ejpmr, 2016,3(1), 66-71

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

THE CORRELATION BETWEEN XANTHINE OXIDASE, LACTATE DEHYDROGENASE AND NITRIC OXIDE LEVELS IN SERA OF PATIENTS WITH MINOR B-THALASSEMIA

[*1]Namama Soran. H., [2]Govand Ali A. and [1]Dlzar D. Ghafoor

[1]Department of Chemistry, Faculty of Science and Science Education, University of Sulaimani.
[2]Department of Biochemistry, Faculty of Medicine, University of Sulaimani.

***Correspondence for Author: Namama Soran H.**

Department of Chemistry, Faculty of Science and Science Education, University of Sulaimani.

Article Received on 13/11/2015 **Article Revised on 04/12/2015** **Article Accepted on 24/12/2015**

ABSTRACT

In the present study, 55 patients with β-Thalassemia minor aged between (9-45 years) and 35 apparently healthy controls aged between (8-40 years) were investigated for Xanthine Oxidase (XO), Lactate dehydrogenase (LDH) activities and Nitric Oxide (NO) level. Correlation between XO, LDH activity and NO levels has been studied to select the best characteristic marker to identify β-thalassemia minor patients and to investigate the relationship between these markers with β-thalassemia minor subjects. The results showed a significant increase in XO, LDH activity and a significant decrease in NO levels compared to control group while no correlation has been noticed between XO, LDH activity and NO levels in both patients and healthy subjects.

KEYWORD: β-thalassemia, Xanthine Oxidase, Lactate Dehydrogenase, Nitric Oxide.

INTRODUCTION

β-thalasemia is a genetic blood disorder characterized by a partial or complete lack of the ability to synthesize beta chains of hemoglobin.[1] The disease is more prevalent throughout the Mediterian region, Middle East, Africa and South Asia. Depending on the severity of the disease, three main groups of β-thalasemia have been described, which are β-thalasemia major, β-thalasemia intermedia and β-thalasemia minor.[2] The process of beta-globin chain synthesis is controlled by a gene that is present in chromosome11.[3] Imbalance between α- and β-globin chains synthesis may be compensate for by increased γ-globin chain and fetal hemoglobin (HbF) synthesis which 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. This led to defective hemoglobin synthesis, severe anemia, excessive iron absorption and iron overload in the patient tissues.[4,5]

Excess iron that is produced from dietary absorption or multiple blood transfusion catalyze the production of a variety of reactive oxygen species such as hydrogen peroxide and superoxide anion. XO has been shown to be a major radical generating enzyme in biological system under ischemic conditions. The enzyme is expected to mainly be found in damaged tissues.[6,7,8] The enzyme is involved in the metabolism of purines, catalyzing the conversion of both hypoxanthine and xanthine to uric acid.[9,10] XO contains two Fe_2S_2 clusters and bound FAD, in the dehydrogenase NAD^+ is the electron acceptor that oxidizes the bound $FADH_2$. Xanthine dehydrogenase, in the absence of thiol compounds, is converted spontaneously into xanthine oxidase, propably as a result of a conformational change and formation of a disulfide bridge within the protein. Evidently in the oxidase form the NAD^+ binding site has moved away from the FAD, permitting oxidation of $FADH_2$ by O_2 with formation of hydrogen peroxide.[11,12]

Lactate dehydrogenase (LDH) is an enzyme found in nearly all living cells, catalyzes the reversible conversion of pyruvate to lactate, as it reversibly converts NADH to NAD^+. In medicine, LDH is often used as a marker of tissue breakdown as LDH is abundant in red blood cells and can function as a marker for hemolysis.[13,14]

Nitric oxide (NO) is a potentially toxic gas and a highly reactive inorganic free radical produced by most mammalians. NO have manifold physiologic functions including modulation of vascular tone, platelet aggregation, inflammation and neurotransmission.[15] Under normal condition NO combines with superoxide anion ($O_2^{\cdot-}$) radical to protect organs but when NO and superoxide anion radical are produced in high quantities they react very rapidly to form peroxynitrite (NOO^-) which may contibute to organ injury. NO itself is derived from L-Arginine by the action of NO synthase.[16]

The relationship among these parameters (XO, LDH and NO) is difficult to interpret in term of cause, though correlation between LDH, XO activity and NO levels in serum of minor β-thalassemia patients have not been studied before. So, the aim of the present study is to investigate the activity of these enzymes and the level of NO in β-thalassemia patients as indicators of the relationship between these parameters and the complication of the disease.

