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Original Article

Evaluation of Peripheral B Lymphocytes Alteration in COVID-19 Patients with Different Severity

Ishad Mazhar[1], Chandan Kumar Roy[2], Tasnim Khanam[3], Sharmin Akter[4], Sharmeen Ahmed[5]

ABSTRACT

BACKGROUND: *The corona virus disease 2019 (COVID-19) outbreak has posed a threat to global health. Lymphocytes are the important immune system components in controlling severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) infection.* ***OBJECTIVE:*** *This study aimed to compare the differences in lymphocyte subsets, particularly B lymphocytes among COVID-19 patients and healthy controls.* ***METHODOLOGY:*** *A total of 85 COVID-19 patients and 20 healthy controls were enrolled between March 2020 and January 2021 from Bangabandhu Sheikh Mujib Medical University. The COVID-19 patients were divided into two groups: mild-moderate (n=38) and severe-critical (n=47), according to severity. Total lymphocyte, T lymphocyte and B lymphocyte numbers were measured by flow cytometry.* ***RESULTS****: Compared to healthy individuals, the COVID-19 group had a significant decrease in both total lymphocyte and T lymphocyte percentages (P<0.001). However, the B lymphocyte percentage was increased in the COVID-19 group (P<0.001). Furthermore, the severe-critical COVID-19 group had a significant decrease in B lymphocyte count compared to the mild-moderate COVID-19 group (P<.001) and the healthy group (P<.001). However, there was no significant difference in B cell count between mild-moderate COVID-19 group and healthy group.* ***CONCLUSION:*** *Peripheral B lymphocytes were lower in severe COVID-19 patients compared to healthy individuals and those with mild symptoms. B lymphocyte immunophenotyping could be used as an indicator of COVID-19 severity.*

Key words: COVID-19, B lymphocytes, disease severity.

INTRODUCTION

In December 2019, corona virus disease 2019 (COVID-19) was first reported in Wuhan, China and then rapidly spread throughout the world.[1] Since the beginning, it has been necessary to understand the interaction between SARS-CoV-2, the causative agent of COVID-19, and the immune system of host.[2] The majority of COVID-19 patients exhibit mild to moderate symptoms, but depending on their immune responses, some patients could experience severe complications that could result in death.[3]

In viral infection, innate immunity is responsible for the initial non-specific response through neutrophils, dendritic cells, natural killer (NK) cells, and macrophages, while the adaptive immune system is responsible for the specific antiviral immunity through the T lymphocytes and B lymphocytes.[2,4,5] Natural killer cells and T lymphocytes reduce viral load by destroying virus-infected cells. Conversely, humoral immunity is controlled by B lymphocytes, which produce neutralizing antibodies.[4,5] In COVID-19, follicular helper T cells activate B

Authors affiliation:

1. *Dr. Ishad Mazhar, Assistant Professor, Department of Microbiology, Medical College for Women and Hospital, Dhaka, Bangladesh. Phone: 01924824716, Email: ishadmazhar21304@gmail.com
2. Dr. Chandan Kumar Roy, Associate Professor, Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.
3. Dr. Tasnim Khanam, Lecturer, Department of Microbiology, Rangpur Medical College, Rangpur, Bangladesh.
4. Dr. Sharmin Akter, Lecturer, Department of Microbiology, Shaheed Tajuddin Ahmad Medical College, Gazipur, Bangladesh.
5. Dr. Sharmeen Ahmed, Professor, Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.

***For correspondence**

cells. After interacting with T cells, B cells rapidly multiply and differentiate into plasma cells, and produce neutralizing antibodies and memory B cells. Memory B cells and specific antibodies enter the blood circulation to defend against viral infection and re-infection.[3,6] So, ineffective clearance of SARS-CoV-2 in COVID-19 patients largely depends on proper and sufficient humoral responses.[6]

Lymphopenia, a reduction in the total number of lymphocytes in the blood, can result from viral infections. The clinical outcome of acute viral infections can be impacted by lymphopenia.[7] In COVID-19, immune dysregulation and excessive pro-inflammatory mediator production can result in total lymphopenia mostly in severe COVID-19 cases.[5,8] Further evaluation of lymphocyte subsets has also revealed that T cell and B cell counts are altered between severe and non-severe COVID-19 cases.[9-11] Immune alteration in B lymphocytes is the prominent characteristic of COVID-19 and is strongly associated with the severity of the disease.

