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TO STUDY THE EFFECT OF IRON LOAD ON PLASMA MINERALS AND HEMATOLOGICAL PARAMETERS IN THALASSEMIA PATIIENTS

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ABSTRACT

Thalassemias are genetic disorders that involve the decreased and effective production of hemoglobin a molecule found inside all red blood cells (RBCs) that transport oxygen throughout the body. There are two types of thalassemia, alpha-thalassemia and beta-thalassemia. Their names describes which part of the hemoglobin molecule is affected, the alpha or the beta chain. Thalassemia can cause ineffective production of RBCs and their destruction. As a result, people with thalassemia often have a reduced number of RBCs in the bloodstream (anemia), In addition, thalassemia can cause the morphological change in RBCs and the life of red blood cells reduces. The destruction of RBCs occurs and iron released in the plasma. B-thalassemia is one of the commonest inherited Hemoglobin disorder in Pakistan. In a population of nearly 130 million, in northern areas, 83% of the children suffering from refractory anemias have β-thalassemia. Alpha thalassemia carrier frequency estimated by Hemoglobin Bart's in cord blood samples is 2.4% showing that both α and β-thalassemia are present in Pakistan. Fifty blood samples of children (age 3-13 years) having thalassemia were selected for the study. The serum was analyzed for the determination of the electrolytes (sodium, potassium, calcium, chloride, magnesium and phosphorus). Plasma sodium and potassium were analyzed by flame photometer, while calcium, chloride, magnesium and phosphorus were determined by the Merck Assay kit.The aim of present study was to estimate the effect of iron load on plasma minerals (sodium, potassium, calcium, magnesium, chlorides and phosphorus) and hematological parameters (RBC, WBC, Platelets, Hemoglobin, Neutrophills, Eosinophills, Basophiles, Lymphocytes, Monocytes, PCV, MCV, MCH, MCHC) in thalassemia patients. This study comprisedof children having thalassemia belonging to urban and rural areas of sindh province of Pakistan.

Key words: Thalassemia, Malnutrition, Disease, Platelates

INTRODUCTION

Thalassemia is the world's most common monogenic disorder. It has been estimated that worldwide there are 270 million carriers of mutant globin alleles which can potentially cause severe forms of Thalassemia and hemoglobinopathy (including sickle cell diseases) (1). Over the past 50 years, as the world economy improves and the overall infant mortality rate falls, Thalassemia is rapidly emerging as a major economic and health burden in developing countries since infants with Thalassemia who would otherwise have died from infection and malnutrition now survive and require lifelong treatment. Therefore, worldwide, Thalassemia is an enormous and ever-increasing hematologic problem in both developed and developing countries (2).

On global scale, Thalassemia cause far more ill health than any other group of inherited conditions. The frequency of different forms of Thalassemia varies from area to area. In South-East Asian countries α- Thalassemia and HbE-thal are most common, while in Africa, the Mediterranean region, Indian sub continent and South-East Asia, β- thal is common (3).

Although α-thal is found throughout the world, its distribution varies among different populations. The prevalence of deletion of one α-globin gene is reaching over 80% in some area of Nepal, Nepal and Southwest Pacific (4).

β- Thal is one of the commonest inherited Hb disorder in Pakistan. (5). In a population of nearly 130 million, in Northern areas, 83% of the children suffering from refractory anemias have β-thal (6). The carrier frequency is estimated to be 5.4% (7), although the incidence of β-thal trait varies from 1.4% (8) to 7.96% (7). A higher prevalence of the disease occurs in areas along the Arabian Sea Cost in the south and the North West Frontier Province (NWFP) bordering Afghanistan, where people from the Middle East, the Mediterranean and the central Asia have settled after invasions during various periods in history. Alpha thal carrier frequency estimated by Hb Barts in cord blood samples is 2.4% (9) showing that α and β thal are present in Pakistan.

Many social factors such as preference to marry within ethnic groups and consanguineous marriages have also contributed to the increased incidence of this disease in the Pakistani population (10).

The extant and magnitude of the problem of Thalassemia in Pakistan is alarming. Life expectancy and quality of life are low for β-thal patients in this country because provision of good quality, screened blood increases the cost which is serious problem and the facilities for bone marrow transplant are so far not available. Genetic counseling and prenatal diagnosis can play an important role in reducing the incidence of this disease. (11).

In 1925, Cooley and Lee first describe a severe form of anemia occurs in children and associated with splenomegaly and characteristic bone changes. This condition was called Cooley's anemia but later named Thalassemia (12).

MATERIALS AND METHOD

Equipments were calibrated daily with quality control (QC) materials, and instrumentation standards were maintained within 2 standard deviations (SD) of those QC values. The research work was carried out in the clinical research laboratory of the institute of biochemistry, University of sindh Jamshoro.

The blood samples were collected into well labeled set of sample bottles without anticoagulant and were kept in refrigerator at about 4^{o}c to aid sedimentation. The samples were later spun in a centrifuge machine at 3000 rpm for 10 minutes and the serum minerals (Sodium, Potassium, phosphorous, Calcium, Chloride and Magnesium).

