ONLINE ISSN :3007-309X
PRINT ISSN :3007-3081

Journal of Medical & Health Sciences Review

RETROSPECTIVE ANALYSIS OF IMMUNOGENIC CELLS AMONGST COVID-19 PATIENTS FROM TERTIARY CARE HOSPITALS OF PAKISTAN

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ARTICLE INFO

Keywords: Blood, COVID-19, WBCs, Lymphocytes and Neutrophils.

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ABSTRACT

Background: The co-relation between lymphocytes and neutrophils is significant in COVID-19 patients. Change in blood composition is the major risk in COVID-19 patients. Arterial blood is the medium to transport nutrients to cells and take away wastes from cells. We have conducted this retrospective cohort study to find correlation between blood components in COVID-19 patients.

Methods: The reports collected from COVID-19 ward in various tertiary care hospitals of Lahore included 60 patients of which 32 male and 28 female. The median age of the patients is 55 (32-81). All these patients show huge difference in the components of peripheral white blood cells among lymphocytes and neutrophils.

Results: The reports show the changes in neutrophils in 6 patients are in range of 60-70 of, 14 patients are in 71-80 and the rest of 37 patients are above from 80. The lymphocyte ratio in 33 patients is below the 15, 26 patients are in the range of 15-30 and 2 patients onlyhave 31-40 of total WBCs.

Conclusion: Hence, the results of data collected from Pakistan population also have similar case in all the patients of COVID-19 that have suffered with low level of lymphocytes and higher level of neutrophils. However, the early signals of disease development indicated by peripheral white blood cells and can be chosen as an effective parameter for the treatment of COVID-19.

Retrospective Cohort Study of Co-relation between Lymphocytes and Neutrophils in COVID-19 patients

Background: A corona virus is a common virus that is causing nose and lung infection in human. It also affects throat and other body parts. Most of the coronas Viruses were not dangerous in 2019; the first case of coronavirus examinedwas called COVID-19 in the city of China, Wuhan. This infection spread in China first and then all over the world. On 30th January 2020, the world health organization announced the epidemic and in February 2020 world health organization (WHO) named this disease as corona virus disease 2019 (COVID-19)[1].

During that time, patients may have normal or low levels of white blood cells, lymphopenia, or thrombocytopenia, with increased duration of stimulated thromboplastin and a level of C-reactive protein. In simple, we can say that COVID-19 should be blamed, if a person gets high fever and symptoms in respiratory tract and also in a patient with lymphopenia and leukopenia, specifically those persons who came from Wuhan or were in touch with those people. Blood is important component of our body and major connective tissue [2]. Arterial blood contains a specific type of nutrition and oxygen that delivers these nutrients to the whole body, and venous blood contains carbon dioxide and all kinds of wastes away from the cell. In a healthy person, blood is a homogeneous non crystalline substance, which has tiny particles present in it. Blood is present 7% of the total body weight. The1% of blood is white blood cells that work as immunity of our system. Others are 150,000 to 300,000 platelets in each ml of blood. But when we talk about COVID-19 patients this composition of blood is disturbed by the virus in many ways. It has infected more than 79 million people around the world[3]. The key immune cells that are responsible for complete destruction of virus is lymphocytes. Lymphocytes in peripheral blood are generally increased in any kind of infection or viral inflammation. Although, peripheral lymphocytopenia was treated with acute H1N1 cold and severe acute respiratory syndrome (SARS)[4]. Against influenza A infection, T lymphocytes work as a major role in immunization and can show the minimum symptoms of infection. Studies suggest that

the number of lymphocytes that can be CD3,4,8 +T-cells suddenly decrease in the case of influenza virus [4].

