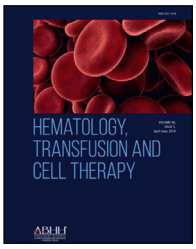
HEMATOLOGY, TRANSFUSION AND CELL THERAPY

www.htct.com.br



Original article

Prevalence of hypogonadism in transfusion-dependent β-thalassemia patients of Bangladesh: investigating the role of serum ferritin level as a diagnostic tool

Romana Chowdhury [a], *Mohammad Azmain Iktidar* [b,c,f,*], *Mushfiq Newaz Ahmed* [d], *Mohammad Mehedi Hasan* [e], *Md. Mazharul Hoque Tapan* [f], *Sheikh Saiful Islam Shaheen* [e], *Atiar Rahman* [e], *Ayesha Khatun* [e]

[a] *Sirajul Islam Medical College, Dhaka, Bangladesh*
[b] *North South University, Dhaka, Bangladesh*
[c] *Public Health Professional Development Society (PPDS), Dhaka, Bangladesh*
[d] *National Institute of Diseases of the Chest and Hospital (NIDCH), Dhaka, Bangladesh*
[e] *Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh*
[f] *Director General Health Services (DGHS), Bangladesh*

ARTICLE INFO

Article history:
Received 26 January 2022
Accepted 29 June 2022
Available online 11 August 2022

Keywords:
Beta-thalassemia major
Gonadal function
Hypogonadotropic hypogonadism
Hypergonadotropic hypogonadism
Normogonadotropic hypogonadism

ABSTRACT

Introduction: Hypogonadism is one of the most frequent complications in transfusion-dependent thalassemia patients and early recognition and treatment is the core element in restoring impaired gonadal function. Despite the high burden of disease, relevant studies are scarcely addressing the gonadal function of such patients in Bangladesh. The pattern of gonadal function in transfusion-dependent thalassemia patients must be characterized before planning a generalized management plan. Moreover, since iron overload is a key reason behind hypogonadism in thalassemia patients, investigating the role of serum ferritin level as a diagnostic tool for hypongadism was also an aim of this study.

Methods: This cross-sectional study was conducted at the Department of Transfusion Medicine of the Bangabandhu Sheikh Mujib Medical University. According to the inclusion and exclusion criteria, a total of 94 patients were enrolled in this study. A detailed history and thorough clinical examination were carried out in each patient and recorded using a pre-tested structured questionnaire. In addition, the laboratory assessment of serum ferritin, luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone and estradiol in serum were also performed. The data were analyzed using the STATA (v.16).

Results: The mean age of the patients with transfusion-dependent thalassemia was 18.81 ± 4.65 (SD), with 53.3% of the patients being male. The overall prevalence of hypogonadism was 35.11%, 18.1% being normogonadotropic, 11.7% being hypogonadotropic and 5.3% being hypergonadotropic. The serum ferritin level was significantly higher ($p < 0.001$) in patients with hypogonadism (Eugonadal: 2,174.79 (± 749.12) ng/ml; Hypogonadal: 3,572.59 (±

* Corresponding author at: Department of Public Health, North South University, Plot # 15, Block # B, Bashundhara R/A, Dhaka, 1229, Bangladesh.
E-mail address: sazmain@gmail.com (M.A. Iktidar).

https://doi.org/10.1016/j.htct.2022.06.010

2531-1379/© 2022 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1,199.49) ng/ml). The area under the receiver operating characteristic (ROC) curve of serum ferritin was high (0.83) and the p-value was highly significant (< 0.001).

Conclusion: Therefore, the serum ferritin level and gonadal hormone analysis of transfusion-dependent thalassemia patients can be considered a screening tool for assessing gonadal function and early detection and prevention of hypogonadism.

© 2022 Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Thalassemia is one of the most common genetic hemoglobinopathies that can result in severe anemia.[1,2] It is highly prevalent in Southeast Asia, the Indian subcontinent and Mediterranean and Middle Eastern countries, collectively known as the 'World Thalassemia Belt'. In Bangladesh, 6 to 12% of the population are carriers of a gene causing thalassemia.[3] Thalassemia is characterized by the partial or complete deficiency in the synthesis of α or β-globin chains that compose the major adult hemoglobin ($\alpha_2\beta_2$). Patients with an absolute deficiency of the α or β-globin chains require lifelong blood transfusion and are denoted as the transfusion-dependent thalassemia group.[4] With regular transfusion, the life expectancy and survival rate of thalassemia patients dramatically improved from the first to fifth decades of life.[5] However, it leads to iron overload, which is accumulated within different tissues, including endocrine glands, resulting in a functional imbalance, among which gonadal dysfunction is the most common.[6,7] In addition to the iron overload, factors, such as ferritin level, genotype, transfusion frequency, starting age and iron chelation efficiency, also play a significant role.[8] Hypogonadotropic hypogonadism, or secondary hypogonadism resulting from iron deposition in the pituitary gonadotrope, is more commonly found, whereas gonadal iron deposition in ovaries or testes occurs less frequently.[9]

