HAEMATOLOGICAL PARAMETERS IN COVID-19 INFECTION WITH EMPHASIS ON NEUTROPHIL-LYMPHOCYTE RATIO (NLR) AND PLATELET-LYMPHOCYTE RATIO (PLR)

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ABSTRACT

Objective: The objective of our study was to assess and evaluate the various hematological parameters including the alterations in new CBC parameters like Neutrophil to Lymphocyte Ratio (NLR) and Platelet to Lymphocyte Ratio (PLR) in patients with laboratory confirmed COVID-19.

Material and Methods: A single-centre tertiary care hospital-based cross-sectional prospective study was conducted at Pathology Department, Pakistan Institute of Medical Sciences (P.I.M.S), Islamabad. Haematological profiles of one hundred and fifteen hospitalized, moderate to severely affected cases of COVID-19 confirmed on Polymerase Chain Reaction of nasopharyngeal and oropharyngeal swabs were included in the study.

Results: This study included 115 patients with SARS-CoV2 confirmed on RT-PCR on respiratory specimens over a 3-month period, and several haematological abnormalities were studied. Neutrophilia was seen in 52 (45.2%) patients, lymphopenia was observed in 50 (43.5%) patients. New CBC parameters, NLR and PLR were evaluated and found as raised in our cases, 10.08 ± 12.63 and 283.0 ± 310.07 respectively. Out of 115 positive cases enrolled in our study, 89(77%) patients had normal platelet counts, 11(9.5%) patients had mild thrombocytopenia. Hemoglobin and mean red cell indices were within normal limits. The mean PT was 16.63±7.11 sec, and mean APTT was 39.69 ± 13.58 sec.

Conclusion: COVID-19 is accompanied with a wide-ranging array of haematology-related abnormalities; neutrophilia, lymphopenia along with increased NLR and PLR were noted in our study.

Key Words: SARS-CoV2, COVID-19, Lymphopenia, Neutrophil-lymphocyte ratio, Platelet-lymphocyte ratio.

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INTRODUCTION

A number of cases of pneumonia of unknown cause have been reported from Wuhan city of China, since December 8, 2019 [1]. In the month of January 2020, the disease disseminated to other areas in China, and now it has become a pandemic spreading all over the globe and affecting millions of people [2]. The first two cases of coronavirus were reported in Pakistan on February 26, 2020, in Karachi and Islamabad. Now it has spread all over the country, affecting thousands of people [3]. The culprit virus is named as Severe Acute Respiratory Syndrome Coronavirus, SARS-COV-2 and the resultant infection is known as “coronavirus disease 2019” [4]. This microorganism is a member of the genus Beta coronavirus in the family of Coronaviridae. Transmission of the virus occurs mainly through respiratory droplets and human to human contact [5].

The disease is linked with a vast spectrum of clinical manifestations ranging from asymptomatic infection, mild upper respiratory symptoms to critical pneumonia with respiratory insufficiency, and even mortality [6]. The most frequent symptoms at the beginning of the disease are fever, cough, body aches, dyspnoea, hemoptysis, and diarrhoea. The severity of symptoms is related to an increased rate of mortality [7]. The worsening of inflammatory process has a significant role in the progression of several viral types of pneumonia, and COVID-19 [8]The epidemiological and clinical features of individuals suffering from COVID-19 show that this type of infection can cause severe respiratory ailment resulting in admissions to intensive care units and high fatalities[9]. Hence, prevention, early diagnosis, and on-time treatment of critical cases have primary importance [2].

In COVID-19 patients, multiple haematological abnormalities have been reported, including lymphopenia, neutrophilia, increased levels of D-dimer and fibrinogen. Additionally, a significant prothrombotic phase has also been reported [9].

There is increasing evidence that inflammation and dysregulation of immune responses play an important role in the progression of COVID-19 [10]. In connection to this mechanism, the NLR, which can be conveniently computed from a simple

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blood test by dividing absolute count of neutrophils by absolute count of lymphocytes, has been described as having a high value in demonstrating patients' inflammatory status [11].Similarly, platelet to lymphocyte ratio, obtained by dividing platelet count to absolute lymphocyte count is another simple parameter that can predict systemic inflammatory status. They can also predict disease severity [12]. There can be a contributory role of these two for early intervention and improving mortality rates [10].

Therefore, in this paper, we have evaluated the haematological aspects with special reference to NLR and PLR in COVID-19 in-patients presenting to Pakistan Institute of Medical Sciences, Islamabad.

