Influence of Gender and Subjects' Condition on Haemostatic Parameters in Sickle Cell Anaemia and Control Subjects

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

Aim: The aim of this study was to assess the influence of gender and subjects' condition on haemostatic parameters in sickle cell anaemia and control subjects.
Study Design: This study is a cross-sectional observational study.
Place and Duration of Study: University of Port Harcourt Teaching Hospital, Rivers State, and the Federal Medical Centre, Yenagoa, Bayelsa State, between the months of February and August, 2020.
Methodology: A total of four hundred and fifty (450) subjects with age range of 1-50 years were randomly selected. Out of the four hundred and fifty (450) subjects, 269 (59.78%) were males and

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181 (40.22%) were females. The subjects were further grouped into three based on the subjects' condition, which include steady state (n=150), vaso-occlusive crisis (n=150); and the control (n=150). There were 200 registered patients (adults and children alike) at the sickle cell clinics of the University of Port Harcourt Teaching Hospital, and the Federal Medical Centre, Yenagoa, with an average of 4 new patients per month. The sample size was obtained using a prevalence of sickle cell anaemia of 2% and was calculated using Cochrane formula. Five milliliters (5ml) of venous blood sample was withdrawn. The haemostatic parameters (vWF, FVIII, D-dimer, L-arginine, fibrinogen, ADAMTS13) were assayed quantitatively with Bioassay Technozym kit using Microplate Reader (Labtech microplate auto ELISA plate reader, an IS0 13485:2003 CE and WHO compliance Co., Ltd. Shanghai International Holding Corp. GmbH; Europe) calibrated to a wavelength of 450 nm with strict adherence to the manufacturer's instructions, while PT and APTT were analyzed with Fortress reagent and Uniscope SM801A Laboratory using water bath. Data management and statistical analyses were conducted using Statistical Analyses System SAS 9.4 (SAS Institute, Cary, North Carolina, USA) and p values <0.05 were considered statistically significant.

Results: The results showed that There was significant variability in the haemostatic parameters by subjects' condition. The comparison of HbF and haemostatic parameters showed significant (p<0.05) increase and decrease in VOC and steady state respectively compared with the control group. Subject condition as an independent factor predicts significantly high 72.91% and 86.00% for ADAMTS13 and for vWF activations respectively compared to other factors.

Conclusion: Subjects with SCA, particularly during VOC, undergo significant haematologic alterations that increase their risk of developing coagulation activation-related complications. Thus, though selected markers of coagulation were significantly different between the subject conditions, they were often significantly higher in the SCA.

Keywords: Haemostatic parameters; sickle cell anaemia.

1. INTRODUCTION

Recently, studies have suggested that haemostatic factors relating to endothelial dysfunction were considered to be associated with SCA [1]. von Willebrand factor (vWF) is an important biomarker of endothelial dysfunction. It plays a crucial role in primary haemostasis and thrombus formation by mediating platelet adhesion and aggregation. Upon released from endothelial cells, ultra large vWF multimers which are the most thrombogenic are cleaved into the less active forms by a disintegrin and metalloproteinase with a thrombospondin type 1 motif member 13 (ADAMTS13) [2]. Reduction in ADAMTS13 activity and elevated vWF antigen are associated with vascular complications [2] and other thrombotic diseases development such as ischemic stroke, [2] acute myocardial infarction, [3] and coronary artery disease [4].

Sickle cell anaemia (SCA) is one of the most common severe monogenic disorders worldwide [5]. It is the most frequent variant (homozygous SS disease) and is caused by a single amino acid substitution at the sixth residue of the β-globin subunit gene on chromosome 11p15.5 [6], (β^6-Glu →Val) which results in the production of the characteristic sickle haemoglobin [7]. In order words, the homozygous state is referred to as sickle cell anaemia (SS), where the red blood cells lacking normal adult haemoglobin are replaced by sickle cell haemoglobin [8,9]. This genotype expresses severe haemolytic anaemia among other manifestations [8]. The sickle cell trait is a heterozygous state (AS), where the red blood cells contain both normal adult haemoglobin (HbA) and sickle cell haemoglobin rarely have a phenotypic expression of clinical significance [8]. There is also the double heterozygous state in an allele carrying HbS, and the other allele carrying other abnormal haemoglobins such as HbC, HbE or alpha or beta chain quantitative variant (thalassaemic gene Hb products) [8,9].

