Original Article

ISSN: 1735-0344 *Tanaffos 2023; 22(4): 389-394*

TANAFFOS

Status of Inflammatory and Coagulation Factors in COVID-19 and Its Relation with the Disease Severity

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Received: 15 November 2022
Accepted: 22 July 2023

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Background: The role of activation of inflammatory processes in the exacerbation of COVID-19 disease has been fully confirmed. In addition, the occurrence of thromboembolic events in patients with COVID-19 is expected even long after recovery from the disease. However, which factors are essentially prognostic for this disease is still not theoretically agreed upon. What we did in the present study was to evaluate the prognostic role of some inflammatory and coagulation factors in predicting the severity of COVID-19 disease. In this study, the need for ICU admission was considered as a symbol of disease severity.

Materials and Methods: Forty-six cases were studied in this cross-sectional study. Patients over 18 years of age with a definitive diagnosis of COVID-19 were assessed in terms of coagulation profiles and inflammatory and cytokine markers. Regarding laboratory data, serum levels of D-dimer, protein S, protein C, FDP, and fibrinogen were measured using an automated coagulation analyzer, and serum levels of interleukin-6 were measured using the ELISA technique.

Results: In total, 21 patients (45.7%) were admitted to the ICU due to the severity of the disease. In comparing inflammatory and coagulation factors between the two groups of patients, with and without ICU admission, a significant difference was revealed between fibrinogen (P=0.023), D-dimer (P=0.047), protein C (P=0.001), and protein S level (P=0.014). The decrease in protein C level had the highest value for predicting the severity of the disease and therefore the need for ICU admission.

Conclusion: Among various inflammatory and coagulation factors, the role of fibrinogen, D-dimer, protein C, and protein S in predicting the severe form of COVID-19 and the patient's need for ICU admission was confirmed.

Keywords: COVID-19; Coagulation factors; Protein C and S; D-dimer; Fibrinogen; Interleukin 6

INTRODUCTION

COVID-19 Pandemic has been associated with significant morbidity and mortality for the past two years. Despite the provision of standard treatment protocols and preventive approaches such as vaccination, we still face frequent mutations of the disease-causing virus and successive waves. The death toll from the disease has surpassed six million and continues to plague many countries (1). In this regard, COVID-19 is associated with a significant rate of patients' hospitalization in intensive care units (2-4). One of the potential and confirmed

complications of COVID-19 is coagulation disorders (5-11). The incidence of thrombotic complications in arterial and venous circulation in these patients, especially in patients admitted to the ICU, is significantly higher and is estimated to be about 31% (12). Also, coagulation-related markers such as D-dimer and fibrinogen levels are significantly higher in patients with severe COVID-19 disease (13). In some studies, in severe cases of the disease, a 7 to 10-fold increase in the level of D-dimer and therefore, an increased risk of thrombotic events in patients with COVID-19 has been observed (14).

On the other hand, a decrease in the number of blood cells such as platelets, as well as the activation of several inflammatory processes, along with coagulation disorders, has increased the disease mortality. Therefore, the set of these markers has always been considered a factor to predict the prognosis of the disease. In this regard, it seems that in the initial evaluation of patients with COVID-19, especially in severe cases, evaluation of inflammatory and coagulative markers, especially thrombotic markers is strongly recommended in predicting the prognosis of the disease as well as choosing the best therapeutic and managerial approach to patients.

In fact, evaluation of markers such as cytokines, serum inflammatory factors, coagulation factors, and blood cell counts seems necessary to determine the severity of the disease and its consequences, especially in patients admitted to intensive care units (15, 16).

In this study, we test the hypothesis that certain inflammatory and coagulation markers are significantly increased in patients with severe COVID-19 disease and thus, the evaluation of these markers can be considered as a prognostic factor in these patients.

MATERIALS AND METHODS

This cross-sectional study was conducted on patients over 18 years old with a definitive diagnosis of COVID-19 (based on clinical manifestations, disease-related virus molecular test results, and CT scan of patients' lungs) who were assessed in terms of coagulation profiles and inflammatory and cytokine markers. Patients who were treated with anticoagulants, or OCPs, patients with a BMI higher than 30, patients with a history of coagulative diseases, pulmonary disease or malignancy, and pregnant women were excluded. Patients' background information was collected at the beginning of the study and at the time of admission including demographic characteristics, patients' clinical and pharmacological records, and clinical manifestations. Also, laboratory findings were recorded by reviewing their hospital records.

