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Plasma haemostatic levels in COVID-19 patients and healthy controls: A comparative study at Two tertiary Hospitals in Zambia

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Abstract

COVID-19 is a novel coronavirus disease that has caused a global pandemic with millions of confirmed cases and deaths worldwide. One of the major complications associated with COVID-19 is the development of a hypercoagulable state, leading to thrombotic events such as venous thromboembolism, pulmonary embolism, stroke, and myocardial infarction. The pathophysiology of COVID-19-associated coagulopathy involves a complex interplay between viral infection, host immune response, endothelial dysfunction, and inflammatory cytokine storm.

The main objective of this study was to evaluate Plasma Haemostatic Levels in COVID-19 Patients and Healthy Controls and its association with COVID-19 severity levels.

This was a mixed-methods research project that employed cross-sectional and case-control study designs. The study population consisted of SARS-CoV-2 positive patients at Ndola Teaching Hospital (NTH) and Levy Mwanawasa University Teaching Hospital (LMUTH). The laboratory tests included the assessment of haemostatic profiles in COVID-19 patients compared to control subjects. Additionally, this study explored the use of haemostatic profiles in classifying COVID-19 severity in relation to the clinical methods currently in use. Data analysis was performed using SPSS version 21.

Our study observed elevated plasma levels of haemostatic profiles such as D-dimer, Von Willebrand Factor (VWF), VWF/ADAMTS13 ratio, Factor VIII, Plasminogen Activator Inhibitor (PAI), and Soluble P-selectin in COVID-19 patients compared to the control group. Additionally, COVID-19 patients exhibited a higher prevalence of hypercoagulability (57.2%) compared to control participants (3%). The study also found that the frequency of coagulability increased with COVID-19 severity. Furthermore, statistically significant differences in mean haemostatic plasma concentration were observed in relation to COVID-19 disease severity.

In conclusion, our study found that COVID-19 patients exhibited elevated haemostatic parameters compared to healthy controls. These parameters were observed to correlate with COVID-19 severity levels. The study provides valuable insights into the haemostatic mechanisms of COVID-19 and identifies potential biomarkers for hypercoagulability. These findings may have implications for the diagnosis, prognosis, and management of COVID-19 patients. Clinicians can utilize this information to identify patients with a poor prognosis and assess disease severity, enabling early intervention.

Keywords: COVID-19; Sars-Cov-2; D-dimer; Hypercoagulability; Biomarker; Endothelium

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1. Introduction

COVID-19 is a novel coronavirus disease that emerged in late 2019 and has since caused a global pandemic with over 200 million confirmed cases and 4 million deaths as of August 2021 (Sampath et al., 2021). COVID-19 is characterized by a wide range of clinical manifestations, from asymptomatic or mild respiratory symptoms to severe acute respiratory distress syndrome (ARDS), multi-organ failure, and death (Ragab *et al.,* 2020). One of the major complications of COVID-19 is the development of a hypercoagulable state, which can lead to thrombotic events such as venous thromboembolism, pulmonary embolism, stroke, and myocardial infarction (Sampath et al., 2021). The pathophysiology of COVID-19-associated coagulopathy is not fully understood, but it is likely to involve a complex interplay between viral infection, host immune response, endothelial dysfunction, and inflammatory cytokine storm (Kang & Kishimoto, 2021).

Plasma haemostatic levels are important biomarkers that reflect the coagulation status of COVID-19 patients. Several studies have reported abnormal plasma levels of fibrinogen, D-dimer and other markers of hypercoagulability in COVID-19 patients compared to healthy controls (Contoli et al., 2021; Burke et al., 2020; Zhu et al. 2020). Moreover, these markers have been shown to correlate with disease severity, clinical outcomes, and mortality in COVID-19 patients. However, most of these studies were conducted in single centers or with limited sample sizes. Moreover, according to our knowledge no study of this kind has been done in Zambia and hence a need for comprehensive and comparative studies to determine the haemostatic profiles of COVID-19 patients in comparison to healthy controls across different settings and locations in Zambia.

The main objective of this study was to compare the plasma haemostatic levels of COVID-19 patients and healthy controls at two tertiary hospitals in Zambia: Ndola Teaching Hospital (NTH) and Levy Mwanawasa University Teaching Hospital (LMUTH). Additionally, we aimed to determine the prevalence of hypercoagulability among COVID-19 patients in comparison to healthy control individuals. We also investigated the association between these markers and disease severity in COVID-19 patients. Our hypothesis was that COVID-19 patients would have higher plasma levels of haemostatic markers than healthy controls, and that these markers would be associated with worse disease severity in COVID-19 patients.

2. Material and methods

This research was a Hospital based study and utilised cross sectional and case control study designs. These study designs were chosen because of being relatively cheap and results were obtained in a quickest possible time. The study was conducted at Ndola Teaching Hospital and Levy Mwanawasa University Teaching Hospitals (LMUTH). Ndola Teaching Hospital is a third level referral hospital for Copperbelt and Northern part of Zambia. The Hospital is located at the Corner of Broadway and Nkana Roads in Ndola, the Provincial headquarters of the Copperbelt province. It is the second largest Hospital in Zambia. The Hospital has a bed capacity of 851 and acts as a referral Hospital for the Northern part of Zambia. Ndola Teaching Hospital was chosen because of its close proximity to Tropical Diseases Research Centre (TDRC), which was able to undertake confirmatory Molecular Techniques for SARS-CoV2.

The LMUTH is situated along the Great East Road around Chainama Hills area in Lusaka. LMUTH functions as a Provincial hospital with 3rd level services and was chosen because the Hospital served as a COVID-19 referral centre in Lusaka.

The study included Hospitalized or Outpatients at NTH and LMUTH with a confirmed diagnosis of COVID-19 using a reverse transcriptase–polymerase chain reaction (RT-PCR) assay on nasopharyngeal swab samples. For each patient, clinical history and some laboratory findings were obtained from the patients' hospital records. The study also included non-COVID-19 non-hospitalized individuals to serve as controls. Control subjects selected were those with an initial clinical suspicion of COVID-19, but with mild clinical presentation and a negative RT-PCR result.

The study recruited a total number of 340 participants comprising of 87 and 86 SARS-Cov-2 positive patients at NTH and LMUTH respectively while 84 and 83 SARS-Cov-2 negative individuals were recruited at NTH and LMUTH respectively. This study adopted the simple random sampling technique to recruit 173 COVID-19 positive patients and 167 COVID-19 negative patients. This type of technique was adopted in this study because it is easy to conduct and when conducted properly, a simple random sample represents an unbiased sample, and therefore is a fair and accurate representation of the population.

2.1. Inclusion criteria for COVID-19 patients and control participants

This study enrolled individuals who tested positive for COVID-19 by RT-PCR as cases and healthy individuals of both genders aged 18 years or older testing negative for Sars-Cov-2 as control subjects. Only those who provided informed consent were included in the study

2.2. Exclusion criteria for COVID-19 patients and control participants

Participants who had a history of venous thromboembolism or known inherited coagulation disorders, Cancer and hyperthyroidism were excluded from the study. Others excluded include, those who were Pregnant, had recent surgery, those taking standard anticoagulant treatment, less than 18 years and those not willing to consent.

