Thrombocytopenia Secondary to COVID-19: Outcomes Analysis in Terms of Thrombotic Microangiopathy, Acute Kidney Injury, and Mortality

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ABSTRACT
Background: COVID-19 usually complicates respiratory failure; microvascular, macrovascular, and renal complications are common. Both micro and macrovascular complications are associated with multi-organ dysfunction and in-hospital mortality. Thrombotic microangiopathy (TMA) causes microvascular thromboses associated with organ failure, including acute kidney injury (AKI).
Materials and Methods: This Retrospective Cohort study included 100 COVID-19 in patients with thrombocytopenia, followed up in a university hospital's intensive care unit (ICU). The primary endpoints were in-hospital mortality or discharge from the hospital and assessing the occurrence of TMA and AKI during the hospitalization. The effect of thrombotic microangiopathy and acute kidney injury on mortality was investigated using logistic regression models in Stata software version 12.1.
Results: The TMA and AKI were associated with in-hospital mortality in COVID-19 patients presenting with thrombocytopenia in multivariate regression analysis, adjusted for other variables. The effect of AKI on mortality was obtained (adjusted OR 4.09, 95% CI: 1.33–12.53, p = 0.01). Moreover, the odds of mortality due to TMA were ten-fold higher in the patients who had TMA than those who did not (adjusted OR 10.26, 95% CI: 1.26–83.76, p = 0.03).
Conclusion: We outlined TMA in COVID-19 patients, which could be responsible for kidney injury and mortality in critically COVID-19 patients.

Keywords: SARS-CoV-2; COVID-19; Acute kidney injuries; Thromboses; Thrombocytopenia; Thrombotic microangiopathy

INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused due to infection by the severe acute respiratory syndrome – coronavirus – 2 (SARS-CoV-2), has grown out of proportion to a state of an erosive pandemic[1]. With symptoms ranging from mild to severe, its multisystem involvement makes it a challenge to manage in the intensive care setting[2]. The disease is categorized into three broad levels of severity. An initial study from China showed the disease to be milder in 81%, moderate in about 14%, and severe in 5% of the cases[3]. Critically ill patients often experience hypoxic respiratory failure, shock, and multiple organ dysfunction. COVID-19 is known to cause abnormalities in certain

hematological parameters, lymphopenia, thrombocytopenia, thromboses, and coagulopathy[4-6]. Thrombotic phenomena such as deep venous thrombosis and pulmonary thromboembolism were also seen in critically ill patients[7]. Macrovascular thrombotic complications, especially deep venous thrombosis, are common in patients with COVID-19[8]. Scarce evidence also exists for the presence of thrombotic microangiopathies (TMAs), particularly in critically ill COVID-19 patients. Some reports also suggested TMAs may be associated with widespread multi-organ disease complications, including acute kidney injury (AKI)[8-10]. TMAs are a syndrome of microvascular thromboses characterized by microangiopathic hemolytic anemia, thrombocytopenia, and organ damage[11]. Endothelial cells are damaged due to various pathological factors, leading to fibrin and platelet-rich thrombi accumulation in microcirculation[12,13]. TMAs are principally divided into hereditary and acquired types. Hereditary TMAs occur due to genetic defects in genes such as ADAMTS13, complement regulatory genes, and genes of specific factors involved in the process of coagulation (DGKE, PLG)[14]. Acquired TMAs result from exogenous factors, such as infections, pregnancy, drugs, malignancy, bone marrow transplantation, or autoimmune diseases [14, 15]. These classifications are not absolute due to genetic variants observed in secondary microangiopathies related to exogenous factors. Although mortality due to TMAs has declined with the use of plasma exchange and complement inhibitors in recent years, it remains a life-threatening condition due to thrombosis and organ dysfunction; among them, renal failure is common[7, 10, 16]. Kidneys are more frequently involved in TMAs other than thrombotic thrombocytopenic purpura (TTP) and are prone to develop microvascular thromboses in the glomerular circulation[10]. Before plasma exchange, as an effective treatment for TMA, the mortality rate was high, and renal failure, amongst others, was a principal factor contributing to mortality [17]. Most of the reports on the association of COVID-19 with TMA mortality are published as case reports, case series, or reviews, and there is little or no published original research with a sufficient sample

in this area [12, 14, 15, 18, 19]. Responding to this gap, the present study was designed to investigate this relationship with the original design, with a larger sample size, and using epidemiological study design and statistical assessment methods. The present study aims to describe the occurrence of TMAs and acute kidney injury (AKI) and its associations with mortality in thrombocytopenic COVID-19 patients.

