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Original Research Paper

Estimation of Na+/K+ Atpase and Some Metal Ions Due To Effect of Deferoxamine in Beta-Thalassemia Patients

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Abstract: Thalassemia is a genetic disease that is autosomal in nature. The condition is marked by an impaired capacity for the synthesis of polypeptides. The presence of chains of normal hemoglobin has been observed to result in the development of anemia. This phenomenon persists as a significant health concern. The Mediterranean region is characterized by a distinct culinary tradition, with a variety of dishes influenced by the region's geographical and historical context. **Aim of Study:** This study was designed to evaluate the activity of sodium potassium ATPase in the red blood cell membrane of thalassemia patients as well as some metal ions (sodium, potassium, magnesium, total iron) as a result of the effect of deferoxamine treatment. **Methods:** A total of 130 individuals were involved in this case-control study:70Patients with beta thalassemia aged (1±25years), 60 healthy controls aged (2±25yaers). The initial group was carefully selected based on the patients' clinical symptoms. Evaluation of sodium, potassium, magnesium, and iron levels was completed by spectrophotometry. Enzyme activity was expressed as micrograms of phosphate concentration per gram of total protein concentration in red blood cells. **Results:** The average enzyme activity was significantly ($P<0.001$) higher in beta thalassemia patients (1000 ±248) µg Pi/mg protein. min compared to healthy individuals (481±160) µg Pi/mg protein. min. Also, sodium levels were significantly ($P<0.001$) higher in thalassemia patients compared to the control group. We also noticed that iron levels were higher compared to the control group, while noting that there was no significant ($P<0.05$) change in magnesium and potassium levels in patients compared to healthy individuals.**Conclusions:** Elevated sodium potassium phosphate (ATPase) levels result from changes in the cell membrane and the breakdown of red blood cells, which increases sodium permeability and increases the activity of this enzyme. Elevated sodium levels are often caused by kidney disorders due to iron overload, a condition caused by repeated blood transfusions. The body lacks an effective mechanism for disposing of excess iron, leading to its accumulation in the blood and organs (iron overload). In thalassemia, the body also increases iron absorption from the intestine due to false signals from hemoglobin deficiency, which exacerbates the condition**.** It was also noted that there was no change in potassium and magnesium levels in thalassemia patients, due to the fact that these elements are not affected by increased iron levels except in very advanced cases of organ failure

Keywords: Na/K Atpase, Sodium, Potassium, Magnesium, Total Iron and Deferoxamine

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1.Introduction

The β-thalassemia major syndromes constitute a group of inherited blood disorders. The distinguishing characteristic of this condition is the reduced or absent synthesis of the beta globin chain. There are three types of beta thalassemia: Thalassemia Major, also referred to as "Cooley's Anemia" and "Mediterranean, β-thalassemia intermedia, and β-thalassemia minor. Beta thalassemia patients have high sodium potassium ATPase activity [1]. The enzyme known as "Na/K ATPase" The enzyme designated as "EC3.6.1.37" is membrane-bound. The process of hydrolyzing an ATP molecule results in the externalization of three sodium ions ($3Na^+$) [2]. The cell and 2-K+ within the cell. This phenomenon, characterized by its ability to transport individuals to other realms, is a subject of considerable interest. It is imperative to acknowledge the indispensable role of pathways in numerous biological processes. This process may involve the generation of membranes and the presence of nerves. Conduction is associated with the maintenance of sodium (Na+) and potassium (K+) levels. The phenomenon of concentration gradients across the cell membrane has been observed. [3]. Na/K ATPase is a membrane-bound enzyme that plays a pivotal role in regulating membrane potential, cell size, and membrane flux in Ca. Additionally, it is essential for the normal cell-growth cycle and the differentiation of nervous tissues. The preservation of sodium (Na) and potassium (K) ions across the cell membrane is imperative for the maintenance of normal cell activities [4]. Iron overload is a life-limiting complication that is commonly observed in individuals with thalassemia. This phenomenon may be attributed to a number of factors, including ineffective erythropoiesis, increased gastrointestinal absorption, an absence of a physiologic mechanism for excreting excess iron, and, most notably, multiple blood transfusions [5]. Given the absence of effective mechanisms for iron elimination within the body, the only viable approach to address excess iron is through the utilization of iron binders, also known as chelators, facilitates the absorption of iron [6]. The process of excretion is defined as the elimination of waste products from the body through the urine and/or stool. As a consequence of the aforementioned, it is generally accepted practice that patients should initiate iron chelation therapy. The patients will undergo treatment subsequent to receiving 10-20 transfusions. An alternative scenario is the occurrence of elevated ferritin levels, with values surpassing 1000 nanograms per milliliter [7]. Iron-chelating agents, including deferoxamine, the f this study is to determine the efficacy of different treatment modalities in reducing iron overload in patients with the condition, thereby reducing morbidity and mortality, including cardiac complications[8]. Magnesium ions ($Mg^{+\ 2}$) represent the second greatest abundant positive ion found within cellular structures, and the fourth most abundant ion overall. The human body contains positive ions and has a body size of over 1,000 millimoles in the context of thalassemia patients undergoing deferoxamine treatment, there is frequently an observed decline in magnesium levels. However, it is noteworthy that these levels typically remain within the normal range when compared to those observed in healthy individuals [9].

