Original Article

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Pathology Section

Neutrophil Lymphocyte Ratio and Serum Ferritin Levels in COVID-19: A Cross-sectional Study

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ABSTRACT

Introduction: Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2) causing Coronavirus Disease (COVID-19) has challenged the world. A complete blood workup as well as continuous tracking of haematological parameters play a vital role in revealing the risks of disease progression and eventually help in better treatment and outcome.

Aim: To access the haematological parameters {Complete Blood Count (CBC), Neutrophil Lymphocyte Ratio (NLR) and Serum Ferritin (S. ferritin) levels} in COVID-19 patients to correlate its association with the severity of the disease.

Materials and Methods: The present study was a cross-sectional study in which 200 patients who were confirmed as COVID-19 positive by real time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) in the month of July-August 2020 were included. Blood was collected from patients of COVID-19 using the routine methods and was evaluated for CBC and S. ferritin levels. Neutrophil Lymphocyte Ratio (NLR) was also calculated.

Results: The NLR was positively correlated with severity of COVID-19. Patients with higher NLR levels were admitted to Intensive Care Unit (ICU) because of severity in their condition. In the present study, 42 of 47 (89.4%) ICU patients had S. ferritin levels >1000 ng/mL whereas only 8 (5.2%) of non ICU patients had S. ferritin levels >1000 ng/mL.

Conclusion: The NLR and S. ferritin positively correlated with the severity of COVID-19 disease.

Keywords: Complete blood count, Coronavirus disease-2019, Serum inflammatory markers, Severe acute respiratory syndrome 2

INTRODUCTION

The SARS-CoV-2 causing COVID-19 has rapidly evolved around the world. The disease has infected more than 100 million individuals all over the world, whereas billions of other individuals have been affected by measures of social distancing and the socio-economic impact of the pandemic [1]. Tracking haematological parameters plays a vital role in revealing the risks of disease progression and can eventually help in better treatment and outcome. Blood tests have an important role in early diagnosis of the disease [2]. The S. ferritin is an iron storage protein that is widely measured as an indicator of iron status, but it also a well-known inflammatory marker. The S. ferritin levels can be increased significantly in response to inflammation. The S. ferritin level has been reported in COVID-19 patients at risk and a predictor for Acute Respiratory Distress Syndrome (ARDS) [3] and was correlated with the degree of systemic and pulmonary inflammation, it is reasonable that hyperferritinaemia is associated with disease severity in patients with COVID-19 [4]. Hence, the present study was done with an aim to access haematological parameters in COVID-19 patients and to correlate its association with the severity of the disease.

MATERIALS AND METHODS

Present study was a cross-sectional study of laboratory confirmed cases from July-August 2020 carried out on 200 patients who were confirmed as COVID-19 positive by real-time-PCR. During the study, the Declaration of Helsinki ethical principles for medical research was followed and patients' anonymity was maintained. Patients from whom a throat swab was obtained and those who were thereafter hospitalised with an initial diagnosis of COVID-19 were studied. Only patients above 18 years of age were included in the study. Inclusion and exclusion criteria are described in [Table/Fig-1]. Both ICU and non ICU patients were included in the study.

Blood was collected from patients with COVID-19 after informed consent using the routine methods and were evaluated for haematological parameters. The blood samples were run in the Central laboratory of Doon Hospital, Dehradun, Uttarakhand by the laboratory technician and the CBC and S. ferritin results obtained were studied and approved by a Consultant Pathologist. The CBC was determined by using Beckman LH750 haematology analyser and S.ferritin levels (reference range 30-400 ng/mL) were determined using Cobas e411 analyser.

Inclusion criteria	Exclusion criteria
Clinically diagnosed COVID-19 (SARS-CoV-2 positivity in real time-polymerase chain reaction)	Age <18 years
	Anticoagulant medication
	Participants with hypersensitivity or intolerance or contraindication to the use of standard treatment
	History of having received any investigational drug in the preceding one month
	History of taking any kind of formulation or any other form of therapy for COVID-19 prophylaxis

[Table/Fig-1]: Inclusion and exclusion criteria.

