Original Article

Correlation Between the Serum Lactic Dehydrogenase Levels with Clinical Severity Score of Sickle Cell Anemia in Adults

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ABSTRACT

Introduction : LDH is considered as a marker of hemolysis and indicator of risk of morbidity and mortality in sickle cell anaemia. Lactate dehydrogenase and plasma hemoglobin are usually elevated to about twice normal in the steady state of sickle cell anaemia . The identification of level of LDH is an important tool for the early detection of the severity of the disease in SCA individuals.

Aims & objectives : To study the correlation between serum LDH with clinical severity score of SCA in adults, steady state and crisis state. & to see the correlation between serum LDH with haematological variables in adults with SCA steady state & crisis state.

Material & Methods : In this Cross-sectional observational study, total 130 consecutive diagnosed patients of Sickle cell Anemia SS) in Steady & Crisis states attending OPD & IPD were included. Equal number of nonsickle controls were also included. Cases with Sickle cell trait (Hb AS), cases on antibiotics treatment prior to seeking medical care, received previous blood transfusion in the 3 months prior to the study, Patients taking hydroxyurea & Patients under chronic transfusion programme were excluded from the study.

We calculated clinical phenotype score based on recording the individual scores related to the most relevant medical history parameters. LDH assay were performed in all cases & controls. Hematological variables were assessed.

Results : Mean age at presentation of cases was 3.26 ± 4.24 years and controls was 23.24 ± 6.27 years. Mean serum LDH was 592.86 ± 231.98 U/L in cases and 192.85 ± 28.06 U/L in controls which was statistically significant. (p=0.001). Among the cases mean serum LDH in crisis state was 782.50 ± 167.85 U/L compared to 403.21 ± 83.09 U/L in steady state and according to clinical severity score serum LDH was 368.68 ± 49.56 U/L, 563.02 ± 96.65 U/L and 894.63 ± 69.42 U/L in asymptomatic, moderate and severe clinical phenotype respectively. (p=0.001). Among hematological variables mean values of haemoglobin, haematocrit, RBC count and MCHC were less in crisis state than steady state and in SCP than MCP and ACP. On the other hand, mean value of WBC, platelets, reticulocytes and MCV were more in crisis state than steady state and in SCP than MCP and ACP. (p=0.0001). Correlation coefficients of haemoglobin suggest independent correlation of Hb with other hematological parameters. (P=0.0082). Cholelithiasis was the overall most common complication among 130 cases followed by pneumonia and hip joint AVN in 16,11 and 3 cases respectively. Complications were significantly correlated with increased LDH levels. In intragroup analysis, complications were more common in crisis state compared to steady state. According to clinical score complications were present more in severe phenotype than moderate phenotype.

Conclusion :

1. Serum LDH is a good predictor of clinical severity in patients of sickle cellanaemia.

2. Increased levels of serum LDH well correlates with hematologicalvariables in steady state and crisis state.

Key words : Sickle cell anemia, Steady & Crisis state serum LDH, Clinical severity score, Asymptomatic moderate, Severe Phenotype.

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Introduction :

Sickle cell disease has high prevalence in India, especially in central and western regions. About 30% of children of SCD in tribal population die before they reach adulthood. Although knowledge about SCD in India is increasing it is very difficult to establish burden of the problem accurately in the

absence of effective screening programmes, nationwide reporting systems or registries. True incidence of SCD in India is still not known and most of our knowledge about natural history of diseases comes from the developed countries[1,2]

As the basic pathophysiology of sickle cell disease is destruction of sickled RBC, complete blood count and reticulocyte count are basic and important part of evaluation of the same.[3]

The degree of haemolysis is inversely related to the haemoglobin and packed cell volume in sickle cell patients. Many complications of sickle cell disease are associated with leucocytosis. Unlike the red and white blood cells, effects of sickle cell on platelets are not well understood.

Serum lactate dehydrogenase (LDH) is a marker of tissue damage occurring in conditions such as myocardial infarction, lung or liver disease, or in acute pancreatitis. It's relevance as a marker of intravascular hemolysis is of most interest in hemoglobinopathy, especially in sickle cell disease (SCD)[4].

LDH is considered as a marker of haemolysis and indicator of risk of morbidity and mortality in sickle cell anaemia[5,6] Lactate dehydrogenase and plasma hemoglobin are usually elevated to about twice normal in the steady state of sickle cell anaemia. It further increases during painful vaso-occlusive crisis because of hyperhaemolysis as showed by RBC survival studies. Elevated LDH levels at admission for VOC were associated with bad outcome, including death or requiring transfer of patients to intensive care unit[6].

