ORIGINAL RESEARCH PAPER

Pathology

A STUDY OF VARIATIONS IN COAGULATION PROFILE AND HEMATOLOGICAL PROFILE OF COVID-19 PATIENTS- IS IT USEFUL IN DECIDING SEVERITY OF DISEASE ?

KEY WORDS: Coagulation profile, haematological profile, Covid-19.

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ABSTRACT

Background: Since covid has emerged as the most dreadful global health crisis and data on diagnostic profile and morbidity mortality indicators in it is still limited, we propose to study the correlation of morbidity and mortality with values of Inflammatory markers in these patients. **Aim:** To STUDY variations in COAGULATION AND HEMATOLOGICAL PROFLE IN COVID-19 PATIENTS and correlating it with severity of disease. **Materials and Methods:** using STA Satellite automated coagulation analyzer we study PT, INR, APTT. With the help of Snibe maglumi 800 we studied D-dimer. For hematological analysis we use Hemax 330 hematological analyzer for HB, TLC, Neutrophil and Lymphocytes count, Neutrophil lymphocyte ratio, Platelets count. **Results:** In hematological profile leucocytosis was associated with severity and mortality. In coagulation profile D-dimer was associated with severity and mortality. Overall only leukocytosis and raised D-dimer values were associated with death. **Conclusion:** On the basis of findings in this study coagulation profile and hematological profile Leucocytosis and D-dimer concluded as important predictors of disease severity and outcome.These parameters can be used to identify high-risk patients at resource-limited settings.

INTRODUCTION

In late December 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified in Wuhan, China. Since then, this virus has rapidly spread with more than 260 million confirmed cases of COVID-19 worldwide and 30 milion cases in India.

Coagulopathy may play a role in COVID-19 pathogenesis, with an increased risk of developing deep vein thrombosis (DVT) and pulmonary embolism (PE) among patients with severe COVID-19 infection,specially those admitted in intensive care units (ICU), despite adequate thromb oprophylaxis.

Also, several studies have reported abnormal coagulation parameters and hematological parameters , notably in patients with COVID-19 associated pneumonia and acute respiratory distress syndrome (ARDS).

Considering this, we studied coagulation and hematological parameters of patients with COVID-19 pneumonia and determine whether is this useful in deciding on the severity of disease.

MATERIALS AND METHODS :

Study Design :

In this retrospective study we studied 400 confirmed COVID 19 positive patients aged 18 years and above admitted in Gajra Raja Medical College, Gwalior upto 31st June 2022 for a period of One and a half year .Ethical clearance was obtained from ethical committee of Gajra Raja Medical College, Gwalior .

Inclusion Criteria :

Cases confirmed with covid by RT-PCR were included in this study.

Exclusion Criteria :

COVID suspect and Non COVID home quarantine patients were excluded from the study.

Following laboratory investigations were performed and correlated with clinical findings :

Coagulation Profile Analysis -PT, INR, APTT, D-Dimer Haemoglobin and cbc profile-HB, TLC, Neutrophil and Lymphocytes count , Neutrophil Lymphocyte Ratio (NLR), Platelet count.

Coagulation Profile :

- We received samples in Citrate vials.
- Sample was checked for adequacy.
- Clotted and hemolyzed samples were discarded and requisition for repeat sampling was sent to ward.
- Each sample was centrifuged for 15 minutes at 2500rpm.
- Plasma was drawn into a clean plastic tube and stored at 4°C until testing was done within 24 hrs from collection.
- PT,INR and APTT testing was carried out in the laboratory on plasma samples on STA satellite analyser.
- PT and APTT were expressed in seconds.

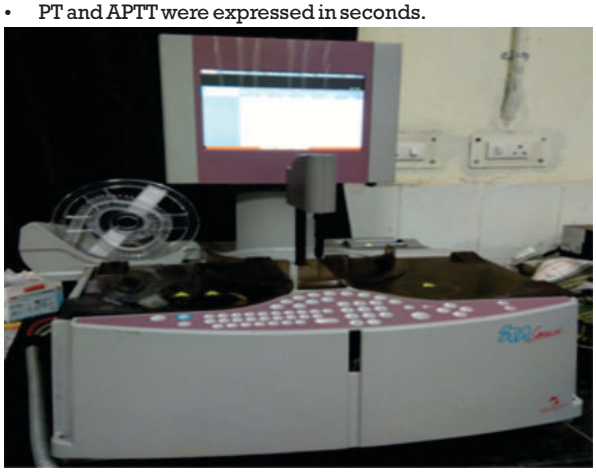


Figure: STA Satellite Automated Coagulation Analyzer

D-Dimer:

For D-dimer testing sample was taken from Citrate vial same as for PT, APTT, INR.

