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Original Research Article

HEMATOLOGICAL PARAMETERS IN COVID-19 AND THIER ASSOCIATION WITH SEVERITY: A CROSS SECTIONAL STUDY

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Abstract

Background: To date, the Coronavirus illness 2019 had affected approximately 100 million people globally. COVID-19could cause a variety of symptoms that led to disease manifestation, ranging from mild and moderate cases to a life threatening illness requiring critical care support. In spite of substantial research, the relevance of numerous haematological and biochemical indicators in prognosis remains unknown. Hence this study was undertaken to analyse the haematological parameters in COVID- 19 infection and its association with disease severity. The aim is to compare haematological parameters between COVID-19 positive patients and apparently normal Reverse Transcription-Polymerase Chain Reaction (RT-PCR) negative individuals, and to assess their association with COVID-19 severity. **Materials and Methods:** A cross-sectional study was conducted in isolation wards and Intensive Care Units (ICUs) at a Tertiary Care Hospital in Government Medical College Manjeri, Kerala, India. The study was conducted over six months, from August 2020 to January 2021. A total of 70 patients diagnosed with COVID-19 and 35 RT-PCR negative healthy subjects, aged between 18-75 years, were included. Haemoglobin, total count, erythrocyte sedimentation rate and neutrophil to lymphocyte ratio were measured and compared between COVID-19 patients and apparently normal RT-PCR negative individuals. The quantitative parameters were evaluated using the Mann-Whitney U test, and the categorical values were analysed using the Chi-square test. Statistical significance was defined as a p-value of ≤0.05. **Result:** All COVID-19 patients were between the ages of 18 and 75 years, with a mean age of 55.03±13.01 years in symptomatic COVID-19 positive (group A), 57.89±10.85 years in COVID-19 positive with pneumonia (group B), and 40.89±15.89 years in the control group (group C). In group A, there were 18 (51%) males and 17 (49%) females, while in group B, 23 (66%) were males and 12 (34%) were females. Out of the 35 control group participants, 17 (49%) were males and 18 (51%) were females. No significant differences were reported when comparing the genders of patients with healthy subjects. The results of the present study showed significantly altered median values of total count (1962.50) N/L ratio (9.40) and ESR (30) in COVID-19 patients, as compared to non-COVID-19 individuals, which were 7170, 1.82 and 14 respectively (p-value<0.001). Also significant difference can be noticed in N/L ratio between symptomatic COVID which is 7.10(4.75, 11.10) and 10.87(7.45, 18) in patient with pneumonia. **Conclusion:** Altered levels of total count, N/L ratio and ESR in COVID patients suggested that they could be used for the early detection of the disease. Moreover, higher levels of N/L ratio in individuals with severe symptoms, as opposed to those with mild symptoms, defined their involvement in illness severity.

INTRODUCTION

In 29 December 2019, a series of acute atypical respiratory disease was occurred in Wuhan, China which rapidly spread to other areas. It was soon identified that a novel coronavirus was responsible for this. Due to its high homology to SARS-CoV, which caused acute respiratory distress syndrome (ARDS) and high mortality during 2002–2003, the novel coronavirus was named as the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2, 2019-nCoV). The initial outbreak of SARS-CoV-2 was started as a zoonotic transmission associated with the seafood market in Wuhan, China, which later became human to human transmission in the subsequent outbreak The disease caused by this virus was named as Coronavirus disease 19 (COVID-19) and a pandemic was declared by the World Health Organization (WHO). It is now widely recognized that respiratory symptoms of COVID-19 are extremely heterogeneous, ranging from minimal symptoms to significant hypoxia with ARDS. Early diagnosis is vital when we consider the short time of onset of acute respiratory distress syndrome after admission to hospital and the high mortality rates in COVID-19.[1]

Disturbance of the immune system in patients had been considered as one of the hallmarks for COVID-19.An effective immune response against SARS-Cov-2 required innate immune system with granulocytes, monocytes and macrophages and adaptive immune system with B and T cells. Tissue resident immune cells recognize viral infection and resulted in local immune response leading to recruitment of further innate immune cells and viral clearance. This handling of viral infection in the lung itself with active immune response prevents further viral dissemination in the lung and pulmonary injury.[2] The majority of the infiltrating adaptive immune cells were T cells, most likely primary cytotoxic T cells, which could kill viruses but also contributed to lung harm. Circulating monocytes responded to GM-CSF produced by pathogenic T cells. Patients with severe illnesses had higher plasma concentrations of proinflammatory cytokines such as IL-6, IL-10, monocyte chemoattractant protein1 (MCP1), granulocyte-colony stimulating factor (G-CSF), tumour necrosis factor (TNF)- and macrophage inflammatory protein (MIP)1. GM-CSF could help to differentiate innate immune cells and improve T cell activity, but in high doses, it could cause tissue damage. Another intriguing result was the presence of aberrant pathogenic CD4+ T cells co-expressing granulocyte-macrophage colony-stimulating factor (GM-CSF) and interferon (IFN) in COVID-19 patients with severe illness.It was showed that virus infected lung epithelial cells produced IL-8 in addition to IL-6 which was a well-known chemo attractant for neutrophils and T cells.[3]

