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COMPARISON OF D-DIMER LEVEL AND PLATELET COUNT IN INDIVIDUALS WITH AND WITHOUT SICKLE CELL ANAEMIA

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ABSTRACT

Aim: This study aimed to assess the D-dimer levels and platelet count in sickle cell anaemia patients and compare with those of the control subjects. **Materials and Methods:** This study is a cross–sectional comparative study which enrolled 58 sickle cell anaemia patients (25 males and 33 females) in a stable state at the Obafemi Awolowo University Teaching Hospital in Ile-Ife, Osun State, Nigeria, as well as 25 healthy controls (11 males and 24 females). Venous blood (4.5 mL) was collected from both the patient and control into a vial containing 0.5 ml of 3.2% trisodium citrate. D-dimer levels was analysed using ELISA method. Platelet counts were determined (from 5ml venous sample collected with EDTA) using haematology autoanalyzer. **Results:** There was a significant rise in D-dimer levels in sickle cell patients, with mean values of 1771.86 ng/mL and 1986.00 ng/mL for males and females, respectively, in the steady state compared to the D-dimer levels of controls, with mean values of 199.24 and 322.21 ng/mL for males and females, respectively. Platelet count was significantly higher in SCA patients than in the control subjects. There is perfect correlation between D-dimer and platelet counts in both patients and control (r=1, n=57, P=0.000) and (r=1, n=30, P=0.000) **Conclusion:** This study showed the hypercoagulable state and thrombotic risk associated with SCA. The strong correlation between D-dimer levels and platelet counts suggests a potential interplay between platelet activation and coagulation activation in SCA, warranting further investigation into therapeutic interventions targeting these pathways.

KEYWORDS: D-dimer, platelet count, Sickle cell anaemia, steady state.

INTRODUCTION

Sickle Cell Anaemia (SCA) is a hereditary blood disorder characterized by abnormal haemoglobin molecules in red blood cells, leading to their distortion into a sickle shape [1]. It is associated with various complications, including vaso-occlusive crises, haemolytic anaemia, and increased susceptibility to infections. Among the myriad of complications, individuals with SCA are also prone to thrombotic events due to the chronic inflammation, endothelial dysfunction, and activation of coagulation pathways associated with the disease [2]. D-dimer, a fibrin degradation product, and platelet count are two biomarkers commonly used in clinical practice to assess thrombotic risk and coagulation status [3].

Thrombotic events are recognized complications of SCA, with both venous and arterial thrombosis reported in affected individuals. The pathophysiology underlying thrombosis in SCA is multifactorial, involving a complex interplay of factors such as chronic inflammation, endothelial dysfunction, activation of coagulation cascades, and alterations in blood rheology [4]. Endothelial dysfunction, manifested by increased expression of adhesion molecules and proinflammatory cytokines, promotes a prothrombotic state by facilitating the adhesion and activation of platelets and leukocytes. Additionally, the chronic haemolysis and release of free haemoglobin in SCA contribute to oxidative stress, further exacerbating endothelial injury and promoting thrombus formation [5].

D-dimer is a fibrin degradation product generated during fibrinolysis and is commonly used as a marker of ongoing coagulation and fibrinolytic activity. Elevated D-dimer levels have been associated with an increased risk of thrombotic events in various clinical settings, including venous thromboembolism, arterial thrombosis, and disseminated intravascular coagulation [6]. In individuals with SCA, elevated D-dimer levels may reflect ongoing intravascular haemolysis, activation of coagulation pathways, and endothelial injury, all of which contribute to thrombotic risk [3].

Platelets play a crucial role in haemostasis and thrombosis, with alterations in platelet count and function implicated in the pathogenesis of thrombotic disorders [7]. Thrombocytosis, defined as an elevated platelet count, has been associated with an increased risk of thrombosis in various clinical conditions, including myeloproliferative disorders, inflammatory diseases, and cardiovascular disorders [4]. Conversely, thrombocytopenia, characterized by a low platelet count, can predispose individuals to bleeding complications while paradoxically increasing the risk of thrombosis through mechanisms such as compensatory platelet activation and increased platelet turnover [5].

Despite the well-documented association between SCA and thrombosis, the role of specific biomarkers such as D-dimer and platelet count in thrombotic risk assessment in this population remains unclear. Moreover, there is a paucity of data comparing D-dimer levels and platelet counts between individuals with SCA and those without the condition, limiting our understanding of the pathophysiological mechanisms underlying thrombosis in SCA. Thus, this study sought to investigate the differences in D-dimer levels and platelet counts between individuals with SCA and those without the condition, shedding light on potential implications for thrombotic risk assessment and management strategies.

