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COAGULATION PROFILES OF ADULT SICKLE CELL PATIENTS IN STEADY STATE

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ABSTRACT

Background: Sickle cell disease is considered to be a prothrombotic state and this has a lot of influence on the associated complications of this disease condition if not put into consideration during the course of treatment. Therefore, the knowledge of the coagulation profiles in these patients will improve the treatment outcome especially during the periodic crises and perioperative period. **Objective**: To evaluate coagulation profiles of the adult sickle cell patients in steady state. **Methods:** This is a comparative cross sectional study. Forty HbS patients in steady state attending Haematology clinic and 40 age and sex matched healthy HbAA controls were recruited. Liver function (LFT) was done with Hitachi 912. **Results:** Both males and females HbS patients had relatively higher median APTT than their male and female counterparts in the control subjects. (P=0.025). Male HbS patients had higher median (25-75 percentiles) Prothrombin Time than their counterparts in the control group. The female HbS patients had similar median Prothrombin Time with the control subjects. The median test shows no statistically significant difference in the prothrombin time between the HbS patients and the control HbA patients (P=1.000). **Conclusion**: Coagulation profile in sickle cell patients in steady state is higher compared with the HbA control subjects but this is not statistically significant. The prolonged coagulation profile may be as a result of increased consumption of coagulation factors following activation of coagulation and fibrinolytic systems in sickle cell patients even at steady state and not necessarily due to hepatic dysfunction.

KEYWORDS: Sickle cell anaemia; coagulation profile; steady state; adult patient; liver function.

INTRODUCTION

Sickle cell haemoglobin was the first hemoglobin variant discovered. It was first described in the medical literature at the beginning of the twentieth century. Over 500 structural variants of haemoglobin have since been discovered. The earliest known medical paper with the original name of sickle cell disease was published in 1874 by Africanus Horton.[1] Herrick's was the first to describe the elongated crescent shaped cells in 1910 and this led to a variety of in vitro experiments which attempted to explain this phenomenon.[2] Hydrophobic Valine is substituted for the normal more hydrophilic Glutamic acid at the sixth residue from the NH_3 terminal of the Beta-globin chain in HbS. This substitution is due to a nucleotide mutation (GAG/GTG) in the sixth codon of the beta globin gene of the DNA in the long arm of chromosome 16.

Sickle cell is a germ line mutation and hence it is passed down intact over generations.[3] The mutation can be found in five different haplo types (Senegal, Benin, Bantu, Arab India and Cameroon) leading to the conclusion that the mutation appeared independently five times in five different founders in human history.[4] However, their highest frequency occurs in tropical areas but the population migrations have ensured that they are encountered in most different countries.[4] It apparently arose repeatedly in region where there is malaria endemicity particularly in Africa and Middle East.

It is interesting to note that a single copy of sickle cell gene helps the carrier to survive malaria infection.[5] The sickled configuration of red blood cells occurs when haemoglobin is deoxygenated. The loss of potassium and water associated with the sickling phenomenon provides inhospitable environment for the plasmodium falciparum parasite.[5]

The vicious cycle of the erythrocyte membrane damage of sickle cell disease is well documented.[5] The sickling phenomenon results from the formation of deoxyhaemoglobin S and the transition from a sol to a gel is accompanied by a dramatic increase in blood viscosity. The consequent increase in haemoglobin concentration accelerates and potentiates the rate of deoxygenation of the erythrocytes at which further

polymerization can occur. This marks the beginning of the numerous structural and functional abnormalities of sickle red blood cells.[6,7]

The clinical manifestations of sickle cell disease vary enormously ranging from asymptomatic subjects to patients disabled by recurrent pain and chronic complications. Virtually every organ system in the body is subject to vaso-occlusion. This accounts for the characteristic acute and chronic multisystem failure in the sickle cell disease patient. A case of neuro-ophthalmological sequelae of sickle cell disease reported by Adeuja *et al* revealed multiple cerebral, pontine and cerebellar infarcts at autopsy of a 16yr old sickle cell anaemia patient who presented with impairement of vision.[9]

