Original article:

Lymphocyte subsets and their association with the severity of patients with major depressive disorder (mdd)

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Abstract

Background: Major depressive disorder (MDD) has been associated with dysregulation of the immune system. While many studies on activation of innate immune response currently dominates the research area, the dysregulation in adaptive immune system especially in circulating lymphocyte subsets has rarely been explored. Some studies suggested that the severity of MDD is importance with respect to the extent of the immune changes in MDD patients. ***Objectives:*** This study aims to compare the percentage and absolute count of T helper cells ($CD4^+$ T cells), T cytotoxic cells ($CD8^+$ T cells), natural killer cells ($CD16^+$ 56^+ NK cells) and B cells ($CD19^+$ B cells) between MDD patients and healthy controls and their association with the severity of the disease. ***Materials and methods:*** This study involved 47 MDD patients and 47 healthy controls. MDD patients were diagnosed according to Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (DSM-5) criteria, and the severity of MDD was assessed using Beck Depression Inventory. Ten ml of peripheral blood were drawn from each subject. The percentage and absolute count of each lymphocyte subsets were determined using immunophenotyping method by using flow cytometry. The statistical analysis was done using Mann-whitney test and Kruskal-Wallis non-parametric test. ***Results and discussion:*** The results showed that there were no significant difference in the percentage and absolute count of T helper cells (p=0.148; p=0.190), T cytotoxic cells (p=0.316; p=0.783), NK cells (p=0.731; p=0.530), and B cells (p=0.136; p=0.148) between MDD patients and healthy controls. The percentage and absolute count of the lymphocyte subsets were also not significantly associated with the severity of MDD. ***Conclusion:*** There were no significant difference in the percentage and absolute count of lymphocytes subsets between MDD patients and healthy controls. The percentage and absolute count of the lymphocyte subsets were also not significantly associated with the severity of MDD. In conclusion, there were no alterations of lymphocyte subsets in our MDD patients

Keywords: major depressive disorder, dysregulation, lymphocyte subsets, percentage, absolute count

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INTRODUCTION

Major Depressive Disorder (MDD) is the most common mental disorder reported in Malaysia[1] and it is forecasted to become the second leading cause of worldwide disabilities by the year 2020[2]. Nowadays, MDD is not only recognized as one of the major psychiatric diseases, but is also associated with immune system disorder[3,4]. Since the past two decades, many characteristics of immune dysregulation have been reported in patients with MDD [5-7].

There has been increasing interest in the role of an immune response and inflammation in the development of MDD. Intriguing data showed that the impairment of immune system in MDD were not only reflected by the alteration of inflammatory cytokines level, but also by the changes in the level of various lymphocytes subsets[6].

The positive findings of these lymphocyte subsets

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are important to determine the pathophysiology of MDD. Decreases in the number of the relevant T cell subset may directly contribute to the development and maintenance of MDD. Enhancement of the relevant lymphocyte subset may represent an interesting and novel approach for the treatment of this disorder.

However, there were only few studies done on the lymphocyte subsets in MDD and showed contradictory results. In addition, most of the studies were done more than a decade ago. New data with better technologies are needed to confirm the finding. No study has been done regarding the association of lymphocyte subset with the severity of MDD. The association between lymphocytes subsets and disease severity are important as they might be considered as biomarkers of severe MDD. Therefore, this study was conducted to evaluate a wide range of lymphocyte subsets including T helper cells ($CD4^+$ T cells), T cytotoxic cells ($CD8^+$ T cells), B cells ($CD19^+$B cells), and NK cells ($CD16^+$ 56^+NK cells) in MDD patients and compared them with healthy controls. This study was also done to evaluate the association of these lymphocyte subsets with the severity of MDD.

