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Research Article

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PLATELET HYPERREACTIVITY RELATED WITH COVID-19 DISEASE SEVERITY

COVID-19 HASTALIK ŞİDDETİ İLE İLİŞKİLİ TROMBOSİT HİPERREAKTİVİTESİ

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Öz

Amaç: SARS-CoV-2 enfeksiyonunda bir hiperkoagulasyon durumu rapor edilmiştir. Trombositler geleneksel rollerinin yanı sıra bağışıklık hücreleri olarak da adlandırılır. Çalışmanın amacı, COVID-19'da trombosit aktivasyonunu ve agregasyonunu incelemekti.

Materyal ve Metot: Bu vaka-kontrol çalışması SARS-CoV-2 enfeksiyonu olan 61 hasta ve 18 sağlıklı bireyden oluşmuştur. Hastalar yoğun bakım ünitesinde (YBÜ) tedavi ihtiyacına göre gruplara ayrıldı. Tüm gruplarda CD41, CD61, CD42a ve CD42b saptandı ve trombosit agregasyon testleri incelendi.

Bulgular: Trombosit CD41, CD61, CD42a ve CD42b ekspresyonları, YBÜ hastalarında sağlıklı donörlere YBÜ olmayan hastalara kıyasla önemli ölçüde yüksekti. YBÜ grubundaki hastalar, YBÜ olmayan hastalar ve kontrollere göre trombosit agregasyonlarında artışa sahipti. Ek olarak, trombosit aktivasyonu ve trombosit fonksiyon testleri, C-reaktif protein, interlökin-6, nötrofil-lenfosit oranı, trombosit-lenfosit oranı, monosit-lenfosit oranı, D-dimer ve fibrinojeni içeren inflamatuar ve pıhtılaşma belirteçleri ile korelasyon göstermiştir.

Sonuç: YBÜ COVID-19 hastalarında artmış trombosit aktivitesi ve daha hızlı trombosit agregasyonu gözlendi. Trombosit hiperreaktivitesinin SARS-CoV-2 enfeksiyonunun ilerlemesine katkıda bulunması olasıdır. Trombosit aktivasyon ve fonksiyon testlerinin inflamatuar ve pıhtılaşma belirteçleri ile arasındaki ilişkiler, sistemik inflamasyonun ve sitokinlerin YBÜ'deki COVID-19 hastalarında hiperkoagulasyonu tetikleyebileceğini veya hiperaktif trombositlerin inflamasyonu artırabileceğini göstermektedir.

Anahtar Kelimeler: COVID-19, inflamasyon, trombosit aktivasyonu, trombosit agregasyonu, SARS-CoV-2.

Abstract

Objectives: A hypercoagulability status has been reported in SARS-CoV-2 infection. Beside their traditional roles, platelets are referred to as immune cells. The purpose of the study was to examine platelet activation and aggregation in COVID-19.

Materials and Methods: This case-control study comprised 61 patients with SARS-CoV-2 infection and 18 healthy individuals. The patients were separated into groups with respect to the need for treatment in the intensive care unit (ICU). CD41, CD61, CD42a, and CD42b were determined as platelet activation markers, and platelet aggregation tests were analyzed in all groups.

Results: Platelet CD41, CD61, CD42a, and CD42b expressions were significantly elevated in ICU patients compared to non-ICU patients and healthy donors. Patients in the ICU group had increased platelet aggregations than those in non-ICU patients and controls. Additionally, platelet activation and platelet function tests correlated with inflammatory and coagulation markers involving C-reactive protein, Interleukin-6, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, monocyte to lymphocyte ratio, D-dimer, and fibrinogen concentrations.

Conclusion: Enhanced platelet activity and faster platelet aggregation were observed in ICU COVID-19 patients. It is possible that platelet hyperreactivity may contribute to the progression of SARS-CoV-2 infection. The relationships between platelet activation and functions tests with inflammatory and coagulation markers show that systemic inflammation and cytokines may trigger the hypercoagulability in COVID-19 patients in ICU, or hyperactivated platelets could augment the inflammation.

