PATTERN OF COAGULATION PARAMETERS IN PATIENTS WITH COVID-19 -A SINGLE CENTRE BASED STUDY

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ABSTRACT

Objective: To determine prognostic significance of coagulation parameters in patients with COVID-19.
Study Design: A prospective comparative study.
Place and Duration of Study: Department of Haematology, Army Medical College, Pak Emirates Military Hospital, Rawalpindi, from Apr to May 2020.
Methodology: A total of 248 patients diagnosed with COVID-19 of all ages irrespective of gender were enrolled. Their coagulation parameters were assessed and comparisons were made between patients with mild/moderate (non-critical) disease against those with severe/critically ill (critical).Performa was designed and data was analyzed using SPSS 26.
Results: Patients in the critical group revealed constantly elevated levels of Domain-dimer (D-dimer, ng/ml - 73.7% vs. 50.5%, 89.5% vs. 39%, 78.9 vs. 41.9%, 77.8% vs. 42%), increased activated partial thromboplastin time (APTT - 34.68 vs. 32.17 sec, 38.84 vs. 32.40 sec, 37.58 vs. 32.50 sec , 37.94 vs. 32.61 sec) and prothrombin time (PT - 14.26 vs. 14.20 sec, 14.79 vs. 14.08 sec, 14.68 vs. 14.10 sec, 15 vs. 14.25 sec) compared to noncritical group (p<0.05). Moreover, higher fibrinogen levels were associated with severe disease (296.32 vs257.92 mg/dl, 280.53 vs. 262.64 mg/dl, 274.74 vs. 264.42 mg/dl, 270.56 vs. 263.10 mg/dl).
Conclusion: Deranged coagulation parameters were observed in patients with COVID-19 and significantly higher in those with severe cases. Regular monitoring of D-dimer, fibrinogen, APTTand PT can provide good accuracy in predicting the severity of disease.

Keywords: Activated Partial Thromboplastin Time (APTT), Coronavirus Disease - 2019 (COVID-19), Domain-dimer (D-dimer), Prothrombin Time (PT).

INTRODUCTION

Human history has been shaped many times by famines, wars and many other geological disasters. As humans continue to evolve on this planet, so do microbes. From Antonin plague in 165AD, to the most recent SARS (severe acute respiratory syndrome), MERS (Middle East respiratory syndrome) and Ebola; there has always been a sudden flare up of infectious diseases. Recently another outbreak, caused by novel Coronavirus started in Wuhan, the capital of Hubei province of China in November 2019[1]. Few more cases appeared in December, all pointing towards an unknown virus, finally gaining attention worldwide and the genome identified as 2019-n COV on 7th January[1] and later COVID-19/SARS-COV2 on February 11th, 2020 by WHO[2]. By now it has crossed borders with confirmed 6,194,533 cases along with 376,320 deaths (last updated June 2nd, 2020).

SARS-COV-2 belongs to the family of coronaviruses, genus Beta coronavirus. Despite the origin of disease from the wild animal market in Wuhan, and later close to 90% whole genome sequence resemblance with the bat coronavirus Isolate RaTG13 and bat-SL-CoVZC45 and bat-SL-CoVZXC21[3,4], person-to-person transmission was considered responsible for the major rapid spread of the disease[2]. Respiratory droplets, close contacts, fomite handling and healthy carriers were also modes of transmission[3]. With the entry of pathogen inside body; viraemia develops with generalized features like fever (88.7%), cough (67.8%) and diarrhea[5]. Followed by spread to

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many vital organs mainly those with ACE2 receptors. This roughly coincides to 7-14 days after onset of initial symptoms. Worsening of primary lung injury causes imaging lesions to become more apparent, associated with a drop in lymphocytes and a surge in leucocytes/inflammatory markers eventually leading to cytokine storm and hyper coagulable state. At this point the D-dimers and fibrinogen levels show major variations; along with increasing prothrombin time and mild drop in platelet[6]. Severe COVID-19 patients gradually develop condition that resembles DIC per ISTH criteria. Clinically the patient presents with symptoms that range from mild, moderate, severe to critically ill ultimately leading to multiorgan failure[6,7].

