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Red Cell Na-K-ATPase Activity and Electrolyte Homeostasis in Thalassemia

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Abstract: Oxidative damage induced by free globin chains has been implicated in the pathogenesis of the membrane abnormalities observed in β thalassemia. We determined whether thalassemia could account for abnormal cation transport system. During a study of clinical and laboratory features in 70 patients with thalassemia (age between 2-20 years) along with same number of healthy teenagers, we have evaluated erythrocyte Na, K content, membrane bound Na-K-ATPase activity and PCV (packed cell volume). Serum levels of Na, K, Mg^{+2}, Ca^{+2} and Li were also measured. With the exception of K, altered levels of cation were observed. We found significant decrease in the PCV values. Red cell Na-K-ATPase activity also reduced significantly, where as red cell Na was significantly increased. We also observed a significant reduction in the concentrations of serum Ca^{+2}, Mg^{+2} and Li. Serum Na was increased significantly. We can conclude that defective membranal transport is responsible for observed changes of electrolytes in red cell and serum. These results may help to understand the altered electrolyte homeostasis in thalassemia but there is still need of many future studies to clarify their mechanism of generation and pathological significance.

Key words: Thalassemia, Na-K-ATPase, sodium, potassium, magnesium, lithium, calcium

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INTRODUCTION

Thalassemia is a congenital hemolytic disorder caused by a partial or complete deficiency of alpha- or beta-globin chain synthesis[1]. Homozygous carriers of beta-globin gene defects suffer from severe anemia and other serious complications from childhood. The major abnormality within red blood cell (RBC) of patient with severe form of thalassemia results from the precipitation of unstable hemoglobin chain, which is present in excess[2,3]. The RBC membrane is severely damaged by the excess precipitating hemoglobin chains. Almost every component of the thalassemic RBC membrane is altered: lipids, proteins, sialoglycoproteins and glycolipids[4]. This membrane damage represent an important mechanism leading to anemia in thalassemia[5].

Impairment of anion and cation transport in thalassemia is very obvious. Thalassemic RBCs particularly from splenectomized patients[6] loss K because of an increase in selective permeability of the membrane to K, which results in shrinkage of RBCs and increased cellular rigidity[7,8]. Na-K-ATPase activity(membrane bound enzyme) is reduced in thalassemia like cells, where as it is increased in severe α- and β-thalassemic cells[8]. This reduced membrane-associated ATPase activity is also due to the premature destruction of red cells both in the bone marrow and by the reticuloendothelial system[9].

Intracellular Ca^{+2} concentration in RBCs of β-thalassemia is markedly elevated[10,2]. Increased serum level of K in thalassemia is attributed to the rapid erythrocyte turnover[11].

Distribances in magnecium balance should always be suspected in association with other fluid and electrolyte disturbances[12]. Serum level of Mg^{+2} is found to be normal in hemoglobinopathies specially in thalassemia[13]. The present study was designed to investigate membrane bound Na-K-ATPase activity, intracellular sodium, potassium and serum electrolytes in thalassemia.

MATERIALS AND METHODS

Study Protocol: Seventy individuals, 2-20 years of age with thalassemia (major) diagnosed by standard methods were eligible for the study if they had normal renal and liver function. Seventy healthy individuals with same age were selected for control group.

Samples: From Fatmid blood transfusion centre in the year 2001-2002 blood samples of patients were collected in lithium heparin coated tubes for analysis of erythrocyte membrane Na-K-ATPase activity and intra erythrocyte sodium and potassium, an aliquot was taken in another tube to get serum for the estimation of serum electrolytes. Blood samples were processed the same day for estimation of electrolytes in serum and red cells.