In female patients, gonadal hormones, such as the luteinizing hormone (LH), estradiol, follicle-stimulating hormone (FSH), anti-mullerian hormone (AMH) and prolactin, are lower in transfusion-dependent thalassemia patients.[7,10] Nearly half of these patients show a low to undetectable LH/FSH ratio.[11] As a result, amenorrhea, anovulation and infertility are commonly found in adults. In younger females, the low gonadal function manifests as delayed puberty, delayed menarche or primary amenorrhea and short stature.[12] In male patients, testicular functions are seen to be reduced, evidenced by the high anti-mullerian hormone and low testosterone levels.[13] Lower sperm concentrations, a lower percentage of sperm with normal morphology, sperm DNA damage and reduced testis volume are also seen in transfusion-dependent thalassemia patients.[14] The adult male clinical features are lack of facial and body hair, decreased muscle mass and appearance of fine facial wrinkles, gynecomastia, diminished libido, fatigue and ejaculatory dysfunction.[15,16]

Early assessment of gonadal dysfunction and its prompt treatment by chelation therapy may reduce its incidence, improve the quality of life in already burdened thalassemic patients and prevent further complications.[17] Despite the high prevalence of thalassemia in our context, baseline information on gonadal function is not well addressed. Therefore, this study aimed to find the status of gonadal function in transfusion-dependent thalassemia patients and assess the role of the serum ferritin level in predicting hypogonadism.

Methods

Study design, site & duration

This cross-sectional study was conducted from June 2020 to June 2021. Department of Transfusion Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, was selected as the study site, as it is located in the capital and receives patients from all over the country.

Study participants

β-thalassemia patients diagnosed by hemoglobin electrophoresis, who were dependent on transfusion and providing consent, were included in the study. The β-thalassemia patients with other chronic illnesses that can cause gonadal dysfunction (*e.g.*, connective tissue disease), under hormonal replacement therapy or taking an oral contraceptive pill (OCP), with congenital gonadal malformations, taking a regular antipsychotic, antidepressant and other drugs that hamper gonadal function, were excluded from the study. A total of 400 transfusion-dependent β-thalassemia patients, who visited the Department of Transfusion Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, during the study period, were approached for enrolment. Among these, 277 were excluded for meeting one or more of the exclusion criteria and 29 were excluded as they/their guardian did not provide consent to enter the study (Figure 1). Finally, a total of 94 patients having an average transfusion frequeny of 1 unit per month, of whom where 80 were β-thalassemia major (β^0/β^0)(85%) and 14, severe HbE/β-thalassemia (β^0/HbE)(15%), were included in the study.

Data collection

The data were collected by face-to-face interviews of patients/guardians using a pretested structured questionnaire. Background information, previous medical records, physical findings and laboratory reports were also assessed. Secondary sexual characteristics were assessed using the criteria proposed by Tanner.[18] Patients in the pre-pubescent stage (stage 1 or stage 2) were classified as sexually undeveloped