2. Methodology

Study Design

Blood samples were obtained from patients with beta thalassemia who were registered at the Thalassemia Center at the Maternity and Children's Hospital in Babylon, Iraq, to conduct this case-control study. The ages of the patients who participated in the study ranged from 1-25 years.

A total of 130 cases were categorized into two groups: -70Patients with beta thalassemia aged (1±25 years), 60 healthy controls aged (2±25 years), All clinical data were documented, including the duration of treatment, the number of doses, and repeated blood transfusions. Patients with a history of heart and kidney disease, and patients who had had their spleen removed, were excluded and divided according to the duration of treatment.

The collection of blood samples

The sample collection was conducted in accordance with established protocols. Two millilitres of blood were extracted and transferred into an EDTA tube.

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Subsequently, the contents of the EDTA tube were subjected to gentle agitation to ensure homogeneity. The blood sample was stored at -20°C. Subsequently, an additional 2 millilitres of blood were transferred into an EDTA tube at -20°C, and the remaining 3 millilitres of blood were collected in a gel tube. The gel tube was subsequently transferred to the laboratory for immediate centrifugation at 3,000 x g. Following this procedure, the plasma and serum were separated into distinct tubes, in accordance with standard laboratory protocols. Meanwhile, the lower cellular layer had immediately treated for the preparation of red cell membrane ghosts, while the serum was employed to determine the levels of potassium, while the plasma was employed to determine the levels of total iron, magnesium, and sodium.

Determination of Na/K-ATPase Activity

Principle

The activity of Na-K ATPase in RBC (ghost) membranes was determined to be The inorganic phosphate that is formed during the process of enzymatic hydrolysis of ATP is expressed in milligrams of phosphate per gram of protein that is released during 30 minutes of incubation (Pi) [10].

Preparation of ATPase Reagent: The ATPase reagent was prepared by combining the following materials:

The Tris-HCl solution (100 mM) was prepared by precisely measuring and mixing 1.2 g of Tris and 1.2 g of HCl. A quantity of 0.095 g of $MgCl_2$ (10 mM) was prepared. The prepared weight of KCl (15 mmol) was found to be 0.11 g. Sodium chloride (85 mM) was prepared by weighing 0.4 g of the substance. The compound under investigation is Na2-EDTA, with a concentration of 1 mM and a prepared weight of 0.036 g. ATP (2 mM), prepared with a weight of 0.1 All materials were dissolved in 100 ml of distilled water, and the pH of the reagent was subsequently adjusted to 7.4 by the addition of NaOH.

