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**Brief communication (Original)**

# Testicular function in patients with regular blood transfusion for thalassemia major

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***Background:*** Regular blood transfusion and iron chelation therapy have improved the quality of life of patients with thalassemia and increased their longevity, but transfusion also increases the frequency of endocrine complications, possibly because of iron deposition in the pituitary gland or the gonads, or both.

***Objective:*** To evaluate testicular function in patients with thalassemia major by basal hormonal study, and identify risk factors for dysfunction.

***Methods:*** We performed a cross-sectional study of 28 patients with thalassemia major aged 11.7 ± 1.8 (8–14.9) years (15 in prepuberty, 13 in puberty with no delayed puberty) who had regular blood transfusions. A normal control group comprised 64 boys who were matched for age and Tanner genital stage.

***Results:*** The mean level of serum ferritin in the previous year was 1,575 ± 642 ng/mL, and the onset of blood transfusion was at 3.8 ± 2.3 years and iron chelation therapy was 6.6 ± 2.8 years. The trend for anti-Müllerian hormone levels in patients and controls was similar with age, and although higher in the patients, particularly at Tanner stage II, was not significantly different. Testosterone levels were lower in the patients compared with controls; particularly at Tanner stages IV–V (290.88 vs. 537.4 ng/dL, $P < 0.05$). Serum follicle-stimulating hormone and luteinizing hormone levels were not significantly different between the groups at any Tanner stage.

***Conclusion:*** Patients who received regular blood transfusions had normal Sertoli cell function. Leydig cell dysfunction may occur, even though the patients had a normal pubertal onset.

***Keywords:*** Anti-Müllerian hormone, follicle stimulating hormone, hypogonadism, luteinizing hormone, regular blood transfusion, thalassemia major

Thalassemia is the most common hematologic disease in Thailand [1, 2]. Treatment of thalassemia, which including regular blood transfusion and iron chelation therapy have improved quality of life of these patients and increased their longevity. However, regular blood transfusion may lead to iron deposition in various organs, causing their dysfunction and failure [3, 4]. Hypogonadism and delayed puberty are the most common endocrine complications. These result from iron deposition in the pituitary gland, testicular tissue, or both. Prevalence is 40%–80% [5-9]. Iron overload [10-12] is considered one of the main factors for developing endocrine complications. Other factors are the genotype of thalassemia and the time for onset of blood transfusion and chelating therapy [4, 7, 13, 14].

Testicular functions can be assessed by the basal hormonal study (follicle stimulating hormone (FSH) luteinizing hormone (LH), and testosterone), the gonadotropin-releasing hormone (GnRH) stimulation test, the human chorionic gonadotropin (hCG) stimulation test, and seminal fluid analysis. However, it is difficult to evaluate testicular function during the prepubertal period when serum gonadotropins (LH, FSH) and testosterone levels are not informative because the hypothalamic–pituitary gonadal axis during prepuberty is still inactive. Serum anti-Müllerian hormone (AMH) level can be used as a marker of testicular function without any need for a stimulation test. AMH is primarily secreted by the Sertoli cells, which function during prepuberty. Serum AMH level

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is high during childhood and declines during puberty as a result of down-regulation by increasing testosterone level [15, 16]. Serum AMH level is a useful tool with which to assess testicular function in various conditions such as isolated cryptorchidism, anorchia, childhood cancer survivors and after hematopoietic cell transplantation [17-20].

The aim of this study was to evaluate testicular function in patients with thalassemia major who have been receiving regular blood transfusions, by Tanner assessment and by measuring serum levels of LH, FSH, testosterone, and AMH and to identify risk factors for developing testicular dysfunction.