Intraerythrocyte sodium and potassium estimations: Heparinized blood was centrifuged and plasma was separated. Buffy coat was aspirated and discarded erythrocytes were washed three times at room temperature by suspension in the magnesium chloride solution (112 mmol L^{-1}), centrifugation at 450 x g at 4°C for 5 min and the aspiration of the supernatant as described earlier[14]. Final supernatant was retained for the estimation of intraerythrocyte sodium and potassium concentration neither electrolyte was detectable in the final wash. Erythrocytes were then lysed and used for the estimation of intraerythrocyte sodium and potassium.

Erythrocyte membrane preparation: The packed red cells extracted by centrifugation at 4°C, 450 x g for 15 min. were resuspended and diluted in 25 volumes of 0.011 mol L^{-1} Tris-HCl buffer at pH 7.4. The hemolysed cells were then centrifuged for 30 min. at 12,000 rpm at 4°C and the membrane pellet was resuspended in 30 ml of 0.011 mol L^{-1} Tris-HCl buffer. This centrifugation step was repeated three times. The final concentration of the membrane suspension was ~4 mg protein ml $^{-1}$ of Tris buffer. The membrane suspension was stored at -80°C until the assay was performed.

Erythrocyte Na-K-ATPase activity measurement[15]: ATPase activity was measures in a final volume of 1 ml as follows: Membrane (400ug) were preincubated for 10 min. at 37°C in a mixture containing 92 mmolL^{-1}-HCl(pH=7.4), 100 mmol L^{-1} NaCl, 20 mmol L^{-1} KCl, 5 mmol L^{-1} $MgSO_4.H_2O$ and 1 mmol L^{-1} EDTA. Assays were performed with or without 1 mmol L^{-1} Ouabain, a specific inhibitor of Na-K-ATPase. After incubation with 4 mmol L^{-1} ATP(Vanadate free, Sigma) at 37°C for 10 min. the reaction was stopped by adding of ice-cold trichloroacetic acid to a final concentration of 5%. After centrifugation at 4°C, 5500 g for 10 min. The amount of inorganic phosphate in the supernatant was determined[16]. Na-K-ATPase activity was calculated as the difference between inorganic phosphate released during the 10 min. incubation with and without ouabain. Activity was corrected to a nanomolar concentration of inorganic phosphate released/milligram protein/hour.

Serum sodium, potassium, calcium and lithium were estimated by flame photometer (Corning 410).Serum magnesium by the method of Hallry and Sky Peck[17].

Statistical analysis: Results are presented as mean ± SE. Level of significance from control and SS patients were evaluated by student's t-test.

RESULTS

Two-way ANOVA showed a significant difference ($P<0.01$) for all measures (sex, disease, interaction) of red cell Na, their PCV values and Na-K-ATPase activity. For red cell K two-way ANOVA showed a significant difference ($P<0.01$) for sex and interaction, for disease two-way ANOVA showed no significant change in red cell potassium (Table 1).

Lower mean levels of the red cell PCV values in both male and female patients were observed ($P<0.01$), as compared to control group. There is a significant increase in the mean value of red cell Na for male patients ($P<0.05$), where as mean value of female patients raised non-significantly than control. In thalassemic group mean value of female patient showed a significant decrease ($P<0.05$) in red cell sodium as compared to male patients. The mean values of red cell K concentration in both male and female patients were non-significantly raised as compared to control. Mean values of Na-K-ATPase activity in both male and female patients showed a significant decrease ($P<0.01$) than control group. Mean values of intraerythrocyte sodium, potassium, Na-K-ATPase activity and PCV values in male control and female control did not show any significant variation. In the thalassemic group except Na, mean values of red cell K, PCV values and Na-K-ATPase activity in both male and female patients showed no significant variation.

According to Table 2, two-way ANOVA showed a significant difference ($P<0.01$) for all measures (sex, disease, interaction) of serum Ca^{+2} and Li. In serum Na and K two-way ANOVA showed a significant difference for sex and interaction ($P<0.01$), for disease there was no significant difference observed. In serum Mg^{+2}, two-way ANOVA again showed a significant difference for sex and interaction ($P<0.01$); as well as for disease ($P<0.05$).

