Original Article

Decreased CD4 and CD8 Count are Responsible for Severity of COVID-19 Infection

Muhammad Omar Malik, Yasir Ishaq, Yasar Mehmood Yousafzai and Awal Mir

ABSTRACT

Objective: The goal of this research was to measure and compare CD4:CD8 ratio in critically ill vs. non-critically ill COVID-19 patients.

Study Design: Prospective, and one Pool Cross-sectional Observational study.

Place and Duration of Study: This study was conducted at the Pathology department of Rehman Medical Institute (RMI), Peshawar from March 2021 to August 2021.

Methods: Peripheral blood samples were taken from 26 critically sick and 26 non-critically sick COVID-19 individuals of comparable age and sex. Absolute WBC count, absolute lymphocyte count, and platelet count were checked and flow cytometry was performed to calculate the absolute CD4 and CD8 T cells counts.

Results: The critically ill COVID-19 patients were older ($p<0.001$) than non-critically sick COVID-19 individuals. In patients who got critically ill, absolute lymphocyte count ($p=0.004$), absolute CD4 count ($p=0.002$) and absolute CD8 counts ($p=0.014$) were low. However, the CD4:CD8 ratio did not differ substantially across the groups ($p=0.538$). The two groups did not differ in terms of gender.

Conclusion: When compared to COVID-19 patients who weren't in critical condition, the absolute lymphocyte count, CD4 count, and CD8 count of critically sick COVID-19 patients were significantly lower. This revealed that the lack of adequate cellular immune responses in critically sick COVID-19 patients may be the cause of the disease severity.

Key Words: Coronavirus, COVID-19, CD4 lymphocyte, CD8 lymphocyte.

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INTRODUCTION

Coronavirus was isolated for the first time from a group of atypical lung infection patients in city of Wuhan, China in December 2019[1]. Because of its precipitous global spread like wildfire, WHO classified it as a pandemic on March 2020[2].

Seven infectious coronaviruses have been identified so far. Some particularly virulent human CoVs include SARS-CoV, SARS-CoV-2, and MERS-CoV. These lead to epidemics with a variety of clinical severity levels, including symptoms of the respiratory and extra-respiratory systems. Common human coronaviruses include HCoV-OC43, HCoV-HKU1, HCoV-229E, and HCoVNL-63. They have the ability to cause upper respiratory tract infections and colds in immunocompetent individuals[3].

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COVID-19 is confirmed in an individual if the PCR turns out to be positive for viral RNA. Most of the time, nasopharyngeal or throat swabs are used to obtain the sample but in certain conditions, sputum and bronchoalveolar lavage can be used for sample collection[4].

The immune mechanisms of our body protect us from the invasion of microorganisms, tumour cells, and various toxins. The innate and adaptive immunity works hand in hand to recognize and abort the obnoxious material[5].

For the host to react to any virus that enters the body, innate and acquired immunity must work together. Due to the body's immunological response to viral infections, the number of T lymphocytes, particularly CD4 T cells and CD8 T lymphocytes, varies[6]. For instance, acute HIV-1 infection, cytomegalovirus infection, and glandular fever are known to cause the reversal of the CD4:CD8 ratio. This behaviour, on the other hand, does not occur in those who have HIV non-convertase[6]. Numerous investigations have demonstrated that the immune response brought on by these infections reduces the CD4:CD8 ratio. As a result, Immunosenescence occurs and the patient's immunity gradually deteriorates[7].

In the perspective of COVID-19, recent observation and research has shown that there is a decline in lymphocyte count in patients with COVID-19 (6). Due to this, all of the individuals who had this viral infection showed changes in the usual CD4:CD8 ratio (8,9). In previous studies, it was noted that viral infections lead to the initiation of T-cell responses, which results in changes in the immune response in affected individuals[6].

As a result, in this investigation, we measured the T-cell CD4 and CD8 counts by flow cytometry and assessed the CD4:CD8 ratio in COVID-19 infected patients, and also checked if was related to disease severity. This research will advance our knowledge of the immune reactions to COVID-19 infection, with potential implications for treatment and diagnosis.