2.3. Data Collection

Good Laboratory Practice (GLP) principles according to the Ministry of Health laboratory quality manual was observed to ensure uniformity, consistency, reliability and reproducibility of all the laboratory test results in the study. Quality control measures were observed in all the laboratory procedures. Laboratory results of some COVID-19 patients who were once admitted to NTH and LMUTH but had been discharged were retrieved from the laboratory Disalab electronic system. Blood for Full Blood Count was collected in EDTA tubes. The Sysmex XT-2000i was used for Full blood count analysis.

2.4. Prothrombin and Activated Partial Thromboplastin Time (PT AND APTT) Analysis

Three (3) ml of venous blood for PT and APTT was collected from each of the study participants in sodium citrate containers and centrifuged at 1500 g for 15 minutes. Plasma was then separated and transferred into siliconized glass tubes and stored at 4° C in a fridge until analysis. The reagents for PT and APTT were sourced from SPINREACT Diagnostics Company of Spain. PT and APTT results were reported in seconds.

Blood for fibrinogen determination was collected from the study participants in sodium citrate containers and centrifuged at 1500 g for 15 minutes. Plasma was then separated and transferred into plastic or siliconized glass tubes and stored at -20°C until analysis. The reagents for this test were sourced from SPINREACT Diagnostics Company of Spain. The kit used utilizes the thrombin clotting time assay based on the method originally described by Clauss (Burtis, 1994).

The same participants' plasma samples for PT and APTT analysis as prepared above was used for VWF analysis. VWF was determined by the Human ELISA kit manufactured by Abnova of USA. The detailed procedure for VWF estimation was as provided by the manufacturer of the kit. Plasma VWF concentration results were reported in international units/ml (IU/ml).

2.5. ADAMTS13 Assay

The Chromogenic Activity assay was used for the analysis of the ADAMTS13. This assay employs a recombinant fragment of the A2 domain of VWF and which encompasses the cleavage site [Tyr1605-Met1606] for ADAMTS13. The recombinant fragment is tagged with Glutathione--transferase (GST). A microtitre plate is coated with an antibody to GST and which then immobilises the GST-tagged rVWF fragment. A test plasma sample is added and the ADAMTS13 will cleave the rVWF fragment at the ADAMTS13 cleavage site The plate is washed and an HRP-conjugated monoclonal antibody targeted to part of the VWF protein that is released when VWF is cleaved by ADAMTS13 is added. A substrate for the HRP is added and the colour change recorded. The change in OD is proportional to the cleavage of the VWF fragment by ADAMTS13 and therefore is a measure of ADAMTS13 activity (Lotta *et al.,* 2014).

2.6. D-Dimer analysis

D-dimer is a degradation product of cross-linked fibrin formed during activation of the coagulation system. Ichroma™ II automated equipment manufactured by Boditech Med Incorporated of the Republic of Korea was used for the analysis of plasma D-dimer levels. It is a fluorescence and Europium nanoparticle scanning instrument used in conjunction with various Ichroma™ Immunoassay Tests which are based on antigen-antibody reaction and fluorescence technology. Ichroma™ II uses a semiconductor diode laser as the excitation light source for illuminating the test cartridge membrane (pre-loaded with the clinical specimen duly processed as per the standard test procedure prescribed by Boditech Med Inc.) thereby triggering fluorescence from the fluorochrome molecules present on the membrane. The test uses a sandwich immunodetection method; the detector antibody in buffer binds to antigen in sample, forming antigen antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip. The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of

fluorescence signal on detector antibody, which is processed by Instrument for Ichroma™ tests to show D-Dimer concentration in sample.

2.7. Plasminogen Activator Inhibitor analysis

Type I plasminogen activator inhibitor (PAI-1) is a 50 kDa serpin family member that inhibits tissue- and urokinase-type plasminogen activators (t-PA, u-PA). This protein appears to be an important regulator of plasminogen activation by t-PA and extracellular proteolysis by u-PA.

Ab108894 Human PAI-1 Chromogenic Activity Assay Kit was used to determine PAI-1. This kit was developed to determine human PAI-1 activity in plasma samples. A fixed amount of tPA is added in excess to undiluted sample, which allows PAI-1 and tPA to form an inactive complex. The assay measures plasminogen activation by residual tPA in coupled assays that contain tPA, plasminogen, and a plasmin specific synthetic substrate. The amount of plasmin produced is quantitated using a highly specific plasmin substrate releasing a yellow para-nitroaniline (pNA) chromophore. The absorbance of the pNA at 405 nm is inversely proportional to the PAI-1 enzymatic activity.

2.8. Plasma P-Selectin estimation

P-selectin is a protein from the lectin family and a cell adhesion molecule. It is the first upregulated glycoprotein on activated endothelial cells and platelets and has procoagulant properties. P-selectin, stored in the platelets (alpha granules) and in the endothelial cells (Weibel-Palade bodies), is translocated to the cell surface after activation and partially released into the circulation in its soluble form.

The kit used for estimation of Plasma P-selectin in plasma was based on sandwich enzyme-linked immune-sorbent assay technology. Capture antibody was pre-coated onto 96-well plates. And the biotin conjugated antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of target was calculated.

We compared the average levels of these biomarkers in patients with different levels of disease severity to determine if there was a correlation between the two.

WHO acceptable classification was used for COVID-19 severity classification (Buonsenso et al., 2021) and is as follows;

2.8.1. Asymptomatic Infection

Individuals who tested positive for SARS-CoV-2 using nucleic acid amplification test (NAAT) or an antigen test) but who have no symptoms that are consistent with COVID-19.

2.8.2. Mild Illness

Individuals who had any of the various signs and symptoms of COVID-19 (e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell) but who did not have shortness of breath, dyspnea, or abnormal chest imaging.

2.8.3. Moderate Illness

Individuals who showed evidence of lower respiratory disease during clinical assessment or imaging and who had oxygen saturation (SpO2) ≥94% on room air at sea level.

2.8.4. Severe Illness

Individuals with clinical signs of pneumonia (fever, cough, dyspnoea, fast breathing) plus one of the following: respiratory rate > 30 breaths/min; severe respiratory distress; or SpO2 < 90% on room air.

2.8.5. Critical Illness

Individuals who have respiratory failure, septic shock, and/or multiple organ dysfunction.

Plasma D-dimer and Soluble P-selectin levels were used as biomarkers for hypercoagulability in Covid-19 patients. A Hypercoagulable state is the medical term for a condition in which there is an abnormal increased tendency toward blood clotting. COVID-19 Patients were considered to be hypercoacoagulable when the D-Dimer and Soluble P-selectin (sP-Selectin) concentration were above 500 ng/mL and 3.2 ng/ml respectively (Pagana et al., 2019; Fenyves et al., 2021). Soluble P-selectin levels were chosen besides D-dimer to discriminate between hypercoagulable and Non hypercoagulable Covid-19 patients in reference to Fenyves et al., (2021) who reported Plasma P-selectin as an early marker of thromboembolism in COVID-19 patients and when used with D-dimer improved its ability to detect hypercoagulability.

