Original Article

Protein C and Antithrombin Levels in Patients with Sickle Cell Anemia in Ahmadu Bello University Teaching Hospital Zaria, Nigeria

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Background: Alterations in the components of hemostasis, namely platelet function, the procoagulant, anticoagulant, and the fibrinolytic systems, are observed in sickle cell anemia (SCA) and are in favor of a procoagulant phenotype. Therefore, study of protein C and antithrombin (AT) levels in patients with SCA in steady state may be used in the treatment and/or prevention of SCA-related thrombotic complications. We studied the changes of these naturally occurring anticoagulants in patients with SCA attending the sickle cell clinic in Ahmadu Bello University Teaching Hospital, Zaria. Methods: We conducted a case–control study involving 50 SCA (HbSS) patients in the steady state as cases and 25 healthy volunteers with normal hemoglobin (HbAA) as controls. Protein C and AT levels were estimated by semi-automation using Diagnostica Stago hematology coagulation analyzer. Frequencies, proportions, and independent t test were performed using SPSS version 20. Results: The mean ages of both the patients and controls were 23.80 ± 7.46 and 24.28 ± 3.48 years, respectively, and study participants comprised 40 (53.0%) women between the ages of 15–50 years and 15–34 years (P = 0.76). The mean values of protein C and AT levels in patients with SCA in the steady state and the control group were 60.26 ± 20.58% versus 81.30 ± 19.74%, 95% CI 11.13–30.96, and 42.11 ± 5.01% versus 61.88 ± 11.27%, 95% CI 16.03–23.51 with P values (P < 0.001), respectively. Conclusions: This study showed that there was a significant decrease in the levels of protein C and AT between the SCA patients in the steady state and the controls. We recommend baseline investigations of these naturally occurring anticoagulants in patients with SCA, especially in those with frequent vaso-occlusive crises. This will give us an insight into the additional pathophysiologic mechanism in SCA-related thrombotic complications for better patient management and outcome.

Keywords: Antithrombin, hemostasis, protein C, sickle cell anemia, steady state

INTRODUCTION

Sickle cell anemia (SCA) is an inherited condition resulting from the inheritance of two sickle genes.[1] Sickle cell disease (SCD) is one of the most common genetic disorders worldwide. Based on the World Health Organization (WHO) published global prevalence of SCD and other data, an estimated 20–25 million individuals have homozygous SCD worldwide.[2] About 12–15 million in sub-Saharan Africa, the highest gene frequency is in tropical Africa.[2] Nigeria bears the highest burden of SCD in the world with about 3% of the population being homozygous.[3]

Because of the alterations of nearly every component of hemostasis, namely platelet function, the procoagulant, the anticoagulant, and the fibrinolytic systems in favor of a procoagulant phenotype in the disease, SCD is
frequently referred to as a “hypercoagulable state.”[4] Abnormal exposure of phosphatidylserine (PS) in sickle erythrocytes may occur due to repeated cycles of sickling and unsickling linked to the polymerization and depolymerization of HbSS that results in the production of terminal spicules or microvesicles with exposed PS.[5] Moreover, external exposure of PS alters the adhesive properties of sickle blood cell (RBC) and appears to be involved in the hemostatic changes observed in SCD.[6-8] It has also been observed that plasma levels of prothrombin fragments 1.2 (F 1.2) are associated with the number of circulating PS-positive RBC.[8]

Patients with SCD have increased levels of markers of thrombin generation and thrombin antithrombin (AT) complexes (F 1.2, TAT complexes) in the noncrisis state.[9,10] Decreased levels of natural anticoagulant proteins are also observed in SCD.[10,11] Therefore, the levels of protein C and S are decreased in the noncrisis steady state and perhaps even more so during acute pain episodes. These may be a consequence of the following conditions:

- Chronic consumption of proteins C and S due to increased thrombin generation, resulting from intravascular tissue factor (TF) expression and red blood cell (RBC) prothrombinase activity.[13]
- Increased binding of protein S by sickle RBC due to membrane PS exposure.[5]
- Inhibition of the binding of protein S to β₂-glycoprotein I by antiphospholipid antibodies,[14] resulting in inactivation of protein S by circulating C4b-binding protein.

Significantly decreased levels of proteins C and S were reported in patients with SCD who developed thrombotic strokes,[15] as compared to neurologically normal children with SCD.

