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Original Article

Lactate Dehydrogenase as a Biomarker for Early Renal Damage in Patients with Sickle Cell Disease

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ABSTRACT. Among many complications of sickle cell disease, renal failure is the main contributor to early mortality. It is present in up to 21% of patients with sickle cell disease. Although screening for microalbuminuria and proteinuria is the current acceptable practice to detect and follow renal damage in patients with sickle cell disease, there is a crucial need for other, more sensitive biomarkers. This becomes especially true knowing that those biomarkers start to appear only after more than 60% of the kidney function is lost. The primary purpose of this study is to determine whether lactate dehydrogenase (LDH) correlates with other, direct and indirect bio-markers of renal insufficiency in patients with sickle cell disease and, therefore, could be used as a biomarker for early renal damage in patients with sickle cell disease. Fifty-five patients with an established diagnosis of sickle cell disease were recruited to in the study. Blood samples were taken and 24-h urine collection samples were collected. Using Statcrunch, a data analysis tool available on the web, we studied the correlation between LDH and other biomarkers of kidney function as well as the distribution and relationship between the variables. Regression analysis showed a significant negative correlation between serum LDH and creatinine clearance, R (correlation coefficient) = -0.44, P = 0.0008. This correlation was more significant at younger age. This study shows that in sickle cell patients LDH correlates with creatinine clearance and, therefore, LDH could serve as a biomarker to predict renal insufficiency in those patients.

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Introduction

Sickle cell disease is an incurable, inherited form of hemolytic anemia. The red blood cells become crescent shaped and rigid and therefore not only is their ability to carry oxygen impaired but also they can occlude small vessels and block the blood flow.[1]

Among many complications of sickle cell disease, renal failure is the main contributor to early mortality.[2] It is present in 4–21% of patients with sickle cell disease.[3,4] For different reasons, there is a gradual infiltration of the glomerulus, resulting in glomerular sclerosis and proteinuria, which eventually leads to renal failure.[5]

Many biomarkers have been studied to detect early renal involvement, including estimated glomerular filtration rate (eGFR), microalbuminuria and proteinuria. Estimated GFR was found to be highly affected by aging; therefore, it is an unreliable biomarker. Also, both microalbuminuria and proteinuria are common findings in patients with sickle cell disease in both adults (up to 45%) and children (up to 30%).[6-9]

The risk for microalbuminuria and proteinuria was found to vary between patients with sickle cell disease. One study showed that the risk for both microalbuminuria and proteinuria was related to age and hemoglobin (Hb) level, with the risk becoming higher as the patient ages and the risk becoming lower when Hb rises. In the same study, microalbuminuria and proteinuria were found to develop during childhood, with both being amenable to treatment with drugs, namely hydroxyurea and angiotensin-converting-enzyme inhibitor (ACEI).[7]

The primary purpose of this study is to determine whether lactate dehydrogenase (LDH) positively correlates with other, direct and indirect biomarkers of renal insufficiency in patients with sickle cell disease and, therefore, could be used as a biomarker for early renal damage in patients with sickle cell disease. Our hypothesis is that LDH correlates with biomarkers of renal insufficiency.

In most parts of the world where neither dialysis nor transplantation is available or is extremely expensive, sickle cell disease nephropathy means an end-stage disease. Hence, a large number of people die from renal failure. Practicing preventive nephrology, along with education, is the most cost-effective practice. This is especially true knowing that there are approved agents for preventing or delaying the progression of renal failure, such as angiotensin-receptors blockers and aldosterone.[7] This makes efforts in investigating biomarkers for the early detection of renal failure not only useful but rather essential.