Preparing ghosts of red blood cells

Fresh blood was collected in EDTA-K3 tubes, which serve as an anticoagulant, and subsequently placed in a centrifuge at 4000 rpm for a period of 10 minutes. RBCs were obtained by centrifuging 250 µl of the erythrocyte sediment. In a 1-ml volume of a commercially available phosphate-buffered saline (PBS) solution (comprised of 10 mM phosphate buffer, 150 mM NaCl, and a pH of 7.4), 250 µl of red cell sediment was incorporated. Subsequent to this addition, the mixture underwent three cycles of centrifugation and the decantation of the residual normal saline. The washed red blood cells (RBCs) obtained through the aforementioned procedure were then subjected to lysis by adding a Tris-HCl buffer (5 mM, pH 7.4) to a volume of 500 milliliters and centrifuging at 4,000 rpm for 10 minutes.

Measurements of Na-K ATPase Activity

In this experiment, 10 µL of red cell ghosts were added to 500 mL of ATPase reagent, which had been prepared in section B and subsequently incubated for precisely 30 minutes. Following the incubation period, the samples were subjected to a centrifugation process at a speed of 4,000 revolutions per minute for a duration of 10 minutes. Subsequently, 50 µl of the remaining fluid should be extracted for the purpose of identifying the inorganic phosphate.

The inorganic phosphate was determined spectrophotometrically according to the method described by Baykov et al [11]. In order to ensure standardization, the protein concentration in red cell phantoms was estimated using the standard biuret method.. The enzyme activity was expressed with the concentration of inorganic phosphorous to the ghost protein of red cells as follows: The enzyme activity (in milligrams per gram) is directly proportional to the concentration of inorganic phosphorus and the concentration of total protein. Potassium, magnesium, sodium and total iron levels were estimated CT spectra from (Enzyme Egypt).

Statistical study

The SPSS-26.0 version of the Software Package for Social Science was used in data analysis. The mean and standard deviation (SD) presentations of the data

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Using ANOVA and linear regression analysis—which helped to find noteworthy variations between the groups—the normalcy of continuous variables was evaluated. P. values were considered highly significant if less than 0.001 and significant if less than 0.05.

3. Results and discussion

The average enzyme activity was significantly (P<0.001) higher in beta thalassemia patients (1000 ±248) µg Pi/g protein. min compared to healthy individuals (481±160) µg Pi/mg protein. min.

Also, sodium levels were significantly (P<0.001) higher in thalassemia patients compared to the control group. We also noticed that iron levels were higher compared to the control group, while noting that there was no significant (P<0.05) change in magnesium and potassium levels in patients compared to healthy individuals

Table 1: The Mean and Standard deviation of N a/K ATPase

Parameter	**Groups**	**No.**	**Mean ±Std.**	**p. value**
N a/K ATPase	patients	70	559.9 ±170	<0.001
	controls	60	325.84±61.2	

Table 2: The Mean and Standard deviation of Na, K, Mg and Iron

Parameter	Groups	N	Mean ±Std.	P. value
Na mmol/L	Patients	70	178.47 ±16.07	<0.001
	Controls	60	147.41 ±8.72	
Mg mmol/L	Patients	70	2.098 ±0.23	0.251
	Controls	60	2.043 ± 0.31	
Iron (µmol/L)	Patients	70	52.51 ±12.77	<0.001
	Controls	60	22.73 ±6.22	
Potassium (mmol/L)	patients	70	5.58 ± 0.640	<0.001
	controls	60	4.1 ±0.73	

In table 3, there are no significant correlation among parameters.

Table 3: the correlation of study parameters in patient group.

