International Journal of Academic Health and Medical Research (IJAHMR)
ISSN: 2643-9824
Vol. 5 Issue 3, March - 2021, Pages: 84-88

Assessment of Plasma Protein C Level among Sudanese Children with Sickle Cell Anemia

Tibyan A. Mustafa[1]. Rayan G. Abdelnabi[2]. Mohamed A. Omer[3], May Mohamed Ali[4] and Ahmed Elhadi[5*]

[1] Department of Haematology, Test Laboratory, Khartoum, Sudan.

[2, 5] Department of Haematology, Faculty of Medical Laboratory Sciences, Sudan International University, Khartoum, Sudan.
[3] Diagnostic Laboratory, Sharg Elneel hospital, Khartoum, Sudan.

[4] Department of Haematology, College of Medical Laboratory Sciences, Sudan University of Science and Technology, Khartoum, Sudan.
E-mails: *Mayhussen1976@gmail.com[4]*, *aabdlla3@gmail.com[5]*.

Abstract: *Protein C (PC) deficiency has been reported in sickle cell anemia (SCA) and postulated to contribute to the pathogenesis as well as clinical manifestations of the disease. However, there is paucity of data on PC level in Sudanese children with SCA. This is a case control study conducted in Jafar Ibn Auf Pediatric Hospital, Khartoum-Sudan during the period from February 2019 to May 2019.A total of 100 Sudanese children were enrolled for this study, 50 SCA children as cases group and 50 apparently healthy children as control group who were matched with cases in regards to age and gender. PC level was tested by (AESKULISA) assay Kit and measured by ELISA (Human Reader, HS). The results showed that, mean PC level in cases was (79.71±11.97) which is significantly lower than in controls (84.38±10.37) (p. value = 0.039). Moreover, PC level showed significant differences between study groups according to the history of blood transfusion, thrombotic event and anti-thrombotic therapy of patients (p. values = 0.006, 0.019 and 0.019 respectively). On the other hand, no significant differences of PC level were observed between males and females (p. value = 0.78). In addition, a significant positive correlation was observed between the age of patient and PC level (p. value = 0.010, R = 0.359). In conclusion, children of SCA had lower PC level compared with healthy subjects. Those children need to be investigated for PC level in their early stage of the disease in order to minimize the risk of hypercoagulability.*

Keywords--Sickle cell anemia, Protein C, hypercoagulability.

1. Introduction:

Sickle cell anemia (SCA) primarily affects those of African descent and Hispanics of Caribbean ancestry, but the trait has also been found in those with Middle Eastern, Indian, Latin American, American Indian, and Mediterranean Heritage. It has been estimated that 90,000 to 100,000 People in the U.S. are affected by sickle cell anemia and that approximately 3 million people, and 10% of African-Americans, have Sickle cell trait. Millions of Worldwide suffer complications from sickle cell anemia [1]. SCA is a group of inherited red blood cell autosomal recessive hemoglobinopathies characterized by sickle hemoglobin in the red blood cell genotypes od this disease includes Heterozygous (Sickle-cell Trait) and Homozygous (Sickle Cell Disease
'SS disease') [2]. HbS results from single base-pair point mutation (GAG to GTG) leading to substitution of the amino acid; glutamic acid substituted to Valine in the 6th position near the N-terminal end of the β globin chain [3]. Sickle cell disease is a multi-system and multi-organ disorder characterized by acute and chronic complications which occur when fetal haemoglobin (HbF) drops to adult level by five to six months of life [4]. SCD is considered to be a hypercoagulable state with chronic activation of coagulation and an increased incidence risk of thrombosis [5],patients exhibit high plasma levels of thrombin generation, abnormal activation of the fibrinolytic system ,increased platelet activation, , depletion of natural anticoagulant proteins and increased tissue factor expression, even in steady state the non-crisis status [6,7].
Protein C (PC), a glycoprotein synthesized in the liver, circulates in the blood as an inactive zymogene. Activated protein C (APC) exerts its anticoagulant activity primarily through the inactivation of coagulation factors Va and VIIIa, which are required for factor X activation and thrombin generation. This may explain; in part, why PC deficiency appears to be associated primarily with venous thrombosis [8].
The deficiency of natural coagulation inhibitors including (PC) and increased thrombin generation in SCD has been postulated to contribute to the pathogenesis and clinical manifestations of SCA [9, 10]. Some researchers have reported reduced levels of natural coagulation inhibitors (including PC) and increased thrombin generation in SCD [11, 12, and 13].
The deficiency of natural coagulation inhibitors including protein C has been postulated to contribute to the pathogenesis and clinical manifestations of SCA. Thereafter, the aim of this study was to investigate the level of protein C in Sudanese children with SCA.

2. Materials and Methods:

2.1. Study design:

This was a case-control study.

2.2. Study area and study period:

This study was conducted in Jafar Ibn Auf Pediatric Hospital, Khartoum state in 2019.

2.3. Sample size:

A total of 100 children were enrolled for the study. Among them; 50 children were clinically diagnosed with SCA, and 50 apparently healthy children as a control group. Cases and controls were matched according to age and gender. Patients on oral anticoagulant therapy and those with renal disease were excluded.

2.4. Methods:

Venous blood samples (2.5 ml) were collected from all participants in Tri-sodium citrate tubes, and plasma was separated by centrifugation. PC level was estimated according to manufacture instructions using (AESKULISA) kits and sandwich ELISA (Human Reader, HS).