The whole blood samples were collected into well labeled set of sample bottles with anticoagulant (K2EDTA, 1mg/ml of blood) was used for the determination of hematological parameters (Hemoglobin, Red Blood Cells Count, White Blood Cells Count, Platelets, Neutrophils, Esinophils, Basophils, Lymphocytes, Monocytes Packed cell Volume, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin concentration).
During the study Micro lab 200 (GERMANY) and the flame photometer technique were used for the estimation of electrolytes.

Equipment Used
Sodium and potassium were estimated by using flame photometer (Jenway-Clinical PFP7). And magnesium, calcium, chloride and phosphorus were estimated by ELI TECH, BIO MERIEUX, DIALAB and DIASYS Gmbh respectively.
Hemoglobin, total leukocyte count, red blood cells count, differential leukocytes count, platelets count and absolute indices (PCV, MCV, MCH, and MCHC) were determined by using hematological analyzer.
Procedure
Maximum absorbance: 505nm
Measuring range: 490-515nm, eg.492nm
Light path: 1 cm
For every sides of determination one regent blank is required.

One or two standards are necessary for each series.

	Sample(s)	**Standard(ST)**	**Reagent blank**
Buffer(1)	1000 micro liter	1000 micro liter	1000 micro liter
Serum(diluted)	20 micro liter	----------	----------
Standard (3)	----------	20 micro liter	----------
Bidest. water	----------	----------	20 micro liter
Color reagent(2)	200 micro liter	200 micro liter	200 micro liter

Mix and incubate for 2 to 20 minutes. Measure the absorbance of the sample (AS) and the absorbance of the standard (AST) against the reagent blank.
Dilution Limit:-
5.3mg/dl or 2.2mmol/l

RESULTS AND DISCUSSION

Table 1 shows the mean and standard deviation of plasma minerals (Phosphorous, Calcium, Magnesium, Chloride, Sodium and Potassium) in Thalassemia patients. Our data reveals that Phosphorous and Chloride are found to be increased in Thalassemia patients and decreased concentration of Calcium while concentration of Magnesium, Sodium and Potassium are found to be within the normal range.
Table 2 shows the mean and standard deviation of Hematological parameters (Hemoglobin, Red Blood Cells Count, White Blood Cell Count, and Platelets Count) in Thalassemia patients. Our data reveals that there is decreased concentration of RBCs and Hemoglobin, while concentration of Platelets and WBCs are found within the normal range.
Table 3 shows the mean and standard deviation of Differential Leucocytes Count (Neutrophils, Eosinophills, Basophills, Lymphocytes and Monocytes) in Thalassemia patients. Our data reveals that there is no significant alteration is found in differential leucocytes count including Neutrophills, Lymphocytes, Basophils, Eosinophils and monocytes.

Table 4 shows that the mean and standard deviation of Absolute Indices parameters (Packed Cell Volume, Mean Corpuscular Volume, Mean corpuscular Hemoglobin and Mean Corpuscular Hemoglobin Concentration) in Thalassemia patients. Our data reveals that there is decreased in PCV, MCV, MCH and MCHC is found to be normal in Thalassemia patients.

TABLE 1 SHOWING THE MEAN AND STD. DEVIATION OF PLASMA MINERALS IN THALASSEMIA PATIENT

Parameters (n=27)	**Normal Values**	**Mean± Std. Deviation**
Phosphorous	0.80 – 1.45mmol/l	2.39 ±0.506
Calcium	2.2 – 2.53mmol/l	1.76 ±0.311
Magnesium	0.7 – 0.99mmol/l	0.889 ± 0.621
Chlorides	90 – 147mmol/l	121 ± 142.1
Sodium	137 – 147mmol/l	135 ± 2.69
Potassium	3.8 – 5.4mmol/l	5.05 ±0.353

TABLE 2 SHOWING THE MEAN AND STD. DEVIATION OF HEMATOLOGICAL PARAMETERS (HEMOGLOBIN, RBCS, WBCS AND PLATELETS COUNT) IN THALASSEMIA PATIENTS.

Hematological Parameters (N=39)	**Normal Values**	**Mean ± Std. Deviation**
Hemoglobin	13.7 – 16.3 g/dl	7.19 ± 1.72
Red Blood Cells Count	4.5 – 6.5 x 10^{12} /l	2.7 ± 0.65
White Blood Cells Count	4 – 10 x10^{9} /l	8.08 ±4.01
Platelets Count	150 – 450 x 10^{9} /l	157 ±102.60

TABLE 3 SHOWING THE MEAN AND STD. DEVIATION OF DIFFERENTIAL LEUCOCYTES COUNT (NEUTROPHILS, EOSINOPHILS, BASOPHILLS, LYMPHOCYTES AND MONOCYTES) IN THALASSEMIA PATIENT.