**Materials and Methods**

It was a case-control study involving 75 participants, which comprised 50 patients with SCA (HbSS) and 25 healthy volunteers (HbSS) as controls after ruling out inherited thrombophilias from both groups using inclusion and exclusion criteria. The adult participants with HbSS only in the steady state were included in the study, whereas those with hypercoagulable comorbid states (hypertension, diabetes mellitus, pregnancy), patients on contraceptives, aspirin, and those with known bleeding disorders or thromboembolism were excluded from the study. The research was carried out at hematology clinic of the Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Nigeria, during 2014. Ethical approval was obtained from the Health Research Ethic Committee (HREC) of the ABUTH, Zaria. Venous blood of 4.5 mL was dispensed into a bottle containing 0.5 mL of 3.2% trisodium citrate (blood anticoagulant ratio of 9:1) for the determination of protein C and AT levels using semi-automated hematology coagulation analyzer Cedex (Diagnostica Stago ST art 4, coagulation analyzer 2009, France). Control plasma normal (N) and prolong (P) were first used to obtain a calibration curve for protein C and AT levels with strict adherence to the manufacturer’s instructions. Frequencies, proportions, and independent t test were performed using the statistical package for social sciences (SPSS) version 20.0 and the level of significance was set at P ≤ 0.05.

**Results**

The mean ages of both the patients and controls were 23.80 ± 7.46 and 24.28 ± 3.48 years, and study participants comprised 40 (53.0%) women between the ages of 15–50 years and 15–34 years (P = 0.76). The mean values of protein C and AT in patients with SCA in steady state and the control group were 60.26 ± 20.58% versus 81.30 ± 19.74% and 42.11 ± 5.01% versus 61.88 ± 11.27% with 95% CI 16.0326–23.5074 and 11.1307–30.9573 (P < 0.0001), respectively [Table 1].

**Discussion**

In this study, the reduction in protein C levels among the majority of patients with SCA as compared with the controls [Table 1] was similar to the finding of Wright et al.,[11] In studies by Peters et al.,[16] Liesner et al.,[17] Wood et al.,[18] Bashawri et al.,[19] and colleagues working independently reported that the mean protein C levels in SCD individuals were significantly lower than protein levels of the controls. The mechanism of

**Table 1: Protein C levels of all the participants**

<table>
<thead>
<tr>
<th>Protein C levels (%)</th>
<th>HbSS</th>
<th>HbAA</th>
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<tbody>
<tr>
<td>50 – 69</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>70 – 89</td>
<td>9</td>
<td>36</td>
</tr>
<tr>
<td>90 – 109</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>110 – 129</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>130 - 149</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

**Table 2: Antithrombin levels of all the participants**

<table>
<thead>
<tr>
<th>Antithrombin levels (%)</th>
<th>HbSS</th>
<th>HbAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 – 40</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>41 – 60</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>61 – 80</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>81 - 100</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
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low protein C levels remains unknown, but this may be due to consumptive coagulopathy. However, a similar study in Nigeria by Ibijola et al.,[21] though with smaller sample size, showed significant decrease in protein C levels in adult patients with SCA in steady state. This may be the result of low-grade inflammation in patients with SCA.[14]

It may also be due to sustained tissue destruction associated with either hemolysis or continued vaso-occlusion or the liver being frequently affected in SCD, tissue being a site of protein C production. Therefore, impaired hepatic function due to chronic hepatic sickling may affect its production (it is also a vitamin K-dependent protein).[11] All of the studies on protein C discussed above were carried out on adult patients with SCA using different types of coagulation analyzer and with sample size similar to this study.

All of the patients with SCA (100%) had reduced AT levels compared with 28% in the control group [Table 2]. This was similar to a previous study done by Onyemelukwe et al.[22] in ABUTH, Zaria, who reported a reduced AT levels among the children with SCA compared with the normal children (HbAA). However, other independent studies by Leslie et al.[23] Bashawri et al.[19] and Karayalcin et al.[24] reported significantly reduced levels of AT in all the adults with SCA. On the contrary, Lieszner et al.[17] and Karayalcin et al.[24] reported normal AT levels in two different studies in all the patients with SCA. Devine et al.[25] separately reported normal AT levels in two different studies in all the patients with SCA. Again, another study in Nigeria by Ladu et al.[26] demonstrated a reduction in AT levels in patients with SCA in steady state.

Normal levels of AT in the studies above could be due to the differences in the methods, where they used thrombin-based assay method as against factor Xa method used in this study. Assays based on thrombin lead to overestimation of AT levels because of the interference of thrombin with heparin cofactor (HCII). Heparin cofactor II inhibits thrombin, leaving most of the AT untreated and resulting in overestimation.

Majority of studies above observed reduction in AT levels, which may be due to the background inflammatory state of SCA because of the impaired synthesis resulting from negative acute phase response, degradation by elastases from activated neutrophils, and, most importantly, consumption as a consequence of ongoing thrombin generation.[29,30] Some studies also recruited children even though they observed reduced AT levels in patients with SCA.[17,21]

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Conflicts of interest
There are no conflicts of interest.

REFERENCES