The mean value of serum Na concentration increased significantly ($P<0.05$) in male patients where as it was decreased significantly ($P<0.01$) in female patients as compared to their controls. The mean level of serum K showed no significant change in both male and female patients of thalassemia as compared to control group. Mean values of serum Na concentration in female control increased significantly ($P<0.01$) as compared to male control, similarly mean value of serum K in female control increased significantly ($P<0.05$). There was a significant decrease observed in the mean levels of both male and female patients of serum Ca^{+2} ($P<0.01$). The mean value of serum Li decreased significantly in both male ($P<0.05$) and female ($P<0.01$) patients as compared to their contol. Mean value of serum Mg^{+2} showed no significant variation in male thalassemic patients where as in female patients Mg^{+2} level was significantly decreased ($P<0.01$) as compared to female control. Mean values of serum Ca^{+2}, Li and Mg^{+2} in male and female control did not show any significant change. In the thalassemic group, again there was no significant variation observed in the mean values of serum Ca^{+2}, Na, K, Li and Mg^{+2}.

DISCUSSION

The results presented in Table 1 and 2, shows differences of serum and erythrocytes cation transport in thalassemia.

The PCV(packed cell volume) measurements of the thalassemic patients showed marked abnormalities as compared to controls. The PCV value of both male and female thalassemic patients decreased significantly ($P<0.01$). This may be generated due to low mean corpuscular hemoglobin concentration (MCHC), because the lack of normal quantities of intracellular hemoglobin, which serves as a substrate for the potent oxygen radicals and could result in an excess of free radicals which oxidize various membrane components lead to membrane damage in thalassemic RBCs[18].

Disturbances in monovalent cation transport are manifested by osmotic swelling or shrinkage and this can be a consequence of rare genetic defects in cation transport[19]. The Na-k-ATPase activity in both male and female subjects of thalassemia decreased significantly ($P<0.01$)[9]. The elevated concentrations of intracellular Na and K is also associated with a lower activity of red cell Na-K-ATPase activity[20]. The red cell Na concentration only in male thalassemic patients increased significantly ($P<0.05$), may be due to reduced pump activity. The group mean of red cell K did not show any significant variation for both male and female thalassemic patients unlike previous studies[9].

Enhanced permeability of cations in thalassemia has been described previously[10]. Increased serum level of potassium in β-thalassemia major was attributed to the rapid erythrocyte turnover[21]. There is also a relationship between abnormal K leak and hemoglobin precipitation on the membrane[24]. Oxidative damage is responsible for the K-loss in β-thalassemia by increasing the activity of K-Cl cotransport[9]. Unchanged serum K concentration in both male and female thalassemic patients not seems to be in agreement with earlier studies. In both male and female patients of thalassemia the K was non-significantly increased, did not show any variation as compared to control subjects.

For the preservation of the intracellular Ca^{+2} against a very steep electrochemical gradient should be necessary[22] and it is mediated both by a low membrane permeability to Ca^{+2} in the inward direction and by an active efflux mechanism catalysed by the membrane

Table 1: Erythrocyte Sodium, potassium, sodium-potassium-ATPase activity and PCV values in thalassemic patients and control subjects

	Male		Female		Two-way-anova (Df 1, 136)		
Parameters	Control	Test	Control	Test	Sex	Disease	Interaction
PCV	42.54±2.89	21.80±7.05**	41.57±2.68	20.54±9.14**	F=1803.02	F=407.96	F=1804.20
Na (mmol L^{-1})	8.91±4.49	12.61±7.59*	7.98±3.36	9.89±6.26^{+}	F=170.77	F=8.74	F=175.41
K(mmol L^{-1})	104.11±38.21	112.36±51.69	120.50±43.30	122.76±55.64	F=450.98	F=0.42N.S	F=453.87
Na-K-ATPase Activity(nm mg^{-1} hr^{-1})	422.14±287.54	72.40±71.45**	446.25±340.98	48.21±42.45**	F=83.13	F=95.00	F=83.52