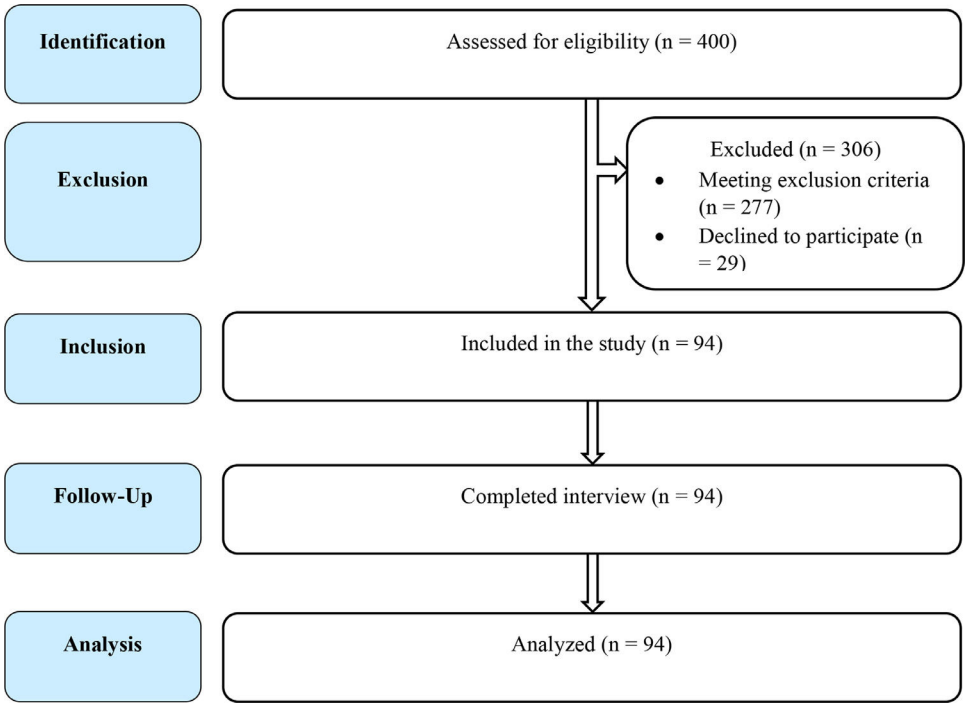


Figure 1 – STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) flow chart of study participants.

according to the Tanner staging. Patients in the pubescent (stage 3) or post-pubescent (stages 4 and 5) stages, on the other hand, were deemed sexually developed.[19] The gonadal function of the patient was evaluated by history, physical examination and laboratory tests, including the serum luteinizing hormone (LH), serum follicle-stimulating hormone (FSH), serum testosterone for males and serum estradiol for females. The serum ferritin level was also measured to assess the iron overload status of the patients. Blood samples (3 - 5 ml) were collected from the patients, put into labelled test tubes and left for clotting, with all available aseptic precautions. The serum was then separated by centrifugation at 6,000 rpm after the blood had clotted. The assessment of hormone and ferritin levels was performed in the Department of Biochemistry and Microbiology, BSMMU, by the VEG-4000 Fully Automated ELISA Processor (manufactured by ESSE 3 Medical Equipment, Italy).

Statistical analysis

After the data collection, the data were checked for errors and analyzed using the STATA (version 16). Continuous variables were presented as mean and standard deviation and categorical variables were presented as frequency and relative percentage. In addition, Pearson's chi-square test was performed to explore a bivariate relationship. A two tailed *p*-value of < 0.05 is considered statistically significant and all the reporting is performed according to the STROBE guidelines.[20]

Ethics

This study was approved by the Institutional Review Board of the BSMMU. The 1964 Declaration of Helsinki and later modifications and comparable ethical standards were followed, whenever feasible. Informed consent has been obtained from each participant/guardian.

Results

The mean age of the participants was 18.81 ± 4.65 years, with the maximum being between 13 to 18 years of age (60.6%). There was a male (53.2%) preponderance, with a male-to-female ratio of 1.14:1. Most of the patients were from a rural area (57.4%) (Table 1).

The most common features of sexual underdevelopoment among males were long downy pubic hair (22%) (Tanner stage

Table 1 – Background information of study participants (n = 94).

Characteristics	Entire Study Cohort (N=94)	
Age (in years), mean ± SD	18.81 ± 4.65	
13 to 18	57	(60.64)
19 to 24	24	(25.53)
25 to 30	13	(13.83)
Gender		
Male	50	(53.19)
Female	44	(46.81)
Residence		
Rural	27	(54)
Urban	23	(46)

Values are expressed as n(%), unless otherwise mentioned.
Abbreviations: SD, standard deviation.

Table 2 – Secondary sexual characteristics of study participants according to Tanner Staging (n = 94).

Tanner Stage	Male (n = 50)				Female (n = 44)			
	External Genitalia		Pubic Hair Growth		Breast Growth		Pubic Hair Growth	
I	5	(10%)	4	(8%)	3	(6.82%)	2	(4.55%)
II	11	(22%)	10	(20%)	11	(25%)	10	(22.73%)
III	2	(4%)	3	(6%)	1	(2.27%)	3	(6.82%)
IV	24	(48%)	21	(42%)	22	(50%)	22	(50%)
V	8	(16%)	12	(24%)	7	(15.91%)	7	(15.91%)

Values are expressed as n (%).

II) and testes 2.5 to 3.2 cm (Tanner stage II). In the case of females, the prominent features of sexual underdevelopment were long downy pubic hair (10%) (Tanner stage II) and breast-budding (25%) (Tanner stage II) (Table 2).

Among the 94 patients, the mean serum FSH of all patients was 4.52 ± 2.83 IU/L, of which the majority (89.36%) had a normal level of FSH and only 8.51% of the patients had an FSH below the normal level. Among the males, 4% had an FSH above the normal level, while none were below. Among females, 18.18% had an FSH below normal and none, above (Table 3). The mean serum LH of all the patients was 3.93 ± 2.82 IU/L, 11.70% having below the normal level of LH and the rest being within and above the normal. Among the males, 22% had an LH level above normal and 10%, below normal. Among the females, 13.64% had an LH below normal, while none were above normal. Among all the male patients, the mean serum testosterone was 390.29 ± 287.93 ng/dL, of which the majority (64%) had a normal testosterone level of and the rest (36%), below normal (Table 3). The mean serum estradiol of female patients was 85.05 ± 153.47 pg/mL, of which most (52.27%) had a normal level. Among the rest, 13.64% had above the normal level of estradiol and 34.09 %, below normal.

