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LUPUS ANTICOAGULANT IN NIGERIAN CHILDREN WITH HOMOZYGOUS SICKLE CELL DISEASE

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Key words: Lupus anticoagulant, Nigerian children, homozygous sickle cell disease

Abstract
Background: Lupus anticoagulant (LA) is an antiphospholipid antibody (APLA), which
recognise combinations of phospholipids or phospholipid -binding proteins or both and interfere with coagulation reactions dependent on protein phospholipid complexes in vitro. LA has paradoxically been associated with thrombosis and a myriad of clinical conditions such as cerebrovascular accidents (CVA). APLA have been described in adult homozygous sickle cell disease (SCD) patients and adults with β- thalassaemia. Our objective was to determine the presence or otherwise of LA in children with homozygous SCD and to see if they were more prone to developing LA compared to normal HbAA controls.
Method: A total of 57 children with homozygous SCD between the ages 1-15 years were prospectively screened for the presence of LA using the kaolin clotting time (KCT), while 52 healthy HbAA children served as controls. KCT was performed in duplicates on all 109 subjects. Kaolin clotting time ratio was calculated to determine the presence of LA. A ratio greater than or equal to 1.2 was taken to signify the presence of LA.
Results: One (1.8%) child with homozygous SCD had prolonged KCT, and this was not corrected by normal plasma, the KCT ratio was greater than 1.2, signifying the presence of LA. None of the control subjects had LA ($p>0.05$).
Conclusion: One (1.8%) child in this study had LA; this value was not statistically significant when compared with HbAA controls. In spite of the fact that secondary LA is more common in females, the only patient with LA in our study was male and had no prior history of CVA or thrombosis.

Mots clés : Anticoagluant de lupus, enfants nigérians, le drépanocytose homozogote

Résumé
Fond : L'anticoagulant de lupus (AL) est un anticorps antiphospholipide (AAPL), qui identifie les combinaisons des phospholipides ou du phospholipide protéines liantes ou toutes les deux et qui interfère avec des réactions de coagulation qui dépendent sur les complexes phospholipide de protéine en vitro. L'AL a été paradoxalement associée à la thrombose et à une myriade de conditions cliniques tels que les accidents cérébrovasculaires (ACV). AAPL ont été décrits dans des malades adultes de drépanocytose homozygote et adultes de drépanocytose homozygote et des adultes avec la thalassémie de b. Notre objectif était de déterminer la présence ou autrement d'AL chez les enfants avec drépanocytose homozygote et pour voir s'ils étaient plus en pronation au développement d'AL en comparaison avec les commandes normales de HbAA.
Méthode : Un total de 57 enfants avec drépanocytose homozygote entre les âges 1-15 ans étaient prospectivement examinés pour la présence de l'Al en

utilisant le temps de coagulation de kaolin (TCK), tandis que 52 enfants en bonne santé de HbAA servaient de commandes. Le TCK a été exécuté en reproductions sur chacun des 109 sujets. La proportion de temps de coagulation de kaolin a été calculée pour déterminer la présence de l'AL. Une proportion supérieure ou égale à 1,2 a été prise pour signifier la présence de l' AL.

Les résultats : Un (1,8 %) enfant avec drépanocytose homozogote avait prolongé le TCK, et ceci n'a pas été corrigé par le plasma normal, la proportion de TCK était plus grande que 1,2, signifiant la présence de l'AL. Aucun des sujets de contrôle n'avait AL (p>0,05).

Conclusion : Un (1,8%) enfant dans cette étude a eu l'AL ; cette valeur n'était pas statistiquement significative en comparaison avec des commandes de HbAA. Malgré le fait que l'AL secondaire est plus commune dans les femelles, le seul malade avec l'AL dans notre étude était un mâle et n'a eu aucune histoire antérieure de ACV ou de thrombose.

Introduction

Sickle cell disease (SCD) patients are generally considered to have a hypercoagulable state[1] and are thus prone to vasocclusion and thrombotic episodes. [2] This has usually been attributed to small blood vessel obstruction by sickle erythrocytes. However, the wide variability of the clinical spectrum of sickle cell disease makes it pertinent to consider other possible mechanisms.

