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An analytical study of the risk factors affecting the platelet aggregation in patients with sepsis

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ORIGINAL STUDY

An analytical study of the risk factors affecting the platelet aggregation in patients with sepsis

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Abstract

Objectives: **(a) To determine the correlation of platelet count and aggregation with sepsis severity and (b) to find the risk factors affecting the platelet aggregation in sepsis.**

Patients and methods: **The present observational cross-sectional study was conducted on 30 patients of sepsis over a period of 18 months. All patients underwent complete blood counts, and platelet aggregation to adenosine diphosphate (ADP). Pearson correlation coefficient was used to assess the association of Sequential Organ Failure Assessment (SOFA) score and aggregation to ADP and platelet. Linear regression was done to determine the risk factors affecting platelet aggregation in sepsis. A P value of less than 0.05 was considered statistically significant.**

Results: **The age of the patients ranged from 19 to 70 years with 12 females and 18 males. The mean (SD) SOFA was 7.73 (3.92). Thrombocytopenia (<150 000/μl) was observed in 17 (56.6%) patients. Platelet count was statistically significantly negatively correlated with severity of sepsis as assessed by SOFA scoring system ($r = -0.492$, $P = 0.006$). Platelet aggregation was decreased, that is, less than 65%, in 15 (50%) patients, and was normal in the rest of 15 (50%) patients. No statistically significant correlation was observed between ADP-induced platelet-aggregation abnormality and SOFA score in patients with sepsis ($r = -0.0354$, $P = 0.55$).**

Conclusion: **Since most of the cases in the study had mild sepsis, it can be concluded that alteration in the platelet aggregation is more with the severe sepsis and thrombocytopenia is an initial finding that begins even in the mild cases of sepsis and increases proportionately with the severity of sepsis.**

Keywords: Platelet aggregation, Sepsis, Sequential organ failure assessment score

1. Introduction

Sepsis is one of the public health problems, which encompasses a syndrome of immune dysregulation and nonimmune mechanisms. It carries a high rate of hospital admissions, morbidity, and mortality [1]. The growing attention of the studies on the pathophysiology and the interlinked connection with inflammation and coagulation dysfunctions has brought the contribution of the platelets into attention [2–4].

Thrombocytopenia has been encountered in patients with sepsis and is itself an independent entity leading to many ICU admissions [2–4].

In addition to the decrease in the number of the platelet counts postsepsis, various platelet functions are also altered during sepsis namely activation, adhesion, aggregation, and secretion of inflammatory molecules [1,2]. Davies et al. [5], in their study showed that low platelet count, white cell count, and platelet aggregometry by collagen activation were significantly associated with severe sepsis and its mortality, however, this was not the case when adenosine diphosphate (ADP) was used as the agonist.

ADP is the first known and a significant agonist that stimulates both primary and secondary platelet aggregation. It induces change in the platelet shape, promotes secretion from storage granules, influx of Ca^{2+}, and inhibition of stimulated adenylyl cyclase activity [6].

In view of the findings that show a significant association of low platelet count and decline in

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platelet functions during sepsis, the association of platelet parameters with the severity of sepsis remains a concern. Since limiting platelet functions carry an adverse prognosis, its evaluation remains critical to predict the prognosis of the sepsis patients. Besides, it becomes important to know the risk factors that may affect platelet aggregation (specially to ADP) in patients with sepsis.

This study was conducted on patients of sepsis with an objective (a) to determine the correlation of platelet count and aggregation with sepsis severity and (b) to find the risk factors affecting the platelet aggregation in sepsis.

2. Patients and methods

The present observational cross-sectional study was conducted in the Department of Medicine and Haematology over a period of 18 months from November 2016 to April 2018. The study was performed on 30 participants of all sexes, aged more than 18 years and suffering from sepsis. Sepsis was defined as the presence (probable or documented) of infection together with systemic manifestations or infection. Severe sepsis was defined as sepsis plus sepsis-induced organ dysfunction or tissue hypoperfusion [7]. Patients with primary hematological disorders, primary platelet disorders, receiving drugs affecting platelet function, and having hepatic or renal disease, were excluded from the study. The institutional ethical clearance was obtained for the study.

The sample-size calculation was based on the previous study, Yaguchi et al. [4], where platelet dysfunction was significantly more in septic patients (100%). Taking these values as reference, the minimum required sample size with 90% power of study and 5% level of significance is one patient in each study group. So the total sample size taken is 30 patients from sepsis.