Mean while the epidemiological and other demographic risk factors are being explored, a significant number of recently published articles have highlighted the role of laboratory parameters in estimation of risk associated. Various studies found the association of severe disease with deranged coagulation parameters[3,7-9].

Explosive growth has been observed in confirmed cases of COVID-19 in Pakistan, since reporting of first case on 26th February 2020. Patient zero was followed by many other pilgrims returning from Iran[10]. When submitting this article there were 76, 398 reported cases of COVID-19 with 1,621 deaths. In a developing country with limited health care resources, strict guidelines need to be established to minimize the disease burden. No study has been yet conducted in our population to establish the association of coagulation markers with disease severity and progression of COVID-19. Based on this background, we attempted to recognize the abnormalities of coagulation marker which might be helpful in better understanding of progression of disease and timely management of COVID-19 patients in future.

METHODOLOGY

This comparative study was conducted at Department of Haematology, Army Medical College, Pakistan Emirates Military Hospital (PEMH), Rawalpindi during Apr to May 2020. COVID-19 patients were diagnosed according to the WHO interim guidance at the department of Virology, Armed Forces Institute of Pathology, Rawalpindi. Nasopharyngeal swabs were obtained and diagnosis was made using RT-PCR (real-time polymerase chain reaction). Sample size was calculated using WHO calculator[2] (with a prevalence of 7 and confidence interval of 95%). A total of 248 patients of all ages irrespective of gender with a hospital stay of ≥7 days were part of study. Patients were excluded based on hospital duration less than 7 days, and unavailability of laboratory data. The study was conducted after approval of the Institutional ethics review committee. Informed consent was taken and patient proforma was filled. All the data pertaining to patients was kept confidential and code numbers were assigned to all participants. Data was not accessible to anyone outside the research team. Demographic data of all patients were noted from the hospital records.

For diagnosis of COVID-19, pharyngeal swabs were obtained from patients who were either symptomatic, had a contact history or travelled from abroad. During hospital stay patients were categorized by treating physicians according to hospital devised criteria: patients with mild fever, shortness of breath and flue like symptoms with or without comorbidities (mild), <50% infiltrates on lung fields in chest x-ray with respiratory rate of <20/min (moderate), evidence of pneumonia, oxygen saturation less than 93% at rest, respiratory rate >30 and lung infiltrates >50% (severe) and no response to oxygen therapy with multiorgan involvement (critical disease).In our study, patients were divided into two groups. Group included non-critical cases with mild/ moderate disease (samples obtained from wards) while patients in critical group had severe to critical disease (sent from intensive care/ treatment units).

Coagulation tests including the prothrombin time (PT), activated partial thromboplastin time (APTT), D-dimers and fibrinogen were recorded at three days interval from day 1 to day 10 after

admission and comparisons were made. PT and APTT were done though Stago coagulation analyzer with normal values of 14 seconds and 32 seconds respectively. Latex agglutination method was used for detection of D-dimers with a cut off value of 250ng/ml. Fibrinogen was estimated using tube method with a normal range of 200-450 mg/dl. Older age was marked at 60 years or older.

Categorical variables were expressed as frequencies whereas means and standard deviation were calculated for quantitative data. Comparison of means was performed using independent sample t-test, while chi-square test was used to analyze differences of frequencies among groups. The *p*-value of ≤0.05 was considered statistically significant. Data was analyzed using SPSS 26.0 for Windows (SPSS Inc.).

RESULTS

A total of 248 patients hospitalized in PEMH, Rawalpindi by May 2020 were included in the study and their clinical features, laboratory data and outcome were followed through their admission. Non-critical group had 210 while critical had 38. Among all COVID-19 patients 228 (91.94%) were males and 20 (8.06%) were females. Age ranged from 20-75 years with an overall mean age of 42.28 ± 13.6 years. Overall 222 patients (89.5%) recovered, 10 deaths (4%) were reported from critical group and 16 patients (6.5%) were still under treatment. Comorbidities were present in all of the critical patients 38 (100%) and only 16.52% of non–critical group. Mean stay in hospital was longer in critical group than non-critical (18.37 ± 6.36 days vs. 15.74 ± 4.16 days). Most commonly reported clinical symptom was fever followed by cough; frequencies of these are given in table-I. Clinical outcome of these patients are given in fig-1.

