Original Article

Plasma Lactate Dehydrogenase Level as Indicator of Severe Homozygous Sickle Cell Disease

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ABSTRACT

Background: Homozygous sickle cell disease (SCD) (SS), also called sickle cell anemia (SCA) is the most common SCD in Nigeria. Studies done in the developed world showed that serum lactate dehydrogenase (LDH) levels correlated positively and significantly with clinical severity of the disease. This study attempts to provide information on the relationship between the plasma LDH level and the clinical severity of SCA in Nigerian children.
Objective: The objective of this work was to assess plasma LDH level in Nigerian SCA children, aged 5–15 years, as a clinical indicator of disease severity.
Methods: Plasma LDH level was measured quantitatively using Randox LDH reagent kit. A semi-quantitative assessment of clinical severity of SCA was carried out on all the SCA subjects using the Bienzle *et al.* assessment profile modified for this study. Subjects were classified as having mild, moderate, or severe disease based on their clinical severity score. Data were analyzed using Statistical Package for Social Sciences (SPSS) version 21 software.
Results: Eighty SCA children, 40 in steady state and 40 in hemolytic (with or without vaso-occlusive) crises were studied. Mean plasma LDH level was significantly higher in SCA subjects in crises than for those in steady state ($P = 0.0284$). Only steady state plasma LDH level correlated positively and significantly with clinical severity score ($P = 0.0151$).
Conclusion: Plasma LDH appears to be a reliable indicator of SCA disease severity in the steady state in Nigerian children.

Key words: Lactate dehydrogenase, Nigeria, sickle cell anemia, surrogate marker

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INTRODUCTION

Sickle cell anemia (SCA), is by far the most common form of sickle cell disease (SCD) in Nigeria.[1] Studies from Nigeria estimated 2–3% of children to be homozygous for the sickle mutation.[1] The World Health Organisation (WHO) estimated that 60% of 250,000 babies born annually with SCD are from Africa.[2]

Various biochemical manifestations of SCA such as hyperbilirubinemia, hyperuricemia, hypocalcemia, and hyperphosphatemia are now well documented and the possible roles that these play in the pathophysiology of the disease have been proposed.[3-12] Clinical reports have shown that total plasma lactate dehydrogenase (LDH) is increased in SCA patients in the steady state and is further increased when they are in crises.[3,4,10-12] This significantly higher level of LDH in crisis reflects the severity of the event.[3,11,12] The increase in LDH is an inherent manifestation of SCA due to the continuous hemolytic events and release from infarcted bone marrow.[3] The strong correlation of LDH_1 and LDH_2 isoenzymes with clinical markers of hemolytic severity in some studies however, strongly implicates intravascular hemolysis as the dominant source of serum LDH in patients with SCA.[4,12]

Based on several studies done in the developed world, it has been hypothesized that serum LDH level may be a clinically useful prognostic factor in patients with SCA.[12-15] It has also been suggested that the steady state LDH level can be a convenient risk marker for clinicians to use in identifying patients with SCA who are at increased risk for mortality and who might otherwise have treatment options overlooked because of infrequent crises.[12-16] This however, has not been documented in Nigeria. This study assessed plasma LDH levels in Nigerian SCA children, aged 5–15 years, related its levels

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with the clinical severity of SCA and evaluated its usefulness as an indicator of clinical severity.

