The Correlation between Lactate Dehydrogenase, Creatine Kinase and Total thiol Levels in Sera of Patients with β-Thalassemia

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Abstract

Lactate dehydrogenase (LDH), creatine kinase (CK) activities and total thiol levels had been measured in serum samples for 30 β-Thalassemia patients, aged 7-35 years who had been treated at Thalassemia center in the Babylon Maternity and children Hospital for the period between April 2008 to July 2008, compared with 30 healthy controls aged 7-35 years. The purpose of this study was to investigate the activity of these enzymes and the levels of total thiol in β-Thalassemia patients as indicators of the relationship between these parameters and complication of Thalassemia. There was a significant increase of LDH, no significant decrease of CK activity. There was no statistical significant difference between patients and controls as regards to total thiol levels.

Key words: β- Thalassemia, Lactate dehydrogenase LDH, Creatine kinase CK, Total thiol.

الخلاصة

تم دراسة كل من اللاكتيت ديهايدروجينيزLDH و الكرياتين كاينيزCK والثايول الكلي لمرضى البيتا ثلاسيميا (30 مريض) تتراوح اعمارهم بين 7-35 سنة ومقارنة النتائج بمجموعة الاصحاء(30 شخص) اعمارهم 7-35 سنة . وقد استنتج من البحث ان هناك ارتفاعا معنويا في قيم اللاكتيت ديهايدروجينيز وانخفاضا غير معنويا في قيم الكرياتين كاينيز في حين لوحظ انخفاضا معنويا في قيم الثايول الكلي لدى المرضى مقارنة بالأصحاء. ويمكن الاعتماد على العلاقة بين المتغيرات الثلاثة LDH, CK, total thiol لدراسة تطور الحالة المرضية.

Introduction

The term β–Thalassemia refers to a group of inherited hemoglobinopathies characterized by a reduced or absent synthesis of the β-globin chain. In homozygous β-thalassemia, the relative excess of insoluble α -globin chains precipitates within the red cell, which causes ineffective erythropoiesis and severe anemia[1]. Increased γ-globin chain and fetal hemoglobin (HbF) synthesis is capable of compensating for the imbalance between α- and β -globin chains and may improve the clinical manifestations of β-thalassemia. The membrane damage induced by the excess of free α-chains plays a crucial role in the shortening of erythrocyte life span and ineffective erythropoiesis lead to defective hemoglobin synthesis, sever anemia and excessive iron absorption then leading to iron overload in the patient tissues [2,3]. Transfusion is the main treatment of major thalassemia, prolonged and repeated transfusion result in iron overload and hemosidrosis, currently, cardiac complications are reported to cause 71% of death in patients with beta thalassemia, therefore the heart is the target lethal organ in thalassemia[4,5].

The complication of iron overload especially in heart includes pericarditis, arrhythmia, myocarditis and resistant heart failure[6].

There are many definitive diagnostic method for myocardial heamosidrosis, echocardiography, high ESR(Erythrocyte sedimentation rate) and enzymes and cardiac protein in serum may be seen following acute and chronic inflammatory processes of myocard. The relation between myocardial iron and other measures such as serum ferritin and liver iron has been unknown until recently and therefore assessment of cardiac risk these measures has at best been uncertain [7].

Creatine kinase and lactate dehydrogenase are specific cardiac enzymes that are used for evaluation of heart involvement and in patients when there is injury or inflammation[7,8]. Creatine kinase, CK(EC 2.7.3.2) is a cytosolic and mitochondrial enzyme with wide tissue distribution, and catalysis the phosphorylation of Creatine with ATP as followed: [9]

Creatine + ATP $\rightleftharpoons$ Creatine phosphate + ADP

Lactate dehydrogenase, LDH(EC 1.1.1.27), a pyridine linked enzyme found in virtually all animal tissues, functions primarily in the metabolism of glucose, catalyzing the reduction of free pyruvate to lactate during the last step of glycolysis, as well as the conversion of lactate to pyruvate in gluconeogenesis.

Pyruvate +NADH $\rightleftharpoons$ Lactate + NAD^+

LDH has long been considered a useful clinical marker of intravascular hemolysis, its serum levels are elevated in intravascular hemolysis, such as immune hemolytic anemia, but are substantially elevated with intravascular elevated with intravascular hemolysis, such as thermobotic theromo- cytopenic purpura and paroxysmal nocturnal hemoglobinuria[10]

Total thiol are contain potential sulfhydral group (SH) in their structure, these can range from the simply amino acide, cysteine, and protein contain them, thiol exist in two pools protein and non protein[11]

The aim of this study is to investigate the Lactate dehydrogenase (LDH), creatine kinase (CK) activity and total thiols in serum of beta Thalassemia patients compared with healthy controls.

