Assessment of the Immune System Activity in Iranian Patients with Major Depression Disorder (MDD)

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ABSTRACT

Background: Major Depression Disorder (MDD) is a common disorder with prevalence of 15% among men and up to 25% among women. In recent years the association of immune system alterations and MDD has been investigated. Assessments of immunologic and inflammatory responses in these patients enhance our knowledge of the etiology and pathogenesis of this disease. **Objective:** To investigate the changes in immunoglobulin and cytokine serum levels and lymphocyte subsets in patients with MDD. **Methods:** We studied 37 adult patients with MDD, diagnosed based on DSM-IV diagnostic criteria, and 15 healthy controls matched with the patients. Plasma concentration of interleukin-4 (IL-4), IL-10, TNF α, and IFN γ were measured by ELISA and serum immunoglobulins by SRID. Total number of NK cells (CD16 and CD56), B cells (CD19), and T cells (CD8, CD4, and CD3) were determined by flow cytometry. **Results:** We found no significant differences in plasma concentration of IL-4, IL-10, TNF-α, IFN-γ, and immunoglobulins as well as total number of NK cells, B cells, and T cells between major depressed patients and healthy control subjects. **Conclusion:** We conclude that in our patients, there were no significant differences in immune system activity between MDD patients and controls.

Keywords: Major Depression, Cytokines, Immunoglobulin

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INTODUCTION

The contribution of immune effector mechanisms to the pathogenesis, pathophysiology and treatment of psychiatric disorders has been investigated during the past decades. At the present time, there is a circumstantial evidence suggesting that major depression is associated with disregulation of the immune system.

It has been suggested that overproduction of cytokines may be involved in the pathophysiology of depressive disorders. Cytokines are a large and diverse group of interrelated molecules with important and established functions in central nervous system (CNS). Cytokines have been reported to be involved in majority of CNS associated functions such as sleep, cognition, behaviour, food intake, body temperature and neuroendocrine dysfunction (1).

Major depression disorder has been associated with various alterations in the immune system and inconsistent data have previously been reported. Both suppression and activation of the immune effector mechanisms have been reported particularly in severely depressed (melancholic) patients (2-4).

To evaluate the role and function of cytokines in depression disorder one has to consider the complexity of the disease, the clinical pattern, genetic backgrounds, demographic characteristics and the severity of the disease. There is evidence that severity of depression will have impacts on the immune defence mechanisms, possibly through the activation or interaction with the cytokine network (5). For instance, NK cell disturbances, as well as increased cytokine production and their soluble receptors, were marked in melancholic (severely depressed) patients, but were less notable in moderately depressed patients (6, 7). As the symptoms of depression and the hypothalamic pituitary adrenocortical (HPA) alterations are more profound in melancholic than in typical major depression, particular attention ought to be devoted to the possible contribution of neurovegetative features, hormonal imbalance and altered cytokine levels. Additionally, as severe depression often requires hospitalization, it has been considered that hospitalization and other related factors such as a change in diet, diurnal factors, social buffering, etc may be responsible for the altered immune and cytokine functioning (8).

As most cytokines and chemokines can be expressed in the central nervous system, it becomes impractical to study or to review all of them at the same time.

The aim of this study was to investigate the association of MDD with the change in serum levels of immunoglobulins, lymphocyte subsets, cytokines and serum proteins.

MATERIALS AND METHODS

Subjects. This cross-sectional study included 37 outpatient adults with MDD (27 females, 10 males) who referred to the Psychiatry Clinic of Imam Reza Hospital, Mashhad University of Medical Sciences from October 2004 to October 2005 and 15 healthy blood donors, as control group, who were matched for sex, age and socioeconomic status. After taking a comprehensive medical history and complete physical examination, the patients were recruited based on criteria for major depression, indicated in the *Diagnostic and Statistical Manual for Psychiatry* version IV (DSM-IV). Only patients and controls between the age of 18 and 55 years were included to prevent the effects of age on immunological parameters. All patients were receiving standard treatments including pharmacological agents for depression. Exclusion criteria included: presence of

depressive phase of Biphasic Mood Disorder (BMD), familial history of BMD, co-morbid conditions associated with depression, receiving medications such as clomipramine, sertraline, monoamine oxidase inhibitors and lithium during the last 6 months and any other drug therapy that might have affected immune system activity (e.g. glucocorticoids) and melancholic symptoms. The Severity of depression was determined by the Hamilton Depression Rating Scale for Depression (HAM-D: 21-item), according to which depression is classified into mild (score<20), moderate (20<score<30) and severe (score>30). A complete blood assay was performed on every patient and healthy control subjects between 8 and 11 o'clock in the morning to reduce variations caused by the normal diurnal fluctuations in lymphocyte subset levels.