## Patients and methods

Were enrolled 28 patients with thalassemia major who had received regular blood transfusions at King Chulalongkorn Memorial Hospital. They had received blood transfusion every 4–5 weeks. Patients who had received sex hormone replacement therapy or had a history of testicular surgery or cryptorchidism were excluded. A normal control group comprised 64 healthy boys who were age matched. The clinical data, age, height, weight, the age at diagnosis, the type of thalassemia, the age at initiation of blood transfusion, the age at initial chelation therapy, a history of splenectomy, the previous hemoglobin and serum ferritin levels, were retrospectively reviewed from medical records.

At the time of blood sampling, pubertal status was assessed by genital staging according to Tanner's classification. Testicular volume was measured using a Prader orchidometer. Testicular volume <4 mL was considered as a prepubertal and ≥4 mL defined as post puberty. An absence of secondary sexual characteristics with a testicular volume <4 mL at the age of 14 years was defined as delayed puberty. Blood samples for hormonal study were collected before blood transfusion. Serum concentrations of LH, FSH, testosterone, and AMH were measured. Informed ascent was obtained from all participants and informed consent was obtained from their parents before being included in the study. The study protocol was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (approval number 268/2013).

The concentrations of FSH, LH, and testosterone were measured by using a electrochemiluminescence immunoassay (Elecsys and Cobas e analyzers, Roche Diagnostics, Indianapolis, IN, USA). The detection limits of the LH, FSH, and testosterone assays were 0.10, 0.10 mIU/mL and 0.025 ng/mL, respectively. The concentrations of AMH were measured using a two-side enzyme immunoassay (AMH Gen II ELISA, Beckman Coulter, Pasadena, CA, USA).

### *Statistical analysis*

All data are expressed as mean ± SD if the distribution was normal and median and interquartile range (25th; 75th percentiles) if the distribution was not normal. The comparisons between groups were performed using an independent Student *t* test if the distribution was normal and a Mann–Whitney *U* test if the distribution was not normal. The relationship between hormonal profiles and other variables to develop testicular dysfunction were determined using a Spearman correlation. $P < 0.05$ was considered significant. All analyses were performed with the SPSS software for Windows, version 17.0 (SPSS Inc, Chicago, IL, USA).

## Results

### *Patient characteristics*

We included 28 patients with thalassemia major (β-Thal/HbE) (89.3%) and homozygous β-thalassemia in the study. There were 15 prepubertal patients, and 13 had started puberty, none has delayed puberty (n = 0). The iron chelating agents used were deferiprone (GPO-L-one; 96.4%) and deferasirox (Exjade; 3.6%). Five of the 28 patients (17.9%) underwent splenectomy. Patient characteristics are summarized in **Table 1**.

### *Hormone profiles*

The trend of AMH levels vs. age in patients with thalassemia major and normal controls were similar, and showed high serum AMH levels in prepuberty that then gradually declined with age (**Figure 1**). When classified by Tanner genital stage, serum AMH levels were not significantly different between the thalassemia major and the control groups. The serum LH and FSH levels were also not significantly different between both groups as shown in **Table 2** and **Figure 2**.

The serum testosterone levels were lower than the cut-off limit (<2.5 ng/dL) in both prepubertal thalassemia major and the control group. The serum testosterone levels during the pubertal period gradually increased in both groups for each Tanner stage (II–V), although the levels in thalassemia major group were lower than the control group particularly in Tanner IV–V (290.88 vs. 537.4 ng/dL, $P < 0.05$).

**Table 1.** The demographic characteristics of patients with thalassemia major

| Characteristic | Mean ± SD (range) |
|---|---|
| Recent age (y) | 11.7 ± 1.8 (8–14.9) |
| Height (SDS) | –0.4 ± 0.87 (–2.3–1.5) |
| Weight (SDS) | –0.04 ± 0.87 (–1.2–2.2) |
| Age at diagnosis of thalassemia (y) | 1.9 ± 1.1 (0.4–4.4) |
| Onset of blood transfusion (y) | 3.8 ± 2.3 (0.6–9.1) |
| Onset of chelation therapy (y) | 6.6 ± 2.8 (2–12.1) |
| Serum ferritin level before start chelation therapy (ng/mL) | 1,645 ± 536 (1,019–2,846) |
| Mean ferritin level in 1 year (ng/mL) | 1,575 ± 642 (534–3,207) |
| Mean hemoglobin level in 1 year (g/dL) | 8.9 ± 0.7 (7.1–10) |
| Maximum ferritin level (ng/mL) | 3,169 ± 1,184 (1,419–6,220) |
| Age of maximum ferritin level (y) | 9.7 ± 2.4 (5.3–13.8) |

