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Lactate dehydrogenase level and reticulocyte count in sickle cell anaemia children

AO Salako[1], OW Aworanti[2], CE Okoli[3], OO Odubela[1], SP Ogundeji[2], T Adeyemo[4] and EO Temiye[4].

Clinical Science Department[1], Nigerian Institute of Medical Research, Yaba, Lagos State, Department of Haematology[2], University College Hospital, Ibadan, Ibadan, Oyo State, Lifeline Children Hospital[3], Lekki Lagos and College of Medicine[4], University of Lagos, Idi-Araba, Lagos

Abstract

Introduction: Sickle cell anaemia (SCA) is a lifelong chronic haemolytic disorder with episodic acute manifestations and chronic end organ damage. Serum lactate dehydrogenase levels and reticulocyte count are predictors of disease severity. This study determined the serum lactate dehydrogenase (LDH) levels in children with SCA in steady state and crisis; and to correlate the serum LDH levels, reticulocyte counts and haematological parameters.

Methods: Across-sectional study conducted over a nine-month period, involving 212 children with SCA (106 subjects in steady state and another 106 subjects in crisis). Blood samples were taken from the subjects in the two groups, full blood counts (Haematocrit, leucocyte counts & differentials and platelet counts), reticulocyte counts and serum lactate dehydrogenase (LDH) levels done for each sample taken.

Results: There was no significant difference in the mean age [8.0±4.3 vs 8.0± 4.0 years, p=0.67], Body Mass Index [14.6 ±3.2 vs 15.6kg/m^{2,} p=0.07], and LDH levels [(740.0±270.3IU/L) vs (770.4±198IU/L), p=0.323]] for crisis and steady states respectively However, the mean reticulocyte count [0.59±0.48 vs (0.98±0.62%/ p<0.001) was significantly lower in steady state than in crisis state. In the crisis group, there were positive correlations between serum LDH and total white cell counts (r=0.14, p=0.05), serum LDH and platelet count (r=0.03, p=0.747), serum LDH and reticulocyte count (%) (r=0.11, p=0.26). There was a significant negative correlation between serum LDH and haemoglobin levels (r=-0.16, p=0.01).

Conclusion: Lactate dehydrogenase, a marker of haemolysis, was elevated in both steady and crisis states which further supports the fact that SCA is a chronic haemolytic disorder. Increased reticulocytes count as found in patients in crisis state is suggestive of increased erythropoiesis as a response to the pathology present in this clinical state. We recommend routine reticulocytes count checks as a measure to distinguish steady from crisis states.

Correspondence: Dr. O.W. Aworanti, Department of Haematology, College of Medicine, University of Ibadan, Ibadam. Nigeria. Email: aworantioladapo@yahoo.com.

Keywords: *Children; Sickle cell; Crisis, Lactate Dehydrogenase, Reticulocyte.*

Résumé

Introduction: La drépanocytose (ACS) est une maladie hémolytique chronique qui dure toute la vie , avec des manifestations aiguës épisodiques et des lésions chroniques des organes cibles. Les taux sériques de lactate déshydrogénase et le nombre de réticulocytes sont des prédicteurs de la gravité de la maladie. Cette étude a déterminé les taux sériques de lactate déshydrogénase (LDH) chez les enfants atteints d'ACS à l'état d'équilibre et en crise ; et de corréler les taux sériques de LDH, le nombre de réticulocytes et les paramètres hématologiques.

Méthodes : Une étude transversale menée sur une période de neuf mois, impliquant 212 enfants avec ACS (106 sujets en état d'équilibre et 106 autres sujets en crise). Des échantillons de sang ont été prélevés sur les sujets des deux groupes, des numérations globulaires complètes (hématocrite, numération et différentiels de leucocyte et numération plaquettaire), des numérations de réticulocytes et des taux sériques de lactate déshydrogénase (LDH) effectués pour chaque échantillon prélevé.