2.9. Ethical considerations

The study was conducted under a protocol that was reviewed and approved by the Tropical Diseases Research Centre (TDRC) Ethics Review Committee and National Health Research Authority (NHRA). Written permission was obtained from the Permanent Secretary in the Ministry of Health as well as from the Senior Medical Superintendent of Ndola Teaching Hospital and Levy Mwanawasa University Teaching Hospital. The study participants were informed about the study, its purpose, and their rights to participation. Privacy and confidentiality were maintained by using codes instead of names on the forms, lockable cabinets for storage, and password-protected computers. Only qualified medical professionals such as nurses and laboratory staff working in COVID-19 isolation centers were involved in collecting venous blood samples from study participants. Public health measures of social distancing and masking up to mitigate the transmission of COVID-19 was adhered to. Free masks were distributed to all the study participants. All research assistants were required to be fully vaccinated against COVID-19 and underwent a one week training in laboratory safety with a focus on COVID-19.

2.10. Data Analysis

The data was entered and cleaned in Excel sheets before analysis. Data cleaning involved removing duplicates and outliers, correcting inconsistencies, combining data sets, and standardizing data formats. Statistical analysis was performed using SPSS version 21, and the results were summarized in tables and graphs. All statistical tests were performed at a 5% significance level or 95% confidence interval with a p-value of less than 0.05 to determine statistical significance. The distribution of the data was analyzed using the Kolmogoroff-Smirnoff test. Normally distributed results were reported as the mean +/- standard deviation. The independent T-test was used to compare the mean haemostatic and inflammatory parameters of COVID-19 patients for each patient and correlate with COVID-19 disease severity. Analysis of Variance (ANOVA) was used to determine the average levels of Haemostatic profiles in relation to COVID-19 disease severity. The Tukey post-hoc test was employed to ascertain the significant pairwise differences in haemostatic profile concentration among different COVID-19 severity levels.

3. Results

Table 1 Independent T-test for analysis of Haemostatic profiles in Sars-CoV-2 patients and control Subjects

BIOMARKER	SARS-COV-2	CONTROLS	t-value	P-Value
D-Dimer	2147.0±1780.0 ng/ml	226.0±190.5 ng/ml	8.0	0.001
VwF	16.53 ±12.71 Iu/ml	0.65±0.48 Iu/ml	9.0	0.002
ADAMTS13	0.80±0.52 45 Iu/ml	1.03±0.45 Iu/ml	4.2	0.801
VwF/ADAMTS13	38.49±22.54	0.89±1.24	6.7	0.002
Fibrinogen	467.8±271.4 mg/dl	226.1±54.5 mg/dl	11.3	0.000
Factor VIII	4.10±4.00 Iu/ml	0.55±0.29 Iu/ml	11.2	0.000
PAI	57.33±38.28 Iu/ml	13.55±10.24 Iu/ml	6.4	0.000
INR	1.19±0.79	0.69±0.25	7.7	0.059
APTT	33.0±10.0 Seconds	32±4 Seconds	1.9	0.061
P-Soluble Selectin	30.48±16.67 pg/ml	3.16±2.68 pg/ml	8.26	0.000
Platelets	169.0±71.1 $x10^9$ /l	305.0±83.4 $x10^9$ /l	13.8	0.000

An independent-sample t-test was conducted to compare the haemostatic profiles in Sars-CoV-2 Patients and control participants. Table 1, reveals that mean D-dimer concentration for Sars-Cov-2 patients(2147.0 ±1780.0 ng/ml) was significantly higher than control participants (226.0±190.5 ng/ml) t-value = 8.0; P-value = 0.001.

The mean VwF concentration for Sars-Cov-2 patients (16.53 ±12.7 IU/ml) was significantly higher than control participants (0.65±0.48 IU/ml), t-value= 9.0; P-value = 0.002.

The mean ADAMTS13 for Sars-CoV-2 patients (0.80±0.42 Iu/ml) was lower than control participants (1.03 ±0.45 Iu/ml) but the difference was not significant t-value=4.2; P-value = 0.801.

The mean VWF/ADAMTS13 ratio for Sars-Cov-2 patients (38.49±22.54) was significantly higher than control participants (0.89±1.24), t-value= 6.7; P-value = 0.002.

Table 1 further reveals that the mean Fibrinogen concentration for Sars-Cov-2 patients (467.8±271.4 mg/dl) was significantly higher than in Sars-Cov-2 negative participants (226.1±54.4 mg/dl), t-value 11.2; P-value = 0.000.

The mean serum Factor VIII concentration for Sars-Cov-2 patients (4.10±2.00 Iu/ml) was higher than control participants (0.55±0.29 Iu/ml) and this difference in the mea concentration was significant, t-value = 11.2; P-value = 0.000.

The mean plasma concentration of Plasminogen Activator Inhibitor (PAI) for Sars-CoV-2 patients (57.33±38.28 Iu/ml) was significantly higher than in the control subjects (13.55±10.24), t-value 6.4; P-value 0.000.

The mean International Normalised Ratio (INR) and Activated Partial Thromboplastin Time (APTT) for Sars-Cov-2 patients was higher than in the control subjects but this difference was not statistically significant, P-value = 0.059 and 0.061 respectively.

Results further show that the mean P-Soluble Selectin Concentration for Sars-Cov-2 patients (30.48±16.67 pg/ml) was significantly higher than the mean for the control subjects (3.16±2.68 pg/ml), t-value = 8.26; P-value = 0.000.

The mean platelet concentration for Sars-Cov-2 patients (169.0 ±71.1 x 10^9 /l) was significantly lower than the mean platelets count for the control subjects (305.0 ±83.4 x 10^9 /l), t-value = 13.8; P-value = 0.000.

3.1. Frequency of hypercoagulability in SARS-Cov-2 patients and control participants.

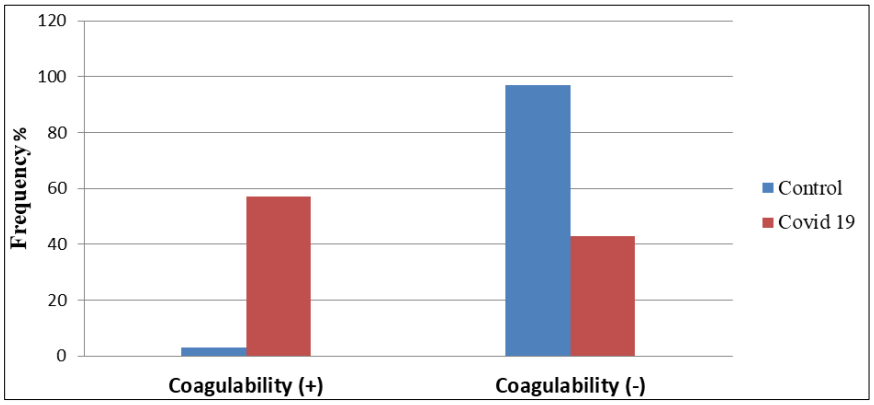

Figure 1 Frequency of Hypercoagulability in SARS-CoV-2 Patients and Control Subjects

D-dimer and P-selectin plasma concentrations, which were continuous variable in SPSS, were recoded so as to categorise the results into two categories; those who had D-Dimer and P-Selectin plasma concentration of greater than 500ng/ml and 3.2 ng/ml respectively were categorized as hypercoagulable and those whose D-dimer and P-selectin plasma concentrations were less than or equal to 500ng/ml and 3.2 ng/ml respectively were regarded as normal. Thereafter a chi-square test of independence was done to determine the proportion of hypercoagulability in Sars-CoV-2 patients and control subjects. Figure 1 reveals that Sars-CoV-2 patients had higher prevalence of hypercoagulability [99(57.2%)] than control participants [5(3%)]. The difference was significance P=0.002.