MATERIAL AND METHODS

Sample collection

Patients with β-thalassemia minor who applied to the hematology polyclinic of central lab in Sulaimanyah city/Iraq between January 2015 and July 2015 were included in the study. Fifty five β-thalassemia minor patients aged between 9-40 years and 35 healthy subjects aged 7-42 years as a control group were recruited for this study. Pregnant, lactating women, history of tuberculosis, HIV, severe pancreatitis and malignancy were excluded from this study. Blood samples were collected from patients before blood transfusion. After clotting, serum was separated by centrifugation and the analytical determinations described below were either performed immediately, or serum was stored at -25^{o}C.

Statistical Analysis

Data were expressed as mean ± standard deviation. Student t-test of two independent samples was used to compare between means. The statistical analysis was carried out using the statistical package for social sciences (SPSS version 16) and p values ≤ 0.05 between β-thalassemia minor patients and control group was considered as statistically significant. Bivariate analysis was carried out to find out the correlation among the parameters.

Estimation of Xanthine Oxidase activity

XO assay was performed by quantifying the rate of formation of uric acid by measuring the absorbance at 290 nm. The reaction mixture was prepared by mixing 0.1 ml serum with (1.9 ml 0.05 mol/L potassium phosphate buffer, pH 7.5 and 1ml of 2% w/v% hypoxanthine substrate). Time dependent increase in the absorbance at 290 nm was recorded against blank.

The activity of xanthine oxidase is expressed as IU/L serum. One international unit of xanthine oxidase will metabolize one micromole of hypoxanthine per minute.[17]

Lactate Dehydrogenase Assay

Lactate dehyderogenase activity was determined by following the decrease in the absorbance at 340 nm resulting from the oxidation of NADH to NAD^{+}. One unit of the enzyme causes the oxidation of one micromole of NADH per minute at 25°C and pH 7.3, under the specified conditions. The reaction mixture composed of (2.8 ml of 0.2 mol/L Tris HCl, pH 7.3, 0.1 ml of 6.6 mmol/L NADH, and 0.1 ml of 30 mmol/L sodium pyruvate) was mixed with 0.1 ml of diluted serum. The absorbance was recorded at 340 nm after 5 min to establish temperature calibration.[18]

$$\text{Units/mg} = \frac{\Delta A_{340}/\text{min}}{6.22 \times \text{mg enzyme/ml reaction mixture}}$$

Nitric Oxide Assay

Since NO is unstable and rapidly converted to nitrate and nitrite, so it is necessary to determine both nitrate and nitrite in serum samples. In this study serum nitrite/nitrate levels were measured spectrophotometrically at 545 nm using the Griess[19] reaction to convert all nitrate to nitrite. Serum nitrate was measure by subtracting direct nitrate from total nitrate. The results were expressed as micromoles per litre serum.