After obtaining informed consent from the patients or their legal guardians, a detailed history about fever, physical examination (blood pressure, peripheral edema, and bowel sounds), Glasgow coma scale, and investigations comprising of total leukocyte, platelet count, C-reactive protein (CRP), random blood sugar, serum bilirubin, capillary refill time, procalcitonin, urine output, and serum creatinine was carried out. Blood samples were drawn within 24 h of admission of patients presenting with sepsis or diagnosis of sepsis if it developed during hospital stay. Besides routine investigations required for the management of patients, platelet aggregometry was done in all participants in the study. The severity of organ dysfunction was assessed with Sequential Organ Failure Assessment (SOFA) [8]. The sample was also obtained for blood culture to determine the organism and antibiotic sensitivity for the initiation of appropriate treatment.

2.1. Platelet aggregometry

Platelet aggregometry was done on Chronolog aggregometer available in the Haematology Department of the hospital. For conduction of the test, whole-blood sample was mixed with 0.11 M sodium citrate (9 : 1) and centrifuged at 100 g for 10 min at room temperature. The upper layer was collected as platelet-rich plasma (PRP). The PRP was then added with ADP to induce platelet aggregation. Platelet aggregation was then studied using an automated aggregometer. The aggregometer works on the principle of light transmission. Initially PRP is cloudy as there is no platelet aggregation, but as ADP induces aggregation, the PRP becomes clear, allowing more transmission of light through the sample. The normal value of platelet aggregation was taken as above 65%.

2.2. Statistical analysis

The data were entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for the Social Sciences (SPSS), IBM manufacturer, Chicago, USA, version 21.0. Categorical variables were presented in number and percentage (%), and continuous variables were presented as mean ± SD and median. Normality of data was tested by Kolmogorov–Smirnov test. If the normality was rejected, then nonparametric test was used.

Pearson correlation coefficient was used to assess the association of SOFA score and aggregation to ADP and platelet. Linear regression was done to determine the risk factors affecting platelet aggregation in sepsis. A P value of less than 0.05 was considered statistically significant.

3. Results

The age of the patients included in the study ranged from 19 to 70 years. The mean and median age of the cases was 34.73 ± 15.77 and 34 years, respectively. Most of the cases were between 20 and 50 (53.3%) years of age. The study population consisted of 12 female and 18 male patients. The duration of fever at the time of presentation ranged from 4 to 25 days in these cases with a mean temperature being 101.17 ± 1.32F. Mean systolic pressure in these patients was 92.73 ± 17.42 mmHg, while diastolic pressure was 56.92 ± 15.59 mmHg, and most of them were on vasopressor support (73%). The observed

range of total leukocyte count varied from 5500 to 44 000/μl and mean total leukocyte count was 21 963 ± 8854.52/μl. The serum procalcitonin was found to be between 9.59 ± 2.77 ng/ml with a median of 10.15 ng/ml. Capillary refilling time was delayed in 46.67% of patients and mottling was present in one patient. Random blood sugar ranged from 102 to 330 mg/dl. The mean glucose was 162.07 ± 52 mg/dl. The mean CRP was 49.87 ± 20.82 mg/l with a median 49.5 mg/l. Serum lactate was found to have between 1.1 and 2 mmol/l with a mean of 1.39 ± 0.21 mmol/l. Peripheral edema was present in 60% (20) of the cases. Bowel sounds were normal in 29 of 30 patients, while ileus was present in one of the patients. Serum bilirubin was normal in 10 patients (<1.2), while the rest 20 were between 1.2 and 9 mg/dl. Serum creatinine mean value was 2.92 ± 1.69 mg/dl with a median of 2.45 and a range of 0.5–8 mg/dl. Urine output over 24 h was between 780.67 ± 399.19 ml with a median of 800 ml. Sensorium was normal in 11 patients, while altered in the remaining altered patients (Table 1).

In 23 of the 30 cases, culture from various sites was found to be positive. The most common microorganisms isolated were *Escherichia coli* (13.04%), *Staphylococcus aureus* (17.39%), *Streptococcus* (13.05%), and *Pseudomonas aeruginosa* (26.09%).

SOFA scoring ranged from 1 to 17 with a mean of 7.73 ± 3.92 and a median of 7 (Table 2).

Thrombocytopenia (<150 000/μl) was observed in 17 (56.6%) patients. The mean platelet count was 137 936.67 ± 76 285.37 μl with a median of 133 000/μl. Platelet count was statistically significantly negatively correlated with severity of sepsis as assessed by SOFA scoring system ($r = -0.492$, $P = 0.006$) (Fig. 1).