METHODS

Study population: From March 19th to August 6th, 2021, a prospective and one-pool cross-sectional observational study was undertaken in two major hospitals in Peshawar, Rehman Medical Institute (RMI) and Hayatabad Medical Complex (HMC). In total, 52 patients infected with COVID-19 were recruited; 26 critically ill patients and 26 non-critically sick patients, all of whom had COVID-19 and were of comparable age (range 20 - 75 years) and gender (M=27,F=25). Patients who were severely ill were admitted to isolation wards or the intensive care unit (ICU), while those who were not critically ill were managed through the pulmonology outpatient clinic for diagnosis and treatment. Patients with ongoing viral, autoimmune, or oncological disorders as well as those with a history of pre-existing chronic diseases particularly those who had been treated with immunosuppressive drugs before contracting COVID-19 infection were excluded from the study. Patients who had taken steroids before the blood sample were also excluded from the study. After taking informed written consent, information like demographics and clinical information was recorded on the purposefully designed questionnaire. The Institutional Research Ethical Board of IBMS KMU Peshawar gave its approval under the Ref. No. KMU/IBMS/IRBE/10th meeting/2024/1753-H. After that the samples were all collected in compliance with the WHO criteria for coronavirus sample collection from human beings (10).

Complete Blood Count: Three milliliters of venous blood were tested for absolute WBC count, absolute lymphocyte count, absolute RBC count, HCT%, HGB, MCH, MCHC, MCV, MPV, and platelet count using a fully automated haematology analyzer (XN-1000, Sysmex, Japan).

Flow Cytometry Analysis: Flow cytometry was performed using anti-human directly conjugated antibodies on Beckman-Coulter Cytoflex (Beckman-Coulter, MA, USA). 50 µl of blood sample of each individual was shifted to a round-bottom tube to count CD4+ and CD8+ T cells. After the addition of 3µl cell surface antibodies anti-CD4-FITC, anti-CD8-PE, CD45-ECD and anti-human CD3-PC7 each, the sample was incubated for 15 minutes in a dark place. Then after the addition of 1ml of lysing solution, it was incubated again in a dark place. After centrifuging the sample for 5 minutes at 2000 rpm, the supernatant was thrown away. Thereafter 2ml of PBS was added to the sediment and it was then re-centrifuged and the supernatant was discarded. Later, after addition of 500 µl PBS, the sample was analyzed by using Beckman Coulter Navios software (Figure 2). The resultant CD4 & CD8 percentages and the absolute lymphocyte count were used to calculate the absolute CD4 & CD8 counts.

Statistical Analysis: SPSS Version 23 was used to record and analyze the data. For all numerical variables, descriptive statistics were employed to derive the Mean and Standard Deviation (SD). The differences between the two groups in terms of CD4, CD8, and CD4/CD8 were compared using an independent sample t-test. The means of the two groups were compared using Fisher's exact test for categorical variables (gender). P-values under 0.05 were considered significant.

RESULTS

In total, absolute WBC count, absolute RBC count, HCT%, HGB, MCH, MCHC, MCV, MPV, and platelets count was not reduced significantly ($p>0.05$) in critically sick COVID-19 infected patients compared with non-critically sick COVID 19 patients (Table 1). Critically ill patients were mostly older ($p=0.001$) (Table 1). Patients who were critically ill had lower absolute lymphocyte count ($p=0.004$), absolute CD4 count ($p=0.002$), and absolute CD8 counts ($p=0.014$), as compared to non-critically ill patients (Fig.1). However, the CD4/CD8 ratio did not change significantly across the groups ($p>0.05$), (Fig. 1). Moreover, no discernible difference was seen between critically sick and non-critically sick across genders.