RESULTS

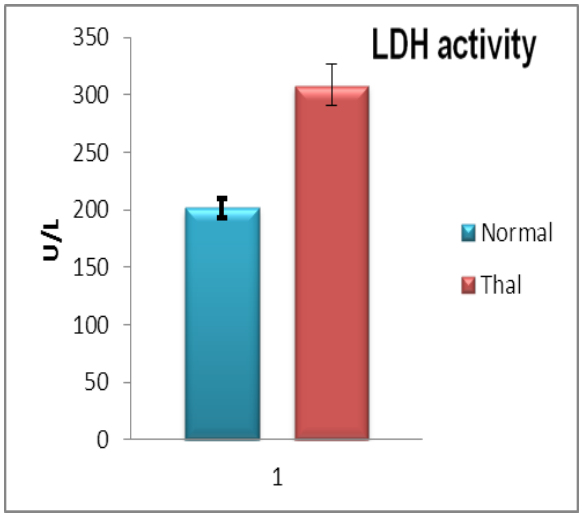
Serum XO activity in both β-thalasemia minor patients and normal individuals are shown in figure 1. A significant increase ($p \leq 0.05$) in XO activity was noticed in patients (28.618 ± 5.98) U/L compared to control group (10.226±1.326) U/L.

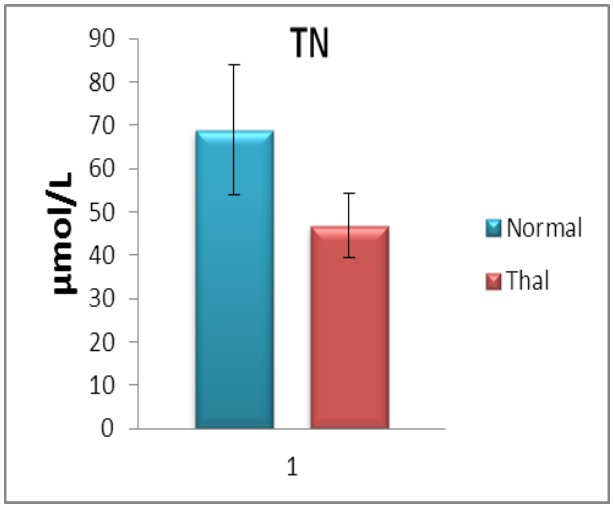
The activity of LDH activity in serum of β-thalassemia minor patients compared with healthy control is shown in figure 1. The activity of the enzyme was significantly increased ($p \leq 0.05$) in β-thalassemia minor patients (308.4± 18.11) U/L compared to control group (201.46±7.94).

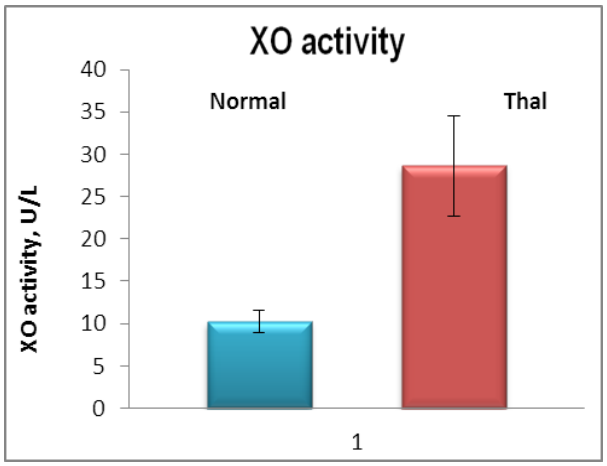
Serum direct nitrate, total nitrite and nitrate levels were (10.115±1.47), (46.836±7.35) and (31.109±2.77) µmol/dl in β-thalassemia minor patients versus (28.874±4.11), (68.94±14.944) and (42.654±3.788) µmol/dl in control, respectively and the difference between the two groups were statistically significant ($p \leq 0.05$).

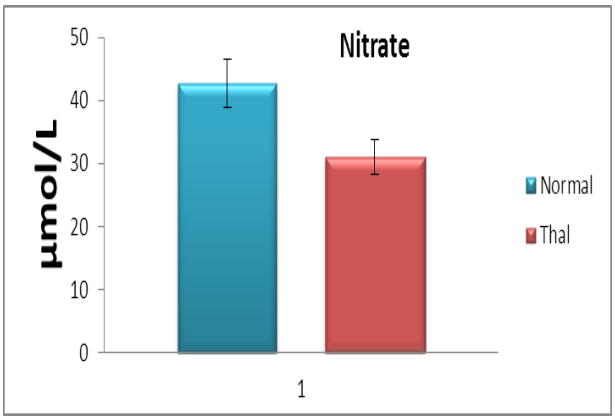
Correlation analysis was performed among the parameters XO, LDH and nitric oxide levels represented by total nitrite. Scatter plot (figure 2) was used to find whether there is positive or negative correlation among parameters. Correlation coefficient, shown in table 1, was calculated using statistical analysis.