Table 3 – Laboratory findings for gonadal profile in study participants (n = 94).

Hormones	Male n = 50	Female n = 44	Total n = 94
S. FSH (IU/L), mean ± SD	4.58 ± 3.34	4.44 ± 2.16	4.52 ± 2.83
Below normal	0 (0)	8 (18.18)	8 (8.51)
Within normal	48 (96)	36 (81.82)	84 (89.36)
Above normal	2 (4)	0 (0)	2 (2.13)
S. LH (IU/L), mean ± SD	4.19 ± 3.4	3.63 ± 1.97	3.93 ± 2.82
Below normal	5 (10)	6 (13.64)	11 (11.70)
Within normal	34 (68)	38 (86.36)	72 (76.60)
Above normal	11 (22)	0 (0)	11 (11.70)
S. Testosterone (ng/dL), mean ± SD	390.29 ± 287.93	-	-
Below normal	18 (36)	-	-
Within normal	32 (64)	-	-
Above normal	0 (0)	-	-
S. Estradiol (pg/mL), mean ± SD	-	85.05 ± 153.47	-
Below normal	-	15 (34.09)	-
Within normal	-	23 (52.27)	-
Above normal	-	6 (13.64)	-

Values are expressed as n (%), unless otherwise mentioned.
Abbreviations: S., serum; SD, standard deviation; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

Hypogonadism was found in 33 patients (35.11%), afflicting 36% of all male and 34% of all female patients. Normogonadotropic hypogonadism, in 17 patients (18%), was the most prevalent type, followed by hypogonadotropic hypogonadism in 11 (12%) and hypergonadotropic hypogonadism in 5 (5%). The majority of the male patients with hypogonadism had the normogonadotropic type, found in 10 patients (20%); in contrast, the majority of the female patients with hypogonadism, 8 in all (18%), had the hypogonadotropic type. There was a significant difference in the distribution of the types of hypogonadism between males and females ($p = 0.023$) (Figure 2).

The mean serum ferritin level among the thalassemia patients was 2,665.51 (± 1,143.25) ng/ml. The serumferritin level was significantly higher ($p < 0.001$) in patients with hypogonadism (Eugonadal: 2,174.79 (±749.12) ng/ml; Hypogonadal: 3,572.59 (± 1,199.49) ng/ml) (Figure 3).

Using receiver operating characteristic (ROC) curve analysis, a serum ferritin of 2,757 ng/ml was found to be the best threshold (Sensitivity = 69.70% and Specificity = 72.13%) for discriminating the presence of hypogonadism (Figure 4). The area under the curve (AUC) was high (0.83) and the *p*-value was highly significant (< 0.001). Both lower and upper bound areas were also above the area of 0.5, indicating that serum ferritin level could accurately predict hypogonadism.

In the multiple logistic regression model, after adjusting for age and gender, patients with a serum ferritin level ≥ 2,757 ng/ml were eight times (AOR: 7.90, 95% CI: 2.63 to 23.69, $p < 0.001$) more at risk of developing hypogonadism, compared to those with a serum ferritin level < 2,757 ng/ml (Table 4).

Discussion

In our study, the overall prevalence of hypogonadism was 35.11%, 18.1% being normogonadotropic, 11.7% being hypogonadotropic and 5.3% being hypergonadotropic. In a study among 21 male patients with transfusion-dependent beta-thalassemia (TDT), the prevalence of hypogonadotropic hypogonadism was 33.3%, which is consistent with our study.[21] Furthermore, a cohort study in Taiwan among 454 TDT major patients showed a lower prevalence of hypogonadism (23.1%).[22] Another study carried out by Daraghmeh et al., in the thalassemia ward at the AL-Wattani hospital in Nablus