Table No. 1: Comparison of Immunological/ Haematological markers between critically sick and non-critically sick COVID 19 patients

Factors	Non-Critically ill	Critically ill	P Value
Age	44.15 ± 14.74	57.85 ± 11.66	0.001
Gender (male/female)	14/12	13/13	
Absolute Lymphocyte Count	1.85 ± 0.97	1.12 ± 0.74	0.004
CD4%	52.26 ± 11.67	45.70 ± 18.53	0.134

Absolute CD4 Count	0.98 ± 0.48	0.52 ± 0.52	0.002
CD8%	45.25 ± 11.63	49.92 ± 16.51	0.244
Absolute CD8 Count	0.85 ± 0.52	0.54 ± 0.32	0.014
Absolute WBC Count(10^3/µl)	8.54 ± 3.83	9.72 ± 4.57	0.315
Absolute RBC Count(10^6/µl)	4.90±0.86	4.99±0.66	0.681
HCT%	42.27±8.68	44.09±8.03	0.436
HGB (g/dl)	13.10±2.12	13.32±1.83	0.686
MCH (pg)	26.96±3.33	26.97±2.54	0.996
MCHC (g/dl)	31.55±5.08	30.15±3.07	0.233
MCV (fL)	86.07±8.16	89.70±7.79	0.107
MPV (fL)	11.87±1.06	11.72±0.87	0.579
Platelets count (10^3/µl)	268.73± 88.83	243.15±100.79	0.336

Mean ± SD values of immunological/haematological markers. P for independent T test comparison of critically ill versus non-critically sick individuals. Indices with significant P values (≤0.05) is shown in bold. HCT: Haematocrit. HGB: Haemoglobin. MCHC: Mean Corpuscular Haemoglobin concentration. MCHC: Mean Corpuscular Haemoglobin Concentration. MCV : Mean Corpuscular Volume. MPV: Mean Platelet Volume

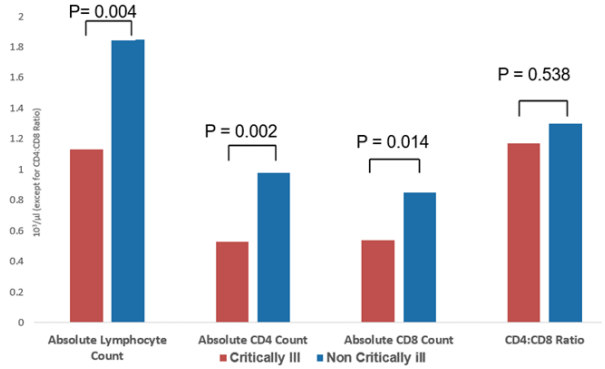

Figure No. 1. Comparison of absolute lymphocyte, CD4, CD8 counts and CD4:CD8 ratio in critically sick vs non-critically sick COVID-19 patients.

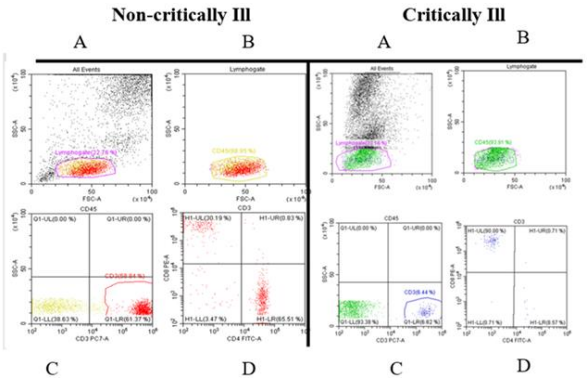

Fig. 2. Flow cytometry analysis graphs of critically sick vs non-critically sick COVID-19 patients

(A) Forward and side scatter plot of red cell lysed whole blood. Lymphocytes are identified by forward and side scatter profile. (B) Precise identification of leukocyte/lymphocytes by staining with CD45 marker. (C) T cell percentages were determined by staining with CD3 marker. (D) The expression of CD4 and CD8 markers on the two-parameter density plot was used to identify and gate the CD3 positive T cells.

P for independent T test comparison of two groups.
Absolute Lymphocyte Count in Critically Ill Patients = 1.12 ± 0.74 10^3/µl; in Non-Critically Ill Patients = 1.85 ± 0.97 10^3/µl; Absolute CD4 Count in Critically Ill Patients = 0.52 ± 0.52 10^3/µl; in Non-Critically Ill Patients = 0.98 ± 0.48 10^3/ µl; Absolute CD8 Count in Critically Ill Patients = 0.54 ± 0.32 10^3/ µl; in Non-Critically Ill Patients = 0.85 ± 0.52 10^3/ µl; CD4:CD8 Ratio in Critically Ill Patients = 1.17 ± 0.89; in Non-Critically Ill Patients = 1.30 ± 0.66

