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Original Article

Assessment of the Natural Anticoagulant Profile in Patients with COVID-19.

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Abstract

Coronaviruses are important pathogens in humans that can cause diseases ranging from the common cold to more severe and even fatal respiratory illness, Studies have emphasized that patients diagnosed with COVID-19 are susceptible to hyper-coagulation and thrombotic events, the symptoms range from mild disorders of coagulation indicators to disseminated intravascular coagulation (DIC).

Objective: Aim of the work in this study, was to investigate the effect of COVID-19 disease on coagulation cascade of COVID-19 infected patients, and to analyze the coagulation parameters of patients with COVID-19 pneumonia admitted to Sohag university hospital.

Patients and Methods: This study was conducted on forty COVID-19 patients diagnosed by PCR, the patients were classified into 3 subgroups according to the severity of pneumonia mild, moderate and critical groups, compared with 20 persons of healthy control group.

Results: The results of the current study illustrated that the absolute lymphocyte was significantly lower in moderate and critical groups comparing to healthy control group, prothrombin concentration was significantly lower in moderate group comparing to healthy control group, concerning d-dimer and von Willbrand factor antigen vWF:Ag demonstrated significant elevations moderate and critical group comparing to healthy control group. Regarding protein C and Anti-Thrombin show lower level in moderate and critical groups comparing to healthy control groups.

Keywords: COVID-19; coagulation factors; coagulopathy; D-dimer; SARS-CoV-2 infection

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Introduction:

At the beginning of 2020, the 2019 coronavirus disease (COVID-19) was deemed a pandemic of global concern posing an unprecedented challenge for national health systems (Cucinotta, D. and Vanelli, M., 2020). In humans, Coronaviruses are Significant pathogens that can cause illnesses ranging from the common cold to more serious and potentially lethal respiratory illnesses. More than 2 million cases have been found worldwide Since the December 2019 outbreak of the new COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Wuhan, China, and the rate of rise is accelerating (Zhou F et al., 2020). According to Guan W-J et al. (2020), patients infected with COVID-19 proceed from a mild, self-limiting respiratory infection to severe, progressive pneumonia with multiple organ failure. In clinical settings, COVID-19 patients typically have coagulation disorders, with symptoms ranging from disseminated intravascular coagulation (DIC) to moderate coagulation marker abnormalities. Direct SARS-CoV-2 attack on vascular endothelial cells, cytokine storm-mediated inflammation-coagulation cascades, hypoxia, or sepsis can all result in COVID-19-associated coagulopathy. Thrombocytopenia or coagulation dysfunction is closely associated with the severity and unfavourable prognosis of COVID-19 patients. Analysing the coagulation parameters of patients with COVID-19 pneumonia and examining the impact of COVID-19 disease on the coagulation cascade in patients with mild, severe, and critical COVID-19 infections are the goals of this study patients with COVID-19 pneumonia who admitted to Sohag university hospital, determine whether coagulationfactorsconsumptionishappening.

Patients and Methods

Patients

Adult patients with SARS-CoV-2 pneumonia (group I) who were placed under hospital quarantine between February 2021 and September 2022 were the subjects of this case-control research at Sohag University Hospital. Hospitalized patients who were 18 years of age or older and had pneumonia and a nasopharyngeal swab polymerase chain reaction (PCR) positive for SARS-CoV-2, followed by a radiological finding of COVID-19, were eligible to participate in the trial. A history of deep vein thrombosis or pulmonary embolism, hormone replacement therapy, autoimmune diseases like systemic lupus erythematosus and antiphospholipid syndrome, prolonged immobility, cancer or its treatments like tamoxifen, bevacizumab, thalidomide, and lenalidomide, or any clinical condition that may be linked to a hypercoagulable state—such as pregnancy—are among the exclusion criteria. Patients were classified into 3 subgroups according to the severity of pneumonia based a specified criteria shown in table **(1).**

Table (1). Patients' subgroups based on the severity and their criteria (The Novel Coronavirus Pneumonia Emergency Response, Epidemiology Team, 2020).