The whole blood viscosity is a function of both the number of erythrocytes, their deformability and of the levels of plasma protein.[5]The plasma proteins have a mediating effect with RBC-RBC adhesive interaction at lower sheer rates. This is mediated by large plasma proteins e.g. fibrinogen.[7,8]

A wide number of interrelated factors influence the micro and macro rheology of the sickle blood.[10] The plasma components, the unsickling-sickling red cell cycles, the cellular dehydration, the erythrocyte deformability and mechanical fragility, the white cell populations, the environment and the alterations of the Virchow triad on the haemostatic system are responsible for the adhesive interaction of sickle RBC with the vascular endothelium.[1,8]

Sickle cell disease is considered to be a hypercoagulable state that is characterized by vaso-occlusive crises ranging from bone pain, acute chest syndrome and stroke.[11] This vasooclusion is known to be one of the major factors that contribute to end organ damage in sickle cell disease.[12,13] All components of haemostasis, platelets, procoagulants, anticoagulants and fibrinolytic systems have been reported to be perturbed in sickle cell patients.[14,13]

This study therefore is designed to evaluate coagulation profiles in adult Nigerian patients with sickle cell anaemia in steady state compared with normal HbA individuals so as to increase the knowledge base of this condition and better management of the disease. Sickle cell anaemia patient is said to be in steady state when he or she is asymptomatic or free of any acute illness for at least two weeks.

MATERIALS AND METHODS

Study design

This was a comparative cross sectional study. Sickle cell anaemia patients who met the inclusion criteria were included in the study.

Patient population

The test population involved forty (40) adult HbS patients in steady state and the control population involved forty (40) normal individuals with Hb phenotype A, age and sex matched.

Inclusion criterion

Adult sickle cell anaemia patients in steady state (asymptomatic for at least two weeks) who presented at the Haematology day care clinic.

Exclusion criteria

1. Patients with other haemoglobinopathies apart from HbS and 2. Female patients on oral contraceptives or pregnant.

Sample collection

9.5mls of venous blood collected from each subject and distributed as follows: 4.5ml of blood into 0.5ml of Trisodium Citrate at ratio of 9:1 for the determination of PT, APTT and 5ml of blood into Lithium Heparin specimen bottle for LFT.

Statistical analysis.

Data collected were analyzed using the Statistical Package for Social Science (SPSS) version 20 for the statistical analysis. The categorical data were summarized with frequencies and percentages, while the quantitative data were summarized with median (25^{th} percentile and 75^{th} percentile) because their observations were not normally distributed; the quantitative variables were also pictorially presented in Histograms.

The inferential analyses were therefore carried out using non parametric methods such as median test, Spearman rank correlation and chi-square test to test for the significance of the relationship. All the statistical tests were two tailed and were done at 5% level of type 1 error.

RESULTS

Biodata

A total of 80 subjects participated in the study. Half of the 80 subjects constituted the HbS patients while the other 40 constituted the control HbA subjects. Table 1 presents the gender distributions of the subjects. There were slightly more females (55%) in HbS group than males; while males (57%) were slightly more in the control group than females. The chi-square statistics however shows that the HbS patients had similar sex distributions with the control HbA Subjects ($p>0.05$).

Table 1: Gender distribution of HbS patients and Controls (HbA subjects).

	HbS patients	Control HbA	χ^2	p value
	N (%)	N (%)		
Male	18 (45)	23 (57)	1.25	0.371
Female	22 (55)	17 (43)		
Total	40 (100)	40 (100)		