Table 2 Frequency of Coagulability according to Disease severity

Coagulabillity (+) D-Dimer (>500ng/ml)			**Coagulability (-) D-Dimer (≤500ng/ml)**
P-value 0.002			
	Total	**N (%)**	**N (%)**
Asymptomatic	26	2(7.7)	24(92.3)
Mild	32	9(28.1)	23(71.9)
Moderate	61	38(62.3)	23(37.7)
Severe	39	35(89.7)	4(10.3)
Critical	15	15(100)	0 (0)

Table 2 show results of frequency of hypercoagulability according to Sars-CoV-2 disease severity. The results indicate that frequency of hypercoagulability was higher in the critical Sars-CoV-2 patients 15(100%) than the Asymptomatic Sars-CoV-2 patients 2(7.7%), Mild 9(28.1%), Moderate 9(28.1%) and Severe 35(89.7%). These differences in the frequency of hypercagulability according to disease severity was significant, P<0.05.

3.2. Haemostatic profiles of Covid-19 patients according to disease severity

Table 3 Analysis of variance for the means of Haemostatic profiles in Sars-CoV-2 Positive individuals according to disease Severity

Blood Parameter	**Sars-Cov-2 Asymptomatic (N = 26)**	**Sars-Cov-2 Mild Cases (N=32)**	**Sars-CoV-2 Moderate cases(N=61)**	**Sars-CoV-2 Severe (N=39)**	**Sars-CoV-2 Critical (N=15)**		
Haemostatic Profiles	Mean	Mean	Mean	Mean	Mean	Anova (F)	P-value
D-dimer (ng/ml)	310.3	493.3	1461.2	3884.5	6940.0	32.939	0.000*
VwF (Iu/ml)	4.98	4.80	13.91	29.6	38.13	13.801	0.000*
ADAMTS13 (Iu/ml)	0.85	0.80	0.70	0.59	0.36	6.858	0.000*
VwF/ ADAMTS13 Ratio	6.48	9.53	20.67	84.00	110.81	16.886	0.000*
Fibrinogen (mg/dl)	335.2	348.2	388.5	588.2	962.1	35.547	0.000*
Factor VIII (Iu/ml)	1.02	1.57	3.90	6.16	10.27	30.276	0.000*
PAI (Iu/ml)	22.1	31.0	38.4	59.8	242.7	33.437	0.000*
INR	0.73	0.88	0.96	1.46	2.91	49.160	0.000*
APTT (Seconds)	28	28	32	38	54	46.875	0.000*
P-Selectin (pg/ml)	5.04	8.48	20.73	54.61	97.16	32.350	0.000*
Platelets ($x10^{9)}$	244.6	210.9	153.3	133.6	103.8	26.040	0.000*

*= Significant at p<0.05

Table 3 show results of Analysis of Variance (ANOVA) for the means of Haemostatic profiles in Sars-CoV-2 patients according to Covid-19 disease severity. We found statistically-significant differences in mean D-dimer plasma concentration in relation to Covid-19 disease severity (F) =32.939, p < 0.05). Critical patients had the highest mean D-dimer concentration of 6940.0 ng/ml. Tukey post-hoc test revealed significant pairwise D-Dimer concentration differences between Critical Sars-CoV-2 patients and Asymptomatic, Mild, Moderate and Severe Covid-19 patients, with

an average difference of 6629.9, 6446.7,5478.8 and 3055.5 ng/ml D-Dimer concentration respectively ($p < 0.05$). Furthermore, statistically-significant differences in mean D-dimer plasma concentration was found between severe and Asymptomatic mild, and moderate Covid-19 patients with an average difference of 3574.2, 3391.2, 2423.3 ng/ml D-Dimer concentration respectively ($p < 0.05$).

Table 3 further show statistically-significant differences in mean Vonwillebrand (VwF) plasma concentration in relation to Covid-19 disease severity (F) =13.801, $p < 0.05$). Sars-CoV-2 patients in critical state had increased VwF plasma concentration of 38.13 ng/ml. Tukey post-hoc test revealed significant pairwise VwF concentration differences between Critical Sars-CoV-2 patients and Asymptomatic, Mild, Moderate and Severe Covid-19 patients, with an average difference of 33.2, 33.3, 24.2 and 8.5 Iu/ml plasma VwF concentration respectively ($p < 0.05$). Furthermore, statistically-significant differences in the average VwF plasma concentration was found between severe and Asymptomatic mild including moderate Covid-19 patients with an average difference of 24.6, 24.8, and 15.7 Iu/ml VwF concentration respectively ($p < 0.05$).

The table further indicate statistically-significant differences in the average ADAMTS13 plasma concentration in relation to Covid-19 disease severity (F) =6.858, $p < 0.05$). Sars-CoV-2 patients in critical state had the lowest ADAMTS13 concentration of 0.36 Iu/ml in comparison to the asymptomatic patients who had the highest ADAMTS13 plasma concentration of 0.85 Iu/ml.

We found statistically-significant differences in the average VwF/ADAMTS13 ratio in relation to Covid-19 disease severity (F) =16.886, $p < 0.05$). Sars-CoV-2 patients in critical state had the highest VwF/ADAMTS13 ratio of 110.81. Tukey post-hoc test revealed significant pairwise VwF/ADAMTS13 ratio differences between Critical Sars-CoV-2 patients and Asymptomatic, Mild and Moderate Covid-19 patients, with an average difference of 104.3, 101.3 and 90.1 VwF/ADAMTS13 ratio respectively ($p < 0.05$). Additionally, statistically-significant differences in the average VwF/ADAMTS13 ratio was found between Severe and Asymptomatic, Mild and moderate Covid-19 patients with an average difference of 77.5, 74.4, 63.3 VwF/ADAMTS13 ratio respectively ($p < 0.05$).

We further found statistically-significant differences in mean Fibrinogen plasma concentration in relation to Covid-19 disease severity (F) =35.547, $p < 0.05$). Critical patients had the highest mean Fibrinogen concentration of 962.1mg/dl. Tukey post-hoc test revealed significant pairwise Fibrinogen concentration differences between Critical Sars-CoV-2 patients and Asymptomatic, Mild, Moderate and Severe Covid-19 patients, with an average difference of 627.0, 613.9, 573.6 and 373.9 mg/dl Fibrinogen concentration respectively ($p < 0.05$). Furthermore, statistically-significant differences in mean Fibrinogen plasma concentration was found between Severe and Asymptomatic mild and moderate Covid-19 patients with an average difference of 253.1, 240.0 and 199.7 mg/dl Fibrinogen concentration respectively ($p < 0.05$).