Among innate immune cells, we expected the majority to be neutrophils. Neutrophils could act as double-edged weapon as neutrophils could induce lung injury. An increase in the circulating neutrophil versus decrease in lymphocytes was a hall mark of severe COVID 19.The reason for lymphopenia in severe illness remains unclear. It had been postulated that this association might be due to direct lymphocyte infection, destruction of lymphatic tissue, inflammation leading to lymphocyte apoptosis, or inhibition of lymphocytes by metabolic disorders such as lactic acidosis.[4]As a result of the inflammatory process erythrocyte sedimentation rate (ESR) was also found to disrupted in covid-19.[5]It was the time required for RBCs to settle down in the bottom of column of capillary tube per hour.[6] In inflammatory conditions, fibrinogen was abundantly released, causing RBC to form colonies and stick to each other, resulting in a high level of ESR due to increased viscosity of blood, which was also observed in conditions such as renal disease, pregnancy, in females due to menstrual cycle, geriatric, degenerative disease, and some types of cancer.[7]

MATERIALS AND METHODS

A cross-sectional study was conducted in the isolation ward and ICU in a tertiary care hospital at Govt. Medical college Manjeri, kerala, India for a period of six months, from August 2020 to January 2021. The Institutional Ethical Clearance was obtained [IEC number IEC/GMCM/48] and written informed consent was obtained from all the subjects.

Inclusion Criteria

All symptomatic patients aged between 18 to 75 years, who tested positive for COVID-19 were included. Symptoms include fever and/or sore throat or cough. Those presenting with fever and/or respiratory symptoms, spO2<94% and chest x-ray findings suggestive of lung infection were diagnosed as having COVID-19 pneumonia.[8] Subjects, aged between 18 and 75 years, who were admitted for diseases other than COVID-19 and RT-PCR negative were chosen as controls.

Exclusion Criteria

Those with associated co-morbidities such as uncontrolled diabetes, hypertension, or heart disease, were excluded from the study population and comparison group.

Sample Size: Considering the power of 80% and an error of 5%, based on the previous study,[9] sample size calculated was 33 subjects in each group.

$$n = \frac{(Z\alpha + Z\beta)^2 \, SD^2}{d2}$$

$Z\alpha = 1.96$ for an error of 5%

$Z\beta = 0.84$ for a power of 80%

SD, the pooled standard deviation=13

d,the difference in means=7

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Study procedure

70 COVID patients were divided in to 35 symptomatic COVID patients (group A) and 35 COVID patients with pneumonia(Group B) and compared against 35 RT-PCR negative healthy individuals(Group C).After obtaining written informed consent, 5 ml venus blood was collected in Ethylenediamine Tetraacetic Acid (EDTA) tubes from both cases and control for the assessment of haemetological parameters. Complete blood count was assessed by automated five part haematology analyser. NLR was calculated and further subdivided on the basis of severity in each group as mild (< 3.5), moderate to severe (3.5- 5).

Statistical Analysis

IBM SPSS version 25 was used to analyze the data. Since data were not normally distributed they were presented as median. The quantitative parameters were evaluated using the Man Whitney U test, and the categorical values were analyzed using chi-square test. A p-value of <0.05 was considered to be statistically significant.

RESULTS

All the 70 COVID patients were aged between 18-75 years with a significantly higher mean age as compared to healthy individuals [Table/Fig 1]. In group A, there were 18(51%) males and 17(49%) females while in group B, 23(66%) were males and 12(34%) were females. Out of 35 control group 17(49%) were males and 18(51%) were females. No significant differences were reported when comparing gender of patients with healthy people [Table1].

The median values of total count, neutrophil lymphocyte ratio and ESR were significantly altered in COVID patients,as compared to healthy individuals [Table2].There were no significant differences when comparing haemoglobin values in COVID12.50(10,13.45) with 12.40 (12,13) in nonCOVID.

