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A CROSS-SECTIONAL CONTROLLED STUDY OF GONADAL FUNCTION AND PUBERTAL DEVELOPMENT IN THALASSEMIA MAJOR

A.A. VAHIDI, M.D., M.H. TORABINEJAD, M.D., A. AHMADI, M.D., Z. FARAHMANDINIA, M.D., G. KIANI MOGHADDAM, M.D., AND B. MEGHDADI, M.D.,

From the Department of Pediatrics, Kerman University of Medical Sciences, Kerman, I.R. Iran.

ABSTRACT

Early and regular blood transfusion therapy in patients with homozygous beta-thalassemia decreases the complications of severe anemia and prolongs survival. In the long term, however, the beneficial effects of transfusions are limited by the organ damage resulting from iron overload. Endocrine complications in patients with thalassemia major in developing countries may be frequent due to suboptimal iron chelation.

The goal of this study was to investigate the gonadal function and secondary sex characteristics in thalassemic patients. We studied 71 randomly selected adolescent thalassemic patients and 30 age- and sex-matched controls. Sexual maturity rating (SMR), height and weight, and gonadotropin, sex steroid, and ferritin levels were evaluated.

Cases had significantly lower mean height and weight. Also, serum levels of gonadotropins and sex hormones were significantly lower in cases with thalassemia than in controls. Gonadotropin and sex steroid levels were lower in cases with thalassemia who had not used deferoxamine regularly compared to those with a regular chelation therapy regimen. All of the control subjects had sexual maturity ratings of II or above, while 36.6% of thalassemic cases were in Tanner stage I. About 53.3% of controls had surpassed all levels of sexual maturity, while only 2.8% of cases were in the stage SMR V. Distribution of SMR ratings was significantly different in cases and controls.

These findings clearly show that a high percentage of thalassemic patients in this part of the country suffer from various endocrine abnormalities, especially impairment in height growth and sexual maturity. It is wise to consider more sophisticated treatment modalities in these patients, including the administration of sex hormones for the compensation of hormonal abnormalities resulting from hemosiderosis.

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Correspondence should be addressed to: Dr. Ali-Asghar Vahidi, Department of Pediatrics, Kerman University of Medical Sciences, University Hospital No. 1 (Kerman Darman Hospital), Jehad St., Kerman, 76194, Iran. Phone: 0098 341 232021, Fax: 0098 341 268656 Email: ghasemkiani@yahoo.com

INTRODUCTION

In the past years, the use of regular blood transfusions and of chelation therapy with deferoxamine has led to the transformation of thalassemia major from a fatal disease in early childhood to a chronic illness associated with relatively prolonged survival. Transfusion regimens maintaining pretransfusion hemoglobin above 9-10 g/dL are effective in suppressing erythroid marrow expansion. Repeated transfusions in thalassemic patients and subsequent hemosiderosis often result in a variety of complications including growth retardation, gonadal dysfunction, and delayed appearance of secondary sex characteristics.[1,2] Long term deferoxamine therapy using subcutaneous infusions at least 4-6 days a week have clearly demonstrated major effects on iron overload complications. Deferoxamine treatment reduces excessive iron and prevents cardiac, hepatic and endocrine diseases.[3,4] Nonetheless, compliance is difficult for many patients and availability problems limit its use in some other patients.[5]

Endocrine disorders related to secondary hemosiderosis such as short stature, delayed puberty and hypogonadism are major problems in both adolescent and adult patients. With modern regimens of comprehensive care for these patients, many of these complications can be prevented and others ameliorated and delayed in their onset.

The aim of this study was to evaluate the height, weight, gonadal function, and sex maturity rating, as well as their relationship to the pattern of deferoxamine usage, in thalassemic patients in Kerman Province.

MATERIAL AND METHODS

This was a cross-sectional, controlled study conducted at University Hospital No. 1 of Kerman University of Medical Sciences and the Thalassemia Center of Kerman Province. The objective of this investigation was to determine gonadal function and pubertal development in patients with thalassemia major.