Table 3 further show statistically-significant differences in mean FVIII plasma concentration in relation to Covid-19 disease severity (F) =30.276, $p < 0.05$). Sars-CoV-2 patients in critical state had the highest FVIII plasma concentration of 10.27 Iu/ml. Tukey post-hoc test revealed significant pairwise Factor 8 concentration differences between Critical Sars-CoV-2 patients and Asymptomatic, Mild, Moderate and Severe Covid-19 patients, with an average difference of 9.2, 8.7, 6.4 and 4.1 Iu/ml plasma FVIII concentration respectively ($p < 0.05$). Furthermore, statistically-significant differences in the average FVIII plasma concentration was found between severe, asymptomatic, mild and moderately ill Covid-19 patients with an average difference of 5.1 ,4.6 and 2.3 Iu/ml FVIII concentration respectively ($p < 0.05$). Results further show Pair-wise statistically-signifiant differences in the average Factor 8 plasma concentration between moderate and Asymptomatic including mild Covid-19 patients with an average difference of 2.88 and 2.33 Iu/ml Factor 8 concentraion respectively ($p < 0.05$).

Table 3 further show statistically-significant differences in the average Plasminogen Activator Inhibitor (PAI) plasma concentration in relation to Covid-19 disease severity (F) =33.437, $p < 0.05$). Sars-CoV-2 patients in critical state had the highest PAI plasma concentration of 242.7 Iu/ml. Tukey post-hoc test showed significant pairwise PAI concentration differences between Critical Sars-CoV-2 patients and Asymptomatic, Mild, Moderate and Severe Covid-19 patients, with an average difference of 220.6, 212.0, 204.3 and 182.8 Iu/ml plasma PAI concentration respectively ($p < 0.05$).

Furthermore, results of our study indicate that statistically-significant differences in mean International Normalised Ratio (INR) in relation to Covid-19 disease severity (F) =30.276, $p < 0.05$) was obtained. Sars-CoV-2 patients in critical state had the highest INR of 2.91. Tukey post-hoc test revealed significant pairwise INR differences between Critical Sars-CoV-2 patients and Asymptomatic, Mild, Moderate and Severe Covid-19 patients, with an average difference of 2.18, 2.02, 1.94, and 1.45 INR respectively ($p < 0.05$). Furthermore, statistically-significant differences in the average INR was found between severe and asymptomatic, mild and moderately ill Covid-19 patients with an average difference

of 0.72, 0.57 and 0.49 INR respectively ($p < 0.05$). Results further show Pair-wise statistically-signifiant differences in the average Factor 8 plasma concentration between moderate and Asymptomatic including mild Covid-19 patients with an average difference of 2.88 and 2.33 Iu/ml Factor 8 concentraion respectively ($p < 0.05$).

Table 3 further show statistically-significant differences in the average Activated Partial Thromboplastin Time (APTT) in relation to Covid-19 disease severity ($F = 46.875$, $p < 0.05$). Sars-CoV-2 patients in critical state had the prolonged APTT of 54 seconds. Appendix 25 report results of a Tukey post-hoc test which revealed significant pairwise APTT differences between Critical Sars-CoV-2 patients and Asymptomatic, Mild, Moderate and Severe Covid-19 patients, with an average difference of 26, 26, 22, 16 seconds respectively ($p < 0.05$). Furthermore, statistically-significant differences in the average APTT was found between severe and Asymptomatic mild including moderate Covid-19 patients with an average difference of 9.3, 9.6, and 5.3 seconds respectively ($p < 0.05$).

Table 3 further show statistically-significant differences in mean P-Selectin plasma concentration in relation to Covid-19 disease severity ($F = 32.350$, $p < 0.05$). Sars-CoV-2 patients in critical state had the highest P-selectin plasma concentration of 97.16 pg/ml. Tukey post-hoc test revealed significant pairwise P-selectin concentration differences between Critical Sars-CoV-2 patients and Asymptomatic, Mild, Moderate and Severe Covid-19 patients, with an average difference of 92.12, 88.67, 76.463, and 42.55 pg/ml P-selectin concentration respectively ($p < 0.05$). Furthermore, statistically-significant differences in the average P-selectin concentration was found between severe and Asymptomatic mild including moderate Covid-19 patients with an average difference of 49.57, 46.13 and 33.88 pg/ml P-selectin concentration respectively ($p < 0.05$).

Table 4 further show statistically-significant differences in the average Platelet count in relation to Covid-19 disease severity ($F = 26.040$, $p < 0.05$). Sars-CoV-2 patients in critical state had the lowest average platelet count of 103.8×10^9 /l in comparison to the asymptomatic patients who had the highest platelet count of 244.6×10^9 /l. Tukey post-hoc test revealed significant pairwise Platelet count differences between Critical Sars-CoV-2 patients and Asymptomatic, Mild and Moderate Covid-19 patients, with an average difference of -140.8 ,-107.1 and -49.6 platelet count respectively ($p < 0.05$). Furthermore, a statistically-significant difference in the average platelet count was found between severe and Asymptomatic inclusive of patient with mild Covid-19 with an average of -111.1 and -77.3 respectively ($p < 0.05$).

4. Discussion

Hypercoagulability is a condition where the blood clots more easily than normal, which can lead to serious complications such as deep vein thrombosis, pulmonary embolism, stroke, and heart attack. COVID-19 is a viral infection that can cause inflammation and damage to the blood vessels, making the blood more prone to clotting. The current study reported a higher frequency of hypercoagulability in Sars-Cov-2 patients (57.2%) than the healthy control individuals (3.0%). These results are in agreement with a systematic review and meta-analysis of 66 studies involving 28,173 COVID-19 patients which found that the overall prevalence of venous thromboembolism (VTE), a common manifestation of hypercoagulability, was 17%, with higher rates in intensive care unit (ICU) patients than in non-ICU patients (Khaire et al.,2022). A prospective cohort study of 400 COVID-19 patients admitted to a hospital in New York City found that the prevalence of thrombotic events, including VTE, arterial thrombosis, and thrombotic microangiopathy, was 16%, with higher rates in ICU patients than in non-ICU patients (Re et al., 2022). A retrospective cohort study of 3,334 COVID-19 patients admitted to a hospital in China found that the prevalence of coagulopathy, defined as an elevated D-dimer level (>0.5 mg/L), was 46%, with higher rates in severe cases (60%) than in mild cases (43%) ((Cuker et al., 2021).

According to the current study, D-dimer levels in COVID-19 patients were significantly higher than those in controls. This finding is consistent with the results of Campbell et al. (2021) and Devreese (2021), who also reported elevated D-dimer levels in COVID-19 patients. Furthermore, we found a strong correlation between D-dimer levels and COVID-19 severity, which is consistent with the findings of Li et al. (2021), Kim et al. (2021), Varikasuvu et al. (2020), and Nasif et al. (2022). Guan et al. (2020) conducted a retrospective study that revealed a significant association between elevated D-dimer levels and COVID-19 severity. They found that severe COVID-19 cases had a significantly higher proportion of D-dimer levels above 0.5 mg/L than mild or moderate cases. Similarly, Tang et al. (2020) reported that severe COVID-19 cases had a median D-dimer level of 3.5 times higher than mild or moderate cases. The high values of D-dimer reported may result from the activation of the coagulation cascade due to Systemic Inflammatory Response Syndrome (SIRS) in COVID-19 patients. The Sars-CoV-2 may also damage the lung tissue and blood vessels, exposing the subendothelial matrix and activating the extrinsic pathway of coagulation. Moreover, the infection may impair the anticoagulant and fibrinolytic systems, such as lowering the levels of antithrombin, protein C, and plasminogen activator inhibitor-1.