Keywords: COVID-19, inflammation, platelet activation, platelet aggregation, SARS-CoV-2.

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Introduction

Coronaviruses are a group of primary pathogenic RNA viruses that target the respiratory tract in humans. At the end of 2019, a group of cases of pneumonia with unfamiliar etiology was identified. The novel pathogenic agent was designated as acute respiratory syndrome coronavirus-2 (SARS- CoV-2).[1]The infection created by the SARS-CoV-2, commonly recognized coronavirus disease-2019 (COVID-19), has at full speed proceeded overspread throughout the world. The signs and symptoms of COVID-19 range from asymptomatic to severe infection among individuals. [1-2]

Traditionally, platelets have physiologic and pathophysiologic features in hemostasis and thrombosis. Besides this, platelets induct cellular functions that get involved in the immune and inflammatory network system.[3] Activated platelets have essential thromboinflammatory features in the link between coagulation and immune responses in various infections. Platelets are increasingly being accepted as immune cells.[4,5] In addition, platelets interact with a wide variety of immune cells and thus help regulation of the immune response to injury, infections, and inflammatory responses.[3-6] Pathophysiological mechanisms comprising thrombocyte responses in HIV infection, dengue fever, and influenza pneumonia have been reported in naturally infected patients and experimental infection models. [4,7]

The interaction between virus and platelets causes alterations in both innate and adaptive immunity.[4] Viruses can increase platelet production at various phases.[5] Also, they have an impact on the cytokine profile of the host, arriving at the conclusion of altered thrombopoietin production in the liver.[5] Thrombocytopenia, which is rapidly stimulated in response to viral infections, is intermediated by enhanced platelet disruption.[4,5] The fastest metabolic pathway of platelet destruction arises from directly the interaction through platelets and viruses.[5] These immediate interactions frequently end up platelet activation and afterward platelet degranulation, and adherence of activated platelets to leukocytes.[3, 5, 8] Activation of platelets and connection of platelets to neutrophils raises the clearance of platelets in the spleen and liver.[5, 8] The interaction of platelets with B lymphocytes enhances the generation of antiviral IgG. Moreover, platelets trigger the differentiation of T lymphocytes and monocytes. [5, 8]

With the increase in the number of studies performed in COVID-19, obtained data emphasizes that thrombotic complications are closely related to the SARS-CoV-2 infection.[9, 10] Patients suffering severe COVID-19 are observed to have a hypercoagulable state, which is associated with the progression of multiple organ injury.[10] Increased acute phase reactants in severe COVID-19 may also involve in the COVID-related hypercoagulability.[11, 12]

The aim of the study was to investigate platelet aggregation and activation in patients affected by COVID-19 and to evaluate the platelet homeostasis of the patients with respect to the disease severity. The correlation between inflammatory markers and platelet functions was also investigated in order to discuss the pathophysiological mechanisms related to the coagulation status of COVID-19 patients. Although there are currently very few studies examining platelet activation in SARS-CoV-2 infection, to our knowledge, it is the first study that detects CD41, CD 61, CD42a, and CD42b surface molecules that we investigated.

Materials and Methods

Study design

This study was conducted at Ankara City Hospital, which is one of the main pandemic hospitals for COVID-19 in Turkey. Clinical diagnosis and classifications were made in accordance with the directory of WHO for COVID-19. Patients with existing clinical symptoms, the indication of COVID-19 pneumonia with respect to computed tomography, and/or positive RT-PCR test results of oro-nasopharyngeal swab samples for SARS-CoV-2 were enrolled in the study. Patients having an unverified diagnosis of COVID-19 and receiving previously anticoagulants, anti-inflammatory, and antiplatelet drugs were excluded. A group of healthy subjects without existing respiratory diseases and not being under anticoagulant treatment were included. None of the volunteers in the control group had cancer or any other systemic, inflammatory, or infectious disease, and none were taking medication. All the controls had negative RT-PCR test results for SARS-CoV-2. All participants underwent a comprehensive physical examination, oro-nasopharyngeal swab sampling, chest CT and standard clinical laboratory tests. As well as the routine clinical examinations and blood tests, all participants had platelet activation and aggregation tests. Blood samples were taken from all patients within 24 hours of hospitalization after diagnosis with SARS-CoV-2 infection. Demographic and clinical features and radiological and laboratory test results were gained from both electronic laboratory information systems and case report forms.

