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Evaluation of Haemostatic Profile of Homozygous Haemoglobin S Disease in Benin City, Nigeria: Are there any gender differences?Obazelu, P.A.*,¹ Emokpae, M.A.,¹ Danladi, S.B.,² Ajamajebi B.B.¹

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Abstract

In Sickle cell disease (SCD), nearly all components of haemostasis such as platelet function, procoagulant, anticoagulant and fibrinolytic systems are altered with a resultant hypercoagulable state. This study compares some haemostasis parameters in SCD patients in steady clinical state with control subjects and also investigates gender differences in prothrombin time (PT) and partial thromboplastin time (PTT) among SCD patients and control subjects. Plasma (PT) and activated PTT were assayed using reagents supplied by Trinity Biotech, Inc (USA). The measured parameters were compared using unpaired Students-t-test. The PT and aPTT were significantly higher ($p < 0.001$) in SCD patients than controls. The PT and aPTT were significantly higher ($p < 0.001$) in both male and female SCD patients than male and female control subjects. Whereas gender differences were observed in PT ($p < 0.001$) among control subjects, no gender difference was observed in PT and aPTT among SCD patients. Sickle cell disease patients in steady clinical state has prolonged PT and aPTT and no gender differences exist in PT among SCD patients.

Keywords: Haemostasis, activated Partial Thromboplastin Time, Prothrombin Time, Sickle Cell Disease

Introduction

Sickle cell disease (SCD) is an inherited disorder caused by the substitution of valine for glutamic acid at the sixth position in β -chain of the haemoglobin molecule (Nnodim, 2013; Switzer *et al.*, 2006). In individuals with sickle cell disease high level of sickle cell predominate (Nnodim *et al.*, 2013; Mmeremiukwu, 2005; Uwakwe *et al.*, 2002). Sickle cell disease is a significant cause of morbidity and mortality among individuals of African descendants (Nnodim *et al.*, 2014; Ibe *et al.*, 2009). The

prevalence of SCD is very high in sub-Saharan Africa, Middle East and in certain parts of India (Behera *et al.*, 2012).

Nigeria, the most populous country in Africa remains the leading country with high prevalence of the disease. Life expectancy is shortened, with studies reporting an average life expectancy of 42 and 48 years for male and female respectively (Nnodim *et al.*, 2013; Dunlop and Banneth, 2006). Although sickle cell disease is present from birth, clinical symptoms are rare before the age of 3 to 6 months for the facts that large proportion of foetal haemoglobin (HbF) predominates. As more sickle cell haemoglobin replaces HbF in the subject, clinical symptoms such as: anaemia, joints pain, infections and associated crisis begins to manifest due to irreversible sickling of the erythrocytes. HbS molecule polymerizes invariably leading to vaso-occlusion in the small capillaries (Bhagavan and Chung-Eun, 2015; Behera *et al.*, 2012). The sickle haemoglobin is known to interact with diverse genes and environmental factors, producing multi-systemic diseases with several phenotypes (HbSC, HbSD, HbSthal etc.) (Nwaoguikpe, 2010).

Reduced platelet count, decreased levels of anticoagulant, generation of activated thrombin and procoagulant microparticles and other clotting parameters may be affected in sickle cell disease. This strongly suggests a state of deregulation of the coagulation system or clotting profile in individual with sickle cell disease both in steady state and painful crisis, with increase rates of thrombin and fibrin formation constantly higher than normal (Noubouossie *et al.*, 2012). Information regarding the commonly haemostatic profile such as prothrombin time (PT) and aPTT of SCD patients is inconclusive; and reports on the gender differences of haemostatic parameters in SCD patients is limited. This study compares PT and aPTT in SCD patients on steady clinical state with controls and also to

investigate gender differences in haemostatic parameters of SCD patients and control subjects.

Materials and methods

Subjects

This is a prospective case-control study of 150 (83 males and 67 females, age range 10-25 years) subjects with homozygous haemoglobin S (HbSS) in steady state and 150 (81 males and 69 females, age range 10-24 years) with normal haemoglobin (HbAA) as controls. The SCD patients were recruited from Sick cell Disease Centre in Benin City, Edo state and the control subjects were apparently healthy staff and students of the University of Benin.

Sickle cells disease and control samples were obtained after verbal informed consent in accordance with institutional guidelines set by the Edo state Ministry of Health, Benin City, Nigeria as per declaration of Helsinki ethical principles for medical research involving human subjects.

Sample collection and analysis

Standard blood collection method was used to obtain 5mL of venous blood from the patients and control subjects. Three millilitres of blood was delivered into 3.8% sodium citrated and 2mL was emptied into disodium salt of Ethylene Diamine Tetra Acetic Acid anticoagulant containers. Each sample was labeled with a unique identification code. Samples were collected between the hours of 08:00 to 12:00 noon each day and the blood in plasma separated and store

at -20°C prior to laboratory analysis.

The blood samples were subjected to routine coagulation profile analysis (Prothrombin Time, activated Partial Thromboplastin Time) and platelet count using standard laboratory methods under strict internal and external quality control schemes.

