ORIGINAL ARTICLE

ASSESSMENT OF INSULIN LIKE GROWTH FACTOR 1 AND BONE DENSITY IN NORMAL AND β-THALASSEMIA MAJOR CHILDREN

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Background: Bone disease and growth failure is a major problem in patients of β-thalassemia major. IGF-1 deficiency is the major cause of growth failure and maturation delay. Our aim was to compare IGF-1 and bone density between β-thalassemia major and healthy children of 5–11 years of age and to investigate the relationship of IGF-1 with bone density in these children. **Methods:** It was a cross-sectional comparative research which used convenience sampling. One-hundred-thirty subjects were enrolled for the study, 65 healthy and 65 β-thalassemia major patients. Patient samples were taken from Fatimid and Sundus foundation, Lahore. Serum insulin-like growth factor-1 (IGF-1) was measured by ELISA. Bone density was checked by Quantitative Ultrasound (QUS) bone profiler measurements; amplitude dependent speed of sound (Ad-SoS), bone transmission time BTT and Bone profile Z-Score was recorded. **Results:** Serum IGF-1 and bone density were considerably decreased in β-thalassemia major children in our study population as compared to healthy children. Important negative connection between IGF-1 with bone density was also found in our thalassemia children. **Conclusion:** Majority of the thalassemia major children had low IGF-1 which leads to low bone density and increase fracture risk in adulthood. Improvement of IGF-1 levels early in the life through proper nutrition can help prevent bone problems in later life. Easy assessment of bone density by QUS can help prevent the fracture risk and improve the value of life of thalassemia children.

Keywords: Insulin-like growth factor 1, β-thalassemia major, bone density, quantitative ultrasound, nutrition, fracture

INTRODUCTION

β-thalassemia is among the critical inherited autosomal recessive ailments. There is defective synthesis of β-chains which results in β-chain imbalance. WHO studies revealed that it is the most prevalent genetic blood disorder with a carrier inhabitants of 150 million.[1] Cases of transfusion dependent β-thalassemia in Pakistan are almost above 4,000 per annum.[2] Due to great prevalence of contagious marriages, large population size and high birth rate, the carrier ratio is 5–7% with 9.8 million carriers in entire residents.[3]

Bone changes its form and nature during the passage of life. There is increase in bone loss in different kind of illnesses. Bone mass is increased at the time of puberty. By the end of growth period, bone mass reach at its peak. It is important to promote peak bone mass in order to prevent bone related problems.[4] A large number of factors is engaged in promoting peak bone mass. Among them are genetic factors, hormones, nutrients, minerals, calcium, vitamin D, physical activity and body weight.[5]

Bone density is augmented by dietary proteins. Insulin-Like Growth Factor-1 is a small peptide that circulates in serum combined to high affinity binding proteins.[6] Many tissues secrete IGF-1 and the action depends upon the site from where it is secreted. It can act as anabolic, anti-inflammatory and anti-oxidant hormone but its main effect is on actions of growth hormone.[7]

Bone density measurement is useful to determine whether children adopt appropriate aggregate of bone for their body size. Knowingly bone content beforehand prevent childhood fractures and possibly delay osteoporosis in later life.[8]

There are a number of methods for assessment of bone mineralization. Though the most extensively used method for assessment of bone mineralization is dual X-ray energy for the measurement of bone status[9] yet DXA has major limitations. Firstly, there is exposure to ionization radiation and secondly there are no standardized measurements for paediatric age.[10]

Bone density in children and neonates is assessed by ultrasound. Quantitative ultrasound (QUS) is safe, easy to use, free of ionization radiation and non-invasive.[11] Measurements can be taken from radius, tibia and humerus. Speed of sound, bone transmission time and bone profile Z-score are measured by QUS. Recently second shaft of metacarpal bone has been used for assessment due to its easy accessibility in children, good compliance, safety, and early ossification.[12]

The management of thalassemia has improved over the past decades with enhanced transfusion programs and chelation therapy. Therefore, the quality of life and life expectancy has improved and most of the

children attain standard growth in first decade. It is worth mentioning that with improvement in life span, variety of bone deformities appear like growth failure, bone pains, rickets, delayed bone age, nerve compression, spinal deformities, osteopenia and osteoporosis. Major growth disorder occur due to deficiency of IGF-1.[13] IGF-1 deficiency can be due to chronic anaemia, iron overload, and growth hormone deficiency.[14]

A study done in Qatar and Italy deduced IGF-1 deficiency in 67% of adults with thalassemia.[15] Another study done by Iranian population reported lower IGF-1 in thalassemia minor children.[16] None of the study is conducted in our population in thalassemia major to determine the relationship of IGF-1 plus bone density.