Routine Laboratory Tests

Complete blood cell counts were measured on Siemens Advia 2120 Hematology Analyzer (Siemens Healthcare Diagnostics, Erlangen, Germany). C-reactive protein (CRP) tests were detected on Advia Chemistry- XPT systems (Siemens Healthcare Diagnostics, Erlangen, Germany) with an immunoturbidimetric method. The Interleukin-6 (IL-6) tests were determined on an Atellica IM analyzer with chemiluminescence. D-dimer and fibrinogen tests were analyzed on The Sysmex CS-5100 coagulation analyzer. Siemens commercial kits were used in the analysis of routine laboratory tests.

Flow Cytometry

Venous blood samples for flow cytometry were collected in Vacuette® Blood Collection Tubes containing sodium citrate as anticoagulant and studied according to the manufacturer's instructions. In brief, plasma samples (100 uL) were incubated in the dark at room temperature for 15 min with 5 uL of anti-CD41-PC5 (platelet glycoprotein GPIIb; IIb integrin), 5 uL anti-CD42a-FITC (platelet glycoprotein GPIX), 5 uL anti-CD42b-PE (platelet glycoprotein GPIbα), 5 uL anti-CD61-PC7 (platelet glycoprotein GPIIIa) after the centrifugation at 4000 rpm for 10 minutes. After 15-min incubation, a washing procedure was performed according to the instructions of the supplier. The stained samples were analyzed for their immunofluorescence content. A combination of SSC (Side Scatter Channel) and FSC (Forward Scatter Channel) was used to differentiate the platelets, and the antibody expressions on the gated platelets were calculated.

Analyses were performed by using a 10-color flow cytometer (Beckman Coulter, Navios, Miami, FL, USA) within one hour after the sample preparation. In order to perform daily verification of the flow cytometer optical alignment and fluidics system, Flow Check Pro Fluorospheres (Beckman-Coulter) were used. For calibration and standardization of fluorescence detectors, Flow Set Pro Fluorospheres (Beckman-Coulter) were utilized. Settings were optimized, and fluorescence overlap compensation was calculated using single labeling, isotype controls, and Full Minus One (FMO) procedure.

Platelet Function Analyze

Platelet function tests were performed by using Innovance PFA-200 System (Siemens Healthcare Diagnostics, Erlangen, Germany). Citrated blood samples were transferred to disposable cartridges coated with adenosine diphosphate (ADP) and coated with epinephrine (EPI). If platelets were activated, blood plugs were formed, and blood flow in the analyzer was occluded. The closure times were determined for each activator. Platelet reactivity under two different conditions was recorded. The upper detection limit of the closure time was 300 seconds. When the time exceeded the limit, it was counted as 300 seconds.

Statistical analysis

Variables were tested with respect to their distribution via The Kolmogorov-Smirnov test. Normally distributed data were stated with mean and standard deviation. The categorical variables were represented as a number and percentage (%). The significance of difference through categorical variables was assessed by the chi-square or Fisher's exact test (when proper). A one-way ANOVA with a Bonferroni posthoc test was used to identify the differences of parameters among groups. Correlation analyses were conducted by Pearson's correlation for data following a normal distribution. A p-value less than 0.05 was noted as pointing to a significant difference.

The Statistical Package for Social Sciences (SPSS) software program (v.26; IBM, Armonk, NY) was performed for statistical utilizations.

