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Research Article

Comparative Analysis of Complete Blood Count Parameters in COVID-19 Patients with Normal Population

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Abstract

Introduction: World Health Organization (WHO) has proclaimed the new 2019 coronavirus illness (COVID-19) as a worldwide pandemic.

Objective: To report the changes of CBC parameters in COVID-19 cases and to compare the values of the Complete Blood Count (CBC) of COVID-19 patients with normal population.

Methods: A retrospective analysis of 100 COVID-19 hospitalized patients of Jinnah Hospital and 100 healthy individuals from January to May 2021 was conducted. The demographic data and CBC values of the groups were collected and evaluated statistically.

Results: According to demographic analysis, population has an average age of 52 years. In all the cases, CBC results reveal elevated WBC, low lymphocyte count, and neutrophilia.

Conclusion: In comparison to the normal population, there is a significant variation in complete blood count variables.

Keywords: COVID-19; SARS-CoV-2; CBC; Reverse Transcriptase Polymerase Chain Reaction

Abbreviations

CBC: Complete Blood Count; RT-PCR: Reverse Transcriptase Polymerase Chain Reaction; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; SOPs: Standard Operating Procedures; NIH: National Institute of Health; WHO: World Health Organization

Introduction

According to World Health Organization, COVID-19 had been diagnosed in 196,553,009 people worldwide by 30 July 2021 resulting in 4,200,412 deaths. In Pakistan, there have been 1,020,324 cases reported so far, with 23,209 deaths [1]. Coronavirus-2 (SARS-CoV-2) is a single-stranded RNA virus with an enclosed envelope and belongs to the beta coronavirus genus. In December 2019, the first occurrences of COVID-19 cases have been documented in Wuhan and Hube [2,3]. The SARS-CoV-2 pandemic is wreaking havoc on society, the economy, and healthcare systems all across the world, and steps are taken to prevent the spread [4,5]. The most common method for identification is Real-time reverse transcription polymerase chain reaction (RT-PCR) and RT-PCR analysis was used to confirm the diagnosis of COVID-19. Patients with COVID-19 have been found to exhibit lymphopenia and neutrophilia, which are thought to be linked to the severity of the disease [6]. The most crucial parameter in the diagnostics of COVID-19 is the eosinophil count [7]. In COVID-19 patients, low lymphocyte percentage is a marker of disease prognosis. Lymphocyte count implies that this metric could be used to evaluate illness severity [8]. Blood tests play a crucial role in early intervention of the condition as they give knowledge about inflammatory response [9]. Neutrophils are the most distinctive white blood cell type and have a pivotal role in immune system [10]. Inflammatory processes are regulated by platelets [11]. The complete blood count changes have the potential to act as predictors for identification, therapy, and survival of COVID-19 patients.

The goal of this research was to determine the characteristics of changes in the complete blood count measures of COVID-19 patients. We retrospectively investigated the haematology test findings of 100 COVID-19 cases and 100 healthy people to determine the significance of these parameters. Secondly, we compare the CBC parameters of COVID-19 positive cases to the COVID-19 negative cases to see if there is a statistically significant difference between the two groups.

Methods

Study population & design

A retrospective analysis was undertaken on hospitalized patients of Jinnah hospital from January 2021 to May 2021. It is the first hospital to adopt RT-PCR in specialized healthcare to perform diagnosis and treatment of COVID-19. A total of 100 patients with SARS-CoV-2 infection above age of 20 years and 100 patients with negative results of SARS-CoV2 infection were enrolled in study. Demographic and epidemiological data was taken.

Sample collection

Nasopharyngeal swab samples were collected according to the standard operating procedures of NIH and WHO [12]. A plastic swab with a polypropylene fibre tip was used to obtain nasopharyngeal

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samples. The swab was carefully inserted through the nostrils to the posterior wall of the nasopharynx and kept for 3 seconds, twisted 2 or 3 times, softly withdrawn, and placed in 3mL of viral transfer medium. The samples were promptly stored at 4-8 degrees celsius and delivered on ice to the laboratory for PCR analysis. Blood samples were drawn through venipuncture, following disposal of 4mL of blood and Sysmex XP-300™, the automated hematologic analyzer was used to evaluate complete blood count (CBC) samples.

Data collection

Demographic, epidemiological history, comorbidities (diabetes, cardiovascular disease, pneumonia) and disease outcome data were taken from medical records. Demographic data of patients including age and gender was noted. Six complete blood count parameters including white and red blood cell counts, hemoglobin and lymphocytes levels, neutrophils and platelet count were compared.

