Original Article

Endocrinopathy Complications and the Role of Serum Ferritin as a Marker of Endocrinopathy Prediction in Patients with Beta-Thalassemia Major

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Abstract

Background: This study aimed to estimate the prevalence of complications and in β-thalassemia patients, to identify its related risk factors and to determine the optimal thresholds of serum ferritin and disease duration as a predictor of the endocrine disease. **Materials and Methods:** A total of 140 patients with β-thalassemia major, 65 (46.4%) male with a mean age of 21.4 ± 7.5 (range 8–39) years were enrolled in this study. Logistic regression and receiver operating characteristic curve were used to estimate the diagnostic power of ferritin level and determine the optimal cut points. **Results:** The serum ferritin level was 3395 ± 2611 μg/L with stable trend across the last 5 years. Puberty delay was the most common complication with the prevalence of 33.6%. There was a significant association between ferritin levels and hypocalcaemia (odds ratio [OR] = 1.29, $P = 0.001$), short stature (OR = 1.04, $P < 0.001$) and puberty delay (OR = 1.02, $P = 0.002$). A >2100 μg/L and >3400 μg/L optimal cut-off values of serum ferritin level for puberty delay was 2100 area under the curve (AUC = 0.78, $P = 0.004$) and 3400 for short stature (AUC = 0.74, $P < 0.0001$). **Conclusions:** Progressive deterioration of endocrine dysfunction and inadequacy of chelation therapy in older patients are endocrine complications amongst beta-thalassemia major patients that need more attention. Prosperous control of the ferritin levels before puberty with deferoxamine appeared to be an effective treatment to prevent and reduce diabetes and hypothyroidism. The serum ferritin >1500 μg/L along with early second decade of illness is the best predictor for the development the endocrinopathy.

Keywords: Diabetes, hypocalcemia, Iran, Iron overload, pubertal delay

Introduction

Thalassemia, a haemoglobin synthesis-related disease, is one of the most prevalent genetic disorders worldwide. Thalassemia major is hereditary haemolytic anaemia and severs form of β-thalassemia with the ineffective functioning of bone marrow that results in haemolysis and progressive severe anaemia.[1] The β-thalassemia is the most prevalent hereditary autosome disease with, at least, 200,000 new births (homozygotes) and 240 million new carriers (heterozygotes) every year.[2] Estimated frequency of carriers in Iran is 2–3 million where β-thalassemia with the prevalence of 3.2% is the most common type of thalassemia with the highest reported prevalence of 10% from northern and southern regions.[3]

The most confident treatment for thalassemia is through bone-marrow transplantation which is not available for all patients. Repeated blood transfusion is a symptomatic treatment that increases life quality and expectancy and reduces complications such as anaemia.[4] However, it results in hemosiderosis, an inevitable complication followed by liver disorders, heart failure, growth disorders, hypogonadism, hypoactive of thyroid and parathyroid and diabetes.[3] Even though chelation therapy is used to control complications of repeated blood transfusions, the endocrine disorders are prevalent in these patients and may increase with ageing.[5]

Thalassemia major patients treated with repeated blood transfusions are subject to higher risk of hypothyroidism and

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hypoparathyroidism, short stature, hypogonadism and diabetes than general populations.[6] Endocrinopathy disorders that are caused by repeated blood transfusions are of higher importance in thalassemia major patients. They are the second leading cause of mortality, after heart disorders, in this population.[7,8] Hypogonadism is the most prevalent endocrine disorder in thalassemia major patients and is associated with iron sediment in the pituitary.[5]

Studies on the evaluation of chelation therapy effects are not frequent with contradictory results.[6,9,10] Some of this discrepancy could be due to cross-sectional nature of study designs in most reports that rely on information from a single time point. Serum ferritin is a proxy for iron accumulation in the past 3 months, whereas the development of endocrine disorders demands long-term exposure to excess iron. Therefore, estimated associations based on single measurements would be biased.[11]

This study aimed to estimate the prevalence of endocrine complications in β-thalassemia major patients, who are treated with deferoxamine. Predictors of endocrinopathy and their threshold values related to complications are also determined.

