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Evaluation of Protein C in Nigerian Patients with Sickle Cell Anaemia in Steady State

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Authors' contributions

This work was carried out in collaboration between all authors. Author AAI designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author OTO managed data analysis and interpretation, participated in drafting of manuscript. Author WAS participated in the design of the study and protocol. All authors read and approved the final manuscript.

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ABSTRACT

Background: Sickle cell disease which is considered to be a hypercoagulable state has a worldwide distribution. Protein C is a naturally occurring anticoagulant with anti inflammatory and fibrinolytic properties.
Objective: To evaluate the level of Protein C in Nigerian Patients with sickle cell anaemia (SCA) in steady state.
Methods: This is a comparative cross sectional study carried out in Ibadan on 40 HbSS patients in steady state attending Haematology clinic at University College Hospital, Ibadan and 40 age and sex matched healthy normal HbAA control. Protein C was assayed with Amax Destiny Coagulometer using clot based method. Liver function test (LFT) was done with Hitachi 912.
Results: A significant decrease in Protein C was found in HbSS patients in steady state (median value 62.8%) compared with HbAA control Subjects (74.6%) (p = 0.00).
There was no significant difference in the LFT of the HbSS patients and the control HbAA subjects,

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P > 0.05 in all the parameters measured. (Alanine Transaminase (ALT), *P* = 0.82; Albumin, *P* = 0.12; Total Protein, *P* =0.37).
Conclusion: The low Protein C level observed in HbSS patients in steady state may not be due to hepatic dysfunction as LFT in the subjects were found to be normal.

Keywords: HbSS; Protein C; LFT; hypercoagulable state.

1. INTRODUCTION

Sickle cell anaemia is a genetic disease characterized by hypercoagulable state in which various hemostatic systems both in steady state and during vaso-occlusion are perturbed. This contributes to the characteristic acute and chronic multisystem failure associated with the disease [1]. The pathogenesis of hypercoagulability is multifactorial, and include: certain characteristics of sickle cells such as abnormal adhesivity to endothelium and loss of lipid asymmetry; increase in white cell populations and alterations in components of haemostatic system [2,3]. Every component of haemostasis including platelet function, the procoagulant, anti-coagulant and fibrinolytic system is perturbed in SCA [4-5]. Various studies have documented high levels of thrombin, abnormal activation of fibrinolysis and decreased levels of Protein C, Protein S and Anti thrombin III (ATIII) [6-7]. The decreased in levels of natural coagulation inhibitors has been attributed to consumption or hepatic dysfunction [8-10].

Protein C is a vitamin K dependent serine protease which is synthesized by liver parenchymal cells and plays a role in the regulation of haemostasis. Protein C exists in an inactive form and is activated by the thrombin-thrombomodulin complex. Its activated form (activated protein C -APC) controls the coagulation process by cleaving and inactivating factors VIIIa and Va in the presence of protein S, which acts as a cofactor for APC, down-regulating clot formation [11]. APC enhances fibrinolysis by inhibiting plasma inhibitors of tissue plasminogen activator [12].

Aside its anticoagulation role, Protein C exhibits anti-inflammatory effects which include inhibition of neutrophil chemotaxis, reduction of adhesion molecules expression on endothelial cells, suppression of production and release of pro-inflammatory cytokines in monocyte and endothelial cells and increase production of anti-inflammatory cytokines. [13-16]. Considering the close link between coagulation and inflammation on the pathophysiologic processes of SCA [6] protein C would be an important protein to study because of its anticoagulation and anti inflammatory characteristics.

Despite the high prevalence of SCA in Nigeria and the potential role of Protein C in coagulation and inflammation, there is a paucity of data on the levels of Protein C in patients with SCA in the sub-region. The aim of this study is to assess the level of Protein C in Nigerian Patients with sickle cell anaemia in steady state and find influence of hepatic function on it.

2. MATERIALS AND METHODS

2.1 Study Design

The study was carried out at the University College Hospital, Ibadan, Nigeria after obtaining Ethics committee approval. The study population comprised of 40 sickle cell anaemia patients in steady state (point in time with no painful episodes within 1 month before the observation) [17] compared with 40 age and sex-matched healthy HbAA control.

2.2 Blood Collection and Storage

9.5 ml of venous blood was collected from each subject, 4.5 ml of blood was transferred into 0.5 ml of Trisodium Citrate at ratio of 9:1 for the determination of protein C while 5 ml of blood was transferred into Lithium Heparin specimen bottle for LFT. Citrated venous blood was centrifuge at 3,000 revolutions per min for 15 minutes to make platelet poor plasma which was stored at -20ºC until it was used for Protein C Assay.

2.3 Diagnostic Method

Platelet poor plasma obtained from citrated venous blood collected from the subjects is processed by Amax Destiny plus Coagulometer manufactured by Trinity Biotech (2007) using clotting based assay. Protein C clotting assay uses an APTT reagent incorporating a protein C activator, Protein C deficient plasma and calcium chloride. The degree of prolongation of the clotting is proportional to the concentration of

protein C in the patient plasma when patient plasma is mixed with protein C deficient plasma.

Plasma obtained from lithium Heparin specimen bottle is processed by the Hitachi 912 autoanalyser for Liver function tests- alanine transaminase, albumin and total protein.

