ORIGINAL ARTICLE

T-cell Expression of CD 4, 8, and 137 among Naïve Patients with Major Depressive Disorder: A Comparative Study

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ABSTRACT

Background: *Major depressive disorder (MDD) is one of the most prevalent psychiatric and immune system illnesses worldwide. Recent studies have found a possible relationship between MDD and immune dysregulation, which manifests as an alteration in inflammatory biomarkers.* ***Objectives****: We aim to investigate the role of CD4, CD8, and CD137 expressions on T cells among patients suffering from major depressive disorder (MDD) not under treatment in comparison with normal individuals.* ***Methodology****: The current study was comparative and descriptive conducted on middle-aged patients (age range 18-60 years old) who were diagnosed with MDD. Assessing the differences in T cell expression of CD 4, CD 8, and CD 137 molecules between MDD cases and healthy controls was the primary outcome of our study.* ***Results****: In this study, we included 50 patients with MDD and a similar number of healthy controls. In terms of the primary outcomes of the present study. The study results found statistically significant differences between cases and controls in terms of CD4 (P=0.001), CD8 (P=0.001), and CD137 expression (P=0.001). MDD patients had significantly lower expression of CD4 (582.88 ± 133.13 versus 775.04±159.6 in the control group), CD8 (374.24 ±204.8 versus 524.04 ± 195.3 in the control group), and CD137 (391.04 ±158.2 versus 587.52±191.7 in the control group).* ***Conclusion****: We can state that there is a strong association between immune status and patients with MDD, especially naïve patients. This was confirmed according to the significant decrease in the CD4, CD8, and CD137 levels.*

INTRODUCTION

Major depressive disorder (MDD) is one of the most common psychiatric and immune system disorders. Recent epidemiological studies in the United States of America (USA) showed that MDD affects more than 10% of the adult population.[1] It was estimated that 5–10% of men and up to 25% of women are susceptible to this disorder at least once in their life.[1,2] Therefore, it was estimated that the global social cost of MDD is more than 66 million years of life with a handicap.[3] MDD is considered the fourth rank of death and disability worldwide.[4] Approximately 35% of all MDD patients treated with antidepressants failed to respond to the treatment.[5]

Recently, many studies observed the relationship between MDD and immune system dysregulation, which presents in form of disturbance of inflammatory biomarkers of peripheral blood such as Interleukin 2 (IL-2), Interleukin 6 (IL-6), Interleukin 12 (IL-12), C-reactive protein (CRP), and tumor necrosis factor-alpha (TNF-alpha).[6–12]

Previous reports showed that 29% of MDD patients had a CRP level of >5.0 mg/L and 47% had a CRP level of >3.0 mg/L.[13] Moreover, depression and some inflammatory conditions like cardiovascular disease, metabolic syndrome, infections, diabetes, and rheumatoid arthritis are significantly associated with MDD.[14]

T_{reg} cells is a term that means a highly specialized subpopulation T cells ($CD4^+$, $CD8^+$, $CD25^+$, and $CD137^+$) that are responsible for regulation and controlling of inflammation and suppression of autoimmune disorders.[15–17] They act by producing the inflammatory cytokines and inhibiting of pro-inflammatory cellular response.[18] Animal studies showed that Treg cells may play a protective role against depression. In addition, some studies demonstrated that rats who treated with antidepressants were associated with higher level of Treg cells.[19,20] In human, it was observed that adolescent with lower level of T_{reg} cells are associated with higher risk of mood disorder.[21–23] Furthermore, some researchers considered Treg cells as a predictor of treatment in patients with MDD.[24] However, the role of T_{reg} cells in MDD has not been explored yet, and all of these associations mentioned above do not prove causality. Therefore, in the current study, we aimed to investigate the role of CD4, CD8, and CD137 expressions on T cell among

patients suffering from MDD not under treatment in comparison with normal individuals of the middle age group.