Materials and Methods

In this study, sample size determined based on Type I error = 0.05, Type II error = 0.1 and area under receiver operating characteristic (ROC) curve of 0.7. The data from 140 thalassemia major patients' analysed, undergone standard treatments in the Qom Provinces, Thalassemia Center, from 2012 to 2017. During this period, patients received a regular blood transfusion and iron chelation every 3 or 4 weeks with deferoxamine. Disease diagnosis was based on the first haemoglobin electrophoresis in the absence of haemoglobin A band and the transfusion initiation age of fewer than 2 years. All patients who had recorded in the centre were included in the study.

In the absence of inflammatory disease, measuring serial ferritin, which is accumulated the amount of iron in the last 3 months, is a reliable index of iron sediment in the body and a way to assess the quality of chelation therapy.[5] Since the development of endocrine disorders requires long-term exposure to excess iron, biopsy tissue hemosiderin was used to measure the excess iron amount.

Demographic data including gender, age, age transfusion, transfusion intervals, deferoxamine dose and age of disease onset and age of iron removal were obtained from the database of the thalassemia centre. Data on height, weight and pubertal rating (sexual maturity rating) were recorded based on Tanner score criteria for all patients.[12] Fasting blood sugar (FBS), serum calcium and serum ferritin were also available. According to American Diabetes Association, individuals with FBS >126 mg/dl were diagnosed with diabetes and those with a serum calcium of <8 mg/dl were recorded as hypocalcaemic. In individuals of age >10 years, assessment of serum T4 and thyroid-stimulating hormone (TSH) were used to evaluate thyroid performance where TSH >6.3 mUI/L was considered hypothyroidism, T4 >6.5 mUI/L as subclinical and T4 <6.5 mUI/L as manifest hypothyroidism. Short stature was classified as being under 3rd percentile of the stature plots provided by Vital Statistics Center. Based on Marshal-Tanner criteria, individuals with follicle SH >0.4 and luteinising hormone >1.2 along with estradiol 10 pg/ml (measured by ELISA) in females aged >13 years and testosterone <2.8 ng/dl (measured by radioimmunoassay) in males age >14 were classified as hypogonadism. Serum ferritin levels of >500 μg/L were considered as high.

Descriptive statistics were calculated as frequency (%) for categorical and meant ± standard deviation for normally distributed continuous variables. For variables with non-normal distribution, median and interquartile range (IQR) were calculated. Binary logistic regression was used for the evaluation of the association between endocrine disease and serum ferritin levels. According to variance inflation factor results, variables with high correlation were excluded from our analysis. The ROC curve is used to evaluate ferritin for classifying disease status. To estimate ferritin probability cut-off the Youden index (j) is used to maximise the difference between sensitivity (true-positive rate) and 1-specificity (false-positive rate). In a perfect test, Youden's index equals 1, in addition, a test with area under the curve (AUC) equal 1 indicates perfect accuracy, and an area of 0.5 represents a worthless test. All analyses were conducted in SPSS (IBM SPSS Statistics for Windows, Version 22.0. IBM Corp., Armonk, NY, USA). Results with $P < 0.05$ were considered statistically significant. The study protocol was approved by the Ethical Committee of the Qom University of Medical Sciences under number IR.MUQ.REC.1395.111.

Results

A total of 140 β-thalassemia major patients were entered in the study of which 65 (46.4%) were male. Median (IQR) age of patients was 21[15-27] year, median (IQR) ferritin was 2657.8 (1542.4, 3990.1) μg/L and median [IQR] age onset of disease was 7[5-15] months. The trend of ferritin level was stable during the 5 years. However, 24 patients (17.1%) have upward trend of ferritin level. Regarding the 5-year median ferritin level, 8 patients (5.7%) had serum ferritin levels of ≤1000 μg/L, 57 patients (40.7%) with serum ferritin levels of 1000–2000 μg/L and 75 patients (53.6%) had serum ferritin levels of >2500 μg/L. Amongst β-thalassemia major patients, 117 (83.57%) had at least one endocrine disease. The distribution of endocrinopathy complications in the β-thalassemia patients is presented in Figure 1.

Table 1 shows the results from the stepwise logistic regression of endocrine disease as the outcome variable in β-thalassemia patients.

The results suggest the median level of ferritin and disease duration as predictors of hypocalcaemia ($P < 0.05$).

Patients with higher ferritin levels were more likely to be hypocalcaemia where 1000-unit increase in ferritin level was associated with 29% increase in the likelihood of hypocalcaemia. For each year increase in disease duration, the odds of hypocalcaemia increased by 7.4%.

