

## ORIGINAL ARTICLE

## COMPARISON OF SKELETAL AGE WITH CHRONOLOGICAL AGE IN THALASSEMIC PATIENTS OF 9–15 YEARS: ROLE OF GROWTH HORMONE LEVEL

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**Background:** Thalassemia is a single gene disorder with defect in synthesis of alpha or beta globin, causing anaemia. Thalassemia major is evident in early childhood causing growth retardation and visceromegaly. The objective of this study was to compare the bone age at 9–15 years in thalassemic children with normal children of same age through growth hormone estimation. **Methods:** This cross-sectional analytical study was conducted in collaboration with Fatimid Hospital Peshawar and Paediatrics Unit, Khyber Teaching Hospital, Peshawar from Aug 2014 to Jun 2018. The sample size was calculated as 156. After obtaining written informed consent of the parents, blood samples of children were taken for growth hormone assay using commercially available ELIZA kits. Bone age was determined on hand-wrist radiographs by evaluation of ossification centres. Skeletal and chronological ages, and growth hormone level in thalassemic and normal children of same age group were compared. Data was analysed using SPSS-20. **Results:** In total 156 children 97 (62.2%) were male and 59 (37.8%) were female with mean age  $11.9 \pm 2.2$  years. In thalassemia patients, 49 (62.8%) were male and 29 (37.2%) were female. There were significant differences in bone age between the groups ( $p=0.0001$ ) as compared to their chronological age. Growth hormone level among children with thalassemia ( $1.18 \pm 0.56$  ng/ml) were significantly lower ( $p=0.025$ ), compared to the control group ( $5.08 \pm 15.02$  ng/ml). Bone age was significantly correlated to growth hormone ( $r=0.244$ ,  $p=0.031$ ) in thalassemia patients. The correlation between bone age and growth hormone was found non-significant ( $r=0.161$ ,  $p=0.302$ ) in control group. **Conclusion:** Growth hormone level and hand-wrist X-ray should also be used in assessment of thalassemic children for evaluation of possible growth retardation.

**Keywords:**  $\beta$ -Thalassemia major, skeletal age, chronological age, growth hormone, growth retardation

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## INTRODUCTION

Thalassemia is a genetic disorder having a single gene disorder with defect in synthesis of either  $\alpha$  or  $\beta$  globin, causing anaemia. In this type of anaemia there is deletion or mutation of alleles of globin genes.<sup>1,2</sup> Thalassemia is a pathology of childhood, and manifests commonly in early life. In this disease there is formation of abnormal masses due to osteopenia, extra medullary haemopoiesis, or other factors.<sup>3,4</sup>

The life expectancy of thalassemia patients has been prolonged due to modern therapy. Multiple blood transfusions have a significant effect in improvement of patient survival, but besides its effectiveness it has a major side-effect of iron overload.<sup>5,6</sup> Before 9–10 years of age, growth of patients with thalassemia is normal. After that, reduction or absence in growth along with failure of signs of puberty are observed.<sup>5,7</sup> Mostly in thalassemic adolescence, height is less than 3 percentile than mean age 13 or 2 SD less, because of multiple pathologies due to growth failure, impairment of height and secondary sex characteristics.<sup>7,8</sup>

Various methods have been adopted for age determination such as ossification centres of wrist bones, eruption of teeth and their correlation.<sup>9</sup> The

skeletal maturity is one of the vital indicator of biological maturity of human being that is linked to various factors like disease, hormonal imbalance, environment, nutritional status, and genetics.<sup>10</sup> Due to variations in bone maturation, some children enter pubertal stage at the age of 10 years or earlier, while others enter into the puberty after 15–16 years.<sup>11</sup> Skeletal age is similar important as chronological age in evaluation of children and adolescents' physical development and treatment plan.<sup>10</sup> The finding of phalanges and metacarpal bones maturation is an indicator of accelerated pubertal growth.<sup>12</sup>