Platelet aggregation was decreased, that is, less than 65%, in 15 (50%) patients, and was normal in the rest of 15 (50%) patients. The percentage of platelet aggregation ranged from 5 to 125%, while median was 62 with mean being 60.73 ± 24.65%. No statistically significant correlation was observed between ADP-induced platelet aggregation abnormality and SOFA score in patients with sepsis ($r = -0.0354$, $P = 0.55$) (Fig. 2).

In subgroup analysis of patients with low platelet count (<1.5 lacs), mild negative correlation was observed between decreased platelet aggregation (ADP induced) and severity of sepsis, but it failed to reach statistical significance ($r = -0.334$, $P = 0.191$) (Fig. 3).

Univariate linear-regression analysis showed that none of the baseline demographic and clinical parameters were significant risk factors for altering platelet aggregation in sepsis ($P > 0.05$) (Table 3).

Table 1. Baseline, demographic, and clinical characteristics.

Baseline, demographic, and clinical characteristics	*n* (%)
Age (years)	
≤20	8 (26.67)
21–30	6 (20)
31–40	6 (20)
41–50	4 (16.67)
>50	6 (20)
Sex	
Female	12 (40.00)
Male	18 (60.00)
Fever duration at presentation	
0–10 days	14 (46.6)
11–20 days	15 (50)
21–30 days	1 (3.33)
Mean arterial pressure (mmHg)	
>70	6 (20)
≤70	24 (80)
TLC (μl)	
1–10 000	1 (3.33)
10 001–20 000	14 (46.67)
20 001–30 000	10 (33.33)
30 001–40 000	4 (13.33)
40 000–50 000	1 (3.33)
Serum procalcitonin (ng/ml)	
0.5–10	14 (46.66)
>10	16 (53.33)
CRT	
Delayed	46.67 (14)
Normal	53.33 (16)
RBS (mg/dl)	
1–150	17 (56.67)
151–250	10 (33.33)
251–350	3 (10)
CRP (mg/l)	
0–30	6 (20)
31–60	14 (46.67)
61–80	9 (30)
81–120	1 (3.33)
Serum lactate (mmol/l)	
1.1–1.5	24 (80)
1.6–2.0	6 (20)
Peripheral edema	
Absent	18 (60)
Present	12 (40)
Bilirubin (mg/dl)	
<1.2	10 (33.33)
1.2–1.9	8 (26.67)
2.0–5.9	12 (40)
Serum creatinine (mg/dl)	
<1.2	2 (6.67)
1.2–1.9	7 (23.33)
2–3.4	12 (40)
3.5–4.9	6 (20)
>5	3 (10)
Urine output (ml)/24 h	
0–100	1 (3.33)
101–500	7 (23.33)
501–1000	16 (53.33)
1000–1500	6 (20)
Sensorium (GCS)	
5	1 (3.33)

(*continued on next page*)

Table 1. (continued)

Baseline, demographic, and clinical characteristics	*n* (%)
6	1 (3.33)
8	4 (13.67)
9	1 (3.33)
15	23 (76.67)

CRP, C-reactive protein; CRT, capillary refilling time; GCS, Glasgow coma scale; RBS, random blood sugar; TLC, total leukocyte count.

4. Discussion

The study results showed that platelet aggregation was reduced in sepsis patients, however, it failed to reach statistical significance, which may be because the mean SOFA score of the study patients was 7.73 ± 3.92, which was in the lower range, indicating that the severity of sepsis in the study patients was low. Interestingly, we found a mild negative correlation ($r = -0.354$) between platelet aggregation and sepsis severity as assessed by SOFA score, indicating that there was a fall in the platelet-aggregation functions with the increase in the severity of sepsis. This fact was also observed in the subgroup analysis of patients with low platelet counts ($r = -0.334$). The findings are in line with the study by Woth et al. [9], and Yaguchi et al. [4], who showed significant deterioration of aggregation in all patients and subset patients of thrombocytopenia with increasing severity as assessed by SOFA score. The findings have been reinstated in the study by Adamzik et al. [8], where the impedance aggregometry was used to define platelet functions in sepsis. Their results showed that aggregation studies may be a potential marker for diagnosis of sepsis and monitoring of the severity of sepsis. They stated that impedance aggregometry using collagen as the activator carried an OR of 6 for predicting sepsis along with procalcitonin, interleukin-6, CRP, and platelet count. However, our results differ from this aspect as we did not find any significant correlation of deranged platelet aggregation with sepsis severity, nor did we find any significant risk factor for the prediction of derangement in the platelet aggregations. We only found a significant association of low platelet counts with the severity of sepsis, which have been unanimously proven in other previous studies [10,11]. Both these incidents of thrombocytopenia and decreased platelet aggregation hold importance as each of them have individually shown to adversely affect the patient outcomes in terms of mortality and hospital stay [4].