Literature search showed that the elevation of LDH is associated with hemolysis, pain crisis, pulmonary hypertension, leg ulcer, kidney damage and endothelial activation with elevated soluble vascular adhesion molecules.[5] In sickle cell patients LDH correlates with creatinine clearance and, therefore, LDH could serve as a biomarker to predict renal insufficiency in those patients.[7]

The estimation of serum level of LDH is an important tool for the early detection of the severity of the disease in SCA individuals[8]. Despite the high prevalence and severity of the disease, there is scarcity of information about LDH in population suffering from sickle cell anemia in central India. The objective of this study was to see correlation between serum LDH & clinical severity score of SCA in adults, in steady and crisis state. and also to see association between serum LDH and hematological variables in adults with SCA (Steady & crisis state).

Material and Methods :

The study was conducted at a tertiary care hospital in Central India. The study was approved from Institutional Ethics Committee.

In this Cross-sectional observational study 130 consecutive patients of Sickle Cell anemia (Hb SS) in steady state and crisis state attending OPD & IPD were enrolled and were compared with an equal number of non sickle healthy controls.

Selection of cases :

All diagnosed cases of SCA (Hb SS) attending out door Sickle cell clinic in steady state who are free of pain for at least one month and had not been hospitalized or transfused for at least 90 days before the study.7 All SCA (Hb SS) indoor cases hospitalized for complications in crisis state.

The cases with Sickle cell trait (Hb AS), those on antibiotics treatment prior to seeking medical care. Previous blood transfusion in the 3 months prior to the study, Patients taking hydroxyurea and patients under chronic transfusion programme were excluded from the study.

Selection of controls :

One control was recruited for each case, (1:1) with normal adult haemoglobin (Hb AA). Controls were recruited from the patients attending general OPD for minor ailments, from the relatives accompanying admitted patients and from the office working staff from various departments of tertiary care centre. Controls having other haemolytic diseases, with Vitamin B12 or Folic acid deficiency & those who are on medications causing megaloblastic anaemia e.g. antiepileptics were excluded.

Data was collected over 18 months, all the subjects were explained about the purpose of the study and an informed consent was taken.

We calculated clinical phenotype score based on recording the individual scores related to the most relevant medical history parameters & categorized as follows[5] ***(Fig. 1)***

Asymptomatic clinical phenotype (ACP) : score = 5,

Moderate clinical phenotype (MCP) : score 6-15,

Severe clinical phenotype (SCP) : score = 16

Blood samples were taken in all the subjects, (cases &controls) for various haematological parameters. Another 3 ml of venous blood collected in dry tubes for serum LDH assay. LDH assay were performed with ADVIA 1800 spectrophotometer at 340 nm by lactate/NAD method. Reference range was 120-246 U/L. blood samples were collected as early as possible before administration of antibiotics and other treatment measures to avoid possible interference in results. Investigations like x-ray chest and x-ray hip joint, ultrasonography of abdomen 2D echocardiography and CT head were done whereever indicated.

Statistical Analysis :

Results were manually entered into a microcomputer and were analysed using the Excel Version and they were exported on SPSS 17.0 for further analysis. Data are represented as means ± SD when the distribution were normal. The analysis of Student's t-test was used for comparisons of means. ANOVA test was used to compare differences

Fig. 1 : **Clinical criteria and severity score based on phenotype[5]**

Clinical criteria	Variables	Score (points)
Days of hospitalisation/year	= 1	0
	2-7	2
	= 8	5
Severe vaso-occlusive crises/year	0	0
	1-2	2
	= 3	5
Blood transfusion/year	0	0
	1-2	2
	= 3	5
Hip disease (AVN)	Absent	0
	Present	5
Leg ulcer	Absent	0
	Present	5
Hepato biliary complications	Absent	0
	Present	5
	Cholecystectomy	2
Neurological events	Absent	0
	Present	5
Renal disorders	Absent	0
	Present	5
BMI	19-27	0
	< 19	2

Asymptomatic clinical phenotype (ACP) : score = 5,
Moderate clinical phenotype (MCP) : score 6-15,
Severe clinical phenotype (SCP) : score = 16

among categorical variables. Associations between variables and LDH were evaluated using chi-square and fisher exact test (for the cell with expected frequency less than 5 in two by two table more than 20%). Statistical significance level was set at p = 0.05.