Cases

Thalassemic patients who made regular visits to the University Hospital No. 1 and the Thalassemia Center for blood transfusion were eligible for inclusion in this study if their chronological age was beyond 12 years for males and 10 years for females and did not have a history of hormonal drug use. An informed consent was obtained from all the patients or their parents. A sample of 71 patients satisfying the recruitment criteria was enrolled in this study. A complete history, including demographic data and blood transfusion and chelation therapy profile, was obtained from each patient. According to the frequency of deferoxamine injection reported by the patient, the status of deferoxamine use was defined as "regular" (>4 injections a week) or "irregular/absent" (< 4 injections a week). Sexual maturity rating (SMR) was determined according to Tanner method[6] using data collected by history taking and careful physical examination. Height and weight were measured by standard clinical methods. From each patient, a 5-mL sample of venous blood was collected between 8:30 and 9:00 AM for measurement of sex hormones (testosterone in boys, estradiol in girls), FSH, LH, and ferritin by radioimmunoassay (RIA) method. All laboratory measurements were carried out in the Laboratory Department of University Hospital No. 1 of Kerman University of Medical Sciences.

Controls

Thirty healthy subjects with the same age and sex distribution as the experimental group served as the control group. Clinical and laboratory evaluation of controls was performed in the same way as for the cases. In female controls who had experienced menarche, the blood samples for FSH, LH, and estradiol were collected in follicular phase. Serum ferritin levels were not measured in controls.

Statistical analysis

Statistical analysis was performed using SPSS Version 10.0.5 software package. All quantitative results were reported as mean ± standard deviation. Independent samples t tests were used to compare continuous variables between experimental and control groups. To compare the trend of SMR between cases and controls, the Extended Mantel-Haenszel Chi Square Test for Trend[7] was used. Statistical significance was set at 0.05.

RESULTS

The experimental group consisted of 36 male and 35 female thalassemic patients. The control group included 15 males and 15 females. The age range of cases was 10-23 years; controls had an age range of 11-19 years. Comparison of the mean age of cases and controls showed no apparent enrollment bias. Height and weight of thalassemic patients was lower than controls and the difference was statistically significant ($p< 0.001$) (Table I and Table II). Mean levels of FSH and LH were significantly lower in cases than in controls ($p< 0.001$). Serum ferritin level was enormously elevated in thalassemia patients as expected (mean ± standard deviation: 5017±3421 µg/L).

A separate analysis of differences between cases and controls was performed for boys (Table I) and girls (Table II). Values of height, weight, and gonadotropins were lower in male cases than in male controls ($p< 0.001$).

Table I. Descriptive statistics of demographic and laboratory variables in cases and controls (boys).

Variables	Cases (n = 36)	Controls (n = 15)
Age (years)*	15.9±2.6	16.1±1.8
Height (cm)†	142±8	164±8
Weight (kg)†	35.0±6.9	52.5±8.1
LH (mIU/mL)†	0.93±1.00	4.31±1.93
FSH (mIU/mL)†	1.65±1.13	6.22±3.66
Testosterone (nmol/L)‡	2.40±5.39	7.82±5.92

* Not significant.
† $p < 0.001$
‡ $p = 0.003$

Table II. Descriptive statistics of demographic and laboratory variables in cases and controls (girls).

Variables	Cases (n = 35)	Controls (n = 15)
Age (years)*	15.1±2.7	14.9±2.1
Height (cm)†	136±13	158±8
Weight (kg)†	34.5±9.6	50.1±9.0
LH (mIU/mL)†	1.24±1.28	4.72±1.88
FSH (mIU/mL)†	2.67±2.30	6.43±3.92
Estradiol (pmol/L)‡	113±186	335±219

* Not significant.
† $p < 0.001$
‡ $p = 0.003$

Table III. Descriptive statistics of laboratory variables by status of deferoxamine use.

Variables	Deferoxamine Use	
	Regular (n = 30)	Irregular/None (n = 41)
Serum Ferritin (µg/L)*	4400±3432	5468±3383
LH (mIU/mL)†	1.63±1.49	0.69±0.56
FSH (mIU/mL)†	2.81±2.41	1.68±1.14
Testosterone (in boys) (nmol/L)‡	4.04±7.04	0.58±1.02
Estradiol (in girls) (pmol/L)†	217±292	65±81

* Not significant.
† $p < 0.05$
‡ $p = 0.053$

Table IV. Descriptive statistics of laboratory variables by serum ferritin level in thalassemic patients.

