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Red Cell Na^+/K^+- Atpase (NKA) Activity and Serum Electrolyte Concentration of Patients with Major Thalassemia Disease in Babylon Province/Iraq

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Abstract

Background: β-thalassemia is inherited hemoglobinopathies resulting in structural and quantitative changes in the β-globin, characterized by increased red blood cell breakdown, less than its normal life.

Objectives: This study investigated the measurement of enzymeNKA activitypresent in the red blood cell membranein β-thalassemia major patients and measurementthe electrolytes' (sodium, potassium, calcium, iron, magnesium) concentration in serum. And its relationship to the activity of the enzyme NKA.

Methods: This study included 54 patients with β-thalassemia (in steady-state), and a control group of 30 age-matched individuals without blood diseases. Red blood cell NKAactivity and electrolytes were measured for the β-thalassemia and control group. The study found that there is an increase in the activity of theNKAcompared to the control group, and that there are significant differences in terms of the value $P< 0.01$ and that there is an increase in the level of iron in the blood serum of patients compared to the control group as well as the results showed that the level of electrolytes in the blood serum within the references range, except for calcium, there is a decrease from the references range.

Conclusion: β-thalassemia major is associated with alterations in markers of NKA activity and iron of serum but there are no significant relationship between the activity of NKA and electrolyte level. Its level is not affected by thalassemia.

Keywords: Na^+/K^+-ATPase, NKA, β-thalassemia, electrolyte

Introduction

Theβ-thalassemia major syndromes are a group of inherited blood disorders. that are distinguished by reduced or absent beta globin chain synthesis.[1]There are three types of beta-thalassemia: Thalassemia Major, also known as "Cooley's Anemia" and "Mediterranean

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Anemia," Thalassemia Intermedia, and Thalassemia Minor, are also known as "beta-thalassemia carriers," "beta-thalassemia traits," or "heterozygous beta-thalassemia."[2][3] Because of their severe anemia, patients with beta thalassemia major require repeated blood transfusions to survive. Recurrent blood transfusions cause an accumulation of excess iron in the body tissues in beta thalassemia major. Secondary iron overload causes peroxidative damage by increasing the production of reactive oxygen species (ROS) within the erythrocytes, resulting in oxidative stress.[4] Unpaired globin chains and high cellular iron concentrations in beta-thalassemia patients may promote oxidative damage to red blood cells, resulting in decreased bloodstream survival. ROS can combine with polyunsaturated fatty acids (PUFA) in phospholipids to form lipid peroxides, which can damage the human erythrocyte membrane.[6]Enzymatic activity in the erythrocyte membrane is required for the maintenance of normal membrane structure and functions, for normal oxygenated hemoglobin, and for hemoglobin protection against peroxidation Control of the erythrocyte's internal ionic milleu is required to maintain its normal volume and shape. The intracellular concentrations of Ca+2, Na+-K+, and Mg+2 are regulated by active cation transport pumps. The energy required for active transport of these cations is derived from glucose metabolism. This is a cyclic conversion of ATP to ADP and Pi and vice versa.The reaction is catalyzed by a group of enzymes known as Adenosine triphosphatases (ATPases). The reaction catalyzed by this group of enzymes can thus be demonstrated.

$$ATP^{+4} + H_2O \rightarrow ADP^{+3} + HPO_4^{+3} + H^+ .$$

In erythrocyte membranes, there are three different Adinosinetriphosphatases: Ca+2/K+-ATPases, Na+/K+-ATPases, and Mg+2-ATPases are ion-activated or stimulated. Erythrocyte membrane ATPases are transport enzymes that are membrane bound.[7]The Na+/K+-ATPase (NKA) is a P-type pump family essential transmembrane protein that functions as the primary active ion transporter in all eukaryotic cells. NKA, which is powered by ATP, exchanges three intracellular Na+ ions for two extracellular K+ ions in exchange for one hydrolyzed ATP molecule, thereby maintaining proper ion gradients across the plasma membrane.[8]

Iron overload is also responsible for oxidative stress due to an increase in free radical production and altered element levels in serum.Trace electrolytes are important in many biological systems because they act as activators or inhibitors in a variety of biochemical reactions. Before and

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during the treatment of major -thalassemia, serum electrolyte levels must be determined and monitored. Serum levels of may also aid in the early detection of this disorder.[9][10]

SUBJECTS AND METHODS

Subjects

A total of 54 beta-thalassemia major patients diagnosed by Hemoglobin Electrophoresis (28 males and 26 female) and 30 controls (24 males and 6 female) were included in this study. Beta thalassemia major patients were aged between 1 and 25 years old. The control group consisted of healthy children with ages ranging from 2 to 23 years old.Patient samples were collected from the Center for Genetic Blood Diseases at Babylon and Children's Hospital in Babel Governorate Iraq.

