



TO STUDY THE SIGNIFICANCE OF VARIOUS BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS IN COVID CASES IN INDIA IN 2ND WAVE OF COVID-19 PANDEMIC: STUDY FROM PUNE, MAHARASHTRA

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ABSTRACT COVID-19 infection is an ongoing pandemic. The world has already witness two waves and a third one is imminent. Also the COVID 19 infection is now presenting with numerous manifestation besides respiratory symptom. Thus it becomes essential to study surrogate markers or various biochemical and hematological parameters and their significance in these cases. Data available so far is mostly from other nations with only a limited number of studies from Indian context. Thus the present study was conducted to evaluate the significance of laboratory parameters in predicting cases infected with COVID 19 and also the correlation of these parameters with gender and course of disease. 557 subjects were included in this study from Pune, Maharashtra. The groups were divided into cases and controls on basis of RT PCR results and clinical manifestations. The control group had 442 subjects and the cases were 135 in number. The inflammatory markers (CRP, D-dimer and ferritin) were significantly high in cases as compared to controls. Among cases 40 % patients belonged to young age (<45 years), 38% to middle age (45 -59 years) and 22 % to elderly group (>60 years). This shows high infectivity rate in younger group as compared to 1st wave. NLR was higher in cases as compared to controls. CRP, D-dimer and ferritin were significantly higher in cases as compared to controls.

KEYWORDS :

INTRODUCTION

In December 2019, the country office of WHO in China was informed about a cluster of cases of pneumonia of a previously unknown etiology in Wuhan, Hubei Province, China (1). Subsequent laboratory evaluation identified that a new-type coronavirus had caused the pneumonia (2). This newly identified virus, was later found to be related to the deadly Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) coronaviruses(3). Ever since the outbreak in December 2019, the virus has rapidly spread across the globe and was declared a pandemic by WHO on 11 March 2020(4). Two waves of COVID pandemic have already occurred and a third wave is imminent.

The clinical manifestations in COVID infection vary from patient to patient as well as in degree of severity. Symptomatic patients usually present with fever, cough, nasal congestion and fatigue (4). Later these patients may progress to pneumonia in 2nd or 3rd week of infection (5). Various studies have been conducted to compare hematological and biochemical parameters in infected and non-infected as well as between mild and severe cases of COVID-19 (6-10). These can serve as surrogate markers to establish the infectivity status of COVID infection. This becomes extremely important with COVID presenting with several other presentations besides respiratory involvement. Also in view of imminent 3rd wave and limited number of studies it becomes essential to study the significance of these parameters in Indian context.

The present study aims to evaluate the significance of laboratory parameters in predicting cases infected with COVID 19 and also the correlation of these parameters with gender and course of disease.

MATERIALS & METHODS

Study design:

Retrospectively analysis data was collected from all samples received in iGenetic diagnostic laboratory for tru raksha package from a period of 1 July 2020 to 31 July 2021(13 months). . Tru raksha package is a health package offered by the company and includes Complete Blood Count (CBC), Lactate Dehydrogenase (LDH) , C-reactive protein (CRP) , Ferritin and D-dimer.

After excluding the subjects as per exclusion criteria of the study, study group was divided in two groups on basis of COVID- RT PCR tests results. RT PCR is a diagnostic test for detection of Corona virus, and is approved by both WHO and ICMR.

Participants:

INCLUSION CRITERIA:

All samples received in laboratory for tru raksha health package during 1 July 2020 to 31 July 2021 were included in the study after excluding the subjects as per exclusion criteria of present study.

EXCLUSION CRITERIA:

Patients with comorbidities (Diabetes, hypertension, cardiac issues etc.)

Sample Collection:

Venous blood was collected from the study groups into Ethylen ediaminetetraacetic acid [EDTA] tubes, SST tube (Serum separator tubes) and sodium citrate tubes.

ESTIMATION OF CBC:

EDTA blood was used to estimate CBC. The test was performed using fully automated analyzer (Beckman Coulter- DXH800); these parameters analyzed included:

RBC parameters- Hb, HCT

WBC parameters- TLC, DLC, Neutrophil count, Lymphocytes count, NLR.

Platelet parameters: Total platelet count, MPV.

Determination of serum D-dimer:

Citrate tube was used to determine D-dimer level. The kit used for analysis was Mispa i2 -Aggape d-dimer quantitative assay, using Mispa i2 analyzer.

Determination of CRP, LDH, ferritin:

The SST tube was centrifuged to obtain serum which is used for the determination of serum concentration of LDH, CRP, and ferritin.