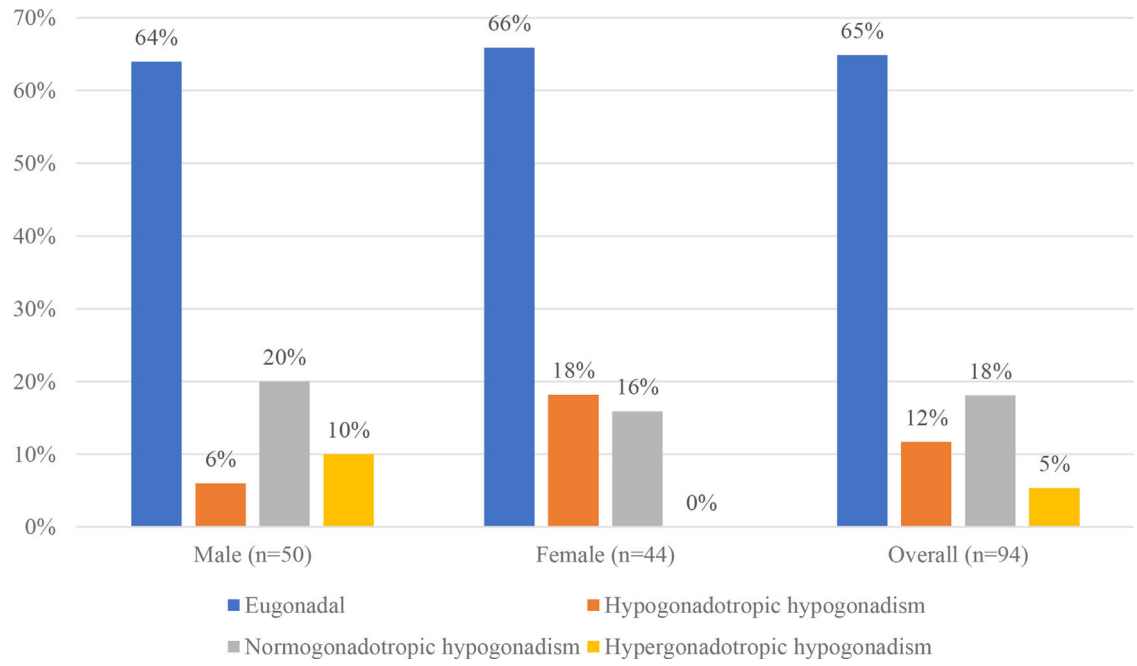


Figure 2 – Status of gonadal function among the study participants.
Pearson's chi-square test revealed significant difference in distribution of types of hypogonadism between males and females (p = 0.023).

among 75 TDT patients, found that hypogonadism was prevalent among 46.7% of the tested patients, including both primary and secondary hypogonadism.[23] However, the prevalence of hypogonadism in this study population was lower than some published reports,[7,24–27] all of which have reported hypogonadism as the most frequent endocrinopathy among TDT patients from different countries. The availability of iron chelation therapy can explain the lower prevalence of hypogonadism in our study, compared to other studies. A study on 382 TDT patients treated with desferrioxamine at the Thalassemia Centre in Dubai showed a significantly lower prevalence of hypogonadism of only 25%.[28] Moreover, chelation therapy with desferrioxamine before puberty has helped patients attain normal sexual maturation in some studies. In a study of 40 patients with TDT, 90% of 19 patients who began treatment with desferrioxamine before the age of 10 years had normal sexual development, compared to only 38% of those treated after the age of 10 years.[29] However, results of a prospective study in patients with thalassemia and secondary amenorrhea suggest that the damage to the hypothalamus is

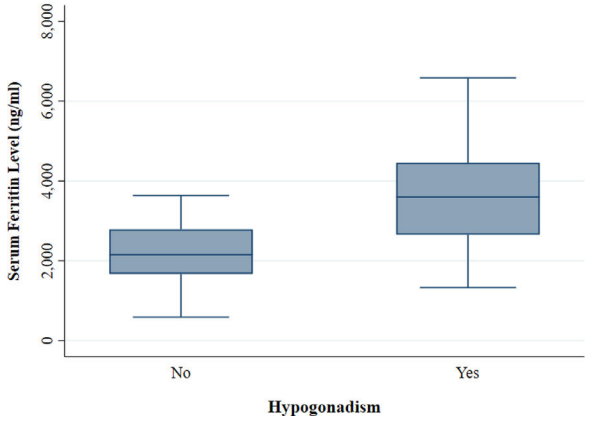


Figure 3 – Comparison of serum ferritin level in thalassemia patients with or without hypogonadism. Patients with hypogonadism had a significantly ($p < 0.001$) higher level of serum ferritin.

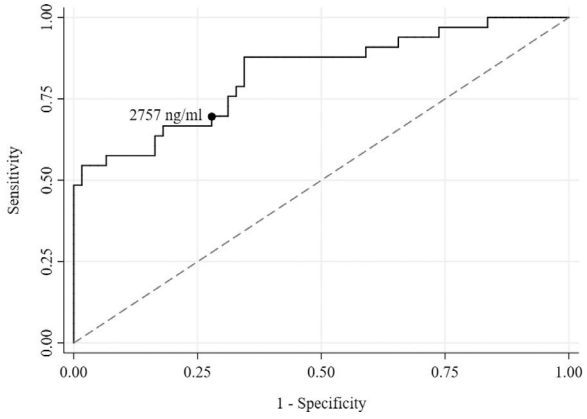


Figure 4 – Receiver operating characteristic (ROC) curve for serum ferritin level.