Our study reported higher mean VWF concentration in COVID-19 patients than in controls. This is consistent with Escher et al., (2020) who reported massive elevation of VWF levels in COVID-19 patients compared to COVID-19 negative individuals. We also found higher VwF levels in critical and severe COVID-19 patients than in asymptomatic, mild, and moderate patients. This agrees with Flaumenhaf et al, (2022) who reported that VWF levels are elevated in COVID-19 patients and correlate with disease severity and mortality. We further reported that VwF levels were correlated with COVID-19 severity and increased in severely and critically ill COVID-19 patients compared to asymptomatic and mild patients. This is in line with several studies that have shown the association of VWF levels with COVID-19 severity and thrombotic risk. For instance, a systematic review and meta-analysis of 66 studies with 28,173 COVID-19 patients reported that VWF levels were significantly higher in COVID-19 patients with VTE than in those without VTE. (Petrilli et al., 2020). A prospective cohort study of 400 COVID-19 patients hospitalized in New York City reported that the prevalence of thrombotic events, including VTE, arterial thrombosis, and thrombotic microangiopathy, was 16%, and higher in ICU patients (31%) than in non-ICU patients (9%). They also reported that VWF activity and antigen ratios to ADAMTS13, which cleaves VWF, were significantly higher in patients requiring mechanical ventilation than in those not requiring it (Madeeva et al, 2021).

These studies suggest that VWF levels can be used as potential markers of endothelial cell activation and immunothrombotic complications in COVID-19 patients. High VWF levels may indicate a higher risk of developing thrombosis and worse outcomes. Therefore, it may be beneficial to monitor VWF levels and provide appropriate anticoagulation therapy for COVID-19 patients who have signs of hypercoagulability. A possible mechanism for the elevated vWF levels in COVID-19 patients is that SARS-CoV-2 infects cells via the transmembrane protein ACE2. ACE2 is expressed on alveolar epithelial cells, and arterial and venous endothelial cells (Guney et al., 2021) The viral infection may induce inflammation and damage of endothelial cells, leading to the release of prothrombotic mediators, mainly vWF from the Weibel–Palade storage bodies (WPB), and the exposure of underlying collagen to which vWF binds. Ultra Large-VonWillebrand (UL-vWF) molecules that remain bound to EC surface may subsequently bind platelets and may facilitate leukocyte interaction (Izuzquiza-Avanzini et al., 2022). The virus may also trigger a severe immune response in some patients, termed a cytokine storm, which may damage the endothelial cells and other organs. This may also increase the levels of VWF and other clotting factors in the blood (Sun et al.,(2020).The increased levels of VWF in COVID-19 may overwhelm the activity of an enzyme called ADAMTS13, which normally cleaves VWF into smaller and less adhesive fragments. This may result in the accumulation of large and adhesive VWF multimers, which may enhance platelet aggregation and thrombus formation (Ward et al., 2021). These factors may contribute to the hypercoagulable state and risk of microvascular thrombosis in COVID-19 patients, which may cause serious complications such as stroke, heart attack, pulmonary embolism, and organ failure. Therefore, measuring VWF levels can be potential markers of disease severity and prognosis in COVID-19.

The mean VwF/ADAMTS13 ratio was increased in COVID-19 patients than in the healthy control individuals and this difference was statistically significant. This is consistent with the findings of Mancini et al., (2021) who found significant alteration of the VWF-ADAMTS13 axis in COVID-19 patients, with an elevated VWF:Ag to ADAMTS13 activity ratio that was strongly associated with disease severity. A study by Marco & Marco (2021) confirmed that the VWF antigen/ADAMTS13 activity ratio was significantly higher in COVID-19 inpatients than in non-COVID-19 inpatients, indicating an imbalance in the VWF/ADAMTS13 system in COVID-19. They also observed a lower ADAMTS13 activity and a reduction in fibrinogen and VWF antigen levels in COVID-19 outpatients compared to non-COVID-19 inpatients. Such an imbalance enhances the hypercoagulable state of COVID-19 patients and their risk of microthrombosis (Mei et al., 2021). Reduced plasma ADAMTS-13 levels have previously been reported in association with other types of sepsis including *Plasmodium falciparum* malaria and Dengue virus. Lower ADAMTS-13 activity in severe COVID-19 is likely to be attributable in part to reduced hepatic ADAMTS-13 synthesis. However, increased ADAMTS-13 consumption or clearance leading to deficiency has also been previously associated with markedly elevated plasma VWF levels, which is a hallmark of severe COVID-19. In contrast to other types of sepsis, severe COVID-19 results in a major increase in the VWF/ADAMTS-13 ratio and a significant inverse correlation was observed between VWF:Ag levels and ADAMTS-13 activity. Moreover, this imbalance between substrate and enzyme under high shear stress is likely to be even more pronounced in the lung microvasculature where endothelial cell damage is most evident. Inflammatory cytokines stimulate endothelial cells to release procoagulant molecules. Specifically, IL-8 and tumor necrosis factor-α (TNF-α) trigger the release of ultra-large von Willebrand factor (ULVWF) multimers from endothelial cells in a concentration-dependent manner (Kichloo et al., 2020). IL-6 soluble IL-6 receptor (sIL-6R) complex triggers release of ULVWF multimers from endothelial cells, but to a lesser extent than IL-8 or TNF-α (Kichloo et al., 2020).. Conversely, IL-6 inhibits a ADAMTS13 and thus reducing ULVWF multimer cleavage and enhancing platelet adhesion and aggregation (Kichloo et al., 2020). Our study revealed that higher mean Vwf/ADAMTS13 Ratio correlated with COVID-19 severity and this is consistent with the study by Madeeva et al. (2020) who reported an increase in VWF antigen and a reduction in ADAMTS13 activity in COVID-19 patients, which is related to disease severity and could predict poor clinical

outcomes. They also suggested that the ADAMTS13 activity reduction could be a marker associated with COVID-19 compared to other non-critical medical conditions.

Mean fibrinogen level among COVID-19 patients was significantly higher than the control individuals. These results are in conformity with the report by Connors et al., (2020) who found a statistically significant increase in values of fibrinogen in COVID-19 compared to controls. The results accords that of Mehrdad et al., (2021) who found statistically significant increase in COVID-19 patients than in the control subjects. Our study revealed that the mean Plasma levels of fibrinogen in severe and critical Covid-19 patients was higher than the asymptomatic and mild COVID-19 patients. These results are consistent with a study by Zou et al (2020) that evaluated the correlation between coagulation function and disease status in COVID-19 patients revealed that abnormal fibrinogen levels were associated with higher mortality risks and critical disease development compared to normal fibrinogen levels.

A study by Kornblith et al (2022) that measured the levels of gamma' fibrinogen, a novel inflammatory marker, in COVID-19 patients. They showed that gamma' fibrinogen levels were significantly higher in COVID-19 patients than in healthy controls, and were also higher in patients with severe disease than in those with mild/moderate disease. They suggested that gamma' fibrinogen may be useful in predicting COVID-19 disease severity and outcomes.