Results

The study included 61 patients who were infected with SARS-CoV-2. The patients were divided into groups regarding the requirement of treatment in the intensive care unit (ICU). Twenty-one (15 male/6 female) of the patients needed ICU support. There were 40 cases (22 male/18 female) in the non-ICU group. The control group consisted of 18 subjects (10 male/8 female). The mean age of the patients who required ICU support was significantly higher than other study groups ($p < 0.001$, for both). Moreover, patients in the ICU group had almost two-fold more comorbidities. The characteristics of participants with COVID-19 are presented in Table 1.

As shown in Table 2 and Figure 1, patients in ICU group displayed increased platelet surface expressions of CD41, CD61, CD42a, CD42b when compared to non-ICU group and controls ($p = 0.013$, $p = 0.012$, $p = 0.009$, $p = 0.011$, respectively). When evaluated based on the platelet function tests, closure times in response to both epinephrine and ADP (agonists) were significantly lower in patients in the ICU group than those of subjects in the non-ICU group and healthy group ($p = 0.007$, $p = 0.004$, respectively). Also, patients had increased platelet aggregations (with epinephrine and ADP agonists) in non-ICU group than control group ($p = 0.650$ and $p = 0.712$, respectively)(Figure 2). There was no significant difference between the non-ICU group and control group in terms of platelet surface expressions of CD41, CD61, CD42a, CD42b. Patients in the ICU group had significantly increased D-Dimer and fibrinogen levels than those in other groups ($p < 0.001$, for both). With regard to inflammatory parameters, C-reactive protein (CRP), Interleukin-6 (IL-6), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and monocyte to lymphocyte ratio (MLR) were significantly higher in the ICU group than non-ICU group and controls ($p < 0.001$, for all). Considering in the way of platelet number and size, platelet counts were lower in the ICU group than those of other study groups; mean platelet volume (MPV) and plateletcrit (PCT) levels were significantly higher in ICU patients with COVID-19 than non-ICU patients and controls ($p < 0.001$, $p = 0.002$ respectively). There were no differences in terms of platelet count and platelet distribution width (PDW) among groups.

The associations of platelet activation and aggregation tests with other parameters were evaluated and shown in Table 3, there were correlations between the platelet surface markers (CD41, CD61, CD42a, CD42b) and inflammatory markers (CRP, IL-6, NLR, PLR and MLR). Also, platelet activation tests had significant relationships with D-dimer and fibrinogen concentrations. Like the platelet activation tests, platelet function tests also had relationships with inflammatory indicators, D-dimer and fibrinogen levels. Additionally, there were associations between MPV and CRP, IL-6, NLR, PLR and MLR ($r = 0.41$, $r = 0.48$, $r = 0.44$, $r = 0.33$, $r = 0.41$

respectively; $p < 0.001$, for all). In addition, significantly positive correlations were found between PCT and CRP, IL-6, NLR, PLR and MLR ($r = 0.35$, $p = 0.012$; $r = 0.34$, p =0.036; $r = 0.31$, p = 0.011; $r = 0.26$, p= 0.042; $r = 0.45$, p < 0.001 respectively).

Table 1. Demographic characteristics of patients with COVID-19

	Non-ICU COVID-19 cases (n = 40)	**ICU COVID-19 cases (n = 21)**	***p***
Age, mean ±SD	47.4 ± 12.48	68.13 ± 15.38	< 0.001
Sex			
Male	22 (55)	15 (71.42)	0.039
Female	18 (45)	6 (28.57)	0.005
Comorbidities	17 (42.50)	17 (80.95)	0.951
Diabetes	7 (17.50)	8 (38.09)	0.754
Hypertension	8 (20)	10 (47.61)	0.042
Cardiovascular disease	6 (15)	5 (23.80)	0.859
Chronic lung disease	6 (15)	5 (23.80)	0.859
Cancer	1 (2.50)	1 (4.76)	0.965
Signs and symptoms			
Fever	25 (62.50)	6 (28.57)	0.056
Cough	21 (52.50)	8 (38.09)	0.058
Dyspnea	19 (47.50)	15 (71.42)	0.047
Myalgia	11 (27.50)	4 (19.04)	0.052
Fatigue	18 (45)	2 (9.52)	0.008

