IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 14, Issue 9 Ver. III (Sep. 2015), PP 87-91
www.iosrjournals.org

Evaluation Of Protein C Levels In Sickle Cell Disease Subjects Seen At The University Of Benin Teaching Hospital, Nigeria.

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Summary:
Background: Protein C (PC) deficiency has been reported in sickle cell disease (SCD) and postulated to contribute to the pathogenesis as well as clinical manifestations of SCD. However there is paucity of data on PC in Nigerian patients with SCD.
Objectives: *This study was aimed at evaluating the levels of Protein C (PC) antigen and activity in SCD subjects; the prevalent type of PC deficiency and association between PC deficiency and parameters such as sex, age, ABO and Rh D blood groups.*
Patients/methods: *A cross sectional study conducted at University of Benin Teaching Hospital, Nigeria. Twenty-nine consenting SCD subjects were consecutively recruited. Protein C antigen and activity levels were determined using immunological (ELISA) and functional assays (Protac method) respectively. Blood groups were determined using standard antisera.*
Results: *Fifteen (51.7%) were males, 19 (65.5%) were <18 years, 18 (62.1%) had blood group O and 26 (89.7%) were Rh D positive. The mean PC antigen and activity levels were 65.7±4.9% and 48.8±2.8% of normal respectively. Type I PC deficiency was found in 6 (20.69%) and type II in 10 (34.48%) of the participants. Protein C antigen was significantly higher in subjects above 18 years (p=0.001). There was no significant association between PC deficiency and parameters such as sex, age, ABO and Rh D blood groups.*
Conclusions: *Protein C levels were reduced in SCD subjects and functional PC deficiency was more prevalent. There was no significant association between PC deficiency and the assessed parameters (sex, age, ABO and Rh D blood groups).*
Keywords: *Protein C Antigen, Protein C Activity, Sickle cell disease, Blood group.*

I. Introduction

Sickle cell disease (SCD) is a group of haemoglobinopathies characterised by the inheritance of a sickle cell gene and a clinical evidence of related symptoms and signs. It includes haemoglobin (Hb) SS, SC, S-thalassemia syndromes among others. The prevalence of SCD in Benin City, Nigeria is 2.4%[1] which mirrors the national prevalence of 2%.[2]

The disease is characterized by a hypercoagulable state with an imbalance in the haemostatic system causing a bias towards activation of the coagulation system and inappropriate fibrin deposition with consequent thrombotic consequences.[3] Increased platelet activation, endothelial damage, vaso-occlusion by sickled cells, depletion of nitric oxide, increased expression of tissue factors and depletion of natural anticoagulant levels are among the mechanisms implicated in the pathogenesis of the disease.[4]

Protein C (PC) is a vitamin K-dependent coagulation inhibitor produced in the liver. Acting together with its cofactor, protein S (PS), activated PC inhibits activated factors V and VIII thus down-regulating thrombin generation. Deficiency of PC is associated with increased risk of thrombotic complications. Protein C deficiency may be genetic or acquired. It could be qualitative or quantitative defect. Genetic PC defects have been classified into type I and type II defects. Type I defect is due to reduced PC antigen and activity levels while type II is due to reduced activity despite a normal antigen level.[5]

Some researchers have reported reduced levels of natural coagulation inhibitors (including PC) and increased thrombin generation in SCD.[6–8] Deficiency of natural coagulation inhibitors including PC has been postulated to contribute to the pathogenesis and clinical manifestations of SCD.[9,10]

There is paucity of data on PC in SCD in our environment. This study therefore sought to determine protein C levels in SCD subjects seen at the University of Benin Teaching Hospital (UBTH), Benin City, Nigeria.

II. Objectives

The objectives of this study were to determine the level of PC antigen and activity in SCD subjects; the prevalent type of PC deficiency and lastly, any association between PC deficiency and parameters such as sex, age, ABO and Rh D blood groups in the study participants.

III. Patients and methods

This was a cross sectional study conducted at UBTH, a government-owned tertiary hospital in southern part of Nigeria. A total of 29 consenting SCD subjects were consecutively recruited from the paediatric and adult haematology Clinics; between the months of June and September, 2013.