Table 4 – Multiple logistic regression results for the factors associated with hypogonadism in thalassemia patients.

Variables	AOR	p-value	95% CI		
Age, years	0.70	0.001	0.58	to	0.86
Gender					
Female	Reference				
Male	2.50	0.125	0.77	to	8.12
Serum Ferritin Level (ng/ml)					
< 2,757 ng/ml	Reference				
≥ 2,757 ng/ml	7.90	< 0.001	2.63	to	23.69

AOR, adjusted odds ratio; CI, confidence interval.

progressive, despite regular transfusion and chelation therapy.[30] These results suggest that the development of hypogonadotropic hypogonadism might be caused by early and progressive damage due to iron loading. We believe that these could be attributed to the possible progressive toxic effect of iron-induced free radicals and/or to some other undefined risk factors involved in the development of hypogonadotropic hypogonadism.[31]

There was no statistically significant relationship between gender and hypogonadism in our study. This result was similar to the studies carried out in Iran and Palestine.[32,33] However, this result was different from the results of a study by Dumaidi et al., which stated that a higher percentage of hypogonadism in males indicates that males are more prone to hypogonadism than females.[27]

In the current study, serum testosterone, estradiol, LH and FSH were lower among 36%, 34.09%, 11.70% and 8.51% of the patients, respectively. The most common symptom of gonadal dysfunction among males was the lack of axillary and pubic hair (6%) (Tanner stage II) and among females, slow or absent breast growth, hot flashes and amenorrhea (6.82% each). Regarding the menstrual status, adult females presented hypogonadism in the form of total absence of spontaneous menarche (primary amenorrhea); this applied to 29% of female participants with TDT, as confirmed by the low level of estradiol. In contrast, the other form is the lack or irregular menses (secondary amenorrhea), found in 42% of female participants with TDT. In males, hypogonadism was in the form of sexual infantilism; this applied to 72% of male participants with TDT, which agrees with contemporary studies.[34,35] Several other studies also revealed that, in the thalassemic group, the baseline and peak levels, after the GnRH test, of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and estradiol, were significantly lower than those in the control group.[36,37] However, the discrepancy in the gonadotropin hormone level and gonadal dysfunction symptoms among males and females, compared to other studies, might be explained by ethnic factors, different availability of therapeutic agents and economic status.[7]

The transfusion-dependent beta-thalassemia (TDT) most often requires regular red blood cell transfusions, which start within the first year of life.[38] The predilection for iron deposition in the pituitary gland and hypothalamus among these patients frequently causes a lack of sexual maturation and loss of gonadal function. Telfer et al. showed that the serum ferritin level is a relevant marker for the evaluation of iron overload.[39] We found that hypogonadal patients had a significantly higher mean serum ferritin level, compared to eugonadal patients (Eugonadal: 2,174.79 (± 749.12) ng/ml; Hypogonadal: 3,572.59 (± 1,199.49) ng/ml). Patients with ≥ 2,757 ng/ml of serum ferritin level were almost eight times (AOR: 7.90, 95% CI: 2.63 to 23.69, $p < 0.001$) more at risk of developing hypogonadism, compared to those with a serum ferritin level < 2,757 ng/ml. This association between hypogonadism and serum ferritin level conforms to the existing evidence.[40,41]

Conclusions

In our study, approximately one-third of the TDT patients were found to be suffering from different hypogonadisms. Hypogonadal patients had a significantly higher serum ferritin level and the serum ferritin level was highly accurate in predicting hypogonadism. Therefore, the serum ferritin level, along with gonadal hormone analysis, of TDT patients can be considered a screening tool for assessing gonadal function and early detection and prevention of hypogonadism. However, further prospective studies with a larger sample size might be necessary to provide better recommendations for patients with TDT.

Conflicts of interest

None.

Acknowledgment

We want to thank all the patients who participated in this study.

REFERENCES

1. Ansari S, Kiumarsi A, Azarkeivan A, Allameh MM, Amir kashani D, Razaghi Azar M. Fertility assessment in thalassemic men. Thalassemia Rep. 2017;7(6362):21–4. https://doi.org/10.4081/thal.2017.6362.
2. Mensi L, Borroni R, Reschini M, Cassinerio E, Vegetti W, Baldini M, et al. Oocyte quality in women with thalassaemia major: insights from IVF cycles. Eur J Obstet Gynecol Reprod Biol X. 2019;3:100048.