MTERIALS AND METHODS

This retrospective cohort study was designed and performed between March 5, 2020, and March 21, 2021, based on registry data of hospitalized patients with COVID-19 infection in Ilam province, southwest Iran. The Ethical clearance was approved by the Ilam University of Medical Sciences (IR.MEDILAM.REC.1399.262). Inclusion criteria included adults (aged 18 years or older), severe COVID-19 infection characterized by dyspnea, tachypnea, hypoxemia, and pulmonary infiltration(20), and having thrombocytopenia on hospital admission (Thrombocytopenia is defined as a platelet count below the 150,000/μL). Moreover, patients who have experience of Thrombocytopenia were excluded from the study. One hundred patients with COVID-19 infection diagnosed with thrombocytopenia were followed up in the hospital's intensive care unit (ICU). The follow-up period is the time from the hospital admission to the recovery or death in the hospital. Peripheral blood smear performed for patients. If Schistocytes were seen on the peripheral blood smear, the thrombocytopenia was considered due to hemolytic anemia; otherwise, bone marrow suppression. We classified hemolytic anemia patients into three groups based on the number of schistocytes in the visual field in the peripheral blood smear (PBS). The presence of ≥1% schistocytes per high-powered field on PBS is an essential criterion for diagnosing thrombotic microangiopathy[21, 22]. The primary endpoints were in-hospital mortality or discharge from the hospital and assessing the incidence of TMA and AKI during the hospitalization. By the KDIGO definition, AKI was defined as an absolute increase in serum creatinine (SCr) 0.3mg/dl within 48 hours or a relative increase in SCr ≥ 50% from baseline over seven days[23]. The effects of TMA,

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acute kidney injury, and other predictor variables on mortality were investigated using Logistic regression models. The multivariate Logistic regression models included all variables with a p-value<0.2 from the univariate analysis[24]. Analyses were performed using Stata software, version 12 (StataCorp LP).

RESULTS

One hundred thrombocytopenic patients infected with SARS-Cov-2 were included in this cohort study. On admission, the mean platelet count was 147 (134-160)*10^3 cells/mm^3 and decreased to 83 (77-90)*10^3 cells/mm^3 during hospitalization. Peripheral blood smear examination was performed for red cell fragments (Schistocytes) on blood smears. Fifty-one patients (51%) did not have any detectable Schistocytes in peripheral blood smears that bone marrow suppression was the cause of thrombocytopenia. In follow-up, 49 patients had schistocytes on PBS. Forty patients (40%) had 1%> Schistocyte per every three high power fields to one Schistocyte per every one high power field. Nine patients (9%) met the criteria for TMA. Of these, seven patients died despite treatment. Fifty-three patients (53%) had raised Creatinine and developed AKI during the hospital stay. The occurrence of acute kidney injury (AKI) within the context of bone marrow suppression was observed in 55% of cases (28 patients), respectively. Among all patients diagnosed with hemolytic anemia, the incidence of AKI stood at 38% (19 out of 49 patients), while among those specifically diagnosed with TMA, it was 33% (3 out of 9 patients). Eighteen patients (18%) responded to treatment and increased their platelet counts, 82 patients (82%) did not improve, and their platelet counts remained less than 150,000/uL during their hospital course. The treatments were mostly supportive but to improve TMA, renal replacement therapy with dialysis and plasma exchange were also used. Mortality for the respond-treatment and non-respond treatment groups was 22% (4 patients) and 59% (49 patients), respectively. Mortality among thrombocytopenic patients with AKI and TMA was 71.7% and 77.8%, respectively. Table 1 summarizes other patient characteristics.