The purpose of the study was to evaluate the changes in lymphocyte subsets in COVID-19 patients compared to healthy individuals. Additionally, it aimed to measure and compare the levels of peripheral B lymphocytes in COVID-19 patients with varying degrees of severity and healthy individuals.

MATERIALS AND METHODS

A total of 85 RT-PCR-confirmed COVID-19 patients and 20 healthy subjects were included in this cross-sectional study from the COVID Unit of Bangabandhu Sheikh Mujib Medical University between March 2020 and January 2021. The COVID-19 patients were classified into mild-moderate and severe-critical groups.[12] Healthy subjects and patients with immunosuppressive drugs, chemotherapy and immunodeficiency disorders were excluded. All healthy controls and patients provide informed consent and the study was approved by the Institutional Review Board of BSMMU (No.BSMMU/2020/7870).

On admission, patient's venous blood was collected for complete blood count and 3ml of blood was taken in EDTA (Ethylenediamine Tetra acetic Acid) tube for flow cytometry. The complete blood count report was collected on the following day for total WBC count. Flow cytometry was performed at Department of Microbiology and Immunology, BSMMU.

For each sample, 50µl of anticoagulated blood was pipetted into a 12x75 FACS tube. Next, a 5µl mixture of AntiCD45-ECD (Energy Coupled Dye), AntiCD3-FITC (Fluorescein isothiocyanate), and AntiCD19-PE (Phycoerythrin) conjugated antibodies was added to detect CD45+ total lymphocytes, CD45+CD3+ T cells, and CD45+CD19+ B cells, respectively. The tubes were then incubated for 10-15 minutes in the dark at room temperature and 200µl of Lysing solution was added to tubes to lyse red blood cell. Following the incubation, 3ml of sheath fluid was added, and the tubes were centrifuged for 5 minutes at 300g. The supernatant was discarded, and the cells were re-suspended in 50µl of sheath fluid. Finally, the tubes were run through a precalibrated flow cytometer (Beckman Coulter Cytomics FC 500), and the data were analyzed using CXP software. For each sample, 10,000 events were counted. Statistical analysis and graphic representation of the data were performed by SPSS software version 27 and Graph Pad Prism 9.0 software. The results were expressed as medians (interquartile range) and the non-parametric test, Kruskal-Wallis test was used for multiple comparisons, while the Mann-Whitney U test was used for two-group comparisons. P-values less than 0.05 were considered statistically significant.

RESULTS

In SARS-CoV-2 infection, lymphocyte number alterations might have a possible association with pathogenic mechanisms. The percentages of total lymphocytes, T lymphocytes and B lymphocytes were compared between COVID-19 patients (n=85) and the healthy group (n=20) in Table I. The median percentage of total lymphocytes was decreased significantly in the COVID-19 group compared to the healthy group [11 (5-19) vs 29 (27-34), P<0.001]. Additionally, the COVID-19 group had a significantly lower percentage of T lymphocytes compared to the healthy group [58 (50-69) vs 70 (68-75), P<0.001]. However, the median percentage of B lymphocytes was increased in COVID-19 group [25 (17-38) vs 18 (10-20)], P<0.001].

Table I: Comparison of peripheral blood lymphocyte subsets among the study groups

Characteristics	Healthy group (n=20)	COVID-19 group (n=85)	P value
Total lymphocytes, %	29 (27-34)	11 (5-19)	<0.001
T lymphocytes,%	70 (68-75)	58 (50-69)	<0.001
B lymphocytes,%	18 (10-20)	25 (17-38)	<0.001

Group medians (IQR) are showed and P values are derived from Mann–Whitney test.

To analyze B lymphocytes in relation to disease severity, the COVID-19 patients (n=85) were divided into mild-moderate group (n=38) and severe-critical group (n=47). B lymphocyte counts were measured and compared among COVID-19 patients with different severity and healthy subjects in Figure 1. The median count of B lymphocytes was significantly decreased in the severe-critical group [154 (71.52-304.5)] compared to the mild-moderate group [323 (230.9-475), P<.001] and the healthy group [269.9 (220-453.6), P<.001]. However, there was no significant difference in B cell count between the mild-moderate COVID-19 group and the healthy group. It is important to note that these findings suggest that severe-critical COVID-19 patients have lower B lymphocyte counts compared to those with milder symptoms.