Variables	Serum Ferritin Level	
	< 2000 µg/L (n = 16)	⩾ 2000 µg/L (n = 55)
LH (mIU/mL)*	1.49±1.55	0.97±0.99
FSH (mIU/mL)*	2.02±1.20	2.20±2.03
Testosterone (in boys) (nmol/L)*	5.08±9.84	1.87±4.07
Estradiol (in girls) (pmol/L)*	79.0±88.3	126±213

* Not significant.

Mean testosterone levels were significantly depressed in male thalassemic cases compared to controls (p= 0.003). The trend in girls also discerned a significant decrease of height, weight, and gonadotropin levels in cases compared to controls (p< 0.001), as well as a lower level of estradiol in female patients compared to female controls (p= 0.001).

The mean serum level of ferritin in thalassemic patients who reported regular deferoxamine injections (4400.27±3431.90 µg/L) was lower than in cases who had irregular/no deferoxamine injections (5467.46±3383.07 µg/L), though the difference did not attain statistical significance (p> 0.05) (Table III). On the other hand, mean levels of gonadotropins and sex hormones were significantly higher in patients with regular chelation therapy compared to cases

Table V. Frequency distribution of sexual maturity rating in cases and controls.

Sexual Maturity Rating	Cases		Controls	
	Number	Percent	Number	Percent
I	26	36.6	0	0.0
II	20	28.2	3	10.0
III	19	26.8	5	16.7
IV	4	5.6	6	20.0
V	2	2.8	16	53.3
Total	71	100	30	100

Extended Mantel-Haenszel Chi Square Test for Trend: $\chi^2 = 44.947$; DF = 1; $p < 0.00001$.

who had irregular/no deferoxamine injections ($p < 0.05$; in the case of estradiol, $p = 0.053$) (Table III). Comparison of laboratory variables between cases with ferritin levels below 2000 µg/L (n= 16) and cases with ferritin levels of 2000 µg/L or higher (n= 55) did not show any significant difference ($p > 0.05$) (Table IV).

A grossly impaired pattern of sexual maturity was found in thalassemic patients (Table V). While 53.3% of control subjects had an SMR of V, this degree of sexual maturity was found only in 2 thalassemic cases (2.8%). None of the female patients had menstruation. On the other hand, none of the controls were in the SMR I group, whereas 26 patients (36.6%) had this rating. An Extended Mantel-Haenszel Chi Square Test for Trend was performed to compare the trend of SMR between the experimental and control groups. This test discerned a significant difference in the trend of SMR between the 2 groups ($p < 0.00001$).

DISCUSSION

With modern treatment and longer survival of patients with homozygous β-thalassemia, endocrine dysfunction assumes greater importance. Short stature, delayed puberty and hypogonadism are major problems in both adolescent and adult patients.[8] The present study revealed a grossly impaired pattern of pubertal development in thalassemic patients of Kerman province, Iran. Due to ethical considerations, particularly in the control group, multiple blood sampling was impractical in the present study; therefore, a single blood sample between 8:00-9:30 AM was obtained for hormonal assays in all cases and controls. The impaired sexual maturity was accompanied by significant decrease in gonadotropin and sex hormone levels, indicating that iron deposition in the pituitary gland could be the primary reason for delayed puberty in these patients. The high levels of ferritin found in our patients are another evidence for this mechanism of pubertal disorder.

Previous studies in Iran have shown decreased growth and endocrine function in thalassemic patients[9,10] In a study in Northern Iran on 110 thalassemic patients 8-18 years of age, sexual maturity was significantly delayed in cases than in sex- and age-matched controls.[10]

Multiple endocrinopathies, including hypogonadism, hypothyroidism and diabetes mellitus, occur mainly in patients who have high serum ferritin levels.[11] Prognosis for survival is greatly improved if the serum ferritin is kept below 2000 µg/L by regular chelation.

Berkovitch et al[12] assessed the endocrine status of 33 thalassemic patients above 15 years of age by clinical examination, a gonadotropin releasing hormone (GnRH) stimulation test, and MRI of the pituitary. Anterior pituitary function (GnRH stimulation test) correlated well with MRI results. Other studies have clearly shown that hypogonadotropic hypogonadism is caused by the selective loss of pituitary gonadotropin function.[13] Rasekhi et al[14] studied 36 thalassemic patients for the presence of hypogonadotropic hypogonadism and MRI findings of pituitary gland and compared them to 20 age- and sex-matched individuals. Seventy-eight percent of their patients had hypogonadotropic hypogonadism. There was a statistically significant correlation between low hormonal level and both low pituitary signal ($p = 0.012$) and small pituitary size ($p = 0.04$).