2.5. Data analysis:

Data were recorded in excel sheet and transferred to statistical package for social sciences (SPSS) version 20 (IBM Washington) for analysis. Descriptive statistics were used to summarize patients' characteristics. Quantitative data were expressed as mean ± SD. Comparisons were made using independent sample *t* test. Pearson's correlation was used to test the correlation. For All tests, *p*. values of < 0.05 were considered statistically significant.

2.6. Ethical considerations:

The study was approved by the faculty of medical laboratory sciences at Sudan International University. Informed consent was taken from each child guard before enrolment in the study.

3. Results:

In this study; 100 Sudanese children were included, 50 SCA patients as a case group, and 50 healthy children as a matching control group. One half of the participants were males and the other half were females. Their ages range between 5 and 13 years (Mean = 7.71±3.72 years).

In this study population, Mean PC level among cases was (79.71±11.97) which is significantly lower than controls (84.38±10.37) (*p*. value = 0.039).

Among the cases group, PC values were compared according to gender, history of blood transfusion, history of thrombotic event and history of anti-thrombotic therapy. Statistically significant differences were observed across the cases groups except for gender. (Table 1)

Regarding the association of PC level with age, a statically significant positive correlation was observed in this study population between PC level and age (*p*. value = 0.010, r = 0.359). (Figure1)

Table (1) Comparison of PC level across the cases groups:

Parameters	PC level (Mean ± SD)	*P-value*
Gender		
Male (n=28)	80.16±12.15	0.781
Female (n=22)	79.25±12.01	
History of blood transfusion		
Yes (n=45)	80.56±12.26	**0.006**
No (n=5)	72.01±4.30	

History of Thrombotic event		
Yes (n=5)	91.47±18.33	**0.019**
No (n=45)	78.40±10.56	
History of Anti-thrombotic therapy		
Yes (n=5)	91.47±18.33	**0.019**
No (n=45)	78.40±10.56	

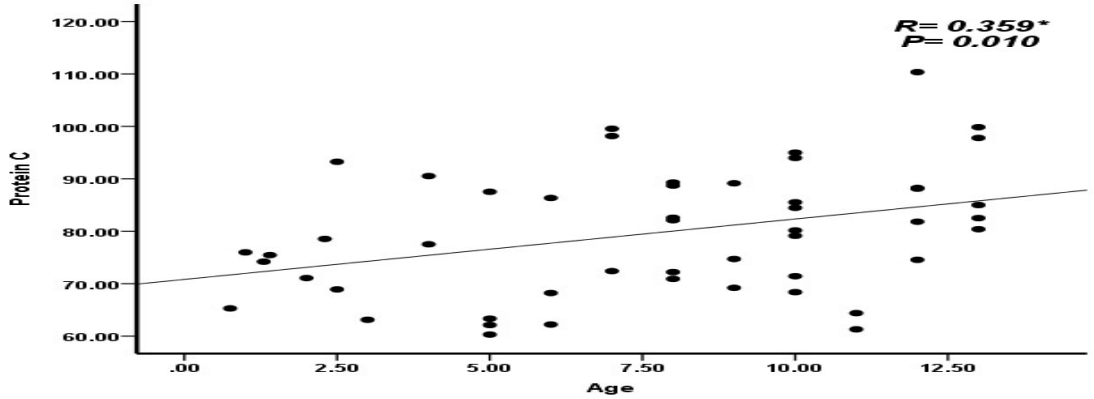

Figure (1) shows linear correlation between PC level and age of participant

4. Discussion:

This study was conducted to evaluate the effect of SCA on PC level among Sudanese children with SCA. The results showed a significant reduction of PC level among children with SCA compared with healthy children. This finding agrees with a Nigerian case-control study that observed a significant reduction of PC level in SCA children [14]. Also, our finding agrees with another two Nigerian cross-sectional studies, which also observed a significant reduction of PC level among SCA patients [15, 16]. Moreover, this finding agrees with another study that reported deficiencies of natural coagulation inhibitors including PC, PS, and anti-thrombin with a high incidence of hypercoagulability among patients of SCA [17]. Also our finding is in agree with Saudi study found that PC levels of SCA patients in steady state and AS were significantly low compared to controls ($p < 0.01$ and $p < 0.05$ respectively) [18] and with Schnog *et al*. who reported significant reduction in both protein S and protein C level among SCA patients [19].

In this study, SCA patients who received blood transfusions had significantly higher PC levels compared with those who did not receive blood transfusions. This denotes that, a regular blood transfusion may improve the general health status of SCA patients and may help to protect them from hypercoagulability by encouraging the synthesis of natural anticoagulants including PC. In the same manner, patients with a previous history of thrombotic events had significantly higher PC levels and this could be explained by the fact that all of them received anticoagulation therapy that may associate with an elevation of PC synthesis and/or activity rate. On the other hand, no significant effect of patient's gender on PC level was observed in this study. This finding agrees with the Nigerian study which showed no significant association between PC and gender [15]. Also this is in accordance with the physiological status, in which no difference exists between males and females and they have the same normal range. Finally, this study observed a significant positive correlation between PC level and patient's age. This finding is in agreement with the Nigerian study which showed that PC level significantly varies with the increasing age of SCA patients [15].

5. Conclusion

Patients with SCA had lower PC levels compared with healthy subjects. This reduction is corrected by increasing age, blood transfusion, and anticoagulant therapy. As lower PC levels may confer a risk of hypercoagulability, SCA children should have PC level tested in their early stages of the disease to minimize this risk.

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