Sampling and data collection

blood samples werecollected over a period of 4 months (fromDecember 2020 to March 2021). Under sterileconditions, about 2 ml of Blood Were Collected in A Tube Containing Anticoagulant EDTA through venipuncture, Which Was Used to Measure NKA Enzyme directly. An aliquot of blood (2.0 mL) was takentransferred to anothertube contains gel The tube containing theremaining 2 mL blood sample was then centrifugedfor 10 min at 3,000 rpm and serum samples werecollected in Eppendorf tubes using Pasteur pipette. stored at -20oC untilcompletion of blood count. It is then used to measure electrolytes.

Biochemical assays

All the reagents that had been used were analytical grade supplied from Fluka AG, Buchs, Switzerland and BHD Chemical Ltd, Poole, England.

The activity of NKA in erythrocyte membranes was measured in terms of inorganic phosphate liberation during enzymatic ATP hydrolysis and was expressed in g phosphate per g of protein released during a 30-minute incubation. (Pi).[11] Serum level of magnesium and iron wasdetermined using the kits and procedures of Dirui industrial Co., Ltd P.R. China.data were read usingauto- chemistry analyzer(CS-T180). And serum level of potassium, sodium and total calcium date were read using electrolyte analyzer (GE 300).

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Statistical analysis.

Statistical analysis was carried out using SPSS 25.0 Statisticalsignificance was evaluated by two tailed *t*-test and thelevel of statistical significance was set to P<0.01. Data are presented as Mean ± SD (standard deviation). The results were analyzed using analysis of variance (ANOVA) followed by the independent t-test. Correlations were calculated by the Pearson's correlation coefficients.

Result and discussion.

The mean age of beta-thalassemia major patientsand controls is 13.2 and 11.5 years, respectively, mean value ± standard deviation in the activity of the enzyme NKA for the twoStudy Group (β-thalassemia Patients Group and the Control Group). As the results showed that the rate of enzyme activity in thalassemia patients(147.03 ± 513.68) In the control group (147.91 ± 363.84) it was found that there is an increase in the activity of the enzyme in patients with thalassemia compared to the control group and there is a significant difference of statistical significance as the P value<0.001 as shown in the figure 1.it is identical to a previous study conducted by Omar et. al. on thalassemia patients and sickle cell anemia patients and the results showed an increase in the two groups and a P < 0.01 in thalassemia patients compared to the control group. Patients with reduced oxygenation and its physiological role in the cell.[12]

