

Immunological Changes in Patients with Mania: Changes in Cell Mediated Immunity in a Sample from Egyptian Patients

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The immune system and its relation to psychiatric disorders has attracted many investigators, but there is still great controversy about the extent of immunological changes associated with different psychiatric disorders. Bipolar disorder is the least studied among the three major psychiatric disorders: schizophrenia, major depression and bipolar disorder. The current study was conducted on 20 patients with mania in comparison to 20 healthy controls, to evaluate changes in percentage of lymphocytes, active T-lymphocytes and natural killer (NK) cells. We found a significant decrease in the percentage of lymphocytes in mania patients as compared to controls. Assessment of Active T lymphocyte (HLADR⁺) and NK cell (CD56⁺) by flowcytometry using a battery of paired (FITC and PE) monoclonal antibodies, revealed significant decrease in NK cells ($P < 0.001$), while no significant difference was found between patients and controls as regards percentage of active T lymphocytes. It is concluded that the immune response is altered in manic patients. However, further studies on large number of cases using various immunological parameters related to CMI are needed to confirm these findings.

Psychoneuroimmunology is a fast growing field of research attracting interest of many investigators. A large number of literature is now emerging that indicates interaction between the central nervous system and the immune system (Kokai et al., 2002).

Bipolar disorder is a mood disorder that may manifest psychosis in manic episode (Tsai et al., 1997b).

Kronfol and House (1988) reported that an impairment in cell mediated immunity (CMI) was found in a small group of manic patients as reflected by a reduced in vitro lymphocyte response to mitogen stimulation. Reduced cellular immune function in manic patients was also supported by lower antibody-dependant cellular cytotoxicity (Barsi et al., 1989). Soluble interleukin-2 receptor (sIL-2R) is released from activated T cells into the blood which was significantly higher in manic patients than in normal control subjects suggesting activation of CMI in mania (Maes et al., 1995a). Interestingly, elevation of plasma sIL-2R level is not present in minor

psychiatric disorders, such as panic disorder, social phobia, obsessive compulsive disorder, post-traumatic stress disorder, anorexia nervosa or stress induced anxiety (Tsai et al., 2001).

Natural killer (NK) cells are predominantly large granular lymphocytes (LGL) which express CD16 and/or CD56 surface antigens and comprise 10-15% of the human peripheral blood lymphocytes (Robertson and Ritz, 1990). NK cells are considered key players in the initial defense against virally infected or malignant cells (Barlozzari et al., 1983). In a study on young adults (18-35 years) with major depression, they found increased circulating leukocytes, decreased CD56⁺ (natural killer) cells and possibly decreased natural killer cell activity (NKCA) (Schleifer et al., 1996). In another study on pubertal children with major depression, increased concanavalin (Con A) mitogen response and decrease NKCA, with no alterations in the numbers of leucocytes, lymphocytes or NK cells were found. Yovel et al., (2000) reported

higher natural killer cell activity in schizophrenic patients.

Bipolar disorder is the least studied among the three major psychiatric disorders of schizophrenia, major depression and bipolar disorder. Furthermore, investigations on infection and immunity in bipolar disorder make up only a small portion of sparse research done on this disorder. However, there are reports that modulations of the immune system might be associated with bipolar disorders (Selch et al., 2002).

The aim of this work was to evaluate some immunological changes in patients with mania as regards percentage of lymphocytes, activated T lymphocytes and NK cells in peripheral blood.

Subjects and Methods

Subjects

The present study was conducted on 40 individuals divided into 2 groups: group I included 20 patients with bipolar disorder current episode mania according to DSM-IV criteria (American Psychiatric Association, 1994), they consisted of 11 males and 9 females with age range (19-51 years) \pm 31.8 and group II "control group" consisted of 20 apparently healthy persons matched with sex (11 males and 9 females) and age [range (19-52 years) \pm 36.35].

All were attending Ain Shams University hospitals, "psychiatric department and blood bank". From October 2005- December 2005.