We also compared these parameters between groups A & B, only significant difference could be seen in N/L ratio. There was no statistically significant difference in haemoglobin, total count, or ESR between groups A and B [Table3].

While categorising COVID patients using chi-square test, on admission it showed that N/L ratio of 8(18%) COVID patients belonged to mild and 62(100%) to moderate to severe category whereas 35(78%) of healthy people belonged to mild category might be due to some nonCOVID respiratory tract infections and this difference between cases and controls was found to be statistically significant [Table4]. Also 39(95%) of COVID patients had leucopenia, 27(48%) had normal count and 4(50%) had leucocytosis which was significant when compared to group C, which had 2(5%), 29(52%) and 4(50%) respectively[Table5].In addition 49(96%) of COVID patients had high ESR ,21(39%) had normal ESR against 33(61%) normal and 2(4%) high ESR in healthy group [Table6]. Based on Hemoglobin values, 31(57%) had normal level of Hb, 29(73%) had mild to moderate anemia and 10(100%) had severe anemia but in RT-PCR negative group, 24(43%) had normal level of Hb and 11(27%) had mild anemia might be due to some nutritional deficiency, but none of them suffering from severe anemia,this difference between cases and controls was found to be statistically significant [Table7].

Table 1: Comparison of Age between group A , B and group C by One way ANOVA and Gender distribution by Chi-square test

Variables	Group A (n=35) Mean ±SD	Group B (n=35) Mean ±SD	Group C (n=35) Mean ±SD	p-value
Age in years	55.03±13.01	57.89±10.85	40.89±15.89	<0.001
Gender Distribution	n (%)	n (%)	n (%)	
Males	18 (51%)	23 (66%)	17 (49%)	0.303
Females	17 (49%)	12 (34%)	18 (51%)	

Table 2: Comparison of Hemoglobin, Total count, Neutrophil lymphocyte ratio and Erythrocyte sedimentation rate between Covid Positive (group A+B) and normal subjects (group C) on admission using Man Whitney U test

Variables	Groups		p-value
	Group A+B (n=70)	Group C (n=35)	
	Median (Q1, Q3)		
Hb	12.50(10.13.45)	12.40(12,13)	0.301
TC	1962.50(1377,7355)	7170.00(5200,8872)	<.001
N/L	9.40(5.22,15.89)	1.82(1.61,2.21)	<.001
ESR	30.00(20.75,38.25)	14.00(12,18)	<.001

Table 3: Comparison of Hemoglobin, Total count, Neutrophil Lymphocyte ratio and ESR in Symptomatic Covid Positive and covid with Pneumonia patients using Man Whitney U test

Variables	Groups		p-value
	Group A	Group B	
	Median (Q1, Q3)		
Hb	12.5(7.9,14)	12.5(11.1,13.3)	0.860
TC	4700(1630,8320)	1801(1088,6280)	0.083

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N/L	7.10(4.75,11.10)	10.87(7.45,18)	<.001
ESR	28(20,36)	33(21,45)	0.088

Table4: Categorization of COVID patients using Neutrophil Lymphocyte ratio on the day of admission by Chi square test

Group	N/L ratio		χ2	p value
	Mildn (%)	Moderate to Severen (%)		
Group A+B (n=70)	8(18)	62(100)	75.69	<.001
Group C (n=35)	35(78)	0(0)		

Table 5: Categorization of COVID patients using Total count on the day of admission by Chi square test

Group	TC			Chi square	p value
	Leucopenian (%)	Normaln (%)	Leukocytosisn (%)		
Group A+B (n=70)	39(95)	27(48)	4(50)	24.51	<.001
Group C (n=35)	2(5)	29(52)	4(50)		

Table6: Categorization of COVID patients using Erythrocyte sedimentation rate on the day of admission by Chi square test

Group	ESR Level		Chi square	p value
	Normaln (%)	Highn (%)		
Group A+B (n=70)	21(39)	49(96)	38.60	<.001
Group C (n=35)	33(61)	2(4)		

Table7: Categorization of COVID patients using Hemoglobin on the day of admission by Chi square test

Group	Hemoglobin			χ2	p value
	Normaln (%)	Mild to Moderaten (%)	Severen (%)		
Group A+B (n=70)	31(57)	29(73)	10(100)	8.24	.016
Group C (n=35)	24(43)	11(27)	0(0)		

DISCUSSION

COVID19 had recently emerged as one of the leading causes of morbidity and mortality for past few years. Early monitoring of haematological markers was critical for determining the severity of patient's condition and guiding treatment choices. In the current study, there was a substantial difference in age between patients and controls, which might be attributed to a low incidence in young adults and a larger requirement for hospitalisation among middle aged and elderly people compared to young persons.