Results :

Total 130 cases of sickle cell anaemia (Hb SS) steady state and crisis state and equal number of controls with normal adult haemoglobin Hb AA were enrolled for the study. Cases were analysed in two groups (Steady & Crisis state) comprising 65 subjects in each group. Mean age at presentation of cases was 23.26 ± 4.24 years and controls was 23.24 ± 6.27 years. Mean serum LDH was 592.86 ± 231.98 U/L in cases and 192.85 ± 28.06 U/L in controls with difference being statistically significant. **(p=0.001)**

Among the cases mean serum LDH in crisis state was 782.50 ± 167.85 U/L compared to 403.21 ± 83.09 U/L in steady state ***(Table 1).***

According to clinical severity score serum LDH was 368.68 ± 49.56 U/L, 563.02 ± 96.65 U/L and 894.63 ± 69.42 U/L in asymptomatic, moderate and severe clinical phenotype respectively which was statistically significant. **(P=0.001)** ***(Table1)***

Results of hematological variables revealed mean values of haemoglobin, haematocrit, RBC count and MCHC were significantly decreased in cases than controls. ***(Table 2)*** similarly hematological variables were (Hemoglobin, hematocrit, RBC count & MCHC) more decreased in crisis state than steadystate. ***(Table 3)***

Table 1 : **Comparison of mean Sr. LDH in steady state & crisis state & according to clinical severity score**

Sr. LDH (U/L)	Steady state (n=65)			Crisis state (n=65)			P-value
Mean	403.21			782.50			0.001*
	ACP (n=50)	MCP (n=15)	SCP (n=0)	ACP (n=0)	MCP (n=24)	SCP (n=41)	
Mean	368.68	518.33	-	-	590.95	894.63	
SD	49.56	77.76	-	-	894.63	69.42	
Min.	260	400	-	-	412	412	
Max.	487	651			734	991	

LDH : Lactate dehydrogenase, SD : standard deviation, ACP : Asymptomatic Clinical Phenotype, MCP : Moderate Clinical Phenotype, SCP : Severe Clinical Phenotype

Table 2 : **Comparison of haematological variables in cases & controls**

Hematological Variable	Cases (N=130)		Control (N=130)		p-value
	Mean	SD	Mean	SD	
Haemoglobin (g/dl)	8.22	2.39	14.2	1.02	0.0001*
Haematocrit (%)	28.22	5.09	41.73	2.28	0.0001
RBC count (×106)	2.88	0.89	4.24	0.52	0.0001*
WBC count (×103)	11.32	6.43	5.67	0.97	0.0001*
Reticulocytes (%)	6.24	3.2	1.27	0.39	0.0001*
Platelets (×103)	310.75	119.78	273.02	54.74	0.0012*
MCV (fl)	80.69	9.73	81.47	3.9	0.3987
MCHC (g/dl)	29.75	3.5	36.26	1.76	0.0001*

* Significant p-value, RBC: Red blood cell, WBC: White blood cell, MCV : Mean corpuscular volume, MCHC: Mean corpuscular haemoglobin concentration, SD : Standard deviation, fl : femtolitre, g/dl : gram per deciltre Mean

As per severity score hematological variables were abnormal in SCP than MCP and ACP. On the other hand, mean value of WBC, platelets, reticulocytes and MCV were more in crisis state than steady state and similarly same results in SCP than MCP and ACP. **(p=0.0001)** ***(Table 3)***

Correlation coefficients between LDH and hematological variables according to clinical severity score showed independent correlation of Hb with other hematological parameters.

In relation with Hemoglobin, significant correlations were observed between Hb and WBC in ACP and SCP, & Hb and RBC in MCP. No significant correlations were observed between Hb and reticulocytes in any group. ***(Table 4)***

Out of 130 cases Cholelithiasis was the overall most common complication observed followed by pneumonia and hip joint AVN 16, 11 and 3 cases respectively. Complications were significantly correlated with increased LDH levels. In intragroup analysis, complications were more common in crisis state compared to steady state. **(p=0.0001, p=0.0044, p=0.0411)**

(Table 5) According to clinical score complications were present more in severe phenotype than moderate phenotype. Asymptomatic phenotype had no complications. ***(Table 6)***

Table 3 : **Comparison of hematological variables according to clinical severity score**

Hematological Variable	Steady state (n=65)			Crisis state (n=65)			P-value
	ACP (n=50)	MCP (n=15)	SCP (n=0)	ACP (n=0)	MCP (n=24)	SCP (n=41)	
Haemoglobin (g/dl)	10.31	6.98	-	-	7.69	6.42	0.0001 *
Haematocrit (%)	33.10	26.42	-	-	27.56	23.31	0.0001*
RBC count (×106)	3.27	3.16	-	-	3.10	2.18	0.0001*
WBC count (×103)	5.84	13.36	-	-	12.61	16.84	0.0001*
Reticulocytes (%)	3.14	6.26	-	-	6.35	9.95	0.0001*
Platelets (×103)	287.18	234.06	-	-	367.37	334.41	0.1646
MCV (fl)	78.17	78.83	-	-	80.90	84.08	0.0221*
MCHC (g/dl)	32.09	30.42	-	-	28.06	27.54	0.0001*