Hematological Parameters N=26	**Normal Values**	**Mean ± Std. Deviation**
Neutrophils	41.9 – 48.7%	27.3 ± 12.06
Eosinophils	01 – 6 %	0.96 ± 0.156
Basophils	0 – 1 %	0.30 ± 0.47
Lymphocytes	40 – 70 %	65.42 ± 13.33
Monocytes	2 – 10 %	6.53 ± 3.24

TABLE 4 SHOWING THE MEAN AND STD. DEVIATION OF ABSOLUTE INDICES PARAMETERS (PCV, MCV, MCH AND MCHC) IN THALASSEMIA PATIENTS

Hematological Parameters N=40	**Normal Values**	**Mean ± Std. Deviation**
Packed cell Volume	41.9 – 48.7%	21.9 ± 5.03
Mean Corpuscular Volume	76 – 96 cμ	75.57 ± 4.87
Mean Corpuscular Hemoglobin	26 – 32 μg	24.91 ±2.08
Mean Corpuscular Hemoglobin Concentration	32 – 36%	32..87 ± 1.50

Discussion

The goal of the present study was to study the effect of iron load on plasma minerals (calcium (1.76 ± 0.311,2.2-2.53 mmol/l), Phosphate (2.39± 0.5.6, 0.80 -1.45 mmol/l), Magnesium (0.889 ± 0.621, 0.7 -0.99 mmol/l), Chloride (121± 142.1, 90 -110 mmol/l), Sodium (135±2.69, 137 -147 mmol/l), Potassium (5.05± 0.353, 3.8 -5.4 mmol/l) and hematological parameters (Hemoglobin (7.19± 1.72, 13.7 -16.3 g/dl), Red Blood Cells (2.7±0.65,4.5 – 6.5 x 10^{12}/l), White Blood Cells (8.08± 4.01, 4-10x 10^{9} /l), Platelates (157± 102.6, 150 – 450 x 10^{9}/cmm), Neutrophills (27.3± 12.06, 20 – 40 %), Eosinophills (0.96±0.156,1 – 6%), Basophills (0.30± 0.47, 0 – 1"%), Lymphocytes (65.42±13.33, 40 – 70%), Monocytes (6.53±3.24, 2 – 10%), Packed Cell Volume (21.9± 5.03, 41.9 – 48.7 %),Mean Corpuscular Volume (75.57± 4.87, 76 – 96 cμ), mean Corpuscular hemoglobin (24.91±2.08, 26 – 32 μg), Mean Corpuscular Hemoglobin Concentration (32.87± 1.50, 32 - 36 %)) in Thalassemia Patients.

The result were obtained suggest that alteration in plasma minerals particularly increased concentration of phosphorous and chloride and decreased concentration of calcium, but no significant differences was found in case of serum sodium, potassium and magnesium when compared with the reference values. Hematological parameters are affected by many factors, including age, sex, diet recent nutritional status, consumption of medications or illicit drugs and the chronic disorder (48-51)

There was a statistical significant difference in serum calcium between the control group and β-thalassemiac patents. The hypocalcaemia in thalassemia patents also reported by other workers (5, 6).

The case of a thalassemiac patent with severe cardiac failure has been reported due to hemosiderosis, hypomagnesaemia, and hypocalcemia, refractory to conventional cardiac therapy. Calcium and magnesium supplementation may improve the myocardiopathy (52).

The same observation for calcium level was also reported by Sake et al (53). Hypocalcaemia is a well known complication of iron overload . Iron overload occurs either from the transfusion of red blood cells or because there is increased absorption of iron from the digested tract. Both of these occur in thalassemia. Iron overload also cause pituitary damage with hypogonadism, endocrine complication, hypothyroidism and hyperparathyroidism is also seen (54). Parathyroid hormone which is secreted by the parathyroid gland mobilizes calcium from bone (54, 55).

The concentration of phosphorous increased in thalassemia due to rapid lysis of red blood cells. Cell membrane is made up of phospholipids and due to rupture phosphorous comes out of the cell and thus its concentration increase in the plasma.

Homozygous β-thalassemia is a sever heredity disorder characterized by decreased synthesis of the β-polypeptide chain of hemoglobin, which leads tom the development of a hypo chromic microcytic anemia (10). Persons with β-thalassemia minor are usually symptomless but the peripheral blood picture reveals minimal anemia and microcytosis (56).

The expansion of the ineffective elytroid mass that occurs in β-thalassemia, estimate to be between 10 and 30 times normal in some cases (Finch et al, 1970) (59). On the other hand frequent blood transfusion in turn can lead to iron overload (60, 61).

As iron loading progresses, the capacity of serum transferring, the main transport protein of iron, to bind and detoxify iron may be exceeded and a non transferrin- bound fraction of plasma iron may promote the generation of free hydroxyl radicals, propagators of oxygen related damages (63, 64).

In patients who are not receiving transfusions abnormally regulated iron absorption results in increases in body iron burden ranging from 2 to 5 g per year, depending on the severity of erythroid expansion (63).

Combined measurements of iron absorption and ferrokinetics have demonstrated a consistent relationship between the magnitude of increased erythropoiesis and radioactive iron absorption in patients with β-thalassemia.

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