Patients

30 patients with β -thalassemia aged 7-35 years, and 30 healthy subjects aged 7-35 years, as a control group were recruited for this study. Blood samples were collected from patients before transfusion. After clotting, serum was separated by centrifugation, the analytical determinations described below were either performed immediately, or serum was stored at -20°C and used within 72 hours.

Methods

Pyruvate is reduced to lactate by Lactate dehydrogenase (LDH) at PH 7.4 and at 30 ºC. The progress of the accompanying oxidation of NADH to NAD^+ is monitored continuously by measuring the rate of absorbance decrease at 340 nm in a spectrophotometer[12].

Creatine kinase(CK) determination by using commercially available kit (Randox - U.K.)

The total thiol group were evaluated in 200 µL serum sample by colorimetric reaction with 5,5'- dithiobis(2-nitrobenzoic acid) as reported[13].

Statistical analysis.

All results are expressed as a mean ± SD(standard deviation), comparison between patients and controls were preformed by the student's t- test. Person's correlations were used to determine relationship between parameters studied. A value of $p \leq 0.05$ was considered statistically significant.

Results and Discussion

The levels of LDH and CK activity in serum of patients compared with healthy controls are shown in Table 1, 2 respectively. The LDH activity were significantly increased(P=0.03) while the CK activity were no significantly decreased(p= 0.4) in β- thalassemia patients.

Table 1:-LDH Activity IU/L in Serum of Patients and That of Healthy Controls.

	Mean	SD	SE	P -Value	Sign.
Control	208.97	26.8	4.9	------	------
Patients	365.72	221.4	40.4	0.03	sign.

Table 2:-CK Activity IU/L in Serum of Patients and That of Healthy Controls.

	Mean	SD	SE	P -Value	Sign.
Control	134.25	28.5	5.2	------	------
Patients	123.25	41.6	7.6	0.4	NS.

Leakage of intracellular CK or LDH is indicative of local tissue damage induced by free radicals or oxygen lack or trauma [14]. Increased cellular LDH activity reflected a shift towards anaerobic metabolism and increased glycolysis in the cytoplasm of Thalassemia cell accompanied by a high turnover rate. In conditions with tissue damage and rapid cell turnover, the serum LDH may rise, and the isoenzyme distribution usually reflects that of the damage organ and cell [15]. These data begin to suggest that LDH elevation may be a marker of hemolytic-associated with thalasemia.

Decreased of ATP, increased of lactate, increased lactate/ pyruvate ratio and the overall decreased of mitochondrial metabolism activity are indicated of oxidative damage in thalassemia patients due to the iron overload and the complication of this disease.[16]

Results suggest that the CK and phosphocreatine shuttle may play an important role in protection against free radicals damage and ATP depletion[17,18], phosphocreatine/ Creatine kinase system plays a key role in buffering of ATP levels in the cell under stress conditions, and Creatine kinase isoenzymes are very sensitive to oxidative modifications[19, 20]. As well as CK is a protein contain thiol groups that is particularly susceptible to oxidation, and the decreasing of serum CK activity are results from the oxidation of SH group of CK by free radicals that generated in thalassemia patients due to hemolytic anemia [21].

A significant decrease in levels of serum total thiols group for thalassemia patients(P=0.05) compared with healthy controls were observed in Fig. 3. this result may be due to the oxidized of sulfhydryl group in proteins by the overproduction of free radicals such as superoxide anione , thus, the oxidize and reduced glutathione ratio GSSG/GSH is increased . it has been noted that serum SH groups are susceptible to oxidative damage in patients suffering from thalassemia ,hence the estimation of thiols group may serve as an adjutant for the diagnosis for the disease complications [22].

Table 1:-Total thiol levels µmol/L in Serum of Patients and That of Healthy Controls.

	Mean	**SD**	**SE**	**P -Value**	**Sign.**
Control	365.13	106.2	19.4	------	------
Patients	277.25	54.2	9.9	0.05	sign.

Oxidative damage especially due to iron overload and depleted of antioxidant play an important role in pathogenesis of thalassemia, increased oxidative damage in thalassemia may be due to the depletion of antioxidants such as CK and total thiol groups

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