The Relationship Between COVID-19 Suspected Patient's Coagulation and Platelet Parameters and Polimerase Chain Reaction Results

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Abstract

Aim: Our aim was to investigate the relationship between prothrombintime(PT), activated partial thromboplastin time (aPTT), INR (International Normalized Ratio) and D-dimer levels, platelet (PLT)levels at admission to hospital and positivity or negativity of Polymerase Chain Reaction (PCR) result in patients with suspected COVID-19 followed at COVID-19 services. Material and Method: 238 people who applied with suspicion of COVID-19 in March-April-May of 2020 were included in the study. According to COVID-19 results, PCR negative 119 participants and PCR positive 119 patients were included in the study. PT, aPTT, Ddimer, INR and PLT levels were examined. Results: While PCR negative participants had a mean PT value of 11.46±0.86 sec, PCR positive patients had a mean PT value of 12.97±3.65 sec ($p<0.001$). There was no significant difference in mean aPTT values of PCR positive and negative patients. Whereas INR, D-dimer increased significantly in PCR positive patients. PLT value decreased from mean value of 266.75±71.36*109/L in PCR negative participants to 241.18±96.64*109/L in PCR positive patients ($p=0.002$). Conclusion: In our study, it was found that in patients who were admitted to Samsun Training and Research Hospital with COVID-19 suspicion and followed up in COVID-19 services, PT, D-dimer, INR, PLT values were important in detecting Coagulopathy and Thrombocytopenia in the group who were PCR positive according to COVID-19 results. Keywords: COVID-19, PT, aPTT, D-dimer, INR, PLT

Introduction

Coronavirus disease- 19 (COVID-19), which shares similarities with SARS (Severe Acute Respiratory Syndrome) and Middle East Respiratory Syndrome (MERS) viruses responsible for endemic diseases in 2003 and 2012, is a novel beta corona virus [1-2]. In addition to being a critical disease for health, coronavirus 2019 (COVID-19) poses a global threat [3].

First, pneumonia cases of unknown etiology occurred in Wuhan, Hubei province of China; the cases were reported to WHO (World Health Organization) in December 2019 and in March 2020 WHO declared this new infection as a pandemic [4-5].

COVID-19 is described as a new beta coronavirus. It is closely related to Severe Acute Respiratory Syndrome (SARS). It is a new infectious disease in which the virus is the causative pathogen[5]. Despite the presence of pulmonary pathophysiology during the disease, severe COVID-19 infection which is not fully understood is associated with pronounced alveolar inflammatory cell infiltration and it is accompanied by systemic cytokine storm[6]. One of the most important poor prognosis indicators is the development of coagulopathy in patients [7-8-9]. Increased plasma levels of fibrin degradation D-dimers with COVID-19 infection constitute

an independent biomarker for poor prognosis.9 In the COVID-19 pathogenesis of coagulation activation, significant pathological changes involving lung microvasculature including widespread micro thrombus and significant hemorrhagic necrosis have been highlighted, particularly in line with post mortem studies[10-11].

Severe COVID-19 increases the risk of developing significantly associated deep vein thrombosis and pulmonary embolism[12-13]. For estimating the severity of COVID-19, monitoring of useful screening parameters for PLT, PT, aPTT and D-D (D-dimer) and daily changes in coagulation function is important in patients with COVID-19 [14].

Poor prognosis that continues with COVID- 19 and continuation of D-dimer increase are precursors of multi organ failure and development of DIC (Disseminated Intravascular Coagulation) [15]. Especially, high D-dimer level is associated with increased mortality. In patients who lost their lives, the increase is evident from the fourth day of hospitalization. Despite coagulopathy, hemorrhage findings are not a common biomarker [15-16-17].

Materials and Methods

Study design and participants

This study presents a retrospective evaluation of the 238 patients admitted to Health Sciences University Samsun Training and Research Hospital who applied with suspicion of COVID-19 between March 11 and May 30, 2020. PCR results are determined in two categories, PCR positive group, and PCR negative group in line with The World Health Organization's guide titled "Laboratory Testing for 2019 Novel Coronavirus (2019-nCoV) in Suspected Human Cases" published on March 2, 2020 and TC Ministry of Health COVID-19 Guidelines.

All samples were studied with the SARS CoV-2 Double Gene RT-q PCR Kit (BioSpeedy, Turkey) in accordance with the manufacturer's instructions. Briefly, after nucleic acid isolation in nasopharyngeal lavage/aspirate, broncho alveolar lavage, nasopharyngeal suture, oropharyngeal swab and sputum samples, detection by single-step reverse transcription (RT) and Real-Time PCR targeting the ORF1ab and N gene regions were performed.