Inflammatory biomarkers are found in blood to distinguish the white blood cells count, lymphocyte to neutrophil ratio platelet to lymphocyte ratio and C reactive protein that bis serum level have been calculated independently of systemic diseases that should be inflammatory [5]. After entering into the host cell, coronavirus first binds to host receptor cells by the spike protein. This spike protein is structurally trimer in which S1 heads sitting on top of trimeric S2 stalk. S1 contain receptor binding domain that help for recognizing the angiotensin-converting enzyme 2 as its receptor. These spikes need proteolytic activity at S1 and S2 boundary and S1 dissociate and S2 perform such activities that are needed for structural change by this protease enzyme activated on cell surface by TMPRSS2 and lysosomal proteases cathepsins. These are the key factors for entry and rapid spread & more fatality rates in infected patient [6]. In COVID-19 patients, lymphocyte decrease with various subsets (CD3+ CD4 +, CD8 +, CD19 +and CD16/56 +) &become less after the first week of disease [7].

During SARS-CoV-2 infection, the immune homeostasis is maintained by Lymphocytes that plays an important role. Most of the patients have low numbers of lymphocytes and greater number of leukocytes count and neutrophils-lymphocytes ratio (NLR). Lymphopenia was also observed as a determinant of diagnosis in COVID-19 patients[7].

This study was conducted to analyze how WBCs are affected by SARS-CoV-2. The main objective of this study was to find out the effect of peripheral white blood cells components of lymphocyte to neutrophil ratio in COVID-19 patients and with the statistical analysis to know which part of the world population has higher proportions of neutrophil to lymphocyte ratio in COVID-19 patients and how this statistical analysis relates with Pakistan's population. The results will expand the knowledge about the correlation of blood with COVID-19.

Materials and Methods

Study design: The complete blood count (CBC) reports were collected from a tertiary care hospital of Lahore, Pakistan. This retrospective cohort study was implemented from August to October for the year 2021. The cohort included 87 patients in total M/F (35, 52), who volunteered for this study having COVID-19. After screening, for negative and duplicate reports, the remainder was 72, out of which 40 were female and 32 were male. The clinical data which was extracted from reports included, demographic information such as name, gender, and age; the COVID-19 ward type in which patients were admitted; the white blood cell count, lymphocyte, neutrophil, and eosinophils count. We categorized the patients in severe and mild severe groups. The mild severe group contained patients who were in general COVID-19 ward or were not admitted. And the severe group contained the others who were in ICU/CCU or isolation ward. In this way we had a ratio of 60 and 40 percent between mild and severe groups.

Statistical analysis: Data were summarized, medians were calculated, and Wilcoxon test was performed for analysis. Frequencies and percentages were reported for dichotomous or nominal categorical variables, and Chi square test was performed for analysis. All other blood components were not included in this study because of their similar count in all patients. All the data were recorded into an excel sheet and crosschecked by three experienced technicians independently.

Co-relation Analysis: The changes were observed in white blood cells, lymphocytes and neutrophils in all the recorded data of patients. Moreover, the co-relation was observed in between lymphocytes and neutrophils and then compared this co-relation with the literature of other countries.

Results

Demographic information: A total of 72 patients (40 females and 32 male) consented for this study, who had confirmed COVID-19. In demographic information, the median age and gender distribution were calculated. The median age was 64 (26-85). These patients were admitted in different wards i.e., cardiac surgery, ICU (ventilated), ICU (non-ventilated), isolation and COVID-19 ward. According to their wards we categorized these in two groups i.e. sever and mildly severe. Male and female had the same frequency in severe group while there were 3 more females than males in mildly severe group. Hematologic profiles of these COVID-19 patients were detected by their complete blood count reports, which showed a huge difference in WBCs 12.20 (3.2062.10) among lymphocytes 1.18(0.32-9.31), neutrophil 10.11 (23.3-47.81). N/L ratio in severe group was higher 8.5(3.5-98) than in mildly group 5.46(4.87-47.5). In severe patients, WBCs are greater in numbers 13.80(3.50-3.420) as compare to less sever 12.20(3.20-62.10). On the other side lymphocytes are lower number in severe patients
0.91(0.32-3.54) than mildly 1.30(0.38-9.31). Eosinophils in total patients are 0.23(0.061.24) and observe a minor difference between severe and mildly patients, in mildly severe patients these numbers are little bit greater 0.24(0.06-1.24) than severe 0.20(0.100.59). Same in the case of RBCs mild group have larger number 4.7(3.2-11.2) than severe. The information about age, gender, and severity is listed in Table 1. At data cutoff, 664 (260 females, 39%; median age 70 (56-81) years) patients completed their hospital in-stay: 221/664 patients (33.3%) had died and 453/664 (66.7%) had been discharged. The median time to death was 6 days [2–10], while the median time to discharge was 8 days [5–14] ($p < 0:001$). Table 1 reports the main features of the study population. The proportion of males was higher among those who died in-hospital; moreover, the age was significantly higher in this group of subjects, who also showed increased white blood cells (WBC), absolute neutrophil count (ANC), neutrophiltolymphocyte ratio (NL ratio), eosinophil count, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and red cell distribution width (RDW). Conversely, survivors had significantly higher platelet count, haemoglobin concentration, and absolute lymphocyte count (ALC). **Laboratory findings in COVID-19 patients**