A study by Sui et al (2021) that investigated the association between plasma fibrinogen levels and excessive inflammation in COVID-19 patients, reported that fibrinogen levels were commonly elevated in COVID-19 patients, especially in those with severe disease, and were correlated with pro-inflammatory cytokines and chemokines. They concluded that fibrinogen may be involved in the pathogenesis of COVID-19 by promoting inflammation and coagulation. The mechanism of fibrinogen elevation in COVID-19 is not fully understood, but it may be related to the intense inflammatory response, intravascular coagulation activation, and microvascular thrombosis caused by the viral infection1. Fibrinogen may also contribute to the pathogenesis of COVID-19 by promoting platelet aggregation, endothelial dysfunction, and vascular inflammation4. Moreover, fibrinogen has different isoforms, such as gamma' fibrinogen, which may have different biological functions and clinical implications in COVID-194. For example, gamma' fibrinogen levels were higher in COVID-19 patients than in controls, whereas total fibrinogen levels were not significantly different (Iba et al., 2020).

The mean Factor VIII concentration in COVID-19 patients was significantly higher than in the control subjects. This higher Factor VIII concentration found in the Sars-CoV-2 Positive group agrees with Escher's report (2020) of higher Factor VIII in the COVID-19 patients than the Sars-CoV-2 patients. The findings also accords that of Fan et al., (2021) who reported increased Factor VIII concentrations among Chinese Sars-CoV-2 patients. Our study reported increased mean Factor VIII activity in correlation with COVID-19 severity. Severe and critical COVID-19 patients had increased mean Factor VIII plasma concentration than the asymptomatic, mild and moderate COVID-19 patients. The results accords that of Zhang et al.(2020) who found that COVID-19 patients had significantly higher levels of factor VIII, von Willebrand factor, and fibrinogen than healthy controls, and that these levels correlated with markers of inflammation and disease severity. A study by De Cristofaro et al. (2021) found that COVID-19 patients with severe disease had higher levels of factor VIII and D-dimer than those with mild disease, and that factor VIII was an independent predictor of mortality in COVID-19 patients. These studies suggest that factor VIII may play a role in the hypercoagulability and thrombotic complications of COVID-19. However, more research is needed to understand the mechanisms and implications of this association. Direct infection of the endothelial cells through the ACE2 receptor leads to endothelial activation and dysfunction, expression of tissue factor, and platelet activation and increased levels of VWF and FVIII, all of which contributes to thrombotic risk in COVID-19 patients.

Plasminogen activator inhibitor-1 (PAI-1) is a protein that inhibits the breakdown of blood clots by blocking the activity of plasminogen activators, such as tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) (Keragala et al., 2021). In the current study, the mean Plasminogen Activator Inhibitor (PAI) concentration was higher in COVID-19 patients than the control participants. This accords the results obtained by Baycan et al., (2023; Zuo etal., 2021), who found increased PAI levels in Sars-CoV-2 patients. The plasma level of PAI was increased in severe and critical COVID-19 than in the asymptomatic, mild and moderate COVID-19 patients. These results are consistent with a study published in Scientific Reports (Zuo et al., 2021) who measured plasma antigen levels of tissue-type plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) in hospitalized COVID-19 patients and healthy controls. The study found markedly elevated tPA and PAI-1 levels in patients hospitalized with COVID-19. High levels of tPA and PAI-1 were associated with worse respiratory status. High levels of tPA, in particular, were strongly correlated with mortality and a significant enhancement in spontaneous ex vivo clot-lysis. The authors suggested that fibrinolytic homeostasis in COVID-19 is complex with a subset of patients expressing a balance of factors that may favor fibrinolysis. A study published in the Journal of Thrombosis and Thrombolysis (Baycan et al., 2023) evaluated the association between PAI-1 levels and severity and mortality for COVID-19. The study included 101 patients with confirmed COVID-

19 infection and 50 healthy controls. The study found that PAI-1 levels were significantly higher in COVID-19 patients than in controls, and higher in severe cases than in mild cases. PAI-1 levels were also positively correlated with inflammatory markers, such as C-reactive protein, interleukin-6, and ferritin. Moreover, PAI-1 levels were significantly higher in non-survivors than in survivors, and higher PAI-1 levels were associated with increased risk of death. The authors suggested that PAI-1 levels could be used as an indicator of severity and mortality for COVID-19.

There are several possible reasons why PAI-1 levels are elevated in COVID-19 patients. One reason is that PAI-1 is induced by inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha), which are also increased in COVID-19 patients (Keragala et al., 2021). Another reason is that PAI-1 is produced by activated platelets, endothelial cells, and adipose tissue, which are all involved in the pathophysiology of COVID-19 (Keragala et al., 2021). A third reason is that PAI-1 is regulated by hypoxia-inducible factor-1 alpha (HIF-1 alpha), which is activated by low oxygen levels in the tissues, a common feature of COVID-19 patients with respiratory distress (Keragala et al., 2021). . The high levels of PAI-1 in COVID-19 patients may have several negative consequences. First, PAI-1 may impair the fibrinolytic system, which is responsible for dissolving blood clots and preventing excessive coagulation. This may lead to the formation of microthrombi in the lungs and other organs, causing organ damage and failure. Second, PAI-1 may interfere with the clearance of misfolded or aggregated proteins, such as amyloid-beta and alpha-synuclein, which are associated with neurodegenerative diseases (Zuo et al., 2021). This may worsen the neurological symptoms and outcomes of COVID-19 patients (Zuo et al., 2021).. Third, PAI-1 may modulate the immune response and inflammation by affecting the activation and migration of leukocytes, such as neutrophils and macrophages. This may contribute to the cytokine storm and tissue injury seen in COVID-19 patients. Therefore, PAI-1 levels may serve as an indicator of severity and prognosis in COVID-19 patients, as well as a potential target for therapeutic intervention. However, more studies are needed to confirm the role and mechanism of PAI-1 in COVID-19 and to evaluate the safety and efficacy of PAI-1 inhibitors or plasminogen activators in this context.

Our study found increased International Normalised Ratio (INR) and Activated Partial Thromboplastin Time (APTT) in COVID-19 patients than in the control participants; however these differences were not statistically significant. These results are in contrast with Araya et al., (2021), who reported statistically significant prolonged APTT and elevated INR in COVID-19 patients than the COVID-19 Negative individuals. Several studies have investigated the role of INR and APTT in COVID-19 disease severity and prognosis. A study from China by Gerber et al., (2021) found that INR and APTT were significantly higher in COVID-19 patients than in healthy controls, and that INR was positively correlated with disease severity and mortality. The study suggested that INR above 1.13 had 71% sensitivity and 63% specificity for predicting death in COVID-19 patients. A study from Ethiopia by Tekle, et al., 2022). found that INR and APTT were significantly different among mild, moderate, and severe COVID-19 patients, and that both factors were associated with poor outcomes, such as ICU admission, mechanical ventilation, and death. The study suggested that INR above 1.2 and APTT above 35 seconds had high accuracy for predicting poor outcomes in COVID-19 patients.

These studies suggest that INR and APTT may be useful indicators of disease severity and prognosis in COVID-19 patients, as well as potential targets for anticoagulant therapy. However, more research is needed to determine the optimal ranges and strategies for coagulation management in COVID-19 patients.

