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Pubertal status and its relation with serum ferritin level in thalassemia major patients

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Abstract

Background: Thalassemia major (TM) is one of the most common hereditary anemia with multiple endocrinopathies (especially hypogonadism). So, we evaluated the rate of delayed puberty (DP) and its relation with serum ferritin level in patients.

Materials and Methods: This cross-sectional (descriptive-analytical) study was conducted on 100 patients with TM between 14-64 years old, admitted to Amirkola Thalassemia Center, Babol, Iran, in 2016. The pubertal status, (Marshall-Tanner scale), existance of DP, and its different types were evaluated. Mean serum ferritin level was measured and the data were classified to three groups of <1500, 1500-2500, and >2500 ng/ml. Data were analyzed using SPSS (version20).

Results: Out of 100 patients, 64 (64%) and 36 (36%) were female and male, respectively. Considering age, 23, 77 patients (%) were under and over 20 years old, respectively. Totally, 69 (69%) of them had DP, of whom 64 (92.8%) ones had secondary (central) hypogonadotropic hypogonadism. Mean serum ferritin level (±SD) was 2707.94±1683.42 ng/ml. In addition, 26, 29, and 45 patients had ferritin level <1500, 1500-2500, and >2500 ng/ml, respectively. Thirty two patients with DP (46.4%) had ferritin level above 2500 ng/ml (p-value= 0.623).

Conclusion: The results showed a high frequency of DP in TM patients, requiring careful examination and follow-up in terms of puberty for early diagnosis and proper treatment to improve their quality of life, and prevention of the complications like osteoporosis. We couldn't find any significant relationship between serum ferritin level and hypogonadism, even for cases who received enough iron chelators.

Keywords: Delayed Puberty, Ferritin, Hypergonadotropic Hypogonadism, Hypogonadotropic Hypogonadism, Thalassemia Major

Introduction

Thalassemia major (TM) is one of the most common hereditary anemia, requiring blood transfusion (1, 2). In these patients, due to regular blood transfusions, large amounts of iron accumulate in different parts of the body, causing hemosiderosis in the long term. In addition, the endocrinopathies are common in these patients because of the replacement of iron in the endocrine system. The most common iron overload complications are hypogonadism (35-55%), hypothyroidism (9-11%), hypoparathyroidism (6-10%), liver fibrosis, heart failures (33%), and diabetes (6-10%) (1, 3, 4). The pituitary gonadotropic cells are very sensitive to iron overload and free radicals. The most studied patients suffered from hypogonadotropic hypogonadism (secondary or central) and few of them experienced hypergonadotropic hypogonadism (primary or peripheral) related to direct iron deposition in the gonads (5, 6). Delayed growth and puberty in TM patients who need regular blood

transfusions are common problems. In the course of thalassemia, regular blood transfusion and the occurrence of hemosiderosis along with problems associated with anemia can lead to multiple endocrinopathy, including hypogonadism. Although multiple blood transfusion can maintain normal levels of hemoglobin, iron overload and endocrinopathy are still common problems in this regard (7-9). Mostly, endocrinopathy gradually occur due to iron overload which can lead to complications in the second decade of life (10). DP is one of the most common endocrinopathy in TM patients, which is associated with the lack of the appearance of secondary sexual characteristics by 13 and 14 years of age for girls and boys, respectively (11, 12). Based on previous studies, endocrinal complications in TM cases are more common in developing countries (12) with prevalence of 40% in some studies (13). Endocrinal dysfunction has direct relationship with serum ferritin and iron levels of thalassemia patients (14). Ferritin is iron protein reservoir. MRI T2* of liver and heart is the best non-invasive method to evaluate iron overload, but this equipment is not available in Mazandaran Province. Therefore, we had to evaluate periodic serum ferritin level to evaluate body iron overload and iron chelator efficacy (15, 16). DP has various etiologies such as iron deposition in the pituitary or gonads, and as a result, reduces the function of the gonads (13). Sexual dysfunctions have also been observed in both men and women after puberty (8, 9, 13, 14).

Primary (peripheral) DP, related to hypergonadotropic hypogonadism, was determined by the increased level of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and decreased level of sexual hormones (12, 15). In the secondary (central) DP that is related to central nervous system (CNS) involvement, hypergonadotropic hypogonadism is determined by decrease of LH and FSH levels as well as sexual hormones (12). Amenorrhea is two kinds: primary amenorrhea defined as the absence of menarche in women before the age of 16 and secondary amenorrhea, which is the absence of menstrual periods for more than three months in a woman who was previously menstruating (16). DP has negative effects on the quality of life and also osteoporosis which itself, causes bone pains and sometimes bone fractures; therefore, the aim of this study was to determine the pubertal status and its relation to ferritin level in these patients. If the occurrence of DP is associated with high serum ferritin level, early diagnosis, appropriate treatment, and better using of ferritin chelation therapies are recommended.