METHODOLOGY

We certify that the current study was conducted in accordance with the Declaration of Helsinki's guiding principles and all relevant national or local laws. The local ethics and research committee of Misr University for Science and Technology University Hospital gave their permission to the study's protocol. Prior to research recruitment, each eligible patient provided a signed, informed consent.

Study Design, Setting, and Patients:

The current study was a descriptive, comparative study carried out from March to September 2019 at the Misr University for Science and Technology's Outpatient Clinic for the Department of Psychiatry. We included middle-aged people (between the ages of 18 and 60) who had been given a DSM-IV diagnosis of MDD and hadn't used any antipsychotic drugs in the previous two months. Additionally, a control group of age- and sex-matched healthy volunteers was added. Patients having other psychiatric comorbidities, a history of a chronic medical condition, a history of alcohol use, and/or a history of illicit drug, anti-inflammatory, cholesterol-lowering, or other potentially immune-modifying drug usage were excluded in the study. Pregnant women were also excluded. To find suitable patients, a non-probability consecutive sampling strategy was applied.

Data collection and Measurement of CD Molecule Expression:

Every patient participated in the study had their demographic information and the T cell expression of CD 4, CD 8, and CD 137 molecules were recorded. Venous samples were withdrawn from the study subjects. Samples were stored at 18–20 °C. We conducted all tests within 4 hours after collection. Flow cytometric analysis was conducted after adding EDTA to the samples. 50 µL of EDTA treated samples were combined with 5 µL of an antibody for each target cell. FITC conjugated mouse anti-human CD4 targeted CD4-positive (helper T cell), PE conjugated mouse anti-human CD8 targeted CD8-positive (suppressor T cell), and 4B4-1 conjugated mouse anti-human CD137 targeted CD137-positive cells. All mixtures were intubated for 30 minutes in a dark room. Red blood cells were destructed by adding 2 ml of lysing solution (Immunotech, Marseille, France), then the erythrocytes sediments were removed. Triple strain was used for CD4, CD8, and CD137 staining during flow cytometry (FACScan, Becton–Dickinson, CA, USA) performance. Positive cells from each type were separated from the gating cells. Besides, we stained the isotype controls (Immunotech, Marseille, France) to perform as negative controls. Eventually, we estimated the positive signal group in the gated lymphocytes.

Study Outcomes:

The differences in T cell expression of CD 4, CD 8, and CD 137 molecules between MDD cases and healthy controls were the study main outcomes.

Statistical Analysis:

SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 22 for Microsoft Windows and Microsoft Excel 2007 (Microsoft Corporation, NY and the USA) were used for data entry, processing, and statistical analysis. While qualitative data were expressed as frequencies (number of cases) and relative frequencies, quantitative data were described in terms of mean and standard deviation (SD) (percentages). Unpaired Student's t-tests for parametric data or Mann-Whitney Rank Sum tests for non-parametric data were used to compare quantitative variables. Categorical variables were subjected to the Chi-square test. Statistical significance was defined as a probability value (p-value) less than 0.05

RESULTS

In this study, 50 MDD patients and a comparable number of healthy controls were included. The included patients were primarily female and had a mean age of 29.5±5.2 (62 %). In terms of age (P = 0.37) or gender (P = 0.41). there were no significant differences between cases and controls. The demographic traits are displayed in Table.1.

Table 1: The pre-fasting characteristics of the included patients

Variables	Patients (n =50)	Control (n =50)	P-value
Age in years, mean ±SD	29.5 ±5.2	28.6 ±5.12	0.37
Male (%)	19 (38%)	20 (40%)	0.41
Female (%)	31 (62%)	30 (60%)	0.84

Regarding the study's main findings, there were statistically significant variations in CD4 (P = 0.001), CD8 (P = 0.001), and CD137 expression between patients and controls (P = 0.001). Figures 1 & 2 show that the expression of CD4 was (582.88 ± 133.13 compared with 775.04 ± 159.6 in the control group), CD8 (374.24 ± 204.8 versus 524.04 ± 195.3 in the control group), and CD137 (391.04 ± 158.2 versus 587.52 ± 191.7 in the control group) significantly lower in MDD patients. (Figure 3)