Table (1) shows the correlation analysis between the XO and the LDH activity and NO levels. Results showed no correlation among parameters in both control group and healthy individuals. Table 2 show Pearson correlation for each parameter in both control group and healthy individuals.









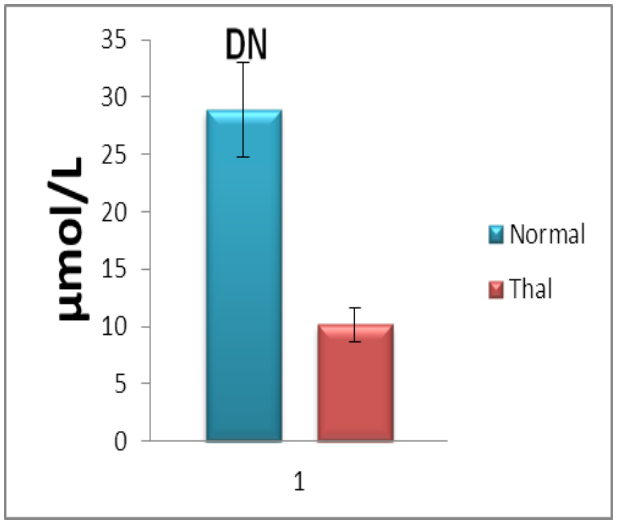


Figure 1: XO and LDH activities, DN, TN and Nitrate levels as (Mean ± SD).

Table 1 Correlation coefficients (r) among the parameters in both healthy individuals (a) and patients (b).

a.

parameters	XO	LDH	TN
XO	- - - - -	-0.177	0.225
LDH	-0.177	- - - - -	-0.203
TN	0.225	-0.203	- - - - -

b.

parameters	XO	LDH	TN
XO	- - - - -	-0.016	-0.063
LDH	-0.016	- - - - -	0.035
TN	-0.063	0.035	- - - - -