ICU, Intensive Care Unit

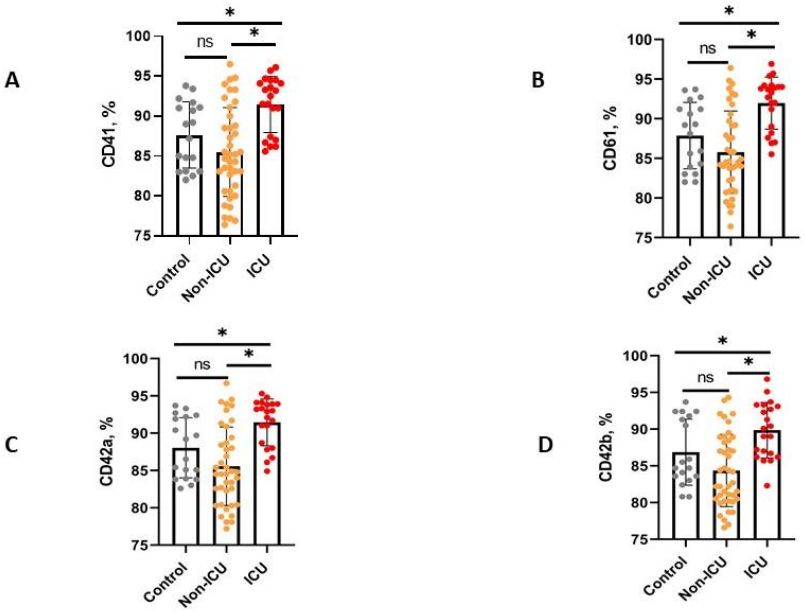

Figure 1 Elevated platelet activation in ICU COVID-19 patients (A-D)
The percentage expressions of CD41 (A), CD61 (B), CD42a (C) and CD42b (D) surface molecules of healthy individuals and patients with COVID-19. The scatter plot with the bar represents the mean with standard deviation. ICU, Intensive Care Unit; * indicates p < 0.05 between selected groups, ns means nonsignificant.

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Table 2. Laboratory findings of participants in study groups

	ICU COVID-19 cases (*n* = 21)	Non-ICU COVID-19 cases (*n* = 40)	Healthy individuals (*n* = 18)	*p* value
CD41, %	91.51 ± 3.37 [b]	85.56 ± 5,57 [c]	87.38 ± 4.12	= 0.013
CD61, %	92.02 ± 3.28 [b]	85.83 ± 5.19 [c]	87.62 ± 4.18	= 0.012
CD42a, %	91.59 ± 3.13 [b]	85.43 ± 5.26 [c]	88.06 ± 4.07	= 0.009
CD42b, %	89.98 ± 3.78 [b]	84.6 ± 4,91 [c]	86.87 ± 4.5	= 0.011
EPI CT, s	93.69 ± 10.62 [a, b]	102.75 ± 9.54 [c]	109.66 ± 8.42 [c]	= 0.037
ADP CT, s	78 ± 8.14 [a, b]	88.31 ± 7.31 [c]	90.53 ± 8.54 [c]	= 0.042
D-dimer, mg/L	1.46 ± 0.59 [a, b]	0.59 ± 0.30 [a, c]	0.28 ± 0.08 [b, c]	< 0.001
FIB, g/L	5.05 ± 1.07 [a, b]	3.93 ± 0.97 [a, c]	3.01 ± 0.52 [b, c]	< 0.001
WBC, x10^9/L	10.03 ± 3.27 [a, b]	6.94 ± 2.25 [c]	5.89 ± 1.26 [c]	< 0.001
NEU, x10^9/L	8.54± 1.95 [a, b]	4.22 ± 1.23 [c]	3.55 ± 0.80 [c]	< 0.001
LYM, x10^9/L	0.72 ± 0.34 [a, b]	1.61 ± 0.43 [c]	1.89 ± 0.5 [c]	< 0.001
NLR	11.61 ± 4.7 [a, b]	3.06 ± 1.12 [c]	2.08 ± 0.31[c]	< 0.001
HGB, g/dL	13.79 ± 1.69	13.4 ± 1.62	14.37 ± 0.7	= 0.078
PLT, x10^9/L	226.11 ± 64.49	229.35 ± 57.71	238.05 ± 33.6	= 0.614
MPV, fL	9 ± 0.55 [a, b]	8.27 ± 0.57 [c]	8.1 ± 0.76 [c]	< 0.001
PCT, %	0.25 ± 0.1 [a, b]	0.19 ± 0.05 [c]	0.20 ± 0.04 [c]	= 0.002
PDW, %	55.56 ± 8.93	51.84 ± 6.9	50.37 ± 7.21	= 0.134
MLR	0.74 ± 0.26 [a, b]	0.26 ± 0.08 [c]	0.19 ± 0.05 [c]	< 0.001
PLR	483.22 ± 89.49 [a, b]	174.96 ± 40.22 [c]	135.76 ± 24.58 [c]	< 0.001
IL-6 pg/mL	106 ± 25.7 [a, b]	20.9 ± 8.3[a, c]	2.72 ± 0.14 [b, c]	< 0.001
CRP, g/L	0.14 ± 0.06 [a, b]	0.030 ± 0.011[a, c]	0.0018 ± 0.0008 [b, c]	< 0.001

