

Effect of Zinc Status on Sexual Maturation in Children with Transfusion Dependent Thalassemia

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ABSTRACT

Background: A child with thalassemia major has a particular growth pattern, which is relatively normal until age of 9–10 years; after this age a slowing down of growth velocity and a reduced or absent pubertal growth spurt are observed. Zinc is known to affect normal growth and sexual development and an estimation of 20% to 30% of patients with thalassemia are zinc deficient. Our aim was to determine the relationship between zinc status and Tanner stage to evaluate the effect of zinc deficiency on sexual maturation in transfusion dependent thalassemia.

Methods: we included 40 children with transfusion dependent β -thalassemia and 40 sex and age matched apparently healthy children as control group. All children were subjected to thorough history taking and full medical examination with body mass index calculation and pubertal stage assessment using Tanner scale, serum zinc and ferritin levels were assayed.

Results: there was a significant delay in sexual maturation assessed by Tanner staging in children with thalassemia than controls ($p=0.003$). Mean age of menarche in cases was 15.1 ± 1.7 years, this was significantly higher than that in controls which was 11.9 ± 1.3 years ($p=0.001$). Serum zinc in cases was 58.01 ± 10.58 $\mu\text{g/dl}$, which was significantly lower than serum zinc of controls (68.37 ± 8.67 $\mu\text{g/dl}$, $p = 0.001$). Tanner stage was found to positively correlated with BMI and serum zinc ($r = 0.54$, $p = 0.04$ and $r = 0.22$, $p = 0.012$ respectively) and negatively correlated with serum ferritin ($r = -0.34$, $p = 0.03$).

Conclusion: Reduced serum zinc and iron overload in children with thalassaemic patients was associated with delayed sexual maturation.

Keywords: Serum zinc; sexual maturation; thalassemia

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INTRODUCTION

Beta-Thalassemia major usually causes severe anemia with several health problems like enlarged spleen, bone deformities, short stature, diabetes, hepatitis infection and requires regular life-long transfusion, therapy and medical supervision [1,2], and frequent blood transfusion in turn can lead to iron overload which may result in hypogonadism, diabetes mellitus, hypothyroidism, hypoparathyroidism and other endocrine abnormalities [3]. Children with thalassemia major have particular growth pattern, which is relatively normal until age of 9–10 years; after this age a slowing down of growth velocity and a reduced or absent pubertal growth spurt are observed [4]. With current chelation therapy, the most prominent endocrinal complications are failure of normal pubertal development and growth retardation [5]. Sexual complications in thalassemia (TM) present the commonest endocrine complication in almost all studies, these include delayed puberty, arrested puberty and hypogonadism [3]. Delayed puberty in TM is almost always due to hypogonadotropic hypogonadism because of pituitary dysfunction, which still remains the most stressful complication. [6-8]

Adolescent girls with TM often present with primary Amenorrhea and boys fail to become well virilized. The damage to the hypothalamus and pituitary is progressive, even when intensive chelating therapy is given and the appearance of hypogonadism in both sexes is often unavoidable. [5,9,10] Most women with TM manifest secondary amenorrhea at some stage in their life and men

develop hypogonadism in their 3rd decade after being normal for some years and even becoming fathers. [9,11,12] Researchers suggested that early initiation of deferoxamine (DFO), i.e. before the age of 10 years, with effective long-term chelation therapy, assures normal puberty in the majority of patients [13]. However, the initiation of DFO at a young age is associated with bone toxicity and, consequently, decreased growth. [14]

Zinc deficiency in Children with thalassemia has global health impact because its manifestations become less visible and usually begins to show when the condition is severe and has already led to serious health burdens. [15]

Zinc deficiency is associated also with cognitive deficits among children impaired growth (stunting), genetic disorders, decreased resistance to infectious diseases. An estimated 20% to 30% of patients with thalassemia are zinc deficient. [16] The high prevalence is thought to be related to a combination of increased urinary losses compounded by elevated requirements. [17] Since zinc is known to affect normal growth and sexual development, and children with thalassemia have shown to have lower zinc levels when compared to their counterparts with normal children. [18]

Urinary zinc excretion is increased with hemolysis, as seen in thalassemia and other hemoglobinopathies. [19]

It becomes necessary to assess the serum zinc levels of these children and see if it has any relationship with their sexual maturation pattern. In this study, our aim was to determine the relationship between zinc levels and Tanner stage and their matched controls to evaluate the effect of zinc deficiency on sexual maturation

in transfusion dependent thalassemia. This will provide important information on the possible need for zinc supplementation on some of these children with delayed sexual maturation.