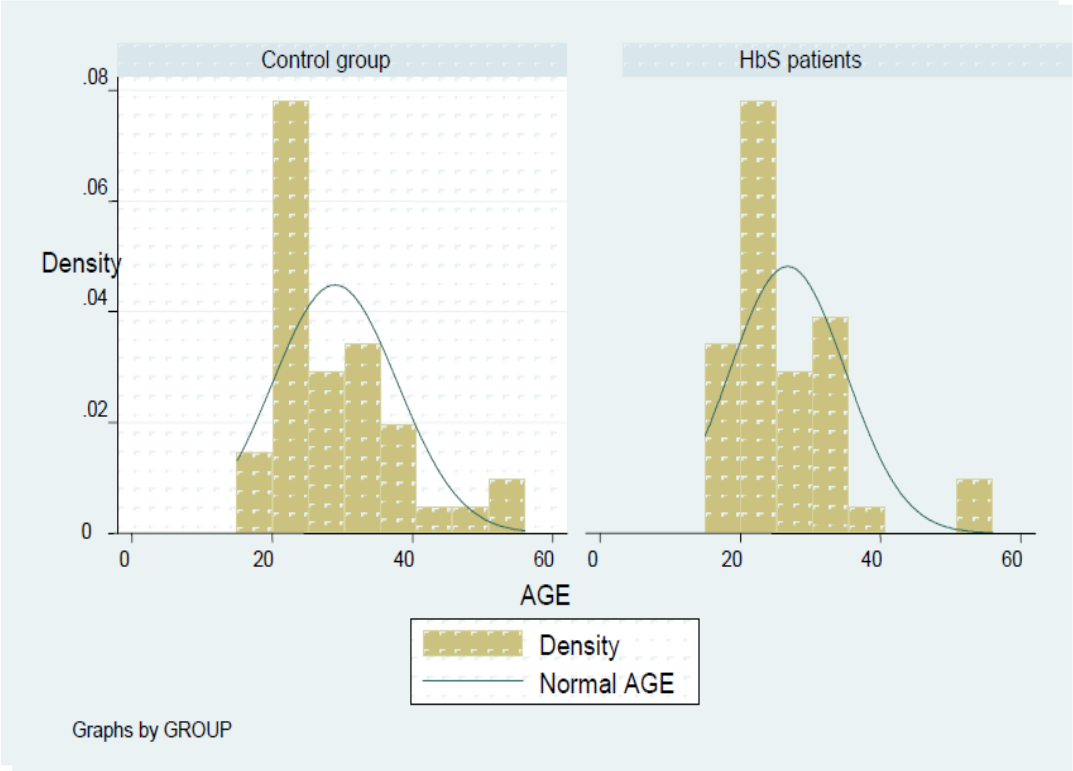

Figure 1: Age distribution of the study group.

Table 2: Median comparison of Coagulation profile and Liver function tests of the HbS patients and Controls.

Variables	HbS patients	HbA subjects (control)	Median test	
	median	median	chi-square	p-value
Age (years)	24.5	26.0	0.802	0.370
PT (s)	15.0	15.0	0.000	1.000
APTT (s)	48.5	44.0	5.051	0.025
INR	1.2	1.2	0.000	1.000
Total protein (mg/dl)	7.4	7.2	0.800	0.371
Albumin (mg/dl)	3.7	3.9	2.452	0.117
ALT (i.u/L)	11.5	11.0	0.050	0.823

Table 2 presents the median estimates (25-75percentile) of the Age, Prothrombin time, Activated Partial Thromboplastin Time (APTT), International Normalizes Ratio (INR), Total protein, Albumin and Alanine Transaminase (ALT) of the HbS patients and control HbA subjects. The median age estimates of the HbS patients and the control subjects which stood at 24.5 and 26years respectively were not significantly different from each other (p=0.370). Similarly, the median Prothrombin time of the HbS patient (15s) was the same (p=1.000) as that of the control HbA subjects (15s).The two groups had similar INR (P=1.000), Albumin (P=0.117), Total protein (P=0.371) and ALT (p=0.823).

However, the APTT on the other hand, differed significantly between the groups; the HbS patients significantly had longer APTT (48.5s) than the control HbA subjects (44.0s) (P=0.025).

Coagulation profile of the HbS patients and the control HbA subjects.

The coagulation profile of the Subjects was assessed by Prothrombin Time (seconds), International Normalized Ratio (INR), (seconds) and Activated Partial Thromboplastin Time (APTT).