In order to confirm the diagnosis of mania patients, they were assessed by the Structured Clinical Interview for DSM-IV (SCID) (First et al., 1998). The severity of the manic symptoms was rated with the Young Mania Rating Scale (YMRS) (Young et al., 1978). To be eligible for inclusion, patients should be either medication-naïve or had been free of medication for at least one month, gave an informed consent, have a score of \geq 26 in the YMRS, and no history of administration of drugs affecting immune system e.g. corticosteroids or history of medical disorders e.g. malignancy, diabetes mellitus.

Methods

3 ml venous blood sample was taken "on EDTA" from every subject and divided into 2 parts. On one part CBC "WBC count and differential count" was done by standard technique, while the other part was analyzed within 6 hours as follows: 50 μ L of diluted whole blood sample was added to 5 μ L of the PE and FITC labeled monoclonal antibody (mAb), vortexed and then incubated in dark at room temperature for 15 min. 1.5 ml of Lyse solution "NH₄CL buffered with KHCO₃ at PH 7.2" was added and vortexed and subjected to flowcytometric processing by dual immunofluorescence Flowcytometry "Coulter EPICS XL".

To identify NK cells, anti CD56 was detected using PE labeled (mAb). For identification of activated T lymphocytes, paired expression of anti-CD3 and anti-HLADR was detected by FITC and PE labeled mAbs respectively. Labeled monoclonal antibodies used were supplied from "Caltag Laboratories Burlingame".

Statistical Analysis

Statistical analysis was performed with the software version 10 of the Statistical Package for Social Sciences (SPSS – 10). Values were expressed as mean \pm SD for all variables except for the variable Gender, which was expressed as a proportion. An α -value less than 0.05 was considered statistically significant. We used independent, two-tailed student t-test and the chi-square test to compare differences between bipolar patients and controls. Spearman's correlation coefficient (r) was used to correlate the severity of the manic episode with the immunological parameters.

Results

The present study was conducted on 40 persons, 20 manic patients "group I" and 20 apparently healthy persons "control group II". They were 11 males and 9 females in each group "with no statistically significant difference between them". Range of age was (19-51) with mean \pm SD [31.8 \pm 9.6] in patients versus (19-52) with mean \pm SD [36.35 \pm 10.2] in controls. The difference between them was not statistically significant. The above data are shown in table (1).

Table 1. Characteristics of subjects of the study "patients and controls"

	Manic patients	Controls	P-value
<u>Age</u>			
Mean \pm SD	31.8 \pm 9.6	36.35 \pm 10.2	NS
Range	19 - 51	19 - 52	
<u>Gender</u>			
Males	11	11	NS
Females	9	9	

P<0.05 is significant., NS= not significant.

Table (2) shows comparison between manic patients and controls as regards different measured immunological parameters e.g. WBCs, percentage of lymphocytes, percentage of active T. lymphocyte "measured by measuring CD3 and HLADR on T. lymphocytes" and NK cells "measured by detection of CD56 expression".

WBCs percentage was nearly the same both in patients and controls "7.2 \pm 2.53" versus "7.0 \pm 1.35" respectively with non statistically significant difference between them. Percentage of lymphocytes was lower

in manic patients (20.71 \pm 8.35) than in controls (28.37 \pm 9.53) with statistically significant difference between them. The percentage of active T lymphocytes "CD3⁺ HLADR⁺" was nearly the same both in patients and controls "19.62 \pm 17.62" versus "18.73 \pm 17.2" respectively with no statistically significant difference.

As regards percentage of NK cells "CD56⁺" it was lower in manic patients than in controls "17.23 \pm 7.46" versus "25.35 \pm 6.74" respectively with highly statistically significant difference.

Table 2. Comparison between manic patients and controls as regards percentage of circulating lymphocytes, active T cells and natural killer cells.