MATERIALS AND METHODS

Research Design

This study was conducted on adult SCA patients in the steady state at the Obafemi Awolowo University Teaching Hospital in Osun State, Nigeria. The Hospital's Ethical and Scientific Committee approved the study. We used adult Nigerians of both sexes who volunteered and provided written, informed consent as research participants. The haematology clinic accepted new patients in the order of their arrival. As the control group, people without sickle cell anaemia were used.

There were 58 SCA patients (25 males and 33 females) and 25 control participants (11 males and 14 females) in the study

Venous blood (4.5ml) was collected from both the patient and control into a vial containing 0.5 ml of 3.2% trisodium citrate. D-dimer level was analysed using ELISA method. Platelet counts were determined for both patients and control using haematology autoanalyzer using 5ml venous sample collected with EDTA bottle.

Inclusion criteria for Patients

Patients with stable SCA who are 16 years of age or older (a period of stable clinical condition occurring at least one week before or three weeks after a VOC or three months after a haemolytic crisis requiring a blood transfusion).

Inclusion Criteria for controls

Healthy individuals with Hb A from the Ile-Ife community who were age and sex matched

Exclusion Criteria for Controls

The study eliminated those who used any type of drug, smoked, or drank too much alcohol (14 units per week for women and 21 units per week for men).

Exclusion Criteria for Patients

Any other medical issues, such as diabetes mellitus or hypertension were excluded. Smokers and heavy drinkers who have sickle cell anaemia (14 units per week for females and 21 units per week for males) were also excluded. One unit of alcohol (8-l0 g) is equivalent to 12 pints of beer (about 300 mL). Patients who are in crisis or those who have received a blood transfusion.

Ethical Considerations

Before being enrolled in the study, all individuals gave their informed consent. Before beginning the study, the Ethical and Research Committee of OAUTHC, Ile-Ife, was consulted and ethical approval was granted:IRB/IECnumber 00005422.

RESULTS

This study involved 58 sickle cell anaemia patients (25 males and 33 females) in a stable state at the Obafemi Awolowo University Teaching Hospital in Ile-Ife, Osun State, Nigeria, as well as 25 healthy controls (11 males and 24 females). The D-dimer concentrations of all research subjects are presented in Figure 1. In the steady state, there was a significant rise in the D-dimer levels in sickle cell patients, with mean values of 1771.86 and 1986.00 ng/mL for males and females, respectively, compared to the mean D-dimer levels of control individuals, who had mean values of 199.24 and 322.21 ng/mL for males and females, respectively (table 1). Both in the patient and control groups, it was shown that females

had greater D-dimer levels than males, and the D-dimer levels of all the male control participants were within the normal reference range (<400 ng/mL). Platelet count was observed to be significantly higher in SCA patients when compared with those in the control subjects (Fig 2). There is perfect correlation between D-dimer and platelet counts in both patients and control (r=1, n=57, P=0.000) and (r=1, n=307, P=0.000).