Based on our observation overall mean PT was 14.2 ± 0.59 sec, but the values were significantly raised in critical vs. non-critical group (table-II).

On comparison of mean APTT levels between critical and non-critical group respectively, significant difference was observed (34.68 vs. 32.17 sec $p<0.001$ on Day 1, 38.84 vs. 32.40 sec $p<0.001$ on Day 4, 37.58 vs. 32.50 sec $p<0.001$ Day 7 & 37.94 vs. 32.61 sec $p<0.001$ on Day 7).

D-dimer levels which were initially raised in 50.5% of the patients in noncritical group, decreased to 42% by day 10. However, the patients in critical group showed persistently elevated levels on all four occasions. As per our findings, a gradual decline was seen in fibrinogen levels in 32.26% of the patients from day 1.

The trend of elevated PT (2-3 sec), raised APTT (as high as 60 sec) and a decreasing fibrinogen levels was seen in patients, who succumbed to the disease. By the end of our

Table-I: Demographic characteristics of Patients with Coronavirus Disease 2019.

Parameters	Critical	Non Critical	*p*-value
Participants	38	210	<0.001
Age (years)	59.89 ± 9.96	39.10 ± 11.58	
Gender			
Male	30 (78.9%)	198 (94.3%)	<0.001
Female	8 (21.1%)	12 (5.7%)	
Contact History			
Unknown	36 (94.7%)	122 (58.1%)	<0.001
Travel	-	16 (7.6%)	
Contact	2 (5.3%)	72 (34.3%)	
Fever			
Yes	38 (100%)	194 (92.4%)	<0.079
No	-	16 (7.6%)	
Cough			
Yes	38 (100%)	130 (61.9%)	<0.001
No	-	80 (38.1%)	
Shortness of Breath			
Yes	32 (84.2%)	56 (26.78%)	<0.001
No	6 (15.8%)	154 (73.3%)	
Comorbid			
Yes	38 (100%)	34 (16.52%)	<0.001
No	-	176 (83.48%)	
Outcome			
Recovered	24 (63.2%)	198 (94.2%)	
Under Treatment	04 (10.5%)	12 (5.8%)	
Expired	10 (26.3%)	-	
Days of Admission	18.37.42 ± 6.36 (8-31)	15.74 ± 4.16 (7-25)	<0.001

observation period, 24 patients had recovered and 4 were still under treatment with an overall mortality rate of 4%.

ratio of 11[1]. This may be because of the cultural and social circumstances as in majority of the house-holds male are still the main bread earner

Table-II: Laboratory Indices of Patients with Coronavirus Disease 2019 (n=248).