Résultats: Il n'y avait pas de différence significative dans l'âge moyen [8,0 ± 4,3 vs 8,0 ± 4,0 ans , p = 0,67], l'indice de masse corporelle [14,6 ± 3,2 vs 15,6 kg/m^{2,}p = 0,07] et taux de LDH [(740,0 ± 270,3 UI/L) vs (770,4 ± 198 UI/L), p = 0,323]]respectivement pour les états de crise et d'équilibre. Cependant, le nombre moyen de réticulocytes [0,59±0,48 vs (0,98±0,62%/ p<0,001) était significativement plus faible à l'état d'équilibre qu'à l'état de crise. Dans le groupe de crise, il y avait des corrélations positives entre LDH sérique et le nombre total de globules blancs (r = 0,14, p = 0,05), LDH sérique et la numération plaquettaire (r = 0,03, p = 0,747), LDH sérique et le nombre de réticulocytes (%) (r=0,11, p=0,26). Il y avait une corrélation négative significative entre les taux sériques de LDH et d'hémoglobine (r=-0,16, p=0,01).

Conclusion **:** Lactate déshydrogénase, un marqueur d'hémolyse, était élevée à la fois dans les états d'équilibre et de crise, ce qui confirme davantage le fait que l'ACS est un trouble hémolytique chronique. L'augmentation du nombre de réticulocytes observée

chez les patients en état de crise suggère une augmentation de l'érythropoïèse en réponse à la pathologie présente dans cet état clinique. Nous recommandons des contrôles de numération des réticulocytes de routine comme mesure pour distinguer les états stables des états de crise.

Mots clés : *Enfants ; Drépanocytose; Crise, Lactate Déshydrogénase, Réticulocyte.*

Introduction

Sickle cell anaemia (SCA) isthe homozygous form of sickle cell disease, which is an inherited autosomal recessive disorder [1,2]. The disease is prevalent amongst people of Sub-Saharan Africa, Mediterranean basin, Arabian Peninsula and India sub-continental ancestral origin. Sub-Saharan Africa accounts for the largest disease burden worldwide having 200,000 of the 300,000 live births delivered with sickle cell disease yearly [3-5]. Nigeria accounts for the highest disease burden and mortality in children below five years with SCA [6,7].

It is characterized by lifelong chronic haemolysis with ongoing risk of acute manifestations and multi-organ dysfunctions [1,8,9]. Clinical features (dactylitis, frequency of acute painful crisis and blood transfusions/year etc.) and laboratory parameters including haemoglobin concentration, fetal haemoglobin fraction, reticulocyte count and C-reactive protein have been used as predictors of the severity of sickle cell anaemia [10-12].

Serum LDH and reticulocyte count are important biochemical and haematological parameters used as markers of haemolysis and marrow response in haemolysis [13, 14]. High levels of these markers have been associated with severity of sickle cell anaemia [15, 16]. They have been described as predictors of pulmonary hypertension, priapism, leg ulcer, multi-organ dysfunction and death [16-21].
Elevated serum LDH levels and reticulocyte counts have been established in children with SCA in steady state [22, 23]. The value and the clinical significance of these parameters in children with SCA in crisis remain unknown despite the high burden of disease and its impact on the morbidity and mortality in the paediatric age group.

This study was undertaken to determine the values of serum LDH levels and reticulocyte count in children with SCA in crisis and to compare with those of children with SCA in steady state. It was hoped that the findings from this study would contribute to knowledge of the disease in children with SCA and the investigated parameters may become important markers of severity of crisis.

Methods

This was a cross-sectional study involving 212 children living with Sickle cell anaemia aged 1-18years (106 in acute painful crisis and another 106 in steady state) who presented at emergency room, or out-patient units of the Paediatrics Department of the Lagos University Teaching Hospital, Lagos, Nigeria. The study was conducted over a nine-month period, from March 2015-November 2015. Vaso-occlusive crisis was defined as occurrence of pain in the extremities, back, chests or any other parts of the body that led to hospital presentation and/or admission and could not be explained by any other disease entity except sickle cell disease [19]. Steady state refers to a point when patients do not experience acute painful episodes or any changes due to therapy [24]. All children with sickle cell anaemia with confirmed chronic disease [CVA, CKD, chronic heart disease], newly diagnosed SCA children in acute painful crisis, and known patients with fever and bacteremia were excluded. A case record form (CRF) was developed to collect data on detailed clinical information which included age, gender, and educational status of the participants. The anthropometric measurements of weight (kilograms), height (centimetres) and body mass index (kilograms per square metre) were obtained.