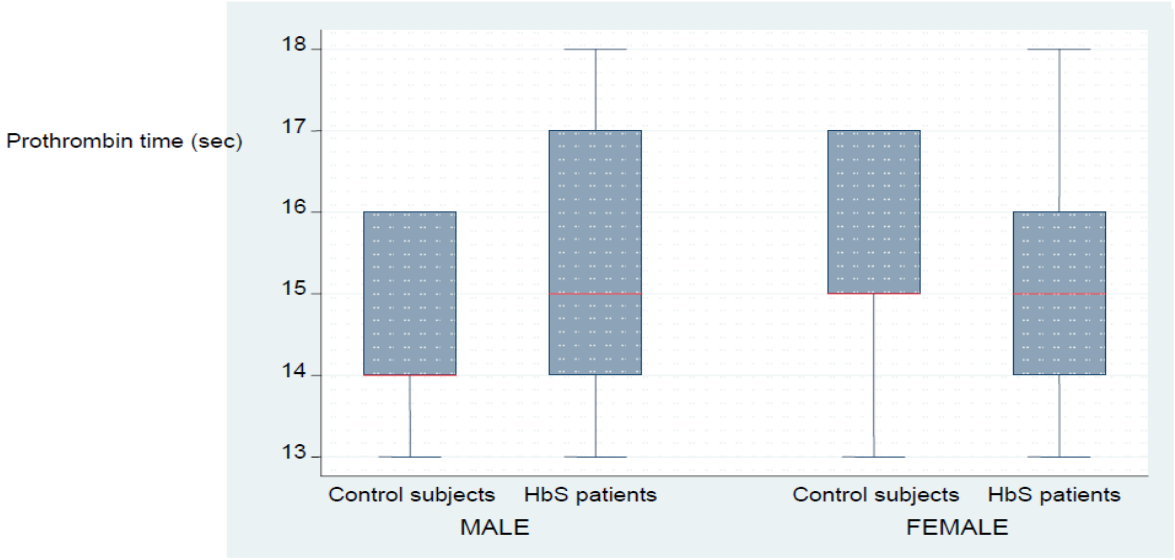

Figure 2: Prothrombin time of the HbS patients versus controls (HbA subjects).

As shown in Figure 2, male HbS patients had higher median (25-75 percentiles) Prothrombin Time, 15s (14-17s) than their counterparts in the control group, 14s (14-16s). Meanwhile, the female HbS patients 15s (14-17s) had similar median Prothrombin Time, 15s (14-16s) with the control subjects. However, the median test (Table 2) shows no significant difference in the prothrombin time between the HbS patient and the control HbA (P=1.000).

