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Evaluation of coagulation parameters: Coronavirus disease 2019 (COVID-19) between survivors and nonsurvivors

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SUMMARY

OBJECTIVE: This study aims to investigate and compare the coagulation parameters of coronavirus disease 2019 (COVID-19) patients with mortal and nonmortal conditions.

METHODS: In this study, 511 patients diagnosed with COVID-19 were included. Information about 31 deceased and 480 recovered COVID-19 patients was obtained from the hospital information management system and analyzed retrospectively. Whether there was a correlation between coagulation parameters between the mortal and nonmortal patients was analyzed. Descriptive analyses on general characteristics of the study population were performed. Visual (probability plots and histograms) and analytical methods (Kolmogorov–Smirnov and Shapiro–Wilk test) were used to test the normal distribution. Analyses were performed using the SPSS statistical software package.

RESULTS: Out of 511 patients, 219 (42.9%) were females and 292 (57.1%) were males. There was no statistically significant difference between males and females in terms of mortality (p=0.521). In total, the median age was 67 (22). The median age was 74 (13) in the nonsurvivor group and 67 (22) in the survivor group, and the difference was statistically significant (p=0.007). The D-dimer, prothrombin time, international normalized ratio, neutrophil, and lymphocyte median age values with p-values, in the recovered and deceased patient groups were: 1070 (2129), 1990 (7513) µg FEU/L, p=0.005; 12.6 (2.10), 13.3 (2.1), p=0.014; 1.17 (0.21), 1.22 (0.19), p=0.028; 5.51 (6.15), 8.54 (7.05), p=0.001; and 0.99 (0.96), 0.64 (0.84), p=0.037, respectively, with statistically significant differences.

CONCLUSIONS: As a result of this study, D-dimer, prothrombin time, and international normalized ratio increase were found to be associated with mortality. These parameters need to be closely monitored during the patient follow-up.

KEYWORDS: Coronavirus infections. Blood coagulation. International normalized ratio. Prothrombin time. Fibrin fibrinogen degradation products.

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INTRODUCTION

The SARS-CoV-2 virus responsible for the COVID-19 pandemic first appeared in the city of Wuhan, China. Similar to SARS-CoV and MERS-CoV, it is also believed to be transmitted from animals to humans. On February 11, 2020, the World Health Organization (WHO) named this disease, caused by the novel coronavirus, as COVID-19 and recognized as a pandemic[1].

The incubation period of SARS-CoV-2 infection is between 2 and 10 days. Flu-like symptoms, such as fever, chills, cough, myalgia, and headache, are the main symptoms in this infection. High fever was not always present in the elderly patients. Respiratory symptoms appear approximately after 3 days of the onset of fever. Cough, shortness of breath, and oxygen deficiency are among the most significant symptoms[2].

Severe illness was reported in 16% of the cases hospitalized due to COVID-19. Venous thromboembolism (VTE) increased in patients with severe respiratory distress[3]. Coagulopathy and associated complications are common in patients with severe COVID-19[4]. The lifespan of platelets in circulation is about 7–10 days. Of them, one-third are found in the spleen and two-third are found in circulation. Thrombocytopenia, which occurs due to decreased production of platelets, may occur due to the involvement in the spleen or increased degradation. Infections can often cause thrombocytopenia since they suppress hematopoiesis[5].

The D-dimer is one of the important tests of the coagulation system and is formed by the activation of this system for any reason and degradation of the cruciate ligament by plasmin. In clinical studies, the D-dimer is mostly used in the diagnosis and follow-up of VTE and disseminated intravascular coagulation (DIC)[6]. In the literature, the D-dimer levels were also reported to be high in patients with severe COVID-19[7]. The D-dimer levels were found to be high in 59.6% of the COVID-19 patients[4]. It has also been stated that macrophage activation syndrome (MAS) may occur during the course of the infection in COVID-19 patients. The presence of persistent fever, elevated D-dimer levels in repeated measurements, lymphopenia, and thrombocytopenia in spite of treatment show that the disease is accompanied by the MAS[7]. The D-dimer levels were high in approximately half of the patients hospitalized with COVID-19 infection, and the reported levels were ≤0.5 µg/mL in 32% of the patients, 0.5–1 µg/mL in 26% of the patients, and >1 µg/mL in 42% of the patients[8].