Ethical approval was obtained from Lagos University Teaching Hospital Health Research and Ethic Committee [LUTH HREC]. Assent were given by older individuals (10-18years) while written informed consent were taken from parents and caregiver of the patient who were not old enough to give assent.

Laboratory Parameters

Ten milliliters of venous blood sample was collected from all the participants at the time of presentation at either the emergency room or outpatient clinic for haematological parameters and serum Lactate Dehydrogenase levels (LDH). Five millilitres of the sample was aliquoted into EDTA vacutainer bottle for haematological parameters (haemoglobin, reticulocyte count, leucocyte & differential count, and platelet count) and another 5ml was dispensed into lithium heparin bottle for serum LDH. The complete blood count was analyzed immediately or refrigerated at 2-4℃ and analysis done within 6 hours using the Sysmex auto analyzer model KX-21N (Sysmex Corporation, Kobe, Japan) while the reticulocyte count in percentage was determined by manual counting. Serum LDH assay was analyzed by Turbidometry using Roche/Hitachi cobas ™ 912 ACN 206 auto analyzer (Roche/ Hitachi corporation, Indianapolis, USA.

Data Analysis

Data collected was recorded, validated, and analysed using the Statistical Package for Social Sciences (SPSS) software version 23. Descriptive and Inferential statistics were applied in the course of the analysis. Univariate statistic for continuous normally distributed data was presented as mean and standard deviation while proportions were used to summarize categorical data. Pearson Correlation was done to determine the relationship between serum Lactate Dehydrogenase levels and haematological parameters (haemoglobin levels, reticulocyte counts, platelet counts, leukocyte and differential counts). A p-value of <0.05 was considered statistically significant.

Table I: Socio-demographic Characteristics

Parameters	Crisis (n=106)	Steady state (n=106)	p-value
Age Mean±(SD)years			
Age group (years)	8.0(4.0)	8.0 (4.3)	0.67
1-7	58(54.7)	51(48.1)	
8-18	48(45.3)	55(51.9)	
Sex			
Male	59(55.7%)	53 (50%)	
Female	47(44.3%)	53 (50%)	
Body Mass Index (BMI)			
Mean(±SD)*(kg/m²)*	14.6 (3.2)	15.4 (3.2)	0.07

Results

Of the 212 participants, 106 were in each of the steady and crisis states. The mean(±SD) age of the children in the steady state was 8.0(±4.3) years while those in crisis was 8.0(± 4.0) years (p =0.67). There was no significant difference in the Body Mass index between the two groups, this is as shown in Table I.

The haematological parameters and serum lactate dehydrogenase levels (LDH) in the study participants are as shown in Table (II). Participants in crisis had higher levels of serum lactate dehydrogenase [770.0±198.0IU/l], reticulocyte count [0.97±0.6%], white cell count [18.3±8.3×$10^9/l$], platelet count [389±135×] and lower haemoglobin level at presentation [7.1±1.5 g/dl] compared to the steady state group with serum LDH [740.4±198.0 IU/l], reticulocyte count [0.59±0.48%], white cell counts [13.3±5.0×and haemoglobin level at presentation [7.5±1.2 g/dl]. The total WBC, absolute neutrophil counts and reticulocyte count were significantly higher in crisis sate than in steady state [18.3±8.3 x10^9/l vs 13.1±5.2 x10^9/l, p<0.001; 10.9 ±6.9 x10^9/l vs 5.6±2.4 x10^9/l, p<0.001 and 0.97±0.62% vs 0.59±0.48%, p<0.001respectively]