Subgroup	Severity	Criteria
Ia	Mild	Respiratory symptoms No radiological findings for pneumonia
Ib	Moderate	Fever Respiratory symptoms Radiographic Evidence of Pneumonia
Ic	Severe	Dyspnea, Respiratory rate ≥ 30/min, blood oxygen saturation ≤ 93%, PaO_2/FiO_2 ratio < 300, with or without lung infiltrates > 50% within 24–48 hours, respiratory failure, septic shock, and/or multiple organ dysfunction/failure.

Furthermore, 20 persons of age- and sex-matched apparently healthy people who were negative for SARS-CoV-2 infection were chosen as controls (group II).

Sample were collected from all patients included in the study at hospital quarantine and transferred to the Hemostasis unit of Sohag university hospital laboratory according to the COVID-19 protocol of sampling and transfer .

METHODS

For each patient and control, was estimated for prothrombin concentration (PC), D-dimer, von Willebrand factor antigen (vWF:Ag), protein C, free protein S (PS), and antithrombin and for performing the complete blood count.

The complete blood count including Hemoglobin, RDW-CV, WBCs, platelet indicators such as mean platelet volume (MPV), plateletcrit (PCT) and platelet distribution width (PDW) was analyzed using Sysmex XN-1000 Hematology Analyzer (Sysmex, Wakinohama, Japan) which is a fluorescent flow cytometry uses a semi-conductor laser and hydrodynamic focusing in dedicated channels to count blood cells and calculate the related indices.

Hemostatic assays including prothrombin concentration (PC), D-dimer and von Willebrand factor antigen (vWF:Ag) coagulation factors, and physiological coagulation inhibitors such as protein C (PC), free protein S (PS), and antithrombin (AT) were carried out using Sysmex CS-1600 automated hemostasis analyzer (Sysmex, Wakinohama, Japan); a compact analyzer that incorporates coagulation, chromogenic and immunoassay methodologies.

This protocol of this research was corrected by the Scientific Ethical Committee of Sohag University Hospital. Informed written consents were taken from all patients and controls included in the study.

2.3. Statistical Analysis:

Statistical analysis was done by SPSS v26 (IBM Inc., Chicago, IL, USA). Shapiro-Wilks test and histograms were used to evaluate the normality of the distribution of data.

The study data was quantitative parametric data and was presented as mean and standard deviation. The detection of statistically significant difference utilized the use of unpaired Student's t-test (when comparing between two groups) and analysis of variance (ANOVA) test followed by Tukey's post-hoc test (when comparing between more than two groups). A two-tailed P value <0.05 was considered statistically significant.

Results

The present study was conducted on 40 patients aged from 18 to 85 years and diagnosed as COVID-19 patients based on SARS-CoV-2 PCR who attended the Sohag University Hospital starting from February 2021 to September 2022. In addition a healthy control group comprising 20 healthy individuals who were negative for SARS-CoV-2 PCR whose age ranged from 18 to 85 years.

Confirmed cases of COVID-19 were sub-classified based on the severity of disease into the 3 groups: mild (A), moderate (B), and critical (C) case groups according to the protocol predefined criteria (The Novel Coronavirus Pneumonia Emergency Response, Epidemiology Team, 2020)

Our results showed the collected CBC data of all study groups. Using ANOVA tests to compare between these groups, statistically significant differences were found only in certain parameters namely, neutrophils and lymphocytes count, neutrophils-to-lymphocytes ratio, mean platelet volume (MPV) and plateletcrit (PCT).

Using post-hoc tests, no significant difference was found when comparing white blood cells, between individual groups.

The neutrophil count and the absolute lymphocyte counts of groups B and C were significantly lower when compared with that of HC group (p-values were 0.003 and < 0.001 respectively), (p-values were 0.018 and 0.002 respectively).

When comparing the neutrophils to lymphocytes ratio (NLR) a significant difference was noticed when comparing the NLR of HC group with groups B and C (p-values were 0.002 and < 0.001 respectively).

As regards the MPV a highly significant increase was noticed when comparing the MPV of each of these groups with HC group (all p-values were < 0.001). Significant differences were found when comparing the PCT of group A with that of group B and HC (p-values were 0.024 and 0.007 respectively).

Studied groups hemostatic parameters demonstrates that by using ANOVA test to compare between

these groups, statistically significant differences were found in all hemostatic parameters except the free protein S. As regards prothrombin concentration, the group B was significantly lower when compared with HC group (p-value was 0.004) with no other significant differences between comparing individual groups.