MATERIALS AND METHODS

This was a prospective, descriptive study conducted over a period of 1 year (April 30, 2008–March 30, 2009) at the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, Osun State, South-Western Nigeria. Subjects were children with SCA in crises and steady state aged 5–15 years. More than 90% of SCA children seen at the pediatric sickle cell clinic of the OAUTHC were in this age bracket. Less than 10% of them were below 5 years of age. Children 15 years of age and above attend the adult hematology clinic. A total of 80 patients seen consecutively either at the sickle cell clinic or children emergency ward (CEW) were studied. Those that were seen at the sickle cell clinic were forty patients in the steady state, attending clinic regularly at intervals of 6–8 weeks. Forty patients with features that denote hemolytic or a combination of hemolytic and other crises were recruited from CEW. Each of the 80 SCA subjects was on routine folic acid supplementation of 5 mg daily and malaria prophylaxis (100 mg daily of proguanil). Patients were deemed to be in steady state if (i) at recruitment they were not clinically ill and remained so for at least 1 week thereafter (ii) the last crisis event was at least 3 weeks before recruitment and (iii) the last blood transfusion was at least 3 months before recruitment. The first of these three conditions was critical since the rise in plasma LDH level usually starts during the prodromal phase of SCA crises when patients may still appear to be in steady state clinically, and this antedates established SCA crises by at least 1 week.[11] Forty healthy age and sex-matched children with hemoglobin genotype AA served as controls. They were recruited from among children that came to the hospital for routine clinic follow-up for simple ailments (uncomplicated malaria fever and upper respiratory tract infections) from which they had fully recovered. Ethical clearance was obtained from the OAUTHC Research and Ethical Committee. Signed or thumb-printed informed consent and assent (where applicable) were obtained from the parent(s) or primary caregiver(s) of subjects after a discussion session on study's rationale was conducted in their best-understood language.

Clinical methods

Each child (subject or control) was thoroughly reviewed and relevant aspects of their history, physical examination, and anthropometry (weight and height) recorded. For the SCA subjects the, past medical history detailed the number of past hospital admissions, blood transfusions, history of osteomyelitis, and they also had their steady state packed cell volume (PCV) recorded. In addition, their liver and spleen sizes below the costal margin were measured and recorded. A semi-quantitative assessment of the severity of SCA was carried out on all the SCA subjects based on the Bienzle *et al*.[17] assessment profile modified for this study. There were seven assessment indices including steady state PCV and splenomegaly. The latter's significance in SCA children seen at the center had earlier been reported by Adekile *et al*.[18] and Adeodu and Adekile.[19] The steady state PCV and other clinical parameters were additively compounded to yield a score, on the basis of which subjects were arbitrarily classified as having mild disease (score below 6), moderate disease (score between 6 and 10), or severe disease (score above 10) (as follows: [1] Steady-state PCV: <20%, 6; 20–25%, 4; 26–30%, 2; >30%, 1. [2] Liver enlargement below costal margin: >6 cm, 2; 2–6 cm, 1; <2 cm, 0. [3] Spleen enlargement below costal margin: >6 cm, 2; 2–6 cm, 1; <2 cm, 0. [4] Blood transfusion: 1 point per transfusion. [5] Osteomyelitis: 1 point per site involved. [6] Hospital admission: 1 point per each admission. [7] Subjective feeling: frequent complaints, 2; infrequent complaints, 1; mostly well, 0.).

Laboratory methods

Ten milliliters of venous blood were collected from each subject and control into dipotassium salt of ethylene diamine tetraacetic acid (K_2 EDTA) bottle (5 ml) and lithium heparin bottle (5 ml). Blood samples in K_2 EDTA bottle were used for the estimation of PCV, reticulocyte counts, red blood cell (RBC) counts and blood film appearance following standard procedure.[20]

Plasma was prepared from the blood sample collected in lithium heparin bottle following standard procedure[21] and the plasma was used for the estimation of total bilirubin (B_1), conjugated bilirubin (B_2) and LDH. B_1 and B_2 were determined using Randox Bilirubin reagent kits (cat. number BR 411) formulated according to standard methods.[21] Unconjugated bilirubin fraction was estimated by subtracting the value of B_2 from the value of B_1. Measurement of plasma LDH activity was done using Randox LDH reagent kit (cat. number LD 401) based on the optimized standard method according to the recommendation of the Society for Clinical Chemisrty.[22] The enzyme activity was determined at room temperature (25.0°C) by measurement of the rate of absorbance change at 340 nm due to the oxidation of nicotinamide adenine dinucleotide (NADH) using ultraviolet spectrophotometer (Norvaspec II, Pharmacia Biotech, Cambridge, England). LDH activity was expressed as U/L.

Data analysis

This was done using the Statistical Package for Social Sceinces (SPSS) version 21 software (IBM Corporation, 2012). Mean, standard deviations (SD), proportions, and percentages were determined as applicable. Means and SDs were compared using paired *t*-test. For simultaneous comparison of more than two mean values, *F*-test was used. Test of associations between plasma LDH levels and clinical severity score was done using Pearson correlation. Values of $P < 0.05$ were accepted as statistically significant.

