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Research Article

BIOCHEMICAL STATUS OF BONE TURN OVER IN TRANSFUSION DEPENDANT B-THALASSEMIA CHILDREN; A COMPARATIVE STUDY

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Abstract:

Background and objectives: *Thalassemia is amongst the most common inborn blood disorders and beta thalassemia is the extreme form. The collective effect of transfusion supported with chelation has increased the life expectancy of thalassemia patients but with the complications of deranged biochemical status. The purpose of the present study was to assess the biochemical status of bone turnover in thalassemia children who are regularly transfused and comparison to normal population of children with same age group.*

Subjects and methods: *In this study, 65 healthy and 65 beta thalassemia major children were recruited of age 5-11 years. Serum content of calcium, phosphate, alkaline phosphatase, albumin and hemoglobin were measured and compared with the controls.*

Results: *Biochemical status was deteriorated in beta thalassemia major children as compared to control group. Serum calcium, phosphate and alkaline phosphatase were significantly raised in beta thalassemia major children as compared to controls. Serum albumin and hemoglobin were reduced in thalassemia group.*

Conclusion: *Our study concludes that beta thalassemia major children and controls have difference in biochemical profile which emphasizes the need for increasing the struggles of regular assessments and follow ups of thalassemia children. This could be used to adjust, improve and enhance the management protocols which ultimately raise the quality of life of these children in future.*

Keywords: *Beta thalassemia major, serum calcium, serum phosphate, albumin.*

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INTRODUCTION:

Among the most common monogenic diseases in humans around the globe, thalassemia is one of them. They belong to the family of anemias which are hereditary in nature, occur due to mutation in the hemoglobin gene which disrupts the synthesis rate of one or more globin chains. [1] Practically, thalassemia is encountered in every geographical location of the world but this disease is most common in Mediterranean region, Middle East, Arabian peninsula, Indian subcontinent, and South East Asia. [2] It is predicted that 4000 to 9000 newborns are being added to the existing pool of disease each year. In our part of the world, the carrier frequency is 5 to 8% and is seen in all ethnic groups uniformly. [3]

Thalassemia is classified according to globin genes with alpha and beta thalassemia forms worldwide. [4] The severity of disease is sub-classified according to mutation in the genes with totally absent or partially reduced globin chain synthesis. [5] But still, beta thalassemia is the most important and common type of blood disorders because affected individuals are unable to make healthy red blood cells throughout their life and dependent upon regular blood transfusions. [6] Above 200 genetic variants of beta globin genes have been identified and different variety of severities of thalassemia is because of mutations in these variants. Among all, point mutations leads to most of the types of beta thalassemia. [7] The major defect in beta thalassemia is due to imbalance in the synthesis of globin chain which leads to presence of excess of alpha chains. These alpha chains are unstable and precipitate in bone marrow precursors of red blood cells, forming large intracellular inclusions leading to ineffective erythropoiesis and various complications. [8]

In Thalassemia patients bone related problems are the commonest cause of morbidity and mortality. Various skeletal problems including osteopenia, osteoporosis, rickets, spontaneous fractures, scoliosis, spinal deformities, nerve compression are reported in transfusion dependent thalassemia patients. [9] A large number of factors contributes to skeletal complications including expansion of medullary cavity, accumulation of iron, abnormal balance of calcium and phosphorus, hormonal insufficiency and hypoxia. [10] Disruption of calcium homeostasis is the due to accumulation of iron which negatively affect the bone metabolism.

Essential elements of bones and teeth in humans are calcium, phosphorus and alkaline phosphatase. [11] Rigidity and strength to skeleton is maintained by hydroxyapatite which is the mineral form of calcium

and phosphorus. For the assistance of hydroxyapatite crystal deposition between the collagen filaments of the bone, alkaline phosphatase is helpful. [12] Major role in skeletal mineralization is played by calcium, which is the 5th most bountiful element of human body. Serum calcium is found in two forms; free and bound with albumin. [13] Phosphorus aids in the development of bones conjoint with the calcium. Bony tissue comprises of 85% of total body phosphorus. Bone mineralization is promoted by alkaline phosphatase. It increases the local concentration of inorganic phosphorus. [14]

There are various specific and sensitive markers which determine the bone turnover. Serum albumin, calcium, alkaline phosphatase and phosphate are predominantly altered in thalassemia with bone destruction. Moreover, repeated blood transfusions leads to citrate toxicity which results in iron deposition in parathyroid gland. This leads to alteration in levels of serum calcium and phosphate. [15] Scarce data is available in Pakistani population of thalassemia children of 5-11 years which shows the serum levels of these biomarkers. The purpose of this study was to assess the serum levels of various biochemical markers including calcium, phosphorus, alkaline phosphatase and albumin in thalassemia children and to compare them with the normal population.