The prothrombin time (PT) and international normalized ratio (INR) tests are primarily used to evaluate the extrinsic pathway of coagulation[9]. These tests were reported to be statistically significant and were at higher levels in COVID-19 patients compared to the healthy control group[10].

According to a review, the use of contraceptives and the women under menopause hormone therapy may have a greater risk of thromboembolism[11]. Hence, ensuring proper use of contraception is very important in COVID-19 period[12].

Since the COVID-19 pandemic is quite a recent phenomenon, studies on the characteristics and treatment of the virus and the disease are yet to be added in the literature. However, despite the numerous published scientific studies, there is not adequate and accurate information available about COVID-19 infection and its treatment. Considering the pathogenesis of the disease, manifestation, and test results in patients, it is observed that coagulation mechanisms/tests are impaired/affected in this infection.

There are a few studies in the literature, investigating the coagulation parameters, D-dimer, PT, platelet count in COVID-19 patients. The present study aims to investigate and compare the coagulation parameters of the deceased and survived COVID-19 patients.

METHODS

This study was approved by the Sakarya University Medical Faculty Ethics Committee (20.05.2020/No. 4637). A total of 511 patients who were diagnosed with COVID-19 were included in this study. Of these, information about 31 deceased patients and 480 recovered patients was obtained from the hospital information management system and analyzed retrospectively. COVID-19 was diagnosed by clinical findings such as computed tomography and SARS-CoV-2 reverse transcriptase polymerase chain reaction (RT-PCR). The coagulation parameters such as platelet, D-dimer, and PT, and hematological parameters such as hemoglobin (HGB), hematocrit (HCT), neutrophil (NEU), lymphocyte (LYM), and neutrophil–lymphocyte ratio (NLR) were evaluated.

Sample collection, nucleic acid isolation, and RT-PCR

Combined nasopharynx and oropharynx swab samples were collected using Dacron swab, placed in viral transport medium immediately, and delivered to the laboratory by keeping them at 2–8°C in accordance with the rules of cold chain with the triple transport system, complying the infection prevention and control procedures.

After receiving the samples in microbiology laboratory, samples were taken to a negative pressure chamber with third-level biosafety. Bio-Speedy® Viral Nucleic Acid Isolation Kit (Bioeksen, Turkey) was used for total nucleic acid isolation from the specimens. The isolation procedure was carried out according to the recommendations of the manufacturer.

Bio-Speedy® COVID-19 RT-qPCR Detection Kit (Bioeksen, Turkey) was used for the RT-PCR assays. The PCR amplification and evaluation of the results were carried out according to the recommendations of the manufacturer.

Complete blood count and coagulation parameters

Hemogram tests were performed on CELLDYN 3700 (Abbott, USA) device, and D-dimer and PT tests were performed on DIAGON COAG XL (DIAGON, Hungary) device.

For the PT standardization, a model was defined based on the INR. The INR value can be calculated using the following equation: INR=(PT patient/PT Average Normal) ISI.

Statistical analysis

Descriptive analyses were performed to provide information on general characteristics of the study population. Visual (probability plots and histograms) and analytical (Kolmogorov–Smirnov and Shapiro–Wilk test) methods were used to test the normal distribution. Descriptive analyses were presented using medians and interquartile range (IR) for the non-normal distributed variables. The Mann–Whitney *U* test was used for nonparametric tests to compare these parameters. Pearson's χ^2 test was used to compare the categorical variables between two groups. The categorical variables were presented in frequencies (%). A p-value<0.05 was considered significant. Analyses were performed using the SPSS statistical software package (IBM SPSS Statistics, Version 22.0, IBM Corp., Armonk, NY). The result of post hoc power analysis of INR is 18%, and PT is 21%.