STATISTICAL ANALYSIS

Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) version 21.0. The p-value of <0.05 was considered to be statistically significant.

RESULTS

Demographic and haematological details are mentioned in [Table/Fig-2,3]. A total of 141 141 (71%) patients were males and 59 (29%) were females. There were 88 (44%) cases in the age group of 18-35 years, 47 (23.5%) cases in the age group of 36-50 years and 65 (32.5%) cases were above 50 years of age. Of the 200 patients, 47 (23.5%) patients were severely ill and were admitted to ICU while 153 (76.5%) patients were moderately ill clinically and were admitted to general ward. The mean age for patients admitted to ICU was 51 years while the mean age of patients admitted to wards was 39 years.

Parameters	Patients admitted in ICU (N=47)		Patients admitted in Non ICU (N=153)	
	Male/ Number (%)	Female/ Number (%)	Male/ Number (%)	Female/ Number (%)
Symptoms				
Sore throat	10/31 (32.26)	5/16 (31.25)	29/110 (26.36)	14/43 (32.56)
Shortness of breath	31/31 (100)	15/16 (93.75)	20/110 (18.18)	3/43 (6.98)
Co-morbidities				
Chronic respiratory disease	28/31 (90.32)	11/16 (68.75)	14/110 (12.73)	2/43 (4.65)
Cardiovascular disease	20/31 (64.52)	8/16 (50)	21/110 (19.09)	9/43 (20.93)
Diabetes	30/31 (96.77)	12/16 (75)	25/110 (22.73)	2/43 (4.65)
Chronic kidney disease	3/31 (9.68)	1/16 (6.25)	5/110 (4.55)	1/43 (2.33)
Chronic underlying conditions				
Obesity	12/31 (38.71)	10/16 (62.5)	09/110 (8.18)	7/43 (16.28)
Hypertension	10/31 (32.26)	6/16 (37.5)	34/110 (30.90)	10/43 (23.26)
Cancer	1/31 (3.23)	0/16 (0)	0/110 (0)	0/43 (0)
Liver disease	4/31 (12.90)	1/16 (6.25)	3/110 (2.73)	0/43 (0)
Haematological parameters	**ICU**	**Non ICU**	**p-value (Student's t-test)**	
Total Leukocyte count (10^9/L; mean±SD)	13166±2743	6356±6126	<0.001*	
Neutrophil (10^9/L; mean±SD)	87.58±12.02	68.14±8.22	<0.001*	
Lymphocyte (10^9/L; mean±SD)	9.02±10.78	25.51±6.88	<0.001*	
Eosinophils (10^9/L; mean±SD)	1.76±1.55	2.77±0.86	<0.001*	
Monocyte (10^9/L; mean±SD)	1.62±2.86	3.54±1.19	<0.001*	
Serum Ferritin (10^9/L; mean±SD)	1494±374.15	298.22±561.86	<0.001*	
Lymphopenia (Reference range 18-45% of total Leukocytes)	10.628±3.38	7.6±4.23	<0.001*	
Neutrophil Lymphocyte ratio (NLR)	13.79±4.02	4.09±3.88	<0.001*	

[Table/Fig-2]: Demographic and haematological details of COVID-19 patients.
*statistically significant

Severity of disease	Number of patients (%)			p-value
ICU patients	47 (23.5)			<0.001*
Non ICU patients	153 (76.5)			
Age range (years)	**18-35**	**36-50**	**>50**	Kruskal-wallis test
Number of patients (%)	88 (44)	47 (23.5)	55 (32.5)	
Leucocytosis number (%) Reference value: >11.0×10^9/L	8 (9)	7 (14)	27 (42)	>0.05
Leukopenia number (%) Reference value: <4.00×10^9/L	9 (10)	1 (2)	2 (3)	>0.05
Neutrophilia number (%) Reference value: 2.5-7.5×10^9/L	49 (55)	23 (49)	50 (77)	>0.05
Lymphopenia number (%) Reference value: <1,500/µL	35 (39)	17 (36)	42 (65)	>0.05
Serum ferritin (>1000 ng/mL) number (%) Reference value: 30-400 ng/mL (cobas e411)	27 (30.6)	6 (12.7)	34 (52.3)	<0.01*