Growth is a vital feature which differentiates a child from an adult. Growth denotes a net increase in the size or mass of tissues, whereas development specifies maturation of function.<sup>13</sup> Growth among children and their puberty is linked with the endocrine changes which follow a well-coordinated schemes of changes but the rates of maturation differ broadly as a result these events may be associated with physical maturity as compared to chronological age.<sup>14</sup> Thalassemic children with pubertal failure have short stature.<sup>15</sup> Biological/skeletal age, also known as 'developmental age' or 'physiological age', reveals the individual maturity

levels. Numerous methods are available for assessment of biological age which provide information to the health professionals/physician.<sup>16,17</sup> Frequent blood transfusions in patients with thalassemia lead to excessive iron accumulation; serum ferritin estimation is an indicator of iron overload. Some studies have shown association between serum ferritin level and bone mineral density.<sup>18</sup> Growth deficit occurs in thalassemia syndrome which depending on the severity of disease. In thalassemia, growth failure has multifactorial aetiology such as chronic anaemia, chelation toxicity, endocrinopathies like deficiency of growth hormone, hypothyroidism and hypogonadism.<sup>19</sup>

For making generalized evaluation of growth to documented populations ethnicity must be considered; however, only ethnic difference does not explain the intensity of the shortfalls observed. A few reports from investigators suggest that substandard and insufficient nutrition plays a major role in growth and pubertal failure.<sup>20</sup> The purpose of this study was to develop a steady, attainable and advantageous method to archive growth retardation in children with thalassemia through bone age and growth hormone.

## MATERIAL AND METHODS

This study was conducted in Paediatrics Department Khyber Teaching Hospital, and Fatimid Hospital Peshawar after approval from Ethical Review Committee. It was a cross-sectional and analytical study conducted from Aug 2014 to Jun 2018. Beta thalassemia major patients of 9–15 years age, with no other illness like cardiac, hepatic, renal disease, or diabetes mellitus were included. Children whose exact birth dates could not be verified, or having metabolic or bone deformities not due to thalassemia were excluded.

Parents of the Thalassemia major patients, visiting Fatimid Hospital were contacted. Written informed consent was obtained after explaining the purpose of the survey. Patients' detailed history of the disease was taken. A structured data collection checklist was used. The check list was broadly divided into two parts consisting of demographic data and biochemical findings, i.e., growth hormone level. The patients were advised to fast for at least 12 hours before the blood sample for biochemical assessment. Blood sample of 3 ml was collected in an EDTA tube, stored at 2–8 °C and transferred to the laboratory for analysis. Hormonal assay was performed using commercially available ELIZA kits. Bone age was determined on hand-wrist radiographs by evaluation of ossification centres.

WHO sample size calculator and Epi-info 6 were used for calculations of sample size and data analysis. Data analysis was done on SPSS-20. Mean±SD, frequency, ratio, proportion, and percentages were calculated. Student's *t*-test was used to compare the means of biochemical values between the two

groups, and  $p \leq 0.05$  was considered statistically significant.

## RESULTS

Total 156 children, 97 (62.2%) males and 59 (37.8%) females, were included in the study with mean age 11.9±2.2. Out of the thalassemic patients, 49 (62.8%) were male and 29 (37.2%) were female. In the non-thalassemic children, 48 (61.5%) were males and 30 (38.5%) were female.

Among the control group mean chronological age was 11.86±2.18 years while it was 12.01±2.2 years among thalassemia patients. The differences were not significant ( $p=0.67$ ).

Bone age in normal children was 11.7±2.5 years whereas it was 9.9±1.6 years in thalassemic children. The bone age had significant differences ( $p=0.0001$ ) between the two groups.

The growth hormone level in normal children was 5.08±15.02 ng/ml, and it was 1.18±0.56 ng/ml in thalassemic children. The differences in the mean growth hormone level among normal and thalassemic children were significant ( $p=0.025$ ). (Table-1).

**Table-1: Comparison of bone age and laboratory findings among the study groups**

Characteristics	Group A (Thalassemic)	Group B (Normal)	CI	<i>p</i>
Chronological age (Year)	12.01±2.26	11.86±2.20	1.09–2.4	0.67
Bone age (Year)	9.9±1.6	11.7±2.5	0.55–0.85	0.0001
Growth hormone (ng/ml)	1.18±0.56	5.08±15.02	0.54–7.3	0.025

Pearson's correlation analysis revealed that the bone age had positive correlation with growth hormone ( $r=0.244$ ,  $p=0.031$ ) in thalassemic patients. There was no significant correlation between growth hormone and bone age of the control group ( $r=0.161$ ,  $p=0.302$ ).