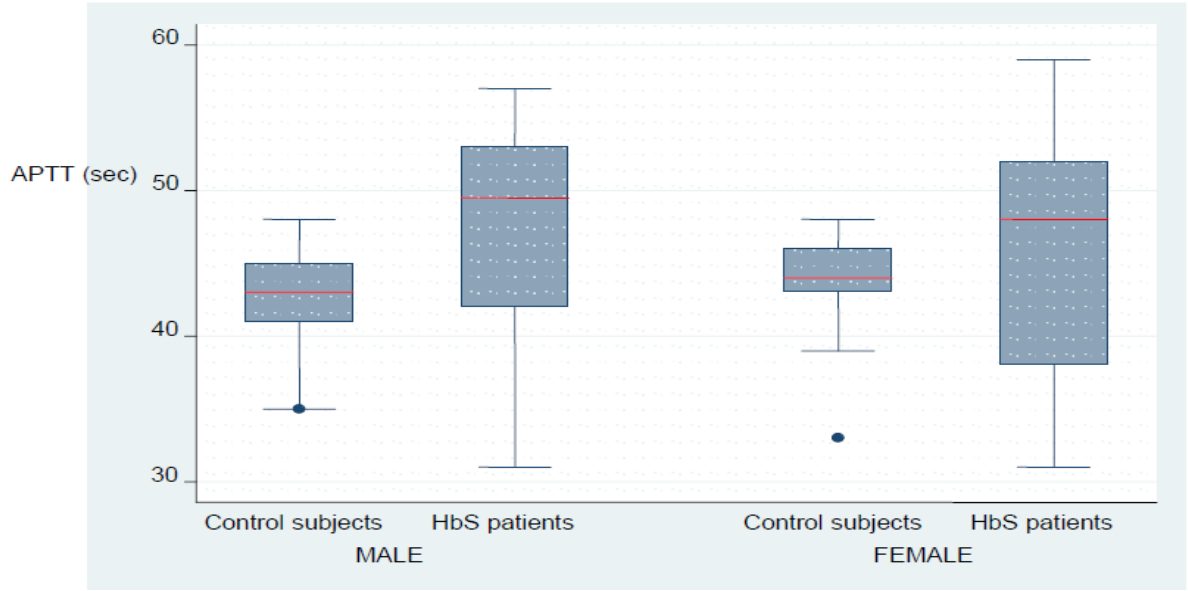

Figure 3: Activated partial thromboplastin time of the HbS patients and controls (HbA subjects).

Figure 3 shows the median APTT of the HbS patients and control HbA subjects. Both male (49.5s, 42-53s) and female (48s, 38-52s) members of the HbS patients had relatively higher median APTT than their male (43s, 41-45s) and female (44s, 43-46s) counterparts in the control subjects. However, there was an outlier among the female patients in the control group. The median test (Table 2) however shows that the HbS patient had significantly higher APTT than the control HbA (P=0.025).

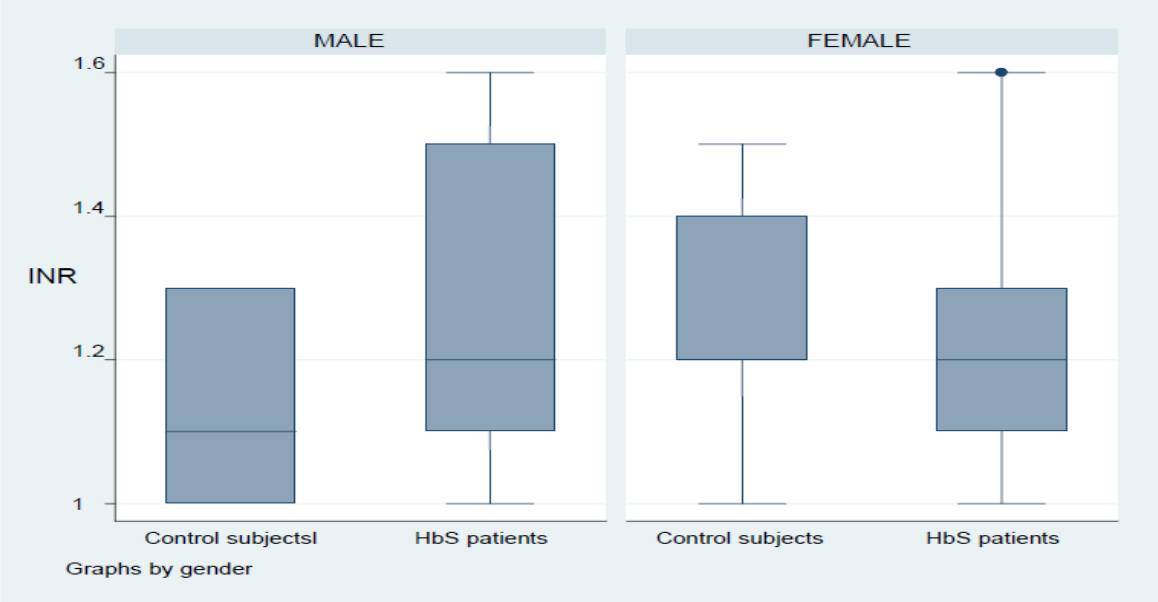

Figure 4: INR of the HbS patients versus Controls (HbA).

The male HbS patients had relatively higher median (25-75 percentiles) INR 1.2(1.1-1.5) than the male control subjects 1.1 (1.0-1.3), meanwhile the female HbS patients had median INR, 1.2 (1.1-1.3) which was similar to that of the median INR 1.2 (1.2-1.4) of female in the control HbA subjects. According to the median test (Table 2), there is no significant difference in the INR between HbS and the Controls (p-value =1.000)

Liver function tests of HbS patients and Control HbA subjects.