The WBCs count was observed in 17 patients to be in the range of 0-10× 10^9/L, while, in 33, 6 and 3 patients, it was observed to be in the range of 11-20× 10^9/L, 21-30×
10^9/L and above 30× 10^9/L respectively. The white blood cells varied in neutrophils and lymphocytes while in other components, they were in normal range. The reports showed the following changes in neutrophils, in 6 patients the range was between 60-70 percent of overall WBCs, 14 patients were in range of 71-80 percent of total WBCs, while the rest of 37 patients were above from 80 percent of the total count of WBCs. The lymphocyte ratio in 33 patients was below the 15 percent of total WBCs count, 26 patients were in the range of 15-30 percent and 2 patients only had 31-40 percent of total WBCs.

Discussion: To address the issue of blood co-relation, it is essential to have the knowledge about proportions of the problem by gathering the data about co-relation between Lymphocytes and

Neutrophils in COVID-19 patients fromworldwide. This study is the first retrospective cohort based on the correlation of blood in patients with confirmed SARS-CoV-2 infection.

The classification of patients has been done based on gender difference and admission ward. In this retrospective cohort study, the different components of White blood cells which have fluctuating numbers, and their effects were analysed. According to literature review, it was observed that most of the patients had low numbers of lymphocytes and greater numbers of leukocytes counts and also neutrophilslymphocytes ratio (NLR). Lymphopenia was also observed as a determinant of diagnosis in COVID-19 patients [7]. Our data also revealed that the number of lymphocytes were lower in COVID-19 patients while the number of neutrophils were higher. SARS-CoV-2 RNA and proteins may interact with various recognition receptors that can initiate effects of antiviral immune system by the separation and proliferation of various antibodies cells with immune mediator production and release, especially lymphocytopenia and elevated IL-1β levels, IFN-γ, IP-10 and IL-17, which regulate viral replication. SARS-CoV-2 has been proven to promote the regeneration of nearby lymphocytes and a strong humoral immune response causes severe infection. Decreased production, apoptosis and the redistribution of lymphocytes may lead to concussion circulatory lymphopenia [8]. In short, uncontrolled SARS-CoV-2 infections as well the immune system can cause systemic damage, while peripheral cell mutations may work as early symptoms of physical disability in COVID-19 patients[9].

The studies revealed that in the year 2020 the analysis of COVID-19 patients in China has decreased number of lymphocytes and at the first week of illness, the various subsets of lymphocytes (CD3 +, CD4 +, CD8 +, CD19 +and CD16/56 +) were below from the normal range[7]. Additionally, the patients with severe type of COVID19 with decreased lymphocytes have also been noticed in decrease count of eosinophils, basophils but increased counts of neutrophils and these PBSIs were improved in recovery phase while worst conditions in an aggravated phase[10]. Sukrismanet al, in his study reported that the use of neutrophile to lymphocyte ratio and C reactive protein gives an understanding about the severity and non-severity of the patients [11].