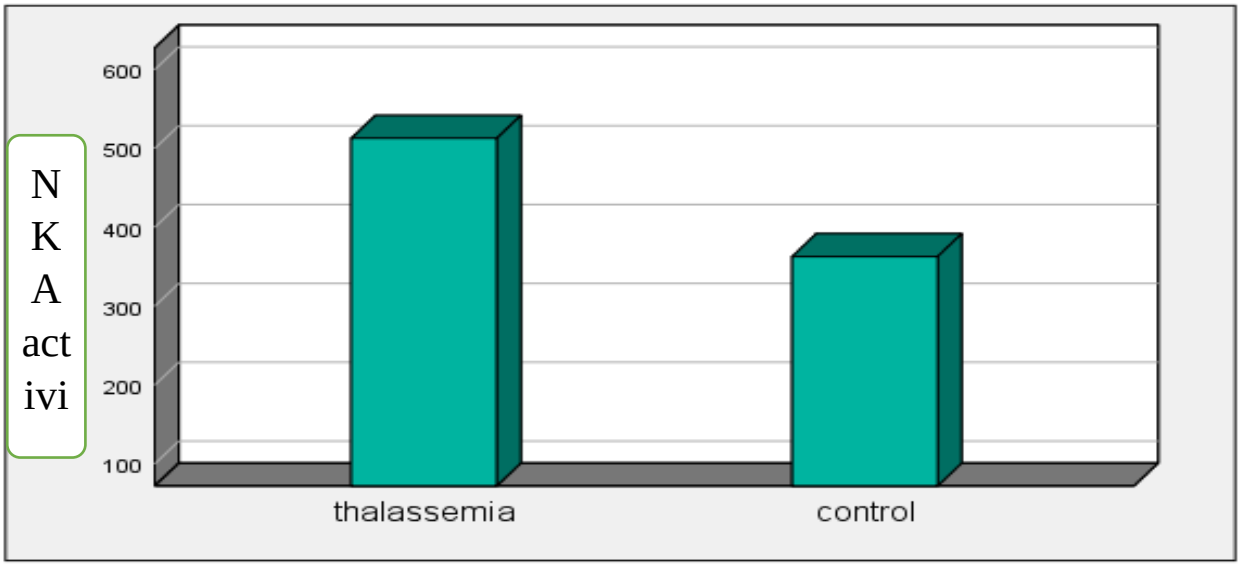

Figure 1 showed the difference in mean values in the enzyme NKA activity (µg Pi/ g protein .min) for the two study groups.

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Jason and et. al. mentioned when they conducted a study to measure the NKA enzyme and compare its activity in sickle cell blood cells increased compared to its activity in normal blood cells due to the increased production of young red blood cells compared to in healthy people who do not suffer from hereditary blood diseases, the red blood cells remain in their normal life.[7]Likewise in study conducted by Madan G. Luthra and David A. Sears on patients suffering from sickle cell anemia it was found that the activity of the enzyme NKA was higher people as a control group. Its age is older so it appears that in the first case the enzyme activity is higher than in the second case.[13]the results showed a significant increase in serum iron concentrationfor thalassemia patients compared to the control groupwith very high significant differences, as the value P< 0.01 is as shown in the table 2. The high levels of iron in thalassemia patients are consistent with previous studies and documented in many studies.[9]iron in excess of normal accumulates in tissues and blood stimulates the formation of harmful compounds from free radicals.[14] The Haber-Weiss reaction, which is catalyzed by excess iron, generates hydroxyl radicals from activated oxygen species.[15]

$$Fe^{+3} + O_2^{\bullet -} \longrightarrow Fe^{+2} + O_2$$

$$Fe^{+2} + H_2O_2 \longrightarrow Fe^{+3} + OH^- + OH^{\bullet} \text{ (Fenton reaction)}$$

$$\text{Net reaction } O_2^{\bullet -} + H_2O_2 \longrightarrow O_2 + OH^- + OH^{\bullet}$$

parameter	Group	N	mean	Std. Deviation	95% confidence interval for mean		Sig.value
					Lower bound	Upper bound	
NKA (μg Pi/g protein)	Thalasmic patient	54	513.86	147.03	409.61	529.65	.000**
	Control	50	363.84	147.91	331.11	478.85	
Fe^{+2}(μg/dl)	Thalasmic patient	54	161.83	63.57	127.99	182.50	.000**
	Control	50	73.4	39.86	97.28	56.11	
	**. Correlation is significant at the 0.01 level (2-tailed).						