SUBJECTS AND METHODS

Subjects

This Prospective case-control study included 40 children with transfusion dependent β -thalassemia major with an age range between 8 and 18 years. They were on a regular monthly or bimonthly blood transfusion program to keep hemoglobin around 9 gm/dl. They were enrolled from hematology outpatient clinic, Minia University Children Hospital, from the period of January 2016 till January 2017. Forty sex and age matched apparently healthy children were enrolled as control group. We excluded from this study children younger than 5 years, children suffering from any chronic illness other than thalassemia and children who change an iron chelator drug in the last 6 months prior to inclusion into this study.

The protocol of the study was approved by the Institutional Research Committee of the Faculty of Medicine, Minia University. The study complied with the Declaration of Helsinki principles. All included children were subjected to thorough history taking and complete medical examination with anthropometric measures evaluation, included weight measurement, height measurement and BMI determination. Pubertal stage assessment by applying Tanner's classification of pubic hair and testicular development. The Tanner scale (also known as the Tanner stages or Sexual Maturity Rating (SMR) is a scale of physical development in children, adolescents and adults. The scale defines physical measurements of development based on external primary and secondary sex characteristics, such as the size of the breasts, genitals, testicular volume and development of pubic hair.^[1]

Methods

We collected 10 ml of venous blood samples which were taken for serum zinc & ferritin assessment by ELISA. The samples were collected using aseptic technique. All blood samples were collected between 9 am and 1 pm to eliminate the effect of diurnal variation in the Serum zinc^[20]. The blood samples for zinc estimation were handled in zinc free materials.

Statistical analysis

The numerical data were presented as means – standard deviations while non numerical data were presented as percentage. Two tailed t-tests were used to analyze differences between the control and patients groups. *P-values* less than 0.05 were considered statistically significant. The magnitude of correlations was determined by Spearman's correlation coefficient. All the data were analyzed by statistical package Prism 3.0 (Graph Pad software, San Diego, CA,USA). Figures were done by Microsoft Office Excel 2007.

RESULTS

In this study, we included 40 children with thalassaemia (subjects). They were 19(47.5%) male and 21(52.5%) females, and their ages ranged between 8 and 18 years with a mean of 13.7 ± 3.78 years. We also included 40 age and sex matched controls with a mean age of 12.7 ± 2.77 years for the controls. They were 24 (26%) males and 16 (40%) females ($p > 0.05$ for age and sex). The mean age of menarche in cases was 15.1 ± 1.7 years, this was significantly higher than that in controls which was $11.9 \pm$

1.3 years ($p = 0.001$). (Table 1). Children with thalassemia had significantly lower body mass index (BMI) ($p = 0.001$) and were significantly more frequently suffering from sexual maturation delay than controls ($p = 0.003$) (Table 1). In this study, 18 children with thalassemia had delayed sexual maturation, while 22 children with thalassemia had normal sexual maturation.

When we compared children with thalassemia who had delayed sexual maturation with those who had normal sexual maturation, we found that these children had significantly higher mean serum ferritin level ($p = 0.009$) and were significantly more frequently to have a serum ferritin level above 2500 ng/ml than children with normal sexual maturation ($p = 0.003$) (table 2). Also, their overall mean serum zinc level was (55.67 ± 19.23 $\mu\text{g/dl}$), which was significantly lower than that of children with thalassemia who had normal sexual maturation (81.82 ± 25.23 $\mu\text{g/dl}$) ($p = 0.0001$) (Table 2). Furthermore, children with thalassemia who had delayed sexual maturation were significantly more frequently to be not receiving chelation therapy than children with normal sexual maturation ($p = 0.02$) (Table 2). Tanner stage in thalassemia cases had positive correlations with BMI and serum zinc ($r = 0.54$, $p = 0.04$ and $r = 0.22$, $p = 0.012$ respectively) and had significant negative correlation with serum ferritin ($r = -0.34$, $p = 0.03$) (Table 3).