(Values are expressed as mean ± SD. *p* value < 0.05 considered significant. *p* value*, One-way analysis of variance [ANOVA]. [a] Statistically significant difference between healthy subjects vs. the other groups; [b] Statistically significant difference between the non-ICU(Intensive Care Unit) group vs. the other groups; [c] Statistically significant difference between the ICU vs. the other groups. EPI CT, induced by epinephrin, cloture time; CT, ADP, induced by adenosine, cloture time; FIB, fibrinogen; WBC, white blood cell; NEU, neutrophils count; LYM, lymphocyte count; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; and MLR, monocyte to lymphocyte ratio; HGB, hemoglobin; PLT, platelet count; MPV, mean platelet volume; PCT, plateletcrit; PDW, platelet distribution width; CRP, C-reactive protein; IL-6, Interleukin-6.)

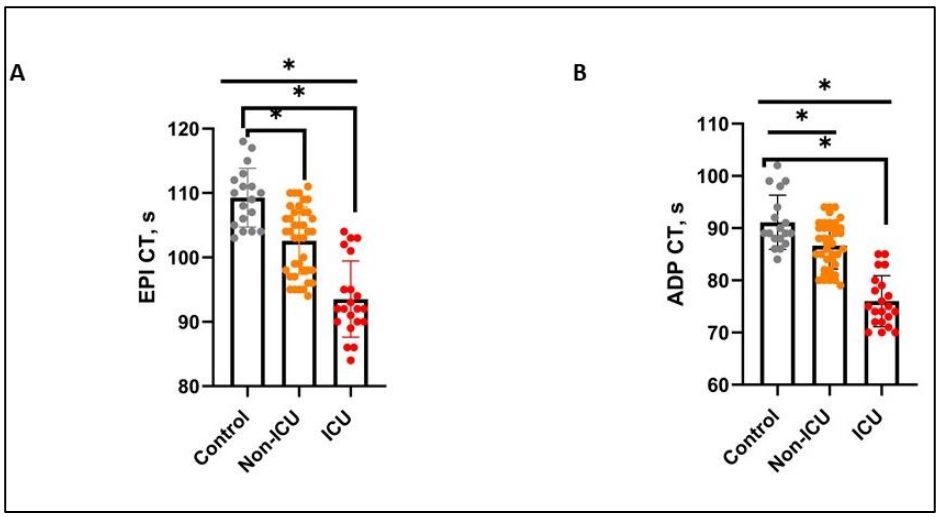

Figure 2. Increased platelet aggregation in COVID-19 patients (A-B)

(Closure times in response to both epinephrine (EPI) and adenosine diphosphate (ADP) (agonist). The scatter plot with the bar represents the mean with standard deviation. ICU: Intensive Care Unit, *indicates p<0.05 between selected groups.)