LDH was estimated using Erba LDH-P diagnostic kit using Mispa vivo analyzer, CRP quantitative assay was done using mispa agape quantitative kit and mispa i2 analyzer).

Serum Ferritin was analyze using fully automated analyzer DXI800 (Beckman Coulter).

STATISTICAL ANALYSIS:

The results of the current study are analyzed using SPSS software(20.0). Descriptive analysis was performed on all variables. Data was stratified into cases and controls. Cases were further analyzed with respect to gender, age and severity of disease.

The mean and standard deviation for each parameter was calculated in both case and control group. Statistical significance was defined as a $P \leq 0.05$. Statistical tests such as independent t-test, percentage were used for data analysis.

RESULTS:

The 557 subjects included in study, were divided in two groups on basis of RT PCR test results and sign and symptoms for COVID. The positive group was labelled as cases and the RTPCR negative group with no signs and symptoms was taken as control group. The control group had 442 subjects and the cases were 135 in number.

The cases and controls were comparable in terms of sex distribution, however a significant difference in ages was noted.

Among the CBC parameters, no significant difference was noted in cases and controls. The mean D-dimer results were 0.82 µg/ml in cases as compared to controls (mean = 0.38, p values= 0.0). The mean ferritin results were 204.26 ng/ml in cases as compared to controls (mean = 153.42, p values=0.03). The CRP values were higher in cases, mean 29.79 mg/L in cases, mean 13.14 in control group, p values= 0.01).

All these parameters showed significant difference with higher values in cases as compared to controls. No significant difference was seen in LDH values in the two groups.

Table 1 shows demographic details and laboratory parameters analysis of cases and controls.

Parameter	Unit	Cases	Control	P value
Age	Years	51.76 ± 16.88	46.09 ± 15.97	0
Sex ratio		1.45	1.29	
Hemoglobin(Hb)	g/dl	12.83 ± 1.79	13.17 ± 1.79	0.06
HCT	%	39.95 ± 5.04	41.34 ± 5.44	0.01
WBC	X 10 ³ /µl	7.33 ± 3.80	6.94 ± 3.29	0.31
ANC	X 10 ³ /µl	4.94 ± 3.60	4.42 ± 2.99	0.14
ALC	X 10 ³ /µl	1.65 ± 0.79	1.84 ± 0.93	0.02
NLR		4.21 ± 5.87	3.24 ± 4.21	0.09
Platelet count	X 10 ³ /µl	235.81 ± 78.85	241.95 ± 73.98	0.44
MPV	fl	8.46 ± 0.94	8.44 ± 1.00	0.77
D-dimer	µg/ml	0.82 ± 0.84	0.38 ± 0.48	0
LDH	U/L	560.54 ± 281.54	527.86 ± 271.73	0.24
Ferritin	ng/ml	204.26 ± 239.96	153.42 ± 186.83	0.03
CRP	mg/L	29.79 ± 72.86	13.14 ± 25.98	0.01

Out of 135 patients 80 (59%) were males and 55(41 %) were females. Among the cases, the mean age was 50.8 years in males and 53.07 years in females. The ages were comparable with no statistically significant difference (p= 0.46). Though the COVID disease was more prevalent in males as compared to females however the correlation was not significant. Also Males had more severe disease as compared to females. Among the hematological parameters, statistically significant difference was noticed only in hemoglobin and hematocrit (p=0 for both). Among the markers of inflammation ferritin was significantly lower in females as compared to males (p=0).

Table 2 shows the demographic characteristics and laboratory findings on basis of gender in cases.

Parameter	Unit	male	female	P value
Age		50.86 ± 16.55	53.07 ± 17.41	0.46
Hemoglobin (Hb)	g/dl	13.54±1.7	11.88±1.45	0
HCT	%	41.95±4.74	37.28±4.12	0
WBC	X 10 ³ /µl	7.16±3.9	7.54±3.7	0.58
ANC	X 10 ³ /µl	4.93±3.8	4.96±3.35	0.96
ALC	X 10 ³ /µl	1.5±0.74	1.85±0.82	0.01
NLR		4.82±6.86	3.4±4.12	0.15
Platelet count	X 10 ³ /µl	225.37±82.61	249.81±71.89	0.08
MPV	fl	8.43±1.02	8.51±.84	0.63
D-dimer	µg/dl	0.82±0.84	0.82±0.86	0.98
LDH	U/L	571.97±277	544.22±289.73	0.58
Ferritin	ng/ml	268.35±267.25	113.38±156.73	0
CRP	mg/L	29.49±47.78	30.23±97.43	0.96

Patients were trichotomized into three groups: young age ≤ 45 years (n= 54), middle age 45- 59 years (n=51) and elder age >60 years (n=20). Patients were further divided into mild moderate and severe cases. 88 cases were mild, 44 moderate and 3 were severe on basis of clinical conditions. No significant difference was found in ages or gender of mild, moderate and severe cases. The mean ages were 51 years for mild cases, 53.2 years for moderate cases and 50 years for severe cases. No significant difference was noticed in demographic profile or hematological parameters except for platelet count which showed significantly lower values in severe cases as compared to mild cases. The CRP values were higher in severe cases as compared to mild and moderate cases, but the difference was not statistically significant.