In patients with thalassemia major living in developing countries, endocrine complications may be frequent due to suboptimal iron chelation. In a prospective Indian study by Gulati et al,[15] 10 of 11 thalassemic adolescents/young adults had hypogonadism. These results are in accordance with our finding of a very high frequency of pubertal delay and low gonadotropin and sex hormone levels in thalassemic patients. On the other hand, in a study[12] performed in an industrialized country, 28 out of 33 thalassemic patients achieved normal puberty. This indicates a shortage of modern treatment modalities in developing countries. Nevertheless, some other studies in industrialized countries have shown that a regular and homogeneous transfusion and chelation management often does not prevent pubertal failure; it is related with the degree of liver fibrosis and often it is due to hypothalamic and/or pituitary dysfunction.[16]

Chatterjee et al[17] studied GnRH-gonadotropin secretory dynamics in 28 male β-thalassemia major patients with failed puberty and compared them to 5 healthy, non-thalassemic prepubertal males. The thalassemic group had lower basal FSH ($p < 0.01$), LH ($p < 0.01$) and GnRH stimulated FSH ($p < 0.001$) and LH levels ($p < 0.001$) than the controls. Serum ferritin levels in GnRH-non-responders were higher than those in the responders (9,052.63 ± 579.14 μg/L vs 5,933.33 ± 1,819.65 μg/L; $p < 0.05$). The latter statement is in agreement with our finding of lower hormonal profile in patients with irregular chelation therapy compared to those who used regular deferoxamine injections. It is nevertheless noteworthy that we did not find a statistically significant relationship between serum ferritin level and hormonal profile of the patients. This may be due to the recent establishment of Thalassemia Center in Kerman province, which has dramatically improved availability of chelation therapy for thalassemic patients. It is possible that some patients have experienced a recent short-term decrease in serum ferritin level without corresponding changes in the hormonal profile.

In cases of delayed puberty, sexual development should be induced at an appropriate age.[11] In patients with both GH deficiency and hypogonadism, low dose sexual steroid treatment should be considered either as an alternative or an additional treatment before starting GH therapy.[13] Low dose long acting sex steroid treatment in boys with delayed puberty, delayed bone age and without GH deficiency for a year or more is safe and can produce similar results to those obtained with rhGH therapy.[8]

Retarded growth was another complication found in our cases with β-thalassemia. Growth failure has been attributed to growth hormone (GH) deficiency (hypothalamic or pituitary), hypothyroidism, delayed sexual maturation, hypogonadism, diabetes mellitus, zinc deficit, low hemoglobin levels, bone disorders and deferoxamine toxicity.[8] In a study by Gulati et al,[15] height standard deviation (SD) score of patients (-2.2 ± 1.5) was significantly lower than that of normal controls (-1.0 ± 0.7, $p < 0.001$). This confirms our finding of a significantly lower mean height in cases than in controls. The absence of a pubertal growth spurt during spontaneous or induced puberty is detrimental to the achievement of a normal final adult height.[11] In another study by Theodoridis et al,[8] 12% of thalassemic boys and 15% of thalassemic girls without endocrinopathies had height below the third percentile. This incidence was 29% when endocrinopathies were present.

In an Italian study in 1998, Caruso-Nicoletti et al.[18] showed that about 18% of thalassemic patients exhibit short stature. They found that short stature in these patients is due to a spinal growth impairment that starts in infancy and progressively aggravates.

Beta-thalassemia is associated with bone abnormalities characterized by bone marrow expansion of the medullary cavity, and osteopenia with cortical thickening and trabecular coarsening. Good nutrition with adequate vitamins and trace elements intake, along with calcium and vitamin D supplementation, can increase bone density and prevent bone loss. Endocrine abnormalities should be monitored carefully and a thorough endocrine evaluation should be carried out yearly in every patient to detect subclinical endocrinopathies.[19]

CONCLUSION

There is a gross impairment of pubertal development as well as a high prevalence of growth failure in our thalassemic patients. This is accompanied by a significant decrease in gonadotropin and sex hormone levels, and very high levels of ferritin. It is wise to consider more sophisticated treatment modalities in these patients, including the administration of sex hormones for the compensation of hormonal abnormalities resulting from hemosiderosis.

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