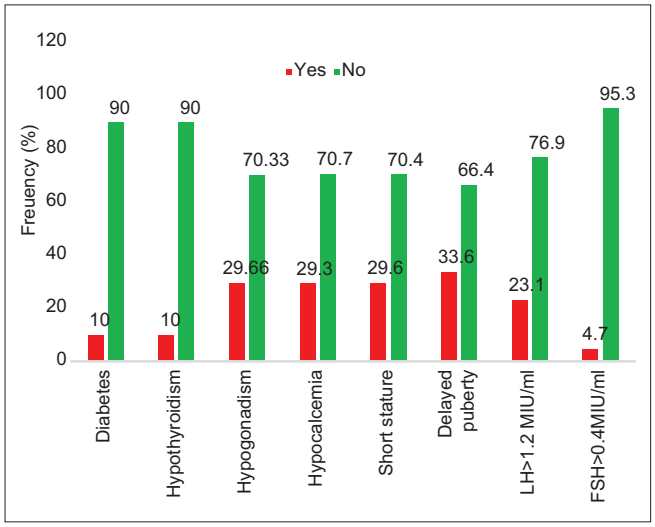

Figure 1: Distribution of the factors in the β-thalassemia patients.

In the multiple logistic regression model, short stature was negatively associated with weight (adjusted odds ratio [OR] = 0.92, 95% confidence interval [CI]: [0.87, 0.97]) and positively associated with ferritin level (OR = 1.41, 95% CI: [1.2, 1.61]) and disease duration (OR = 1.008, 95% CI: [1.002, 1.016]) compared to the normal height group [Table 2]. No significant associations were detected between short stature and other factors [Table 2].

Furthermore, failure of puberty was positively associated with ferritin level (OR = 1.27, 95% CI: [1.09, 1.48]) and gender compared to the mature group. Patients with higher ferritin level were more likely to have delayed puberty where 100-unit increase in ferritin level increased the likelihood of delayed puberty by 2.5%. Likewise, women were found to be 2.44 times more likely to have pubertal delay than men (P = 0.033, 95% CI: [1.07, 5.55]) [Table 2]. Associations

Table 1: Factors associated with endocrine disease based on multilevel logistic regression model

Factor[a]	*B*	SE	Wald	*P*	OR	95% CI for OR	
Hypocalcemia							
Ferritin	0.029	0.008	11.665	0.001	1.29	1.01	1.04
Disease duration	0.071	0.029	6.171	0.013	1.074	1.015	1.136
Short stature							
Ferritin	0.04	0.01	15.33	<0.0001	1.041	1.02	1.06
Disease duration	0.115	0.04	7.78	0.005	1.12	1.03	1.21
Pubertal delay							
Ferritin	0.025	0.008	9.65	0.002	1.026	1.009	1.042
Disease duration	0.054	0.036	2.3	0.129	1.055	0.984	1.13
Diabetes							
Ferritin	0.0001	0.01	0.001	0.97	1	0.98	1.02
Disease duration	0.189	0.05	12.55	<0.0001	1.208	1.08	1.34

[a]Categories (yes/no), Ferritin level is based on per 100 unit. SE: Standard error, OR: Odds ratio, CI: Confidence interval

Table 2: Receiver operating characteristic curve analysis of ferritin level and disease duration in classifying endocrine disorder

	Estimate criterion	AUC	Sensitivity	Specificity	*P*
Hypocalcaemia					
Ferritin	>4921	0.66	59.9	90.1	0.004
Disease duration (year)	>19.83	0.71	78.05	62.59	<0.0001
Short stature					
Ferritin	>3431	0.749	59.46	82.52	<0.0001
Disease duration	>24	0.651	66.76	71.84	0.005
Pubertal delay					
Ferritin	>2100	0.782	95	56.2	0.004
Disease duration	>26	0.597	36.59	87.01	0.097
Diabetes					
Disease duration (year)	>20	0.806	100	55.56	<0.0001
IGF-1					
Ferritin	>2223	0.628	70.33	57.1	0.0091
Disease duration (year)	>14.5	0.672	80.22	48.07	0.0003

IGF-1: Insulin-like grow factor-1, AUC: Area under the ROC curve, ROC: Receiver operating characteristic

between pubertal delay and other variables were not significant.