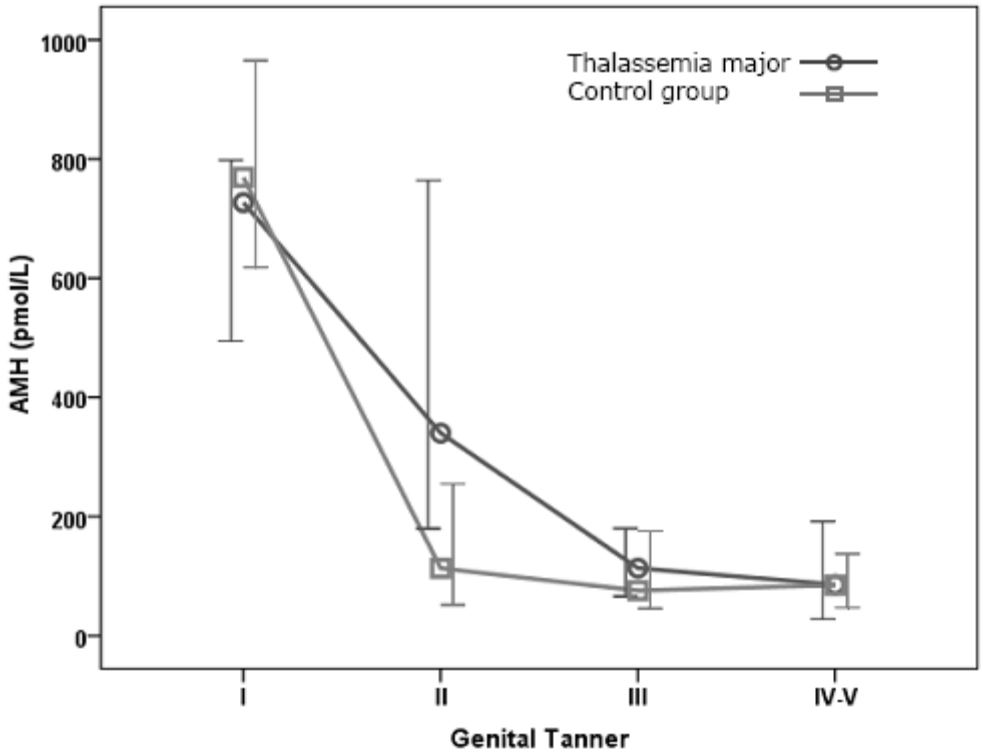


**Figure 1.** The median and interquartile ranges (25th; 75th percentiles) of serum AMH levels according to the Tanner genital stage.

***Factor related to testicular dysfunction***

Serum testosterone levels had a positive correlation with age of onset of chelation therapy ($r$ = 0.46, $P$ = 0.016). Serum AMH levels had a negative correlation with age of maximum serum ferritin level ($r$ = –0.53, $P$ = 0.004).

