Evaluation of Bone Density and Leptin in Thalassemic Children

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ABSTRACT

Background and Objective: *β-thalassemia is inherited blood disorder which affects the metabolism of whole body including bones. Absence of globin chain leads to anemia which results in high serum ferritin levels. It affects the levels of serum leptin. This study is designed to evaluate serum leptin and its correlation with bone density in healthy and β-thalassemia children.*

Methods: *It is a comparative cross-sectional study which includes 65 normal and 65 β-thalassemia major children. Their demographic data was recorded. All children were examined for bone status with the help of Quantitative Ultrasound Bone Profiler. Z-score, Speed of Sound (Ad-SOS) and transmission time through bone (BTT) were recorded. Plasma sample was collected to determine serum Leptin level by Enzyme Immunosorbent Assay (ELISA) method.*

Results: *In normal children mean AD-SOS was 1906.86 ± 49.53 m/sec and of β-thalassemia major children was 1893.62 ± 57.88 m/sec. The mean serum Leptin of normal children was 3.12 ± 2.84 ng/ml and of β-thalassemia children was 1.38 ± 1.17 ng/ml.*

Conclusion: *Bone density is reduced in β-thalassemia children in contrast to healthy children. Serum Leptin is significantly decreased in β-thalassemia major children than in healthy controls. Positive correlation of serum leptin with bone profile was observed, though not statistically significant, which indicates that leptin deficiency is a cofactor leading to decline in bone density at early age.*

KEYWORDS*: β-thalassemia major, Bone density, Leptin.*

INTRODUCTION

Leptin has an emerging role in regulating bone mass. Sex hormones helps in regulating this hormone as women have higher levels of leptin than men, shown by studies in different populations.[1,2] This hormone has a diurnal variability while its peak being at night. It is regulated by intricate complex including glucocorticoid, thyroid and insulin.[3,4] Leptin is a cytokine-like hormone, derived from fat, mainly expressed in adipose tissue and encrypted by OB gene.[5] Leptin is a multifunctional hormone and leads to increased energy expenditure and affects angiogenesis, hematopoiesis and inflammation.[6,7] It is a polypeptide of 146 amino acids and has a direct correlation with body mass index (BMI).[8,9]

A large number of papers published on leptin revealed its complex relationship with bone and CNS. Initially work done by Ducy et al.[10] disclosed that leptin is a powerful inhibitor of bone formation when acted centrally. According to Śato et al.[11] serum leptin is inversely associated with bone density and bone formation in fit adult men after adjustment of body weight. Cornish et al.[12] stated that leptin is osteogenic when affect the bone peripherally. It improved the bone strength in mice and also accelerates proliferation of osteoblasts in vitro. Martin et al.[13] demonstrated the dose dependent effect of leptin on bone. Low leptin doses stop loss of bone at both trabecular and cortical compartment. While high dose leptin decrease formation of bone and increase bone resorption. Additionally, a high dose of leptin lea-ds to decrease in abdominal fat mass, IGF-1 levels and body weight.[13]

Miraglia del Giudice et al.[14] have demonstrated the low leptin levels in beta thalassemia patients. Ded-oussis et al.[15], Perrone et al.[16] and the study done on Iranian population by Choobineh et al.[9] also found out the same results of low leptin levels. Iron overload leading to iron deposition in fat cells causes toxic effects of iron which results in free radical formation which inhibits the activity of adipose tissue. This leads to decrease serum leptin levels.[17] These results are similar to the recent studies.[18,19]

Leptin is positively correlated with age as demonstrated by various studies.[20,21] This is because of increase in adipose tissue with age.

Bone health is now documented to contribute to overall lifetime management of children and adolescents with β-thalassemia. There is a severe paucity of data on the effect of leptin on bones in β-thalassemia major patients. According to a report published from Iran, serum leptin was lower in these

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patients.[9] A study done by Shahramian et al.[19] found the reduced levels of leptin in these patients but its correlation with bone mineral density was not studied.