The lupus anticoagulant (LA) is one of the antiphospholipid antibodies (APLA), which are defined as antibodies that exhibit a broad range of target specificities and affinities, recognizing various combinations of phospholipids, phospholipid -binding proteins or both and thus prolong the phospholipid – dependent coagulation tests by interfering with the coagulation reactions dependent on protein-phospholipid complexes in vitro.[3] Paradoxically, LA has been related to thrombotic events [4] but could lead to bleeding in the presence of thrombocytopaenia.[5] Occurrence of APLA can either be primary when it is found in patients without clinical evidence of autoimmune disease, or secondary in association with autoimmune or other diseases.[3] Primary APLA occurs equally in both sexes, while the secondary form is more frequent in females.[6]

Repeated sickling has been shown to produce a disruption and rearrangement of red cell membranes.[7] The exposure of the negatively charged phospholipids may result in the induction of antibodies against cell membrane constituents.[8] It has been shown that APLA formation can be induced in mice by phospholipid in a hexagonal II phase but not by phospholipid in a bilayer phase, sickle red cell membranes have increased hexagonal II phase content.[9]

APLA has been found in adult SCD patients by various investigators and the prevalence has ranged from 8-68%.[8,9]LA has been described in children,[10] adults with β-thalassaemia[11] and unpublished data from our hospital has shown a prevalence of 11.4% in adult Nigerians with homozygous sickle cell disease. Since the possibility of LA contributing to thrombotic episodes in paediatric SCD patients has not been investigated, we aim therefore, to determine the presence or otherwise of LA in children with homozygous sickle cell disease (HbSS) using the kaolin clotting time (KCT).

KCT has been shown to have a specificity of up to 93% for LA[12] and it is able to detect LA at a greater dilution in normal plasma than the tissue thromboplastin inhibition test (TTI) or the dilute Russell's viper venom time (DRVVT).[13]

Patients and Methods

Patients

From 1st November 2002 to 31st May 2003, all homozygous sickle cell disease patients seen at the department of child health in UBTH and whose parents or guardian gave informed consent were enrolled in this study. The study population was made up of 57 HbSS children between the ages of 1-15, for purposes of comparison 52 healthy HbAA children were also recruited from the follow up clinic and from those who came for routine Hb genotype determination at the haematology laboratory; these were matched for age and sex and served as control. Informed consent was also obtained from the parents or guardian of the controls.

Clearance was obtained from the hospital ethical committee. Only children with homozygous SCD (HbSS) seen in U.B.T.H. within the study period were included in the study. Patients with other variants of SCD and those diagnosed with autoimmune disorders such as systemic lupus erythematosus were excluded. Those on drugs, which have been associated with LA such as phenytoin and chlorpromazine, were also excluded. The children that served as control were screened for SCD using haemoglobin electrophoresis, only HbAA children served as controls.

There was a marked reluctance on the part of most parents or guardians to have the blood samples of their wards collected, despite the fact that they initially consented to participate in the study, for this reason a number of patients could not be included in this study.

Blood Samples

4.5 ml of blood was collected by clean venepuncture into clean plastic tubes containing 0.5ml of 0.129m trisodium citrate in a ratio of one part anticoagulant to nine parts of blood. Platelet poor plasma was prepared by centrifuging the citrated blood twice at 2,500g for 15 minutes at room temperature. The plasma samples were preserved on ice blocks and were analyzed within two hours.

Coagulation tests

Kaolin clotting time (KCT) was performed as described previously[14, 15] by pre- incubating 0.2ml of citrated plasma with 0.1ml Kaolin suspension 20g/l tris buffer at a pH 7.4 for 3 minutes at 37 ^{0}C, 0.2ml of 0.025 M calcium chloride was then added.

The time from addition of calcium chloride to the formation of a clot was recorded; the procedure was carried out in duplicates for each sample and the average was taken as the clotting time.

Plasma that had prolonged KCT were subjected to mixing studies using the KCT on prolonged plasma (PP) and normal plasma (NP) in the following proportions of NP/PP 100/0,90/10,80/20, 50/50, 20/80,10/90 and 0/100 as earlier described.[14]

Plasma from individual healthy volunteers with normal coagulation tests were pooled together and used as normal plasma. The KCT ratio, which is the ratio of KCT at 20% prolonged plasma to KCT at 100% normal plasma of greater than or equal to 1.2, was taken to signify the presence of LA.[15]

$$\frac{\text{KCT (80\% Normal Plasma: 20\% Prolonged Plasma)}}{\text{KCT 100\% Normal Plasma}} = />1.2$$

Graphs were plotted from the results of the mixing studies for those that had prolonged KCT, by plotting the clotting times against the proportion of normal: patient's plasma.