The result as shown in Table III demonstrated that there was significant negative correlation between serum LDH and haemoglobin (r=-0.16, p=0.01), while no significant correlation between serum LDH and WBC (r=0.14, p=0.05), serum LDH and neutrophil (r=0.11, p=0.31), serum LDH and lymphocyte (r=0.15, p=0.13), serum LDH and Platelet (r=0.03, p=0.747), serum LDH and reticulocyte count (r=0.11, p=0.26). However, in steady state, WBC was significantly higher in males than females (14.2 ±5.5 vs .12.1 ±4.1, t=2.2, p=0.03)

There was no significant correlation between reticulocyte count and age (r=0.003, p=0.92), LDH (r=0.05, p=0.452), Lymphocyte count (r=0.06, p=0.33) but there was a significant but weak correlation with PCV (r=0.25, p=0.003) and Neutrophil count (r=0.14, p=0.05)

Table 2: Comparison of the haematological parameters and serum lactate dehydrogenase levels [LDH] between study participants in crisis and steady state

Laboratory Parameters	*Crisisstate(n=106)*	*SteadyState(n=106)* mean(±SD)	p-value mean(±SD)
Haemoglobin[g/dl]	7.1 (1.5)	7.4(1.4)	0.195
White Cell Count [$10^9/l$],	18.3 (8.3)	13.1(5.2)	<0.001*
Neutrophil Count [],	10.9(6.9)	5.6(2.4)	<0.001*
Lymphocytes Count [],	5.8(3.5)	6.2(3.2)	0.399
Platelet Count [],	389.8(163.6)	384.4 (135.4)	0.792
Serum Lactate Dehydrogenase Levels (LDH)[IU/l]	770.4(198.0)	740.0(270.3)	0.352
Reticulocyte (%)	0.97(0.62)	0.59(0.48)	<0.001*

**Statistically significantIndependent sample T-test.*

Table 3: Correlation between serum LDH and haematological parameters among participants in crisis (n=106).

Variables	Correlation coefficient (r)	p-value
Haemoglobin [g/dl]	-0.16	0.01
Reticulocyte count (%)	0.11	0.26
White cell count [$10^9/l$]	0.14	0.05
Neutrophil count [$10^9/l$]	0.10	0.31
Lymphocyte count [$10^9/l$]	0.15	0.13
Platelet count [$10^9/l$]	0.03	0.75

Discussion

This study noted that the reticulocyte counts of children with SCA in crises were significantly higher than the reticulocyte counts of the children in steady state. This finding agrees with earlier reports, irrespective of the differences in the duration used in the definition of steady state [21,25,26]. It has been shown that in crisis state, tissue hypoxia, red cell aggregation and lysis lead to increased bone marrow response with resultant continuous release of reticulocytes into the circulatory system on the background of a chronic haemolytic state [27,28]. The released reticulocytes are important in

Table 4: Comparison of LDH and haematological parameters in steady state and crisis.

	Group	N	Mean	Std. Deviation	T	P value
Retic	Crises	106	0.973%	0.6216%		
	Steady	106	0.590%	0.4815%	5.09	0.000
LDH	Crises	106	740.019	270.2835		
	Steady	106	770.386	198.0397	2.97	0.352
PCV	Crises	106	21.537	4.6017		
	Steady	106	22.872	3.8261	2,83	0.023
Hb	Crises	106	7.125	1.4503		
	Steady	106	7.456	1.2010	3.60	0.000
WBC	Crises	106	18.332	8.3018		
	Steady	106	13.282	5.0067	14.29.	0.000
Neut	Crises	106	10.883	6.9435		
	Steady	106	5.712	2.3840	3.78	0.000
Lymphocytes	Crises	106	5.812	3.4696		
	Steady	106	6.276	3.1750	1.98	0.311

Independent sample t- test.
LDH- Lactate dehydrogenase; PCV- Packed Cell Volume Hb- Haemoglobin; WBC- White blood cell count; Neut- Neutrophil