DISCUSSION

Adolescence is an important developmental period of childhood. Good health and adequate nutrition consisting major food constituents and trace elements like zinc are fundamental for optimal sexual maturation. Children and adolescents with thalassemia are prone to zinc deficiency more than their normal peers due to compound factors of urinary losses with increase requirements. So, in this study we aimed to assess the effect of zinc deficiency on sexual maturation. Children with thalassemia in this study had significantly lower BMI, this was in agreement with the study of *Pantelakis et al. (2014)* which showed a tendency for height and weight gain to fall off after the age of 8 years for boys and 11 years for girls.^[4] *Harrington and Palmert's study in 2012* showed that growth retardation was more pronounced between the age of 10 and 15 years in females and between 15 and 20 years in males.^[21]

Patients with thalassemia showed significant sexual delay and older age of menarche in girls than control group in this study. This was in agreement with results of *Ananti and Chinenye in 2014* revealed most patients with thalassemia have delayed sexual and growth maturation and stated that the pubertal growth spurt is often absent or delayed, and that even patients with normal growth spurts can have delayed sexual maturation and found that patients with growth retardation, hypogonadism is often present.^[22] Also, we found that children with delayed sexual maturation had significantly longer duration of transfusion than those with normal sexual maturation. This was in agreement with *Dhouib et al., 2018*, who reported that delayed growth and sexual maturation was observed in most of the patients who had received longer transfusions.^[23] Also, *Borgna-Pignatti et al. 2011*. stated that the pathogenesis of late impairment of growth and sexual maturation in transfused patients with thalassemia major is not yet well classified and it is directly related to iron toxicity, especially of the endocrine glands.^[24] Regarding the iron overload, children with delayed sexual maturation had significantly higher serum ferritin than children with normal sexual

maturation ($p=0.009$). Furthermore, children with delayed sexual maturation had significantly more frequently a serum ferritin >2500 ng/ml than children with normal sexual development. This is in accordance with **Surapon and Tangvarasittichai 2011** study, who reported that the exact mechanism through which iron overload or the chelating agent produces growth retardation is not known. Although, abnormal growth and delayed puberty can be partly overcome by early initiation of chelating therapy. [25] Also, **Surapon and Tangvarasittichai 2011** study showed that intensive chelation therapy has beneficial effects in restoring height velocity, promoting the development of sexual characteristics, and inducing acceleration of growth and sexual maturity after several years of pubertal delay and stunted growth. [25] Also, **Toumba et al., 2012** stated that prompt chelation therapy before pubertal age and before extremely high levels of ferritin are reached can help thalassemic children to attain normal sexual maturity (but also chelation therapy may not always prevent or ameliorate late growth failure and/or delayed or absent puberty. [26]

The influence of chronic zinc deficiency on gonadal growth and function has been considered important [19] the mean serum zinc levels of the subjects were significantly lower than those of the controls. The overall mean serum zinc levels of subjects (55.67 ± 19.23 µg/dl) was significantly lower than those of the controls (81.82 ± 25.23 µg/dl) ($p = 0.0001$) and lower serum zinc levels were associated with lower Tanner scores for both breast and pubic hair development. Similar findings were documented by **Mashhadi et al. (2014)** and **Sultan et al.(2015)** These suggest that zinc could play a role in the pattern of breast and pubic hair development as well as age of menarche in children with thalassemia. [16-17] Our study showed significance in delayed sexual maturity in thalassemic children on desferrioxamine therapy. This could be attributed to desferrioxamine use increases urinary zinc excretion. [19]

CONCLUSION

This study supports that thalassemic patients have low rate of growth and BMI and have high prevalence of pubertal delay, which is related to low hemoglobin and high ferritin levels, also reduced serum zinc and iron overload in children with thalassmia was associated with delayed sexual maturation. Hence optimization of zinc supplementation and chelation therapy can be implemented so that such complications can be partly or totally prevented.

Limitation of the study: The smaller sample size which due to refusal of many patients to share in this study.