Sample collection: Venous blood was collected from their antecubital veins for the determination of their ABO and Rh D blood groups, PC antigen and PC activity.

Samples for PC studies were collected into a 3.2% citrate bottle. A total of 4.5ml of blood was dispensed into a bottle containing 0.5ml of citrate. The sample was centrifuged at 2000g for 15 minutes. The citrated plasma was separated and stored at -70^0C until time of analysis.

Another 2ml of blood was collected into a sample bottle containing ethylene di-amine tetra-acetic acid (EDTA) anticoagulant. This was used to determine blood groups using standard antisera. The Hb phenotypes of the participants were already determined and the results obtained from their case notes.

Protein C Assays

PC Activity was measured using TECHNOCHROM protein C reagent kit (The Protac method) by Technoclone GmbH, Vienna, Austria. LOT: OK12B01. REF: 5341013. The assay was carried out in accordance with the manufacturer's directives.

PC Antigen was assessed using enzyme immunoassay test kit – TECHNOZYM protein C ELISA by Technoclone GmbH,Vienna, Austria. LOT: 12021122. REF: TC12021. The assay was equally carried out in accordance with the manufacturer's directives.

Interpretation of Assays: Using the percentage of normal, the results were interpreted as follows:[11]

Normal – 70% to 140%

Borderline – 55% to 70%

Deficient - < 55%

Combined deficiencies of PC antigen and activity were classified as PC deficiency - Type 1 while the deficiency of PC activity alone was taken as Type 2.

Inclusion Criteria: Sickle cell disease subjects in steady state.

Exclusion Criteria: Subjects on oral anticoagulant therapy, those with liver disease (confirmed by deranged liver function test) or proven viral hepatitis, pregnant subjects or those on oral contraceptive therapy and those with renal disease were excluded.

Ethical approval: The study was approved by the ethics committee of UBTH.

Statistical Analysis: The data were analyzed using the statistical package for social science (SPSS) version 16. The associations were tested using chi-square or Fishers Exact test as appropriate. P value < 0.05 was considered significant.

IV. Results

A total of 29 SCD subjects comprising 26 (89.7%) Hb SS and 3 (10.3%) Hb SC subjects were recruited. The mean age of the participants was 15.2 ± 2.4 years with a median of 10 years. There were 15 (51.7%) males and 14 (48.3%) females. Eighteen (62.1%) of the participants had blood group O, 5 (17.2%) blood group A and 6 (20.7%) blood group B. Twenty six (89.7%) of them were Rh D positive and 3 (10.3) Rh D negative.

The mean PC antigen and activity levels were 65.7±4.9% and 48.8±2.8% of normal references respectively. Type I PC deficiency was found in 6 (20.69%) and type II in 10 (34.48%) of the participants. Details of PC antigen and activity levels are shown in Table 1.

Protein C antigen was significantly higher in subjects above 18 years (P = 0.001). There was no significant association between PC deficiency and parameters such as sex, age, ABO and Rh D blood group system (Tables 2 and 3).

Table 1: Mean values and grading of Protein C antigen and activity levels.

Protein C	**Antigen(%)**	**Activity(%)**
Mean ± SD	65.73 ± 4.89	48.82 ± 2.82
Percentage of Normal	**Frequency (%)**	**Frequency (%)**
Deficient (<55%)	8 (27.59)	18 (62.07)
Borderline (55 – 70%)	8 (27.59)	10 (34.48)
Normal (>70%)	13 (44.82)	1 (3.44)
Type of PC abnormality	**Frequency (%)**	
Type I defect	6 (20.69)*	
Type II defect	10 (34.48)*	
Normal	13 (44.83)	

*Not all subjects with PC antigen deficiency simultaneously had PC activity deficiency and vice versa.

Table 2: Associations between protein C antigen and other variables – sex, age and blood groups.

	PCAntigen(%)	**P value**
Age (years)		
<18	54.71 ± 21.46	
≥18	86.66 ± 22.30	0.001
Sex		
Female	68.21 ± 31.28	
Males	63.41 ± 21.65	0.633
Blood Group		
ABO		
O	69.67 ± 29.12	
None O	59.28 ± 20.74	0.312
Rh D		
Pos	67.64 ± 26.09	
Neg	49.13± 27.55	0.257

Table 3: Associations between protein C activity and other variables – sex, age and blood groups.