Table 2. Distribution of Sequential Organ Failure Assessment score.

SOFA score	*n* (%)
0–6	11 (36.67)
7–9	12 (40)
10–12	4 (13.3)
13–24	3 (10)
Total	30 (100)

SOFA, Sequential Organ Failure Assessment.

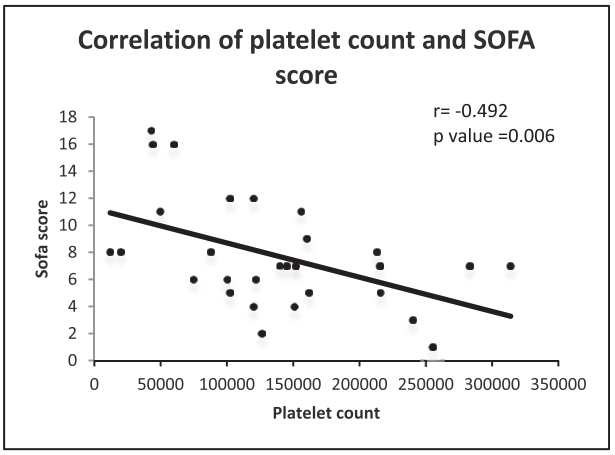

Fig. 1. Correlation of platelet count and SOFA score. SOFA, Sequential Organ Failure Assessment.

The negative results of our study hold importance as we demonstrate that in the early phases of mild sepsis, thrombocytopenia may occur, but the significant effect on the platelet aggregation arises in the later period of severe sepsis. Platelet aggregation might not be affected in mild early cases of sepsis because thrombocytopenia may not be severe enough to cause overall defect in aggregation. The model of derangement of platelets and its functions in sepsis was also shown in the study by Yaguchi et al. [4], who proposed that with the ongoing sepsis, thrombocytopenia instates due to activation and

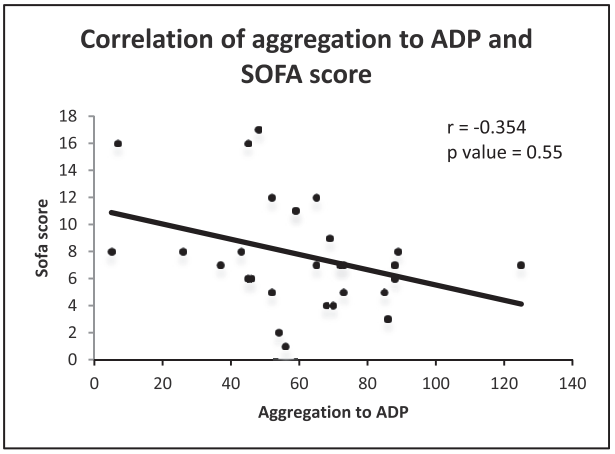

Fig. 2. Correlation of aggregation to ADP and SOFA score. SOFA, Sequential Organ Failure Assessment.

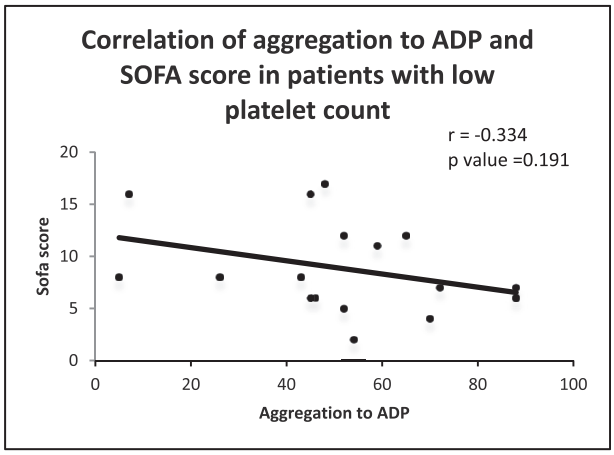

Fig. 3. Correlation of aggregation to ADP and SOFA score in patients with low platelet count. SOFA, Sequential Organ Failure Assessment.

consumption, subsequently leading to decreased aggregation but preserved secretory functions. This could be due to altered transcription pathways inside the platelets that disturb the aggregation [3]. It was also stated that platelet activates and aggregates in-vivo in sepsis without any altered behavior, and thus only the ex vivo response to platelet agonists is reduced [3,4].