MATERIAL AND METHODS

This was a single-center tertiary care hospital-based prospective study carried out in the Department of Pathology, Pakistan Institute of Medical Sciences (P.I.M.S), Islamabad for a period of 3 months from 1st April 2020 to 30th June 2020. This study included 115 individuals of COVID-19 confirmed by RT-PCR on swab specimens of nasopharynx/oropharynx, admitted to isolation ward of PIMS Hospital with moderate to severe symptoms of disease according to World Health Organization guidelines. Moderate cases were defined as adolescents or adults with clinical signs of pneumonia (that is fever, dyspnea, cough or rapid breathing) but no evidence of severe pneumonia, including SpO2 ≥ 90% on room air. Severe cases were defined as adolescents or adults with clinical signs of pneumonia (fever, dyspnea, cough or rapid breathing) along with one of the following: respiratory rate of more than 30 breaths / min; severe respiratory distress; or SpO2 < 90% on room air [13]. Adult patients of both genders over the age of 12 years were included in the study. Prior consent from all patients was taken and approval from Institutional Ethical Committee was sought. Asymptomatic COVID-19 PCR positive cases and those with mild disease (symptomatic patients experiencing fever, fatigue, cough, anorexia, shortness of breath, myalgia or non-specific symptoms meeting the case definition for COVID-19 without evidence of viral pneumonia or hypoxia) [13], patients younger than 12 years and those not giving consent were excluded from the study. The demographic data and hematological laboratory profile of all patients at the time of admittance in the hospital were recorded on a proforma, which included Complete Blood Count parameters analyzed on Mindray BC-6200 Automated Hematology Analyzer, and Prothrombin time (PT)/International Normalized Ratio and Activated Partial Thromboplastin Time (APTT) analyzed on Mindray C3100 fully Automated Coagulation Analyzer, (Shenzhen Mindray Biomedical Electronics Co., Ltd. China) No patient had received any treatment before blood sample collection.

The entry of data and statistical analysis was done using SPSS version 20. Qualitative variables were expressed as percentage whereas quantitative variables were measured as Mean ± Standard Deviation and presented in form of tables.

RESULTS

This study included 115 adult cases infected with SARS-CoV2 confirmed on RT-PCR on respiratory specimens over a period of 3 months. The majority of patients in our study, 92(80%) comprised of males. The age range of patients was 13 to 90 years, with a mean age of 47.9 ± 17.62 years. The mean age of female patients was 42.87 ± 20.73 years, and of males it was 49.2 ± 16.63 years.

Table-I: represents the various hematological findings observed in patients in our study.

CBC Parameters	Reference intervals	Range of patients' values	Mean±SD
Red Cell Count (x 10^6/µl)	3.5-5.6	2.0-6.94	4.58±0.85
White Cell Count (x 10^3/µl)	4.0-11.0	0.9-35.0	10.47±6.13
Hemoglobin (g/dl)	13-17 (males) 12-15 (females)	6.6-17.6	13.17±2.32
Hematocrit (%)	40.0-50.0 (males) 36.0-46.0 (females)	20.8-53.8	39.55±6.90
Mean Corpuscular volume (fl)	80.0-100.0	68.0-103.9	86.74±6.58
Mean corpuscular hemoglobin (pg)	27.0-32.0	21.5-36.4	28.87±2.58
Mean corpuscular hemoglobin concentration (g/dl)	31.5-35.5	27.9-36.3	33.27±1.35
Red Cell distribution Width (CV %)	11.0-14.0	12.4-29.1	14.94±2.59
Platelet Count (x 10^3/µl)	150-450	8-597	236.0±110.9
Neutrophil (%)	45-70	47-98.7	77.0±13.7
Lymphocyte (%)	25-40	0.4-48.0	16.0±11.28
Monocyte (%)	2-11	0.2-25.9	1.19±1.87
Eosinophil (%)	1-6	0-8.2	5.44±3.19
Absolute Neutrophil Count (x 10^3/µl)	2.0-7.0	2.01-33.40	8.54±5.82
Absolute Lymphocyte count (x 10^3/µl)	1.0-3.0	0.13-4.75	1.37±0.83

Among our 115 cases, neutrophilia was seen in 52 (45.2%) patients while none showed neutropenia. Lymphopenia (ALC <1 x 10^3/µl) was observed in 50 (43.5%) patients.

The characteristics of platelet count are shown in **figure-I**. Normal platelet count was taken as 150-450 x 10^3/µl; mild thrombocytopenia as 100 – 149 x 10^3/µl, moderate thrombocytopenia as 50- 99 x 10^3/µl, severe thrombocytopenia as less than 50 x 10^3/µl and thrombocytosis as more than 450 x 10^3/µl.