However, Jun Sugihara et al, reported that the number of neutrophils is increasing in the blood circulation of COVID-19 patients and this quantity is correlated with the severity of disease revealed in USA of the year 2021 [12]Guillaume Carrisimo et al, reported that two factors involves in cytokines storm are elevated IL-6 and IP-10 levels associated with neutrophils [13]. Murat Seyit et al, revealed that C reactive protein and platelet to lymphocytes and neutrophil to lymphocyte ratio was significantly greater [14]

Conclusions/Recommendations

This is the only systematic and retrospective cohort study that analyse correlation of lymphocytes and neutrophils with COVID-19. Overall, the patients from different countries have changing in the lymphocyte to neutrophil ratio. Hence, the results of data collected from Pakistan population also have similar case in all the patients of COVID19 that have suffered with low level of lymphocytes and higher level of neutrophils. However, the early signs of disease development indicated by peripheral white blood cells can be chosen as an effective parameter for the treatment of COVID-19.

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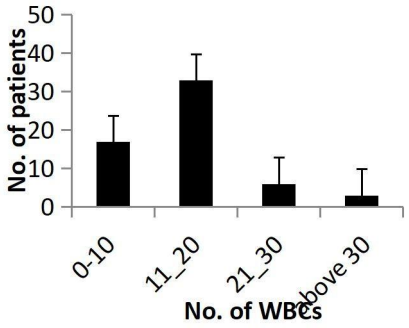
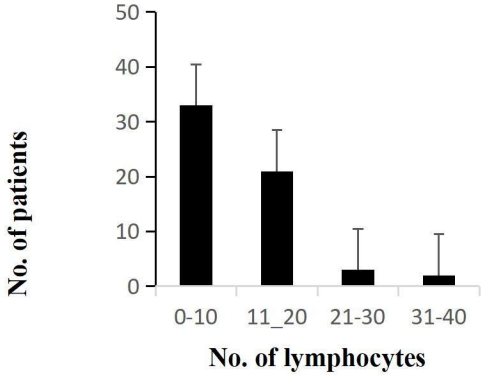


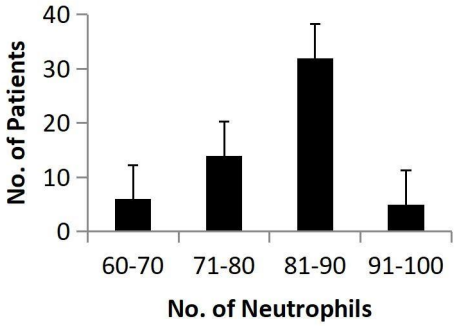
A

No. of WBCs count in various no. of patients: 0-10 WBCs count was observed in 17 patients while, 11-20, 21-30 and above 30 WBCs count were observed in 33, 6 and 3 patients respectively.



B

No. of lymphocytes count in various no. of patients: 0-10 lymphocytes count was observed in 33 patients while, 11-20, 21-30 and 31-40lymphocytes count were observed in 21, 3 and 2 patients respectively.



C

No. of Neutrophils count in various no. of patients:60-70neutrophils count was observed in 6 patients while, 71-80, 81-90 and 91-100 neutrophils count were observed in 14, 32 and 5 patients respectively.

Figure 1: Variation in Immunogenic Cells in COVID-19 Patients

Table 1: Blood Composition data of COVID 19 patients.

