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ORIGINAL RESEARCH

Study of haematological parameter and infection biomarker to assess the severity of disease in covid-19 positive patients

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ABSTRACT

Background:*Corona virus disease 2019 (COVID 19), caused by the novel corona virus is a rapidly spreading and devastating infection which has become a global pandemic. The present study assessed the severity of disease in Covid-19 positive patients.*

Aims and objective: *To study the different haematological parameters and infection biomarkers in COVID-19 patients. To evaluate the role of hematological parameters in determination of COVID-19 disease severity.*

Materials & Methods: *600 Adult (age >18 years old) patients who were diagnosed with COVID- 19 according to WHO interim guidance were screened, between Feb 2020 and Feb 2021 (one year). Data of complete hematological parameter and infection biomarker was collected from records of Central Pathology Lab at Bundelkhand Medical College Sagar, M.P., India.*

Results: *Out of 600 patients, males were 380 and females were 220. Out of 600 patients, 540 survived and 60 died. There was significant difference in value of Hb, TLC, ANC, NLR, PLT, MCV, RDW, PT, aPTT, D- Dimer, Ferritin and CRP level in patients admitted in ward, HDU, ICU, between genders and those survived and died.*

Conclusion:*The study concluded that leukocytosis, neutrophilia, elevated Neutrophil to lymphocyte ratio, APTT, D- Dimer, serum ferritin, CRP and RDW-SDassociated with severity of covid-19 disease.*

Key words: *Corona virus disease, D- Dimer, haematological*

Introduction

Corona virus disease 2019 (COVID-19), caused by the novel corona virus, is a rapidly spreading and devastating infection which has become a global pandemic. The first case of severe acute respiratory syndrome-corona virus-2 (SARS-CoV-2) disease (COVID-19) emerged in Wuhan City of Hubei Province China, in December 2019. Samples of bronchoalveolar lavage fluid from a patient in Wuhan was confirmed as the cause of severe pneumonia.[1]

Corona viruses are RNA viruses with glycoprotein spikes that give them a crown‐ like appearance. Four species have been in circulation for a long time and cause mild respiratory disease.[2]They have a huge genetic diversity and have jumped the species barrier, leading to
severe respiratory disease (SARS virus in 2002–2003 and the MERS virus in 2012–2013).There was a continuous growth in the cases and the virus was spreading globally which forced the World Health Organization to declare a global pandemic on March 11, 2020.[3]The hematological parameter and infections biomarker has been suggested as a marker of the systemic inflammatory response in Covid 19 patients. It has also been reported these markers are potential predictor of clinical risk and outcome in many diseases.[4]Identifying the prognostic predictors of mortality in patients with COVID- 19 might be useful for assessing the disease severity and making optimal treatment decisions. To our knowledge, the utility of these markers to predict mortality in patients with COVID- 19 has not been studied yet.[5,6] The present study assessed the severity of disease in Covid-19 positive patients.

Aims and objectives

- To study the different haematological parameters and infection biomarkers in COVID-19 patients.
- To evaluate the role of hematological parameters in determination of COVID-19 disease severity.

Materials & Methods

The present retrospective study comprised of 600 Adult (age >18 years old) patients who were diagnosed with COVID- 19 according to WHO interim guidance were screened, between Feb 2020 and Feb 2021 (one year). Data were collected from patients who were cured and discharged, or who died without a cure.Patients with hematologic malignancy and any other malignancy and Covid negative patients were excluded.

Data collection: -The study as approved by institutional ethical committee with reference no. **IECBMC/2021/20 ON 18/6/2021**.At the time of admission three whole blood sample were collected & tested at the institutional laboratory.**EDTA vial** –for Hemogram-(fully automated hematology analyzer – mindray BC 5300 ,**Citrate vial** –for Coagulation profile-(Coagulometer STAGO-SATLLITE Analyser,**Plain vial** – for biomarker - Erba XL 640 Clinical chemistry analyzer. Data of complete hematological parameter and infection biomarker was collected from records of Central Pathology Lab at Bundelkhand Medical College Sagar, M.P., India. Demographic, clinical, and laboratory investigation data were collected from patient's medical record from the medical record section. The severity of patients with COVID-19 was classified as mild, moderate, and severe at the time of admission as per Indian Council of Medical Research (ICMR) guideline version-3, published on date 13 June 2020 for patient with COVID-19.