Statistical analysis with logistic regression was used to analyze variables to determine risk factors for mortality in patients with thrombocytopenia (Table 2).

In this study, the univariate analysis showed that the following variables were associated with mortality in thrombocytopenic patients: age, Acute Kidney Disease, Heart failure, Lung involvement, TMAs, and Dialysis in TMAs patients. In univariate analysis, the odds ratio (OR) for in-hospital mortality was 5.41 (CI 95 %:2.29 − 12.73) times greater for AKI patients than non-AKI patients, and in-hospital mortality was higher in patients with TMA than patients without Schistocyte in PBS (OR; 5.89, 95% CI: 1.11 − 31.34, p =0.04) (Table 2). Significant variables with p-value <0.2 in univariate analysis were exported to the multivariate logistic regression model. In the multivariate model, the effect of AKI and TMAs was adjusted according to the main variables, such as age, heart failure, Lymphocyte count, and dialysis treatment (Table 2). The multivariate logistic regression analysis showed that AKI, Lung involvement, and TMA were independent predictors of thrombocytopenic patient mortality. The OR for the effect of AKI on mortality was obtained at 4.09 with a 95% confidence interval of 1.33 to 12.53. TMAs were associated with higher odds of mortality in thrombocytopenic patients in the adjusted model, exhibiting an adjusted odds ratio (95% CI) of 10.26 (1.26 − 83.76) and a p-value of 0.03. This effect was statistically significant and indicated ten-fold higher mortality odds due to TMA than those without TMA (Table 2).

Table 1. Characteristics and outcome of thrombocytopenia in patients with COVID-19 infection: AKI, TMA, and mortality

Characteristics	Total patients(n=100)	No schistocytes in Field* (n=51)	< 1% schistocytes in high-powered Field** (n=40)	≥ 1% in high-powered Field** (n=9)
Age (mean), years	65.9 (1.6)	63.5 (2.5)	69.3 (2.1)	64.4 (4.4)
Sex (%)				
Male	72 (72%)	41 (80.4%)	28 (70%)	3 (33.3%)
Female	28 (28%)	10 (19.6%)	12 (30%)	6 (66.7%)
Comorbidities				
Diabetes	28 (28%)	16 (31.4%)	10 (25%)	2 (22.2%)
Hypertension	36 (%)	17 (33.3%)	16 (40%)	3 (33.3%)
Ischemic heart disease	18 (18%)	11 (21.6%)	6(15%)	1 (11.1%)
Heart failure	9 (9%)	5 (9.8%)	3 (7.5%)	1 (11.1%)
Acute Kidney Disease	53 (53%)	28 (54.9%)	16 (40%)	3 (33.3%)
Lung involvement based on computed tomography				
One lobe	24 (24%)	14 (27.5%)	9 (22.5%)	1 (11.1%)
Two lobe	15 (15%)	6 (11.8%)	5 (12.5%)	4 (44.5%)
Three lobe	13 (13%)	6 (11.8%)	7 (17.5%)	0 (0%)
Four lobe	8 (8%)	2 (3.9%)	3 (7.5%)	3 (33.4%)
Five lobe	1 (1%)	0 (0%)	0 (0%)	1 (11.1%)
diffuse unilateral	0 (0%)	0 (0%)	0 (0%)	0 (0%)
diffuse bilateral	24 (24%)	13 (25.5%)	11 (27.5%)	0 (0%)
little	24 (24%)	10 (19.6%)	5 (12.5%)	0 (0%)
length of hospital stay (mean±SE), (Day)	10.4 (0.7)	9.1 (0.9)	11.6 (1.2)	12.8 (2.7)
in-hospital mortality	53 (53%)	19 (37.3%)	27 (67.5%)	7 (77.8%)
Lymphocyte count (%) (mean±SE)	17.7 (1.1)	19.6 (1.5)	15.1 (1.4)	19.0 (5.2)
Lactate dehydrogenize (U/L) (mean±SE)	1315.4 (246.4)	1245.8 (416.4)	1320.1 (298.7)	1688.8 (504.8)
White blood cells (cell/mm3) (mean±SE)	10035.0 (639.7)	10419.6 (949.0)	9477.5 (949.9)	10333.3 (2130.1)
Indirect bilirubin (mg/dl) (mean±SE)	0.41 (0.1)	0.24 (0.03)	0.29 (0.04)	1.96 (0.2)
Hemoglobin (g/dl) (mean±SE)	13.0 (0.3)	13.1 (0.4)	12.9 (0.4)	12.2 (0.8)
RReticulocytecount (%)				
<2.5	87 (87%)	49 (96.1%)	38 (95%)	0 (0%)
>2.5	13 (13%)	2 (3.9%)	2 (5%)	9 (100%)