Concerning d-dimer and vWF:Ag, groups A, B and C demonstrated significant elevations when compared to HC group (all p-values were < 0.001).

As regards protein C levels, groups B and C showed significant differences depression when compared to group A (p-values were 0.008 and < 0.035 respectively). Finally, the anti-thrombin levels of groups B and C were significantly lower when compared to group A (p-values were < 0.001) and to HC group (p-values were < 0.001).

No correlation was found between the NLR and D-dimer levels among study groups (r=0.201; p-value=0.123)

Discussion

Coronaviruses (Co-Vs) are enveloped, single-stranded ribonucleic acid viruses that can contract infection to birds, mammals, and humans. According to (Singh., *2016).*Human CoVs can result in respiratory, enteric, and neurological conditions

In February 2020, the World Health Organization identified SARSCoV-2 as the causative agent of the Coronavirus Disease 2019 (COVID-19). According to early reports indicate that very high levels of D-dimer are common in COVID-19 pneumonia and are linked to with a poorer prognosis (*Tang et al., 2020).*

It is known that what the pathophysiology of COVID-19 and the underlying mechanism of its clinical manifestations are unclear. However, a number of studies have documented abberent coagulation parameters, especially in patients with COVID-19 associated pneumonia and acute respiratory distress syndrome (ARDS) (Fogarty et al., 2020).

Among the hemostatic system changes identified, elevated D-dimer levels seem to be a seperate biomarker of poor prognosis in COVID-19, as initially documented in initial reports from China and confirmed in several of patients series in the worldwide (Tang et al., 2020).

The most typical symptoms in patients with COVID-19 are fever, fatigue, and cough, which are mostly accompanied by less common symptoms such as headache, dyspnea, sore throat, anosmia, and nausea (Guan et al., 2020).

With reference to this study, it was found that concomitant diseases are mainly in critical (C) group, (Diabetes mellitus and hypertension) and Ischemic heart diseases occupy the highest percentage (22.22%).

In the meta-analysis, 10014 patients infected with SARS-CoV-2 were included. It was found that presence of at least one comorbidity of hypertension,

diabetes, cardiovascular.diseases, malignancy, chro nic kidney disease or chronic liver diseases individually significantly increased the severity of COVID-19 p < 0.00001 (Barek and Islam, 2020).

Data collected from our routine laboratory tests revealed that Neutrophil levels were significantly higher in moderate (B) group and critical(C) group than healthy control (P value =0.003 and<0.001 respectively).

This study also found that, lymphocytes levels were significantly lower in moderate (B) group and critical (C) group than healthy control (HC) (P value =0.018 and 0.002 respectively).

In particular, the NLR was significantly higher in critical(C) group than mild(A) group A Vs C and healthy control (HC) (P value=0.007 and <0.001 respectively), significantly higher in moderate(B) group than healthy control (HC) group B vs HC (P value=0.002).

As (Chen et al, 2021) found that, the lymphocyte count of the severe COVID-19 group was considerably to be significantly lower than of the COVID-19 group (P < 0.001).

While the researches of (Peng et al. (2020) and (Sun et al. (2020) found that patients with COVID-19 tend to have lower leukocyte count than healthy people. However, during observation patients with severe disease have higher leukocyte count than those with mild-moderate disease (P < 0.001).

Contrary to the opinion of (Pozdnyakova et al., 2020) who said that, positive COVID-19–patients had significantly lower values of White Blood Cells than patients with negative COVID-19– (*P* = .0130).

(Usul et al., 2020) In another study, no statistically significant difference was observed between negative and positive COVID-19 patients, respectively, in terms of lymphocyte (P = 0.081).

Contrary to (A.P. Yang et al., 2020) who said that NL ratio has already been described to be highly associated with prognosis of COVID-19.

This study also found that, PCT was significantly higher in mild(A) group than in the moderate(B) and healthy control (HC) group) (P value=0.024 and 0.007 respectively).

MPV was significantly higher in mild(A) group, moderate(B) group and critical(C) group than healthy control (HC) (P value <0.001).