Materials and Methods

This cross-sectional (descriptive analytical) study was conducted on 100 thalassemia patients in 2016. Thirty six male over 14 years of age and 64 female cases over 13 years old, referred to Amirkola Thalassemia Center (Babol-Iran). Patients with genital ambiguity related to adrenal endocrinopathy, or in genetic chromosomal abnormality and heart failures were excluded from the study.

Non- probability method was used for sampling . We decided to compare serum ferritin level in two groups (in delayed and non-delayed puberty) with preference to estimate mean serum ferritin level in delayed puberty cases. Regarding the 95% confidence, σ:1600, standard deviation, and maximum estimated error of 340 , the serum ferritin level in the DP group was evaluated. Considering the probability of missing subjects, the sample size of the study was increased to 100. In this study, first, the pubertal status of each patient was evaluated. Then, their maturity staging was classified based on the Marshall-Tanner scales (table I & II) (17). The presence of DP, the type of hypogonadism (primary or secondary), and its severity were

determined based on the clinical examination and LH, FSH, and testosterone serum levels for male, and LH, FSH, and estrogen serum levels for female. If the levels of LH, FSH, and sexual hormones were low, hypogonadism was defined as secondary or central (hypogonadotropic hypogonadism) and if the levels of LH and FSH were high and sexual hormones were low, hypogonadism was considered as primary or peripheral (hypergonadotropic hypogonadism). We considered normal levels of LH in adult and prepubertal males as 1.8-12 miu/ml and 0.3-6 miu/ml, respectively. The norma range of LH in adult and prepubertal females were 0-5 and 0-4 miu/ml, respectively. Normal levels of FSH in adult and prepubertal males were 1.5-12.4 and 0-5 miu/ml, respectively. The normal range of FSH for adult and prepubertal females were 0.3-10 and 0-4 miu/ml, respectively. Normal level of testosterone was 3-10 ng/ml in males. The normal level of estrogen for females were 10-60, 20-400, and 5-25 pg in pubertal/adult, premenoupusal, and postmenopusal stages, respectively. A sample of 2 ml of venous blood was taken to measure LH, FSH, and testosterone level in males as well as LH, FSH, and estradiol in females by a kit of Monobind Inc. (made in USA) and using chemiluminescence method. We evaluated hormones level at least 15 days after the last blood transfusion. The serum ferritin level was measured using 2-cc blood volume and kit (made Padtan Elm Company, Iran) and applying ELISA method. Due to false results as well as errors in laboratory equipment, the ferritin level was evaluated in three stages, once every two months within 6 months; eventually, the mean ferritin level was calculated and considered as the ferritin level for each patient. Then, patients were divided into three groups based on the ferritin level: less than 1500, from 1500 to 2500, and more than 2500 ng/ml.

This research project was reviewed and approved by the Ethics Committee of Babol University of Medical Sciences (MUBABOL.REC.1394.199). Written informed consent was obtained from each patient and his/her family. All information of patients was kept confidential.

Statistical analysis

In this study, data were carefully collected, recorded, and reviewed. Possible errors were minimized and the validity of the measurement tool (checklist) was studied by a few trained persons. Data were analyzed using SPSS (version 20). The findings were expressed as descriptive statistics (frequency, relative frequency and so on), and chi-square and t-test were used to find out the relationship.

Results

In this study, 100 patients were evaluated with mean age (±SD) of 27.69±8.57 (the oldest was 64 and the youngest was 14 years old). Among 64 females, 16, 48 patients were under and over 20 years old, respectively. Among 36 males, 7, 29 patients were under and over 20 years old, respectively. Mean of serum ferritin level (±SD) was 2707.94±1683.42 ng/ml. In respect to serum ferritin levels, 26, 29, and 45 patients (percent) had <1500, 1500-2500, and >2500 ng/ml, respectively. Thirty two patients with DP (46.4%) had serum ferritin level above 2500 ng/ml (p-value: 0.623). The characteristics of these patients are shown in Table I. From 69 patients (69%) with DP, 64 (92.8%) of them had hypogonadotropic hypogonadism (secondary DP). Before initiating the appropriate treatment for DP cases, no maturity index was appeared in 45 (65.2%) of DP cases. There was no statistical significant relationship between frequency of DP cases and mean age and gender (Table II). According to Table III, the majority of patients with DP (females and males) had secondary DP (24 out of 25