Table 2: Serum electrolytes (sodium, potassium, calcium, magnesium and lithium) in patients with thalassemia and control subjects

	Male		Female		Two-way-anova (Df 1, 136)		
Parameters	Control	Test	Control	Test	Sex	Disease	Interaction
Na (meq L^{-1})	109.689±18.52	119.93±21.37*	135.46±15.41^{++}	123.11±20.22**	F=3212.38	F=0.10NS	F=3244.85
K (meq L^{-1})	3.30±0.83	4.00±1.19	3.98±1.33^{+}	4.08±1.66	F=683.03	F=3.42NS	F=687.99
Ca^{+2} (meq/L^{-1})	0.48±0.08	0.39±0.07**	0.44±0.11	0.37±0.08**	F=1430.58	F=22.86	F=1434.19
Mg^{+2} (meq L^{-1})	3.72±0.56	3.31±1.90	3.60±0.80	3.59±2.52**	F=106.91	F=4.81	F=106.99
Li (meq L^{-1})	2.14±0.46	1.79±0.96*	2.33±0.74	1.65±0.71**	F=504.74	F=16.83	F=506.50

Values are mean±SD(n=140). Following two-way ANOVA, Significant difference by Newman Keuls test, From respective controls P<0.05*, P<0.01**, From male and female thalassemic patients +P<0.05^{+}, P<0.01^{++}

bound Ca^{+2}-Mg^{+2} ATPase[23]. Previously a significant increased Ca^{+2} concentration in red cells of thalassemic patients is reported[2]. As we did not estimate erythrocyte Ca^{+2}, but on the basis of a significant decrease in serum Ca^{+2} both in male and female patients with thalssemia (P<0.01), we can hypothesized that due to membranal defects Ca^{+2} ions influx increases and more Ca^{+2} shift from extracellular to intracellular fluid. Another possibility of this hypocalcemic state is the frequent blood transfusions which is essential for thalassemia major patients. For the preservative purpose the stored blood is anticoagulated with citrates, which binds Ca^{+2} ions to form salts, in this way Ca^{+2} might be decreased in serum of thalassemic patients.

Serum Li concentration showed a significant variation. The serum lithium of both male (P<0.05) and female(P<0.01) thalassemic patients were decreased significantly. In human red cells Li is extruded against its own concentration gradient if the external medium contains Na as a dominant cation[25]. Extracellular lithium concentration affected RBC lithium accumulation[26]. As it is obvious, for Na-Li-counter transport, both Na and Li should be bound to their ligands, while according to our result thalassemic patients shows hyponatremia, therefore no Na for this counter transport. So we can hypothesized that pump activity might be reduced and no lithium out flux occur showing decrease extracellular lithium which coincides to our results.

Disturbances in magnesium balance should always be suspected in association with other fluid and electrolyte disturbances[12]. Previously serum level of Mg^{+2} was found to be normal in hemoglobinopathies specially in thalassemia[13]. Our results also did not show any significant variation in serum Mg^{+2} in male thalassemic patients where as in female patients it is decreased significantly(P<0.01).

The decreased serum level of Na in female patients(P<0.05) of thalassemia seems to be in agreement with earlier studies[10], where as hypernatremic condition in male patients (P<0.01) is associated with increased plasma osmolality, contrasts with previously reported normal concentration[21].

Abnormal membrane function plays a relevant role in the alteration of membrane cation transport as observed in thalassemic RBCs. The defective sodium, potassium transport in red cell and serum is associated with disturbed Na-K-ATPase (membrane bound) activity. Changes in the levels of serum sodium, potassium, lithium, magnesium and calcium reflects the defective membranal transport of the cations in the red cell membrane of thalassemia. These results provide a confirmation that abnormal cation homeostasis may contribute to the pathogenesis of thalassemia.

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