Statistical analysis:-Data were entered in Microsoft Office 2007 Excel spreadsheet and analyzed by using the Statistical Package for the Social Sciences (SPSS) software program, version 26.0 (IBM, Armonk, New York). Chi-square test was used to analyze association between two variables. The quantitative variables such as hematological parameters, coagulation profile, and inflammatory biomarkers were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to assess difference between two means (a value of $P < 0.05$ was considered statistically significant). Fisher's least significant difference (LSD) was also used to find out association with in the group.
The diagnostic cutoff values of the parameters for differentiating severe cases of patients with COVID-19 from non severe were calculated by receiver-operating characteristic (ROC) and area under the ROC curve (AUC). The parameters with AUC >0.8 and with statistical significance ($P < 0.05$) were considered to have good discriminative precision. Best diagnostic cutoff was selected with values corresponding to maximum sensitivity and specificity. Results were tabulated and assessed statistically. P value less than 0.05 was considered significant.

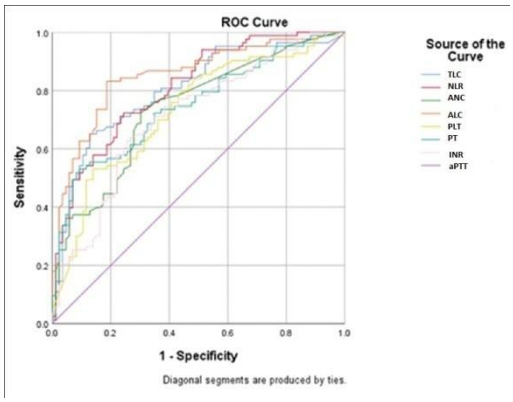

Image-1

Results

Figure I Gender wise distribution of COVID-19 cases

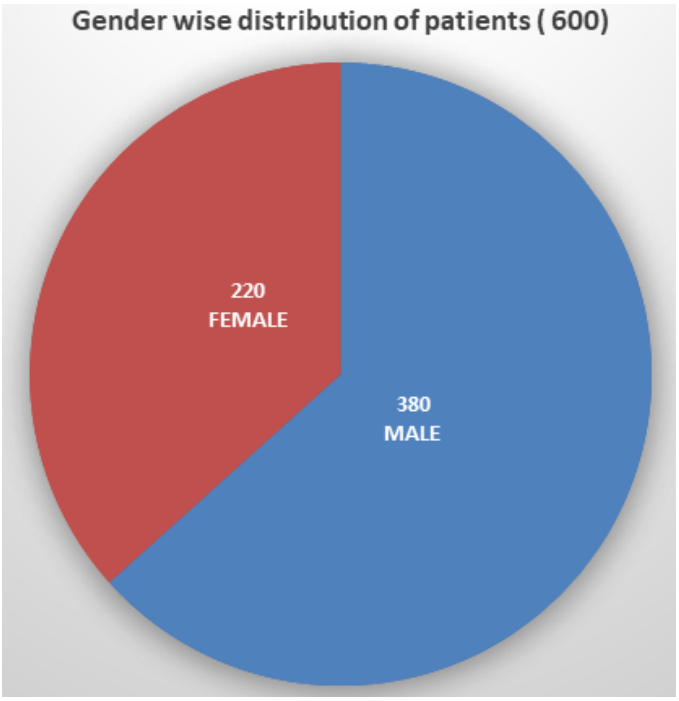

Figure I show that out of 600 patients, males were 380 and females were 220.

Table I: Age wise distribution of COVID-19 cases as per severity of disease

Age Group (years)	Ward	HDU	ICU	TOTAL
18-40	62	24	43	129
41-60	62	56	77	195
>60	71	54	151	276
TOTAL	**195**	**134**	**271**	**600**

Table I shows age wise distribution of COVID-19 cases as per severity of disease

Table II: Outcome of treatment

Total patients	Survived	Died
600	540	60

Table II shows that out of 600 patients, 540 survived and 60 died.

Table IIIHaematology parameters of Covid 19 patients in different wards

Parameters	Ward	HDU	ICU	Survived	Died	P value
Hb	12.1	11.6	9.4	9.5	8.1	0.04
TLC	7212.4	8048.1	9932.6	7015.6	9922.4	0.04
ANC	4969.8	4992.3	5012.5	5014.6	5215.6	0.02
ALC	2180.9	1630.3	1510.1	2050.7	2110.8	0.02
NLR	3.21	4.15	5.01	3.11	5.08	0.04
PLT	221576.3	235617.2	243215.6	214574.3	224587.9	0.03
MCV	93.2	92.5	94.2	92.5	95.7	0.04
RDW	48	49.5	50.6	47.1	51.4	0.02
PT	153.4	184.2	190.6	152.6	189.1	0.04
INR	1.31	1.62	1.92	1.43	1.99	0.04
aPTT	37.5	39.0	41.2	36.2	41.1	0.03
D- Dimer	2770.8	2813.4	2913.5	2542.3	3015.6	0.02
Ferritin	892.5	840.5	910.4	884.5	856.3	0.03
CRP	25.4	26.7	29.6	26.7	29.8	0.03

Table III shows that there was significant difference in value of Hb, TLC, ANC, ALC,NLR, PLT, MCV, RDW, PT,INR,aPTT, D- Dimer, Ferritin and CRP level in patients admitted in ward, HDU, ICU, and those survived and died. The difference was significant ($P< 0.05$).