## DISCUSSION

Our study signifies that the bone age of thalassemia patients is significantly less than the control group. Due to modern therapy the mean survival age of patients with thalassemia has been prolonged but as the thalassemia patients approach puberty age, many of them develop pubertal and growth retardation.<sup>23</sup> Thalassemic patients are at risk of disproportionate trunk shortening due to platyspondyly resulting from a combination of factors like desferrioxamine toxicity, deficiency of trace elements, or haemosidrosis.<sup>24</sup> Although deficiency of growth hormone and neurosecretory dysfunction have been described in thalassemic patients, it may not be the direct cause as normal growth hormone reserve has also been observed in majority of short-statured thalassemic patients.<sup>25</sup>

Subjects of the same chronological age were included in this study in order to avoid bias. There were significant differences in bone age of the study groups. Thalassemia patients had mean bone age less than the

control group. Bone mass density is responsible for chronological age and bone age dissimilarity. Other studies also predicted low bone mass density in thalassemia patients as compared to normal children.<sup>1,2</sup>

In this study, significantly low level of growth hormone was observed in thalassemia patients compared to normal children. Other workers have also observed pronounced decrease in growth hormone level in thalassemia patients.<sup>1,19</sup> Significantly lower values were found for weight, height and sitting height in thalassemia patients than controls in other studies suggesting delayed puberty and growth retardation.<sup>27,28</sup>

## CONCLUSION

Multiple transfusions to thalassemic children cause iron overload in various organs including endocrine glands resulting in decreased secretion of growth hormone from pituitary gland, short stature, delayed puberty, and other metabolic and skeletal deformities. Growth hormone level and hand-wrist X-ray should also be used in assessment of thalassemic children for evaluation of possible growth retardation.