[Table/Fig-3]: Basic clinicpathological findings, haematological parameters and Serum ferritin levels in COVID-19 patients.
*statistically significant

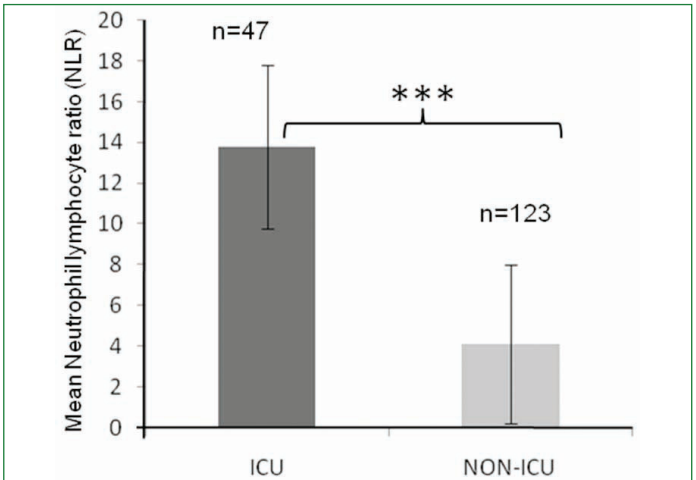
In the age group of 18-35 years, out of 88 cases, leucocytosis was observed in 8 (9%) cases, leukopenia in 9 (10%) cases, neutrophilia in 49 (55%) cases and lymphopenia was observed in 35 (39%) cases. S. ferritin level was less than 500 ng/mL in 53 (60.2%) cases, 500-1000 ng/mL in 8 (9%) cases and in 27 (30.6%) cases the S. ferritin levels were above 1000ng/ml. Out of 47 patients, leucocytosis was observed in 7 (14%) cases (age group of 36-50 years) and leukopenia in 1 (2%) case, neutrophilia in 23 (49%) cases and lymphopenia was observed in 17 (36%) cases. In 32 (68%) cases, S. ferritin levels were below 500 ng/mL, in 9 (19.1%) cases S. ferritin levels were between 500-1000 ng/mL whereas in 6 (12.7%) cases the S. ferritin levels were above 1000 ng/mL.

Of the 65 cases in the age group above 50 years, leucocytosis was observed in 27 (42%) cases, leukopenia in 2 (3%) cases, neutrophilia in 50 (77%) cases and lymphopenia was seen in 42 (65%) cases. Of the 47 cases admitted in ICU, it was observed that 36 (77%) cases had leucocytosis and all 47 cases (100%) cases had neutrophilia and corresponding lymphopenia. In contrast, out of 153 moderately ill non ICU patients leucocytosis was seen in only 9 (5%) cases, leukopenia was seen in 12 (7%) cases, 70 (46%) cases showed neutrophilia and 49 (32%) cases had lymphopenia. Out of the 47 critically ICU patients, in 42 (89.4%) cases S. ferritin levels were above 1000 ng/mL, in 5 (10.6%) cases levels were between 500-1000 ng/mL. All the cases had S. ferritin level more than 500 ng/mL. In non ICU patients the level of S. ferritin was observed to be below 500 ng/mL in 125 (81.6%) cases, between 500-1000 ng/mL in 20 (13%) cases and in only 8 (5.2%) cases the level were above 1000 ng/mL [Table/Fig-4].