Table 3. Correlations between the platelet function tests and inflammatory and coagulation factors

n = 79	D-Dimer	FIB	CRP	IL-6	NLR	MLR	PLR
CD41	*r* = 0.33 *p*= 0.035	*r* = 0.30 *p* = 0.024	*r* = 0.40 *p* =0.003	*r* = 0.41 *p* = 0.024	*r* = 0.28 *p* = 0.039	*r* = 0.28 *p* = 0.037	*r* = 0.29 *p* = 0.033
CD61	*r* = 0.31 *p* = 0.047	*r* = 0.31 *p* = 0.021	*r* = 0.44 *p* = 0.001	*r* = 0.42 *p* = 0.021	*r* = 0.31 *p* = 0.022	*r* = 0.32 *p* = 0.017	*r* = 0.31 *p* = 0.018
CD42a	*r* = 0.33 *p* = 0.037	*r* = 0.26 *p* = 0.048	*r* = 0.40 *p* = 0.003	*r* = 0.46 *p* = 0.01	*r* = 0.28 *p* = 0.037	*r* = 0.29 *p* = 0.032	*r* = 0.29 *p* = 0.028
CD42b	*r* = 0.35 *p* = 0.027	*r* = 0.36 *p* = 0.006	*r* = 0.47 *p* = 0.001	*r* = 0.46 *p* = 0.015	*r* = 0.35 *p* = 0.012	*r* = 0.32 *p* = 0.023	*r* = 0.30 *p* = 0.033
CT, EPI	*r* = - 0.31 *p* = 0.045	*r* = - 0.21 *p* = 0.136	*r* = - 0.17 *p* = 0.262	*r* = - 0.23 *p* = 0.069	*r* = - 0.39 *p* = 0.009	*r* = - 0.30 *p* = 0.43	*r* = 0.35 *p* = 0.021
CT, ADP	*r* = - 0.20 *p* = 0.153	*r* = - 0.28 *p* = 0.048	*r* = - 0.22 *p* = 0.084	*r* = - 0.20 *p* = 0.161	*r* = - 0.37 *p* = 0.007	*r* = - 0.34 *p* = 0.01	*r* = - 0.41 *p* = 0.02

(The *r*-value is the Pearson correlation coefficient. The *p*-value is significance. CT, EPI, cloture time, induced by epinephrin; CT, ADP, cloture time, induced by adenosine diphosphate; FIB, fibrinogen; CRP, C-reactive protein; IL-6, Interleukin-6; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; and MLR, monocyte to lymphocyte ratio.)

Discussion

The results of our study not only provide information about the underlying reasons for the tendency to hypercoagulability in ICU patients with various platelet function tests but also verified the relationship between the inflammatory markers and platelet functions for COVID 19. To the best of our knowledge now, very few studies evaluating platelet activation in COVID-19 are present. Thus, our work constitutes the first study in this area to determine CD41, CD61, CD42a, and CD42b surface molecules.

The novel coronavirus has reached pandemic rates leading to notable raised morbidity and mortality all over the world.[13] However, contributing factors to life-threatening situations in patients suffering from COVID-19 are multifactorial, high incidence of thrombotic complications including venous, arterial thromboembolism, ischemic cerebrovascular stroke, and myocardial infarcts may contribute to poor outcomes in these patients.[14] Platelets are recognized to have possible major contributions in hypercoagulation and thrombotic events in SARS-CoV-2 infection.[9-12]

Besides the conventional opinion, platelets are known to express several receptors and surface molecules triggering the cellular functions existing in the inflammatory and immune system network against various pathogens, including viruses.[5, 15] Platelet-virus interaction, antiviral effects of platelets, and activation of platelets in the pathophysiologic mechanisms of viruses were discussed before.[4-6] In recent studies, increased

platelet activations in viral infections such as human immunodeficiency virus (HIV), dengue, and influenza have been reported. [4,7]