Three parameters (Total protein, ALT, and Albumin) were used in assessing the liver function of the study subjects.

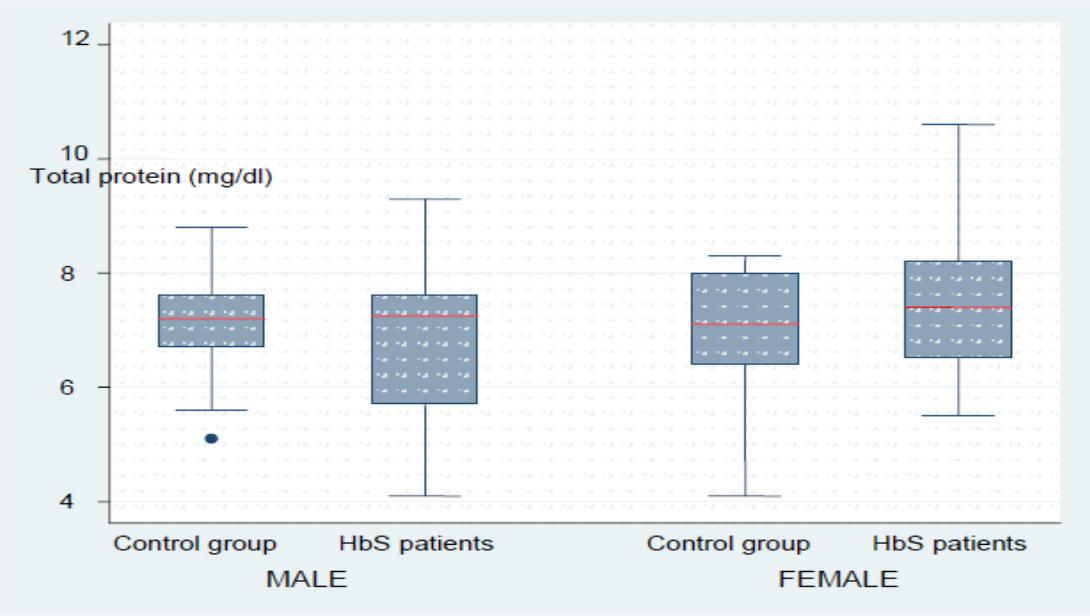

Figure 5: Total protein profiles by gender in the HbS group versus controls (Hb A subjects).

Although, there was an outlier among the male subjects with a total protein of 5.7mg/dl in the control group as depicted in Figure 4.5, the median estimate, 7.25mg/dl (5.7-7.6mg/dl) of the Total protein of the male subjects in the HbS patients was similar to the median estimate 7.2mg/dl (6.7-7.6mg/dl) of the control subjects. On the other hand, the median estimate, 7.4mg/dl (6.5-8.2mg/dl) of Total protein of the female subjects in the HbS patients was marginally higher than the median estimate, 7.1mg/dl (6.4-8.0mg/dl) of the control subjects.

The median test (Table 2) shows no significant difference between the HbS total protein and the control HbA.