Laboratory Parameters		Critical (n=38)	Non-Critical (n=210)	*p*-value
Prothrombin Time (sec)	Day 1	14.26 ± 0.55 (14-16)	14.20 ± 0.59 (14-17)	<0.54
	Day 4	14.79 ± 1.17 (14-18)	14.08 ± 0.36 (14-17)	<0.001
	Day 7	14.68 ± 1.51 (14-19)	14.10 ± 0.31 (14-15)	<0.001
	Day 10	15.00 ± 1.17 (14-17) n=36	14.25 ± 0.83 (14-20) n=200	<0.001
Activated Partial Thromboplastin Time (sec)	Day 1	34.68 ± 5.94 (32-56)	32.17±0.68 (32-36)	<0.001
	Day 4	38.84 ± 10.26 (32-63)	32.40 ± 1.93 (32-48)	<0.001
	Day 7	37.58 ± 10.16 (32-68)	32.50 ± 2.93 (32-60)	<0.001
	Day 10	37.94 ± 9.39 (32-60) n=36	32.61 ± 2.42 (32-46) n=200	<0.001
D-dimer (ng/ml) Day 1	>250	28 (73.7%)	106 (50.5%)	<0.008
	<250	10 (26.3%)	104 (49.5%)	
Day 4	>250	34 (89.5%)	82 (39%)	<0.001
	<250	4 (10.5%)	128 (61%)	
Day 7	>250	30 (78.9%)	88 (41.9%)	<0.001
	<250	8 (21.1%)	122 (58.1%)	
Day 10	>250	28 (77.8%)	84 (42%)	<0.001
	<250	8 (22.2%)	116 (58%)	
Fibrinogen (mg/dl)	Day 1	296.32 ± 52.94 (200-350)	257.92 ± 41.98 (200-455)	<0.001
	Day 4	280.53 ± 38.69 (240-360)	262.264 ± 37.52 (220-455)	<0.006
	Day 7	274.74 ± 39.10 (200-360)	264.42 ± 32.50 (200-350)	<0.083
	Day 10	270.56 ± 39.35 (200-340) n=36	263.10 ± 34.10 (140-360) n=200	<0.24

DISCUSSION

In our study, we followed up on diagnosed cases of COVID-19 admitted in our setup over a period of around six weeks. Most of these cases were symptomatic as mass screening is not being carried out in our country and only symptomatic or high risk susceptible individuals are being screened for COVID- 19.

A total of 248 patients were admitted to our hospital during this period with a male to female

and hence more social interaction. This male preponderance was also observed in other studies[2,4,11-13]. Among the non survivors 60% were male while 40% were female. This was in concordance with the findings of Zhou *et al*[2], according to which 70% of the male, while 30% of the female succumbed to the disease.

Age was observed as an independent risk factor for severe disease. Mean age of 39.10 years as compared to 59.89 years was observed in non-

critical and critical groups respectively. Similar findings were observed by Wang *et al*[11], who observed the median age of non ICU patients at 51 years compared to 66 years for ICU patients. Another fact to be noted was that elderly patients were more at risk of dying of the disease when comparing the mean age of non survivors versus

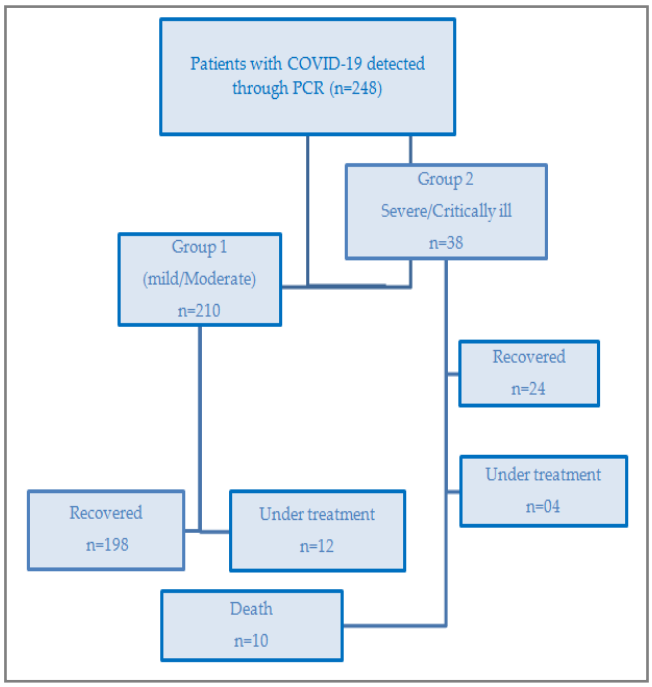

Figure: Clinical outcome of Patients with Coronavirus Disease 2019.

survivors (61.60 ± 5.4 years vs. 41.47 ± 13.17 years, p<0.001). Similar findings were observed in various studies 69 vs. 52 years[2], 68.5 vs. 50 years[4].