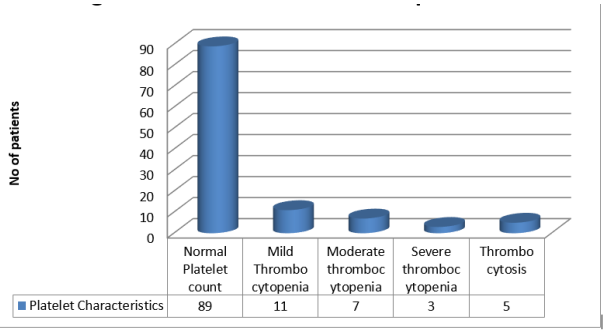

Figure-I: Platelet Characteristics of patients.

The mean hemoglobin, hematocrit and red cell indices were found to be within reference ranges in our study. New CBC parameters, Neutrophil to Lymphocyte ratio (reference range: 1-3) and Platelet to Lymphocyte ratio (reference range: 36.63-149.13 in males and 43.36-172.68 in females) were calculated of each patient, and the mean of both parameters was found to be raised in our cases, as given in **table-II**.

Table-II: NLR and PLR of COVID-19 patients

New CBC Parameters	Range of patients' values	Mean ± SD
Neutrophil to Lymphocyte Ratio (NLR)	0.9–72.01	10.1± 2.6
Platelet to Lymphocyte Ratio (PLR)	2.6–2246.0	283.0±310.0

The basic coagulation profile of patients is depicted in **table-III.** Reference range of PT is10-16 secs, with control value of 13 seconds. Reference range of APTT is 30-40 secs, with control value of 33 seconds. Derangement in coagulation parameters and frequency in each group is mentioned in **table-IV**.

Table-III: Basic Coagulation Profile of COVID-19 patients.

Coagulation Parameters	Range of Patients' Values	Mean±SD
Prothrombin Time (PT) in sec	8.41 to 60.07	16.63±7.11
International Normalized ratio (INR)	0.64 to 5.52	1.36±0.64
Activated Partial Thromboplastin Time (APTT) in sec	24.52 to 150.00	39.69±13.58

Table-IV: Frequency of deranged basic Coagulation Profile of COVID-19 patients.

Deranged Coagulation Parameters	No of patients (%)
Prolonged PT	42 (36.5%)
Shortened PT	02 (1.7%)
Prolonged APTT	45 (39.1%)
Shortened APTT	12 (10.4%)

DISCUSSION

COVID-19 causes a widespread corporal infection with noteworthy ramifications on the hematopoietic tissues and also on hemostatic pathways. Haematological profile has a role not only for predicting diagnosis of this infection but also for monitoring disease progression and prognosis [14].

Recent studies conducted world-wide predict that leucocyte count was either normal or reduced in diagnosed COVID-19 patients. However, in more severe cases, white cell counts were higher as compared to non-severe cases. In ICU settings, leukocytosis was observed more frequently in non-survivors [14]. Our study which included patients with moderate to severe illness showed that the mean total leucocyte count was 10.47 ± 6.13 x 10^3/ µl, which was within normal limits. The presence of neutrophilia in differential leucocyte count as a predictive marker of disease severity has been emphasized by Zhang *et al*., who showed that neutrophilia was seen in 74.3% cases [14]. Lymphopenia is considered as another essential laboratory finding and is associated with poor prognosis [15]. Thus, decreased lymphocyte count should be vigilantly gauged and checked on a regular basis in COVID-19 patients to monitor their disease progression [16]. The results of a study conducted by Mardani *et al* [17] revealed low levels of white blood cell counts, higher values of neutrophil counts and lower lymphocyte counts in RT-PCR positive COVID-19 patients. They showed a mean differential neutrophilic count of 60.7%. Our study showed neutrophilia in 45.2% cases, with a mean neutrophil count of 77.0 ± 13.7%. In our study, lymphopenia (ALC <1 x 10^3/µl) was observed in 50 (43.5%) patients that was also comparable with the study conducted by Chen *et al* that depicted lymphopenia (ALC <1 x 10^3/µl) to be one of the common findings [8]. Thus, regarding white cell characteristics, our findings support that neutrophil percentage might not be influenced by SARS-CoV-2 infection in the initial stage of the disease. Furthermore, we also can suggest that SARS-CoV-2 might mainly act on the lymphocyte series cells [17]

Out of 115 positive cases enrolled in our study, 89(77%) patients had normal platelet counts,

11(9.5%) patients had mild thrombocytopenia which was comparable with the outcomes of study conducted by Yanli *et al* who also revealed that most of the admitted patients had normal platelet counts, only few had decreased platelets [18].

In our study, we noticed mean hemoglobin of 13.17 ± 2.32 g/dl with normocytic, normochromic red cell indices. This is comparable with another study by Arshad *et al*., who also showed a mean haemoglobin value in COVID-19 patients to be 14.4 ± 1.93 g/dl with a range from 8.8 g/dl to 18 g/dl. Red cell indices showed mean MCV of 83 ± 8.4 fl, mean MCH of 28.9 ± 2.66 pg and mean MCHC of 34.4 ± 1.44 g/dl [19].