Percentage	Manic patients	Controls	p-value
<u>WBCs:</u>			
Mean \pm SD	7.20 \pm 2.53	7.0 \pm 1.35	NS
<u>Lymphocytes:</u>			
Mean \pm SD	20.71 \pm 8.35	28.37 \pm 9.53	0.01
<u>Act-T-Lymph (CD3⁺ HLADR⁺):</u>			
Mean \pm SD	19.62 \pm 17.62	18.73 \pm 17.2	NS
<u>NKCs (CD56⁺):</u>			
Mean \pm SD	17.23 \pm 7.46	25.35 \pm 6.74	0.001

Act= activated., P<0.05 is significant., NS= not significant

Table (3) illustrates the effect of gender “among manic patients” on age and various measured immunological parameters “WBCs, lymphocytes, active T. lymphocyte (CD3⁺, HLADR⁺) and NKCs (CD56⁺)”.

There was no statistically significant difference between manic (males and females)

as regard age (30.42 ± 8.52) versus (35.01 ± 10.2), WBCs percentage (7.01 ± 2.32) versus (7.63 ± 3.15), lymphocytes (20.82 ± 9.21) versus (20.45 ± 6.69), active T lymphocytes (CD3⁺, HLADR⁺) (22.97 ± 21.12) versus (21.80 ± 20.32) and NKCs (CD56⁺) (17.14 ± 8.12) versus (17.43 ± 6.31).

Table 3. Percentage of circulating lymphocytes, active T cells and natural killer cells according to Gender in manic patients.

	Males	Females	P-value
<u>Age:</u>			
Mean \pm SD	30.42 ± 8.52	35.01 ± 10.2	NS
<u>WBCs:</u>			
Mean \pm SD	7.01 ± 2.32	7.63 ± 3.15	NS
<u>Lymphocytes:</u>			
Mean \pm SD	20.82 ± 9.21	20.45 ± 6.69	NS
<u>Act-T-Lymph:</u>			
Mean \pm SD	22.97 ± 21.12	21.80 ± 20.32	NS
<u>NKCs:</u>			
Mean \pm SD	17.14 ± 8.12	17.43 ± 6.31	NS

Act= activated., NKCs= natural killer cells., P<0.05 is significant., NS= not significant

In table (4), correlation between various measured immunological parameters “WBCs, lymphocytes, active T. lymphocyte (CD3⁺, HLADR⁺) and NKCs (CD56⁺)” and Young

Mania Rating Scales (YMRS) score. It shows no statistically significant correlations between all parameters and (YMRS) score.

Table 4. Correlation between the various immunologic parameters and young mania rating scale score.

	Pearson correlation r	P-value
WBCs	0.155	NS
Lymphocytes	-0.183	NS
Act-T-lymph	-0.094	NS
NKCs	0.428	NS

Act= activated., NKCs= natural killer cells., P<0.05 is significant., NS= not significant

Discussion

Psychoneuroimmunology is a fast growing field of research attracting interest of many investigators. A large number of literature is now emerging that indicates interaction between the central nervous system and the immune system (Kokai et al., 2002). Bipolar disorder is the least studied among the three major psychiatric disorders of schizophrenia, major depression and bipolar disorder. We evaluated the percentage of lymphocytes, activated T cells and NK in 20 manic patients and 20 apparently healthy persons matched with sex and age.

We found no effect of gender on immunological parameters studied among manic patients. This finding is supported by the study of Tsai et al., (1999) who stated that alterations in cell-mediated immunity in manic patients are not due to difference in gender or age. Rapaport (1994), Schleifer et al., (1989,1996), Barsi et al., (1989), Kronfol and House (1988) and Maes et al., (1995a) supported the effect of age and gender on immunity of patients with mood disorder so they advise to compare these patients with age and gender-matched control as this will lead to consistent results.

