ORIGINAL ARTICLE

Significance of Platelet Activation in Sickle Cell Anaemia

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ABSTRACT

Background: *There is increasing evidence suggesting the contribution of platelets in the vaso-occlusive phenomena found in sickle cell anaemia. This study is aimed at using simple, inexpensive parameters to determine the role of platelets in the steady state and vaso-occlusive crisis state of Nigerian sickle cell anaemia patients.*

Methods: *The circulating platelet aggregate (CPA) ratio, platelet factor-3 availability (PF-3) and platelet counts of 60 adult Nigerian sickle cell anaemia patients were studied.*

Results: *The CPA ratio in the sickle cell anaemia (SCA) patients in steady state (SS) was 0.93 ± 0.05, 0.89 ± 0.04 during vaso-occlusive crisis (VOC) and 0.98 ± 0.02 in the control group (C). The values in the vaso-occlusive crisis and in steady state were significantly lower than in the control group (P<0.05). PF3 availability in steady state and vaso-occlusive crisis were 29.7 ± 4.0 secs and 28.4 ± secs respectively. The times are significantly shorter when compared with the control group with a time of 36.2 ± 4.3 secs (P<0.05). There was however no significant difference between the two sickle cell groups. Platelet count was significantly raised in the steady state patients 224.3 ± 46.3 x 10^9/L when compared with controls of 196.6 ± 39.3 x 10^9/L (P<0.05). There was a significant fall during VOC to 140.6 ± 36.3 x 10^9/L (P<0.05). The difference between the two sickle cell groups is significant (P<0.05).*

Conclusion: *This study indicates varying degrees of partial activation of platelets invivo in the steady state and vaso-occlusive crisis state of sickle cell anaemia. It support's a contribution of platelet to the vascular occlusion that underlies much of the morbidity in the disease.*

KEYWORDS: *Platelet activation; Sickle Cell Anaemia.*

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INTRODUCTION

Vaso-occlusive crisis is a frequent cause of morbidity and mortality in sickle cell disease. Sickling of the red cells at low oxygen tension is a property fundamental to the abnormal sickle erythrocyte, but the mechanisms of vascular occlusion are not fully characterized[1]. In the micro-vasculature there is stasis with increased adherence of sickled red cells to the endothelial cells and platelets[2]. Increasing evidences suggesting the contribution of platelets in the vaso-occlusive phenomena are found in the altered platelet functions in both the steady state and crisis state of the disease. The abnormalities in adult sickle cell anaemic patients include an increase in platelets count, circulating mega-thrombocytes in the steady states and fall during crisis[1,3], shortened platelet survival in both states[1], platelet activation deduced from reduced *invitro* platelet aggregability to various agonist[4,5], elevated levels of platelet secretory products[6], increased levels of circulating platelet aggregates[4,7], increased platelet factor3 availability[3,8], increased expression of activation dependent platelet antigens and micro-particles[9], increased levels of thrombospondin, exposure of platelet surface markers such as CD62 P and CD 40L in both states[10]. The aim of this study was to use simple inexpensive parameters, namely, circulating platelets aggregate ratio, platelet factor-3 availability and platelet count, to investigate platelet functions of adult Nigerian sickle cell anaemia patients and determine their role in the vaso-occlusive phenomena.

MATERIAL AND METHOD

Sixty patients with confirmed sickle cell anaemia (Hb SS) presenting at the sickle cell clinic of Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) and the University of Calabar Teaching Hospital (UCTH) constituted the patients. Thirty patients who did not have any complication of the disease for at least 4-weeks were taken to be in the steady state and patients with bone pain and established to have vaso-occlusive crisis formed the 'Crisis State'. Patients were between the age of 14 and 32-years. The mean age for patients in the steady state was 20.8 ± 4.3 and 20.1 ± 3.8 in the crisis state. 38.3% of the patients were females and 61.6% were males while 13.3% of the controls were females and 86.6% were males. Fifty aged matched healthy adult blood donors and students constituted the controls. Subjects with a history of aspirin or any anti- platelet drug ingestion in the preceding 10 days were excluded.