males and 40 out of 44 females). Most of patients, before treatment, suffered from pubertal delay (19 of 25 males and 26 of 44 females). Thirty-two (71.1%) of 45 patients with ferritin levels >2500 ng/ml had puberty delay, but this relationship was not statistically significant (P=0.623). There was no statistically significant relationship among gender, mean age and DP. Out of 25 males with DP, 13 (52%) ones had ferritin levels >2500 ng/ml (P=0.365) and among 44 females with DP, 19 (43.2%) ones had ferritin levels >2500 ng/ml (P=0.963), which was not statistically significant. Out of 64 cases of secondary DP, 29 (45.3%) had ferritin levels >2500 ng/ml (P=0.350), indicating no significant relationship between secondary DP and ferritin levels >2500 ng/ml (P=0.350). Among 69 patients with DP, 45 (65.2%) of them had no puberty before the start of treatment, 22 (48.9%) had ferritin levels >2500 ng/ml and 13 ones (28.8%) had a ferritin level from1500 to 2500 ng/ml, which was not statistically significant (P=0.666). It is observed that most of the patients (both women and men) had a DP, secondary DP (24 out of 25 men, 40 out of 44) (Tables IV, V). Moreover, there was no statistically significant relationship between mean serum ferritin level in terms of different variables (such as DP, diabetes mellitus, hypothyroidism, sex, age) (Tables VI, VII).

Table I: Classifications of sex maturity states in boys

SMR* Stage	Pubic Hair	Penis	Testes
1	None	Preadolescent	Preadolescent
2	Scanty, long, slightly pigmented	Minimal change/ enlargement	Enlarged scrotum, pink, texture altered
3	Darker, starting to curl, small amount	Lengthens	Larger
4	Resembles adult type, but less quantity; coarse, curly	Larger; glans and breadth increase in size	Larger, scrotum dark
5	Adult distribution, spread to medial, surface of thighs	Adultsize	Adultsize

* Sexual maturity rating

Table II: Classification of sexual maturity states in girls

SMR stage	Pubic Hair	Breasts
1	Preadolescent	Preadolescent
2	Sparse, lightly pigmented, straight, medial border of labia	Breast and papilla elevated as small mound; diameter of areola increased
3	Darker, beginning to curl, increased amount	Breast and areola enlarged, no contour separation
4	Coarse, curly, abundant, but less than in adult	Areola and papilla form secondary mound
5	Adult feminine triangle, spread to medial surface of thighs	Mature, nipple projects, areola part of general breast contour

Table III: Basic characteristics of patients with TM (n=100)

Patient's information	Type		Number (%)
Gender	Male		**36 (36)**
	Female		**64 (64)**
Delayed puberty (DP)	No		**31 (31)**
	Yes		**69 (69)**
		Primary	**5 (7.2)**
		Secondary	**64 (92.8)**
		Prepubertal delay	**45 (65.2)**
		Pubertal delay	**7 (2.9)**
		Postpubertal delay	**22 (31.9)**

Table IV: Relationship between age, gender and serum ferritin level with DP

Factors		Number	With DP	Without DP	P-value
			Number (%)	Number (%)	
Serum ferritin level (ng/ml)	<1500	26	19 (73.1)	7 (26.9)	**0.623**
	1500-2500	29	18 (62.1)	11 (37.9)	
	>2500	45	32 (71.1)	13 (28.9)	
Gender	Male	36	25 (69.4)	11 (30.6)	**0.943**
	Female	64	(%٦٨/٨) ٤٤	20 (31.3)	
Mean age			28.6±7.751	26.19±10.166	**0.267**

Table V: relationship between gender and pubertal status

Pubertal status	Gender	Numbers	P-value
Primary Secondary	Male	1 24	**0.618**
	Female	4 40	**0.448**
Prepubertal delay Pubertal delay Postpubertal delay	Male	19 1 5	**0.478**
	Female	26 1 17	**0.371**

Table VI: Mean of serum ferritin level in terms of different variables

Different variables	Number	Serum ferritin level (ng/ml) mean±SD	P.Value
With DP	69	2630.88±1641.51	**0.497**
Without DP	31	2879.48±1788.89	
With diabetes	16	2645.22±1722.55	**0.872**
Without diabetes	84	2719.89±1686.13	
With hypothyroidism	18	2433.55±1850.04	**0.448**
Without hypothyroidism	82	2768.17±1650.73	
Male	36	2853.13±1965.13	**0.520**
Female	64	2626.28±1512.95	