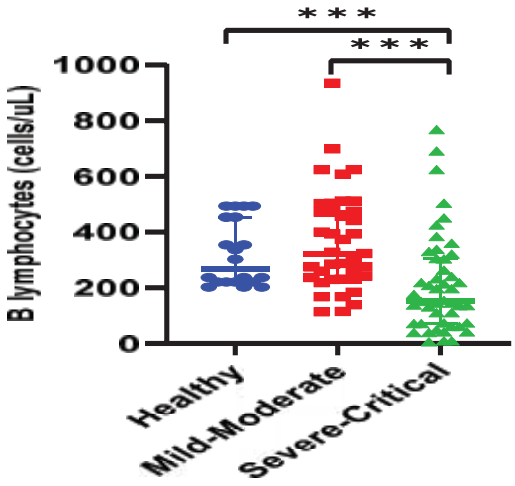

Figure 1: Absolute counts of B lymphocyte in healthy group, mild-moderate and severe-critical COVID-19 groups are showed with scatter plot graph. The longer horizontal line in graph indicates the median value for each group. * indicates P < 0.001.**

DISCUSSION

B lymphocytes are essential components of the humoral immune system. They play a crucial role in fighting viruses by producing neutralizing antibodies. Therefore, they are vital in providing protection against SARS-CoV-2. This study aimed to assess the changes in lymphocyte subsets, particularly B lymphocyte count, in patients with COVID-19.

In the present study, both the percentages of total lymphocytes and T lymphocytes were decreased in COVID-19 patients compared to the healthy group. Similar findings were reported from several previous studies,

suggesting dysregulation of immune response in COVID-19. [9,11,13,14]

The current study showed that there was a significant increase in the percentage of B lymphocytes in the COVID-19 group compared to the healthy group, which is consistent with the results of previous studies.[3,14,15] The possible cause of the relative increase in B cells percentage could be due to the significant decrease of T cells in these patients.[14,15]

Regarding severity of disease, B lymphocyte count was found significantly lower in severe-critical COVID-19 group compared to healthy group and mild-moderate group of COVID-19 patients. This finding is in agreement with several studies conducted in China.[9,10,16] Moreover, a lower count of B-cells was found to be associated with a higher risk of in-hospital death.[11] However, other studies have reported no differences in B lymphocyte count between the mild and severe groups.[17,18] A decrease in B lymphocyte levels was also observed in patients with severe acute respiratory syndrome (SARS) during the large outbreaks of atypical pneumonia in 2003.[19] B lymphocytes were significantly lower in SARS patients who died compared to those who recovered, and in those with severe disease compared to those with non-severe disease.[20]

It was hypothesized that virus can attach and penetrate into lymphocytes that express ACE2 on their surface, which may lead to a depletion of both T cells and B cells. Cell death by apoptosis, inflammatory cytokines, co-inhibitory molecules, and metabolic disorders could be the underlying reason for decreased B lymphocytes in COVID-19.[2,7] Additionally, the severity of COVID-19 is found associated with the changes in the B cell subpopulations, either immature or terminally differentiated B cells.[3]

Notably, immunotherapies that deplete B-cells, such as anti-CD20 treatment, may increase the risk of SARS-CoV-2 infection and prolong the illness. Glucocorticoid therapy can lead to a decrease in circulating B-cells by inducing apoptosis and suppressing B cell activation, proliferation, and differentiation.[21,22] However, it is important to note that a failure in B cell activation or dysfunction can result in a severe form of the disease and also reduce the efficacy of vaccination.[2]

CONCLUSION

The peripheral B lymphocyte count is significantly lower in severely critical patients compared to healthy control and moderate patients. Peripheral blood B lymphocyte count could help identifying the COVID-19 severity. To better understand the role of B lymphocytes in COVID-19 pathogenesis, it is necessary to conduct large scale study that includes follow-up, correlation of antibody titers, cytokine profiling, and analysis of B cell subset populations.

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