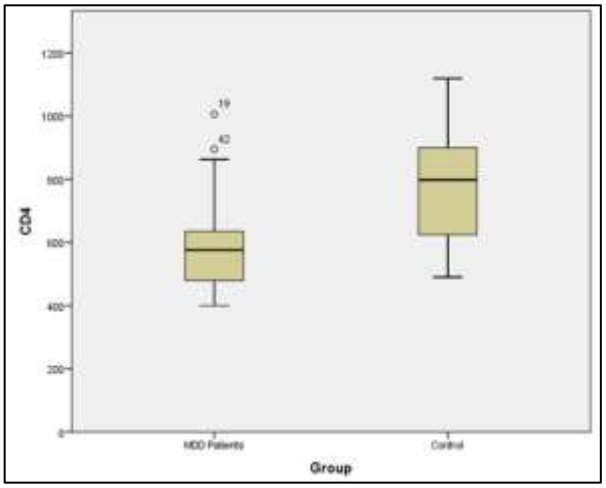

Fig. 1. Box plot showing the difference between MDD patients and controls in terms of serum CD4

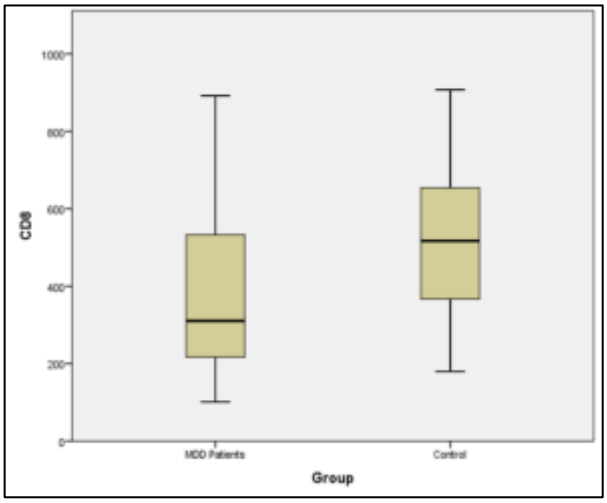

Fig. 2. Box plot showing the difference between MDD patients and controls in terms of serum CD48

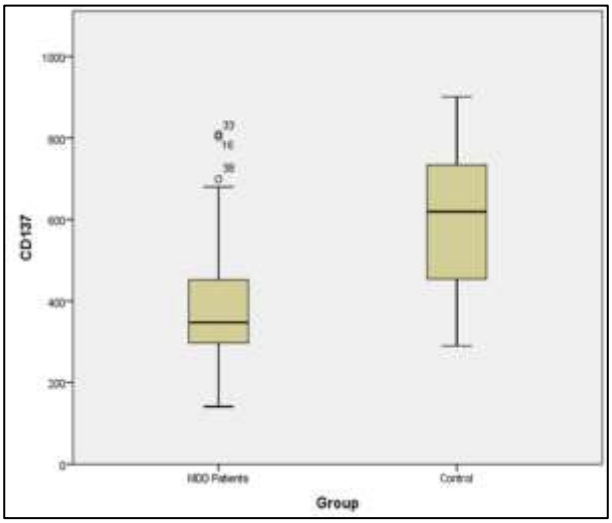

Fig. 3. Box plot showing the difference between MDD patients and controls in terms of serum CD137.

DISCUSSION

In the present prospective, comparative, observational study, we aimed to evaluate the role of CD4, CD8, and CD137 expressions on T cell among patients suffering from MDD not under treatment in comparison with normal individuals of middle age. Interestingly, our analysis demonstrated that CD4, CD8, and CD137 expression were significantly lower in naïve patients with MDD when compared with normal individuals. To the best of our knowledge, this study is one of few studies that investigate the expression of these molecules in naïve patients with MDD. Many studies evaluated expression of these molecules in patients with MDD after antidepressant intervention.