Abnormal coagulation profile (PT, APTT, D-dimers and Fibrinogen) was also correlated with poor prognosis in numerous studies e.g., Liu Y *et al*. showed that non-survivors revealed a significantly higher level of plasma prothrombin times, activated partial thromboplastin times, D-Dimers and fibrin degradation products as compared to survivors (20). Coagulation screen parameters documented in our study were mildly deranged with a mean PT =16.63 ± 7.11s and APTT=39.69 ± 13.58s. These were in contrast to a recent study conducted by Huan *et al* which showed the coagulation screen to be within normal range. (PT =12.65±1.13 sec and APTT=29.53±3.48 sec) [21]. This may reflect relatively severe illness in our patients at the time of admission. It has been observed that in patients with COVID-19 pneumonia, coagulation defects for example mild thrombocytopenia and high D-dimers were observed. Few patients can have abnormally shortened PT and APTT. The short APTT is usually associated with elevated Factor VIII levels as an acute-phase reaction. In rather more severely ill-patients, a disseminated intravascular coagulopathy-like state can ensue with relatively mild prolongation of both PT and APTT [22]. Our study showed Prolonged PT in 36.5% patients, and prolonged APTT in 39.1% patients. A shortened APTT was seen in 10.4% cases. Another study found prolongation of PT in 18.8% patients, prolongation of APTT in 26.3% and shortened APTT in 12.7% patients [23].

Since COVID-19 infection leads to a systemic inflammatory response, various markers can predict the presence and severity of such response. Increase in Neutrophil to lymphocyte ratio being one of them. Neutrophils, the major constituent of the leukocyte population get stimulated and activated by virus-related inflammatory elements, like interleukin-6 and 8, granulocyte colony stimulating factor, tumor necrosis factor-alpha and interferon-gamma, formed by endothelial and lymphoid cells. Conversely, systemic inflammation triggered by virus considerably depresses the cellular immune response namely the helper T lymphocytes. Consequently, virus-triggered inflammatory process augments the NLR. The higher the NLR, the greater is the tendency for disease progression [24]. In the similar manner, there is increased platelet activation and relative lymphopenia due to apoptosis associated with inflammation. Hence integration of these two parameters into one gives us platelet to lymphocyte ratio. High PLR is also associated with increased severity of inflammation [25].

NLR and PLR are measured as new indicators in COVID-19 on which various studies have been done [16,17,24,26,27,28]. These markers are considered as reflection of inflammatory responses. Recently, it has been recommended that PLR and NLR could be observed to predict disease prognostication [26]. Review of the literature shows that NLR may even increase over the course of the COVID-19 infection, particularly in those having severe disease [17]. It was observed that there was more possibility of severe pneumonia in patients having greater difference in the PLR at admission. [16] NLR and PLR observed in our study were 10.1 ± 12.63 and 283.0 ± 310.07 respectively that were comparable with the results obtained in another study done by Yang *et al* which showed the values of 10.8± 15.6 and 255.8±283 respectively [24]. A study from Pakistan also highlights the prognostic importance of NLR, about 90% patients having NLR value less than 3.13 remained stable with recovery of TLC as well as biochemical parameters like liver enzymes and had an uneventful hospital course [27]. Asghar *et al*. [28], reported a mean PLR at the time of admission to ward as 169.81 ± 105.30 which was lower than ours, which may indicate that there may be variation in inflammatory activity at the time of presentation.

LIMITATIONS

It was a single-centered study with small sample size. Classification of patients according to disease severity and comparison of haematological laboratory findings among different groups was not done. Coagulation parameters like D-dimers and plasma fibrinogen levels were not evaluated in our study. Moreover, it was a single time study which evaluated parameters only at admission and patient follow up was not done to assess the trends in values with clinical condition and outcome.

CONCLUSION

Various haematological parameters studied in the context of COVID-19 infection showed

derangement of white blood cell characteristics such as lymphopenia found in 43.5% cases and neutrophilia seen in 52(45.2%) cases. Thrombocytopenia was observed in 18% patients while 4% cases showed thrombocytosis. Mean hemoglobin and red cell indices were not deranged. The recently emerging CBC parameters, which are increasingly used as markers of inflammation namely NLR and PLR were observed to be raised in COVID-19 patients in our study.

AUTHOR CONTRIBUTION

Sundas Ali: Conception, data collection, literature review

Javera Tariq: Data collection, experimentation, literature review

Rabiah Asghar: Literature review, experimentation

Shahzad Ali Jiskani & Summaya Sohail: Discussion & literature review

Maha Tariq: Discussion, conclusion

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