Viral extraction and RT-PCR

Extraction of RNA from nasopharyngeal swab samples was performed by SYSTAAQ SuperExtract NA Universal Auto extraction Kit (SYSTAAQ Diagnostic Products, USA. Catalog # 66205) using Lab-Aid instrument according to manufacturer's protocol. 200 ul of the sample was transferred into a tube containing 200 ul lysis buffer (Poly A solution), followed by the addition of binding buffer. To allow binding of RNA to silica surface, lysates were added with magnetic beads. Then containments were quickly washed away using wash buffers W1 and W2 in a series of washing steps and elution buffer was used to elute pure RNA.

Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) (Kogene biotech Co. Catalog#IR6902) by standard protocol was used for Qualitative RT-PCR. Briefly, 15uL of 2019-nCoV-PCR master mix for one sample was prepared by combining 10μL of 2019-nCoV-PCR mix with 5μL 2019-nCoV-PCR-Enzyme mix. 15μL of master mix was added into each well. The wells were covered and plate was transferred to the sample processing area. 10μL of the extracted RNA was added to the well pre-filled with reagent mix in the following order: 2019-nCoV-PCR-Negative Control, patient sample (s), and 2019-nCoV-PCR-Positive Control. Each well was covered, centrifuged at 12000 rpm for 3 minutes, and placed into Applied Biosystems ABI. PCR conditions were one cycle each for reverse transcription at 50˚C for 30min and melting of cDNA at 95˚C for 1min. 40 cycles of PCR were performed, each including denaturation (95˚C for 15sec), annealing, extension and fluorescence collection (60˚C for 1min). Once PCR is completed, instrument was cooled at 25˚C for 10sec.

To test 2019-nCoV nucleic acid, the FAM (ORF-1 ab region), Cy5 (IC) and JOE (E gene) channels were chosen. Amplification curve of negative control was adjusted to be flat or below threshold. Depending on Ct value, the results were classified as positive or negative. Sample was considered as positive if there was typical S-shape amplification curve detected at FAM and/or JOE channel, and the amplification curve which is detected at cy5 channel(IC), Ct ≤38. For negative sample, there was no typical S-shape amplification curve (No Ct) or Ct >38 detected at FAM and JOE channel, and the amplification curve which is detected at cy5 channel (IC), Ct ≤38. The Ct value for each sample was also recorded for RNA quantification.

Data analysis

Continuous variables were presented as mean and standard deviation. To compare the differences between the two groups the independent sample t-test or Mann-Whitney U-test was used. Statistical analysis was done on (SPSS 22.0, IBM). P-value of less than 0.05 was considered statistically significant.

Ethical approval

Study was authorized by Institutional Ethical Review Committee of Jinnah Hospital, Lahore with Ref No: 118/05/08/2021/S2 ERB. Participants were given an explanation on the purpose of research and those willing to take part in the investigation signed a consent form. Nasopharyngeal swab and blood samples were then taken in accordance with SOPs accepted by ethical review board of the institution.

Results

The 100 COVID-19 cases included 58 men and 42 women, with 100 controls consisting of 39 men and 61 women (mean age, 52 years). Mean and standard deviation of Covid-19 positive patients and controls was reported (Table 1). *p-value comparing these two groups is calculated by independent *t*-test or Mann-Whitney U test and the significance level is 0.05 where p>0.05 (non-significant), p<0.01 (statistically significant) and p<0.001 (highly significant). Average of six variables including white blood cells, red blood cells, haemoglobin, lymphocytes, neutrophils and platelets of control and covid-19 patients was measured as depicted in graph (Figure 1).

Majority of the patients had comorbidities at presentation, which included hypertension (24/60), diabetes (14/60), asthma (7/60), obesity (6/60), pneumonia (5/60) and ischemic heart disease (4/60)

Table 1: Complete blood count profile of covid-19 positive patients and controls (n=200).