Table IV Haematology parameters of Covid 19 patients according to their gender

Parameters	Male	Female	Survived	Died	P value
Hb	10.3	8.5	9.5	8.1	0.04
TLC	7125.7	7083.5	7015.6	7922.4	0.04
ANC	5123.6	5189.3	5014.6	5215.6	0.02
ALC	2250.8	2145.8	2356.8	2790.8	0.02
NLR	3.42	3.16	3.11	3.78	0.04
PLT	223471.9	234261.3	214574.3	224587.9	0.03
MCV	95.1	94.6	92.5	95.7	0.04
RDW	48.5	47.6	47.1	51.4	0.02
PT	15.23	15.37	15.26	15.93	0.03
INR	1.31	1.32	1.34	1.43	0.04
aPTT	38.1	39.1	34.2	38.6	0.03
D- Dimer	2915.2	2809.6	2542.3	3015.6	0.02
Ferritin	884.5	890.5	884.5	891.3	0.04
CRP	27.4	28.2	26.7	28.8	0.03

Table IV shows that there was significant difference in value of Hb, TLC, ANC,ALC, NLR, PLT, MCV, RDW, PT,INR,aPTT, D- Dimer, Ferritin and CRP level in patients admitted in between genders and those survived and died. The difference was significant ($P< 0.05$).

Discussion

The novel coronavirus outbreak, which was first reported in Wuhan, China has now spread worldwide and is characterized by the World Health Organization (WHO) as a pandemic with Europe now being considered a new epicenter of the virus.[7,8] On 14 March 2020, global reports noted a total of 142539 confirmed cases (9769 new) and 5393 deaths (438 new), while in China 81021 (18 new) cases were confirmed with 3194 (14 new) deaths. [9]The 2019 coronavirus disease (COVID-19) shares similar epidemiological characteristics with the severe acute respiratory syndrome coronavirus (SARS-CoV) and

the Middle East respiratory syndrome coronavirus (MERS-CoV).[10] However, their mortality rates differ significantly as COVID-19 presents a lower mortality rate (2.08%) as compared with SARS-CoV (10.87%) and MERS-CoV (34.77%). Despite this fact, the COVID-19 is more contagious. Regardless of the differences noted, these diseases manifest similarly with a cough and fever.[11]The present study assessed the severity of disease in Covid-19 positive patients.

We limit our discussion to basic haematological parameters, coagulation screen and inflammatory biomarkers in Covid 19 positive adults upon hospital admission. Peak age of presentation was > 60 years, with male to female ratio of 1.7:1.There was no significant difference in mean age and sex between the two groups (p>0.05).

We found that out of 600 patients, males were 380 and females were 220. Out of 600 patients, 540 survived and 60 died. Djakpoet al[12] in their study data of 208 mild and common confirmed cases as COVID-19 was analyzed. The median age of subjects used in the present study was 50 years. Data from 107 (51.4%) males and 101(48.6%) females were used for analysis.

Table V:Haematology parameters of Covid 19 patients in severe cases/patient admitted in ICU in various studies

Variables (SEVERE/ ICU)	**Present study (600)**	**Vinay Bharat et al 2021 (140)**	**D.P. Lokwani et al 2021 (300)**	**Pomilla et al 2020 (100)**	**Reetesh et al 2021 (862)**	**Deval B. Dubey et al 2021 (200)**	**Tania pinto et al 2021 (200)**
Hb	9.4 g/dl	10.3	11.9**(NS)**	11.8	12.5	12.03**(NS)**	12.01**(NS)**
4-TLC	9932.6	13490	12670	10713.6	13100	13200	8970
5-ANC	5012.5	9920	9980	8907.5	NA	NA	NA
ALC	1510.1	1850	1510	1087.8	NA	NA	NA
NLR ↑	5.01	6.3↑	9.3↑	10.12↑	10.9↑	6.9↑	7.53↑
PLT**(NS)**	243215	130000 ↓	214420 **(NS)**	210000 **(NS)**	234000↑	173000 **(NS)**	239900 **(NS)**
MCV	94.2	NA	NA	84**(NS)**	84.05	81.1	NA
RDW	50.6	NS	55.31	NS	46.7	NA	NA
PT ↑	19.06	NA	10.85 ↑	14.84 ↑	15.84 ↑	NA	NA
APTT ↑	41.2	NA	27.9 ↑	NA	35.23 ↑	NA	NA
D-Dimer↑	2913.5	NA	2605.6↑	7493.8↑	NA	3813.9↑	NA
Ferritin↑	910.4	NA	875.5↑	701.0 **(NS)**	NA	1275.6↑	NA
CRP↑	29.6	NA	136.4↑	239.4↑	NA	276.2↑	NA