**: Thrombocytopenia due to hemolytic anemia
*: Thrombocytopenia due to bone marrow suppression

Table 2: Logistic regression models of factors for mortality in COVID-19 patients presenting thrombocytopenia in univariate and multivariate analysis

Risk factors	Crude Odds Ratio (95% CI)	P-value-a	Adjusted Odds Ratio (95% CI)	P-value-b
Age	1.02 (0.99 -1.05)	0.14*	1.02 (0.98 -1.01)	0.24
sex				-
male	1**	-		-
female	0.85 (0.35 – 2.03)	0.71		-
Acute Kidney Disease				
no	1	-	1**	
yes	5.41 (2.29 – 12.73)	<0.001*	4.09 (1.33 – 12.53)	0.01*
Heart failure				
no	1	-	1	
yes	3.42 (0.67 – 17.38)	0.13*	1.43 (0.19 – 10.29)	0.72
diabetes				-
no	1	-		-
yes	1.03 (0.43 - 2.48)	0.94		-
hypertension				-
no	1	-		-
yes	1.17 (0.52 – 2.67)	0.71		-
Ischemic heart disease				-
no	1	-		-
yes	1.49 (0.53 – 4.24)	0.45		-
Lung involvement based on computed tomography				
One lobe	1	-	1	-
Two lobe	1.11 (0.31 – 4.03)	0.87	0.38 (0.07 – 2.05)	0.26
Three lobe	3.44 (0.86 – 13.72)	0.08*	3.43 (0.46 – 25.79)	0.23
Four lobe	2.56 (0.54 – 12.26)	0.24	0.68 (0.07 – 6.59)	0.74
Five lobe	4.89 (0.18 – 132.83)	0.35	3.29 (0.85 – 12.66)	0.08
diffuse unilateral	1	No data	1	No data
diffuse bilaterally	4.64 (1.39 – 15.49)	0.01*	4.65 (1.13 – 19.09)	0.03*
little	0.85 (0.23 – 3.17)	0.79	1.01 (0.19 – 5.39)	0.99
Schistocytes in the visual field				
No	1	-	1**	-
<1% in 3 Field - <1% in one Field	3.50 (1.46 – 8.36)	0.005*	3.16 (1.07 – 9.31)	0.04*
≥1% in Field (TMAs)	5.89 (1.11 – 31.34)	0.04*	10.26 (1.26 – 83.76)	0.03*
Dialysis in TMA patients				
No	1	-	1	-
Yes	3.51 (1.26 – 9.83)	0.02*	2.81 (0.72 – 11.03)	0.14
Lymphocyte count (mean)	0.96 (0.92 – 0.99)	0.04*	0.96 (0.91 – 1.02)	0.19

*: significant
**: reference category
a: p-value for crude OR
b: p-value for adjusted OR

DISCUSSION

In this paper, we aimed to evaluate the effect of TMA and AKI on the mortality of confirmed COVID-19 patients presenting with thrombocytopenia. Reduced platelet production, increased platelet destruction, and increased platelet consumption are possible mechanisms of thrombocytopenia in COVID-19 infection[5]. This study showed that thrombocytopenia due to hemolytic anemia and bone marrow suppression was 49% and 51%, respectively. Previous studies have found that the incidence of thrombotic complications is 16–69% in patients with COVID-19 admitted to intensive care. Also, mild thrombocytopenia was presented in 70%-95% of COVID-19 patients[25, 26]. Cases of TMA have been sporadically reported in COVID-19 patients. However, the incidence of TMA is not precisely known [8,12].