RECOMMENDATION

It is thus recommended that children with thalassemia should receive zinc supplementation in addition to iron chelation therapy to avoid delayed sexual maturation. Furthermore, a randomized trial of zinc supplementation in children with thalassmia with delayed sexual maturation should be done.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' Contributions:

MA, LA, AM and SH participated in the study design, data collection an interpretation and wrote the manuscript. AM and NA analyzed the immunological data and SH, LA and MA participated to discuss the results and to write the manuscript. MA supervised the research group. All authors listed in a manuscript have contributed substantially to the work and seen revise and approved the submitted version .

Ethics approval and consent to participate

Written consents were obtained from patients' caregivers for patients less than 16 years old. The study was conducted According to the declarations of Helsinki and Approved from the faculty of medicine scientific committee in Minia University (No: 116-5-2016).

REFERENCES

1. Tubman, Venée N., *et al.* "Guidelines for the standard monitoring of patients with thalassemia: report of the thalassemia longitudinal cohort." *Journal of pediatric hematology/oncology* 37.3 (2015): e162.
2. SHAHEEN, Elham Ahmed Mohammed. *Prevalence of Hypogonadism in Thalassemia major patients in Gaza strip*. 2019. PhD Thesis.
3. Mohammadian, Sakineh, Hamid Reza Bazrafshan, and A. B. Sadeghi-Nejad. "Endocrine gland abnormalities in thalassemia major: a brief review." *Journal of pediatric endocrinology & metabolism: JPEM* 16.7 (2003): 957-964.
4. Arora, Manali, *et al.* "Growth impairment and dental caries in thalassemia major patients." *Indian Journal of Clinical Anatomy and Physiology* Vol 1.1 (2014).
5. De Sanctis, Vincenzo, *et al.* "Growth and endocrine disorders in thalassemia: The international network on endocrine complications in thalassemia (I-CET) position statement and guidelines." *Indian journal of endocrinology and metabolism* 17.1 (2013): 8.
6. Goetsch, Allison L., Dana Kimelman, and Teresa K. Woodruff. "Nonmalignant Hematologic Diseases." *Fertility Preservation and Restoration for Patients with Complex Medical Conditions* (2017): 61-96.
7. Hamidi, Zohreh. *What We Learn from Bone Complications in Congenital Diseases? Thalassemia, an Example*. INTECH Open Access Publisher, 2012.
8. Petrakos, George, Panagiotis Andriopoulos, and Maria Tsironi. "Pregnancy in women with thalassemia: challenges and solutions." *International journal of women's health* 8 (2016): 441.
9. Marbut, Sara Mossa M., and Moafaq Mutlak Zeidan. "The effect of Iron overload on PCV, hemoglobin & serum ferritin of thalassaemia major male patients." *Al-Kufa University Journal for Biology* 8.3 (2016).
10. Olivieri, Nancy F., and David J. Weatherall. "Clinical aspects of b thalassemia and related disorders." *Disorders of hemoglobin: genetics, pathophysiology, and clinical management* (2009): 357.
11. Singer, Sylvia T. "Fertility Issues in Transfusion-Dependent Thalassemia Patients: Pathophysiology, Assessment, and Management." *Pediatric and Adolescent Oncofertility*. Springer, Cham, 2017. 209-229.
12. Gupta, Pankaj, and Ashok Gupta. "The Endocrine Dysfunction in Multitransfused Thalassemic Patients." *Pediatric Education and Research* (2017): 75.