Gender, other factors as well as environmental factors afford to interfere on the characteristics of SCA and also impact on the quality and life expectancy of patients, mainly reducing their social insertion [10,11]. Haemostatic parameters are measurable indices of the blood that serve as markers for sickle cell anaemia diagnosis [12] changes in these parameters constitute the common complications in sickle cell SCA and they play a major role in its pathological presentation [13]. Haemostatic disorder in sickle cell anaemia is a serious condition that is

associated with increase morbidity and mortality due to its hypercoagulable and prothroembolic states. However, this study was to rule out the fact that the activation of the coagulation system is a bystander phenomenon or a main determinant of clinical complications with inconsistency in the available studies. There is paucity of data on this in the local community. Therefore, this study sought to assess the influence of gender and subjects' condition on haemostatic parameters in sickle cell anaemia and control subjects.

2. MATERIALS AND METHODS

2.1 Study Design

This study is a cross-sectional study. Four hundred and fifty (450) subjects were grouped into three as follows: Group 1 (150 sickle cell anaemia subjects at steady that were clinically defined as free of infection, pain or any evidence of active disease at least 2 weeks prior to the next clinical visit and three months after blood transfusion) [14], Group 2 (150 sickle cell anaemia subjects with vaso-occlusive crises which were clinically defined of having pain in the bones, muscles, and joints not attributable to any other cause and requiring parenteral analgesic and hospitalization at the hospital for some hours, and those who still had pain after discharge, including those who did not need hospitalization, but were treated at an acute care facility) [14,9] and finally Group 3 (150 apparently healthy individuals with homozygous haemoglobin A genotype who served as the control subjects).

2.2 Study Area

The study was carried out in two Federal tertiary hospitals, the University of Port Harcourt Teaching Hospital (UPTH) and Federal Medical Centre (FMC) Yenagoa. The two Federal hospitals were formerly located in the same state (old Rivers State). They are referral centres for various health institutions in the respective states, Rivers and Bayelsa, Nigeria.

2.3 Study Population

A total of three hundred (300) subjects with age range of 1-50 years were randomly selected. There are about 200 registered patients (adults and children alike) at the sickle cell clinics of the University of Port Harcourt Teaching Hospital, and 120 registered patients (adults and children alike) at the sickle cell clinics of the Federal Medical Centre, Yenagoa, with an average of 4 new patients per month in both facilities. The clinics run on Thursdays and Fridays respectively with a weekly attendance of between five (5) and ten (10) patients. It was runned by two (2) Consultants, two (2) Senior Registrars, and three (3) Registrars.

2.4 Eligibility Criteria

2.4.1 Inclusion Criteria

Subjects with homozygous haemoglobin S (HbSS) aged between 1 year and above who had been apparently well with no recent drop in the haemoglobin level and there was absence of infection, pain, acute complicating factors or acute clinical symptoms or crisis for a minimum of two (2) weeks before recruitment as established by a careful history and complete physical examination [8,9]. Subjects with homozygous haemoglobin S (HbSS) aged one year and above who had Crises, also referred to the episodes of acute illness attributable to the sickling phenomenon in which there was a sudden exacerbation of symptoms and signs of subjects who had hitherto been in stable condition. The pain was in the form of vaso-occlusive crisis, aplastic crisis, acute sequestration crisis, or haemolytic crises [8,9].

2.4.2 Exclusion Criteria

Subjects who non-homozygous genotype (Hb SS) were excluded from the study. Also, subjects with any type of infective illness (HIV, tuberculosis, SARS-COV 19) or has had recent blood transfusion during the preceding two weeks were excluded from the study. Furthermore, subjects who had recent intake of any myelosuppressive agent (e.g., Hydroxyurea, Ibuprofen, acetaminophen, any narcotics, any other NSAIDS) for the preceding two weeks were excluded. Pregnancy was excluded.