RESULTS

Out of the 511 patients included in this study, 219 (42.9%) were females and 292 (57.1%) were males. There was no statistically significant difference between males and females in terms of mortality (p=0.521). In total, the median age (IR) was 67 (22). The median age was 74 (13) in the nonsurvivor group and 67 (22) in the survivor group, and the difference was statistically significant (p=0.007). The D-dimer, PT, INR, NEU, LYM values (IR), with p values, in the recovered and deceased patient groups were: 1070 (2129), 1990 (7513) µg FEU/L, p=0.005; 12.6 (2.10), 13.3 (2.1), p=0.014; 1.17 (0.21), 1.22 (0.19), p=0.028; 5.51 (6.15), 8.54 (7.05), p=0.001; and 0.99 (0.96), 0.64 (0.84), p=0.037, respectively with statistically significant differences. NLR was 5.3 (9.59) in the survivor group and 11.18 (16.58) in the nonsurvivor group, and the difference between the two groups was statistically significant (p<0.001) (Figure 1). The HGM, HCT, and PLT values (IR), along with p values, in the recovered and deceased patient groups were: 11.9 (2.70), 11.5 (2.40), p=0.691; 37.3 (8.13), 36.2 (7.40), p=0.644; and 207 (129.75), 229 (138), p=0.758, respectively. There was no statistically significant difference (Table 1).

DISCUSSION

COVID-19 infection may have a variety of clinical outcomes ranging from viral pneumonia with severe respiratory failure to mortality[8]. During the infection, abnormalities are observable in some blood parameters in comparison to the pathogenesis of the disease. In this study, some coagulation and hemogram test parameters were investigated in the survived and deceased COVID-19 patients.

According to the previous studies, although individuals in the above-middle age group were affected by COVID-19, older individuals were more likely to develop a severe disease[13]. In this study, it was found that the COVID-19 patients were of old age (median 67 years, 22) and the deceased patients were of advanced age with an average age of 74 (13).

The thrombotic complications cause significant problems in the COVID-19 patients[14] Studies showed that thrombocytopenia was found in 36.2% COVID-19 patients[20]. In severe cases, it was found to be 57.7%[4]. A meta-analysis has shown significant thrombocytopenia in severe cases of COVID-19 patients, and this decreased platelet count was five times higher in some cases[15]. Similar to other viral infections, COVID-19 infection also activates clotting and causes excessive activation of platelets. In addition, it may cause systemic inflammatory response, affecting the procoagulant and anticoagulant mechanisms in hemostasis, thus

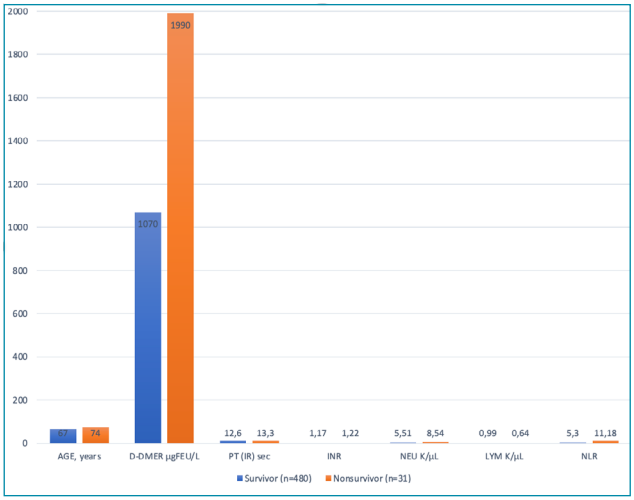

Figure 1. Parameters of the survivor and nonsurvivor patient groups.

distorting the balance between the two[16]. In the autopsies of deceased patients due to COVID-19 disease, there were thrombus in capillaries and small vessels, and numerous microthrombi in the liver venous portal system[17,18]. It may cause hypoxia due to the respiratory distress caused by COVID-19 in the lungs. As a result of this hypoxia, viscosity increases, and thrombus formation accelerates and increases[19]. Recent studies have reported that thrombosis and occlusion occur in small vessels in the lungs of severe cases of COVID-19[20]. The platelet level dropped below 100,000 in 20% of deceased patients, while this ratio was only 1% in survivors (p<0.001)[8]. In the comparison of thrombocytes of COVID-19 patients with that of healthy control group, no statistically significant difference was found[14]. Studies in COVID-19 patients found that the platelet values were low despite treatment[7]. In this study, there was no statistically significant difference between the COVID-19 survivors and nonsurvivors in terms of the platelet level (p<0.758).