3. Hossain MS, Hasan MM, Raheem E, Islam MS, Al Mosabbir A, Petrou M, et al. Lack of knowledge and misperceptions about thalassaemia among college students in Bangladesh: a cross-sectional baseline study. Orphanet J Rare Dis. 2020;15(1):54. https://doi.org/10.1186/s13023-020-1323-y.
4. Taher AT, Weatherall DJ, Cappellini MD. Thalassaemia. Lancet. 2018;391(10116):155–67. https://doi.org/10.1016/S0140-6736(17)31822-6.
5. Cao A, Moi P, Galanello R. Recent advances in β-thalassemias. Pediatr Rep. 2011;3(2):e17. https://doi.org/10.4081/pr.2011.e17.
6. Al-Zuhairy TNAHS, Al-Ali ZAJR. Evaluation of reproductive hormones in patients with β-thalassemia major in Misan province, Iraq. Medico-Legal Update. 2020;20(2):274–9.
7. Albu AI, Albu D. Hypogonadism in female patients with beta thalassemia major. Thalassemia and Other Hemolytic Anemias; 2018. https://doi.org/10.5772/intechopen.73862 Published online.
8. De Sanctis V, Soliman AT, Elsedfy H, Di Maio S, Canatan D, Soliman N, et al. Gonadal dysfunction in adult male patients with thalassemia major: an update for clinicians caring for thalassemia. Expert Rev Hematol. 2017;10(12):1095–106. https://doi.org/10.1080/17474086.2017.1398080.
9. Srisukh S, Ongphiphadhanakul B, Bunnag P. Hypogonadism in thalassemia major patients. J Clin Transl Endocrinol. 2016;5:42–5. https://doi.org/10.1016/j.jcte.2016.08.001.
10. Dumaidi K, Al-Jawabreh A, Al-Assi S, Karmi B. Assessment of gonadal and thyroid function for adult transfusion- dependent- β- thalassemic patients in Palestine. Jordan Med J. 2015;49(1):17–26. https://doi.org/10.12816/0025095.
11. Singer ST, Sweeters N, Vega O, Higa A, Vichinsky E, Cedars M. Fertility potential in thalassemia major women: current findings and future diagnostic tools. Ann N Y Acad Sci. 2010;1202(1):226–30. https://doi.org/10.1111/j.1749-6632.2010.05583.x.
12. De Sanctis V, Elsedfy H. Clinical and biochemical data of adult thalassemia major patients (TM) with Multiple Endocrine Complications (MEC) versus TM patients with normal endocrine functions: a long-term retrospective study (40 years) in a Tertiary Care Center in Italy. Mediterr J Hematol Infect Dis. 2016;8(1):e2016022. https://doi.org/10.4084/mjhid.2016.022.
13. Siripunthana S, Sahakitrungruang T, Wacharasindhu S, Sosothikul D, Supornsilchai V. Testicular function in patients with regular blood transfusion for thalassemia major. Asian Biomed. 2015;9(2):185–91. https://doi.org/10.5372/1905-7415.0902.385.
14. Chen MJ, Peng SS, Lu MY, Yang YL, Jou ST, Chang HH, et al. Effect of iron overload on impaired fertility in male patients with transfusion-dependent beta-thalassemia. Pediatr Res. 2018;83(3):655–61. https://doi.org/10.1038/pr.2017.296.
15. Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, Swerdloff RS, et al. Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2010;95(6):2536–59. https://doi.org/10.1210/jc.2009-2354.
16. De Sanctis V, Elsedfy H. Clinical and biochemical data of adult thalassemia major patients (TM) with multiple endocrine complications (MEC) versus TM patients with normal endocrine functions: a long-term retrospective study (40 years) in a tertiary care center in Italy. Mediterr J Hematol Infect Dis. 2016;8(1):e2016022. https://doi.org/10.4084/mjhid.2016.022.
17. De Sanctis V, Soliman AT, Elsedfy H, Di Maio S, Canatan D, Soliman N, et al. Gonadal dysfunction in adult male patients with thalassemia major: an update for clinicians caring for thalassemia. Expert Rev Hematol. 2017;10(12):1095–106. https://doi.org/10.1080/17474086.2017.1398080.
18. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child. 1976;51(3):170–9. https://doi.org/10.1136/ADC.51.3.170.
19. Shahid Z, Hassan S, Ghazanfar S, Kaneez M, Khan MS, Tariq HT, et al. Investigating the role of ferritin in determining sexual underdevelopment in beta-thalassemia major patients: a cross-sectional analysis from Pakistan. Cureus. 2021;13(6):e15572. https://doi.org/10.7759/CUREUS.15572.
20. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet. 2007;370(9596):1453–7. https://doi.org/10.1016/S0140-6736(07)61602-X.