2.5 Sample Size

Purposive sampling and randomized method were used in the selection of subjects, with due consideration of the total number of patients admitted in the clinic/ward in the University of Port Harcourt Teaching Hospital, and the Federal Medical Centre, Yenagoa. The sample size was obtained using a prevalence of sickle cell anaemia of 2% [15] and the sample size was calculated using Cochran sample size formula [16].

$$N = \frac{Z^2 pq}{d^2}$$

Where N =the desired sample size
Z = the Standard Normal deviate usually set at 1.96 corresponding to the 95% Confidence level
p = the prevalence of target population
q = 1- p
d = degree of accuracy desired set at 0.05

Therefore N = $\frac{(1.96)^2 \times 0.02 \times (1-0.02)}{(0.05)^2}$

p = % or 0.02
N = 30

By adding 10% of non-respondent, the sample size will be 33.

2.6 Sample Collection and Handling

The subjects were made to be comfortable and at ease during the procedure, resting for 15 - 30 minutes prior to phlebotomy. Five milliliters (5mL) of venous blood sample was withdrawn from the peripheral vein in the upper limb of subjects using a standard venipuncture technique with minimum stasis under aseptic conditions from the dorsum of the hand or ante-cubital vein as the case may be as described by [17]. This was done by fastening a soft tourniquet to help locate and define peripheral veins to achieve successful and safe venipuncture, not more than 2 minutes to enable the index finger feel a suitable vein. The punctured site was then cleaned with 70% alcohol (methylated spirit) before collection of samples with 21G dry sterile hypodermic needle with a 5mL syringe, thereafter, 2mL of the whole blood was transferred into EDTA bottle.

The sample was rocked gently to mix and kept at room temperature and 3ml was used for the assay of the haemostatic parameters which was dispensed into sodium citrate anticoagulant container. The separated plasma was stored at -20°C prior to assay. Assay was carried out on the plasma sample thawed only once. The sample bottles were then assigned a study code with a non-water-soluble ink with date, sex and time of collection and logged on to a paper log after dispensing the blood sample into the sample bottles [18-21].

2.7 Laboratory Methods of Analysis

2.7.1 ADAMTS13 Activity Assay

ADAMTS13 activity was assayed quantitatively with Bioassay Technozym kit using Microplate

Reader (Labtech microplate auto ELISA plate reader, an IS0 13485:2003 CE and WHO compliance Co., Ltd. Shanghai International Holding Corp. GmbH; Europe) calibrated to a wavelength of 450 nm with strict adherence to the manufacturer's instructions. All samples and reagents were brought to room temperature before use. Thereafter, 120 µL of the standards (80 ng/mL) was reconstituted with 120 µl of standard diluents to generate a 40ng/mL standard stock solution. It was allowed to stand for 15 minutes with gentle agitation prior to making serial dilutions of the standard stock solution (40 ng/mL) 1:2 with standard diluents to produce 20 ng/mL, 10 ng/mL, 5 ng/mL and 2.5 ng/mL solutions. Standard diluent served as the zero standard, (0 ng/mL). 50 µL of standard was added to standard well only without adding antibody to standard well because the standard solution contains biotinylated antibody. 40µl of sample was added to sample the wells followed with the addition of 10 µL of anti-ADAMTS 13/vWF-cp antibody to the sample wells, followed by 50 µL streptavidin-HRP also to sample wells and standard well with the exception of blank control well.

The content of the microwell plate wells was mixed by gentle agitation and then covered with microplate sealant, and incubated for 60 minutes at 37°C. The sealant thereafter was removed, the plate washed for 5 times with wash buffer. Paper towel was used to blot the plate. 50µL of substrate solution A was added to each well followed by the addition of 50 µL of substrate solution B to each well. Thereafter, the plate was incubated at 37°C for 10 minutes in the dark with a new sealant. At the expiration of 10 minutes, 50 µL stop solution was added to the wells that changed the blue color immediately to yellow. The optical density of each well of the microplates was determined using a microplate reader set at 450 nm within 10 minutes after adding the stop solution. The standard curve range was set at 0.2 ng/mL to 60 ng/mL.