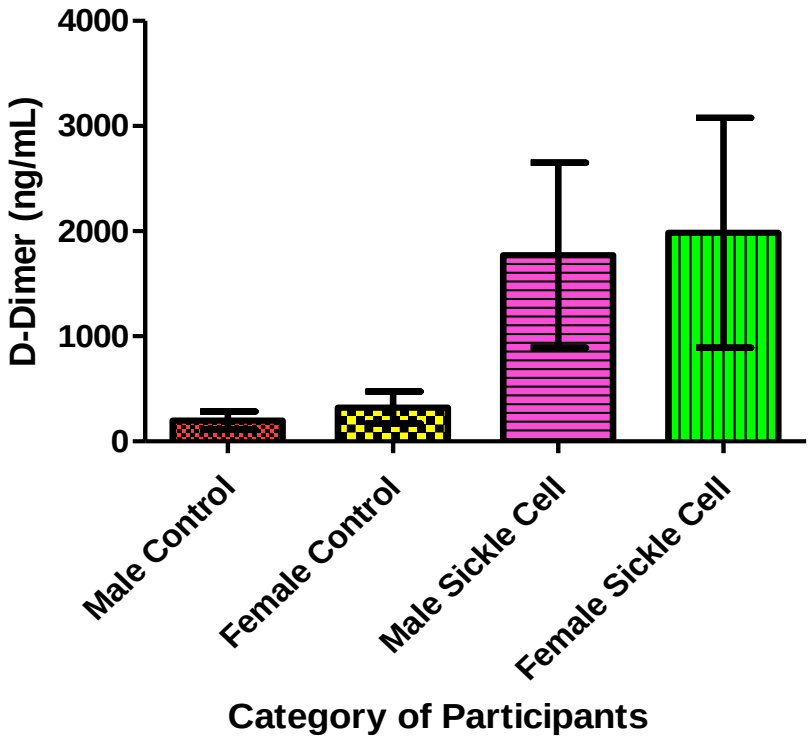

Figure 1 *(Box plot of D dimer levels of all the study participants.)*

Table 1 *(D dimer levels of SCA Patients and Control Participants.)*

D-Dimer Levels (ng/mL)	SCA Patients		Control Participants	
	Male: n (%)	Female: n (%)	Male: n (%)	Female: n (%)
0 – 399	1 (4.00%)	2 (6.06%)	7 (100.00%)	10 (71.43%)
400 – 999	4 (16.00%)	6 (18.18%)	0 (0.00)	4 (28.57%)
1000 – 1499	6 (24.00%)	8 (24.24%)	0 (0.00)	0 (0.00)
1500 – 1999	5 (20.00%)	1 (3.03%)	0 (0.00)	0 (0.00)
2000 – 2499	3 (12.00%)	4 (12.12%)	0 (0.00)	0 (0.00)
2500 – 2999	1 (4.00%)	3 (9.09%)	0 (0.00)	0 (0.00)
3000 – 3499	5 (20.00%)	5 (15.15%)	0 (0.00)	0 (0.00)
3500 – 3999	0 (0.00)	4 (12.12%)	0 (0.00)	0 (0.00)

Normal reference value of D-dimer levels: <400 ng/mL

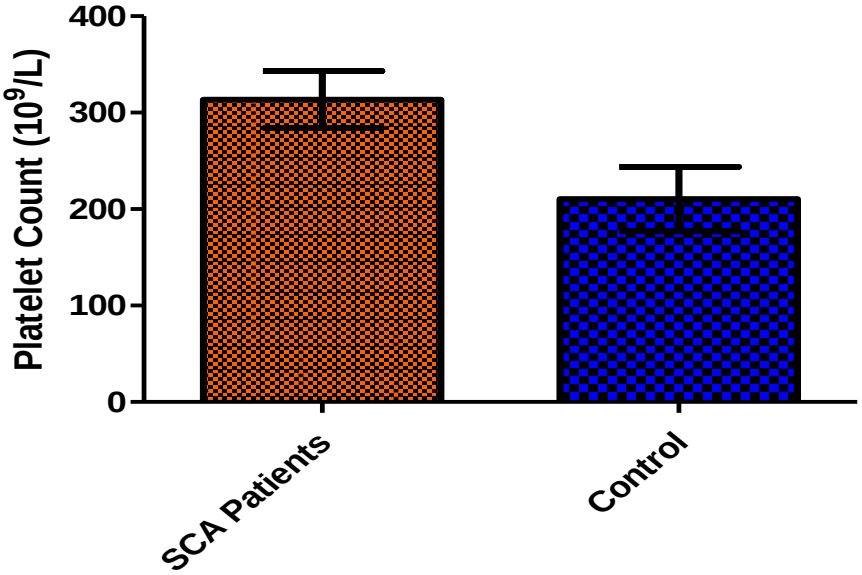

Fig. 2 *(Platelet count in SCA Patients and Control Subjects.)*

Table 2 (*Correlation Analysis of D-Dimer and Platelet count in SCA Patients.)*

		D-dimer of the patient	Platelet counts of the Respondents
D dimer of the patient	Pearson Correlation	1	1.000**
	Sig. (2-tailed)		.000
	N	58	57
Platelet counts of the Respondents	Pearson Correlation	1.000**	1
	Sig. (2-tailed)	.000	
	N	57	57

***. Correlation is significant at the 0.01 level (2-tailed)*

Table 3 *(Correlation Analysis of D-Dimer and Platelet count in Control Subjects.)*

		Platelet counts of the control	D-dimer of the control
Platelet counts of the control	Pearson Correlation	1	1.000**
	Sig. (2-tailed)		.000
	N	33	30

D-dimer of the control	Pearson Correlation	1.000**	1
	Sig. (2-tailed)	.000	
	N	30	30

***. Correlation is significant at the 0.01 level (2-tailed).*

Table 4 *(Correlation of D-dimer and Platelets in steady state Sickle cell anaemia and control.)*

D- dimer	Platelet Count
Sickle cell Patients R-value P-value	 1.00 0.000
Controls R-value P-value	1.00 0.00

DISCUSSION

Despite the fact that SCA is characterised by hypercoagulability, the role of coagulation in the disease's pathophysiology is still poorly understood [8]. D-dimer is high in SCA patients during "steady state," (a non-crisis state), and is elevated even more during acute pain episodes [9]. There is data showing a correlation between D-dimer levels and the frequency of pain episodes measured over the course of the year [10]. Moreover, the time between pain episodes suggests that coagulation activation may be a factor in vaso-occlusion in SCA [9]. There are conflicting data on whether coagulation activation markers increase beyond the steady-state during excruciating crises [11]. The relationship between coagulation activation markers and the incidence of painful crises has also been the subject of conflicting research. Plasma D-dimer levels were shown to be negatively connected with the time of the next episode of pain, and a substantial correlation was found between them and the frequency of pain crises [12].