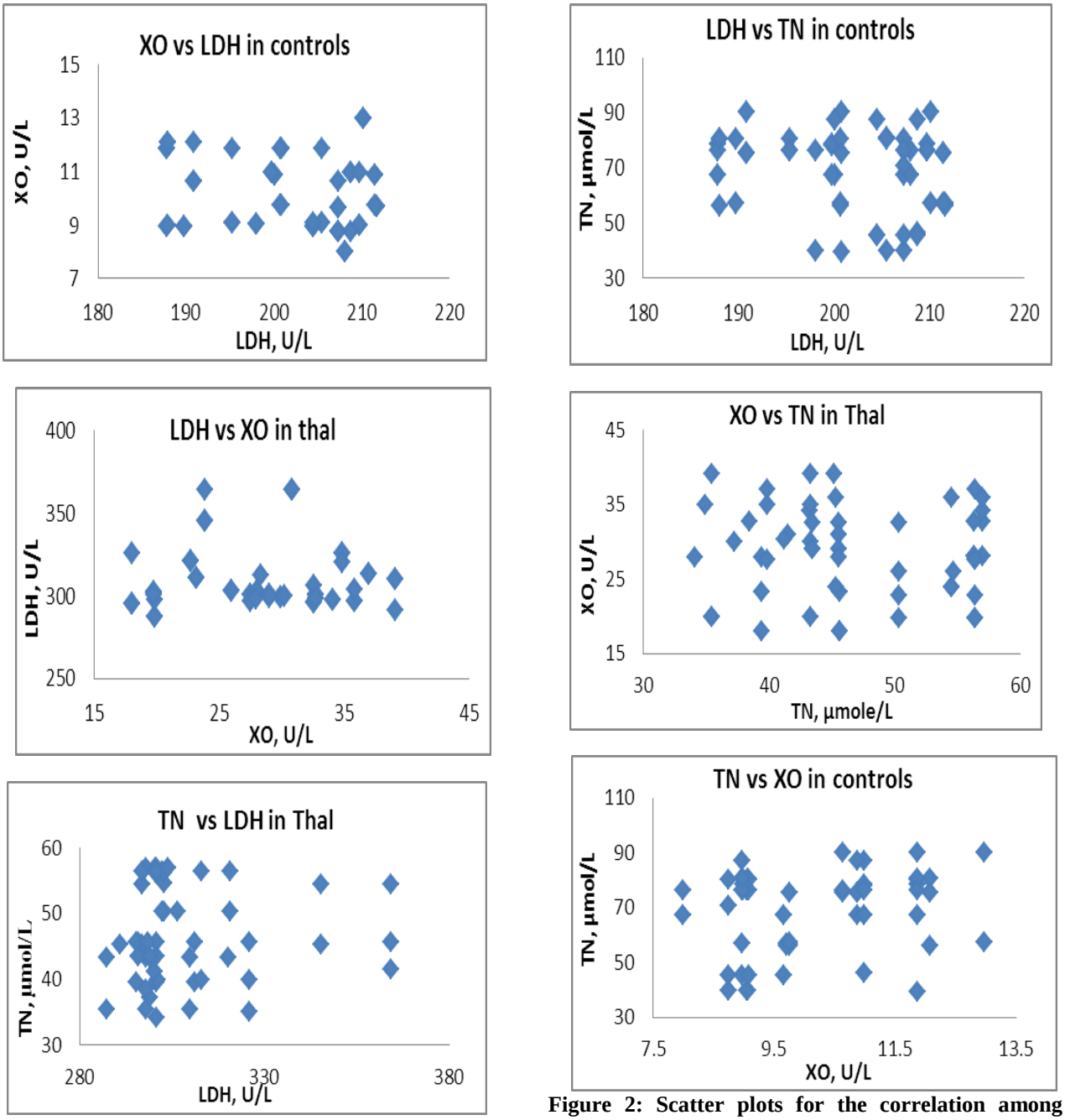


Figure 2: Scatter plots for the correlation among parameters in both patients and healthy individuals.

Table 2: Pearson Correlation for each parameter in both control group and healthy individuals.

Parameters	XO		LDH		DN		TN		Nitrate	
Cases	Normal	Thal.	Normal	Thal	Normal	Thal	Normal	Thal	Normal	Thal
Pearson Correlation	-0.034		0.284		-0.156		0.133		0.069	

DISCUSSION

The activity of XO and LDH in serum of β- thalassemia minor patients was compared with healthy controls. The results of this study showed that XO and LDH are significantly increased compared to the control group. XO is an enzyme that is known to distributed widely in mammalian tissues.[19] There are reports showing the presence XO in the liver, lung, heart, intestine and in the capillary endothelial cells.[20]

Among the tissue components, endothelial cells in micro vascular systems constitute the most abundant source of the enzyme as compared with those in larger vessels[21], because the enzyme reaction that transfers electrons from hypoxanthine to uric acid is coupled with a reduction of molecular oxygen in to superoxide anion, XO has been considered to play a crucial role in the pathogenesis of oxidant induced micro vascular changes and tissue injury.[22]

Several studies have evaluated the oxidant and antioxidants statuses of thalassemia patients.[23] However; most studies have focused mainly on the severe and intermediate states of thalassemia and few data are available for the heterozygote profile.[24] Moreover, the oxidative status has not been evaluated for the different β-thalassemia mutations, although such an assessment is important due to the large genotypic and phenotypic heterogeneity of different populations.[25] With β-thalassemi, auto oxidation reflects the formation of superoxide radicals($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) resulting in oxidative stress.[26]