METHODS

A comparative cross-sectional study was performed after approval by ethical committee and institutional review board of University of Health Sciences, Lahore. The research work was done at the Departments of Physiology, Cell Biology and Biochemistry, University of Health Sciences, Lahore. Sample size was calculated with the following formula.[22]

$$n_1 = \frac{(Z_{1-\beta} + Z_{1-\alpha/2})^2(\sigma_1 2 + \sigma_2 2)}{(\mu 1 - \mu 2)^2}$$

"$Z_{1-\beta}$" is the desired power of study = 90%
"$Z_{1-\alpha/2}$" is the desired level of significance = 5%
"σ1" is the standard deviation of case group = 5.02
"σ2"is the standard deviation of control group = 7.8
μ1 is estimated mean in case group = 5.33
μ2 estimated mean in control group = 9.43
The sample size was calculated at 54 in each group according to above formula.
Established cases of β-thalassemia major of 5-11 years old were selected. Because in this age bone is in growing phase and bone density can be monitored properly. For control, normal children, 5-11 years old, were taken without any apparent disease.
Children who had the history of disease which involve the bone i.e. prolonged liver disease, continuing kidney disease, thyroid or parathyroid or other endocrinological problem or the children who are using drugs that influence the bones i.e. calcium, supplemental vitamin D, steroids were also excluded.
Above mentioned diseases/factors were ruled out by using appropriate questionnaire, general record, complete general physical and systemic examination.

Estimation of Bone Density

For the assessment of bone density, Bone Profiler BP01 was used. The apparatus was switched on for at least half an hour before the test. For even moisturization of the probes, ultrasound gel was applied instantaneously after switching on the device. Name, date of birth, gender, code, weight, dominant hand and height was entered in the bone profiler database before taking measurements. BMI and Age were mechanically designed by the device. Ultrasound gel was applied on dominant hand skin in order to obtain good audile coupling between the hand and the hand. For high precision in measurement, software aided calibration was executed. Initially space of inter-metatarsal of dominant hand was measured. Afterwards probe was applied on the dominant hand, the far ends of the 1st phalanx of finger's condyles. The device calculated the amplitude of sound (Ad-SOS). The closing result was documented as an average of these values altogether in four fingers.

Amplitude Dependent Speed of Sound (Ad-SOS)

It is the digit width by period of flight in m/sec. The analysis is documented in time when the pointer is above 2mV. Greater bone density is labelled when the Ad-SOS values are higher.[23]

Z-Score

This is the average deviation an individual's bone profile fluctuates from the normal bone profile of their age, ethnicity and sex. The measured swiftness minus regular normal speed anticipated agreeing to person's age, distributed by the value of a standard deviation in m/s.

Bone Transmission Time (BTT)

It is the variance of arrival phase of the receiving probe and the arrival interval of the ultrasound through fleshy tissue in μsec. It reduces the confusing result of soft flesh. The greater cortical area of bone is indicated by higher value of bone transmission time.[24]

Estimation of Serum Leptin

Serum leptin was estimated by ELISA (enzyme linked immunosorbent assay) as per recommended protocol.

STATISTICAL ANALYSIS

The data were investigated and entered using SPSS 20.0 version and WHO Anthroplus software.

Shapiro-Wilk's was used to check the normality and data P-value ≤ 0.05 was labelled to be non-normally distributed. In circumstance of normal variables, student "t" test was applied to equate group means with each other. In case of non-normal variables, Mann-Whitney U test was applied to compare various variables between two groups. P-value of ≤ 0.05 was considered significant.

RESULTS

The current research comprised of 5-11 years old, 130 children (65 apparently healthy and 65 β-thalassemia major).

Decline in bone profile was observed in β-thalassemia major children (Table-1).
Considerable reduction in serum leptin levels were observed in β-thalassemia major children (Table-1).