		Na mmol/L	Mg mmol/L	Na/K ATPase	Iron (Mmol/L)	Potassium (mmol/L)
Na mmol/L	Pearson Correlation	1	0.057-	0.013	0.107	0.058
	P. Value		0.641	0.918	0.380	0.631
Mg mmol/L	Pearson Correlation	0.057-	1	0.161-	0.017-	0.021
	P. Value	0.641		0.183	0.890	0.860
Na/K ATPase	Pearson Correlation	0.013	0.161-	1	0.020-	0.148

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	P. Value	0.918	0.183		0.869	0.223
Iron (µmol/L)	Pearson Correlation	0.107	0.017-	0.020-	1	0.024
	P. Value	0.380	0.890	0.869		0.845
Potassium (mmol/L)	Pearson Correlation	0.058	0.021	0.148	0.024	1
	P. Value	0.631	0.860	0.223	0.845	

In this study the activity of Na/K ATPase in patients is may be due to compensation mechanism for adaptation of low oxygen and its physiological role in the cell. This agreed with omer et al. which found the activity in healthy individuals showed a significantly lower values compared to iron deficiency patients and the difference was highly significant and suggested that the activity of Na^+, K^+-ATPase can be used for the diagnosis of individuals with blood diseases/disorders[12]. More specifically, investigations have demonstrated that Na+/K+ ATPase activity is reduced in milder forms of thalassemia (thalassemia-like cells), whereas it shows increased activity in severe alpha-thalassemia and beta-thalassemia cells. This paradoxical increase in Na+/K+ ATPase activity in severe forms may represent a compensatory mechanism in response to the more profound cellular stress [13]. In thalassemia, the enzyme's functionality becomes significantly altered due to oxidative damage induced by free globin chains, which has been implicated in the pathogenesis of membrane abnormalities [14].

Also, the increased of Na concentration attributed to in thalassemia, the enzyme's functionality becomes significantly altered due to oxidative damage induced by free globin chains, which has been implicated in the pathogenesis of membrane abnormalities [15] . Elevated sodium levels in thalassemia are most commonly attributed to increased red cell membrane permeability and renal impairment secondary to iron overload. Regular monitoring of electrolytes and renal function is recommended in the management of thalassemia patients[16].

In this study elevated of iron level as a result of iron overload and repeated transfusion of blood to replace the destructed RBC due to membrane instability. The cornerstone of iron overload pathophysiology in thalassemia is ineffective erythropoiesis, which leads to profound dysregulation of iron homeostasis. Ineffective erythropoiesis occurs when thalassemia mutations cause imbalanced globin chain production, resulting in the premature death of developing red blood cells within the bone marrow [17]. This process triggers a compensatory increase in erythropoiesis and suppresses hepcidin production through specific mediators, particularly erythroferron [18].

The suppression of hepcidin results in two detrimental consequences: increased intestinal iron absorption and enhanced release of recycled iron from macrophages2. In non-transfused patients with severe thalassemia, this abnormal dietary iron absorption increases body iron burden between 2 and 5 grams per year, substantially exceeding the body's natural iron excretion capacity of only 1-2 mg daily [19].

Alongside the elevated of K^+ maybe result from the increased blood hemolysis and repeated blood transfusion. The breakdown of abnormal red blood cells in thalassemia releases intracellular potassium into the bloodstream. In β-thalassemia minor, erythrocytes exhibit altered membrane permeability, leading to excessive potassium efflux during incubation in autologous serum. This phenomenon occurs even in asymptomatic carriers, as thalassemic red blood cells lose 40-60% of their intracellular potassium within 24 hours due to defective ion transport. The hemolytic process in transfusion-dependent thalassemia major further exacerbates this effect, with serum lactate dehydrogenase (LDH) levels—a marker of hemolysis—showing direct correlation with potassium concentrations [20].

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Conclusions

The altered metabolic mechanisms in thalassemia may effect on activity of Na/K ATPase, Na, Iron Na, and magnesium homeostasis.

Ethical approval

The scientific committee of childbirth and children's Hospital and Centre for thalassemia in Babylon province obtained ethical approval. The aims of this investigation were communicated to all study participants to acquire verbal acceptance from attending patients.Approved by the scientific committee of the chemistry department of Babylon College of science. The local ethics committee reviewed and approved the research protocol, subject data, and consent on 5/11/2024 under document number 7801 to get this permission.

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