METHODOLOGY

Subjects Recruitment and Assessment

A total of 47 MDD patients and 47 healthy controls were included into this study. MDD patients were recruited from outpatients Psychiatric Clinic, Hospital Universiti Sains Malaysia. MDD patients were diagnosed according to Diagnostic and Statistical Manual of Mental Disorder 5 (DSM-5) criteria[8]. Patients were excluded if they met one or more of the following criteria: presence of other psychiatric illness, autoimmune diseases, allergic diseases, neoplastic or endocrine diseases, current pregnancy, acute or chronic infection within the past month and immunocompromised or immunosuppressed patients. The severity of MDD patients were assessed using Beck Depression Inventory (BDI)[9] at the time of recruitment. The total BDI score is calculated by summing up the 21 items. A total score of 0-10 is considered as normal depression, 11-20 indicates mild depression, 21-30 indicates moderate depression

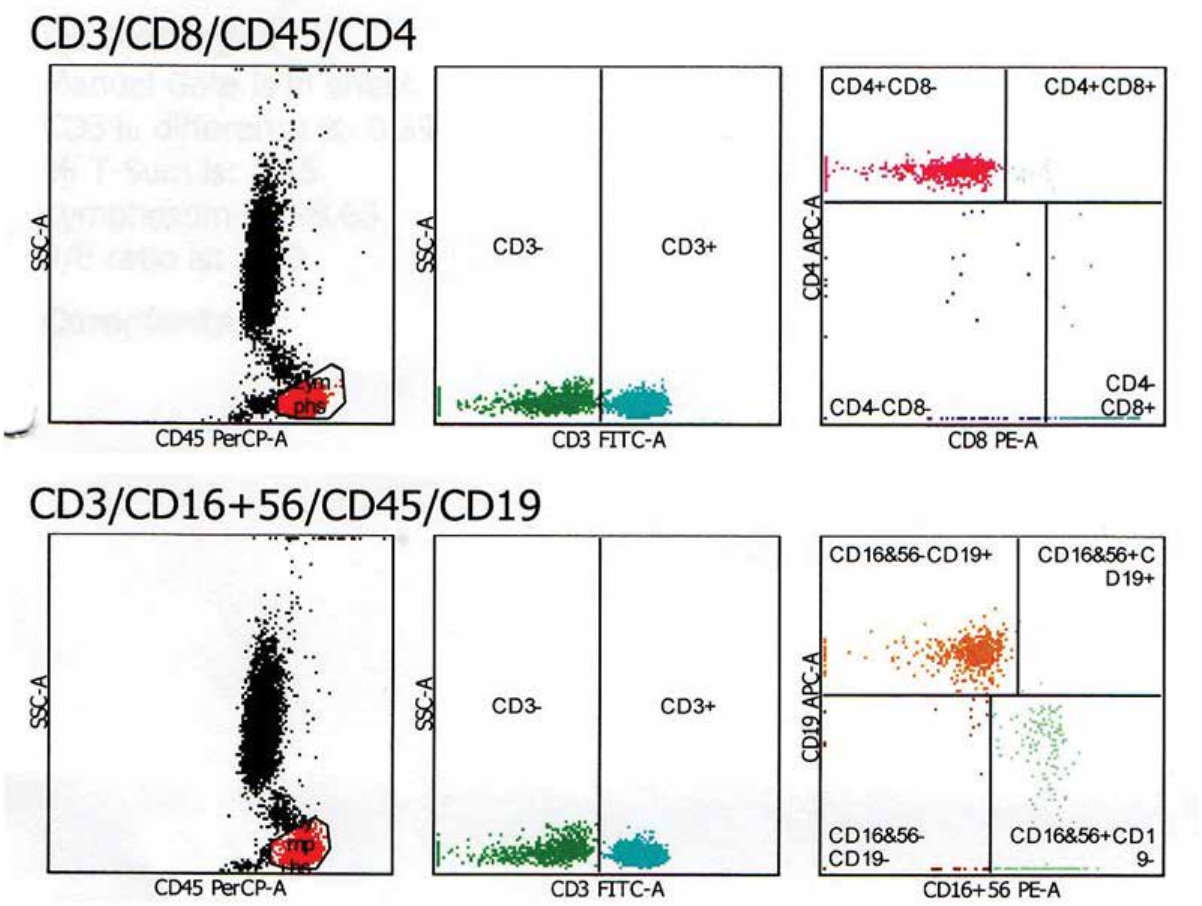

Figure 1 Gating strategy used to identify total T cells (CD3+), T helper cells (CD4+), T cytotoxic cells (CD8+), B cells (CD19+), and NK cells (CD16+ 56+)

and 31 and above indicate severe depression

Healthy controls were recruited from hospital employees and students that were in good general health and had no history of MDD. All eligible subjects were asked to complete Depression Anxiety Scoring System (DASS 21) questionnaire to ensure that they were in good general health and had no depressive symptoms.