Variables	**PC Activity(%)**	**P value**
Age (years)		
<18	48.08 ± 14.87	
≥18	50.24 ± 16.15	0.723
Sex		
Female	45.54 ± 11.55	
Males	51.89 ± 17.80	0.268
Blood Group		
ABO		
O	49.52 ± 15.29	
None O	47.68 ± 15.72	0.758
Rh D		
Pos	49.46 ± 27.55	
Neg	43.33± 22.13	0.519

V. Discussion

Reduced protein C antigen and activity levels were noted in the study participants and this is in agreement with previous reports of low PC in SCD. Westermann et al[6] in Chicago, USA in a case control study, reported a significantly reduced PC antigen and PC activity in SCD subjects. Bayazit and Kilinc[7] in a related study in Turkey, noted that SCD is associated with PC deficiency. Similarly Hagger et al[8] reported a functional deficiency of PC in SCD subjects. There are quite few studies on PC in Nigeria. Ibijola[12] in University College Hospital, Ibadan, Nigeria reported a significantly reduced PC level in SCD patients. They did not however determine the prevalent type of PC deficiency in the subjects. The suggested mechanisms behind PC deficiency in SCD include increased consumption due to the increased activation of coagulation proteins in SCD patients

and the decreased synthesis by the liver due to hepatic dysfunction.[6, 7, 13] The increased proinflammatory activity in SCD patients may contribute to the consumption of PC.[9] However there is also a possibility of an inherent PC abnormality due to genetic defect that can further accentuate the prothrombotic state of SCD. This is a subject for further investigation.

There was a higher frequency of PC deficiency-type II than type I in our study population. It was found that 20.7% of the SCD study participants had type I and 34.5% had type II deficiency state. This is significantly higher than values reported in the general (normal) population.[14] The higher frequency of type II deficiency in the study participants is also contrary to previously documented reports of higher incidence of type I deficiency.[5] This may be because PC deficiency in SCD results mainly from acquired mechanisms rather than a genetic defect in the synthesis of PC. It is noteworthy that the data by Reitsma[5] was apparently for cases of genetic defect and may not be completely applicable to our study populace.

Expectedly this study also found a significantly reduced PC antigen in subjects below 18 years of age. Protein C levels have been reported to vary with age. It is expected to rise with age till about the fifth decade of life.[10] The lack of a significant difference in functional activity between the adult and paediatric age groups is in agreement with Nsiri's observation.[15] This may be due to the wide range of plasma PC activity.[16]

We did not find any significant variation in PC antigen and activity with sex. This was contrary to the observation by other researchers[10,17] who observed lower levels of PC in women in a normal population. Though there were few Hb SC subjects in this study, we noted that there is no significant variation in PC levels with Hb phenotype.

Some haemostatic variables have been noted to vary with blood group phenotype. For instance, blood group O individuals have been noted to have relatively low Von Willebrand factor levels.[18] Dolan[17] also reported that PC levels in females varied with blood group phenotype. However there was no association between PC deficiency and ABO/Rh D blood group phenotype in our study participants.

VI. Conclusion

This study showed that protein C levels were reduced in the SCD subjects and functional PC deficiency was more prevalent in them. There was no significant association between PC deficiency and the assessed parameters (sex, age, ABO and Rh D blood group systems).

Recommendations: Further studies with large sample size will be required to confirm the findings from this study. There is need to access the role of genetic defect in PC levels in SCD subjects. There is also the need for a study to determine if there is any association between PC antigen/activity and the clinical manifestations of the disease in our environment.

Addendum

O. E. Iheanacho recruited the participants and analysed the samples. B. Nwogoh analysed the data. The study design, analyses and final approval of manuscript were done by both authors.