Variables	Covid-19 positive (n = 100)	Control (n = 100)	p-value*
WBC	15.04 ± 10.8	8.96 ± 5.51	0
RBC	4.31 ± 0.61	4.94 ± 0.59	0
Hemoglobin	11.7 ± 2.08	11.5 ± 1.53	0.351
Lymphocytes	11.9 ± 8.49	33.3 ± 8.8	0
Neutrophils	82.1 ± 11.4	60.5 ± 11.07	0
Platelets	218.9 ± 86.9	284.8 ± 91.6	0

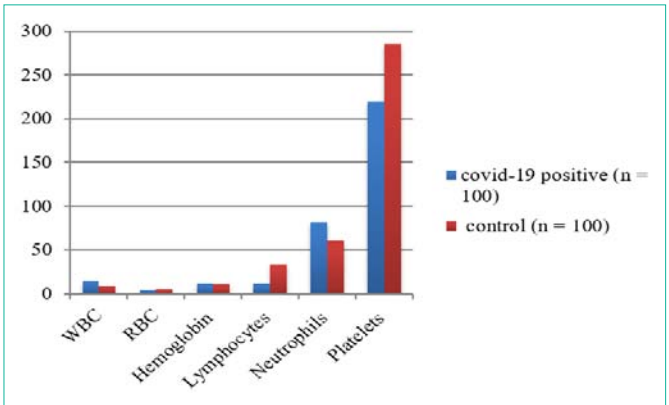

Figure 1: Graph between average of CBC count values including white blood cells, red blood cells, haemoglobin, lymphocytes, neutrophils & platelets of control and Covid-19 patients.

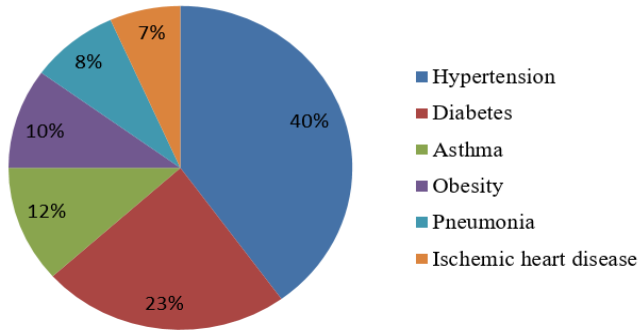

Figure 2: Pie chart showing percentages of comorbidities in Covid-19 patients.

as shown in Figure 2.

Patients with concomitant illnesses had increased WBC (15.04 ± 10.8), hemoglobin (11.7 ± 2.08), neutrophils (82.1 ± 11.4) and lower RBC (4.31 ± 0.61) levels, lymphocytes (11.9 ± 8.49) and platelets (218.9 ± 86.9) according to our findings. The failure of the bone marrow to create enough RBCs to carry oxygen and lung damage caused by COVID-19 which makes gaseous exchange problematic, explains the abnormalities of hemoglobin, RBC seen in individuals with comorbidities. We also noticed a statistical variation in RBC, hemoglobin, neutrophils, and lymphocyte levels across gender groups, which could be attributable to the fact that these parameters are lower in females and RBCs are more quickly impacted by illnesses in females compared to males.

The box-whisker plots for these parameters are presented in Figure 3(Legend: Box-whisker plots of CBC parameters in which combined assessment of variables for baseline values between covid-19 patients and control group show range of variance. The lines exhibit variation beyond the upper and lower limits with random outliers. Each figure contains a central line that depicts the range of dispersion of values and in the composite plots there is variation in all variables of complete blood count).

Discussion

COVID-19 was first reported in Wuhan, China, in December 2019 and has easily expanded and transformed into a disease outbreak in just a few months [13]. The pathogenesis of this virus is unknown; but, SARS-CoV-2, the probable cause of death, has 80 percent genetic similarity to SARS-CoV [14,15].

WHO has approved Real-Time Polymerase Chain Reaction (RT-PCR) assays for COVID-19 screening and treatment [16]. The Open Reading Frame 1 (ORF1), N and E genes are used to amplify in the majority of commercial RT-PCR experiments [17]. The Cycle threshold (Ct) value, which is used to indirectly quantify the viral load, is used to classify COVID-19 based RT-PCR procedures as positive or negative [18].