SAMPLE PREPARATION

Venous blood was collected into ethylenediaminetetracetic acid (EDTA) and 3.8% citrate bottles for heamocytometry and kaolin clotting time of

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platelet rich plasma respectively as described by Dacie and Lewis[11]. Circulating platelet aggregate ratio was carried out by the method of Wu and Hoak[12] as modified by Osim *et al* [13]. 0.5mls of blood each was collected into 2 plastic syringes containing 9.5mls of EDTA/Formalin and EDTA solutions respectively (1:10 dilution). The samples were transferred into plain plastic bottles from which platelet rich plasma (PRP) was prepared. The platelet counts in the PRP are counted. Circulating platelet aggregate, when present will be fixed in the EDTA/Formalin solution, thus the platelet count in the PRP will be reduced whereas in the EDTA solution platelet aggregates are dispersed. The CPA ratio is 1 where there is no aggregation and reduced if aggregation is present. Values may then be converted to percentages (%).

$$\text{CPA RATIO} = \frac{\text{Platelet count in EDTA/Formalin PRP}}{\text{Platelet count in EDTA PRP}}$$

STATISTICAL ANALYSIS

Comparison between the three groups was done by one-way analysis of variance (ANOVA) followed by post hoc least square difference (LSD) t-test. A P value less than 0.05 was considered significant.

RESULTS

The platelet count in the steady state was 224.3 ± 46.3 x 10^9/L this was significantly raised when compared to the 196.0 ± 39.2 x 10^9/L (P<0.05). During vaso-occlusive crisis there was a significant fall to 140.6 ± 36.3 x 10^9/L (P<0.05).

The platelet factor 3-availability was 29.7 ± 4.0 and 28.4 ± 2.5 in the steady state and during crisis. It was significantly reduced in both sickle cell groups when compared to control value of 36.2 ± 4.3 (P< 0.05). There was no significantly difference between the two sickle cell groups.

The circulating platelet aggregate ratio was 0.93 ± 0.05 and 0.89 ± 0.04 in the steady state and in vaso-occlusive crisis. The ratio are significantly reduced in both the sickle cell groups when compared to control value of 0.98 ± 0.02. The reduction in the ratio is greater during vaso-occlusive crisis.

Table I. Platelet Function Test in SS, VOC and Control

PARAMETER	SS	VOC	CONTROL
PLATELET COUNT (X10^9/L)	224.3 ± 46.3	140.6 ± 36.3	196.0 ± 39.2
PLATELET FACTOR 3 AVAILABILITY (SECS)	29.7 ± 4.0	28.4 ± 2.5	36.2 ± 4.3
CIRCULATING PLATELET AGGREGATE RATIO	0.93 ± 0.05	0.89 ± 0.04	0.98 ± 0.02

Table II. Level of Significance

PARAMETER	SS VS VOC	SS VS C	VOC VS C
PLATELET COUNT	P<0.05	P<0.05	P<0.05
PLATELET FACTOR-3 AVAILABILITY	P>0.05	P<0.05	P<0.05
CIRCULATING PLATELET AGGREGATE RATIO	P<0.05	P<0.05	P<0.05

DISCUSSION

This study has shown that platelet counts in steady state SCA was higher than in the control group, this moderate thrombocytosis is attributed to loss of splenic sequestration of platelet as a result of functional asplenia[1]. During vaso-occlusive crisis there was a significant fall in platelet count. This may reflect *in-vivo* activation and consumption of platelets during the vaso-occlusive process[3]. The circulating platelet aggregate ratio is significantly reduced in both steady state and during vaso-occlusive crisis, the reduction is however greater during vaso-occlusive crisis. The reduction in CPA ratio indicates increased circulating platelet aggregate. The finding of increased circulating platelet aggregates in the steady state is at variance with earlier studies which reported normal values[4,7].

The method used in this study has modified the original 1:1 dilution by Wu and Hoak 1974 used by earlier studies to 1:10 dilution[12] as sensitivity studies have shown that increasing the dilution does not change the sensitivity of the test rather it increases the specificity by preventing interactions between formalin and any unspecified factor[14].

Platelet factor 3-availability is significantly increased in the steady state and during crisis. There is no significant difference between the two sickle cell groups. Platelet secretion occurs once platelet are activated. The increased pro-coagulant activity of platelets indicate partial or ongoing activation of platelets. Activated platelets provide a template for the assembly of the prothrombinase reaction that leads to the generation of thrombin[15]. The increase pro-coagulant activity of platelet thus supports the findings of coagulation activation in sickle cell diseases[1,10].

The findings of varying degrees of partial or ongoing activation of platelets and increased pro-coagulant activity of platelets support the contribution of platelets to the mechanism of vaso-occlusion in the disease. Whether this contribution is a primary or secondary effect however needs to be further studied.

It is suggested that treatments that could decrease the activation of platelet may be beneficial in the prevention and treatment of vaso-occlusive complications that dominate the course of the disease.

This study also highlights the use of simple, inexpensive approach to the assessment of platelet functions in developing countries.

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