Serum ferritin (ng/mL)	ICU patients {n=47; number (%)}	Non ICU patients {n=153; number (%)}	p-value Chi-square test
<500	0 (0)	125 (81.7)	<0.001*
500-1000	5 (10.6)	20 (13.1)	<0.001*
>1000	42 (89.4)	8 (5.2)	<0.001*

[Table/Fig-4]: Serum Ferritin levels in ICU and Non ICU patients.
*statistically significant

A positive correlation between was observed NLR and severity of COVID-19. Patients with normal NLR levels (1-3) were concentrated in wards whereas those with higher NLR levels was admitted to the ICU because of severity in condition [Table/Fig-5].



[Table/Fig-5]: Mean Neutrophil Lymphocyte Ratio (NLR) in COVID patients admitted to ICU and non ICU.

Also, neutrophil was positively correlated to ferritin (1 unit increase in neutrophil caused 0.1 unit rise in ferritin) and lymphocytes negatively correlated to ferritin; one unit increase lymphocyte causes- 0.14 units decrease in ferritin. On applying logistic regression with increase in lymphocyte by one unit probability of being in ICU decreases by 0.1%. Similarly, increase in ferritin by 1 unit increases probability of being in ICU by 0.2%. Two-way ANOVA results for ward/ICU patients across ferritin (p=0.002) and neutrophil (p=0.0003) categories exhibits significant differences in admission of ward and ICU patients.

DISCUSSION

Haematological parameters were reviewed and S. ferritin levels in 200 RT-PCR positive COVID-19 patients upon hospital admission. In the present study, males (71%) patients were more affected than females (29%). Earlier study has also shown that 56% of the male patients were affected with COVID-19 [5]. Severely ill patients admitted to the ICU were presented with leucocytosis with significantly higher neutrophil counts and lower lymphocyte counts as compared to the moderately ill non ICU patients. All the patients admitted in ICU showed significant neutrophilia as compared to the non ICU patients (46%). Previous study has reported increased neutrophil counts in severe COVID-19 patients as compared to the patients with the other milder groups [6]. Present study showed that the patients admitted in the ICU showed lymphopenia as compared to the non ICU (32%) patients. In a retrospective study including 52 critically ill patients from Wuhan, China, lymphopenia was reported in 85% of the patients [7].

The NLR was positively correlated with severity in COVID-19. Patients with normal NLR levels (1-3) were admitted in wards whereas those with higher NLR levels had to be admitted to ICU because of severity of disease. Similarly, NLR was significantly higher in the patients in severe group (6.6 versus 3.3) than in the mild group [8]. NLR was the most useful factor affecting the incidence of severe COVID-19 infection and indicated the incidence of severe illness with NLR >3.13 [9]. In the present study, 42 (89.4%) out of 47 patients admitted in ICU had S. ferritin levels >1000 ng/mL. On the other hand out of 153 non ICU patients only 8 (5.2%) had S. ferritin levels >1000 ng/mL showing a positive correlation between severity of severity of disease with S. ferritin levels. Similar findings were reported where individuals with severe and very severe COVID-19 exhibited increased S. ferritin levels (1006.16 ng/mL versus 291 ng/mL respectively) [10]. Thus, present study observations lead to the conclusion that S. ferritin levels are closely related to the severity of COVID-19 disease.

Limitation(s)

The limitations of present study were that the study included COVID-19 patients from Dehradun, Uttarakhand only. The subsequent follow-up of patients was not available. Authors recommend further studies regarding the haematological abnormalities in COVID-19 to identify the actual mechanism of haematological alterations. In-depth statistical analysis needed to explore the maximum risks associated with the pathology.

CONCLUSION(S)

The present study investigated haematological parameters of 200 COVID-19 patients. In COVID patients, NLR and S. ferritin level showed positive correlation with the severity of COVID-19 disease. Our recommendation is that clinicians closely monitor the parameters in hospitalised patients with respiratory distress related to COVID-19 for better management to control the risk of disease.

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