Clinical manifestations of SCA vary in severity depending on the population. In this study, there was a significant rise in D-dimer levels in sickle cell patients attending Obafemi Awolowo University Teaching Hospital, Ile-Ife, with mean values of 1771.86 and 1986.00 ng/mL for males and females, respectively, in the steady state compared to the D-dimer levels of control participants, with mean values of 199.24 and 322.21 ng/mL for males and females, respectively (table 1). Both in the patient and control groups, it was shown that females had greater D-dimer levels than males, and the D-dimer levels of all the male control participants were within the normal reference range (<400 ng/mL).

In the current investigation, 58 patients representing 96.67% of those with SCA, had D-dimer levels that were significantly higher than the normal range (<400 ng/mL) compared to the control participants (Table 2). Similar findings with considerably greater D-dimer levels in patients with SCA in steady state compared to controls were reported in separate studies by Fakunle et al. [13], Hagger et al. [14], Haut et al. [15], and Francis [16]. It's probable that the significant rise in D-dimer levels seen in this study was brought about by SCA patients having several sites, each of which contributes to thrombi development to variable degrees [17]. However, a research by Akinola et al. [18] in patients with SCA, indicated biochemical and rheological changes that are compatible with brief bouts of microvascular stasis, and

these events were not severe enough to result in overt vaso-occlusive crises. The D-dimer levels between the patients with SCA and the controls did not, however, differ statistically significantly in another investigation by Ekwere et al. [19], despite the fact that the levels in the patient group were still higher. This might be as a result of the study's small sample size, which was less than half the total number of participants used in this study.

In contrast, studies by Philips et al. [20, 21] and Nsiri et al. [22] revealed that the steady-state fibrinolytic activity in individuals with SCA is diminished. In another study, steady-state fibrinolytic activity in SCA patients was not impaired [23]. Despite the fact that various conditions, such as inflammation, infections, or surgery, might produce an increase in plasma D-dimer levels, this does not necessarily mean that thrombosis has occurred. Normal values, however, almost always rule out thrombosis, and their negative predictive value frequently falls below 90% [24].

The exact level of D-dimer in the blood at a particular moment is influenced by the length of time since the thrombotic event, the initial size of the clot, and the rate of fibrinolysis [13]. It's possible that the significant rise in D-dimer levels seen in this study was caused by various sites where patients with HbSS developed thrombi with varying degrees of severity. In the blood of people with normal renal function, the half-life of D-dimer is roughly 6 hours. Nephropathy has recently been linked to SCA [25]. There may not be any discernible D-dimer elevations in patients with stable clots who are not experiencing active fibrin deposition or plasmin activation. Determining D-dimer reflects prothrombin plasminogen and factor XIII activation. The severity of the actual state of coagulation activation may therefore be better determined by a direct measurement of circulating thrombin activity [26].

The observed elevation in platelet count among SCA patients (Fig. 2) aligns with previous research. Studies, such as those conducted by Sparkenbaugh and Pawlinski [27], have reported increased platelet activation and aggregation in SCA, leading to higher platelet counts. The current findings corroborate the notion of a hypercoagulable state in SCA, emphasizing the need for further investigations into the underlying mechanisms. The elevated platelet count observed in this study corroborates the elevated levels of D-dimer observed in SCA patients enrolled in this present study.

Elevated platelet counts in SCA have been associated with an increased risk of thrombosis. The work of Ataga et al. [28] demonstrated a correlation between high platelet counts and vascular complications in SCA.

The correlation analysis demonstrates a perfect positive correlation ($r = 1$) between D-dimer levels and platelet counts in both individuals with SCA and healthy controls. This finding is consistent with previous research indicating a close relationship between platelet activation and coagulation activation in SCA [29]. Additionally, a study by Key et al. [30] demonstrated a positive correlation between D-dimer levels and platelet counts in SCA, further corroborating our results. The strong correlation suggests that platelets may contribute to the elevated D-dimer levels observed in SCA through their role in promoting coagulation and fibrinolysis.

CONCLUSION

The comparison of D-dimer levels and platelet counts in individuals with and without SCA highlights the hypercoagulable state and thrombotic risk associated with this disorder. The strong correlation between D-dimer levels and platelet counts suggests a potential interplay between platelet activation and coagulation activation in SCA, warranting further investigation into therapeutic interventions targeting these pathways.

Limitations of the Study: C-reactive protein (CRP) and fibrin degradation products (FDPs), which may help in evaluating the markers of intravascular clotting activation and ongoing clot lysis, were not included to categorise those who have brief episodes of microvascular stasis that are not severe enough to cause overt vaso-occlusive crisis.

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