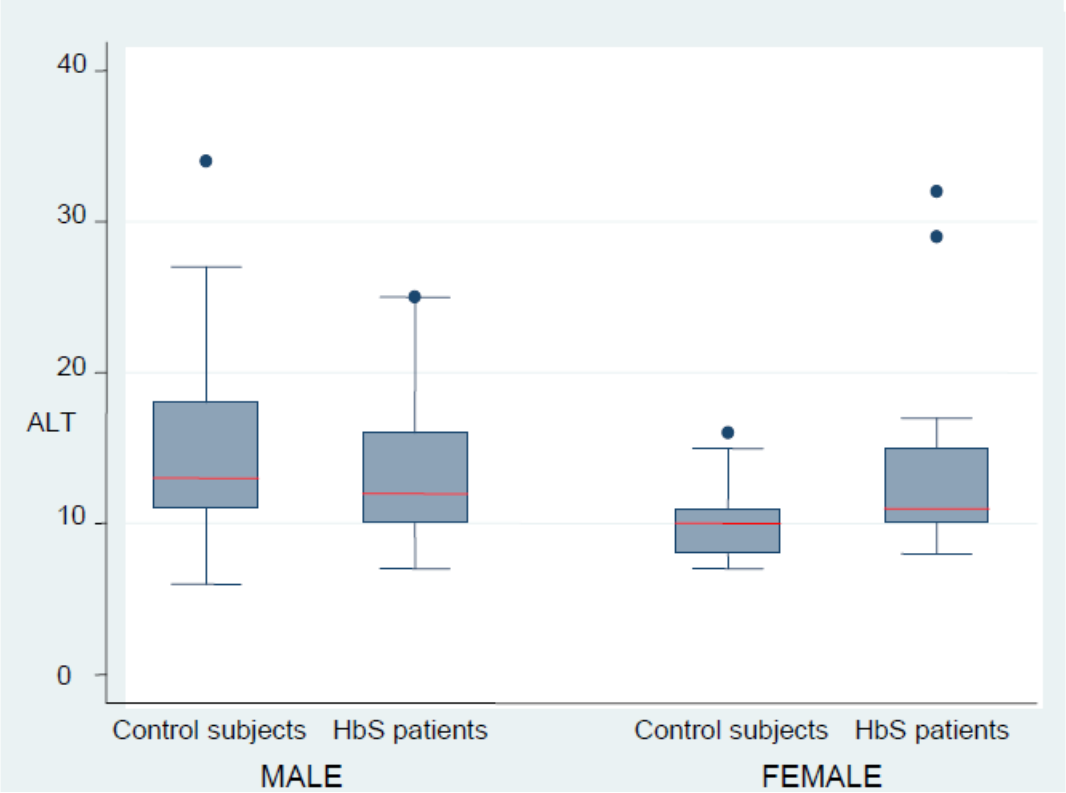

Figure 6: ALT of the HbS patients versus controls (HbA Subjects).

The median ALT estimates of the male HbS patients (12 i.u/L, 10-16 i.u/L) and the control male subjects (13u.i/l, 11-18 i.u/L) were relatively higher than that of the female HbS patients (11 i.u/L, 10-15 i.u/L) and control female subjects (10 iu/L, 8-11 i.u/L) . However, male subjects in the control group still had relatively higher median estimate of ALT than their male counterparts in the HbS patients. In converse, the female HbS patients had marginally higher median estimate than their counterparts female control subjects. However, there were outliers among two HbS patients and two control subject. Median test (Table 2) shows no significant difference in the ALT between HbS patients and the control HbA (P value =0.823).