Determination of Total Numbers of Immune Cells. We determined the circulating populations of T and B-cell subtypes and NK cells in whole blood by flow cytometry immediately after collection. Specifically, 100 µl of fresh whole blood was collected in tubes containing ethylenediamine tetra-acetic acid (EDTA) and mixed with 10 µl of the monoclonal antibody reagent. After 20 minutes of incubation in the dark, the red blood cells were sedimented by centrifugation for 5 minutes, and the supernatant removed. The cells were then washed with 2 ml of phosphate-buffered saline (PBS) containing 0.1% sodium azide and once again centrifuged , the supernatant removed and a fixative (0.5 CC of 0.5% paraformaldehyde in PBS) was added to the pellet and kept at 4°C for 30 minutes . The lymphocytes were subsequently analyzed using a FACSCAN flow cytometer (Calibur Becton-Dickinson, USA). Labeled monoclonal antibodies against CD3, CD4, CD8, CD19, and CD16/CD56 (NK cells) were obtained from IQ-Products, Netherlands. The absolute subset counts were determined as absolute lymphocyte counts by subset percentage (x100).

Determination of Cytokines Concentration. Blood was taken from all subjects and the sera were isolated and frozen at -70°C. concentration of IL-4, IL-10 TNF-α and IFN-γ were determined by quantitative enzyme-linked immunosorbent assay (ELISA). ELISA kits were obtained from Bender Med system, Austria. ELISA was performed according to the manufacturer's procedure.

Determination of Serum Immunoglobulin and Serum Protein Concentrations. Serum IgG, IgA and IgM concentrations were determined by SRID (BN 100, Behringwerke AG Germany). All anti-sera, controls and standards used for protein measurement were obtained from Behringwerke AG (Germany). We used one single batch of antiserum for each parameter and included appropriate controls in each run. Serum total protein, α_1 globulin, α_2 globulin and γ globulin concentrations were also determined by electrophoresis.

Statistical Analysis. Mann-Whitney test was used for statistical analysis of the cytokine concentration. Kruskal –Wallis ANOVA on rank tests were used to determine the relation between lymphocyte subset and cytokine levels in both groups while Student t-test was used to compare cytokines and immunoglobulins. P-values <0.05 were considered significant for all tests. SPSS software (version 10) was used for all statistical tests.

RESULTS

The mean age of the MDD patients was 39.1 ±12.3 years and that of the controls was 38.2 ±12 years. The mean duration of the disease was 14.92 ± 11.85 months. HAM-D test was used to further classify patients based on severity of the disease: 21 cases had moderate and 15 cases had severe depression disorder, one case was presented with mild depression (Table 1).

Table 1. Number, age, sex and psychiatric characteristics of Major Depressive Disorder (MDD) patients

Sex	No (%)	Age(± SD)	HAM-D score(± SD)	Duration of MDD (month) (± SD)
Male	10 (27)	41.25 ± 8.94	31.80 ± 6.34	14.33 ± 7.92
Female	27 (73)	38.36 ± 13.27	30.33 ± 6.23	15.11 ± 13.09

(HAM-D)= Hamilton Depression Scale

The mean absolute number of total lymphocytes and different circulating lymphocyte subsets in MDD patients and controls are shown on Table 2. There were no significant differences in the total absolute number of NK, B and T cells in patients, and among severe, moderate and healthy control subjects ($p > 0.05$).

Table 2. Mean absolute ± SD of numbers of circulating lymphocyte subsets (cell/ml) in depressed patients and controls

	Controls	Patients	Mild	Moderate	Severe
Total lymphocytes	2850 ± 1256	2038 ± 697	1800	2166 ± 762	1856 ± 591
CD3	1811.3 ± 637	1384.5 ± 566	990	1498.5 ± 643	1237 ± 407
CD19	328.1 ± 116	245 ± 92	270	253 ± 82	228 ± 115.3
CD4	1116 ± 349	1218 ± 479	1098	1301 ± 543	1097 ± 368
CD8	643.1 ±153.2	661.3 ± 252.5	648	696.4 ± 278	607 ± 219
CD16/56 (NK)	394 ± 105.4	319.3 ±134	504	324 ± 143	296.5 ± 117

The means of different serum cytokines levels in the patient and control groups were analyzed and no significant differences were observed between the two groups. (data not shown).

The means of cytokine concentration in patients with moderate and the severe disease were compared with the control group as shown in Table 3. Highest concentration of IL-10 was observed in patients with severe disease and the lowest concentration in the control group. However, comparison of cytokine levels between patients and controls did not produce a statistically significant difference.

The concentration of different immunoglobulin classes is shown in Table 4. We found no significant difference in the immunoglobulin concentrations between the patients and healthy controls.