The D-dimer levels were found to be higher in severe COVID-19 patients, indicating its association with mortality. In addition, these patients mostly have a coagulation disorder[4,21]. Fibrin degradation products and a significant increase in the D-dimer values result in the formation of large amounts of microthrombi in the body of patients with COVID-19. The formation of these thrombi and the combination of increased fibrinogen values with other data suggest a rapid increase in coagulation in these cases[22]. Studies have shown that there was an increase in D-dimer values by 46.4% in patients infected with COVID-19[21]. D-dimer prolongation is observed in the COVID-19 cases with poor prognosis[4]. In this study, a statistically significant difference was found in the D-dimer levels between recovered and deceased patients (p<0.005).

Some studies showed prolonged PT and INR[4]. In this study, the PT and INR levels were found increased in the nonsurvivors. In the comparison of the PT and INR values of the nonsurvivors with those of the survivors, a statistically significant difference was found (p<0.014 and p<0.028, respectively).

Studies have shown a higher NLR in COVID-19 patients, and NLR was found to be an independent prognostic biomarker for them[23]. NEUs, LYMs, and platelets play an important role in inflammation and used to identify many infections and types of cancer nowadays. In this study, the NLR values were 5.3 in the survivor group and 11.8 in the nonsurvivor group, with statistically significant difference (p<0.000).

Lymphopenia was detected in COVID-19 patients[23] In this study, lymphopenia was detected in the nonsurvivors group, showing statistically significant difference in LYM values between them and survivors (p<0.037).

There were no comparative studies on the HCB and HCT values in COVID-19 patients. We found that there was no statistically significant difference in the HCB and HCT values between nonsurvivors and survivors.

Owing to the small population size, we were unable to investigate more coagulation parameters (such as, fibrinogen and coagulation factors), which is a limitation of this study.

Table 1. Statistical data of the survivor and nonsurvivor patient groups.

Parameters	Total (n=511)	Survivors (n=480)	Nonsurvivors (n=31)	p
Age, years (IR)	67 (22)	67 (22)	74 (13)	0.007
Female, n (%)	219 (42.9)	204 (93.2)	14 (6.8)	0.521
Male, n (%)	292 (57.1)	276 (94.5)	16 (5.5)	
D-Dimer (IR), µg FEU/L	1090 (2270)	1070 (2129)	1990 (7513)	0.005
PT (IR), s	12.70 (2.15)	12.6 (2.10)	13.3 (2.1)	0.014
INR (IR)	1.17 (0.20)	1.17 (0.21)	1.22 (0.19)	0.028
NEU (IR), K/µL	5.73 (6.24)	5.51 (6.15)	8.54 (7.05)	0.001
LYM (IR), K/µL	0.99 (0.98)	0.99 (0.96)	0.64 (0.84)	0.037
NLR (IR)	5.99 (10.23)	5.3 (9.59)	11.18 (16.58)	0.000
HGB (IR), g/dL	11.9 (2.70)	11.9 (2.70)	11.5 (2.40)	0.691
HCT (IR), %	37.10 (8.10)	37.3 (8.13)	36.2 (7.40)	0.644
PLT (IR)	207 (130.5)	207 (129.75)	229 (138)	0.758

IR: interquartile range; FEU/L: fibrinogen equivalent units/Liter; PT: prothrombin time; INR: international normalized ratio; NEU: neutrophil; LYM: lymphocyte; NLR: neutrophil-lymphocyte ratio; HGB: hemoglobin; HCT: hematocrit; PLT: platelet.

CONCLUSION

We found that advanced age, increases in D-dimer, PT, INR, NEU and NLR values, and decrease in LYM levels were associated with mortality. These parameters need to be closely monitored during the patient follow-up. More comprehensive studies are needed on this subject.

AUTHORS' CONTRIBUTIONS

MO: Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **EC:** Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **SY:** Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **MK:** Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **ACG:** Conceptualization, Data Curation, Formal Analysis, Writing – Review & Editing. **DC:** Conceptualization, Data Curation, Formal Analysis, Writing – Review & Editing. **YA:** Conceptualization, Data Curation, Formal Analysis, Writing – Review & Editing. **AK:** Data Curation, Formal Analysis, Writing – Review & Editing. **AFE:** Data Curation, Formal Analysis, Writing – Review & Editing. **OK:** Data Curation, Formal Analysis, Writing – Review & Editing.

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