*Significant p-value, RBC : Red blood cell, WBC : White blood cell, MCV : Mean corpuscular volume, MCHC : Mean corpuscular haemoglobin concentration, ACP : Asymptomatic Clinical Phenotype, MCP : Moderate Clinical Phenotype, SCP : Severe Clinical Phenotype, fl : femtolitre, g/dl : gram per deciliter

Table 4 : **Correlation coefficients between LDH and haematological variables according to severity score in sickle cell study population**

Variable	Correlation coefficient of HB i.e. "r" (p value)		
	ACP (n=50)	MCP (n=39)	SCP (n=41)
WBC	-0.3697 (0.0082)*	-0.2521 (0.1215)	-0.5137 (0.0006)*
RBC	0.1927 (0.1799)	-0.5455 (0.0003)*	0.7160 (0.0001)
Reticulocyte	0.2370 (0.0975)	-0.0753 (0.6485)	0.0788 (0.6244)

*significant p value, RBC : Red blood cell, WBC : White blood cell, ACP : Asymptomatic Clinical Phenotype, MCP : Moderate Clinical Phenotype, SCP : Severe Clinical Phenotype.

Table 5 : **Correlation of Sr. LDH with complications in cases (steady state & crisis state, n=130)**

Complications	Mean LDH (U/L)		p-value
	Present	Absent	
Cholelithiasis (n=16)	814.88	561.70	0.0001*
Pneumonia (n=11)	781.64	575.41	0.0044*
Hip joint AVN (n=3)	862.67	586.49	0.0411*

*significant p value, RBC : Red blood cell, WBC : White blood cell, ACP : Asymptomatic Clinical Phenotype, MCP : Moderate Clinical Phenotype, SCP : Severe Clinical Phenotype.

Table 6 : **Correlation of Sr. LDH with complications according to clinical severity score**

Complication	ACP (n=50)		MCP (n=39)		SCP (n=41)		p-value
	Mean	SD	Mean	SD	Mean	SD	
Cholelithiasis	-	-	563.25	89.54	898.75	79.50	0.0001*
Pneumonia	-	-	610.40	84.81	924.33	35.24	0.0001*
Hip joint AVN	-	-	-	-	862.66	75.24	0.0001*
PH							
Stroke							

Discussion :

As reported in literature, LDH is an enzyme involved in anaerobic glycolytic pathway with RBC and reticuloendothelial system being the important source of it. During intravascular hemolysis it is released from the destruction of RBC. Major pathophysiological mechanism of sickle cell disease involves premature destruction of RBC and intravascular hemolysis leading to raised LDH. Intravascular hemolysis also leads to decreased availability of Nitric Oxide (NO), release of inflammatory mediators and cytokines and vascular adhesion molecules leading to hemolysis associated vasculopathy. Reports from various previous studies indicate that sickle cell patients in steady state have high serum LDH which further increases during crisis episodes. Elevated LDH is also associated with various complications in sickle cell patients. LDH is considered as a marker of hemolysis and indicator of severity of the disease.[5]

The present study tried to evaluate this fact. Results of our study showed that serum LDH levels are significantly high in cases as compared to controls (592.86 U/L and 192.85 U/L respectively).& also found significantly more in crisis state (782.50 U/L) as compared to steady state (403.21 U/L) in intragroup analysis. These findings of our study are comparable with the results of study carried out by **Tite Minga Mikobi *et al* (2016)**[5] Similar kind of reports regarding increased levels of LDH in crisis state also came from **Adefehinti O *et al* (2009)**[9] **Gregory J kato *et al* (2005)**[8]**.** This elevated LDH levels could be attributed to oxidative stress associated with chronic hemolysis in sickle cell cases and further increased hemolysis in crisis state.

As per clinical severity score, mean serum LDH in ACP, MCP, SCP was 368.68 U/L, 563.02 U/L and 894.63 U/L respectively indicating increased hemolysis in SCP compared to other 2 phenotypes. These observations are well correlating with previous studies. **Tite Minga Mikobi *et al* (2016)**[7] have also reported similar findings of raised serum LDH as per clinical severity score in 211 patients of sickle cell anemia. They observed increased values of LDH in sickle cell patients compared to normal adults and in sickle cell patients they found that LDH was more in SCP than MCP and ACP. Similar results were also reported by **Oula Abdullah Najim et al (2011)**[10] in their prospective descriptive study done in 76 sickle cell patients between 1-18 years & showed significant positive correlation between LDH level and severity of pain ($p<0.05$) on logical regression analysis.