MATERIAL AND METHODS:

This cross-sectional comparative study was conducted at the University of Health Sciences, Lahore. Ethical review committee of the institution approved the study protocols. 65 patients of beta thalassemia major and 65 controls from the government school of age 5-11 years were enrolled in our study after taking written informed consent from the parents or guardians of the patients and the controls.

After taking aseptic measures 5 ml of venous blood was drawn. One ml of blood was stored for hemoglobin estimation in EDTA coated tubes while we secured the remaining blood in non-coated vacutainers for extraction of serum by centrifugation for 10 min at 3000 revolutions per minute. Plasma samples were labelled and collected in Eppendorf tubes. Hemoglobin estimation was done immediately by colorimetric method with the help of spectrophotometer using commercially available kit. Spectrophotometer with commercially available kit was used to evaluate serum calcium, phosphate and alkaline phosphatase by colorimetric method while bromocresol green colorimetric method was used to assess serum albumin

Statistical analysis:

All the data was entered and analysed using SPSS version 20. Normality of the data was checked by Shapiro-wilk test . Mean \pm SD was given for normally distributed variables. Median and IQR was given for non-normally distributed variables. p-value ≤ 0.05 was considered statistically significant.

RESULTS:

Mean age of the patients and the controls was found to be 7.45 ± 1.98 and 8.63 ± 1.64 years respectively.

Serum calcium, phosphate and alkaline phosphatase were significantly raised in beta thalassemia major children as shown in Table 1. However, serum albumin and hemoglobin were significantly reduced in these children tabulated in table 1.

Significant negative correlation of serum calcium with alkaline phosphatase ($p < 0.001$) was observed in beta thalassemia major children as shown in Table 2. We observed significant positive correlation of alkaline phosphatase with serum hemoglobin ($p < 0.001$) (Table 2).

Table 1: Comparison of biochemical parameters in normal and β -thalassemia major children

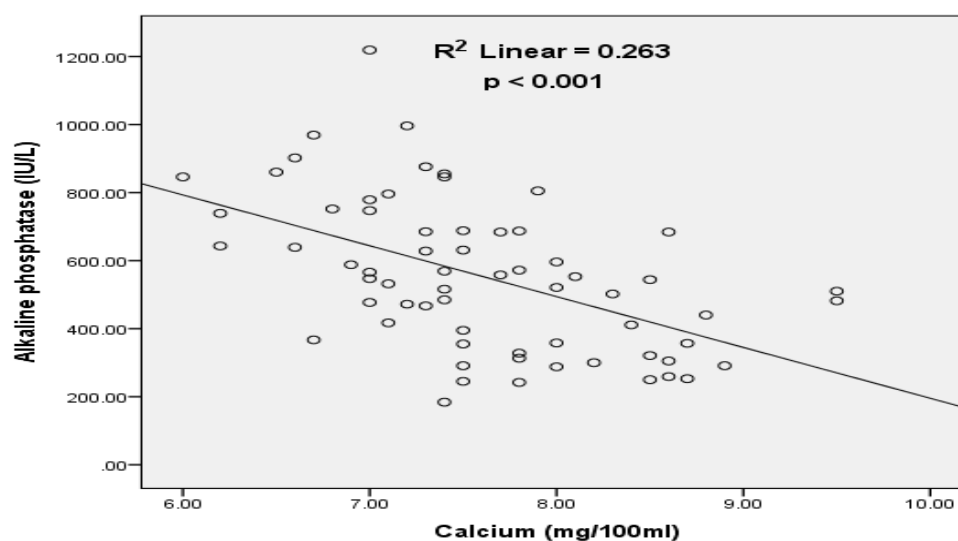
Biochemical Parameters	Apparently healthy children		β -thalassemia major children		p-value
	N	Mean \pm SD	n	Mean \pm SD	
		Median(IQR)		Median(IQR)	
Calcium (mg/100ml)	65	7.22 ± 0.74 7.30 (6.75-7.80)	65	7.60 ± 0.76 7.50 (7.05-8.05)	0.004 ^b
Phosphate (mg/100ml)	65	3.37 ± 0.63 3.30 (2.91-3.75)	65	4.58 ± 1.09 4.40 (3.85-5.10)	<0.001 ^a
Alkaline phosphatase (IU/L)	65	451.38 ± 121.01 457.00 (359.00-520.50)	65	553.58 ± 222.03 544.00 (357.50-687.50)	0.001 ^b
Albumin (g/100ml)	65	3.96 ± 0.48 4.00 (3.60-4.20)	65	3.75 ± 0.52 3.70 (3.35-4.00)	0.019 ^b
Hemoglobin (g/100ml)	65	12.05 ± 1.39 12.00 (11.35-12.70)	65	9.65 ± 2.02 9.40 (8.15-11.00)	<0.001 ^a