Genetics and exogenous factors have been associated with TMA, including malignancies, viral infections, age, pregnancy, transplantation, and genetic variants in complement genes[14]. The mechanism of infections-associated TMA remains unclear; however, direct endothelial damage and complement activation are the most probable mechanisms associated with TMA[12]. Previous studies have shown that COVID-19 increases the risk of both macrovascular and microvascular thrombosis [11, 27, 28]. It seems that COVID-19 infection with complement activation causes endothelial damage, increasing the risk of multi-organ dysfunction and mortality[14, 29]. Although data on this are currently mixed, the SARS-CoV-2 may induce antiphospholipid antibodies followed by coagulopathies and possibly also TMA[8]. The results of this study indicated a relationship between TMA and mortality in the univariate model (OR = 10.26, p-value = 0.03) and adjusted model (OR = 6.59, p-value = 0.004). Patients presenting with TMA had a higher mortality risk in multivariate models adjusted by age, AKI, Heart failure, Lung involvement, Lymphocyte count, and dialysis. The results showed that Schistocytes on the PBS are associated with a higher mortality risk. Although any organ may be involved in all patients with TMA, the kidneys are likely to be affected[30]. Some previous studies reported that the kidneys were

most frequently affected (61%) [19, 28, 31]. In our study, Fifty-three (53%) patients developed AKI during hospitalization. AKI in patients with Schistocyte on the PBS was 36% (19 out of 53 patients). 33.3% (3/9) of TMA patients developed AKI. Alkhalifa et al. noted that thrombocytopenia was closely linked with and significantly predicted AKI[6]. Our results seem to confirm their observation; in fact, AKI in patients with COVID-19 is common, and COVID-19 AKI is associated with high mortality in these patients[6, 32]. Many potential causes, such as interstitial nephritis, collapsing glomerulopathy, TMA, and acute tubular necrosis (ATN), may be considered possible causes of renal failure associated with COVID-19 infection[33]. In one study, the principal diagnosis for mortality in TMA patients was AKI[17]. Our study supports TMA, which seems to be the leading cause of renal injury and is closely linked to mortality in COVID-19 patients. AKI is a form of TMA that is not uncommon among COVID-19 patients. Around 10% of patients during hospitalization develop AKI, which frequently complicates the course of COVID-19 hospitalizations, though the mechanism is unclear. Our results showed that AKI was associated with mortality in the univariate model and adjusted model. The odds of mortality in AKI were 6.5 fold higher than in those with no AKI, adjusted for age, Schistocyte on PBS, Heart failure, Lung involvement, Lymphocyte count, and dialysis. However, the current study was limited by the small sample, which limits generalizability, and caution must be applied.

CONCLUSION

Taken together, we have assisted in understanding the role of TMA and AKI in the increased mortality of COVID-19 patients presenting thrombocytopenia. Therefore, early identification and timely treatment of TMA could reduce kidney injury and decrease mortality in critically COVID-19 patients. Further research in this field is suggested.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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REFERENCES

1. Kaffashian MR, Shirani M, Koupaei M, et al. Clinical Manifestations, imaging procedures and laboratory parameters among hospitalized patients with COVID-19 in Ilam Province, Western Iran. Ethiop J Health Sci. 2022; 32(3):485-496

2. Altowyan E, Alnujeidi O, Alhujilan A, et al. COVID-19 presenting as thrombotic thrombocytopenic purpura (TTP). BMJ Case Rep. 2020;13(12):e238026.

3. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA. 2020;323(13):1239-42.

4. Sukrita B, Banerjee M. Immune Thrombocytopenia Secondary to COVID-19: a Systematic Review. SN Compr Clin Me. 2020;2(11):2048-2058.