In this study, we observed the clinical features; presence of comorbidities and their association in patients with COVID-19. Comorbidities noted were diabetes, hypertension, tuberculosis and ischemic heart disease. Presence of comorbidities was linked with severe disease. This was also observed in study conducted in Wuhan[4], in which majority of patients having severe disease had comorbidities. Wang *et al* reported that the greater number of elderly patients were admitted to the ICU with more comorbid conditions suggesting that age and comorbidity may be risk factors for poor outcome[3,11].

In our study participants presenting to hospital were symptomatic at time of admission; therefore fever was the most common symptom noticed in majority of them (93.5%). This is in comparison to what was observed in other studies which gave a range from 87% to 94%[2,5,14]. The second most common feature observed was Cough (67.7%) followed by shortness of breath (35.48%), which was seen in most of critical patients, ultimately requiring assisted ventilation (noninvasive/invasive). Different studies observed a frequency of around 67% to 81%[2,4,5,15], which is comparable to our observation.

On analysis of laboratory parameters related to coagulation studies we found statistically significant difference among the means and frequencies of these studies (PT, APTT, D-dimer and fibrinogen values) among critical and non-critical group. More so, the non-survivors (n=10) demonstrated maximum derangement of all coagulation parameters with persistently raised D-dimer levels. These findings were in accordance with other published series. Huang *et al*[12], observed raised PT at the time of admission in critical cases. Similar results were seen by Tang[16] who found raised D-dimer, fibrinogen and prolongation of Pro-thrombin count and activated partial thromboplastin time in non survivors. Moreover, 71% of non-survivors fulfilled the International society of Thrombosis and hemostasis criteria of DIC[15,16] with onset of DIC on 4th day of hospital admission.

We observed a persistently elevated D-Dimer levels in critical vs. non –critical group from day 4 onwards. Similar observations were documented by multiple studies, establishing the role of D-dimer in predicting the severity of disease. Fibrinogen and D-dimer level was significantly higher in the severe group than in the mild group in a study conducted on 43 patients[17] and another on 140 patients[15]. Another study conducted in Wuhan established the association of D-dimer with 28 days mortality in 191 patients[2]. Elevated PT and D-dimer were found to be associated with higher risks of development of acute respiratory distress syndrome in a study by Wu *et al*[4]. A small group of medical workers infected with COVID-19 in China were found

to have elevated D-dimer levels[18]. In a study comparing patients with severe COVID-19 pneumonia and severe pneumonia induced by non COVID pathogens, found that patients with higher platelet and D-dimer levels may benefit more from anticoagulant therapy[9].

On the contrary, Han *et al*[8] observed no differences in values of APTT, PT, PT-INR and thrombin time between COVID-19 patients and healthy controls. This may be due to the fact that he used healthy controls as a comparison with diseased group. He concluded by saying that, D-dimer and FDP were more predictive of disease progression[8].

It has been now reported that coagulation cascade is activated and exaggerated in patients with severe COVID-19. Multiple mechanisms involved include presence of ACE 2 - receptor for COVID-19 on endothelial surfaces[2,19], dysfunction of endothelial surfaces[9], surge of pro inflammatory markers leading to cytokine storm, excessive thrombin generation and hyper fibrinolysis[20]. Taking this into account, it is obvious that blood coagulation is clearly affected in patients with COVID-19 and elevated d-dimers, fibrinogen, PT and APTT are significantly associated with poor prognosis of disease. More critical monitoring of these markers should be guaranteed for these patients. The same can be said about the observations made in the current study.

There were some limitations to this study. Firstly, the size of the cohort was relatively small and secondly the duration of study was limited; this might have an influence on interpretation of data. However, by including patients of all ages presenting during the specified time period we believe there was minimum bias. But as it is an evolving situation, multicenter studies are required to study the nature and behavior of this disease.

CONCLUSION

Elevated levels of D-dimer, fibrinogen, PT and APTT can help in predicting the clinical progression/outcome of patients with COVID-19. Therefore monitoring of these markers can be beneficial in clinical settings.

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No funds were taken from any source to conduct this research and data was retrieved from hospital laboratory. Patients were neither given any sort of compensation.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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