In studies of LA using the KCT, four patterns of graphs are possible. Pattern 1 indicates presence of classical LA; pattern 2 indicates a coagulation factor deficiency as well as LA; pattern 3 is seen in plasma containing LA but also deficient in a cofactor necessary for the full inhibitory effect, while pattern 4 is seen in the absence of LA.[15]

Results

A total of 57 homozygous SCD patients and 52 healthy HbAA children were studied. Mean age for SCD patients was 8.09 +/- 4.17 years and 8.00 +/-3.96 years for controls. The age and sex distribution of the SCD patients is shown in Table 1, there were 35(61.4%) male patients and 22(38.6%) were females.

Mean KCT for SCD patients was 81.26 +/- 15.50 s; mean KCT for male SCD patients was 82.03 +/- 15.70 s and 80.05 +/- 15.45 s for females. There was no significant difference in KCT among the sexes. In the control group, mean KCT was 81.21 +/- 10.06 s; it was 79.74 +/- 10.15 s and 82.80 +/- 9.92 s for male and female controls respectively. There was no statistically significant difference in mean KCT between SCD patients and controls.

Table 2 shows the KCT values for different age groups of SCD patients and controls. One (1.8%) child with SCD had prolonged KCT greater than 110 seconds while the 52 controls had normal KCT. Table 3 shows LA and the clinical state of SCD patients, out of the 57 SCD patients in this study, 39(68.4%) were in steady state while 18(31.6%) were in crises. The only patient with LA was a male and was seen in steady state.

The prevalence of LA in children with homozygous sickle cell disease in this study is 1.8%; none of the controls had LA. There was no statistically significant difference between the prevalence of LA in children with homozygous SCD and controls, p>0.05.

Figure 1 shows the graph obtained from mixing experiments in the child with HbSS and prolonged KCT; the prolonged KCT was not corrected by mixing with varying proportions of normal plasma. The KCT ratio was also greater than 1.2 indicating the presence of LA. The graph shows a type 2 pattern, which signifies a coagulation factor defect as well as the presence of LA.[15]

Table 1: Age and sex distribution of patients and controls

Age (Years)	Sex		Total
	Male	Female	
Patient			
1-5	10(17.5)	9(15.8)	19(33.3)
6-10	13(22.8)	5(8.8)	18(31.6)
11-15	12(21.1)	8(14.0)	20(35.1)
Total	35(61.4)	22(32.6)	57(100)
Control			
1-5	13(25.0)	3(5.8)	16(30.8)
6-10	9(17.3)	11(21.1)	20(38.4)
11-15	5(9.6)	11(21.1)	16(30.8)
Total	27(51.9)	25(48.1)	52(100)

Figure in parenthesis are percentages

Figure 1: KCT values of prolonged plasma in various proportion of normal plasma in homozygous SCD patient with KCT ratio >1.2

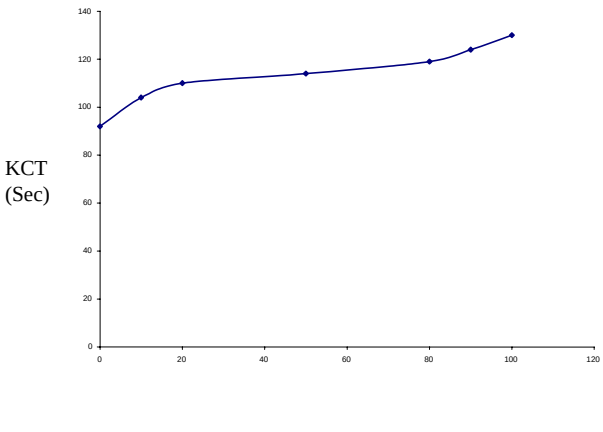

Table 2: Age and Kaolin clotting time (KCT) of patients and controls

Age (Years)	Kaolin clotting time			Total
	<60	60-110	>110	
Patient				
1-5	-	19(33.3)	-	19(33.3)
6-10	4(7.0)	14(24.6)	-	18(31.6)
11-15	4(7.0)	15(26.3)	1(1.8)	20(35.1)
Total	8(14.0)	48(84.2)	1(1.8)	57(100)
Control				
1-5	1(1.9)	15(28.9)	-	16(30.8)
6-10	1(1.9)	19(36.5)	-	20(38.4)
11-15	-	16(30.8)	-	16(30.8)
Total	2(3.8)	50(96.2)		52(100)