Regarding laboratory data, serum levels of D-dimer, protein S, protein C, FDP, and fibrinogen were measured using an automated coagulation analyzer, and serum levels of interleukin 6 were measured using the ELISA technique. Then the severity of the disease was stratified based on the indicators of the respiratory rate more than 30 times per minute, arterial oxygen saturation below 90%, PaO_2/FiO_2 index below 300 mm Hg, or pulmonary infiltration more than 50%, based upon which the patients were classified into two severe and non-severe groups. Patients were followed up during the hospital stay in terms of the need for ICU admission. Finally, in addition to determining the serum levels of inflammatory and coagulation markers, their relationship with disease outcome including disease severity and need for ICU admission were analyzed.

For statistical analysis, results were presented as mean± SD for quantitative variables and were summarized by frequency (percentage) for categorical variables. Continuous variables were compared using the T-test or Mann-Whitney test whenever the data did not appear to have a normal distribution. Comparison between qualitative variables was performed using the Chi-square test. To calculate the diagnostic power and compare this power with the real situation of patients, the ROC curve and the area under the ROC curve (AUC) were used and the cut-off point, sensitivity, and specificity were calculated for all variables. P values of ≤ 0.05 were considered statistically significant. For the statistical analysis, the statistical software SPSS version 23.0 for Windows (IBM, Armonk, New York) was used.

Our study was evaluated and approved by the Research Ethics Committees of the School of Medicine, Shahid Beheshti University of Medical Sciences (IR.SBMU.MSP.REC.1400.793).

RESULTS

In the present study, 46 patients with COVID-19 (26 males and 20 females) were included in the study. In terms of inflammatory and coagulation factors, the mean fibrinogen level was 307.69±79.11, mean D-dimer level was 1.2±1.1, mean FDP level was 22.13±16.62, mean protein C level was 89.80±21.66, mean protein S level was 110.39±39.34, and mean interleukin-6 level was 186.71±50.56.

In total, 21 patients (45.7%) were admitted to the ICU due to the severity of the disease. In comparing inflammatory and coagulation factors between the two groups of patients with and without admission to the ICU, there was a significant difference between fibrinogen level (P=0.023), D-dimer level (P=0.047), protein C (P=0.001) and protein S (P=0.014) (Table 1). In this regard, no statistically significant difference was observed between the two groups regarding FDP and interleukin 6 levels.

Based on ROC curve analysis (Table 2), the decrease in protein C level (AUC=0.78) has the highest value for predicting the severity of the disease and therefore, the need for ICU admission. Accordingly, the best cut-off point for fibrinogen was 285 (with a sensitivity of 76.2% and specificity of 72.0%), for D-dimer was 0.775 (with a sensitivity of 76.2% and a specificity of 68.0%), for protein C equal to 79.5 (with sensitivity of 66.7% and specificity of 84.0%), and for protein S equal to 90 (with sensitivity of 52.4% and specificity of 100.0%).

Table 1. The value of inflammatory and coagulative markers in patients with and without ICU admission

Biomarkers	ICU (+) Mean Rank	ICU (-) Mean Rank	P value
Fibrinogen	28.40	19.38	0.023
D-dimer	27.79	19.90	0.047
FDP	22.19	24.60	0.54
Protein C	16.50	29.38	0.001
Protein S	18.21	27.94	0.014
Interleukin-6	27.71	19.96	0.051

DISCUSSION

The role of activation of inflammatory processes in the exacerbation of COVID-19 disease has been fully confirmed (17, 18). Thus, we expected an increase in the production and activity of inflammatory biomarkers in severe cases of the disease. On the other hand, according to numerous reports, the occurrence of thromboembolic events in patients with COVID-19 is expected even long after recovery from the disease (19). Although rare, it is associated with high morbidity and mortality. In this regard, countless types of inflammatory and coagulation factors can play a role in exacerbating the clinical manifestations of the disease and its consequences, but which factors are essentially prognostic for this disease is still not theoretically agreed upon. Also, although some studies have emphasized the role of inflammatory or coagulation factors in predicting the prognosis of the disease (20-23), in other studies the role of this factor has been rejected (24). Therefore, further studies are still necessary to identify inflammatory or coagulation prognostic factors in COVID-19 disease.