We used a kit which is an in vitro chemiluminescence immunoassay for the quantitative determination of D-Dimer in human plasma using the MAGLUMI 800.

Figure: Snibe Maglumi 800 for D-Dimer, IL-6, Ferritin, TROPONIN-I, PROCALCITONIN

Specimen Collection And Preparation

- Plasma is collected using sodium citrate tubes.
- Collect blood aseptically following the universal precautions for venipuncture.
- After ensuring that complete clot formation in specimens has taken place prior to centrifugation ,specimen is centrifuged before a complete clotting
- Centrifuged specimens with a lipid layer on the top are transferred to a sample cup or secondary tube. Care should be taken to transfer only the clarified specimen without the lipaemic material.
- All samples (Patient specimens or controls) were tested within 3 hours when placed on board the MAGLUMI System.
- The sample volume required for a single determination of D-Dimer is 20 microliter.

Haemoglobin and CBC Examination

- We received samples of covid patients in EDTA vial .
- Individual samples were checked for adequacy.
- Clotted and hemolyzed samples were discarded and requisition for repeat sampling was sent to ward.
- Sample was run in a Haematology analyzer(Hemax) .
- Simultaneously slides were stained with leishman stain and checked for total and differential counts.
- Neutrophil : Lymphocyte ratio was calculated .
- Final values after correlation were taken into conside

Figure: Hemax Hematological Analyzer

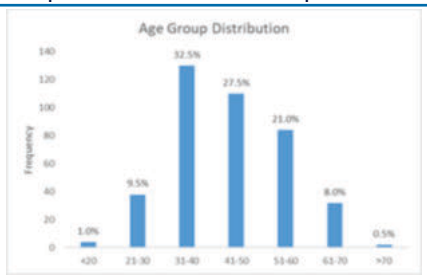
RESULTS

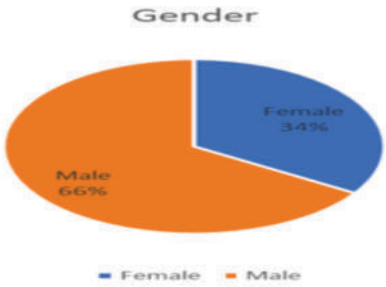
This retrospective study included samples of 400 covid diagnosed patients at the Department of Pathology, Gajraraja Medical College ,Gwalior

1.Population Characteristics:

Overall study population of age was categorised into following groups : <20, 21-30 31-40 , 41-50 , 51-60 , 61-70 and >70 years.

Among 400 covid-19 patients, we observed the highest number of patients in the age group of 31-40 years (32.5%) whereas the least number were observed in the age group of >70 years (0.5%).





Coagulation Profile :

We studied PT,INR,APTT and D-Dimer of covid patients .Among the overall study population, -Increased prothrombin time was seen in 34.5% whereas 2.5% fell below the normal range.

- 32% had INR values above the normal range whereas 24% fell below the normal range.
- 6% had higher APTT values whereas 50% had values below normal range.
- 87% of patients had abnormal(increased) levels of D-dimer.

Haematological Profile:

We observed Hb ,Total leucocyte count ,Differential leucocyte count, neutrophils,lymphocytes ,NLR ratio and platelets of patients.

OBSERVATIONS :

- 58.5% patients having abnormal levels (raised and reduced)values of haemoglobin
- 61.5% of patients had leucocytosis whereas only 3% showed leucopenia .
- 67% of patients had Neutrophilia .
- 68.5% of patients had lymphopenia.
- 79.5% of patients had raised N/L ratio (NLR).
- No major change in platelet counts was observed .Only 21% of patients had reduced platelet counts.

We observed 8.8% mortality among our study population.