Among the various factors that were analyzed, age of the patient may hold critical since older-age patients may have concurrent comorbidities. In our study, the age of the patients ranged from less than 20 to more than 50 years, indicating that there was no specific population that was admitted and studied. The individual category had less patients, which may have been the reason to reach a statistically independent risk factor.

The sex distribution was almost equivalent carrying no altered effects on the platelet aggregation. Inflammatory markers such as procalcitonin and CRP have been independently shown to be the independent risk factor affecting the platelet aggregation in the study by Adamzik et al. [8] In our study, they failed to show any statistically increased risk since the overall absolute values of these markers were low owing to the mildness of the sepsis.

In addition, although we cultured the organisms infecting the patients, but we could not avoid the effect of the antibiotic treatment as they may also play a role in platelet-aggregation dysfunction [6].

The study holds strength in the fact that it helped to resolve the issues about early platelet-aggregation defects in the initial stages of sepsis. Similar to the mechanism of alteration of red blood cell in iron-deficiency anemia where it goes through the steps of microcytosis followed by hypochromia, we propose that platelets also follow steps of activation and consumption, leading to thrombocytopenia, followed by altered aggregation. Another point that the study helps in stressing is that platelets can be portrayed to be a part of the inflammatory process rather than just being considered to perform the functions of clotting. The role of platelets as markers for inflammation has been studied in other inflammatory diseases as well, such as rheumatoid arthritis, Crohn's disease, ulcerative colitis, and inflammatory bowel disease [12]. Platelets seem to be a useful novel marker to diagnose and monitor the disease activity in these sets of conditions. Our study reinstates this fact and shows that platelet number and functions may be a potential marker for monitoring the sepsis severity, which in the future, may be used for management by targeting

Table 3. Univariate linear regression to find out significant factors affecting aggregation to ADP.

Variables	Beta coefficient	SE	*P* value	Lower bound (95%)	Upper bound (95%)
Age (years)	−0.170	0.294	0.566	−0.772	0.431
Fever	−1.210	0.936	0.207	−3.128	0.708
Blood pressure (mmHg)	0.451	0.253	0.086	−0.068	0.970
RBS (mg/dl)	−0.027	0.089	0.762	−0.211	0.156
TLC (μl)	0.001	0.001	0.116	0.000	0.002
CRP (mg/l)	−0.014	0.224	0.950	−0.472	0.444
Serum procalcitonin (ng/ml)	−0.046	1.683	0.979	−3.493	3.402
Urine output (ml)/24 h	0.011	0.012	0.375	−0.014	0.035
Serum creatinine (mg/dl)	−3.513	2.683	0.201	−9.009	1.984
Bilirubin (mg/dl)	−2.585	3.582	0.476	−9.923	4.752
Serum lactate (mmol/l)	3.512	22.102	0.875	−41.762	48.787
Sex					
Male					
Female	−4.000	9.318	0.671	−23.087	15.087
Peripheral edema	0.583	9.348	0.951	−18.565	19.732
CRT					
Normal					
Delayed	2.509	9.168	0.786	−16.271	21.289

CRP, C-reactive protein; CRT, capillary refilling time; RBS, random blood sugar.

certain molecules on the platelet to avoid the defects in the aggregation.

The limitations of the study were that we did not analyze other platelet indices in sepsis such as mean platelet size, platelet distribution width, and aggregation to other substances. Second, due to the cross-sectional nature of the study, the patients' outcome in terms of mortality and hospital stay was not recorded. It would have guided us further with the association of disturbances in platelet aggregation and morbidity. Third, the analytical studies on platelet aggregation such as ours fail to consider thrombocytopenia as a confounding factor in the lowering of the platelet aggregation. Since thrombocytopenia is the first and the foremost incidence, it is possible that the decreased ex-vivo aggregation may be a consequence of thrombocytopenia itself. However, this fact needs to be explored in the future studies.

4.1. Conclusion

Platelet count is reduced in patients with sepsis and there is significant negative correlation between the decrease in platelet count and the severity of sepsis as assessed by SOFA score. Platelet aggregation (ADP induced) was reduced in 50% of the cases but did not reach a statistically significant level. Since most of the cases in the study had mild sepsis, it can be concluded that alteration in the platelet aggregation is more with the severe sepsis and thrombocytopenia is an initial finding that begins even in the mild cases of sepsis.

Conflict of interest

There are no conflicts of interest.

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