5. Xu P, Zhou Q, Xu J. Mechanism of thrombocytopenia in COVID-19 patients. Ann Hematol. 2020;99(6):1205-8.

6. Alkhalifa H, Alsalman Z, Alfaraj A. Thrombocytopenia and clinical outcomes among patients with COVID-19 disease: A cohort study. Health Sci Rep. 2023;6(2):e1111.

7. Tehrani HA, Darnahal M, Vaezi M, et al. COVID-19 associated thrombotic thrombocytopenic purpura (TTP); A case series and mini-review. Int Immunopharmacol. 2021;93:107397.

8. Malgaj Vrečko M, Aleš Rigler A, Večerić-Haler Ž. Coronavirus disease 2019-associated thrombotic microangiopathy: A literature review. Int J Mol Sci. 2022;23(19):11307.

9. Gąsecka A, Borovac JA, Guerreiro RA, et al. Thrombotic complications in patients with COVID-19: pathophysiological mechanisms, diagnosis, and treatment. Cardiovasc Drugs Ther. 2021;35(2):215-229.

10. Brocklebank V, Wood KM, Kavanagh D. Thrombotic microangiopathy and the kidney. Clin J Am Soc Nephrol. 2018;13(2):300-317.

11. Falter T, Rossmann H, Menge P, et al. No evidence for classic thrombotic microangiopathy in COVID-19. J Clin Med. 2021;10(4):671.

12. Tiwari NR, Phatak S, Sharma VR, et al. COVID-19 and thrombotic microangiopathies. Thromb Res. 2021; 202:191-198.

13. Semeraro N, Colucci M. The prothrombotic state associated with SARS-CoV-2 infection: pathophysiological aspects. Mediterr J Hematol Infect Dis. 2021;13(1):e2021045.

14. Palma LMP, Sridharan M, Sethi S. Complement in secondary thrombotic microangiopathy. Kidney Int Rep. 2021;6(1):11-23.

15. Adamski J. Thrombotic microangiopathy and indications for therapeutic plasma exchange. Hematology Am Soc Hematol Educ Program. 2014;2014(1):444-9.

16. George JN, Nester CM. Syndromes of thrombotic microangiopathy. N Engl J Med . 2014;371(7):654-66.

17. Little DJ, Mathias LM, Page EE, et al. Long-term kidney outcomes in patients with acquired thrombotic thrombocytopenic purpura. Kidney Int Rep. 2017;2(6):1088-1095.

18. Jhaveri KD, Meir LR, Chang BSF, et al. Thrombotic microangiopathy in a patient with COVID-19. Kidney Int. 2020;98(2):509-12.

19. Dierkes F, Andriopoulos N, Sucker C, et al. Indicators of acute and persistent renal damage in adult thrombotic microangiopathy. PLoS One. 2012;7(1):e30886.

20. Bell TD. COVID-19 in the critically ill patient. Infect Dis Clin North Am. 2022;36(2):365-377.

21. Zini G, d'Onofrio G, Erber WN, et al. 2021 update of the 2012 ICSH Recommendations for identification, diagnostic value, and quantitation of schistocytes: Impact and revisions. Int J Lab Hematol. 2021;43(6):1264-71.

22. Schapkaitz E, Halefom Mezgebe M. The clinical significance of schistocytes: a prospective evaluation of the International Council for Standardization in Hematology schistocyte guidelines. Turk J Haematol. 2017;34(1):59-63.

23. Sparrow HG, Swan JT, Moore LW, et al. Disparate outcomes observed within Kidney Disease: Improving Global Outcomes (KDIGO) acute kidney injury stage 1. Kidney Int. 2019;95(4):905-13.

24. Jewell, NP. Estimation of Logistic Regression Model Parameters Ch. 13. Statistics for Epidemiology (1st ed.). Chapman and Hall/CRC, USA, 2004, pp 258-259.

25. Nagori EK, Ghantarchyan H, Qadir A, et al. COVID-19-Induced Thrombocytopenia: A Brief Literature Review and Case Report. Cureus. 2022;14(11) : e30993.