RESULTS

Subjects and controls were of the same age range. The male: female ratio of the 120 subjects that were studied was 1:1.

Table I summarizes the characteristics of the three subgroups with respect to weight and height.

Of the 40 SCA subjects studied during crises, 26 (65.0%) had only hemolytic crises while 14 (35.0%) had both hemolytic and vaso-occlusive crises. None presented with combinations of hemolytic and sequestration crises or hemolytic and aplastic crises. Only 8 (10.0%) of the 80 SCA subjects studied belonged to the mild clinical category, and they were all in steady state [Table II].

Laboratory results

Table III shows that the difference between the mean plasma bilirubin levels of the two SCA groups were not statistically significant. However, the difference between the mean hematological values (RBC and reticulocyte count) of the two groups were statistically significant. The mean plasma LDH of the SCA group in crises was over 1½ times higher than that of the SCA group in the steady state. The difference was strongly statistically significant. Table IV shows a comparison of the mean values of the laboratory results of all three sub-groups and *F*-statistics shows statistically significant differences in the mean values of all laboratory parameters of the SCA groups when compared with the hemoglobin (Hb) AA controls. The mean plasma LDH levels were higher than the normal reference value of 120–240 U/L in all the three groups. The highest mean (SD) value of 560.5 (549.4) U/L was obtained among the SCA subjects in crises, followed by 346.8 (254.3) U/L for those in steady state and 309.9 (181.3) U/L for the Hb AA controls. Based on clinical severity of the disease (mild, moderate, and severe), the difference in the mean plasma LDH values of the SCA subjects were statistically significant only in the steady state [Table V]. Furthermore, there was a statistically significant and positive correlation between the plasma LDH level and clinical severity score only in SCA subjects that were in steady state [Figures 1 and 2].

Table I: Mean weight and height of sickle cell anemia subjects and hemoglobin AA controls

	SCA in steady state (*n*=40)	SCA in crises (*n*=40)	Hb AA control (*n*=40)	Total (*n*=120)	*P*
Weight (SD) (kg)	23.5 (9.0)	20.7 (6.2)	22.5 (6.8)	22.2 (7.4)	0.2151
Height (SD) (cm)	125.8 (16.9)	119.8 (16.3)	122.6 (15.0)	122.7 (16.1)	0.2446

Figures in parenthesis are the SDs. SCA: Sickle cell anemia, Hb: Hemoglobin, SD: Standard deviation

Table II: Clinical severity of the sickle cell anemia patients

Clinical severity category	Clinical severity score	SCA in steady state *n* (%)	SCA in crises *n* (%)	Total *n* (%)
Mild	<6	8 (20.0)	0 (0.0)	8 (10.0)
Moderate	6-10	18 (45.0)	13 (32.5)	31 (38.8)
Severe	>10	14 (35.0)	27 (67.5)	41 (51.2)
Total		40 (100.0)	40 (100.0)	80 (100.0)

Figures in parenthesis are percentages of total in each column. SCA: Sickle cell anemia

Table III: Comparison of mean laboratory parameters of sickle cell anemia subjects in steady state with those in crises

Laboratory parameters	SCA in steady state (*n*=40)	SCA in crises (*n*=40)	*P*
Mean (SD) total plasma bilirubin (μmol/L)	36.1 (26.2)	39.0 (41.1)	0.711
Mean (SD) conjugated plasma bilirubin (μmol/L)	13.8 (13.8)	19.4 (31.5)	0.309
Mean (SD) unconjugated plasma bilirubin (μmol/L)	22.3 (20.8)	19.6 (15.9)	0.567
Mean (SD) RBC count ($\times 10^{12}$/L)	3.1 (0.7)	2.2 (0.9)	0.000
Mean (SD) reticulocyte count (%)	8.1 (3.2)	11.9 (6.8)	0.002
Mean (SD) plasma LDH (U/L)	346.8 (254.3)	560.5 (549.4)	0.028

SCA: Sickle cell anemia, SD: Standard deviation, RBC: Red blood cell, LDH: Lactate dehydrogenase

Table IV: Comparison of mean laboratory parameters of sickle cell anemia subjects with hemoglobin AA controls