Materials and Methods

Fifty-five patients with an established diagnosis of sickle cell disease were recruited in this study at the hematology clinic at the King Khalid University Hospital, King Saud University in Riyadh, Saudi Arabia, between September 2008 and January 2009. After obtaining informed consent from all patients, one sample of blood was taken and 24-h urine collection samples were collected, where the patients were given a urine collection storage container and were instructed to keep them refrigerated at all times. The test started by urinating directly in the toilet, not saving this urine, and writing down the time on the storage container. For the next 24 h, urine was collected in the container. This 24-h urine collection sample was sent to the lab at the same institution. Serum samples were analyzed for LDH, blood urea nitrogen (BUN) and creatinine. The urine samples were analyzed for creatinine clearance, creatinine and protein. The study protocol was approved by the Saudi Arabian Institutional Review Board.

The final study population consisted of 55 patients with sickle cell disease, with an average age of 30.7 years. The age range (65 years) was relatively big, with the oldest patient being 78 years of age and the youngest patient being 13 years old. Thirty-six of the patients were male (65.4%).

Statistical Analysis

We summarized the data using means, median, range and standard deviations (SDs). Using Statcrunch, a data analysis tool available on the web, we performed a simple linear regression analysis of the data set, with LDH being the dependant X variable and other biomarkers for renal function, i.e. 24-h urine creatinine clearance, urine proteins, serum BUN and serum creatinine being the independent (Y) variables;

Table 1. Summary statistics (n = 55).

	Mean	Var.	Std. dev.	Std. err	Median	Range	Min	Max	Q1	Q3
Age	30.65	126.23	11.24	1.51	29	65	13	78	23	37
24-h CC	96.07	1491.93	38.63	5.21	94.03	161.53	12.26	173.79	71.21	124.84
BUN	3.30	1.16	1.08	0.15	3	4.7	1.3	6	2.5	4.2
Blood C	51.98	262.76	16.21	2.19	48	69	27	96	40	60
LDH	301.05	12,500.72	111.81	15.08	290	494	119	613	213	340
Urine proteins	0.13	0.029	0.17	0.02	0.08	0.9	0.01	0.91	0.05	0.14
Hb	99.27	271.83	16.49	2.22	98	69	70	139	85	109

CC: Creatinine clearance, BUN: blood urea nitrogen, C: creatinine, Hb: hemoglobin.

Table 2. Simple linear regression results; dependent variable = LDH, independent variable = 24-h creatinine clearance.

Parameter estimates:						
Parameter	**Estimate**	**Std. err.**	**Alternative**	**DF**	**T-Stat**	***P*-value**
Intercept	141.535	13.691	≠0	53	10.337	<0.0001
Slope	-0.151	0.0427	≠0	53	-3.538	0.0008

Sample size: 55, R (correlation coefficient) = -0.4371.

the fitted regression lines were plotted. We studied the correlation between LDH and other biomarkers of kidney function as well as the distribution and relationship between the mentioned variables. *P*-value <0.05 was considered to be statistically significant.

Results

Mean creatinine clearance, with 24-h urine collection, was 96.07 mL/min. The mean BUN was 3.30 mmol/L. Average serum creatinine was 51.98 mg/dL. The mean serum level of LDH was 301.05 uL, with a minimum serum LDH of 119 u/L; the maximum serum LDH was 613 u/L. Mean urine protein was 0.13 g/L and mean Hb was 99.27 g/dL (Table 1).

Regression analysis showed a significant negative correlation between serum LDH and creatinine clearance, R (correlation coefficient) = -0.44, *P* = 0.0008, *P*-values for R not shown in the tables (Table 2 and Figure 1).

Neither correlation between LDH and BUN (R = 0.08) nor correlation between LDH and serum creatinine (R = 0.07) were significant. Also, regression analysis showed no correlation between LDH and urine proteins. In the same manner, there was no significant correlation between LDH and Hb.