This study was approved by Research and Ethics Committee, Universiti Sains Malaysia. After study procedures had been fully explained, written informed consent was obtained from all participants.

Immunophenotying of Lymphocyte Subsets

10 ml of peripheral blood were collected from each MDD patients and healthy controls. The peripheral blood was stored in an EDTA tube and processed within 2 to 4 hours of blood withdrawal. Complete blood count was performed using SYSMEX XS-800i Hematology Automated Analyzer. For immunophenotyping of the lymphocytes subsets, the blood samples were stained with monoclonal antibodies, Multitest™ IMK Kit from BD Biosciences, USA. In brief, 50 µl of whole blood were mixed and incubated for 15 minutes with 20 µl of each monoclonal antibody combination CD3/CD8/CD45/CD4 antibodies and CD3/CD16CD56/CD45/CD19 antibodies in separate tube. The red blood cells were lysed by adding 450uL of 1X lysing solution. The tubes were vortexed and incubated in the dark at room temperature for another 15 minutes. The sample were then analysed using flow cytometer with BD FACSCanto™ software to determine the percentage and absolute count of total T cells ($CD3^+$ T cells), T helper cells ($CD4^+$ T cells), T cytotoxic cells ($CD8^+$ T cells), B cells ($CD19^+$ B cells), and NK cells ($CD16^+$ 56^+ NK cells). The flow cytometric gating strategies used in this study are shown in Figure 1.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 22 software. Since the data were not normally distributed, non-parametric test were applied for all statistical analysis. The percentage and absolute count of lymphocyte subsets between MDD patients and healthy controls were compared using Mann-Whitney test. While, the percentage and absolute count of lymphocyte subsets between different severity of MDD were compared using Kruskal-Wallis test. The result was statistically significant if p value was less than 0.05 ($p<0.05$).

RESULTS AND DISCUSSION

Table 1 summarizes the demographic data for 47 MDD patients and 47 controls subject in this study. The mean age of MDD patients and healthy control were 39.7 years old and 28.0 years old; respectively. In MDD group, 61.7% were females and 38.3% were males, while in healthy control, 68.1% were females and 31.9% were males. Majority of MDD patients and healthy controls were Malays which merely reflects the racial distribution in Kelantan, where majority of the population are Malay ethnicity

Table 1 : Demographics data of MDD patients and controls

	Control	MDD
	n (%)	n (%)
Age (years)		
Mean (S.D.)	27.96 (8.69)	39.72 (13.07)
Sex		
Male	15 (31.9)	18 (38.3)
Female	32 (68.1)	29 (61.7)
Race		
Malay	45 (95.7)	45 (95.7)
Chinese	2 (4.3)	2 (4.3)
Indian	0 (0.0)	0 (0.0)

Table 2 : The percentage and absolute count of lymphocyte subsets

	MDD (n=47)	Healthy control (n=47)	Z statistics	*p*-value
	Median (IQR)	Median (IQR)		
Total Lymphocyte Count (cell/µl)	2500 (700)	2400 (1000)	-0.667	0.505
Total T cells ($CD3^+$ T cells)				
Percentage (%)	67.14 (10.18)	66.92 (8.67)	-0.548	0.584
Absolute count (cells/µl)	1652 (517)	1696 (636)	-0.072	0.943
T helper ($CD4^+$ T cells)				
Percentage (%)	36.47 (7.82)	34.61 (8.63)	-1.448	0.148
Absolute count (cells/µl)	931 (376)	836 (326)	-1.312	0.190
T cytotoxic ($CD8^+$ T cells)				
Percentage (%)	27.12 (10.06)	27.65 (8.88)	-1.002	0.316
Absolute count (cells/µl)	688 (371)	681 (265)	-0.276	0.783
NK cells ($CD16^+$ $CD56^+$ NK cells)				
Percentage (%)	18.26 (10.82)	17.61 (9.15)	-0.344	0.731
Absolute count (cells/µl)	475 (241)	415 (244)	-0.628	0.530
B cells ($CD19^+$ B cells)				
Percentage (%)	15.36 (6.60)	13.54 (5.41)	-1.490	0.136
Absolute count (cells/µl)	377 (190)	345 (208)	-1.448	0.148

Median percentage and absolute count of lymphocyte subsets in MDD patients and healthy controls are shown in Table 2. The median percentage and absolute count of total lymphocytes, total T cells, T helper cells, T cytotoxic cells, NK cells and B cells in healthy controls were within the normal range of healthy adult of Asian population[10] .This validated the results obtained in this study.