The present study further assessed hematological parameters in cases and controls. We found that mean values of haemoglobin, haematocrit, RBC count and MCHC were decreased in cases as compared to controls and mean values of WBC, reticulocytes and platelets were more in cases than controls. These findings were statistically significant. No significant correlation was found for MCV in our study.

The results of intragroup analysis and analysis according to clinical severity score revealed mean values of haemoglobin, haematocrit, RBC count and MCHC were decreased in crisis state than steady state and in SCP. On the other hand, mean value of WBC, platelets, reticulocytes and MCV were more in crisis state than steady state and in SCP than MCP and ACP. Correlations of these hematological parameters with serum LDH were statistically significant. Also, the logistic regression analysis showed significant correlation between Hb and WBC count in Asymptomatic and severe clinical phenotype and between Hb and RBC count in moderate clinical phenotype. This can be explained by basic pathophysiology of sickle cell disease having destruction of RBC leading to intravascular hemolysis causing reduced haemoglobin, RBC count and increased reticulocytes due to ineffective erythropoiesis. These findings of our study are comparable to previous authors reports. **A I Juwah *et al* (2004)[11].** Study conducted by **C. E. Omoti (2002)[12].** Author did not report significant correlation between hematological parameters except for MCV and neutrophil count, which was observed in our study. **Tite Minga Mikobi *et al* (2016)[5]** reported the same observations. According to clinical severity score, average values of Hb, Hb F, hematocrit and red blood cells of patients in ACP clinical phenotype were significantly higher than those of the two other phenotypes MCP and SCP. Variables like white blood cells, platelets, reticulocytes, and serum LDH were significantly increased in patients with SCP clinical phenotype. than those of two other clinical phenotypes. In this study additional parameter like Hb F was measured which was not considered in our study.

The possible correlation between increased LDH and various complications (cholelithiasis, Pneumonia, Avascular necrosis of femur, leg ulcer, Acute chest syndrome) have been reported in previous studies[13,14].

Tite Minga Mikobi *et al* (2016)[7] reported increased complications like leg ulcers, cholelithiasis, femoral head necrosis in SCP than MCP and ACP and significant increase in serum LDH was observed in these patients.

In our study most common complication observed was cholelithiasis in crisis state as compared to in steady state. Next common complications observed were pneumonia. AVN of hip was seen in only few cases of crisis state. Results and analysis of complications as per severity score showed cholelithiasis as most common in severe clinical phenotype and moderate clinical phenotype.. Whereas, pneumonia was found in 06/41(14.63%) cases in severe phenotype & 05/39 (12.82%) in moderate phenotype group. Thus, complications were significantly more in crisis state than steady state.

Observations reported by **Gregory J kato *et al* (2005)[8]** revealed positive correlation between increased serum LDH and hemolysis related complications. The study concluded that LDH, a readily available biochemical laboratory test, which predicts a hemolysis-associated endothelial dysfunction, a sub phenotype of sickle cell disease that includes pulmonary hypertension, priapism, cutaneous leg ulceration, and risk of death. Another study by **García-Morin M *et al* (2016)[14]** tried to see association of serum LDH level as a Marker of the Severity of Vaso-Occlusive Crisis in Children with Sickle Cell Disease. They observed that High serum lactate dehydrogenase (LDH) level was a predictor of severity of crisis and was associated with the need for major opioids during admission and more days of hospital stay in crisis. However, sample size was small (i.e. 29 cases). Our study was conducted in adults and opioid use was not considered.

Thus the results of our study are comparable with previous reports, present study could not establish the causal relationship between increased serum

LDH and hematological parameters & complications in steady state and crisis state.

Conclusion :

1. Serum LDH is a good predictor of clinical severity in patients of sickle cellanemia.
2. Increased levels of serum LDH well correlates with hematological variables in steady state and crisis state.

Study Limitations :

1. It was a hospital-based study and sample was small but adequate enough to decide power of the study.
2. Simultaneous evaluation of Hb F was not done which is considered as a disease modifier in sickle cell patients and can have independent effect on hematological variables.

Study Implications :

1. Serum LDH is considered as an indicator of hemolysis and severity of the disease in sickle cell patients and is readily available and cost-effective investigation.

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