Table 2. The ROC curve valuing parameters to predict ICU admission by measuring biomarkers

Biomarkers	Cut off	AUC	Low limit	Up limit	Sensitivity	Specificity	P-Value
Fibrinogen	285	0.69	0.528	0.864	76.2	72.0	0.023
D-dimer	0.775	0.671	0.509	0.833	76.2	68.0	0.047
FDP	12.4	0.552	0.384	0.721	81.0	40.0	0.544
Protein C	79.5	0.780	0.644	0.916	66.7	84.0	0.001
Protein S	90	0.711	0.553	0.869	52.4	100.0	0.014
Interleukin-6	5.95	0.669	0.507	0.831	76.2	56.0	0.051

What we did in the present study was to evaluate the prognostic role of some inflammatory and coagulation factors in predicting the severity of COVID-19 disease. In this study, the need for ICU admission was considered as a symbol of disease severity.

At the beginning of the study, we showed that among the six evaluated factors, including fibrinogen, D-dimer, FDP, protein C, protein S, and interleukin-6, the highest prognostic ability to predict the intensity of COVID-19 was related to decrease in protein C levels, followed by the prognostic role of decreasing protein S levels and increase in the levels of fibrinogen and D-dimer levels. Interestingly, contrary to our expectations, this study did not find increasing levels of FDP and interleukin-6 as a prognostic factor of COVID-19. Finally, based on the ROC curve analysis, among the studied factors, only the increase in fibrinogen level was sufficient to predict the severity of COVID-19 and the patient's need for ICU admission with acceptable sensitivity and specificity. Therefore, what should be emphasized as a final finding is that the increase in fibrinogen levels during the initial admission of patients in the hospital can be a predictor of the occurrence of morbidities and the need for ICU admission. Thus, the prognostic role of coagulation factors on disease severity is emphasized. It appears that certain inflammatory or coagulation factors did not score high enough to accurately predict the prognosis of the disease. However, there may be some limitations that could have affected the results of the study, such as a small sample size or insufficient calibration of the instrument used to measure these factors.

As mentioned earlier, various studies have examined various pathological and coagulation factors and evaluated their role concerning disease severity. In the study by Corrêa et al., some coagulation markers such as aPTT, PT, and INR did not change during the study and no difference was observed between the two groups with and without disease severity. In both groups, fibrinolysis indices decreased and this decrease was significantly higher in the group with sequential organ failure assessment (SOFA) higher than 10. The D-dimer was higher than normal in both groups while protein S level was decreased in both groups. Patients with SOFA above 10 had lower plasminogen levels and protein C than patients in the other group (25). Therefore, the strong role of coagulation factors in determining the prognosis of COVID-19 was emphasized. In the study of Ibañez et al., there was no significant correlation between the disease severity index and SOFA score with the D-dimer level (26). In the Collett et al. study, an increase in the two parameters of D-dimer and fibrinogen was observed in patients, although other routine coagulation markers were in the normal range in all patients (27).

In another study by Saurabh et al., the levels of D-dimer, fibrinogen, and PT in the group with severe disease were significantly higher than in other patients (28). In the study of Sukrisman and Sinto, patients' D-dimer level was significantly correlated with the disease severity. There was also a significant relationship between ferritin level and disease severity (29). In line with our study results, Leisman et al. showed in their study that COVID-19-induced respiratory failure involves physiologic, clinical, and immunologic phenotypes that are not consistent with either ARDS or cytokine release syndromes. COVID-19 instead reflects immunosuppression and features compatible with vascular disease (30).

Also, Sinha et al. showed that the median values of IL-6 levels are above the normal range in many COVID-19 cases, they are lower than the median values typically reported in ARDS. The median IL-6 values in randomized clinical trials conducted by the National Heart, Lung, and Blood Institute's ARDS network are approximately 10 to 40-fold higher, even when only patients with severe COVID-19 are considered. Median IL-6 levels in patients with the hyperinflammatory phenotype of ARDS are 10 to 200-fold higher than the levels in patients with severe COVID-19 (31). Similarly, it seems that in our population, the role of fibrinogen level changes, D-dimer, protein S, and protein C factors in predicting disease outcome will be much higher than other inflammatory and coagulation factors, and no prognostic roles in IL-6 and FDP levels were observed.

CONCLUSION

In conclusion, among various inflammatory and coagulation factors, the role of fibrinogen, D-dimer, protein S, and protein C in predicting the severe form of COVID-19 and the patient's need for ICU admission has been confirmed; however, doubts remain about the prognostic role of FDP and interleukin-6.

Ethical Approval

Our study was evaluated and approved by the Research Ethics Committees of the School of Medicine, Shahid Beheshti University of Medical Sciences (IR.SBMU.MSP.REC.1400.793).

Acknowledgement

The authors would like to thank the Clinical Research Development Center, Shahid Modarres Educational Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran, for their support, cooperation, and assistance throughout this study.

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