21. Chen MJ, Peng SS, Lu MY, Yang YL, Jou ST, Chang HH, et al. Effect of iron overload on impaired fertility in male patients with transfusion-dependent beta-thalassemia. Pediatr Res. 2018;83(3):655–61. https://doi.org/10.1038/pr.2017.296.
22. Wu HP, Lin CL, Chang YC, Wu KH, Lei RL, Peng CT, et al. Survival and complication rates in patients with thalassemia major in Taiwan. Pediatr Blood Cancer. 2017;64(1):135–8.
23. Daraghmeh NM. Management and complications of thalassemic patients in Palestine: retrospective study. Published online 2016.
24. Habeb AM, Al-Hawsawi ZM, Morsy MM, Al-Harbi AM, Osilan AS, Al-Magamsi MS, et al. Endocrinopathies in beta-thalassemia major. Prevalence, risk factors, and age at diagnosis in Northwest Saudi Arabia. Saudi Med J. 2013;34(1):67–73.
25. Ahmed E, Shaheen M. Prevalence of hypogonadism in thalassemia major patients in Gaza strip. Published online 2019.
26. Belhoul KM, Bakir ML, Saned MS, Kadhim AM, Musallam KM, Taher AT. Serum ferritin levels and endocrinopathy in medically treated patients with β thalassemia major. Ann Hematol. 2012;91(7):1107–14.
27. Dumaidi K, Al-Jawabreh A, Al-Assi S, Karmi B. Assessment of gonadal and thyroid function for adult transfusion- dependent- β- thalassemic patients in Palestine. Jordan Med J. 2015;49(1):17–26. https://doi.org/10.12816/0025095.
28. Belhoul KM, Bakir ML, Saned MS, Kadhim AM, Musallam KM, Taher AT. Serum ferritin levels and endocrinopathy in medically treated patients with β thalassemia major. Ann Hematol. 2012;91(7):1107–14.
29. Srisukh S, Ongphiphadhanakul B, Bunnag P. Hypogonadism in thalassemia major patients. J Clin Transl Endocrinol. 2016;5:42–5. https://doi.org/10.1016/j.jcte.2016.08.001.
30. Chatterjee R, Katz M, Cox TF, Porter JB. Prospective study of the hypothalamic-pituitary axis in thalassaemic patients who developed secondary amenorrhoea. Clin Endocrinol. 1993;39(3):287–96.
31. Chern JP, Lin KH, Tsai WY, Wang SC, Lu MY, Lin DT, et al. Hypogonadotropic hypogonadism and hematologic phenotype in patients with transfusion-dependent beta-thalassemia. J Pediatr Hematol Oncol. 2003;25(11):880–4.
32. Najafipour F. Evaluation of endocrine disorders in patients with thalassemia major. Published online 2008.
33. Ahmed E, Shaheen M. Prevalence of hypogonadism in thalassemia major patients in Gaza strip. Published online 2019.
34. Merchant RH, Shirodkar A, Ahmed J. Evaluation of growth, puberty and endocrine dysfunctions in relation to iron overload in multi transfused Indian thalassemia patients. Indian J Pediatr. 2011;78(6):679–83.
35. Moayeri H, Oloomi Z. Prevalence of growth and puberty failure with respect to growth hormone and gonadotropins secretion in beta-thalassemia major. Published online 2006.
36. Majeed MS. Evaluation of some Biochemical and Endocrine Profiles in transfusion-dependent Iraqi major β-thalassemia patients. Iraqi J Sci. 2017;58(2A):639–45.
37. Safarinejad MR. Reproductive hormones and hypothalamic-pituitary-ovarian axis in female patients with homozygous β-thalassemia major. J Pediatr Hematol Oncol. 2010;32(4):259–66.

38. Chen MJ, Peng SS, Lu MY, Yang YL, Jou ST, Chang HH, et al. Effect of iron overload on impaired fertility in male patients with transfusion-dependent beta-thalassemia. Pediatr Res. 2018;83(3):655–61. https://doi.org/10.1038/pr.2017.296.
39. Telfer PT, Prestcott E, Holden S, Walker M, Hoffbrand AV, Wonke B. Hepatic iron concentration combined with long-term monitoring of serum ferritin to predict complications of iron overload in thalassaemia major. Br J Haematol. 2000;110 (4):971–7. https://doi.org/10.1046/j.1365-2141.2000.02298.x.
40. Belhoul KM, Bakir ML, Saned MS, Kadhim AMA, Musallam KM, Taher AT. Serum ferritin levels and endocrinopathy in medically treated patients with β thalassemia major. Ann Hematol. 2012;91(7):1107–14. https://doi.org/10.1007/S00277-012-1412-7.
41. Shalitin S, Carmi D, Weintrob N, Phillip M, Miskin H, Kornreich 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. https://doi.org/10.1111/J.1600-0609.2004.00371.X.