	Alive (N= 365)	Median	Death(N=35)	Median	p-Value
Age	44.05±11.16	42.00	46.46±10	45.00	0.2135
PT	15.63±2.64	15.00	15.94±2.3	15.50	0.2972
INR	1.15±0.21	1.10	1.18±0.19	1.12	0.2791
APTT	28.04±7.02	26.90	28.77±6.48	28.00	0.1949
D-Dimer	1.96±2.14	1.24	5.03±3.34	4.61	0.0001
HB	11.82±2.24	12.10	12.58±2.4	12.70	0.0801
TLC	13546.03±6563.32	12200.00	16397.15±5464.19	16000.00	0.0130
N	78.37±10.64	80.00	80.09±12.15	85.00	0.0873
L	15.98±9.89	15.00	14.77±10.96	10.00	0.1614
N/L RATIO	7.51±5.72	5.33	8.73±6.06	8.50	0.1400
PLATELETS	2.15±0.84	1.90	2.18±0.84	2.10	0.841

On comparing demographic as well as clinical characteristics of morbid and deceased patients ,Mean PT(p=0.2972) ,INR (p=0.2791), APTT (p=0.1949) ,Mean APTT (p=0.1949), Mean Haemoglobin (p=0.0801), mean Neutrophils (p= 0.0873), mean platelets (p= 0.841), mean Lymphocyte (p= 0.1814), mean N/L ratio (p= 0.1400) had no significant correlation with mortality..

PT, INR and APTT also did not contribute in predicting mortality. According to our equation model, every 0.334 value increase in d-dimer value respectively increased 1.397 times with mortality.[OR=1.397(1.25,1.56).]

Only leukocytosis (p=0.0130) and increased d-dimer (p<0.9172)was significantly associated with mortality.

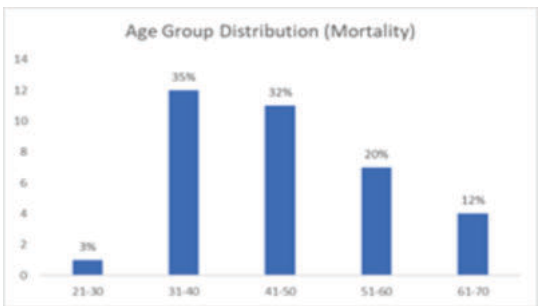
Variables in the Equation

	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
							Lower	Upper
PT	-.044	0.239	0.034	1	0.854	0.957	0.599	1.528
INR	0.742	2.996	0.061	1	0.804	2.101	0.006	745.485
APTT	0.034	0.025	1.926	1	0.165	1.035	0.986	1.086
D-Dimer	0.334	0.057	34.099	1	0.000	1.397	1.249	1.563
Constant	-4.530	1.337	11.487	1	0.001	0.011		

a. Variable(s) entered on step 1: PT, INR, APTT, D-Dimer.

Mortality With Age :

8.75% mortality was observed among our study population. Out of 8.75% mortality patients, highest mortality observed in the age group of 31-40 years whereas least percentage of mortality observed in the age group of 21-30 years.



DISCUSSION

This was a retrospective study done by critical analysis of the information obtained from the pathology and medicine department of Gajra Raja Medical College, Gwalior (Madhya Pradesh) from 1st December 2016 to 30th June 2022. Following laboratory parameters were analysed and correlated with severity and mortality of patients:

- Haemoglobin and cbc profile -HB, TLC, Neutrophils, Lymphocytes, Neutrophilic Leukocytosis Ratio, Platelets
- Coagulation Profile Analysis -PT, INR, APTT, D-Dimer

Patient characteristics :

Overall study population of age was categorised into following groups : <20, 21-30 31-40 , 41-50 , 51-60 , 61-70 and >70 years. Among 400 covid 19 patients, we observed highest number of patients in the age group of 31-40 years whereas least number of percentage observed in the age group of >70 years.Mean age of patient was significantly associated with severity .Among 400 patients, 78% have severe covid -19 whereas 22% have moderate covid-19.We observed 8.8% mortality among our study population. In our study population, 66% were more males compared to females. Highest mortality was observed in age group of 31-40 years whereas least percentage of mortality observed in the age group of 21-30 years.

Haemoglobin and cbc profile -HB, TLC, Neutrophils, Lymphocytes, Neutrophilic Leukocytosis Ratio, Platelets

During covid 19, we observed 58.5% patients having abnormal values (increased or decreased level) of haemoglobin.However it was not significantly associated with severity or mortality.