**Table 2.** The testicular volume and basal LH, FSH, testosterone and AMH levels in patients with Thalassemia major and the controls

| Genital Tanner | n | Age (y) | Testicular volume (mL) | LH (IU/L) | FSH (IU/L) | Testosterone (ng/dL) | AMH (pmol/L) |
|---|---|---|---|---|---|---|---|
| **Tanner I** | | | | | | | |
| Thalassemia | 15 | 10.6 ± 1.4 | 3 (2–4) | <0.1 (<0.1–0.5) | 0.95 (0.3–1.42) | <2.5 | 726.86 (494.29–797.86) |
| Control | 26 | 10.6 ± 1.4 | | <0.1 | 0.44 (0.18–1.1) | <2.5 (<2.5–5.6) | 769.16 (587.7–1,073.4) |
| **Tanner II** | | | | | | | |
| Thalassemia | 5 | 11.5 ± 1.0 | 5 (4–7) | 1.85 (1.1–3.4) | 1.82 (1.3–2.0) | 10.66 (6.3–26.2) | 340.0 (203.82–570.68) |
| Control | 11 | 12.5 ± 0.9 | | 2.3 (1.4–3.5) | 2.2 (1.4–2.4) | 46.1 (19.2–188.8) ($P$ = 0.058) | 113.17 (58.33–256.10) |
| **Tanner III** | | | | | | | |
| Thalassemia | 4 | 13.3 ± 0.8 | 12 (12–14.3) | 3.4 (1.8–5.4) | 2.7 (1.7–3.6) | 139.97 (80.9–290.6) | 113.32 (71.48–169.47) |
| Control | 9 | 13.7 ± 0.8 | | 2.7 (2.1–4.2) | 1.5 (1.3-2.0) | 196.99 (117.0–294.1) | 75.69 (55.25–134.84) |
| **Tanner IV-V** | | | | | | | |
| Thalassemia | 4 | 14.1 ± 1.0 | 20 (14–20) | 1.9 (1.3–2.5) | 4.0 (1.4–6.4) | 290.88 (111.5–426.6) | 85.71 (33.75–173.89) |
| Control | 18 | 15.5 ± 2.0 | | 1.5 (1.2–2.3) | 5.6 (3.5–6.6) | 537.4 (394.6–720.6) $P$ <0.05 | 84.68 (45.61–137.68) |

## Discussion

AMH is a peptide hormone secreted primarily by Sertoli cells, which are active during prepuberty, therefore AMH levels are a useful marker of Sertoli cell function, particularly in the childhood period. The evaluation of this hormone does not require a stimulation test. Serum AMH levels have been reported useful to assess testicular function in many conditions including isolated cryptorchidism, anorchia, childhood cancer survivors, and after hematopoietic cell transplantation [17-20]. Serum AMH levels elevate and become stable during prepuberty and then decrease after the onset of puberty [15, 16]. In our thalassemia major study, they were similar to the normal controls. Furthermore, the levels of AMH in thalassemia were not significantly different from the control group. This suggested that Sertoli cell function is normal in patients with thalassemia major and regular blood transfusions.

Serum gonadotropins (LH, FSH) and testosterone levels in prepubertal patients (n = 15) were not significantly different when compared with controls. The basal levels of serum LH and FSH were not significantly different between pubertal patients (n = 13) and controls suggested that the hypothalamic–pituitary–gonadal axis is intact. Furthermore, these patients had a normal age of pubertal onset and their testicular volumes correlated with Tanner genital stage; the median testicular volume 5 mL in Tanner II, 10–12 mL in Tanner III, and 20 mL in Tanner IV–V. However, serum testosterone levels in pubertal patients were lower than in controls; especially in Tanner IV–V. This suggested that testicular function is diminished, although they have a normal pubertal onset. Rimawi et al. [5] reported that the basal levels of serum testosterone in patients with thalassemia who have a normal puberty, were lower than in normal controls, but this was not significantly different. However, significant differences were seen after a GnRH stimulation test. The basal and peak levels of LH and FSH in patients with β-thalassemia with normal puberty and normal controls were not significantly different. This suggests that Leydig cell function is impaired in β-thalassemia, although puberty is normal. A previous study showed that histological examination of the testicular tissue in thalassemia