13. ElAlfy, Mohsen, *et al.* "Deferiprone and desferrioxamine combined chelation could improve puberty of adolescent males with β -thalassemia major with preserved pituitary and testicular function." *The Egyptian Journal of Haematology* 38.4 (2013): 149.
14. Al-Tayar, Abdullah AK, and Ali Ahmed Mohammed Al-Zaazai. "Growth Pattern Among Yemeni Children Suffering from B Thalassemia Major in Relation to Serum Ferritin the Yemeni Society for Thalassemia and Genetic Blood Disorder-Sana'a Yemen." (2019).
15. Erdoğan, Elvan, *et al.* "The effects of chelators on zinc levels in patients with thalassemia major." *Journal of Trace Elements in Medicine and Biology* 27.2 (2013): 109-111.
16. Mashhadi, Mohammad Ali, *et al.* "The prevalence of zinc deficiency in patients with thalassemia in South East of iran, sistan and baluchistan province." *Iranian Red Crescent Medical Journal* 16.8 (2014).
17. Sultan, Sadia, *et al.* "Effect of iron chelator desferrioxamine on serum zinc levels in patients with beta thalassemia major." *The Malaysian journal of pathology* 37.1 (2015): 35.
18. Fung, Ellen B. "Nutritional deficiencies in patients with thalassemia." *Annals of the New York Academy of Sciences* 1202.1 (2010): 188-196.
19. Dangi, C. B. S., Manpreet Kaur, and Madhulika Singh. "Copper and zinc quotient in haemoglobinopathies." *Biomedical and Pharmacology Journal* 4.1 (2015): 165-173.
20. Mahyar, Abolfazl, *et al.* "Zinc and copper status in children with Beta-thalassemia major." *Iranian Journal of Pediatrics* 20.3 (2010): 297.
21. Harrington, Jennifer, and Mark R. Palmert. "Distinguishing constitutional delay of growth and puberty from isolated hypogonadotropic hypogonadism: critical appraisal of available diagnostic tests." *The Journal of Clinical Endocrinology & Metabolism* 97.9 (2012): 3056-3067.
22. Ananti, Chinenye Helen. "Physical Growth And Sexual Maturation Of Female Adolescent Sick Cell Anaemia Patients Seen In Lagos, Nigeria." *Faculty Of PAEDIATRICS* (2014).
23. Dhoub, Naouel Guirat, *et al.* "Growth and endocrine function in Tunisian thalassemia major patients." *Mediterranean journal of hematology and infectious diseases* 10.1 (2018).
24. Borgna-Pignatti, Caterina, and Maria Rita Gamberini. "Complications of thalassemia major and their treatment." *Expert review of hematology* 4.3 (2011): 353-366.
25. Surapon, Tangvarasittichai. "Thalassemia syndrome." *Advances in the study of genetic disorders* (2011): 101-148.
26. Toumba, Meropi, and Nicos Skordis. "Thalassemia Major and Its Impact on Growth: Clinical Manifestations and Management of the Growing Thalassemic Child." *Handbook of Growth and Growth Monitoring in Health and Disease*. Springer, New York, NY, 2012. 2247-2263.

List of abbreviations

Body mass index (BMI), Deferoxamine (DFO), Sexual Maturity Rating (SMR) Thalassemia (TM)

Table 1: Demographic and clinical data of the two studied groups

Variables	Cases n=40	Control n=40	p-value
Age (years): mean \pm SD	13.7 \pm 3.7	12.7 \pm 2.7	0.1
Sex: Male: n (%)	19(47.5%)	24(60%)	0.2
Female: n (%)	21(52.5%)	16(40%)	
BMI: mean \pm SD	16.6 \pm 3.02	19.1 \pm 3.4	0.001*
Sexual maturity (by Tanner stage):			
Delayed: n (%)	18(45%)	6(15%)	0.003*
Age of menarche (years): mean \pm SD	15.1 \pm 1.7	11.9 \pm 1.3	0.001*

Table 2: Clinical and laboratory criteria of thalassemic children with and without delayed puberty

Variable	Delayed sexual maturation. n=18	Normal sexual maturation n=22	p-value
Age of start transfusion(yrs)	1.6 \pm 2.3	2.07 \pm 3.05	0.2
Duration of transfusion(yrs)	12.3 \pm 4.4	6.9 \pm 4.1	0.001*
Serum ferritin	4062.35 \pm 3645.67	2409.38 \pm 1885.96	0.009*
Serum ferritin >2500(μg/dl): n (%)	7 (38.9%)	4 (18.2%)	0.003*
Serum zinc(μg/dl)	55.67 \pm 19.23	81.82 \pm 25.23	0.001*
Receiving chelation:n (%)	9 (22%)	14(77.8%)	0.02*
Deferoxamine chelation therapy: n (%)	7(38.9%)	3(13.6%)	0.01*

Table 3: Correlation of Tanner stage and different clinical and laboratory parameters in children with thalassemia

	r	p
BMI	0.56	0.04*
Age of start transfusion (years)	-0.4	0.1
Duration of transfusion (years)	-0.33	0.7
Serum ferritin	-0.34	0.03*
Serum zinc(μg/dl)	0.22	0.012*