Laboratory parameters	SCA in steady state (*n*=40)	SCA in crises (*n*=40)	Hb AA controls (*n*=40)	*P*
Mean (SD) total plasma bilirubin (μmol/L)	36.1 (26.2)	39.0 (41.1)	6.3 (4.2)	0.000
Mean (SD) conjugated plasma bilirubin (μmol/L)	13.8 (13.8)	19.4 (31.5)	2.6 (1.5)	0.000
Mean (SD) unconjugated plasma bilirubin (μmol/L)	22.3 (20.8)	19.6 (15.9)	3.7 (3.6)	0.000
Mean (SD) RBC count ($\times 10^{12}$/L)	3.1 (0.7)	2.2 (0.9)	4.2 (0.6)	0.000
Mean (SD) reticulocyte count (%)	8.1 (3.2)	11.9 (6.8)	1.9 (0.5)	0.000
Mean (SD) plasma LDH (U/L)	346.8 (254.3)	560.5 (549.4)	309.9 (181.3)	0.005

Hb: Hemoglobin, SCA: Sickle cell anemia, SD: Standard deviation, RBC:Red blood cell, LDH: Lactate dehydrogenase

Table V: Comparison of mean plasma lactate dehydrogenase values of sickle cell anemia subjects based on the clinical severity of disease

	Clinical severity of disease			*P*
	Mild	Moderate	Severe	
Mean (SD) plasma LDH (U/L) of SCA subjects in steady state	195.5 (181.4)	294.4 (226.1)	500.5 (257.2)	0.009
Mean (SD) plasma LDH (U/L) of SCA subjects in crises	-	372.0 (447.5)	651.3 (577.9)	0.104

SD: Standard deviation, LDH: Lactate dehydrogenase

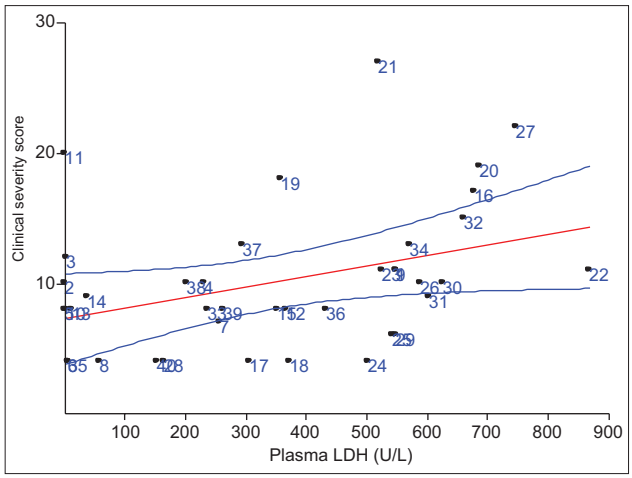

Figure 1: Relationship between plasma lactate dehydrogenase (U/L) and clinical severity score of sickle cell anemia subjects in steady state ($r = +0.3817$, $P = 0.0151$). Key: Numbers on curves are subject study numbers.

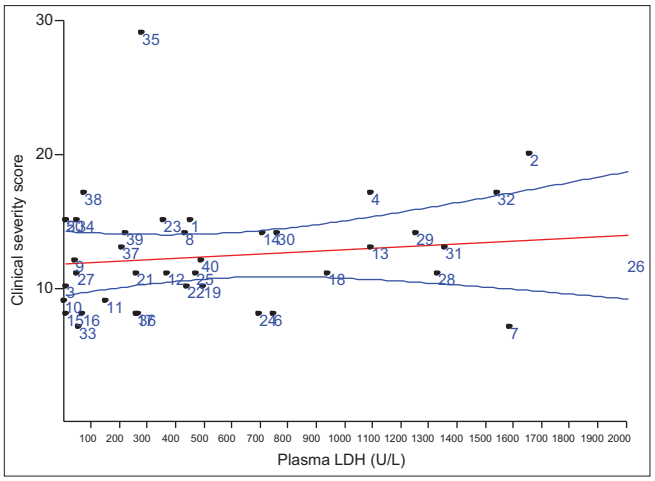

Figure 2: Relationship between plasma lactate dehydrogenase (U/L) and clinical severity score of sickle cell anemia subjects in crisis ($r = 0.1402$, $P = 0.3884$). Key: Numbers on curves are subject study numbers.