This study similar to a study by (Wool and Miller, 2021) reported that, patients with COVID-19 were found to have significantly higher mean platelet volume (MPV) than critically ill non-COVID-19 patients associated with PLT count (fL, P = 0.013)

Also researches by (Wang et al., 2020) and (Guclu et al.,2020) shows that COVID-19 patients tend to have higher MPV and PDW. In addition, patients with higher disease severity and mortality had higher levels of MPV and PDW; therefore these parameters can be used to assess the concition of patients and to predict severity and mortality of the disease.

The evaluation of the coagulation parameters showed that, prothrombin concentration was significantly lower in moderate(B) group than healthy control (HC) (P value = 0.004).

D-Dimer was significantly higher in the mild(A) group, moderate(B) group and critical(C) group than healthy control (HC) (P value <0.001).

(Tang et al., 2020) conducted a meta-analysis, which confirmed a decrease in the concentration of Prothrombin and higher levels of D-dimer in the group of non-survivors in a group of 183 patients.

Also, in concordance with our results, In this recent study by (Adam and Zacharowski, 2020) prolonged PT, PC and INR were more common in severe and critical patients with COVID-19.

Furthermore, (Ichkawa et al., 2020) reported that D-dimer levels were substantially higher in non-survivors P = 0.004, and 69.4% of patients had levels beyond the normal range (0-250 ng/mL).

vWF:Ag; von Willbrand factor antigen was significantly higher in mild(A) group, moderate(B) group and critical(C) group than healthy control (HC) (P value <0.001).

This outcome is in line with the findings of Reyes et al. (2020), who found that vascular damage brought on by SARS-CoV-2 endothelium infection raised von Willebrand factor (vWF) levels. Our findings also showed an increase in factor VIIl and vWF. These alterations might be a reflection of the systemic endothelial damage that Varga et al. (2020) recently reported in COVID-19.

Results in the present study revealed that, regarding physiological coagulation inhibitors by ANOVA test, Protein C and Anti-Thrombin were significantly different among the four groups. Free Protein S was insignificantly different among the four groups.

Protein C was significantly higher in mild(A) group than moderate(B) group and critical(C) group) (P value =0.008 and 0.035 respectively).

It's interesting to note that COVID-19 patients hospitalised to the intensive care unit had higher levels of protein C (von Meijenfeldt et al., 2021). Additionally, (Ichkawa et al., 2020) discovered that patients with DIC had significantly lower levels of protein C (P = 0.001) and antithrombin (P = 0.008) in the severe categories. French Martín et al. (2020) also discovered reduced levels of antithrombin (P=0.008) in non-survivors, which is consistent with our findings. Patients with DIC had significantly reduced antithrombin levels (P = 0.004). Patients with severe SARS-CoV-2 infections who were hospitalised to the intensive care unit (ICU) had a considerable hypercoagulability status, as evidenced by a reduction in fibrinolysis and plasma levels of free protein S (Thiago et al., 2020). Additionally, unwell patients had decreased levels of natural anticoagulant factors such protein C and antithrombin. There is a lot of disagreement among the authors; some research (Ferrari et al., 2021) has shown a connection between severe COVID-19 infection and protein S insufficiency, whereas other research (Tang et al., 2021). 2020) found that non-survivors of COVID-19 had a lower AT content. Furthermore, a further study by Peyvandi et al. (2020) found that hospitalised patients had high amounts of protein C and that there was a relationship between the concentration of protein C and the advancement of COVID-19. The patients in

the intensive care unit had the greatest levels of protein C.

Results in the present study revealed that, there was no correlation between NLR and D. Dimer among patients with COVID-19 compared to control group.

On the contrary, (Fu et al., 2020) reported that The dynamic change of neutrophil to lymphocyte ratio (NLR) and D-dimer level can distinguish severe COVID-19 cases from mild/moderate cases in the ays following admission.

Conclusion

The results of the current study illustrated that the absolute lymphocyte counts and was significantly lower in moderate and critical groups comparing to healthy control group, MPV also show significant increase in mild, moderate and critical group comparing to healthy control group, the PCT also show Significant difference between mild group with moderate and healthy control group, prothrombin concentration was significantly lower in moderate group comparing to healthy control group, concerning d-dimer and vWF:Ag, demonstrated significant elevations among mild, moderate and critical group comparing to healthy control group. Regarding anti-coagulant profile, Protein C show higher level comparing mild group to moderate and critical groups, and Anti-Thrombin was significantly lower in moderate and critical groups comparing to mild and healthy control groups

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