The present study was designed to look into the significance of early evaluation of bone density by QUS and IGF-1 in management of β-thalassemia major children and to compare with healthy controls. Using non-invasive technique for early diagnosis of bone density will help prevent overall risk of future fractures, improve the life quality, relax the economical, psychological and physical limitations of parents and family members.

METHODOLOGY

This cross-sectional comparative study was conducted in Department of Physiology, University of Health Sciences, Lahore after approval from ethical review board. A total of 130 subjects were incorporated in the research. Out of them, 65 were healthy and 65 had β-thalassemia major. Ages of the subjects were from 5–11 years. Sample of thalassemic patients were taken from Fatimid and Sundus Foundation, Lahore, while the samples of healthy children were taken from a government school. Written well-versed consent was taken.

A detailed history was taken. Weight, height and BMI were registered. Serum IGF-1 in ηg/ml were measured by automated EIA analyser by enzyme linked assay. Blood sample was drawn before transfusion to prevent IGF-1 elevation after transfusion.

Bone density was measured by DBM Sonic Bone Profiler (IGEA, Capri, Italy, Model BP01). The device comprises of an emitting and receiving transducer. Adequate amount of gel was applied across each probe and the shaft of metacarpals. Dominant hand was used for measurements of proximal phalanges, distal ends, of all four fingers. The following ultrasound parameters can be measured by the device: 'Amplitude dependent speed of sound' (Ad-SoS), 'bone transmission time' (BTT) and 'bone profile Z-score' (Z-score).

Ad-SoS (m/s) is the ultrasound velocity between receiving and emitting transducers. BTT (μs) measures the difference of receiving probe arrival time

through bone tissue and pulse of ultrasound through only soft tissue. It reduces the confounding soft tissue effect.[17] Z-Score (m/sec) is the difference between normal average speed and measured speed by standard deviation.[18]

The data were examined using SPSS-20. Mean±SD, and median (IQR) were given for normal and non-normally distributed variables. Normality was checked by Shapiro-Wilk test. Student's *t*-test for normally distributed and Mann Whitney U test for non-normally distributed variables were used. Correlation analysis was done through Pearson *r* and Spearman rho for parametric and non-parametric variables respectively. Hypothesis was considered significant with $p \leq 0.05$.

RESULTS

A total of 130 children were recruited in the study. Sixty-five were thalassemics and 65 were normal. Mean age of thalassemics were 7.45±1.98 years and healthy children were 8.63±1.64 years. The mean IGF-1 of healthy children was 424.84±378.86 ηg/ml and β-thalassemia major children was 189.27±270.85 ηg/ml. There was significant difference in IGF-1 of healthy and β-thalassemia major children (*p*=0.002) (Table-1).

The mean Ad-SoS in healthy children was 1906.86±49.73 m/sec and in β-thalassemia major children it was 1893.62±57.88 m/sec. The Ad-SoS was reduced in β-thalassemia major children but not statistically significant (Table-1).

The BTT of healthy children was 0.79±0.20 μsec and β-thalassemia major children was 0.70±0.20 μsec. There was significant difference in BTT of healthy and β-thalassemia major children (*p*=0.012) (Table-1).

Positive correlation of bone density with IGF-1 was observed with healthy children though not statistically significant (Table-2).

Mild significant negative correlation of Ad-SoS was observed with IGF-1 in β-thalassemia major children (Spearman's rho= -0.366, *p*=0.003) (Table-3), (Figure-1). Similarly, mild significant negative correlation of Bone Profile Z-Score was observed with IGF-1 β-thalassemia major children (Spearman's rho= -0.252, *p*=0.044) (Table-3) (Figure-1).

Table-1: Comparison of IGF-1 with Bone density in healthy and β-thalassemia major children

Parameters (n=65)	Healthy Mean±SD	β-thalassemia major Mean±SD	*p*
IGF-1 (ηg/ml)	424.84±378.86	189.27±270.85	0.002[a]
Ad-SoS (m/sec)	1906.86±49.53	1893.62±57.88	0.163[b]
BTT (μsec)	0.79±0.20	0.70±0.20	0.012[b]
Bone Profile Z-score	-0.44±1.22	-0.11±1.62	0.196[b]

[a] p-value created by Mann-Whitney U, [b] Independent *t*-test *p*-value, $p \leq 0.05$ is considered significant.