Extract ion and inhibition control were checked by targeting the human RNase P gene as an internal control. Nucleic acid extraction was validated with the vNAT buffer and this process was conducted without any additional work during sample transfer. Nasopharyngeal swap ororopharyngeal swab samples taken with swabs (dacronor polyester flock) were placed in a sterile transport solution containing vNAT solution and transferred. Reaction components 2X Prime Script mix 10µL, CVD Di Oligo mix 5µL, template nucleic acid 5µL total reaction amount of 20µLvolume was created. Qiagen Rotor Gene (Germany) Real-Time PCR instrument was run on sigmoidal curves under 38 cycles and were evaluated as positive.

Prothrombin time (PT), activated partial thromboplastin time (aPTT), D-dimer, International Normalized Ratio (INR) and platelet (PLT) levels of 119 patients followed in COVID-19 services were taken from hospital information management system. In addition, PT, aPTT, D-dimer, INR and PLT levels of 119 individuals with COVID-19 PCR negative were taken.

D-dimer test was worked on a Beckman Coulter Au 680 device, PT, aPTT and INR tests were worked at Siemens Ca-7000 device and platelet level was worked in Beckman Coulter Dx-800 device by using suitable tubes and kits.

Ethical approval

Ethical committee approval was obtained with the Ethics Committee decision dated 10 September 2020 and No. of Health Sciences University Samsun Training and Research Hospital Ethics Committee.

Data analysis

SPSS 22.0 Windows program was used to analyze the data. One Sample Kolmogorov – Simirnov and the Shapiro-Wilks tests were used to examine whether the differences between the groups and differences

between the groups by gender were normally distributed, and it was found that the data did not show a normal distribution. Therefore, non-parametric analyses were used. Mann–Whitney U-test was used in the comparison of parameters between the groups and ($p < 0.05$) was considered statistically significant. G*Power 3.1.9.7 program was used to calculate the sample size. One hundred ten sample size results were obtained for each group, in this calculation (test=t-test (Wilcoxon-Mann-Whitney test (two groups)), Analysis: A priori: Compute required sample size, Tail(s) =Two, Parent distribution=Normal, Effect size d=0.5, α err prob=0.05, Power (1-β err prob)=0.95, Allocation ratio N2/N1=1).

Results

In our study PT, aPTT, D-dimer, INR, PLT levels of a total of 238 individuals who were admitted to our hospital with a suspicion of COVID-19 were examined and evaluated according to their COVID-19 results. Half of these 238 individuals were individuals with a negative COVID-19 test result and the other half were patients with a positive COVID19 test result. The descriptive information of the study is given in table 1.

The test reference range of the samples analysed in Biochemistry Laboratory was taken as 10-14 seconds (sec) for prothrombin time (PT). While 11.46±0.86 sec. PT mean value was found in PCR negative individuals, 12.97±3.65 sec was found in PCR positive patients. When PT values were examined, a significant difference was found between both groups and the difference was found as statistically significant ($p<0.001$), (Table 2).

The test reference range of the samples analysed in Biochemistry Laboratory was taken as 18-36 seconds (sec) for activated partial thromboplastin (aPTT). While 24.38±3.40 sec. aPTT mean value was found in PCR negative individuals, 24.18±4.83 sec was found in PCR positive patients. No significant difference was found between the two groups (Table 2).The test reference range of the samples analysed in Biochemistry Laboratory was taken as 0-0.5 µg/ml for D-dimer. While 0.23±0.12 µg/ml D-dimer mean value was found in PCR negative individuals, 2.10±5.60 µg/ml was found in PCR positive patients. When D-dimer values were examined, a significant difference was found between both groups and the difference was found as statistically significant ($p<0.001$), (Table 2).The test reference range of the samples analysed in Biochemistry Laboratory was taken as 0.8-1.2 for International Normalized Ratio (INR). While 0.99±0.12 INR mean value was found in PCR negative individuals, 1.14±0.35 was found in PCR positive patients. When INR values were examined, a significant difference was found between both groups and the difference was found as statistically significant ($p<0.001$), (Table 2).The test reference range of the samples analysed in Biochemistry Laboratory was taken as 142-424*10^9/L for platelet (PLT). While 266.75±71.36*10^9/L PLT mean value was found in PCR negative individuals, 241.18±96.64*10^9/L was found in PCR positive patients. When PLT values were examined, a significant difference was found between both groups and the difference was found as statistically significant ($p=0.002$), (Table 2). In addition, the evaluation by gender for all parameters is given in table 2.