Analyzing the activation of platelets by testing the expression of CD41, CD61, CD42a, CD42b in COVID-19 patients, our results showed that the expression of all CD41, CD61, CD42a, and CD42b were significantly higher in ICU COVID-19 patients than in non-ICU patients and controls. CD41 and CD61 are the most abundant surface adhesion molecule of platelets. [3]Upon activation of platelets, this cluster surface molecules take a role in binding adhesion molecules, coherence of platelets, and thrombus growth.[3] Moreover, both CD42a and CD42b are involved in the adhesion of platelets to the proinflammatory endothelium.[15, 16] Platelet surface receptor and molecules and related pathways modulate platelet function.[3, 15, 16] Platelet glycoprotein Ib-IX-V complex including CD42a and CD42b not only triggers platelet adhesion but also mediate in coagulation, arrangement of leukocytes, and be in interaction with viruses.[15, 16] They are recognized as potential risk indicators in several diseases.[16]

Increased platelet aggregations induced by ADP and epinephrine in ICU COVID-19 patients were observed in our study. There were correlations between platelet activation and inflammatory markers, mainly CRP and IL-6. This outcome suggested that inflammation may mediate the platelet activation in SARS-CoV-2 infection. Furthermore, found enhanced D-dimer and fibrinogen levels in ICU patients with COVID-19 represented the activation of coagulation. The relationship found between both the platelet activation and function tests and coagulation factors (D-dimer and fibrinogen) showed that hyperactivated platelets might augment the hypercoagulability in COVID-19 patients. Obtained elevated MPV levels in patients in the ICU group pointed to increased platelet diameter, which can be a reflection of platelet activation. The powerful associations between MPV and PCT levels and inflammatory markers may be a component of the linkage bridge through the circulating platelets and inflammation.

Recent studies have examined platelet activation and aggregation in terms of various molecules.[17-19] Hottz et al. assessed expressions of P-selectin and CD63 surface molecules.[17] They observed elevated P-selectin and CD63 expressions in severe COVID-19 patients than mild group and controls.[17] Also, in the mentioned study, enhanced platelet-monocyte aggregates formation was found in severe COVID-19 patients but not in patients exhibiting mild SARS-CoV-2 infection.[17] Likewise, in this research, Manne et al. evaluated platelet function in COVID-19 patients.[18] Increased P-selectin expressions were obtained in patients with COVID-19 than healthy participants. Additionally, elevated platelet-neutrophil, platelet-monocyte, and platelet- T cell aggregates were determined in COVID-19 patients than controls. The researchers gained faster platelet aggregation in COVID-19 patients.[18] Another study performed by Kalinskaya et al. reported that there was diminished platelet aggregation in patients with SARS-CoV-2 infection at the beginning when compared with the healthy group.[19] After then a significant elevation in platelet reactivity in the course of the disease was observed.[19]

This study has certain limitations. First of all, the study consisted relatively low sample size. Platelet aggregation and activation tests are highly affected by the preanalytical process. Therefore, sample collection for these tests is a somewhat difficult process. In addition, the cost of these tests is high. Although light aggregometry is the gold standard method for platelet aggregation, it could not be used in the study because it is difficult to routinely use in laboratories.

In conclusion, there is rising evidence that hypercoagulability and thrombotic events have a key contribution in the severe SARS-CoV-2 infection. Our outcomes point out that enhanced platelet activation and aggregation are related to the severity of COVID-19. The associations among the markers of platelet activation and aggregation and platelet indices with the indicators of inflammation such as CRP and IL-6 highlight that there is a complex linkage among hyperreactive platelets with inflammation and hypercoagulability, so platelets are probably one of the main participators in immune and inflammatory responses. Our data could be encouraging for improving new therapy approaches targeting platelets in COVID-19.

Ethical Considerations

The study procedure was established in compliance with the basis of the Helsinki Declaration and confirmed by the local ethics board (Number: E1-20-653).

Conflict of Interest

The authors declare no conflict of interest.

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