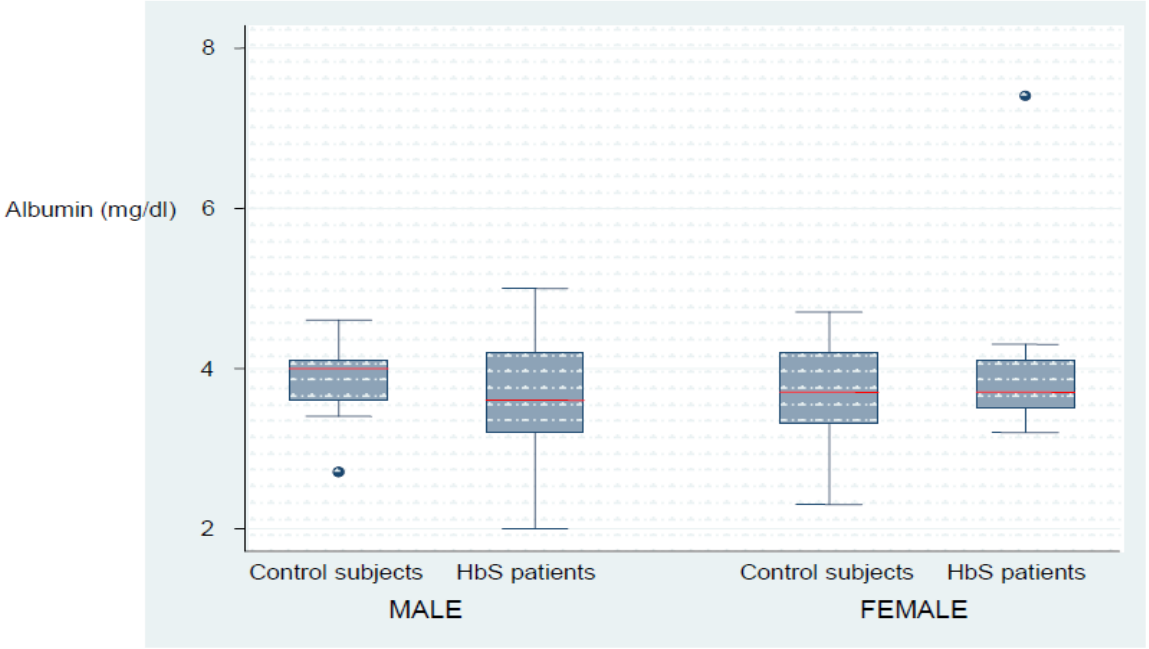

Figure 7: Albumin profiles of the HbS patients versus control (HbA) subjects.

Figure 7 depicts the albumin estimates of the HbS patients and the control HbA subjects by sex. Male control subjects had a relatively higher median Albumin concentration of 4mg/dl (3.6-4.1mg/dl) than the median estimate, 3.6mg/dl (3.2-4.2mg/dl) of the male HbS patients. The female control subjects and the female HbS patients however had relatively similar median estimates of Albumin concentration of 3.7mg/dl (3.3-4.2mg/dl) and 3.7mg/dl (3.5-4.1mg/dl) respectively. The median test (Table 2) shows no significant difference between the HbS patients and the control HbA. (P=0.117).

DISCUSSION

This study corroborated the previous reports of the prolongation of PT, INR and APTT in HbS patients in steady state compared with APTT of HbA healthy individuals. In this study liver function test of the HbS patients were done to rule out primary hepatic dysfunction which may affect the coagulation factors level.[15] The median values obtained in HbS patients for ALT and total Protein were higher than in the control while the subjects median value for Albumin was higher in the control. However none of the differences between these values reached statistical significance. Hence there is no evidence in this study to support the fact that the prolonged coagulation profile found in HbS patient compared to control is due to hepatic disease because there was no abnormality of ALT values in these patients with Sickle cell anaemia which is specific for hepatic injury.

There is evidence that patients with SCA have a state of persistent but low grade inflammation. Acute phase reactants such as C-reactive protein and serum amyloid A are moderately increased in sickle cell patients during steady state and significantly increased during painful vasoocclusive crisis.[16] Elevated IL-1 and TNF-α were found in patients with SCD during steady state.[17]

Previous studies showed Von Willebrand Factor, a marker of injury to the endothelium and an acute phase reactant was found to be significantly increased in HbS patients in steady state compared with the HbA control and this value increased further during bone pain crisis.[18,19] Elevated acute phase reactants during the steady state suggest that patients with SCD have subclinical microvascular ischaemic events that lead to tissue injury, release of inflammatory cytokines and hepatic synthesis of acute phase reactants.

Fakunle et al found significant increase in D-D dimer levels in HbS patients in steady state compared with Hb A healthy individuals.[20] This confirms the activation of coagulation and fibrinolytic systems in steady state. The review of coagulation in patients with Sickle cell disease showed in vivo generation of thrombin even in steady state.[21,22]

The prolonged coagulation profile found in HbS patients in this study and other previous studies may be a consequence of increased thrombin generation and activation of coagulation factors leading to the consumption of the coagulation factors and subsequent prolongation of the coagulation profile.

CONCLUSION

The findings in this study showed the coagulation profile in sickle cell patients in steady state is higher compared with the HbA control subjects but this is not statistically significant. The prolonged coagulation profile may be as a result of increased consumption of coagulation factors following activation of coagulation and fibrinolytic systems in sickle cell patients even at steady state and not necessarily due to hepatic dysfunction.

CONSENT: Informed written consent was obtained from all the participants.

CONFLICT OF INTEREST: Authors declared no conflict interests.

ETHICAL APPROVAL: Institution Ethical approval for the study was obtained for this study.

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