported a stimulatory effect of antidepressants on the production of IL-6 in a study involving the patients with treatment-resistant depression.

Previous studies have suggested that the regulation of the proinflammatory cytokine interleukin (IL)-6 is abnormal in the patients with major depression. Carpenter and collaborators (2004) conducted a study to determine whether IL-6 concentrations in cerebrospinal fluid (CSF) differed between the depressed patients and healthy control subjects. Their findings failed to support the notion that immune activation could be causally involved in the pathogenesis of depression.

Negative emotions such as anxiety and depression confer increased risk for the disorders with an inflammatory etiology, and elevated inflammatory activity may be an important mediator of emotion–disease relationships. Data from O'Donovan et al (2010) indicate that clinically anxious individuals have lower morning cortisol and elevated IL-6 compared with non-anxious individuals, highlighting a potential pathway by which anxiety may increase the risk for inflammatory diseases. This suggests that the anxiety may be associated with inflammatory activity independent of depression and neuroticism, thus, indicating specificity in relationships between the negative emotions and biological responses.

Evidence has been presented that examined the cytokine link to depression. There may be no common molecular mechanism underlying all the depression. Variables such as stress, cytokine immune response, and medical illness probably have bidirectional causality. Proinflammatory cytokines interfere with the body's feedback loop to reduce the circulating corticosteroids during the stress response. Patients with depression have been found to have an activated inflammatory response (Wilson et al., 2008).

Selective serotonin reuptake inhibitors and serotonin noradrenaline reuptake inhibitors are generally considered first-line treatments for the depression. However, it has been reported by Yoshimura et al (2009) that 30–40% of all depressive patients who receive a sufficient dose and duration of antidepressant treatment fail to respond. The plasma levels of IL-6 and TNF-α were significantly higher in the depressed patients than in the healthy controls. These results could suggest that higher plasma IL-6 activity was associated with the refractoriness of depression, and plasma IL-6 levels might be a predictor for the response to selective serotonin reuptake inhibitor or serotonin noradrenaline reuptake inhibitor.

Significant evidence suggests that the immune system is capable of profoundly affecting the central nervous system, functioning in ways that may contribute to the development and expression of neuropsychiatric disorders, including depression. It appears that plasma IL-6 concentrations can play a role in the diagnosis of

schizophrenic patients. The present results suggested that IL-6 could serve as an immunological marker in mood disorder pathogenesis as well.

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