Table VII: Relationship of serum ferritin level and sex in age groups

Variables		Age Groups, N (%)		Total N (%)	P-value
		14-20 years	21-64 years		
Serum ferritin level	<1500	4 (4)	22 (22)	26 (26)	0.382
	1500-2500	9 (9)	20 (20)	29 (29)	
	>2500	10 (10)	35 (35)	45 (45)	
Sex	Female	16 (16)	48 (48)	64	0.526
	Male	7 (7)	29 (29)	36	
Total		23 (23)	77 (77)	100 (100)	

Discussion

In the present study, 69 patients (69%) out of 100 ones suffered from hypogonadism. Before initiating the proper treatment, no secondary sexual characteristics was appeared in 45 (65.2%) of them. In a study by Hadaegh et al., puberty was not appeared in 60% of thalassemic patients until 18 years old (71% and 51% were male and female, respectively) (P<0.05) (15). Soliman et al., reported that the prevalence of hypogonadism was 73% and 42% in male and female, respectively (18). Similar to this study, Abtahi et al., revealed that the prevalence of hypogonadism was 64.5% in thalassemic patients living in Tehran (19). Shamshirsaz et al., evaluated 220 thalassemic patients and found hypogonadism in 22.9% of boys and 12.2% of girls (20), which is less than its prevalence in the current study. DP was investigated in 146 TM patients (84 boys) aged 10-22 years old in a study by Karamifar et al. DP was found in 75.6% of boys and 68.4% of girls aged 12-22 years old (21), the frequency of DP was higher than our results. A multicentre study on the prevalence of endocrinologic complications was performed on 1861 patients in Italy. The prevalence of DP was observed in 51% of male and 47% of female, all were over the age of 15 years old. Secondary amenorrhea was reported in 23% of patients with the mean age of 18.3 years. In the majority of patients, the secondary sexual characteristics was appeared after 13 years old in female and 14 years old in male, and some patients never reached puberty (6), the incidence of secondary amenorrhea was a little higher than that in our patients. A study in the Netherlands evaluated the effect of age at the time of iron chelation therapy initiation on gonadal function in 40 patients with TM who aged > 14 years old. They concluded that starting the chelation therapy with deferoxamine before age of 10 can significantly prevent gonadal dysfunction comparing with the initiation of chelation therapy after age of 10 (90% and 10%, respectively) (22). However, we didn't evaluate the age of starting iron chelators therapy, but in our cases with compliance to use iron chelators, we had some patients with DP. Bazrafshan et al., studied the sexual maturation in TM patients. Their case-control study was performed on 110 patients aged 8-18 years old (divided to three subgroups: 8-11, 12-14 and 14-18 years) and 62 subjects (31 males and 31 females) as a control group with the same age. No significant difference was found between two groups in terms of sexual status, iIn subgroup of 8-11 years female, no significant difference was observed between two groups (case and control). However, significant difference was found between

two groups in the subgroup of 12-14 years female (P <0.01). In subgroup of 14-18 years female, none of them had reached to full sexual maturity. In male cases, secondary sexual characteristics waswere lower than control group. None of the patients had full pubertal status (23). In the current study, we found that 69 (69%) out of 100 patients suffered from hypogonadism; of whom 45 cases (65.2 %) didn't show secondary sexual characteristics before initiating proper hypogonadism treatment. So, the population of females who reached to normal puberty was higher than Bazrafshan's study. Another research was carried out by Gulati et al., on 84 patients with TM. In their study, 83% of cases suffered from hypogonadism (24). In a study by De sanctis et al., (2004), the most common endocrinopathy in thalassemic patients was hypogonadism, which was similar to the results of our study and previous studies (25). Chern et al., in Taiwan evaluated the pubertal status of 29 TM patients (18 girls and 11 boys) who aged ≥15 years old and needed regular blood transfusions. The prevalence of hypogonadotropic hypogonadism was reported in 72% of patients. DP was observed in 5 boys (45%) and 7 girls (39%); and the absence of secondary sexual characteristics was found in 2 boys (18%) and 5 girls (28%) (26). Similarly, the most common type of hypogonadism was hypogonadotropic hypogonadism (secondary) in 64 (92.8%) patients and only 5 (7.2%) cases had hypergonadotropic hypogonadism (primary) in the current investigation.

Conclusion

With respect to findings of current study, hypogonadism is one of the common complications induced by TM, indicating that the issue of DP needs more attention to achieve early diagnosis and suitable treatment to improve sexual status and quality of life among these patients.

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Conflict of interest

Authors declared no conflict of interst.

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