We found decreased percentage of lymphocytes among manic patients in comparison to controls with a statistically significant difference between them, while there was no statistically significant difference between percentage of active T lymphocytes between patients and controls. Our results are supported by Liu et al., (2004), who studied plasma level of some immunological variables as indicators of cell mediated immunity, in acute manic patients, [IFN- γ and IL-2] as indicator of TH1 activity and TH2 [IL-4 and IL-10] cytokines, they found that TH1 cytokines were significantly lower in manic patients in comparison to controls while no significant difference in TH2 cytokines and they suggested that immuno-modulation in patients with bipolar disorder may be

abnormal. Also Su et al., (2002) stated that mean value of IFN- γ in patients with acute mania and in subsequent remission were significantly lower than those of healthy controls. While IL-10 production was not different significantly between manic patients and controls. They suggested that reduced production of IFN- γ without alteration of IL-10 in bipolar mania and subsequent remission may reflect that immune modulation vary in patients with different psychiatric disorders. In the present study we did not differentiate between TH1, TH2 but generally lymphocyte percentage was lower than controls while percentage of active T lymphocytes were the same as controls.

In another study, cell mediated activation in bipolar mania was demonstrated by Tsai et al., (1999). They found that lymphocyte proliferation in response to PHA and plasma level of soluble IL-2 receptor "sIL-2R" levels were significantly higher in acute mania than in controls. But these high levels were not detected in consequent remission. Kim et al., (2004) in their study on symptomatic manic patients, they studied plasma levels of IFN- γ "TH1 cytokine", IL-4 "TH2 cytokine" and transforming growth factor beta-1 "TGF- β 1" "TH3 cytokine, TH3 cytokines play an important role in balancing between TH1 and TH2 cytokines". They found that plasma concentrations of IFN- γ and IL-4 are significantly higher in patients than in controls, while TGF- β 1 level was significantly low and increased significantly after treatment and the change coincided with the relief of symptoms. They also demonstrated that TGF- β 1 concentration might play the most important role in normalizing T cell activation after treatment.

To our knowledge, our study is the first study done to evaluate Natural Killer "NK" cells in manic patients. We demonstrated significant decreased level of NK cells in manic patients in comparison to controls.

Many studies were conducted on level and activity of NK cells in schizophrenics and patients with major depression. In Schleifer et al., (2002) study, they found that activated T lymphocytes "HLADR⁺" and natural killer cells CD56⁺ showed lower percentages in adolescents with major depression. "Maes et al., 1992, 1993 and Schleifer et al., 1996" stated that decreased relative levels of NK cells are consistent with decreased activation of adaptive immune response in depressed adolescents. While Yovel et al., (2000) " in their study on schizophrenic patients", reported higher levels of natural killer cell activity in 29 schizophrenics than 31 controls and 8 non schizophrenic patients [4 bipolar "manic disorder" and 4 personality disorder]. Their results about non schizophrenic, 4 patients with bipolar "manic" disorders are consistent with our results, they found level of NK cells in them nearly the same in controls while we detected a significantly lower level, this difference may be due to very low number "4 cases" of studied group in their study.

In summary, we found lower percentage of lymphocytes and NK cells in manic patients than controls with statistically significant difference, while activated T lymphocyte percentage was the same in both groups.

These findings suggest that there is an interaction between psychiatric disorders and various parameters of immune system. However, there is some controversy between published data about the parameters affected by the psychiatric disorders. Our findings however, suggest similar immune changes in mania and depression adding strength to the current concept of grouping mood disorders together.

Unfortunately, the present study did not evaluate the activity of T lymphocytes or plasma level of cytokines of different T lymphocytes subsets e.g. TH1, TH2 and TH3. Studying these parameters would yield more comprehensive understanding of the

relationship between immunity and mania. Such changes in immunological parameters might be useful in evaluation of the prognosis of the disease by measuring them before and after treatment.

Further studies on this group of patients is recommended to assess the exact role of immune system and its disturbance in psychiatric disorders which may give hope for immunomodulatory therapy. Studying the prevalence of neoplasms in patients with mania to assess the impact of the natural killer cells changes on the development of cancer in those patients is also recommended.

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