No. of Patients	Gender	Age	*WBCs×1/L	**Neutrophils ×1/L	***Lymphocytes ×1/L	Ward Type
1	Female	65	13.8	90	5	COVID-19 ICU (non-ventilated)
2	Male	65	5.4	60	32	CARDIAC SURGERY MALE WARD
3	Female	63	11.4	90	8	COVID-19 Isolation ward
4	Female	63	6.5	80	13	COVID-19 Isolation ward
5	Male	69	16.3	90	8	COVID-19 ward
6	Male	69	14.8	88	10	COVID-19 ward
7	Male	69	11.6	83	11	COVID-19 ward
8	Female	75	6	72	15	COVID-19 ward
9	Male	71	14.5	90	8	COVID-19 ICU (non-ventilated)
10	Male	71	18.4	19	5	COVID-19 ICU (non-ventilated)
11	Female	63	19.8	80	13	COVID-19 ICU (non-ventilated)
12	Female	63	18.1	77	18	COVID-19 ICU (non-ventilated)
13	Female	63	15.9	90	7	COVID-19 ICU (non-ventilated)
14	Female	63	17.5	85	10	COVID-19 ICU (non-ventilated)
15	Female	75	16.8	90	7	COVID-19 ward
16	Female	75	13.9	80	15	COVID-19 ward
17	Female	75	13.9	75	17	COVID-19 ward
18	Female	75	14.7	81	14	COVID-19 ward
19	Male	75	7.5	85	10	COVID-19 Isolation ward
20	Male	75	17.9	66	24	COVID-19 Isolation ward
21	Male	75	16.3	70	20	COVID-19 Isolation ward
22	Female	60	62.1	77	15	COVID-19 ward
23	Male	60	36.9	95	2	COVID-19 ward
24	Female	62	3.2	73	22	COVID-19 ward
25	Male	65	8.7	66	26	COVID-19 ward
26	Female	70	16	85	10	MEDICAL ICU/ CCU (Non-

						Ventilated)
27	Female	70	20.7	89	3	MEDICAL ICU/ CCU (Non-Ventilated)
28	Female	32	12.2	80	16	COVID-19 ward
29	Male	63	21.5	88	9	COVID-19 ICU (Ventilated)
30	Male	39	16.1	83	12	COVID-19 ICU (Ventilated)
31	Male	61	19.5	85	10	COVID-19 ICU (Ventilated)
32	Male	48	11.9	85	10	COVID-19 ICU (non-ventilated)
33	Female	50	10.4	85	10	COVID-19 ICU (non-ventilated)
34	Female	50	9.6	88	6	COVID-19 ICU (non-ventilated)
35	Male	56	5.7	81	14	COVID-19 ward
36	Male	56	5.1	80	15	COVID-19 ward
37	Male	48	13.5	92	6	COVID-19 ICU (Ventilated)
38	Male	48	20	90	5	COVID-19 ICU (Ventilated)
39	Male	81	5.6	85	10	COVID-19 Isolation ward
40	Male	81	5.1	82	10	COVID-19 Isolation ward
41	Male	81	3.5	77	15	COVID-19 Isolation ward
42	Male	81	8.2	84	11	COVID-19 Isolation ward
43	Male	81	16.4	95	2	COVID-19 Isolation ward
44	Male	77	23.6	80	15	COVID-19 Isolation ward
45	Female	75	22.9	85	10	COVID-19 ICU (non-ventilated)
46	Male	46	8.8	70	20	COVID-19 ICU (non-ventilated)
47	Female	55	25.9	83	10	COVID-19 ward
48	Female	35	15.6	83	10	COVID-19 Isolation ward
49	Female	60	7.4	80	12	COVID-19 ICU (non-ventilated)
50	Male	73	12.2	84	10	COVID-19 ward

51	Male	66	12.3	90	8	COVID-19 ward
52	Female	62	12.5	80	13	COVID-19 ward
53	Male	66	14.1	60	35	COVID-19 ward
54	Female	63	34.2	98	1	COVID-19 ICU (non-ventilated)
55	Female	65	15	85	10	COVID-19 ward
56	Female	49	8.1	88	8	COVID-19 ICU (non-ventilated)
57	Male	47	15.5	82	11	COVID-19 ward
58	Male	65	19.4	95	2	COVID-19 ward
59	Male	55	9.2	88	10	COVID-19 Isolation ward
60	Female	26	6.6	52	40	

The normal ranges of WBCs * 4-11, Neutrophils ** 40-80, Lymphocytes *** 15-45