Oxidative stress is not only a simple imbalance between the production and scavenging of ROS (reactive oxygen species), but also a dysfunction of the enzyme involved in reactive oxygen species production. Dysfunction of NAD (p)H oxidase, activation of XO and dysfunction of the mitochondria are underlying signs of oxidative stress, therefore, XO is one of important therapeutic targets.[27]

LDH was negatively correlated with XO and direct nitrate levels. Serum LDH levels are mildly elevated in β-thalassemia major due to infective erythropoeiesis.[28,29] The pathophysiology relates directly to the accumulation of unmatched α chains bearing an obscure toxic effect. Increased cellular LDH activity reflected a shift towards anaerobic metabolism and increased glycolysis in the cytoplasm of thalassemia cell a ccompained 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.[30,13] These data begin to suggest that LDH elevation may be a marker of hemolytic associated with thalassemia.

A patients with β-thalassemia had significantly lower levels of serum NO than control groups. NO have manifold physiologic functions including modulation of vascular tone, platelet aggregation, inflammation and neurotransmission.[31]

Bayraktal et al.[32] have demonstrated that NO levels in β-thalassemia minor patients. Reports have shown that the free heme and the red cell membrane elements that are produced during hemolysis have a negative effects on NO and arginine availability, which in turn promotes vasoconstriction. They also lead to further endothelial dysfunction, resulting in a more pronounced NO reduction.[33] 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 electron transports.

REFRENCES

1. Weatherall DJ, Pressley L, Wood WG, Higgs DR, Clegg JB. Molecular basis for mild forms of Homozygous β-thalassemia. Lancet, 1981; 1(8219): 527-529.
2. Renzo Galanello and Raffaella Origa, Beta-thalassemia, Orphanet Journal of Rare Diseases, 2010; 5(11).
3. Engelborghs S, Pickut BA, De Deyn pp. Recurrent transient ischemic attacks in a 15 year old boy with β-thalassemia minor and thrombophilia. Contribution of perfusion SPECT to clinical diagnosis. Acta Neurol Belg., 2003; 103: 99-102.
4. Simsek F, Ozturk G, Kemahli S, Erbas D, Hasanoglu A. Oxidant and antioxidant status in β-thalassemia major patients. J of Ankara Univer. Faculty of Med., 2005; 58(1): 34-38.
5. Gurpreet D., M.D. Patricia A, Cornett M. and Lawrence M., Tierney JR. American Family physician., 2004; 11: 2606.
6. Aisen P, Enns C, Wessling Resnick M. Chemistry and biology of Eukaryotic iron metabolism. Inter. J of Biochemistry and cell Biology., 2001; 33(10): 940-959.
7. Hershko C. Iron loading and its clinical implications. American J of Hematology, 2007; 82(12): 1147-1148.
8. Emerit J, Beaumont C, Trivin F. Iron metabolism, free radicals and oxidative Injury. Biomedical pharmacotherapy, 2001; 55(6): 333-339.
9. Xia Y, Khatchikian G, Zweier J. Adenosin deaminase inhibition prevents free radical mediated injury in the post ischemic heart. J. Biol Chem., 1996; 271: 10096-102.
10. Rashmi R, Alki K, Pushpa B, Aparna M. and M. Misra. Xanthine oxidase as a marker of Myocardial infraction. Indian J of clin biochemistry, 2007; 22(2): 90-92.
11. David E. Biochemistry the chemical reaction of living cells. Volum 1 and 2 second ed., 2004; 16: 890-891.
12. Muthanna O. Estimation of xanthine oxidase activity related with nitric oxide and lipid profile in serum of patients with atherosclerotic diseases. J of university od anbar for pure science., 2009; 3(2): 1991-8941.
13. Kato G, Me G, Machado J, Little J, Taylor J, Morris C, Nichols J, Wang X, et al. Lactate dehydrogenase as a biomarker of hemolysis associated nitric oxide resistance, priapism, leg ulceration, pulmonary hypertension and death in patients with sickle cell disease. Blood, 2006; 107(6): 2279-2285.
14. Butt A, Michaels S, Greer D, Clark R, Kissinger P, Martin D. Serum LDH level as a clue to the diagnosis of histoplasmosis. AIDS READ., 2002; 12(7): 317-21.
15. Synder S. Bredt DS. Biological roles of nitric oxide. Sci Am., 1992; 266-687.
16. Robert K., Daryl K., Perter A and Victor W. Harper,s Illustrated biochemistry. 26ed., 2006; 49: 571-572.
17. Ackermann E and Brill A. "Xanthine oxidase activity in method of enzymatic analysis" ed Bergmeyer H. 2nd ed. Academic press inc. UA., 512-522.