Statistical analysis

The statistical analysis was done using SPSS version 20 for Windows (IBM statistical software). The results were expressed as Mean ± SD. Statistical significance was set at $p \leq 0.05$.

Results

Table 1 shows the haemostatic parameters of sickle cell anaemia patients and controls. The PT and aPTT were significantly higher ($p < 0.001$) in sickle cell anemia patients than controls. Table 2 shows haemostatic parameters according to gender. The PT and aPTT were significantly higher ($p < 0.001$) among male and female SCD subjects compared to male and female controls. Table 3 indicates the gender differences in haemostatic parameters in SCD and control subjects. Whereas gender differences were observed in PT ($p < 0.001$) among controls, no gender difference was observed in PT and aPTT among SCD patients. Table 4 shows the haemostatic parameters in different age groups. There was a statistically significant increase ($p < 0.05$) in the PT and aPTT in age range 16-20 years compared to other age groups.

Table 1: Haemostatic Parameters of subject and Control Groups

Haemostatic Parameters	Sickle cell disease patients	Control subjects	p-values
Age (years)	15.3.2±3.2	15.9±2.1	>0.05
No of subjects	150	150	>0.05
PT (Seconds)	26.07 ± 5.52	17.72 ± 3.50	0.001
aPTT (Seconds)	46.26 ± 15.31	35.24 ± 8.30	0.001

PT=Prothrombin time; aPTT=activated partial thromboplastin time

Table 2: Haemostatic profile of subjects and controls based on gender

Parameters	Gender	Sickle cell disease patients	Control subjects	p-values
Prothrombin time (Seconds)	Males	25.8 ± 5.47 (n=83)	17.0 ± 3.80 (n=81)	0.001
	Females	26.2 ± 5.70 (n=67)	24.3 ± 12.30 (n=69)	0.01
Partial thromboplastin time (Seconds)	Males	45.9 ± 14.70 (n=83)	34.9 ± 8.10 (n=81)	0.001
	Females	47.0 ± 16.10 (n=67)	36.3 ± 9.40 (n=69)	0.001

Table 3: Gender differences in haemostatic parameters in SCD patients and controls.

Parameters	Subjects	Mean values in males	Mean values in females	p-value
Prothrombin time (Seconds)	Sickle cell disease patients	25.8 ± 5.47	26.2 ± 5.70	p>0.05
	Control subjects	17.0 ± 3.80	24.3 ± 12.30	0.001
aPartial thromboplastin time (Seconds)	Sickle cell disease patients	45.9 ± 14.70	47.0 ± 16.10	p>0.05
	Control subjects	34.9 ± 8.10	36.3 ± 9.40	p>0.05

Table 4: Haemostatic Profile according to the age

Test (s)	Age group (years)	Mean ± SD	p-Value
PT	10-15	25.59 ± 6.24	0.001
	16-20	26.40 ± 5.50	
	21-25	24.90 ± 3.90	
aPTT	10-15	45.19 ± 15.80	0.0001
	16-20	45.86 ± 16.36	
	21-25	44.94 ± 13.50	

Discussion

The main focus of our study was to compare the haemostatic status and gender differences among SCD patients and control subjects. In this study, we present abnormal coagulation profile in individuals with sickle cell anaemia compared to control individuals with normal haemoglobin. This is in agreement with previous studies elsewhere who reported either elevated PT and/or aPTT levels in Hb SS patients (Buseri *et al.*, 2007; Raffini *et al.*, 2006; Wright *et al.*, 1997). Similarly, Richardson *et al.* (1979) observed prolonged PT and APTT in subjects with SCD when compared to haemoglobin AA controls. The possible explanations for the significant increase in PT and APTT values observed in this study includes: hepatocytic dysfunction frequently observed in sickle cell disease, increased consumption of coagulation factors is another alternative plausible explanation for the prolonged coagulation results, vitamin K deficiency due to decreased dietary intake and intrahepatic cholestasis or chronic antibiotic use prescribed for sickle cell anaemia patients (Richardson *et al.*, 2008).

In this study, we observed a significant higher (p< 0.001) PT and APTT in both male and female

subjects with Hb SS compared to Hb AA controls individuals. Noteworthy, the aPTT was significantly high (p< 0.05) than the PT parameter thus, indicating possible defects in the intrinsic clotting factors (I, II, V, VII, IX, X and XII) as compared with the normal Hb AA controls. Gender differences was observed in PT among control group which was absent in SCD patients. We could not assign possible reason for the absence of gender difference in SCD patients.

We observed that PT and aPTT profile of Hb SS subjects in different age groups together with that of control Hb AA group was not consistent. The PT and aPTT were higher (p < 0.05) in the age range 16-20 years, which may indicate that the effects of SCD on haemostatic parameters may be more severe in this age group (16–20 years).

Conclusion

The data presented in this study indicates that individuals with sickle cell disease have prolonged Prothrombin Time (PT) and activated Partial Thromboplastin Time, compared with those of normal haemoglobin AA controls and there are no gender differences in PT among SCD patients.

Conflict of interest

No conflicting interest

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