CD4 T helper cells are very essential for immune system as they interact with other immune cells, stimulate many immune cytokines, and inhibit various immune reaction components. These cells differentiate into several functional subgroups during an active immune response. Out of these subgroups, T helper (h)1 that produces IFN- γ, Th17 produces IL-17 and activate macrophages in cooperation with Th1, and Th2 that secrets IL-4 that stimulates B-cells differentiation to plasma cells. Our results showed significant down grading of CD4 in naïve patients with MDD when compared with control. This finding is similar to those of Patas et al.[25], who reported a lower surface expression of CD4, CC-chemokine receptor type 6 (CCR6), and CXC-chemokine receptor type 3 (CXCR3) in antidepressant-free MDD patients. Moreover, they showed a shift in the $CD4^+$ T compartment toward Treg cells.[25] These findings were also reported in untreated patients with MDD and human immunodeficiency virus (HIV). Amanor-Boadu et al.[26] showed that the count of $CD4^+$ was significantly ($P < 0.004$) lower in those patients compared with patients with good treatment adherence. In Owora study,[27] he classified the MDD patients to four groups; high-chronic, high-episodic, moderate-ascending, and low-chronic group. Lower CD4 count was significantly associated with high-chronic group when compared to moderate group (adjusted Odds Ratio [aOR]: 0.63; 95%CI: 0.49–0.81) and (aOR: 1.53; 95%CI: 1.08–2.19), respectively.

The T cell receptor (TCR) has a co-receptor called CD8, which is essential for T cell signaling and the activation of several transcription factors like NF-B and AP-1. Numerous studies have shown that patients with MDD have lower lymphocyte proliferation by mitogen and consistent lower NK cell activity (NKCA). In this investigation, it was found that patients with MDD had significantly less CD8 expression. In contrast, Jeon and his colleagues [28] discovered that the CD4/CD8 ratio fell from baseline ($p=0.002$) while the number of CD8-positive cells rose ($P = 0.03$) in MDD patients after treatment. They therefore proposed a correlation between the CD4/CD8 ratio and the intensity of

depression, which diminished following treatment.[29] There is no statistically significant difference between the MDD group and the control group for CD8+ (P = 0.107), central memory CD8+ (P = 0.269), naïve CD8+ (P = 0.655), or effector CD8+ (P = 0.638) in cases of MDD with sleep disturbance. However, as compared to the control group, the MDD group revealed a significantly higher level of memory CD8+ (P = 0.007).[30] This increase maybe more related to the sleep disturbance. Another Iranian study by Hosseini et al.[31] demonstrating that there was no significant difference between patients with MDD and normal individuals in terms of CD8, CD3, CD4, and NK cells.

Generally, CD137 was expressed more frequently than CD4 and CD8, as it's found on the surface of B-cells, dendritic cells, NK cells, and wall of inflamed blood vessels. CD137 enhances the immune activity, T cell proliferation, and IL-2 secretion.[31] We found that CD137 was significantly lower in MDD group compared to control group. This is the first study that investigated the relation between CD137 and MDD. The expression of CD137 or 4-1BB is associated with the activation of T-cells and NK cells.[32] A significant correlation between CD137 expression and neuroinflammatory and neurodegenerative disease was observed.[33]

CONCLUSION

A strong association between immune status and patients with MDD especially naïve patients can be stated.

Significant lower level of CD markers was associated with naïve MDD patients.

Immune system dysregulation of MDD is associated with alteration of CD4, CD8, and CD137 levels in MDD patients.

The decreased levels of CD8, CD4, and CD137 in MDD patient suggest involvement in the immune system dysregulation.

Recommendations

Further future research is required to investigate the functional changes of these molecules and its role in treatment prediction.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

Declarations

Conflict of interest: The author confirms no financial or personal relationship with a third party whose interests could be positively or negatively influenced by the article's content.

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