## REFERENCES

- Hashemi AS, Ghilian R, Golestan M, Ghalibaf MA, Zare Z, Dehghani MA. The study of growth in thalassemic patients and its correlation with serum ferritin level. *Iran J Pediatr Hematol Oncol* 2011;1(4):147–51.
- Nasr MR, Ebrahim NA, Ramadan MS, Salahedin O. Growth pattern in children with beta thalassemia major and its relation with serum ferritin, IGF1 and IGFBP3. *J Clin Exp Invest* 2012;3(2):157–63.
- Borgna-Pignatti C, Rugolotto S, De Stefano P, Zhao H, Cappellini MD, Del Vecchio GC, *et al.* Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. *Haematologica* 2004;89:1187–93.
- Mazzone L, Battaglia L, Andreozzi F, Romeo MA, Mazzone D. Emotional impact in beta-thalassaemia major children following cognitive-behavioural family therapy and quality of life of caregiving mothers. *Clin Pract Epidemiol Ment Health* 2009;5:5.
- Adil A, Sobani ZA, Jabbar A, Adil SN, Awan S. Endocrine complications in patients of beta thalassemia major in a tertiary care hospital in Pakistan. *J Pak Med Assoc* 2012;62(3):307–10.
- Mokhtar GM, Tantawy AA, Adly AA, Ismail EA. Clinicopathological and radiological study of Egyptian beta-thalassemia intermedia and beta-thalassemia major patients: relation to complications and response to therapy. *Hemoglobin* 2011;35(4):382–405.
- Thuret I, Pondarre C, Loundou A, Steschenko D, Giro R, Bachir D, *et al.* Complications and treatment of patients with beta-thalassemia in France: results of the National Registry. *Haematologica* 2010;95(5):724–9.
- Borgna-Pignatti C. The life of patients with thalassemia major. *Haematologica* 2010;95(3):345–8.
- Srivastav A, Saraswat PK, Agarwal SK, Gupta P. A study of wrist ossification for age estimation in pediatric group in central Rajasthan. *J Indian Acad Forensic Med* 2004;26(4):971–73.
- Cantekin K, Celikoglu M, Miloglu O, Dane A, Erdem A. Bone age assessment: the applicability of the Greulich-Pyle method in eastern Turkish children. *J Forensic Sci* 2012;57(3):679–82.
- Paxton ML, Lamont AC, Stillwell AP. The reliability of the Greulich-Pyle method in bone age determination among Australian children. *J Med Imaging Radiat Oncol* 2013;57:21–4.
- Negi KS. Reliability of MP3 (middle phalanx of 3<sup>rd</sup> finger) Stages in Assessment of Skeletal Maturation –A Correlative Study. *Orthod Dentofac Orthop J* 2009:1–12.
- Tsushima K, Sone S, Yoshikawa S, Yokoyama T, Suzuki T, Kubo K. The radiological patterns of interstitial change at an early phase: over a 4-year follow-up. *Respir Med* 2010;104(11):1712–21.
- Oh Y, Lee R, Kim HS. Evaluation of skeletal maturity score for Korean children and the standard for comparison of bone age and chronological age in normal children. *J Pediatr Endocrinol Metab* 2012;25(3-4):279–84.
- Kumar V, Sundeep Hegde K, Bhat SS. The relationship between dental age, bone age and chronological age in children with short stature. *Int J Contemp Dent* 2011;2(4):6–11.
- Bala M, Pathak A, Jain RL. Assessment of skeletal age using MP3 and hand-wrist radiographs and its correlation with dental and chronological ages in children. *J Indian Soc Pedod Prev Dent*. 2010;28(2):95–9.
- Rajan RA, Swindells MG, Metcalf JE, Konstantoulakis C. Can orthopaedic clinicians learn to read skeletal bone age? An inter- and intra observer study between specialties. *J Child Orthop* 2011;5(1):69–72.
- Lee BK, Kim Y. Menopause may be the common link that resulted in the association between higher serum ferritin level and lower bone mineral density in women  $\geq 45$  years of age. *Osteoporosis Int* 2014;25(2):787.
- Fung EB. Nutritional deficiencies in patients with thalassemia. *Ann N Y Acad Sci* 2010;1202:188–96.
- Fikry SI, Saleh SA, Sarkis NN, Mangoud H. Study of serum zinc in relation to nutritional status among thalassemia patients in Damanhour Medical National Institute. *J Egypt Public Health Assoc* 2003;78(1-2):73–93.
- Ansari SH, Shamsi TS, Ashraf M, Farzana T, Bohray M, Perveen K, *et al.* Molecular epidemiology of beta-thalassemia in Pakistan: Far reaching implications. *Indian J Hum Genet* 2012;18:193–7.
- Ozer T, Kama JD, Ozer SY. A practical method for determining pubertal growth spurt. *Am J Orthod Dentofacial Orthop* 2006;130(2):131.e1–6.
- Chatterjee R, Bajoria R. Critical appraisal of growth retardation and pubertal disturbances in thalassemia. *Ann NY Acad Sci* 2010;1202:100–14.
- Hattab FN. Patterns of physical growth and dental development in Jordanian children and adolescents with thalassemia major. *J Oral Sci* 2013;55(1):71–7.
- Low LC. Growth of children with beta-thalassemia major. *Indian J Pediatr*. 2005;72(2):159–64.
- Jimenez-Castellanos J, Carmona A, Catalina-Herrera CJ, Vinuales M. Skeletal maturation of wrist and hand ossification centers in normal Spanish boys and girls: a study using the Greulich-Pyle method. *Acta Anat (Basel)* 1996;155(3):206–11.
- Moayeri H, Oloomi Z. Prevalence of growth and puberty failure with respect to growth hormone and gonadotropins secretion in beta-thalassemia major. *Arch Iran Med* 2006;9(4):329–34.
- Shalitin S, Carmi D, Weintrob N, Phillip M, Miskin H, Komreich L, *et al.* Serum ferritin level as a predictor of impaired growth and puberty in thalassemia major patients. *Eur J Haematol* 2005;74(2):93–100.

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