Discussion

Coagulopathy becomes evident with increase in D-dimer and fibrinogen levels and minimal change in prothrombin time (PT), active partial thromboplastin time (aPTT) and platelet count [15]. Hematological laboratory results can be used to determine the severity and prognosis of COVID-19 infection. Thrombocytopenia has been shown to be associated with an increased risk of severe disease and mortality associated with COVID-19 [18]. Platelet count has been accepted as a potential marker for COVID-19 since it is a simple, inexpensive and easily available hematological marker and since it is independent of disease severity and morbidity risk in the intensive care Unit [19].

In a study conducted on thrombocytopenia with patients with COVID-19, mild thrombocytopenia was observed in approximately 5% of patients who had mild disease, thrombocytopenia was observed in 70-95% of patients who had severe disease [5-19]. In a meta-analysis, when platelet count was compared, a significant difference was found between the COVID-19-negative group and the group that had severe COVID-19 in terms of platelet count and the individuals who had severe disease were found to have lower platelet count [18]. In another study conducted on 1476 patients, a direct correlation was found in patients with COVID-19 between the decrease in platelet count and mortality [20]. In another study conducted on patients with

COVID-19, it was reported that low platelet count was associated with increased severe disease and death risk and it could serve as an indicator of worsening of the disease during the hospital stay in COVID-19 [18]. It has been reported that platelet count decreases significantly in patients with COVID-19[21-22] and it is lower in patients who do not survive compared with those who survive [23].

Our study was found to be in parallel with the literature, a significant difference was found between groups and platelet count was found to be lower in the COVID-19 (+) group (U=5435.000, p=0.002, Effect size=0.20)(Table 2).

D-dimer is a fragment produced by the cleavage of fibrin by plasmin during clot breakdown [24]. One of the most common laboratory findings in patients with COVID-19 who require hospitalization is the obvious elevation in D-dimer. A high D-dimer value has been reported as a poor prognostic marker associated with consistent critical course and higher mortality in patients with COVID-19 [25-26]. In a study conducted on 1099 patients with COVID-19 in China, high D-dimer levels were found in almost half of the patients [27].

In an observational study conducted on 183 patients in China, a statistically significant difference was found in the mean D-dimer concentration at admission between patients with COVID-19 who survived and those who did not [8]. In another study conducted, patients with COVID-19 treated in ICU (Intensive Care Unit) were found to have higher D-dimer levels than patients with COVID-19 who were not treated in ICU [5]. Finally, in another study involving 5279 patients with COVID-19, the COVID-19 (+) group was compared with the COVID-19 (-) group. The D-dimer level of the COVID-19 (+) group was found to be four times higher [28].

Our study was found to be consistent with the literature. The difference between groups was found to be significant. D-dimer levels were found to be higher in the COVID-19 (+) group, while they were found to be lower in the Covıd-19 (-) group (U=1457.500, p<0.001, Effect Size=0.69)(Table 2).

Among coagulation parameters, PT is another laboratory parameter with varying consequences in COVID-19. PT and aPTT are exogenous and endogenous coagulation system factors that can be used for the early diagnosis of DIC (Disseminated Intravascular Coagulation). In another observational study conducted on 183 patients in China, a mild prolongation was found in the mean PT concentration at admission between patients with COVID-19 who survived and those who did not and a statistically significant difference was found between the groups[8]. In another study conducted in China, the patients receiving treatment in ICU were found to have higher PT prolongation compared with patients who were not receiving treatment in ICU and a significant difference was found between the groups [5]. In another study conducted on 187 patients diagnosed with COVID-19 and treated in the hospital, patients with high troponin-T level were found to have prolonged PT and aPTT levels [29]. More pronounced prolongation of PT and APTT parameters indicates that patients are in a transition from high coagulation state to fibrinolytic state due to excessive coagulation factor consumption.

Our study was found to be consistent with the literature, a significant difference was found between the groups in terms of PT levels and prolongation was found to be higher in the PCR (+) Group (U=3765.500, p<0.001, Effect Size=0.40) (Table 2).

In conclusion, the relationship between PT, aPTT, DİMER, INR, PLT levels was evaluated according to COVID-19 results and gender. Our study showed statistical significance between groups in PT, D-dimer, INR, PLT values between PCR positive and negative groups in terms of especially COVID-19 results (p<0.01). It is important to find out coagulopathy and thrombocytopenia in Covid-19 patients. These parameters are also important biomarkers for the prognosis of the disease in COVID-19.

The results of this study have shown that hypercoagulation exists in patients with COVID-19 at an early stage and hypercoagulation is closely associated with disease progression and clinical outcome. For this reason, coagulation indicators such as D-dimer and PT should be monitored as early as possible to determine thrombotic complications. It is imperative to take preventive treatment to decrease thromboembolism and DIC risk secondary to coagulation disorder and thus to reduce the morbidity and mortality of patients

infected with COVID-19.

Conflict of interest statement

There is no conflict of interest between the authors.

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