Table V: Comparison of LDH and haematological parameters by gender

	Gender	N	Mean	Std. Deviation	T	P value
Retic	Male	112	0.772%	0.6070%		
	female	100	0.791%	0.5664%	0.007	0.81
LDH	male	112	763.357	250.6195		
	female	100	746.069	221.3324	0.832	0.817
Pcv	male	112	21.840	4.4226		
	female	100	22.612	4.0855	0.141	0.19
Hb	male	112	7.184	1.3756		
	female	100	7.410	1.2923	0.198	0.22
WBC	male	112	16.299	7.4744		
	female	100	15.256	7.0793	0.786	0.3
Neut	male	112	8.434	5.5446		
	female	100	8.145	6.0796	0.109	0.718
Lymphocytes	male	112	6.317	3.6804		
	female	100	5.739	2.8653	1.843	0.202

Independent sample t- test.
LDH- Lactate dehydrogenase PCV- Packed Cell Volume Hb- Haemoglobin

maintaining homeostasis and are also involved in enhancing the cascade of events in vaso-occlusion due to the enriched vascular endothelia adhesion molecules on the immature red cell membrane.

The serum LDH levels of children in crisis were higher than those in steady state; however, the difference was not statistically significant in this study. This finding differs from many other reported studies [15, 25-29]. The difference could be explained by the type of crisis in this study, predominance of acute painful crisis rather than hyper-haemolysis or haemolytic crisis in the present study. Vaso-occlusion has been described as the predominant pathophysiologic mechanism in acute painful crises, though an interplay does exist between the two main pathophysiologic mechanisms, which could account for the rise in serum LDH levels in the acute painful crises. Other reasons for the finding could be the difference in the analytical method and the forms of crises studied in previous reports. While Adefehinti et al. [30] had subjects with predominantly hyper-haemolytic crisis in their research, which explains the significantly higher serum LDH levels reported; it is known that haemolysis is a predominant source of serum LDH.

The haemograms of children with sickle cell anaemia in crisis and steady state in this study were similar to findings reported by different researchers [32-36]. The haemoglobin level at presentation of children in crisis was lower compared to children in steady state but this difference was not statistically significant. This is as a result of increased recruitments of sickled red cell, adherence of the red cell to endothelium and some degree of red cell lysis in the background of chronic haemolytic state in crisis [37, 38].

The white cell count of patients in this study was found to be significantly elevated in crisis compared to steady state group. This is as a result of a possible explanation for this observation is that in crisis, there was increased haemopoetic and inflammatory responses (release of inflammatory mediators, re-distribution of white cell between the marginal and circulating pools, with resultant changes in chemotaxis, adhesion of cell to cell and to vascular endothelium) on a background chronic inflammatory state in SCA [13,39].

Similar to findings from previous work [16,26,29], it was found that in the crisis group, there was a negative correlation between serum LDH levels and haematocrit. The reason for this relationship could be due to increased rate of haemolysis resulting from shortened life span of red blood cell which is hallmark of SCD and associated release of LDH from the damaged red cell and tissue/organ damage.

There existed a weak positive correlation between serum LDH levels and the WBC of participants in this study; this corresponds to findings from previous studies [16,26,29]. This association shows that in acute painful crisis, an interplay exists between the rise in WBC and serum LDH. There is also an exaggerated inflammatory response in crisis characterized by an increased migration of white blood cells from the bone marrow to the blood and altered function as red cell aggregation; lysis and cell turnover/tissue destruction which leads to elevated serum LDH levels in vaso-occlusive crisis [17,38]. However, there was no correlation between the serum LDH levels and platelet count,a similar observation to those reported in previous studies[16, ,26,29].

The positive correlation between reticulocyte counts and the haematocrit levels of the crisis group is also similar to previous reports [25,26, 29]. This is because in crisis state, the reduction in the haematocrit levels potentiates further release of reticulocytes from the bone marrow.

Conclusion

Lactate dehydrogenase and reticulocyte count are significantly higher in SCD in crisis compared to steady state and this suggests increased rate of red cell destruction during the crisis state. Increased reticulocytes count is suggestive of stressed erythropoiesis and is found in SCA more pronounced in crisis. We recommend routine checks of reticulocytes count to distinguish steady from crisis states.

Limitations

The other markers of haemolysis such as serum haptoglobin, bilirubin and urobilinogen were not assayed. We recommend further studies that would include these other parameters so that the relationship of all these makers of haemolysis could be explored in children with SCA.

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