Disease duration and age at onset had direct and reverse associations with developing diabetes, respectively. A year increase in disease duration doubles the likelihood of diabetes. Increase of a month in the age of β-thalassemia onset results in the decreased odds of diabetes by 16% [Table 2].

The median (IQR) serum insulin-like growth factor 1 (IGF-1) level for age <18 years was 92 (78) ng/ml and for age >18 years was 50.8 ng/ml (52.7). The ferritin level in low IGF-I group was significantly higher than normal IGF-1 group ($P = 0.008$).

The results of ROC curve are shown in Table 2, where the cut point of >2100 μg/L for ferritin level is a threshold for discriminating normal from delayed puberty patients; however, low specificity may falsely rule out healthy individuals as delayed puberty [Figure 2]. Regarding fixed sensitivity of 95%, the ferritin level of >1040 μg/L, >1232 μg/L, >1500 μg/L and >1480 μg/L are estimated for hypocalcaemia, short stature, pubertal delay and lower growth factor independence 1, respectively.

Furthermore, at a sensitivity of 95%, the disease duration of >14 years, >9.5 years and >13.3 years were estimated for hypocalcaemia, short stature and pubertal delay, respectively.

There was no significant differences in the AUC across 5 years ferritin for hypocalcaemia ($AUC_{min} = 0.653$, $P > 0.05$), short stature ($AUC_{min} = 0.745$, $P > 0.05$) and delayed puberty ($AUC_{min} = 0.502$, $P > 0.05$).

Discussion

In the studied population, most patients were afflicted with at least one endocrine disorder where pubertal delay was the most common. The results also suggest a significant association between ferritin level and hypocalcaemia, pubertal, hypothyroid, IGF-1 status and short stature in β-thalassemia patients who received chelation therapy.

The prevalence of endocrinopathy in our study was higher than previous reports from different parts of Iran as it was 73.8% in Birjand[7] and 60% in Yazd provinces[15] and the rates in other parts of the world with prevalence range varying from 32% to 100%.[5,9,16] The prevalence of endocrine disorders was higher in older patients.

Pubertal delay was the most frequent hypogonadotropic hypogonadism complication in our study, followed by short stature and hypocalcaemia. Hypogonadism affected 70%–80% of thalassemia major patients that usually are detected during puberty.[13]

The rate in our study was lower than rates reported on Iranian patients, in which, delayed puberty reported to be 69% and 47% at Tehran and Zahedan provinces, respectively.[17,18] Furthermore, pubertal complications are reported to be 25% by Altıncık and Akin[19] and 79% by Casale *et al*.[20] Furthermore, short stature was also present in 52%,[21] 30%[22] and 33.8%[23] of Iranian patients.

We observed a lower rate of hypocalcaemia than rates reported by Najafipour *et al*. (41%).[21] However, hypocalcaemia is reported to be 22% by Mirhosseini *et al*.[22] and 7.8% by Saffari *et al*.[23] This discrepancy could be explained by the distribution of age, Vitamin D levels and type of chelation therapy where high rates of Vitamin D deficiency (60%) have been reported in that population.[24] As it is evident from the ROC analysis, supplementation with Vitamin D along with calcitriol treatment is essential at the second decade of treatment and ferritin level >1000 μg/L.

The lower rate of diabetes and hypothyroidism in our study compares favourably with data from other studies, in which other studies reported hypothyroidism to be 0%–18%.[3] The rate of diabetes reported ranges from 6.4% to 14.1%.[3,17] In agreement with other studies, there was a significant association between ferritin levels and diabetes in our study, while the odds of diabetes was 3.5 times more likely in patients with ferritin >2500 μg/L compared to patients with lower levels of ferritin.[25] Studies show the early occurrence of insulin resistance followed by iron overload in the pancreas is related to developing diabetes;[26] it seems that adequate management of ferritin at early ages in our patients may prevent the development of diabetes.

Similar to results from previous reports, we observed a statistically significant association between endocrine disorders and both serum ferritin level and disease duration.[7,10,13,16] The association between disease duration and ferritin in our study was reasonable, which confirms non-constant variance with funnel shape [Figure 3].