2.4 Statistical Analysis

Data was analyzed using Statistical Package for the Social Sciences (SPSS) version 17. Categorical variables were summarized with frequencies and percentages, while the quantitative data were summarized with median because their observations were not normally distributed.

The inferential analyses were carried out using non parametric methods such as median test, Spearman rank correlation and chi-square test to test for the significance of the relationship and association between the Protein C concentration and the other variables. All the statistical tests were two tailed and were done at a probability level of 5% (P = 0.05)

3. RESULTS

Eighty (80) participants were recruited for the study comprising of 40 HbSS and 40 HbAA as controls.

3.1 Socio-demographic Characteristics of the Study Subjects (Table 1)

Forty HbSS (18 males and 22 females) aged 18 to 50 years were selected for the study and 40 HbAA (23 males and 17 females) aged 20 to 50 years were used as control. The median age of the HbSS patients was 24.5 years which was comparable with that of the control with median age of 26 years (P=0.37). The study groups were similar based on gender also (P = 0.39).

3.2 Comparison of Median Values of Protein C and Liver Function Tests of the HbSS Patients and Controls

Table 1 presents the median estimates of Protein C, Total protein, Albumin and Alanine Transaminase (ALT) of the HbSS patients and control HbAA subjects. The control group had significantly higher median estimate of Protein C than the HbSS patients, however the two groups had similar Total protein, albumin and ALT.

3.3 Relationship of Protein C with LFT in HbSS Patients

Protein C had a weak inverse non significant relationship with Total protein**,** albumin and ALT as shown in Table 2.

4. DISCUSSION

In this study there was a significant decrease in Protein C levels in HbSS patients in steady state compared with the Protein C levels of HbAA healthy individuals. This was consistent with other reports from previous studies [18,19]. The decreased level of Protein C may be partly attributed to the persistent low grade inflammation in SCA [20]. Ischemic damage to tissues as a result of vaso-occlusion elicits an inflammatory response which activates endothelial cells to produce pro-inflammatory cytokines that promote thrombin generation by increasing tissue factor expression that initiates the coagulation cascade [21].

Also reports have shown elevated plasma levels of thrombin-antithrombin (TAT) complexes, prothrombin fragment 1 + 2 (F1 + 2), plasmin-antiplasmin complexes (PAP), D-dimers and fibrinopeptide A which are markers of thrombin generation, in patients with SCA in steady state and even more in bone pain crises [22-25]. These abnormalities of the hemostatic system in SCA confirm activation of coagulation and fibrinolytic systems in steady state which could lead to increase the risk of thrombosis.

The finding of this study is in contrast to report of Pandey et al. [26] where no significant difference was found in similar population. This could be due to the differences in the methods of assay used. Whereas they used chromogenic method, Protein C assay was done using the clotting based method in this study. The Protein C clotting assay measures total functionality of the molecule as well as both Type I and Type II Protein C defects. Assays based on chromogenic method measure only amidolytic activity. Approximately 30-40% of type II Protein C defect involves functional domains not associated with amidolytic activity. The Protein C clotting assay will detect dysfunction in functional domains not associated with amidolytic activity and in addition measures the functionality of the amidolytic domains.

Mohsen et al. [27] and Wright et al. [28] concluded that the lower Protein C level in HbSS

Table 1. Comparison of median values of protein C and liver function tests of the HbSS patients and controls

Variables	HbSS patients	Controls	*P* -value
	median	median	
Protein C (%) activity	62.8	74.6	0.00
Total protein (mg/dl)	7.4	7.2	0.37
Albumin (mg/dl)	3.7	3.9	0.12
ALT (I/U/L)	11.5	11.0	0.82

patients is either due to decreased production or increased consumption. In this study liver function test of the HbSS patients did not differ from the control which may suggest that the lower Protein C level is not due to hepatic dysfunction. The reduced level of Protein C found in HbSS patients in this study and other previous studies may be a consequence of chronic consumption of Protein C; due to increased thrombin generation and activation of coagulation factors as studies have shown further reduction in Protein C level of SCA in acute pain crises [29,30]. However coagulation profile was not done in this study.

Table 2. Correlation of protein C with LFT in HbSS patients

Protein C	Parameter	r	*P* –value
	Total protein (g/dL)	-0.02	0.92
	Albumin (g/dL)	-0.23	0.16
	ALT (IU/L)	-0.10	0.54

5. CONCLUSION

In conclusion, this study has shown that Protein C level is lower in HbSS patients in steady state compared with their controls, this may contribute to increased risk of hypercoagulability and thromboembolic complications. However, Liver function tests did not differ significantly between HbSS subjects in steady state and controls, suggesting that the low Protein C level observed in the HbSS patients may not be due to hepatic dysfunction.

6. RECOMMENDATIONS

The use of Protein C in the treatment of sickle cell disease should be advocated especially when the patients require oral anticoagulation because of its anti-inflammatory and fibrinolytic properties. When used, complications of bleeding tendency, toxicity and other general side effects of currently available anticoagulant agents can also be avoided. However, further studies will be required to substantiate this fact.

CONSENT

Informed written consent was obtained from all the participants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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