2.7.2 von Willibrand Factor Activity Assay

von Willibrand Factor (vWF) Activity was assayed quantitatively with Bioassay Technozym kit using Microplate Reader (Labtech microplate auto ELISA plate reader, an IS0 13485:2003 CE and WHO compliance Co., Ltd. Shanghai International Holding Corp. GmbH; Europe) calibrated to a wavelength of 450 nm with strict adherence to the manufacturer's instructions. All reagents, standard solutions and samples were

prepared as instructed. All reagents were normalized to room temperature before use and the test was performed at room temperature. 50 µL of standard was added to the standard well without adding any antibody to the standard well as the standard solution already contains biotinylated antibody. Thereafter, 40 µL of sample was added to the sample wells and then 10 µl anti-vWF antibody added to the sample wells, before 50 µL of streptavidin-HRP was later added to the sample wells and standard wells [without adding to the blank control well]. The mixtures were thoroughly mixed, and the plate covered with a sealer. The plate was then incubated for 60 minutes at 37 °C. After that, the sealer was removed and the plate was washed five times with the wash buffer [Soaking the wells with 0.35 mL wash buffer for 30 seconds to 1 minute for each wash]. After washing, the plate was blotted with absorbent tissue paper. 50 µL substrate solution A was then added to each well and then 50 µL substrate solution B was also added to each well. The plate was covered with a new sealer and then incubated for 10 minutes at 37 °C in the dark. After the second incubation, 50 µL of the stop solution was added to each well, and the blue colour changed into yellow immediately. The optical density [OD value] of each well was finally determined immediately by the use of a microplate reader that was set at 450 nm within 10 minutes after the stop solution was added.

2.7.3 FVIII Activity Assay

Factor VIII (FVIII) activity was assayed quantitatively with Bioassay Technozym kit using Microplate Reader (Labtech microplate auto ELISA plate reader, an IS0 13485:2003 CE and WHO compliance Co., Ltd. Shanghai International Holding Corp. GmbH; Europe) calibrated to a wavelength of 450 nm with strict adherence to the manufacturer's instructions. All reagents, standard solutions and samples were prepared as instructed. All reagents were brought down to room temperature before use and the test was performed at room temperature. 50µL of standard was added to the standard well without adding any antibody to the standard well as the standard solution already contains biotinylated antibody. Thereafter, 40µl of sample was added to the sample wells and then 10 µL anti-FVIII antibody added to the sample wells, before 50 µL of streptavidin-HRP was later added to the sample wells and standard wells [without adding to the blank control well]. The mixtures were thoroughly mixed, and the plate

covered with a sealer. The plate was then incubated for 60 minutes at 37 °C. After that, the sealer was removed and the plate was washed five times with the wash buffer [Soaking the wells with 0.35 mL wash buffer for 30 seconds to 1 minute for each wash]. After washing, the plate was blotted with absorbent tissue paper. 50µl substrate solution A was then added to each well and then 50µl substrate solution B was also added to each well. The plate was covered with a new sealer and then incubated for 10 minutes at 37 °C in the dark. After the second incubation, 50µl of the stop solution was added to each well, and the blue color changed into yellow immediately. The optical density (OD value) of each well was finally determined immediately by the use of a microplate reader that was set at 450 nm within 10 minutes after the stop solution was added.