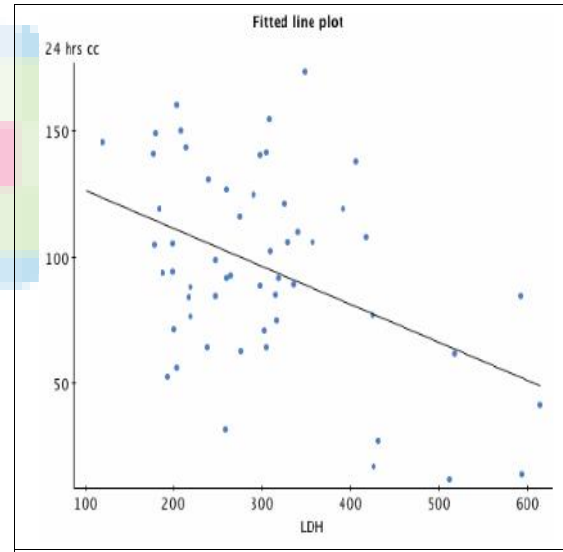

Figure 1. Simple linear regression, Y = creatinine clearance, X = LDH.

In order to study whether correlation between LDH and creatinine clearance at an earlier age would be more significant, we split the data into two groups based on age. The cut-off age was 29 because it is known that LDH becomes less specific at an older age.

The regression analysis for patients <30 years old between LDH and creatinine clearance was

Table 3. Simple linear regression results for patients <30 years; dependent variable = LDH, independent variable = 24-h creatinine clearance.

Parameter estimates:						
Parameter	**Estimate**	**Std. err.**	**Alternative**	**DF**	**T-Stat**	***P*-value**
Intercept	413.949	52.587	≠0	26	7.872	<0.0001
Slope	-1.294	0.492	≠0	26	-2.633	0.0141

Sample size: 28, R (correlation coefficient) = -0.4588.

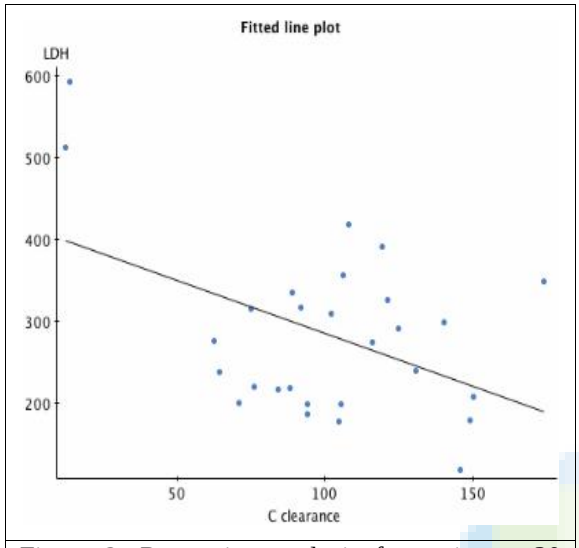

Figure 2. Regression analysis for patients <30 years old, Y = LDH and X = creatinine clearance.

significant at R = -45 (*P* = 0.016) (Table 3 and Figure 2).

Although in this age group the correlation between LDH and BUN was found to be R = -0.34, the *P*-value was 0.076. Nevertheless, no significant correlation was found between LDH and serum creatinine for patients <30 years or age. For patients >30 years old, there was no significant correlation between LDH and the other variables.

Multi-variant analysis for the original group was performed (Table 4), with LDH being the dependent variable, and the independent variables were age, creatinine clearance, serum creatinine, blood creatinine, urine proteins and Hb. The regression equation showed that LDH is only "significantly" affected by creatinine clearance, and for those sickle cell disease patients the average serum LDH increased by 1.28 umol/L per 1 mL/min drop of creatinine clearance.

Discussion

Renal insufficiency is one of the main early complications of sickle cell disease. Unlike other indirect markers of kidney function, creatinine clearance is the most used tool to estimate GFR.

Although screening for microalbuminuria and proteinuria is the current acceptable practice to follow renal damage in patients with sickle cell disease, there is a crucial need for other, more sensitive biomarkers. This becomes especially true knowing that indirect markers of renal function such as microalbuminurea start to appear only after more than 60% of the kidney function is lost.[10]

Five isoenzymes of LDH have been disco-

Table 4. Multiple linear regression results; dependent variable = LDH, independent variable = age, creatinine clearance, serum creatinine, blood creatinine, urine proteins and hemoglobin.