61.5% patients presented with leucocytosis whereas 3% had leucopenia.

Mean TLC was higher with mortality.(p=0.0130). Neutrophilia (p= 0.0873) was observed in 67% of patients. 68.5% of patients had lymphocytes below normal range.We observed 79.5% patients having an increased N/L ratio. There was no major change in platelet counts during covid 19. Only 21% patients had reduced platelet counts.

So we observed leukocytosis, neutrophilia, lymphocytopenia and increased NLR ratio in covid patients in our study.

This study had similar findings with the study done B Abhinam Shetty et al 2021 (1) in which TLC, neutrophils, lymphocytes, and N-L ratio were noted as significant predictors of COVID-19 outcome.

The statistically significant variables noted between the survived and mortality groups were age, TLC, neutrophil, lymphocytes, NLR.However in our study only Leucocytosis was associated with mortality.

Similar findings were seen in study by Singh et al. (2) concluded who concluded NLR to be an independent factor of disease severity. Olga Pozdnyakova et al. also said that Hospitalised patients with COVID-19 should undergo a comprehensive daily CBC with manual WBC differential to monitor for numerical and morphologic changes predictive of poor outcome and signs of disease progression.

Coagulation Profile Analysis -PT, INR, APTT, D-Dimer PT :Among overall study population, 34.5% cases showed increased PT. INR was raised in 32% cases (p=0.2791) and was not associated with mortality.APTT was observed higher in 6% whereas 50% had values below the normal range. Clinical parameters like PT, INR and APTT were not associated with mortality.However 87% patients have abnormal level of D-dimer.This was associated with severity and mortality .Shambel Araya et al concluded prolonged prothrombin time and high INR in COVID-19 patients.Thrombocytopenia and prolonged clotting time assay were dominant in COVID-19 patients older than 55 years. Babak Sayad et al (5) interpreted high PT and INR that were associated with poor prognosis.The abnormal pattern of coagulation parameters was highly associated with comorbidities and mortality.

Hai-Han et al (3) also had similar findings of severe COVID-19 with a higher level of D-dimer than those with non-severe disease, and D-dimer greater than 0.5 μg/ml associated with severe infection in patients with COVID-19. Poudel A et al.(2021) (4) also emphasised on D-dimer as a biomarker for assessment of COVID-19.

Most COVID-19 patients presented coagulation systems, dynamic monitoring of coagulation parameters might be a key in the control of COVID-19 death.Wenjing Ye et al(2020) (6) studied 349 participants and like our study found D-Dimer was higher statistically compared with survivors (P<0.001). The multivariable Cox regression analysis showed that age, the peak D-Dimer were prognostic factors for COVID-19 patients' death which was similar to our study .

SUMMARY

The present study comprises the retrospective study of 400 cases of covid positive patients from 1st December 2020 to 30 th June 2022 in the department of pathology, Gajra Raja Medical College, Gwalior(M.P.) The salient findings of present study (2022) are summarised as follows-

Among 400 patients , 78% had severe covid and mortality was seen in 8.8 % cases. Most common presentation is in the 31-40 yrs age group (32.5 %) followed by the 41 -50 yrs age group (27.5%). Only 1% presented before 20 yrs of age.

Higher aged patients were more severely affected. Males were affected more commonly with the sex ratio of 1.94 :1.

In the CBC profile of the patient,we studied CBC, TLC, haemoglobin ,NLR ratio and platelets. Neutrophilic leukocytosis and increased NLR ratio was observed in the majority of covid patients and was also associated with severity and morbidity.

In the Coagulation profile, we studied PT,INR,aPTT, D-dimer. Out of these D- dimer was increased in 87% patients and raised values were associated with mortality.

Overall only leukocytosis and raised d-dimer values were associated with death.

CONCLUSION

Despite the multimodal strategies of synergism and personalized treatment, available therapies are of limited utility. Patients still have poor prognosis.

The incorporation of haematological , coagulation of patient has paved the way for great prospects regarding determining prognosis of patient and monitoring response to treatment.

On the basis of findings in our study Following investigations can be advised in covid -19 patients :

Total leucocyte count with special emphasis on NLR ratio. Coagulation profile specially D- Dimer should be monitored.

They can be concluded as important predictors of disease severity and outcome.These parameters can be used to identify high-risk patients at resource-limited settings.

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