2.7.4 Fibrinogen Activity Assay

Fibrinogen activity was assayed quantitatively with Bioassay Technozym kit using Microplate Reader (Labtech microplate auto ELISA plate reader, an IS0 13485:2003 CE and WHO compliance Co., Ltd. Shanghai International Holding Corp. GmbH; Europe) calibrated to a wavelength of 450 nm with strict adherence to the manufacturer's instructions. All reagents, standard solutions and samples were prepared as instructed. All reagents were brought down to room temperature before use and the test was performed at room temperature. 50 µL of standard was added to the standard well without adding any antibody to the standard well as the standard solution already contains biotinylated antibody. Thereafter, 40 µL of sample was added to the sample wells and then 10 µL anti-fibrinogen antibody added to the sample wells, before 50 µL of streptavidin-HRP was later added to the sample wells and standard wells (without adding to the blank control well). The mixtures were thoroughly mixed, and the plate covered with a sealer. The plate was then incubated for 60 minutes at 37 °C. After that, the sealer was removed and the plate was washed five times with the wash buffer (Soaking the wells with 0.35 mL wash buffer for 30 seconds to 1 minute for each wash). After washing, the plate was blotted with absorbent tissue paper. 50µL substrate solution A was then added to each well and then 50 µl substrate solution B was also added to each well. The plate was covered with a new sealer and then incubated for 10 minutes at 37 °C in the dark. After the second incubation, 50µl of the stop solution was added to each well,

and the blue color changed into yellow immediately. The optical density (OD value) of each well was finally determined immediately by the use of a microplate reader that was set at 450 nm within 10 minutes after the stop solution was added.

2.7.5 D-dimer Activity Assay

The D-dimer levels was assayed quantitatively with Bioassay Technozym kit using Microplate Reader (Labtech microplate auto ELISA plate reader, an IS0 13485:2003 CE and WHO compliance Co., Ltd. Shanghai International Holding Corp. GmbH; Europe) calibrated to a wavelength of 450 nm with strict adherence to the manufacturer's instructions. All reagents, standard solutions and samples were prepared as instructed. All reagents were brought down to room temperature before use and the test was performed at room temperature. 50 µL of standard was added to the standard well without adding any antibody to the standard well as the standard solution already contains biotinylated antibody. Thereafter, 40 µL of sample was added to the sample wells and then 10 µL anti-D-dimer antibody added to the sample wells, before 50µL of streptavidin-HRP was later added to the sample wells and standard wells (without adding to the blank control well). The mixtures were thoroughly mixed, and the plate covered with a sealer. The plate was then incubated for 60 minutes at 37 °C. After that, the sealer was removed and the plate was washed five times with the wash buffer (Soaking the wells with 0.35mL wash buffer for 30 seconds to 1 minute for each wash). After washing, the plate was blotted with absorbent tissue paper. 50 µL substrate solution A was then added to each well and then 50µL substrate solution B was also added to each well. The plate was covered with a new sealer and then incubated for 10 minutes at 37 °C in the dark. After the second incubation, 50µL of the stop solution was added to each well, and the blue color changed into yellow immediately. The optical density (OD value) of each well was finally determined immediately by the use of a microplate reader that was set at 450 nm within 10 minutes after the stop solution was added.

2.7.6 L-Arginine

L-Arginine activity was assayed quantitatively with Bioassay Technozym kit using Microplate Reader (Labtech microplate auto ELISA plate reader, an IS0 13485:2003 CE and WHO

compliance Co., Ltd. Shanghai International Holding Corp. GmbH; Europe) calibrated to a wavelength of 450 nm with strict adherence to the manufacturer's instructions. All samples and reagents were brought to room temperature before use. Then the 25x wash buffer was diluted inti 1x working concentration with double steaming water. Biotinylated-Conjugate (1x) was diluted by 100-fold dilution by adding 10 µL of Biotinylated-Conjugate with 990 µl of Biotinylated-Conjugate diluents. Standard was reconstituted with 1.0 mL of standard diluents and kept for 10 minutes at room temperature and mixed gently to avoid foaming. Seven tubes containing 0.5 mL standard diluents were then prepared and used to produce a double dilution series. Each tube was mixed thoroughly before transfer such as 100 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, 3.13 µg/mL, 1.57 µg/ml, and the last tube with standard diluents is the blank as 0µg/ml. streptavidin-HRP (1x) was diluted by 100-fold dilution. Then a blank well was set without any solution. Thereafter, 50 µl of the standards or sample was added to sample wells and then 50 µL of Biotinylated-Conjugate (1x) was added to each well. It was then mixed properly by gentle agitation, covered with the adhesive films and incubated for 1 hour at 37⁰C. The sealant was then removed and washed the plate 3 times with wash buffer by allowing the well to soak for at least (250 µL) for 30 seconds for each wash. The plate was then blot onto a paper towel. 100 µL of Streptavidin-HRP (1x) to each well and covered with the adhesive film and incubate for 1 hour at 37⁰C. The sealant was then removed and washed the plate 3 times with wash buffer by allowing the well to soak for at least (250µL) for 30 seconds for each wash and the plate was then blot onto a paper towel. Substrate (90µL) was then added to each well and incubated for 20 minutes at 37⁰C in the dark with a new sealant. Thereafter 50µL stop solution was added to the well that turned blue color immediately. The optical density of each well of the microplates was then determined using a microplate reader set at 450nm within 5 minutes after adding the stop solution. The standard curve range was set at 1.57-100µl/mL.