**Table-2: Correlation of serum IGF-1 with bone density in healthy children**

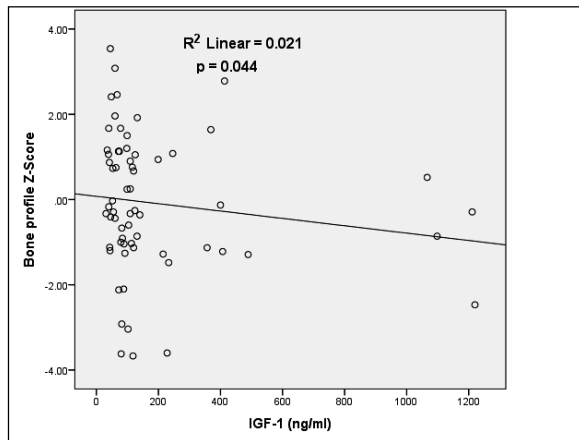| Parameters		Ad-SOS (m/sec)	BTT (μsec)	Bone Profile Z-Score
IGF-1 (ηg/ml)	r/rho	0.145[a]	0.091[a]	0.328[a]
	p	0.554	0.712	0.710
	n	65	65	65

[a] Spearman's Rho

Table-3: Correlation of serum IGF-1 with bone profile in β-thalassemia major children

Parameters		Ad-SOS (m/sec)	BTT (μsec)	Bone Profile Z-Score
IGF-1 (ηg/ml)	Rho	-0.366[a]	-0.176[a]	-0.252[a]
	p	0.003	0.164	0.044
	n	65	65	65

[a] Spearman's Rho Correlation
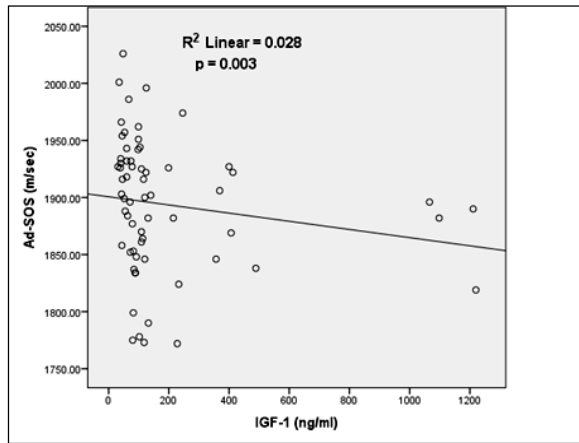
a) Ad-SOS

b) Bone Profile Z-Score

Figure-1: Correlations of IGF-1 with (a) Ad-SOS (b) bone profile Z-Score in β-thalassemia major

DISCUSSION

The results of our study showed significant low levels of IGF-1 in thalassemic patients than in healthy controls. Similar studies done by Riza et al[19] also found low IGF-1 levels with mean age of 11 years. Soliman[20] conducted the study in adults and reported low IGF-1 levels in thalassemics.

Bone density is also low in our thalassemic population compared to controls of same age. Several studies have reported low bone density in thalassemia population. Meena et al[21], has shown decline in bone density of thalassemia population of 2–18 years of age. Similar studies done in Turkey[22] have shown decline in bone density of thalassemia patients. Many factors contributes to low bone density. Among them are multiple blood transfusions leading to iron overload, hypoxia, insufficient usage of chelating agents, hypothyroidism, hypogonadism and deregulation of GH-IGF-1 axis.[23]

IGF-1 stimulates bone formation and osteoblastic activity. It enhances proliferation of osteoblastic cells. IGF-1 helps in upregulation of collagen synthesis which maintain the appropriate level of bone mass and matrix.[24] Hepatocytes undergo into hypoxic state in chronic anaemia. This hypoxia leads to inhibition of protein synthesis in liver, which elevates IGF-binding proteins which bind with IGF-1 and inhibit IGF-1 functions.[25]

There is a risk of osteoporosis in children of thalassemia. To avoid fractures, it is necessary to improve the bone health. Thalassemic children are prone to develop osteoporosis which may lead to bone fractures. IGF levels should increase in early childhood period to improve the bone health and to avoid bone fractures.

Our study found significant and negative relationship of Ad-SOS with IGF-1 (Spearman's rho= -0.366, p=0.003) as compared to apparently healthy controls (rho=0.145, p=0.554), where positive correlation was seen between these two parameters. This indicates that these children are not getting enough nutrition which leads to low IGF-1 levels than in healthy children. This decline in bone density is mainly due to low IGF-1 levels in early part of life which become risk of fractures and osteoporosis in later life. Adequate knowledge of bone density before puberty helps in addressing this problem beforehand to avoid future fractures in later part of life. This helps in improving the life quality of these children.

Major limitation of this study is the small sample size. A large number of subjects from different areas of the city may be included in future studies. In future, QUS measurement should be done to all children early in their life so that supplements can be started in order to prevent future bone complains.

CONCLUSION

IGF-1 and bone density are reduced in β-thalassemia major children. Bone density shows positive correlation with IGF-1 in healthy children indicating that improvement in IGF-1 can improve the growth spurt which decrease the risk of cracks later in lifetime. The use of QUS makes it easier to measure bone profile in

children which helps in improving the quality of life of these children.

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