Variability of serum ferritin at early ages (<18 years) was lower compared to higher age groups that indicates ferritin controlling methods were not adequate in older patients. A similar pattern was observed between disease duration with serum calcium and parathyroid hormone (PTH); however, association between disease duration and IGF-1 was inverse. Ferritin level and age/disease duration indicated as important

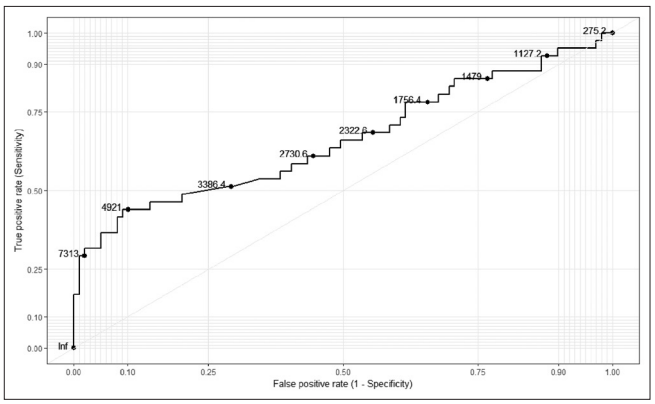

Figure 2: Receiver operating characteristic curve of ferritin level for hypocalcemia.

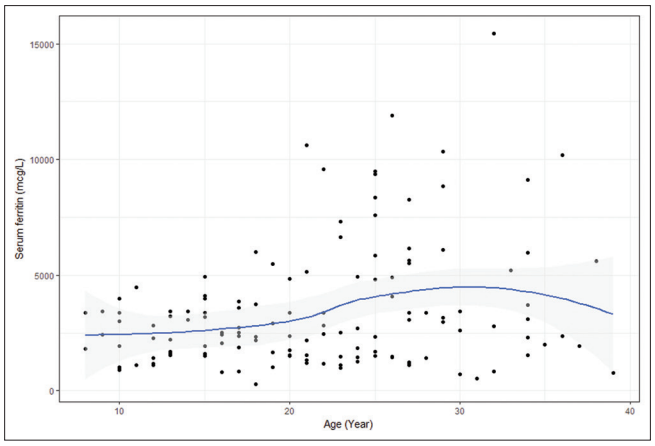

Figure 3: Distribution of ferritin values across age of patients with B-thalassemia major.

measures for monitoring chelation therapy, where a better compliance to chelation therapy was observed in patients with aged <18 years.[16] This suggests that it is essential to revise the supplementary drugs also treatment methods proportionally to change trends of ferritin and disease duration.

There was a contradiction on the best cut point for ferritin. The estimated threshold of ferritin with reasonable sensitivity was 1000–1500 ng/mL. This is the claimed value in some studies and different from reported threshold form some other studies.[5,9,14,27] Ferritin level alone is not an accurate measure of iron load.[28] In addition, initiating chelation therapy at lower ages significantly reduces endocrine disorders.[29] These results suggest that both age and disease duration along with the level of ferritin should be considered in the time of chelation therapy initiation to reduce endocrinopathy disorders. In our study, the serum calcium was negatively associated with age and serum ferritin. Besides serum calcium, PTH, 1, 25-dihydroxyvitamin D_3, calcitonin, growth hormone, oestrogen and fibroblast growth factor-23 have shown to have an effective role in calcium absorption,[30] which are common disorders in β-thalassemia patients.

According to ROC analysis results, the ferritin level was a generally better indicator for diagnosis of positive endocrine than the negative endocrine.

Our study is not without limitations, the main limitation of our study is the non-longitudinal assessment of factors and complications. Regarding the nature of disease duration, point measurements are not without bias, many variables such as blood factors that are time dependent; in addition, lack of information is disadvantage of this studies, so, longitudinal studied through clinical trials recommended to minimise these biases to detect the precise associations amongst factors and trend of disorders across periods.

Conclusions

Management of serum ferritin may be inadequate for young adult thalassemia major patients, and remedial methods are necessary to prevent the development of delayed puberty, short stature and hypocalcaemia in this population. It can be concluded that ferritin level <1500 mg/dl and early years of the second decade of illness are important thresholds. It seems that using drugs with acceptable long-term efficacy and safety is required to control the ferritin level in β-thalassemia patients.

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Conflicts of interest

There are no conflicts of interest.

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