Table 3. Cytokine concentrations in patients with different disease severity and in controls

	Patients (moderate)	Patients (severe)	Controls	
IL-4	7.23 ± 14.60	24 ± 61.66	70.60 ± 131.80	*x^2=1.5, p=0.43
IL-10	8.96 ± 14.30	17.50 ± 19.92	4.20 ± 8.10	x^2=4.1, p=0.12
TNF-α	7.85 ± 5.01	6.37 ± 4.07	7.50 ± 5.40	**F=0.27, p=0.76
IFN-γ	0.12 ± 0.14	0.12 ± 0.13	0.16 ± 0.05	F=0.34, p=0.71

All results were given as mean ± SD, * Kruskal-wallis, ** ANOVA

Table 4. Immunoglobulin concentrations in patients with different disease severity

	Patients (moderate)	Patients (severe)	
IgG	666.33 ± 177.50	570 ± 176.70	t=1.2, p=0.22
IgA	173.66 ± 98.24	152.55 ± 87.50	t=0.53, p=0.6
IgM	204.66 ± 33.13	196.66 ± 60.20	t=0.42, p=0.67

All results were given as mean ± SD

Table 5, shows the concentration of total serum protein, α_1 globulin, α_2 globulin and γ globulin in the patient and the control groups. The mean total protein was significantly higher in patients with either moderate or severe disease. Gamma globulin levels were also higher in patients with moderate or severe disease compared to healthy control subjects ($p< 0.05$).

Table 5. Serum levels of proteins in patients with different disease severity and in controls

	Patients (moderate)	Patients (severe)	Controls
Total protein	58.59 ± 0.89	60.84 ± 2.05	41 ± 9
α_1 globulin	1.93 ± 0.17	1.48 ± 0.40	2.5 ± 1.5
α_2 globulin	8.41 ± 0.33	8.64 ± 0.73	8 ± 3
γ globulin	18.91 ± 0.75	16.32 ± 0.17	11 ± 4

All results were given as mean ±SD

DISCUSSION

In this study, we found no significant differences in total number of T cells, B cells or NK cells between MDD patients and control group. This is in agreement with the report of Robertson et al (9) and Ravindran et al (10, 11). There are contradictory reports of reduced (12, 13), increased (11) and even unchanged (14) blood circulating NK cells in depressed patients. However, cumulative data summarized in two meta-analysis reviews indicated that depressed patients have a decreased number of lymphocytes, reduced mitogen-induced lymphocyte proliferation, and lowered NK cell activity (2, 5, and 15)

In our study, there was a decreased trend (although not significant, $p=0.43$) in serum IL-4 level in patients with moderate disease compared to patients with the severe disease and the controls. Maximal levels of IL-10 were observed in patients with the severe disease, which is in agreement with a study by Seidel et al (16). Increased production of IL-10 in severe disease points to an important finding in respect to the activation state of the proinflammatory cytokine in depressed patients.

No significant difference in immunoglobulin concentrations between MDD patients and controls was detected, similar to a study by Natelson et al. (17), however the mean value of IgG was lower in patients with severe disease than in those with moderate depression or the controls. Total serum proteins and γ globulin levels in 95% of our patients with moderate and severe disease were increased compared with control subjects.

Although, a number of investigators have reported depression-related alterations in peripheral blood immune cell number and function, many others were unable to replicate these findings.

The relationship between depression and the immune system has turned out to be much more complex than was initially anticipated. Factors, which have complicated the interpretation of these investigations, include the heterogeneity of depressed patients, the variability of immune assays, and the clinical relevance of these assays.

The depressed patients are heterogeneous with respect to the state of their immune system, with some components being suppressed whereas others are enhanced. The specificity of these changes is not absolute since some of the immune changes observed in depressed patients have also been reported in patients with other mental disorders. For example, elevated levels of IL-6 and its soluble receptor and biochemical signs of the

acute phase reaction have been observed in patients diagnosed with schizophrenia, mania, and post-traumatic stress disorder.

Concerning immune system activation in depression, illness severity might be important since increased levels of positive acute phase proteins and enhanced production of cytokines have been more consistently found in melancholic and treatment-resistant depressions than in minor cases (7). There was also evidence of a linear relationship between intensity of depression and indicators of cellular immunity (2). There was a limitation to our study, because we only assessed the outpatient and not the hospitalized patients, therefore the melancholic and treatment-resistant depressed patients were not included in the study and this could have an important impact on the results of our study.

It is believed that most of the alterations seen in depression are caused by increases in cortisol levels. Under stressful stimuli high production of cortisol is the result of the hyperactivity of the HPA axis, producing high levels of CRH and ACTH hormones. To understand the mechanism of cytokine production associated with depression, a combined assessment of the hormonal levels and the proinflammatory and anti-inflammatory cytokines is needed.

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