Table 1 T-test of parameters of thalasmic patients and control

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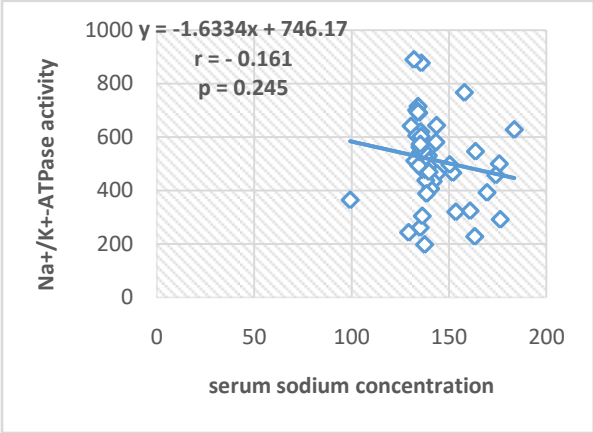
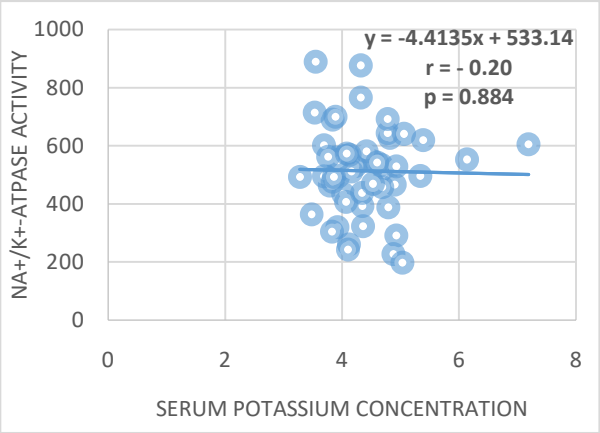
The results were obtained suggest that alteration in serum minerals particularly When compared to the reference values, there was a decrease in calcium concentration but no significant differences in serum sodium, potassium, or magnesium. as in table2.[16][17][18] Hypocalcaemia is a well-known side effect of iron overload. Iron overload occurs as a result of either red blood cell transfusion or increased iron absorption from the digested tract. Both of these are seen in thalassemia.[19]

	mean	SD	Reference range
Na (mmol/l)	142.21	14.47	(146-136) mmol/l
K (mmol/l)	4.36	0.67	(5-3.5)mmol/l
Ca (mg/dl)	8.34	0.76	(10.2-8.6) mg/dl
Mg (mg/dl)	2.16	0.32	(2.2-1.7) mg/dl

Table2 serum electrolytes (sodium, potassium, calcium and magnesium) in thalassemia patients (SD: standard deviation)

No correlations were found betweenelectrolyte levels and NKA enzyme as in figure2.





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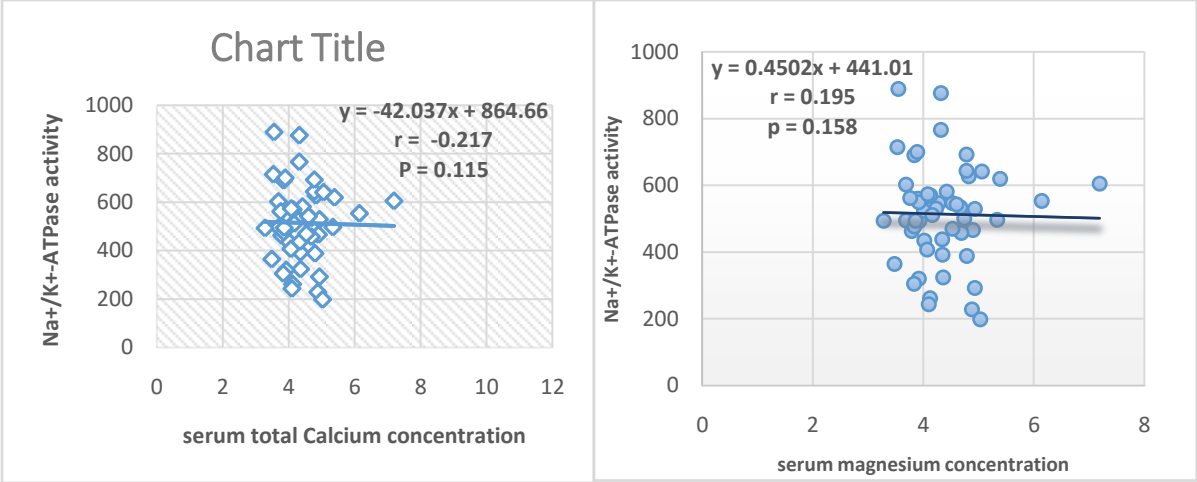

Figure2 Correlations between NKA enzyme and serum electrolytes

Conclusion

The results showed an increase in enzyme NKA activity compared to the control group, we can suggest that the activity of NKA can be used as an indicator for diagnosis ofindividuals with thalassemia. Iron overload in thalassemia patients leads to an increase in the reactive oxygen species (ROS) and thus the destruction of red blood cells which simulates the generation of new red blood cell which causes an increase in NKA activity. The results showed that thalassemia does not affect the concentration of electrolyte in the blood serumbutcalcium decreaseonly.

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