Normal KCT: 60-110 seconds. [16]
Figure in parenthesis are percentages

Table 3: LA and clinical state in HbSS patients

Patients	LA Present	LA Absent	Total
Steady State	1(1.8)	38(66.7)	39(68.5)
Crises	-	18(31.5)	18(31.5)
Column Total	1(1.8)	56(98.2)	57(100)

LA: Lupus anticoagulant
Figure in parenthesis are percentages

Discussion

Sickle cell disease is a major genetic disorder in tropical Africa, [17] homozygous sickle cell disease is the commonest haemoglobinopathy in Nigeria, where about 20 per thousand newborns are affected per year. [18] Cerebrovascular accidents (CVA) occur in SCD most commonly in children aged between 1-15 years, the highest prevalence and incidence rates are found in homozygous SCD patients. [19] Risk factors for CVA include prior occurrence of transient ischaemic attacks, low steady state Hb and recent acute chest syndrome.[20]

LA has been associated with a lot of clinical conditions such as recurrent abortions, arterial and venous thrombosis (including CVA) and thrombocytopeania.[16] Thus the presence of LA in a homozygous SCD patient is likely to predispose such a patient to developing CVA.

The method of detection of LA has remained a source of much debate and disagreement. Several screening methods have been described for the detection of LA, they include the: activated partial thromboplastin time (APTT), kaolin clotting time(KCT), dilute Russell's viper venom time (DRVVT), tissue thromboplastin inhibition test (TTI), APTT correction ratio[21] and platelet neutralization test. The kaolin clotting time (KCT) was used in this study because it is simple, sensitive to the presence of LA, [22] with high specificity[12] and also affordable. Specific immunological assays for LA are also available, these are based on either radioimmuno-assay (RIA) or enzyme linked immunosorbent assay (ELISA).[6]

In this study, a prevalence of 1.8% was found among children with homozygous SCD, which was not statistically significant when compared with normal controls, and is comparable to the 2% reported by Von Landenberg et al among normal control children in their study.[23] While it was much lower than 8% reported among normal non-pregnant multi-parous women in Nigeria.[24].The only patient with LA in our study had no prior history of CVA or thrombosis; the prevalence may be higher in children with past history of CVA, which our study did not include. Thus further study restricted to homozygous SCD patients with CVA may show a higher prevalence.

Again, our findings differ from that of Ceulaer et al and Kucuk et al, who reported a value of 8%and 68% respectively for APLA among adults with homozygous SCD.[8, 9] This difference in the prevalence of APLA in children and adults could be due to the fact that the prevalence of LA like other autoimmune antibodies increases with age.[25]

It may also be that patients with complications of SCD or those in whom the disease runs a severe course are more likely to develop LA. Also, enzyme linked immunosorbent assay (ELISA) was the method used by Ceulaer et al and Kucuk et al; the difference in the sensitivity and specificity of the assay methods may partly explain the differences seen.

In spite of the fact that secondary LA is more frequent in females, the only patient with LA in this study was a male seen in steady state. This may be related to the fact that only 22(38.6%) of the patients seen in this study were females. Also the majority of patients were seen in steady state just 18(31.6%) were

in crises. Screening of a larger population of SCD patients in crises may be required to find out if development of LA in SCD patients is related to whether the patients are seen in crises or steady state.

Despite the fact that the prevalence of 1.8% in this study is not statistically significant, since LA has been described in adults with homozygous sickle cell disease and the exact time of its development is not known, it may be helpful to screen children with homozygous sickle cell disease for LA especially as they grow older, to detect it early, so that appropriate treatment could be instituted where necessary.

In conclusion, we set out to determine the presence or otherwise of LA among children with HbSS using the KCT; one (1.8%) child in our study had LA. We believe that further studies in HbSS patients with past history of CVA may help in determining the actual role of LA in the thrombotic episodes experienced by SCD patients.

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