We found that there was significant difference in value of Hb, TLC, ANC, NLR, PLT, MCV, RDW, PT, aPTT, D- Dimer, Ferritin and CRP level in patients admitted in ward, HDU, ICU, between genders and those survived and died.Pouladzadehet al[13] assessed predictive validation of red cell distribution width (RDW) in COVID-19 patients. In total, 331 COVID-19 patients were classified as 'severe' and 'non-severe' groups based on the WHO standard criteria. RDW-SD ≤43 and ≤47 fl thresholds showed high specificity (90.1–91.4%) for diagnosing non-severe illness and no risk of death. RDW-SD >47 fl indicated severe illness and a high mortality risk while RDW-SD between 43-47 fl indicated severe illness with low risk of death. Similar to us, results published from various studies from Wuhan, China reported elevated aPTT, elevated PT, elevated D-dimer, increased biomarkers of inflammation including interleukin–6 (Il–6), ESR, and CRP from first 99 patients hospitalized in Wuhan and it is same in other various studies as compared in table V.

Tajet al[14] evaluated the role of hematological parameters in determination of COVID-19 disease severity. Total of 101, confirmed cases of covid-19 disease, both genders between 17 and 75-year age were included. Hematological parameters were compared in mild, moderate, severe and critical disease group.

Out of 101 patients, 20.8%, 51.8%, 19.8% and 7.9% were in mild, moderate, severe and critical group respectively. Median (IQR) values of TLC (p-value <0.04), ANC (p-value <0.02), NLR (p-value <0.01) were significantly increased in patients with critical disease. Lymphocytopenia was a prominent (p-value <0.02), and the most consistent feature in all affected patients, although ICU patients suffered from greater lymphocytopenia; thus significantly associated with severity. Lymphocytopenia has been conventionally known to occur in viral diseases. Affinity for the virus for lymphocytic ACE receptors may attribute to its direct cytopathic effect. While lymphocyteapoptosis due to increased granulopoiesis seen as an exaggerated inflammatory response could be another reason. Studies are of the opinion that lymphopenia can be an effective predictor severity in COVID 19, our findings supports this claim. (p-value<0.02). Additionally rise in TLC with high absolute neutrophil count was seen significant (p<0.05) and hence a high NLR and PLR was found. High NLR may be indicative of the patient's response to inflammatory insult, with neutrophils rising in response to stress, which, when overwhelming, induces lymphocyte apoptosis. Neutrophilshere seem act as a double edged sword. There are hypothesis that Neutrophil Extracellular Traps (NETs) released by neutrophils contribute to organ damage and death in COVID–19 patients. Also that development of Acute Respiratory Distress Syndrome (ARDS), thick mucus secretions in the airways and the development of blood clots, were similar to the symptoms of diseases already known to the researchers as being caused by NETs.

The inflammatory markers studied (wherever possible), like CRP AND Ferritin also showed a markedly increased mean value in ICU patients but did not show statistical correlation (p>0.05).Researcher from China assessed the usefulness of CRP levels in the early stage of COVID–19 and found that it positively correlated with lung lesions and could reflect disease severity. Median (IQR) values of APTT (p-value 0.003) and CRP (p- value 0.0001) were suggestively higher in patients with severe disease. Meta-analysis of reports from China have also detailed C-reactive along with D-dimers, coagulation times, and lactate dehydrogenase; with lower platelet and lymphocyte counts a common finding in "Cytokine Storm Syndrome" in hospitalized patients with COVID–19.Other parameters like Hemoglobin, MCV, HCT, ALC, Platelet count, prothrombin time did not show statistically significant association with severity of disease.However, presence of chronic comorbid conditions in many of these patients act as major confounder to establish their role in clinical worsening due to SARS-CoV 2 virus