Parameter estimates:				
Variable	**Estimate**	**Std. err.**	**T-Stat.**	***P*-value**
Intercept	394.188	98.797	3.990	0.0002
Age	0.255	1.333	0.192	0.849
24-h CC	-1.281	0.369	-3.474	0.001
BUN	19.223	14.215	1.352	0.183
Blood C	-0.144	0.987	-0.146	0.885
Urine proteins	-120.795	87.242	-1.385	0.173
Hb	-0.177	0.914	-0.194	0.847

CC: Creatinine clearance, BUN: blood urea nitrogen, C: creatinine, Hb: hemoglobin.

vered.[11] In 1980, Miller et al described for the first time the 6th isoenzyme of LDH that is present and active in individuals whose sickle cell Hb is the greater component (HbS >50%).[12] Later, Sherman et al argued that the observed lesser severity of malaria in individuals with HbS could be attributed to the 6th isoenzyme LDHp in the organism competing directly with the parasite cytoplasm LDH.[13]

Although LDH has a proven diagnostic value in myocardial infarction,[14] its wide distribution in the body limits its specificity. In 1991, Kang et al studied the changes of LDH activity in renal diseases. They studied 44 patients with hemorrhagic fever, ten patients with nephrotic syndrome, ten patients with chronic renal failure on hemodialysis and 15 healthy subjects. They reported that LDH has a positive correlation with BUN but no correlation with serum creatinine,[15] although this study was general for renal disease.

Before that, Nielsen et al investigated the concentration of LDH isozyme in different parts of kidney tissues.[16] The hypothesis was that the isoenzyme pattern reflects an altered synthesis of LDH in the kidneys as an adaptive response to hypoxia in the early phase of acute renal failure. They studied the concentrations in healthy kidney and then studied concentrations in serum from patients with different kinds of kidney disease, i.e. in healthy kidneys; they found that the concentration of a special LDH subunit named M-subunit was higher in the cortex compared with the papilla in healthy kidneys. They found that LDH is high, with an inconclusive isoenzyme pattern in patients with chronic renal failure or renal trauma. However, in acute oligouric glomerulonephritis, serum LDH was moderately elevated in contrast to a very high elevation in acute renal failure of widely different pathogenesis. In both groups, the LDH isoenzyme pattern has a uniform relative increase of the M-subunit concentration.

To further understand those isozyme patterns and whether they could help in distinguishing different diseases by localizing the damaged tissue, as well as excluding other diseases, Cohen et al investigated this and found that in both renal and myocardial infarction there is elevation of serum alfa isozyme. They reported that the two diseases are indistinguishable using solely the pattern of LDH isozymes, and a clinical picture should be taken into consideration when making a diagnosis.[17]

Serum LDH is currently being used as a marker for the risk of vaso-occlusive crisis (VOC) and pain crises in sickle cell disease patients. It is known that LDH is a useful biomarker for intravascular hemolysis, such as, thrombotic thrombocytopenic purpura and paroxysmal nocturnal hemoglobinuria rather than extravascular hemolysis. Although most hemolysis in sickle cell disease is extravascular (70%), in VOC there is more intravascular hemolysis. Therefore, the gold standard is serum LDH.[18,19]

Furthermore, LDH could be a biomarker not only for hemolysis but also for mortality in sickle cell disease. Kato et al studied whether LDH works as a mortality biomarker in sickle cell disease. He found that patients with higher serum LDH than the median range have a higher mortality rate than those with lower than the median range LDH (P = 0.02). Therefore, high, steady levels of LDH could predict early mortality.[20]

O'Driscoll et al are one of the few authors to have studied LDH in sickle cell disease in children, and they have further supported the claim by reporting that LDH elevation is correlated with an increased risk for stroke and that it is related to anemia and low level of Hb. Although O'Driscoll et al did not find evidence that LDH is related to hemolysis, they still suggested that LDH could be used as a biomarker in sickle cell disease in children.[21]