18. Bucolo G, Chang T. and Mc Crosky. Mechanized enzymatic determination of triglycerides in serum. Clin Chem., 1974; 20: 881.
19. Friedewal W. Levy R and Fredric D. Estimation of low density lipoprotein cholesterol in plasma without use of the preparative ultra centrifuge. Clin chem., 1972; 18(6): 499-502.
20. Brudder G,. Heid H,. Jarashi E,. Mather I. Immunological identification and determination of xanthine oxidase in cells and tissue. Differentiation, 1983; 23: 218-25.
21. Shishchbor M,. Aviles R and Brennan M.Association of nitroyrosine levels with cardiovascular disease and modulation by statin therapy. JAMA, 2003; 289: 1675-1680.
22. Friedewald W. Levy R and Fredrictson D. Estimation of low density lipoprotein cholesterol in plasma without use of the preparative ultra centrifuge. Clin chem., 1972; 18(6): 499-502.
23. Cighetti G, Duca L, Bortone L, Sala S, Nava I, Fiorelli G. et al. Oxidative status and malon dialdehyde in β-thalassemia patients. Eur J clin Invest., 2002; 32(1): 55-60.
24. Labib H, Etewa R, Gaber O, Atfy M, Mostafa T, Barsoum I. paraoxonase-1 and oxidative status in common mediterraneam β-thalassemia mutations trait and their relations to atherosclerosis. J clin pathol., 2011; 64(5): 437-442.
25. Beutler E. Discrepancies between genotype and phenotype in hematology: an important frontier. Blood., 2001; 98(9): 2597-2602.
26. Luciana D., Isabeth D., Marina I., Sandro P., Doroteia R. Silva S. et al. Oxidative stress and antioxidant status in β-thalassemia heterozygotes. Rev Brass Hematol Hemoter., 2013; 35(6): 409-413.
27. Schramm A., Maatusik P., Osmenda G., Guzik T. Targeting NADPH oxidases in Vascular pharmacology. Vascular pharmacology, 2013; 56(5-6): 216-231.
28. Amos T., Reuven O., Joseph K., Gabirel C., Memt A., Shimon S. and Arnon N. Normalization of serum lactic dehydrogenase in βthalassemia patients following bone marrow transplantation. American J of Hema., 1996; 15: 166-167.
29. Schwartz E., Benz E. The thalassemia syndromes in Hoffman R, Benz E. shattil S, Furie B, cohen H: Hematology basic principles and practice. Ed 2. New york: churchill livingstone, 1994; 368-392.
30. Kornberg A., Polliack A .serum lactic dehydrogenaselevel in acute leukemia: marked elevations in lymphoblastic leukemia. Blood, 1980; 56: 351-355.
31. Synder S., Bredt D. Biological roles of nitric oxide. Sci. Am., 1992; 266: 6871.
32. Bayraktar N., Erkut M., Aydogdu I., Baflaran Y. The levels of nitric oxide in β-thalassemia minor. Turk J Hematol., 2008; 25: 187-9.
33. Aessopos A., Kati M., Farmakis D. Heart disease in thalassemia intermedia: areview of the underlying pathophysiology. Haematologica, 2007; 92: 658-65.