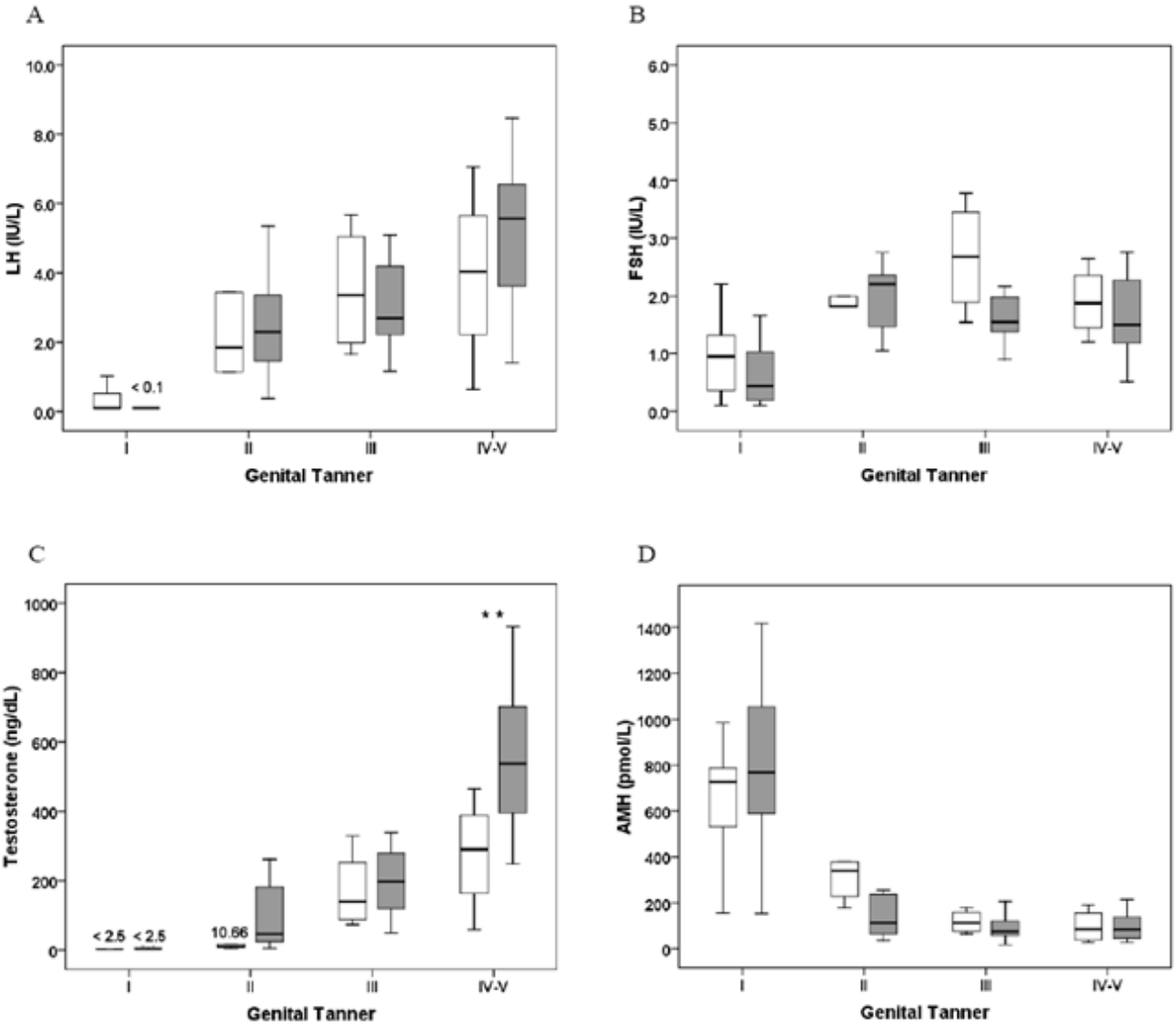


**Figure 2.** The median and interquartile ranges (25th; 75th percentiles) for LH (**A**), FSH (**B**), testosterone (**C**), and AMH (**D**) according to the Tanner genital stage between thalassemia major patients (white boxes) and control group (gray boxes), ** $P < 0.05$.

patients demonstrated varying degrees of testicular interstitial fibrosis with small heavily pigmented undifferentiated seminiferous tubules, hyalinized, and an absence of Leydig cells. This suggested end organ fibrosis secondary to iron overload [21].