^a p-value generated by Mann-Whitney U Test

^b p-value generated by Independent Sample "t"-Test

Table 2: Correlation matrix of serum biochemical parameters with each other in β -thalassemia Major children

Parameters		Phosphate (mg/100ml)	Alkaline Phosphatase (IU/L)	Albumin (g/100ml)	Hemoglobin (g/100ml)
Calcium (mg/100ml)	r / rho	-0.024 ^b	-0.513 ^a	0.110 ^a	-0.029 ^a
	p-value	0.851	<0.001	0.382	0.822
	N	65	65	65	65
Phosphate (mg/100ml)	r / rho		0.130 ^b	0.358 ^b	-0.212 ^b
	p-value		0.302	0.003	0.091
	N		65	65	65
Alkaline Phosphatase (IU/L)	r / rho			0.316 ^a	0.460 ^a
	p-value			0.010	<0.001
	N			65	65
Albumin (g/100ml)	r / rho				0.028 ^a
	p-value				0.827
	N				65

^aPearson correlation^bSpearman rho correlationFigure: Scatter plots showing significant correlations of calcium with ALP in β -thalassemia children

DISCUSSION:

Beta thalassemia is a genetic disease of red blood cells which lead to many biochemical, hematological and systemic deformities. Our study revealed increased levels of serum calcium and phosphate in beta thalassemia children as compared to controls. Current findings of serum calcium are opposite to that of studies done by Modi [16] and Mirhosseini. [17] This difference is due to the fact that our thalassemia children were of lower age group (mean = 7.45 ± 1.98 years) than the above mentioned study results by Mirhosseini i.e. 13.5 ± 3.7 years. Calcium alteration began late in adolescence and this leads to hypoparathyroidism later in life. Multiple blood transfusions lead to iron deposition in parathyroid gland and alter calcium homeostatic mechanism.¹⁵ However studies done by Salama showed no variation in serum calcium in beta thalassemia major children. [18] This means early and effective treatment in controlling and maintaining serum calcium through supplements is essential in the process of improving bone health.

Hyperphosphatemia is an indication of hypoparathyroidism. Excess iron is highly toxic to all body tissues. It is the cause of morbidity and prone to mortality because it causes serious damage like liver fibrosis, heart problems and other endocrine related issues.

Children of beta thalassemia major in this study have increased levels of serum alkaline phosphatase as compared with the controls ($p = 0.001$). This indicates abnormal liver and muscle functions and osteomalacia. Similar results were seen in the studies done by Maher Abdalla. [19] However work done by Goyal did not show significant increase in alkaline phosphatase as compared to controls. [15]

Serum albumin is also significantly reduced in thalassemia population of our children indicating deteriorating liver functions. These findings are similar to the studies done by Saboor. [9]

In short, whole body metabolism is affected by beta thalassemia. Reduced level of beta globin gene leads to anemia. For the correction of anemia, regular blood transfusions are required. It causes iron overload which leads to oxidative stress. Iron got deposited in various organs of the body, deteriorating their functions. Proper chelation therapy and correction of serum biochemical profile can help improve the quality of life of these children.

CONCLUSION:

Children of beta thalassemia major have deranged

biochemical profile. Assertive nutritional support and supplementation of calcium and vitamins are strongly suggested. Balanced nutrition, diet counseling, patient education is required in addition to regular monitoring of biochemical profile. Right monitoring and vigorous treatment will surely improve the bone status and prevent the bone related complications in future.

Limitations:

Limitations of the study was reduced number of sample size and lack of evaluation of parathyroid hormone and vitamin D levels.

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