In general, the elderly were more vulnerable to infections. Persistent viral infections might cause monoclonal T cell growth, resulting in low diversity of memory T cells over time. Because of the decrease in T-cell diversity, this eventually led to immunological fatigue.[10]Mechanical lung defenses such as cough, the barrier function of the mucus and epithelium, and mucociliary clearance in conjunction with the innate immune system aid in the clearance of aspirated or inhaled substances including infectious agents. However, these actions had been shown to decline with age. The severity of the respiratory infection might be associated with these age-related changes in the physical features of the lung as well as a loss in immunological function, known as immunosenescence.[11]Santesmasses et al,[12] also said that SARS-CoV-2 was known to preferentially impact the elderly and those with pre-existing problems due to an increased age-related expression of angiotensin converting enzyme 2 (ACE2) in the lungs of individuals.SARS-CoV-2 infects human cells by affixing its membrane spike (S) protein to ACE2. The depletion of antiviral defences, combined with an age-related rise in the expression of this gene, causes corona virus to cause more damage in the lung. Apart from these, organs differing ACE2 gene expression levels were found to be responsible for problems other than pneumonia, such as diarrhoea in COVID-19-positive patients. However, while ACE2 specifically promotes SARS-corona virus infections, it also protects the lungs from harm, as proposed by Monteil et al.[13]

Our findings were also consistent with recent publications by Guan, W.J. and coworkers.[14] SARS-CoV-2 infection was linked with considerable leucopenia and an enhanced neutrophil lymphocyte ratio when compared to controls. When exposed to stress, leukocytes in the bloodstream increase neutrophils while decreasing lymphocytes. Furthermore, it had been postulated that virus-mediated infection consumes T lymphocyte cells, particularly CD4 and CD8 T lymphocytes, resulting in lymphopenia as supported by Jafarzadeh et al,[15] and that the ratio of these two results might be employed as an inflammatory measure. Furthermore; lymphopenia was thought to be caused by a decrease in bone marrow haematopoiesis.[16] The higher value of N/L in patients with pneumonia in our study suggested that it might be utilized as a blood test to assess the severity in COVID-19, which was corroborated by Wang C and colleagues.[17] Moreover Liu et al. [18] identified

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N/L ratio as a risk factor for COVID-19 mortality in hospitalised patients. Since patients with COVID-19 pneumonia had a normal, a low, or a high leukocyte count, assessing the neutrophil-to-lymphocyte ratio can be utilized as a better biomarker to predict the outcome of an infection.[19] In addition, unlike Ahmed M. E. Elkhalifa et al,[20] no substantial anaemia was observed in our study. The majority of coronavirus nonstructural viral proteins were unlikely to had access to significant amounts of hemoglobin, but rather they localised in infected cells where they played important roles in RNA replication.[21] There was no evidence that the virus infects erythrocytes, which had heme concentrations of 15-20 mM, and these highly specialised cells lack the cellular machinery required to create viral proteins.[22] As a result, intraerythrocytic haemoglobin was likely immune to viral proteins. Although Al-Kuraishy HM and collegues,[23] speculated that viral proteins and haemoglobin might interact in the plasma following immunological hemolysis, considerable hemolysis had not been recorded in COVID-19 patients,[24,25] and we did not observe significant anemia in our patients .However, we found a high ESR, an inflammatory marker that is elevated in COVID-19 due to the inflammatory process.

Limitations

A complete laboratory score consists of hematological, inflammatory and biochemical parameters must be taken into consideration along with the clinical aids to diagnose and predict the severity of COVID-19 patients. In the present study, only hematological parameters were used as an attempt to assess the severity of the disease. As this study was based on a small sample size there may be some limitations and the authors suggest that future research should be conducted on a larger population

CONCLUSION

The present study concluded that total count, ESR and N/L ratio were altered in patients with COVID-19 compared to individuals who tested negative for RT-PCR. Therefore, they could be used for early detection and isolation of suspected patients, which was crucial in controlling the COVID-19 outbreak. Additionally, the level of N/L ratio was higher in patients with pneumonia compared to those with mild symptoms rendering it a better marker of disease severity so that more research was needed to determine the independent predictive role of N/L ratio in the disease process.

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