Fan BE et al[15] found mild thrombocytopenia in 20% of his study cases Together with no significant rise noted in ICU admissions supports the fact that "COVID–19 associated coagulopathy" proposed to be occurring early in infection, reflects abnormalities in tests but does not fulfill the usual definition of a clinical coagulopathy where impaired ability to clot results in bleeding. The development of coagulation test abnormalities seen in SARS-CoV–2 infected patients is most likely a result of the profound inflammatory response than intrinsic procoagulanteffect of virus itself.Considering this disease as highly contagious and pestilent, we could not correlate the coagulation profile with clinical severity of the patients. The study has a limitation that no asymptomatic carrier was included. We could not analyze and study the prognosis of the included cases. Severity of the patients was assumed on the basis of admission to ICU, and not correlated clinically.

Conclusion

The study concluded that leukocytosis, neutrophilia, elevated Neutrophil to lymphocyte ratio, APTT, D-Dimer, serum ferritin, CRP and RDW-SDassociated with severity of covid-19 disease.Also by obtaining these parameters by basic preliminary workup may segregate patients requiring intensive care at the time of admission enabling risk stratification and guide intervention.

References

1. P. Mehta, D.F. McAuley, M. Brown, E. Sanchez, R.S. Tattersall, J.J. Manson, et al., COVID-19: consider cytokine storm syndromes and immunosuppression, Lancet (London, England) 395 (10229) (2020) 1033.
2. N. Tang, D. Li, X. Wang, Z. Sun, Abnormal coagulation parameters are associated with poor prognosis in patients with novel corona virus pneumonia, J. Thromb. Haemostasis 18 (4) (2020) 844–847.

3. Y. Shang, C. Pan, X. Yang, M. Zhong, X. Shang, Z. Wu, et al., Management of critically ill patients with COVID-19 in ICU: statement from front-line intensive care experts in Wuhan, China, Ann. Intensive Care 2020; 1–24.
4. C. Huang, Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, et al., Clinical features of patients infected with 2019 novel corona virus in Wuhan, China, The lancet 2020; 497–506.
5. G. Lippi, M. Plebani, B.M. Henry, Thrombocytopenia is associated with severe corona virus disease 2019 (COVID-19) infections: A meta-analysis, Clin. Chim. Acta 2020; 145–148.
6. A. Amgalan, M. Othman, Hemostatic laboratory derangements in COVID-19 with a focus on platelet count, Platelets 2020; 1–6.
7. J.-M. Jin, P. Bai, W. He, F. Wu, X.-F. Liu, D.-M. Han, et al., Gender differences in patients with COVID-19: focus on severity and mortality, Front. Publ. Health 2020; 152.
8. D. Liao, F. Zhou, L. Luo, M. Xu, H. Wang, J. Xia, et al., Haematological characteristics and risk factors in the classification and prognosis evaluation of COVID-19: a retrospective cohort study, Lancet Haematol. 7 (9) (2020) e671–e678.
9. A.-P. Yang, J. Liu, W. Tao, H-m Li, The diagnostic and predictive role of NLR, dNLR and PLR in COVID-19 patients, Int. Immunopharm. 2020;106504.
10. B.E. Fan, V.C.L. Chong, S.S.W. Chan, G.H. Lim, K.G.E. Lim, G.B. Tan, et al., Hematologic parameters in patients with COVID-19 infection, Am. J. Hematol. 2020; E131–E134.
11. A. Bansal, A.D. Singh, V. Jain, M. Aggarwal, S. Gupta, R.P. Padappayil, et al., A Systematic Review and Meta-Analysis of D-Dimer Levels in Patients Hospitalized with Corona virus Disease 2019 (COVID-19), medRxiv, 2020
12. Djakpo DK, Wang Z, Zhang R, Chen X, Chen P, Antoine MM. Blood routine test in mild and common 2019 corona virus (COVID-19) patients. Bioscience reports. 2020 Aug 28;40(8).
13. Pouladzadeh M, Safdarian M, Choghakabodi PM, Amini F, Sokooti A. Validation of red cell distribution width as a COVID-19 severity screening tool. Future science OA. 2021 Aug;7(7):FSO712.
14. Taj S, Fatima SA, Imran S, Lone A, Ahmed Q. Role of hematological parameters in the stratification of COVID-19 disease severity. Annals of medicine and surgery. 2021 Feb 1;62:68-72.
15. Fan B.E., Chong V.C.L., Chan S.S.W., Lim G.H., Lim K.G.E., Tan G.B. Hematologic parameters in patients with COVID- 19 infection. Am. J. Hematol. 2020;95(6):E131–E134.