DISCUSSION

It was noted in this study that plasma LDH level of children with SCA in crises was significantly higher than the plasma LDH level of their counterparts in steady state. This finding is in agreement with the reports of earlier studies carried out in the USA and Saudi Arabia.[4,5,12,14]

Sources of plasma LDH are multiple. However, the dominant source in SCA patients is the LDH isoenzymes 1 and 2 predominantly in erythrocytes.[4,12,14] The significantly higher plasma LDH level of children with SCA in crises compared to the plasma LDH level of their counterparts in steady state in this present study is understandable. RBC lysis in crises is probably responsible for the LDH elevation. In support of this hypothesis that the severe acute hamolysis that occurred during crises was the cause of the elevated plasma LDH are the significantly low mean RBC count and high mean reticulocyte count of the group compared to the mean RBC count and mean reticulocyte count obtained for the steady-state group. Thrombocytosis is a source of plasma LDH, but this is considered unlikely to be the cause of the elevated plasma LDH in this study because platelet poor plasma was used for the plasma LDH analysis. In addition, megaloblastic anemia from folate deficiency a known cause of elevated plasma LDH was an unlikely reason because all the children with SCA that were studied were on routine folate supplementation of 5 mg daily and none of the subjects studied had hypersegmented neutrophils on blood film appearance. However, bone marrow examination of the children in this study was not indicated and hence not done.

Akenzua *et al*. in 1992 in Benin, Nigeria, did not find a significant difference between alpha-hydroxybutyrate dehydrogenase (α-HBDH) level in steady state patients and patients in crises.[10] α-HBDH comprises the anodal isoenzymes 1 and 2 of LDH with high specificity for the reduction of 2-oxobutyrate in the presence of NADH. The reasons for the difference in observations may be due to the fact that all the children with SCA in crises state that were studied at Benin were mainly in painful crisis as opposed to the children with SCA in crises in this present study in which 65% of the 40 children had only hemolytic crises and 35% had both hemolytic and painful crises. The dual factors of acute hemolysis of RBCs and bone marrow infarction which were thought to be the probable sources of plasma LDH of children with SCA in crises in this study may account for this. The higher plasma LDH level in children with SCA in the steady state compared to the plasma LDH level in the Hb AA children is likely due to chronic hemolysis which is an inherent aspect of the disease. LDH isoenzyme 1 is known to be cleared very slowly from circulation,[4,7,11] hence the higher value recorded in children with SCA in steady state may be due to the residual iso-enzyme 1 fraction in the plasma. The higher mean plasma LDH value than the normal reference value of 120–240 U/L in the Hb AA controls was most probably due to outlier's effect. The LDH enzyme assay was performed following the standard laboratory procedures described by the manufacturer of the LDH reagent kit and all necessary quality control measures were observed. Our study showed that plasma LDH level increases with increased clinical severity score (which is a reflection of the clinical severity of the disease) both in the steady state and during crises. For the steady state, this may suggest the possibility that chronic hemolysis occurred more among the children in moderate and severe clinical categories. For those in crises, it showed that acute hemolytic event is worse and its extent unpredictable among those in moderate and severe clinical categories since none of the children in crises belonged to mild clinical category. These observations are similar to the reports from the National Institute of Health in Collaboration with Cooperative Study of SCD[16] in the USA. Other workers elsewhere had also reported similar findings.[12-14]

Observations from the present study showed that plasma LDH correlated positively and significantly with clinical severity score in children with SCA in steady state. This is consistent with the findings of different workers in the USA, United Kingdom, and Jamaica.[12-14,16]

CONCLUSION

It is well-documented that, the clinical manifestations of SCA vary from one geographical location to another.[23,24] The causes of the diversity in clinical variations are disease modifying factors which may either be genetic or environmental.[23,24] Despite the clinical, genetic, and environmental differences related to SCA generally, the consistency of the relationship between LDH levels and clinical severity scores worldwide suggests that plasma LDH is probably a reliable indicator of SCA disease severity in children if measured in the steady state.

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Conflicts of interest

There are no conflicts of interest.

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