2.8 Statistical Analysis

Data management and statistical analyses were conducted using Statistical Analyses System SAS 9.4 (SAS Institute, Cary, North Carolina, USA). The mean/standard deviation and Analysis of Variance (ANOVA) with interaction effects where significant differences existed in the

subject states or interaction of the states with any of the independent factors were determined. Post Hoc Tests was conducted using the Tukey's Honestly Significant Difference (HSD) procedure to compare all possible pairs of the parameters means. Pearson Correlation analysis was used to determine the direction of relationships within and between the haemostatic parameters by the sickle cell anaemia subjects' state.

3. RESULTS AND DISCUSSION

A total of four hundred and fifty (450) subjects consisting of 269 (59.78%) males and 181 (40.22%) females were recruited for the study, and were statistically not significant (p>0.05) across the gender study groups. The subjects were also grouped into three based on the subjects' condition, which include steady state (n=150), comprising 20.22% (n=91) males and 13.11% (n=59) females (Table 1)

The interactive measures between gender and subject condition showed that there was no statistically significant difference (p>0.05) in the haemostatic parameters (Haemoglobin F; D-dimer; A Disintegrin and Metalloproteinase with a Thrombospondin Type 1 motif, member 13; Factor VIII Inhibitor; von Willebrand Factor; Arginine; Prothrombin Time; International Normalized Ratio; Activated Partial Thromboplastin Time) examined (Table 5).

Sickle Cell Anaemia (SCA) is associated with a hypercoagulable state resulting in a predisposition to venous thromboembolism [22, 23]. Despite being a monogenic disorder [24] SCA presents with extreme complication variability. Haemolytic anaemia, vaso-occlusion and vasculopathy are the hallmarks of SCA protean complications. This study is consistent with the research carried out by Valentine [25] and Pawlinski [26] and has now made it clear that multiple actors including ADAMTS13, leukocytes, platelets, L-arginine which is the building block of nitric oxide (NO) availability, and haemostatic activation play a role in the disease expression. According to global estimates, approximately 5% of the population has some type of haemoglobin variant, and more than 300,000 babies are born each year with haemoglobinopathies, with the homozygous sickle cell disease (HbSS) being the most prevalent type [27-29].

Table 1. Demographic Characteristics of Study Subjects

			Female	*Male*
N			181	269
(%)			(40.22)	(59.78)
	Steady State	**N**	59	91
	(n=150)	**%**	13.11	20.22
	(VOC) (n=150)	**N**	59	91
Subject State		**%**	13.11	20.22
	Control	**N**	63	87
	(n=150)	**%**	14.00	19.33
Test Statistics		**X^2 value**	0.296	
		p-value	>0.05[ns]	

Within characteristic, percentages may not add up to 100 due to rounding. Significance Levels: *=significant (p<0.05), ns=not significant (p>0.05)

Table 2. Influence of Gender and Subjects' State on D-Dimer and Adamts13 Levels in Sickle Cell Anaemia and Control Subjects