Table-1: *Comparison of leptin and ultrasonographic parameters in apparently healthy and β-thalassemia major children.*

Parameters (n = 65)	*Healthy Children*	*β-thalassemia Major Children*	*P-value*
	Mean ± SD Median (IQR)	*Mean ± SD Median (IQR)*	
Leptin (ng/ml)	3.12 ± 2.84	1.38 ± 1.17	< 0.001[a]
	2.19 (1.40-3.50)	1.03 (0.77-1.59)	
Ad-SOS (m/sec)	1906.86 ± 49.53	1893.62 ± 57.88	0.163[b]
	1916.00 (1873.50-1944.00)	1899.00 (1852.50-1931.00)	
BTT (µsec)	0.79 ± 0.20	0.70 ± 0.20	0.012[b]
	0.78 (0.67-0.93)	0.70 (0.57-0.84)	
Bone Profile Z-Score	-0.44 ± 1.22	-0.11 ± 1.62	0.196[b]
	-0.33 (-1.35-0.42)	-0.29 (-1.13-1.07)	

[a]Mann-Whitney U Test
[b]Independent Sample "t"-Test

Leptin was positively correlated with bone profile as confirmed by AdSoS, BTT and Z-Score (Table-2).

Table-2: *Correlation matrix of serum leptin with bone profile in Healthy children.*

Parameters (n = 65)		*Ad-SOS (m/sec)*	*BTT (µsec)*	*Bone Z-Score Profile*
Leptin (ng/ml)	r/rho	0.108[a]	0.336[a]	0.009[a]
	P-value	0.661	0.160	0.972

[a]Spearman's Correlation

DISCUSSION

Bone disorders in thalassemia patients include bone age delay, bone pain and deformity, spinal deformities, failure of growth, rickets, nerve compression, pathologic fractures, scoliosis, osteopenia and osteoporosis.[25,26] Osteopenia and osteoporosis is the main reason of illness in 40-50% of well managed β-thalassemia major patients.[27,28] Bone alterations in thalassemia patients are caused by increased RBCs formation and increased deposition of iron which results in bone marrow cavities expansion and decreased bone volume in trabecular bone. This leads to osteoporosis and decreased bone tissue.[29,30] That is why, it is imperative to maintain adequate bone health early in the life to prevent bone related changes later in life.

Leptin is a fat derived cytokine-like hormone. An OB gene encode it which is mainly expressed in fat tissue.[31] Leptin is a multifunctional hormone and leads to increased energy expenditure and affects angiogenesis, hematopoiesis and inflammation.[6,7] It is a polypeptide of 146 amino acids and has a direct correlation with BMI.[8,9]

Standard reference values of leptin in males are 1.6-10.8 ng/ml and 1.7-10.6 ng/ml in females.[32,33] Results of the current study shows significant (P< 0.001) lower levels of leptin in β-thalassemia major (1.38 ± 1.17 ng/100ml) as compared to healthy controls (3.12 ± 2.84 ng/100ml). This is similar to the studies done earlier. [14-18] This can be explained by the fact that iron overload leads to toxic effects of iron on cell membranes. Free iron causes damage to peroxide on lipid membrane and proteins which results in free radical formation. This production of ROS and the resultant oxidative stress inhibits the activity of adiposities which leads to decrease in leptin levels.[34] Leptin receptors are present on cells of bone marrow, hematopoietic cells and stem cells. In beta thalassemia hematopoietic cells are damaged which results in decrease in serum leptin levels.

CONCLUSION

Thalassemia affects the whole body metabolism. Low leptin levels can contribute to complications related to bone at early age. In our country, parent's education is very essential because no balanced nutrition is given even to the normal healthy population of our children. It is our tradition, if we want to give a good diet; we increase the fat content of the diet which increases the subcutaneous fat and subscapular skinfold thickness in thalassemia children. A balanced nutrition is essential in controlling the disease and also in maintaining good health of children. Creation of school going awareness in children is equally essential.

LIMITATIONS OF STUDY

Limitations of the study were lack of assessment of serum ferritin and vitamin D levels. Also sample size should be large in order to get better results.

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AUTHOR'S CONTRIBUTION
HS: Concept and study design, data collection, literature review and drafting the article.
AF: Data Collection, drafting the article.
KPL: Concept and study design.
RK: Data collection, literature review.
FI: Data analysis, technical guidance.
SJ: Data collection.
IF: Technical guidance.
RK: Data collection.

DISCLAIMER
The manuscript is derived from the M.Phil. thesis of the first author Hira Sohail, submitted for M.Phil. Biochemistry UHS. The degree has already been granted.

CONFLICT OF INTEREST
None to declare.

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