Similar to our research, COVID-19 positive patients had

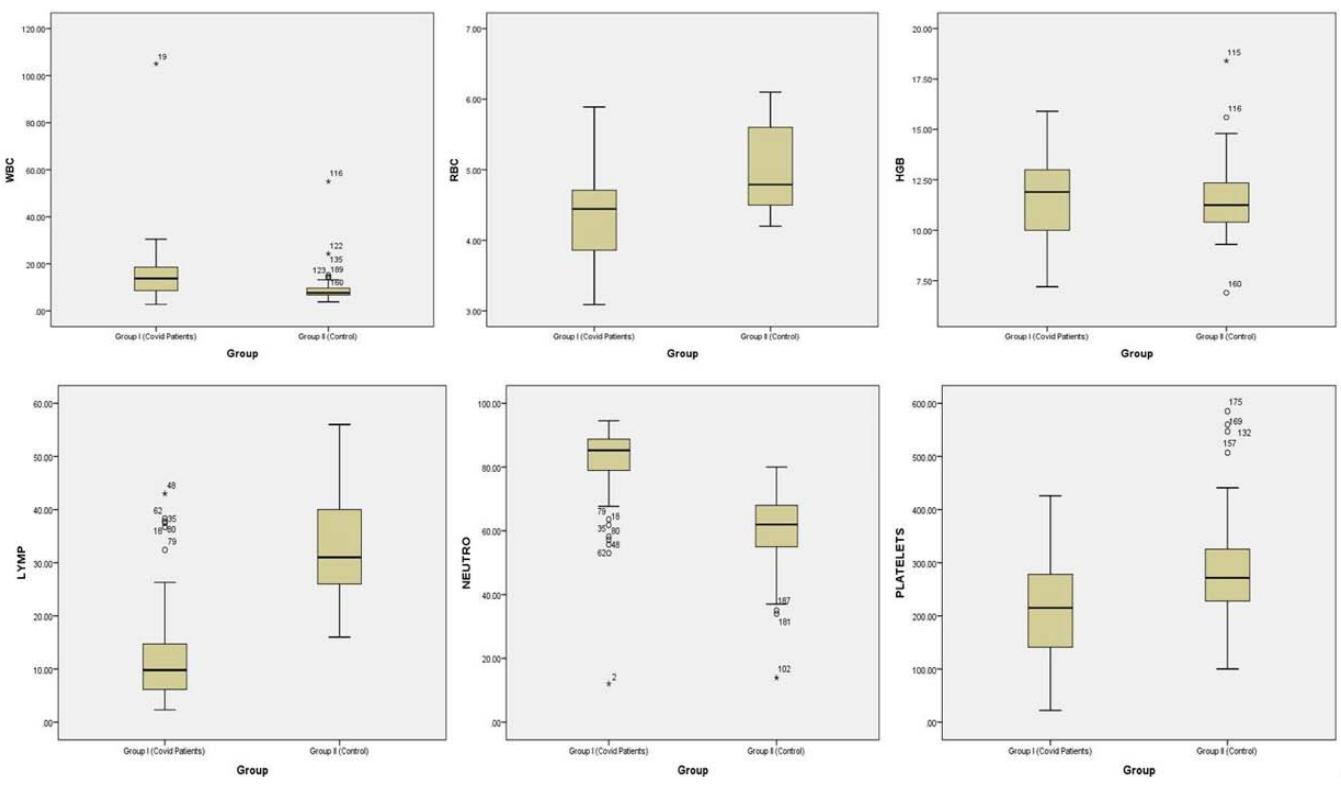

Figure 3: Box-whisker plots of CBC parameters in which combined assessment of variables for baseline values between covid-19 patients and control group show range of variance. The lines exhibit variation beyond the upper and lower limits with random outliers. Each figure contains a central line that depicts the range of dispersion of values and in the composite plots there is variation in all variables of complete blood count.

considerably greater haemoglobin levels compared to COVID-19 negative patients [19]. In investigation by Assiri et al., the low blood platelet count was shown [20].

According to Yang et al. lymphopenia was found in 80% of serious COVID-19 patients [21], while Chen et al. found that only 25% of patients with moderate COVID-19 had lymphopenia, implying that lymphopenia may be related to illness severity [22].

Reduced level of platelets, WBC, and neutrophils were seen in COVID-19 positive patients in a research, which is also similar to earlier findings. As a result, thrombocytopenia, leukopenia, and neutropenia may be signs of COVID-19 infection [23].

According to a study by Usul et al. a complete blood count test revealed low levels of WBC, neutrophils, platelets, and haemoglobin, which was helpful in determining the initial diagnosis of COVID-19 [24].

Furthermore, another study conducted by Mardani et al. showed the number and percentage of WBC, lymphocytes, and neutrophils were significantly different between positive and negative cases for COVID-19. Patients with positive RT-PCR COVID-19 had lower WBC and lymphocytes levels than the normal range, but higher neutrophils counts [25].

Conclusion

SARS has indeed spread over the world, and its increased viral infection is concerning. In the future management of this syndrome, an accurate, quick screening test based on blood samples or nasopharyngeal aspirates is essential. Patients with COVID-19 must have a daily CBC check for numerical and morphologic alterations that could indicate a poor prognosis and disease progression. The measures to prevent such fatal virus are early detection, rapid isolation, and adequate treatment.

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