Interactive Measures			D-Dimer (mg/mL)	ADAMTS13 (ng/mL)
Gender	Subject State	N	Mean ± SD	Mean ± SD
	Steady State	59	113.36±74.744	3.95±2.007
Female	VOC	59	1333.17±74.744	0.83±2.007
	Control	63	46.68±72.333	19.99±1.942
	Steady State	91	96.23±60.184	4.93±1.616
Male	VOC	91	1517.46±60.518	0.82±1.616
	Control	87	48.95±61.909	19.66±1.653
Test Statistics: *F-Ratio, Prob>F*			1.3393; 0.2631[ns]	0.0706; 0.9319[ns]

Significance Levels: *=significant (p<0.05), ns=not significant (p>0.05)

Table 3. Influence of gender and subjects' state on fviii, vwf and fibrinogen levels in sickle cell anaemia and control subjects

Interactive Measures	N	FVIII (ng/mL)	vWF (ng/mL)	Fibrinogen (mg/mL)
Subject State		Mean ± SD	Mean ± SD	Mean ± SD
Steady State	59	3.97±3.576	40.00±9.905	2.06±0.627
VOC	59	53.49±3.576	165.72±9.905	10.26±0.627
Control	63	0.95±3.460	0.85±9.585	0.65±0.607
Steady State	91	7.04±2.879	40.92±7.975	2.00±0.505
VOC	91	54.78±2.879	159.42±7.975	9.62±0.505
Control	87	0.92±2.945	0.86±8.156	0.69±0.516
Test Statistics: *F-Ratio, Prob>F*		0.1153; 0.8911[ns]	0.0960; 0.9085[ns]	0.2095; 0.8110[ns]

*Significance Levels: *=significant (p<0.05), ns=not significant (p>0.05)*

Table 4. Influence of gender and subjects' state on arginine and pt levels in sickle cell anaemia and control subjects

Interactive Measures			Arginine (µg/mL)	PT (sec)
Gender	Subject State	N	Mean ± SD	Mean ± SD
	Steady State	59	18.38±1.574	20.88±0.643
Female	VOC	59	2.43±1.574	30.25±0.643
	Control	63	67.65±1.524	12.56±0.622
	Steady State	91	21.49±1.268	20.11±0.518
Male	VOC	91	3.04±1.268	30.11±0.518
	Control	87	67.95±1.297	12.68±0.529
Test Statistics: *F-Ratio, Prob>F*			0.5842; 0.5580[ns]	0.3116; 0.7325[ns]

*Significance Levels: *=significant (p<0.05), ns=not significant (p>0.05)*

Table 5. Influence of gender and subjects' state on inr and aptt levels in sickle cell anaemia and control subjects

Interactive Measures			INR	APTT (sec)
Gender	Subject State	N	Mean ± SD	Mean ± SD
	Steady State	59	1.89±0.059	35.24±0.778
Female	VOC	59	2.75±0.059	43.56±0.778
	Control	63	1.12±0.057	24.30±0.753
	Steady State	91	1.82±0.047	35.77±0.626
Male	VOC	91	2.74±0.047	43.74±0.626
	Control	87	1.11±0.049	25.72±0.641
Test Statistics: *F-Ratio, Prob>F*			0.2192; 0.8033[ns]	0.4181; 0.6586[ns]

*Significance Levels: *=significant (p<0.05), ns=not significant (p>0.05)*

The trend of statistically non-significance in the independent parameters gender was observed in this study. This finding strongly supports that of a previous study by Sanjay [30] that the incidence of SCA is not strictly gender-related, as it is transmitted as an autosomal recessive disorder. In particular, the gender-related differences in paediatric SCA are not well-characterized. There was also no significant influence of gender on PT, APTT, Haemoglobin F, D-dimer, FVIII, vWF.

4. CONCLUSION

Subjects with SCA, particularly during VOC, undergo significant haematologic alterations that increase their risk of developing coagulation activation-related complications. Thus, though selected markers of coagulation were significantly different between the subject conditions, they were often significantly higher in the SCA.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Appropriate Ethics Committee and have therefore been performed in accordance with the Ethical Standards Laid Down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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