References

[1]. Nwogoh B, Adewoyin A, Iheanacho OE, Bazuaye GN. Prevalence of haemoglobin variants in Benin City, Nigeria. Annals of biomed Sci 2012; 11: 60 – 4.
[2]. Akinkugbe OO. Sickle cell disease. In: Non-communicable diseases in Nigeria. 1st Ed. Akinkugbe OO (Ed), Federal Ministry of Health, Lagos 1992; 45-52.
[3]. Ataga KI, Orringer EP. Hypercoagulability in sickle cell disease: a curious paradox. Am J Med 2003; 115: 721-8.
[4]. El-Hazmi MAF, Warsy AS, Bahakim H. Blood protein C and S in sickle cell disease. Acta Haematol 1993; 90: 114-9.
[5]. Reitsma PH, Bernardi F, Doig RG, Gandrille S, Greengard JS, Ireland H, Krawczak M, Lind B, Long GL, Poort SR, Saito H, Sala N, Witt I, Cooper DN. Protein C deficiency: A database of mutations, 1995 update. Thromb Haemost 1995; 73: 876-89.
[6]. Westerman MP, Green D, Culman-Sachs A, Gilman-Sachs A, Beaman K, Freels S, Boggio L, Allen S, Zuckerman L, Schlegel R, Williamson P. Antiphospholipid antibodies, protein C and S, and coagulation changes in sickle cell disease. J Lab Clin Med 1999; 134: 352-62.
[7]. Bayazit AK, Kilinic Y. Natural coagulation inhibitors (protein C, protein S, antithrombin) in patients with sickle cell anaemia in a steady state. Pediatr Int 2001; 43: 592-6.
[8]. Hagger D, Wolff S, Owen J, Samson D. Changes in coagulation and fibrinolysis in patients with sickle cell disease compared to healthy black controls. Blood Coagul Fibrinolysis 1995; 6: 93-9.
[9]. Shua F, Kobayashia H, Fukudomeb K, Tsuneyoshib N, Kimotob M, Teraoa T. Activated protein C suppresses tissue factor expression on U937 cells in the endothelial protein C receptor – dependent manner. FEBS Lett 2000; 477: 208-12.
[10]. Zhu T, Ding Q, Bai X, Wang X, Kakguelidou F, Alberti C, Wei X, Hua B, Yang R, Wang X, Wang Z, Ruan C, Schlegel N, Zhao Y. Normal ranges and genetic variants of antithrombin, protein C and protein S in the general Chinese population. Results of the Chinese Hemostasis Investigation on Natural Anticoagulants Study I Group. Haematol 2011; 96: 1033-40.
[11]. Aiach M, Borgel D, Gaussem P, Emmerich J, Alhenc-Gelas M, Gandrille S. Protein C and protein S deficiencies. Semin Hematol 1997; 34: 205-16.
[12]. Ibijola AA. Protein C level in Nigerian patients with sickle cell anaemia. Part II Dissertation for National Postgraduate Medical College of Nigeria, 2010.
[13]. Wright JG, Malia R, Cooper P, Thomas P, Preston FE, Serjeant GR. Protein C and protein S in homozygous sickle cell disease: does hepatic dysfunction contribute to low levels? Br J Haematol 1997; 98: 627-31.

[14]. Lane DA, Mannucci PM, Bauer KA, Bertina RM, Bochkov NP, Boulyjenkov V, Chandy M, Dahlback B, Ginter EK, Miletich JP, Rosendaal FR, Seligsohn U. Inherited thrombophilia I. Thromb Haemost 1996; 76: 651-62.
[15]. Nsiri B, Gritli N, Bayoudh F, Messaoud T, Fattoum S, Machghoul S. Abnormarlities of coagulation and fibrinolysis in homozygous sickle cell disease. Hematol Cell Ther 1996; 38: 279-84.
[16]. Bertina RM. Protein C deficiency and venous thrombosis: the search for the second genetic defect. Thromb Haemost 2000; 83: 360-1.
[17]. Dolan G, Neal K, Cooper P, Brown P, Preston FE. Protein C, antithrombin III and plasminogen: effect of age, sex and blood group. Br J Haematol 1994; 86: 798-803.
[18]. O'Donnel J, Laffin MA. The relationship between ABO histo-blood group, factor VIII and von Willebrand factor. Transfus Med 2001; 11:343-51.