In the present study, we did not observe any significant differences in the total number of circulating lymphocytes between MDD patients and healthy controls. The findings were consistent with previous study by Li et al.[11] which indicated no significant differences in total number of lymphocytes between MDD patients and healthy controls. They concluded that the immune imbalance of MDD was related to dysregulation of different subsets of lymphocytes, instead of the whole peripheral lymphocyte population.

Previous studies reported reduced T cell number and percentage in patient with MDD[6,12,13], however our study did not detect any significant differences in the absolute count or percentage of total T cell between MDD patients and healthy controls. Our result was supported by Hosseini et al. [14] which also did not find any significant difference in the absolute count of total T cells between MDD patients and healthy controls.

We also did not observed any significant differences in the percentage or absolute count of T helper cells between MDD patients and healthy controls which are consistent with studies done by Roberson et al.[15] and Basterzi et al.[16]. However, the finding was in contrast with previous studies by Zorrilla et al. [13] and Miller [6] which reported lower

percentage and absolute count of T helper cells in MDD patients compared with healthy controls.

NK cell counts and activity in MDD patients have been determined in a number of studies, but with divergent or even conflicting results. While some studies found reduced numbers of NK cells[17-19], other studies observed an increased[20] or no differences in the percentage and absolute count of NK cells between MDD patients and healthy controls[14, 21]. In this study, we found no significant difference either in the percentage or absolute count of NK cells between MDD patients and healthy controls.

Our result showed that the percentage and absolute counts of B cell in MDD patients did not differ from healthy controls. The investigation of B cell numbers also has yielded contradictory results, Maes et al. [22] found a significantly increased number of B cells, Schleifer et al. [12] detected a lower B cell numbers, while other authors were unable to find any alterations in B cell numbers during depression [14, 23-25].

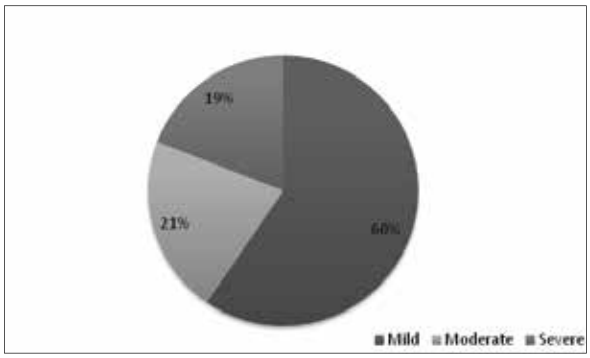

Figure 2: The severity of MDD patients based on BDI scale

Based on BDI scale, 28 (60%) patient were mild, 10 (21%) patients were moderate and 9 (19%) patients were severe MDD (Figure 2). Table 3 shows the median percentage and absolute count of lymphocyte subsets in mild, moderate and severe MDD. This present study showed that there were no significant differences in the percentage and absolute count of T cells, T helper cells, T cytotoxic cells, NK cells, and B cells between mild, moderate and severe MDD (p>0.05). Our findings are in accordance with previous study by Hosseini et al. [14] who also did not find any significant differences in total absolute count of NK cells, B cells and T cells among healthy controls, moderate and severe MDD patients. However, Maes et al. [26] found a significantly decreased NK cell number and activity in severely depressed subjects. We also observed a decreasing trend in median percentage and absolute count of NK cells with increasing severity of MDD, but the differences did not reach statistical significance. This could be due to the small sample size within each severity group especially in severe patients.