In another study, Neely et al studied the LDH and Hb elevation in sickle cell disease. They found that both Hb and LDH are significantly elevated during sickle cell crises. Although Hb in general could be a source of LDH, during sickle cell crises, it is not related or correlated with the rise in LDH. Therefore, LDH level could be a useful tool in diagnosing sickle cell painful crises (no P-values reported).[19] Darbari et al reported the same finding but in children

thus providing evidence that the current practice is valid in children as well.[22]

On searching the PubMed database, we found only one similar study to our proposed plan. Gurkan et al published a study in 2010 with 40 patients aged 5–19 years. They studied the correlation between serum LDH levels and other biomarkers. Microalbuminuria (urine albumin/creatinine >30 mg/ng) was present in six patients (15%) and proteinuria (urine protein/creatinine >0.2 mg/ng) was present in two patients (5%). Despite those small patient numbers, they reported a positive correlation between LDH and microalbuminuria (R = 0.47, *P* = 0.04) and positive correlation between LDH and proteinuria (R = 0.48, *P* = 0.035).[23] This supports our hypothesis that LDH could help indentify risk for kidney involvement in sickle cell disease and thus early intervention.

In a similar manner but in diabetic patients, Mohammadi-Karakani et al in 2007 studied the urine LDH level and its relation to renal injury in diabetic patients. They found that the level of LDH excretion is high in diabetic patients compared with healthy subjects. Therefore, they concluded that LDH excretion has diagnostic validity in detecting renal damage in diabetic patients, with a sensitivity of 62.5% and a specificity of 58.3%.[24]

Investigating LDH isozymes and their potential role in detecting renal injury, Schoenenberger et al used an experimental approach to determine the correlation of hemodynamic changes with increases in urinary LDH as a new parameter reflecting serious renal tissue damages. They found that the urinary LDH in mice was increased by 800% after renal artery constriction. In addition, the isozyme pattern was LDH 1 increased and LDH V decreased. However, neither occlusion of the renal vein nor the ureters gave similar results. They concluded that the kidney responds to hypoxia by excreting LDH in urine.[25] This supports the idea that LDH is observed more in the acute phase rather than in later, chronic renal failure, and that it has potential to be useful as a biomarker in the early detection of sickle cell nephropathy.

In this study, we found that LDH negatively correlated with creatinine clearance. This result indicates more clearly that LDH might be useful for detecting early renal involvement. No previous study that we know of has studied the relationship between such a direct marker of renal function, creatinine clearance and LDH. This is mainly due to the difficulty of collecting urine for 24 h. However, our results confirm what previous studies have suggested. When we split the data based on age, we still had a significant correlation but with a larger slope, i.e. more change in LDH as creatinine clearance changes. This could be explained by LDH being more specific at a younger age. In addition, sickle cell disease patients who are <30 years old generally have fewer co-morbid health conditions.

It is worth noting that those patients have a mean creatinine clearance (96.07 mcmol/L) that is at the high normal (normal reference: 53–106 mcmol/L) level. This could be because they have anemia and higher blood flow to organs, including the kidneys. Although we know that LDH is highly affected by hemolysis, we still find a correlation between LDH and creatinine clearance. This is a strong clue that LDH might carry a high specificity for renal injury in patients with sickle cell disease.

In conclusion, this study shows that in sickle cell patients, LDH is correlated with creatinine clearance and, therefore, LDH could serve as a biomarker to predict renal insufficiency in those patients.

There are some possible limitations for this study. Simple linear regression does not give information about causality or risk. However, this was partially addressed by multivariable linear regression. In our sample, we do not know how acute the renal damage is due to poor follow-up. This is important especially to assess LDH rise as an early response to hypoxia. Not to mention, wide distribution of LDH in the body limits its specificity. LDH levels could be useless in patients with co-morbidities, especially heart disease. In addition, LDH levels are altered during different pathological states in sickle cell disease, such as pain crises

and hemolytic. This limits its specificity for renal insufficiency. We did not measure the LDH isoenzyme pattern, with or without LDHp, which might be useful. We suggest that future investigators consider doing this.

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