One possible explanation for the prolonged APTT and INR in COVID-19 patients is the presence of lupus anticoagulant (LA) or antiphospholipid antibodies (aPL), which are associated with an increased risk of thrombosis (Gerber et al.,2021). Another possible factor is the endothelial damage caused by the viral infection, which may trigger the activation of the coagulation cascade and the consumption of clotting factors (Araya et al.,2021). Additionally, some COVID-19 patients may receive anticoagulant or antiplatelet therapies for underlying conditions or as part of their treatment, which may affect their coagulation parameters (Godino et al., 2021). However, the exact mechanisms and clinical implications of these abnormalities are still under investigation.

In the current study we found statistically significant elevated mean P-soluble Selectin and reduced platelet count in COVID-19 patients than in healthy control individuals. Our results accord that of Comer et al,.(2021) who reported P-Selectin plasma levels 30- to 90-fold higher in COVID-19 patients compared with hospitalised controls. Another study by Osburn et al., (2022) found that plasma P-selectin levels were elevated with increasing severity of pulmonary disease in patients hospitalized with COVID-19. The results are also consistent with the results obtained by Canzano et al. (2021), who found a 10-fold higher P-selectin in COVID-19 patients than in healthy control subjects. These studies suggest that sP-sel is a biomarker of endothelial cell activation and platelet hyperactivity in COVID-19, which may contribute to the increased risk of thrombosis and organ damage.

Furthermore results in our study reveals that Plasma P-selectin levels were elevated in the severe and critically ill COVID-19 patients than Asymptomatic, Mild and moderately ill COVID-19 patients. These results accords that of Agrati

et al. (2021) who reviewed the current literature on P-selectin involvement in COVID-19 coagulopathy and suggested that P-selectin could be used as a severity marker and a therapeutic target. The authors reported that several studies have shown elevated levels of soluble P-selectin (sP-selectin) in the plasma of COVID-19 patients, especially those with severe disease or poor prognosis. They also discussed the potential mechanisms by which P-selectin could promote thrombosis and inflammation in COVID-19, such as enhancing the formation of neutrophil extracellular traps (NETs), activating the complement system, and inducing cytokine release. Soluble P-selectin (sP-sel) is a protein that is released by activated platelets and endothelial cells. It is involved in the process of blood clotting and inflammation. COVID-19 is a disease caused by a novel coronavirus that can affect the lungs and other organs. Some patients with COVID-19 develop severe complications such as acute respiratory distress syndrome (ARDS), venous thromboembolism (VTE), and multi-organ failure. These complications are associated with increased inflammation and coagulation in the blood vessels.

A study by Flaumenhaft et al. (2022) described the vascular pathogenesis of COVID-19 and the role of endothelial dysfunction and immunothrombosis. The authors mentioned that P-selectin is a key molecule that mediates the interaction between platelets, endothelium, and leukocytes, and that its expression is increased in COVID-19 patients. The selectins are a family of calcium-dependent (C-type) lectins best known for mediating immune cell adherence to the endothelium, allowing immune cells to enter secondary lymphoid organs and inflammatory sites. P-selectin is a protein that is involved in the process of inflammation and blood clotting. It is expressed on the surface of activated platelets and endothelial cells, and it can bind to other cells such as white blood cells and red blood cells. P-selectin plays a role in the development of hemostasis disorders in COVID-19. The exact mechanism of why a P-selectin increase in COVID-19 is not fully understood, but some possible explanations could be that SARS-CoV-2, the virus that causes COVID-19, can infect and damage the endothelial cells, causing them to release P-selectin and other inflammatory molecules (Srihirun et al., 2023). The virus can also activate the platelets, either directly or indirectly through cytokines, which are proteins that regulate the immune response. Activated platelets express more P-selectin and form aggregates with leukocytes, which are white blood cells (Agrati et al., 2021). The increased P-selectin expression and platelet-leukocyte aggregates can contribute to the formation of microthrombi, which are small clots that block the blood flow in the capillaries, especially in the lungs. This can impair oxygen exchange and cause respiratory distress (Srihirun et al., 2023).

Therefore, P-selectin is an important marker of coagulation and inflammation in COVID-19, and it may also be a potential target for therapeutic intervention. Some drugs that can inhibit P-selectin or its interaction with its ligands, such as heparin or monoclonal antibodies, may have beneficial effects in reducing thrombosis and improving outcomes in COVID-19 patients (Agrati et al., 2021). However, more studies are needed to confirm the safety and efficacy of these drugs in this setting.

The reported lower mean platelet count in COVID-19 patients in our study is in agreement with the results obtained in previous studies (Liu et al., 2020; Lippi et al., 2020; Guan et al., 2020). Platelet counts correlated very well with COVID-19 severity. Severe and critically ill COVID-19 patients had significantly lower platelet count than the asymptomatic, mild and moderately sick COVID-19 patients. A study from Gerber et al. (2022) found that platelet count was significantly lower in COVID-19 patients than in healthy controls, and even lower in non-survivors than in survivors. Platelet count also correlated with chest computed Tomography (CT) severity scores, which reflect the extent of lung damage. The study suggested that platelet count below 150×10^9/L had 83% sensitivity and 83% specificity for predicting mortality in COVID-19 patients. There are many reasons why there is thrombocytopenia in COVID-19 patients and correlation with the disease severity. Coronaviruses are able to infect bone marrow cells, resulting in abnormal hematopoiesis (Yang et al., 2005). Human aminopeptidase N (CD13) is a metalloprotease that is present on the cell surfaces of epithelial cells in the intestine, kidneys, and lungs and is a receptor for HCoV-229E (Golubeva 2022). CD13 is a marker of granulocytes and monocytes and is ubiquitous in respiratory tract epithelial cells, smooth muscle cells, fibroblasts, epithelial cells in the kidneys and small intestine, activated endothelial cells, lymphocytes, and platelets. HCoV-229E enters bone marrow cells and platelets through CD13 receptors and induces growth inhibition and apoptosis in the bone marrow, leading to aberrant hematopoiesis and thrombocytopenia Golubeva et al., (2022). Thrombocytopenia caused by SARS-CoV-2 infection is similar to that caused by SARS-CoV and HCoV-229E infection. Based on this phenomenon, it is speculated that SARS-CoV-2 similarly inhibits hematopoiesis in the bone marrow through certain receptors to cause decreased primary platelet formation and lead to thrombocytopenia. Damaged lung tissues and pulmonary endothelial cells may activate platelets in the lungs, resulting in aggregation and formation of microthrombi, which increases platelet consumption.

5. Conclusion and recommendation

This study investigated the haemostatic profiles of COVID-19 patients and their association with disease severity. COVID-19 patients had elevated haemostatic parameters compared to healthy controls, and these parameters

correlated with COVID-19 severity levels. This study provides valuable insights into the haemostatic mechanisms of COVID-19 and the potential biomarkers for hypercoagulability. These findings may have implications for the diagnosis, prognosis, and management of COVID-19 patients. Our study can help the clinicians to understand which biomarkers are important in identifying patients with poor prognosis, which may be useful to assess disease severity or to enable early intervention.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The study was conducted under a protocol that was reviewed and approved by the Tropical Diseases Research Centre (TDRC) Ethics Review Committee and National Health Research Authority (NHRA). *Informed consent was obtained from all individual participants included in the study.*

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