In general, the results concerning the lymphocyte subsets in MDD patients have been contradictory and controversial. There are several factors that might be responsible for this phenomenon. One explanation for this could be that most of the previous studies determined the number of lymphocyte cells after separation of mononuclear cells, whereas we determined lymphocyte subsets in the whole blood. Previous study showed lower counts were obtained for total T, T helper and T cytotoxic cells after mononuclear cell isolation[27].

It has been suggested that modulating factors such as the biological and environmental factors also might be responsible for the results variation. Various types of biological rhythms may exert influences on lymphocyte subsets. For example, ultradian rhythms can caused a decline in total numbers of lymphocytes in the peripheral blood at numerous time points during a day and within the lymphocyte population, T cells may be decreased by as much as 50% [28,29]. Hence, to minimize the effects of ultradian rhythm on the lymphocyte subsets count, all blood samples were collected within 8.00 am to 12.00 pm.

The distribution of lymphocyte subsets in peripheral blood can also be altered by exercise. Exercise decreases the number of total T cells and T helper cells in peripheral blood while increasing the percentage of NK cells. After cessation of vigorous exercise, the number of T helper cells return to normal within 2 hours, whereas the NK cell numbers return to baseline only after 24 hours[30]. Other study suggested that T cytotoxic cells count also is increased by vigorous physical activity[31].

Light to heavy cigarette smoking can also alter both total count and percentage of lymphocytes. Among the subsets, decrease percentages of T helper cells with increased T cytotoxic cells have been reported[32]. Tollerud and colleagues reported that the number of NK cells is reduced in the smoker's blood[33]. In addition, environmental factors also can cause variation in the lymphocyte subsets count. The exposure to persistent environmental pollutant such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) caused a significant decreased in

Table 3: The association of lymphocyte subsets with the severity of MDD

	Severity of MDD	Median (IQR)	χ^2 stat[a] (df)	*p*-value
T cells ($CD3^+$ T cells)				
Percentage (%)	Mild	63.27 (9.91)	3.918 (2)	0.141
	Moderate	67.74 (5.11)		
	Severe	70.32 (14.94)		
Absolute counts (cells/µl)	Mild	1647 (521)	0.211 (2)	0.900
	Moderate	1625 (696)		
	Severe	1733 (729)		
T helper ($CD4^+$ T cells)				
Percentage (%)	Mild	35.98 (8.74)	1.908 (2)	0.385
	Moderate	34.85 (9.05)		
	Severe	38.29 (11.43)		
Absolute counts (cells/µl)	Mild	949 (381)	0.682 (2)	0.711
	Moderate	844 (403)		
	Severe	895 (500)		
T cytotoxic ($CD8^+$ T cells)				
Percentage (%)	Mild	26.90 (6.52)	0.416 (2)	0.812
	Moderate	27.87 (4.32)		
	Severe	28.58 (13.74)		
Absolute counts (cells/µl)	Mild	670 (322)	0.012 (2)	0.994
	Moderate	761 (336)		
	Severe	714 (601)		
NK cells ($CD16^+$ $CD56^+$ NK cells)				
Percentage (%)	Mild	18.79 (5.36)	3.091 (2)	0.213
	Moderate	17.78 (12.34)		
	Severe	13.72 (14.13)		
Absolute counts (cells/µl)	Mild	525 (296)	3.618 (2)	0.164
	Moderate	484 (263)		
	Severe	357 (336)		
B cells ($CD19^+$ B cells)				
Percentage (%)	Mild	15.72 (6.96)	0.374 (2)	0.830
	Moderate	14.88 (4.57)		
	Severe	15.70 (9.87)		
Absolute counts (cells/µl)	Mild	441 (192)	1.495 (2)	0.473
	Moderate	358 (276)		
	Severe	345 (160)		

the percentage of total T and T helper cells as well as an increased in the percentage of T cytotoxic cells[34].

CONCLUSION

There were no significant differences in the percentage and absolute count of T helper cells, T cytotoxic cells, NK cells, and B cells between MDD patients and healthy controls. The result also showed that the percentage and absolute count of T helper cells, T cytotoxic cells, NK cells, and B cells were not significantly associated with the severity of MDD. In conclusion, there were no alterations of lymphocyte subsets in our MDD patients.

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Conflict of interest: none

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