Rest of the inflammatory markers did not show any correlation with disease severity.

Table 3 shows the findings in mild moderate and severe cases.

Parameter	Unit	Mild (n=88)	Moderate (n= 44)	Severe (n=3)
Age		51.06 ± 16.86	53.2 ± 17.11	50.0 ± 19.8
Hemoglobin (Hb)	g/dl	13.0±1.68	12.61±2.0	13.0±0.85
HCT	%	40.29±2.87	39.37±3.4	39.9±2.97
WBC	X 10 ³ /µl	6.82±3.48	8.17± 4.23	7.6±1.84
ANC	X 10 ³ /µl	4.54±3.12	5.64±4.24	5.15±3.04
ALC	X 10 ³ /µl	1.57±0.72	1.77±0.90	1.85±1.06
NLR		3.54±3.64	5.37±8.42	3.72±3.65
Platelet count	X 10 ³ /µl	226.75 ± 69.92	250.29± 92.46	259±4.24
MPV	fl	8.47 ±0.99	8.46±0.90	8±0.14
D-dimer	µg/dl	0.84±0.85	0.78±0.86	0.6±0.11
LDH	U/L	529.05±253.44	618.96±325.57	568.21±269.82
Ferritin	ng/ml	214.62±265.66	179.91±177.91	306.95±389.97
CRP	mg/L	29.05±78.93	29.60±60.12	66.36±91.69

Table 4 shows percentages of patients showing abnormal results of the laboratory parameters analyzed in the current study.

Parameter	Percentage of patients showing increase/decrease in biomarkers
WBC	Increased 24/135 decreased 25/135
ANC	Increased 23/135
ALC	Decreased 30/135
D-dimer	Increased 84/135
LDH	Increased 77/135
Ferritin	Increased 5/135
CRP	Increased 68/135

DISCUSSION:

The COVID pandemic is affecting all ages and gender and all geographic locations. The first wave had predominance of elderly patients while the second wave had more affinity for comparatively younger age. The mean age for cases was 51.76 ± 16.88 years. Among the cases group for COVID, majority of the patients were male in the ratio of 1.45:1. The finding of male predominance is present in most of the studies conducted on COVID infection.

In the current study 40 % (patients belonged to young age (54/135), 38% to middle age (51/135) and 22 % to elderly group (20/135). This finding is consistent with age affected in second wave of COVID pandemic (11).

Hematological Markers:

Complete blood count is a basic hematological investigation conducted as first line investigation in majority of groups. The various coulter parameters show an increase or decrease depending on the clinical condition and health of the subject.

Hemoglobin and Hematocrit:

The mean Hb and HCT levels were 12.83 ± 1.79 and 39.95 ± 5.04 in cases which did not show any statistical difference from controls (mean Hb=13.17 ± 1.79, HCT= 41.34 ± 5.44.) Among cases the Hb and HCT was statistically significantly lower in females as compared to males (p= 0.0). This is similar to findings of several other authors (6, 12). The difference can be attributed to the facts that anemia is more prevalent in females.

WBC parameters (WBC count, NLR, absolute neutrophils and lymphocyte count):

The mean values for WBC count, absolute neutrophil count and absolute lymphocytes count was 7.33 ± 3.80, 4.94 ± 3.60 and 1.65 ± 0.79 in cases and 6.94 ± 3.29, 4.42 ± 2.99 and 1.84 ± 0.93 in controls. The WBC count and ANR were high in cases but the difference was not statistically significant. The ALR was significantly lower in cases (p= 0.02). These findings are in consistency with other studies (6). Among the cases the ALR was significantly lower in males as compared to females (p=0.01). This difference could be attributed to the fact that COVID incidence is more in males as compared to females.

NLR:

NLR is a novel marker of systemic inflammatory response. It takes into account both neutrophils and lymphocyte count. An increase in neutrophil count and a fall of lymphocyte count can indicate the intensity of the inflammatory response and damage to the immune system. Various studies have been conducted to evaluate its role in COVID infection.