DISCUSSION

In response to viral infections, both the innate and acquired immune systems are triggered. Modulation of T cell associated immune responses occurs in majority of the infections with viruses[11]. Perforin, granzyme, and interferons are just a few of the molecules that CD8 + cytotoxic T lymphocytes (CTLs) might secrete in order to get rid of viruses from the host body[12]. CD4 helper T cells support cytotoxic T cells and B cells in the elimination of viral infection[13].
In a robust immune system, the CD4:CD8 ratio is about 2:1. According to research on the immunological reaction in human beings, the CD4:CD8 ratio is reversed in some viral infections[14]. According to Sainz et al research, this ratio could be utilized to diagnose various viral diseases[15].
In this investigation, flow cytometry was used to calculate the absolute CD4 and CD8 T cell counts in blood specimens of critically and non-critically ill COVID-19 individuals. Then CD4:CD8 ratio was calculated in these two patient groups. Critically sick COVID-19 patients had a considerably lower absolute lymphocyte count in their peripheral blood than non-critically sick COVID-19 individuals. This is consistent with findings from other studies[6,16,17].
In critically ill COVID-19 individuals, absolute CD4 and CD8 counts were also considerably lower than in non-critically ill COVID-19 individuals. These findings

are in harmony with C Pallotto et al. study[8]. This indicates that T cells are important in COVID-19 defence and reduced CD4 and CD8 counts make an individual more prone to severe infection.

The typical value of 2:1 was observed for the CD4:CD8 ratio in COVID-19 individuals, demonstrating that the two groups of COVID-19 individuals who were critically sick and those who were not did not significantly vary from one another. According to the results of research by Ganji A et al., the CD4:CD8 ratio in COVID-19 individuals is within the normal range[6]. The same CD4:CD8 ratio in both groups may be due to the fact that in critically ill patients both CD4 and CD8 counts were reduced, therefore the ratio remained the same as in non-critically ill patients. If only one count was reduced then the ratio must have changed. So overall CD4 and CD8 ratio is not changed but both counts are reduced in critically sick individuals.

In critically sick COVID-19 individuals, decreased absolute lymphocyte count, absolute CD4 count, and absolute CD8 count were seen, indicating reduced protection in these patients. There is a possibility that the counts may have been less due to overconsumption in critically ill patients, resulting in the depletion of these counts. Another possible explanation is that the patients got critical illness due to reduced CD4, CD8, and lymphocyte counts in first place. LI et. al. showed that the number of peripheral T cell subsets was lower in patients with the severe acute respiratory syndrome (SARS). In recovered patients, peripheral T cell subsets were quickly restored; as a result, peripheral T cell amount can be employed as a reliable COVID-19 diagnostic tool[18].

Furthermore, when compared to non-critically sick COVID-19 individuals, the absolute eosinophil count and absolute monocyte count in critically ill patients were significantly lower, which is in line with prior research findings[20]. This suggests that continuous monitoring of the peripheral blood system, particularly eosinophils and monocytes can help forecast severe COVID-19 cases.

In this study, it was also discovered that the critically sick COVID-19 patients were older than the COVID-19 patients who weren't critically ill. This shows that being older could be a risk factor for a poor outcome. However, the ratio of males and females among COVID-19 patients who were severely ill and those who were not; did not differ. These findings contradict a recent study that found males are more susceptible to COVID-19 infection[17]. COVID-19 infection was previously related to Wuhan seafood wholesale market exposure, and the bulk of the patients were male workers, therefore this could be the possible explanation.

CONCLUSION

The changes in the CD4:CD8 ratio in critically ill COVID-19-infected individuals were not significant when compared to the non-critically ill COVID-19 group. However, absolute lymphocyte counts, absolute CD4 counts, and absolute CD8 counts were all significantly lower in critically ill patients. This suggested that the cellular immune responses were low in critically ill COVID-19 patients and may be the reason for disease severity.

Declaration of competing interest: The authors state that they have no competing interests.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Muhammad Omar Malik, Yasir Ishaq
Drafting or Revising Critically:	Yasar Mehmood Yousafzai, Awal Mir
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Ethical Approval: No.1753/H dated 'nil'

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