In the present study the NLR ratio was higher in cases as compared to controls (4.21 ± 5.87 in cases and 3.24 ± 4.21) but the difference was statistically not significant. The high NLR ratio indicates a rise in neutrophil count and a simultaneously fall in lymphocytes in COVID patients. It did not show significant difference between males and females.

The NLR ratio was higher in moderate cases as compared to mild or severe forms. This can be explained by the fact that the immune system is more activated in moderate cases as compared to mild forms. The lower NLR in severe cases indicates exhausted/spent out condition of the immune system.

Various studies have observed high NLR ratio. This is consistent with studies conducted previously (6, 13, 14)

CRP:

CRP is an acute phase reactant and is predictable marker of inflammatory response [6, 15]. The CRP marker was found to be significantly increased in the initial phases of the infection for COVID-19 patient. In our study we found CRP levels were significantly higher in cases as compared to controls ($p=0.01$). This finding is supported by several studies [6, 16, 17].

D-dimer

D-Dimer is a specific bio-marker for patients with venous thromboembolism, and is also used as an inflammatory marker [18]. In the current study the D dimer levels were significantly higher in cases as compared to controls ($p=0.0$). Also no statistically significant difference was noticed in males and females in case group. The findings are consistent with as reported by other authors (9). The high D dimer values in cases can be attributed to the effect of COVID infection in patient.

Serum Ferritin

Ferritin is an acute phase reactant protein. (19). During this pandemic time, studies have found that elevation of serum ferritin levels were associated with COVID-19 infection and mortality [20]. In our study we found higher mean serum ferritin values in cases as compared to controls, with a significant p value ($p=0.03$). This is consistent with several other studies. (6, 9)

Among the cases the ferritin levels were significant lower in females as compared to males. This is attributed to the presence of in females as indicated by Hb levels of the subjects.

LDH:

Lactate Dehydrogenase is an enzyme which indicates ongoing cell damage. Though several studies have indicated a higher LDH levels in cases as compared to control (Ref). The present study showed a high LDH levels in cases as compared to controls but the values were not statistically significant. Also no significant difference was noticed in male and female covid patients.

Indian Studies:

Though India is one of the nation showing highest COVID infection rates, only a few studies have been conducted on COVID 19 in India so far.

Table 5 shows the comparison of present study with several other studies conducted in India.

S. No	Author	Study design and time period	Location	Study size	Parameters studied which are part of current study	Comments
1	Das B et al(8)	Retrospective (01.03.2020 to 10.07.2020)	Mumbai, Maharashtra	100 COVID cases and 40 controls	Ferritin, LDH, CRP	Significant higher level of ferritin, LDH and CRP in cases as compared to controls
2	Kalyan Kumar PV et al(10)	Observational cross sectional (15.04.2020 to 15.10.2020)	Guntur, Andhra Pradesh	567 COVID cases	WBC, D-dimer, Ferritin, CRP, Platelet, Lymphocytes	Majority of patients infected were men as compared to women. Among the biomarkers D-dimer, serum ferritin were reliable markers increased in COVID 19 infections. CRP was a nonspecific marker also increased in COVID infections. Remaining all blood investigations were normal except in minority of patients where platelet count increased.
3	Lingaih A et al(09)	Retrospective (April 2020 to Oct 2020)	Hyderabad, Andhra Pradesh	115 patients	Hb, CRP, D-dimer, Ferritin	COVID infection was more common in elderly. Mean CRP, d-dimer and ferritin were higher in elderly age patient.
4	Present study	Retrospective (1 July 2020 to 31 July 2021)	Pune, Maharashtra	577 total (135 COVID patients and 442 controls)	Hb, HCT, WBC, Lymphocytes, Neutrophils, NLR, Platelet, CRP, Ferritin, LDH, D-dimer	COVID infection was more common in younger and middle age. Inflammatory markers CRP, Ferritin and D-dimer showed significantly higher values in cases. NLR ratio was higher in cases as compared to controls.

CONCLUSION:

In the current study of COVID infected patients 40 % patients belonged to young age (<45 years), 38% to middle age (45 -59 years) and 22 % to elderly group (>60 years). This shows high infectivity rate in younger group as compared to 1st wave. NLR was higher in cases as compared to controls. CRP, D-dimer and ferritin were significantly higher in cases as compared to controls. Though ferritin levels were high in cases as compared to controls, the difference was not significant. In our knowledge this is the first study in second wave of COVID -19 pandemic in India. Further larger scales studies are needed to confirm the findings and establish surrogate markers for imminent 3rd wave of COVID -19 infection.

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