Improvement of treatment regimens, consisting of regular blood transfusions and adequate iron chelation therapy, has led to decreased morbidity and mortality in patients with thalassemia major. The prevalence of hypogonadism and/or delayed puberty was reported at 40%–80% [3, 4] by previous studies. This prevalence depended on serum ferritin levels, type of thalassemia, the onset of blood transfusion and chelating therapy [4, 7, 13, 14]. We had no patients with abnormal testicular function or delayed puberty in this study. This can be attributed to adequate treatment with blood transfusion at an appropriate time ($3.8 \pm 2.3$ years old) and effective iron chelation shown by a mean serum ferritin level during the past year of $1,575 \pm 642$ ng/mL. Bronspiegel-Weintrob et al. [13] reported that patients with abnormal puberty had higher serum ferritin levels before chelation and had received suboptimal chelation therapy (serum ferritin levels $4,734 \pm 3,081$ and $3,572 \pm 1,870$ ng/mL, respectively). Important factors for developing hypogonadism and/or delayed puberty are a high serum ferritin level

[10-12]. However, this study did not show such a relationship because there was no delay in puberty.

Limitations of our study include the small number of patients in the sample and that it was a cross-sectional study. Our patients were relatively young when compared with other studies. We need long-term follow-up to clarify their testicular function further. Prospective studies of larger participants with other testicular markers such as serum inhibin B are needed to evaluate testicular function in more detail.

## Conclusion

Sertoli cell functions can be preserved by regular blood-transfusions and adequate iron-chelation. Leydig cell dysfunction may occur in the late pubertal period despite a normal pubertal onset at an appropriate age. Therefore, testicular function should be regularly evaluated in the long term to detect early abnormalities and so that appropriate treatment can be initiated if necessary.

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## References

1. Fucharoen S, Winichagoon P. Thalassemia in SouthEast Asia: problems and strategy for prevention and control. Southeast Asian J Trop Med Public Health. 1992; 23:647-55.
2. Pansuwan A, Fucharoen G, Fucharoen S, Himakhun B, Dangwiboon S. Anemia, iron deficiency and thalassemia among adolescents in Northeast Thailand: results from two independent surveys. Acta Haematol. 2011; 125:186-92.
3. Borgna-Pignatti C, Gamberini MR. Complications of thalassemia major and their treatment. Expert Rev Hematol. 2011; 4:353-66.
4. Gamberini MR, De Sanctis V, Gilli G. Hypogonadism, diabetes mellitus, hypothyroidism, hypoparathyroidism: incidence and prevalence related to iron overload and chelation therapy in patients with thalassaemia major followed from 1980 to 2007 in the Ferrara Centre. Pediatr Endocrinol Rev. 2008; 6 Suppl 1:158-69.
5. Al-Rimawi HS, Jallad MF, Amarin ZO, Al Sakaan R. Pubertal evaluation of adolescent boys with β-thalassemia major and delayed puberty. Fertil Steril. 2006; 86:886-90.
6. De Sanctis V, Roos M, Gasser T, Fortini M, Raiola G, Galati MC. Impact of long-term iron chelation therapy on growth and endocrine functions in thalassaemia. J Pediatr Endocrinol Metab. 2006; 19:471-80.
7. 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; 25:880-4.
8. 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; 78:679-83.
9. Soliman AT, elZalabany M, Amer M, Ansari BM. Growth and pubertal development in transfusion-dependent children and adolescents with thalassaemia major and sickle cell disease: a comparative study. J Trop Pediatr. 1999; 45:23-30
10. Borgna-Pignatti C, Rugolotto S, De Stefano P, Zhao H, Cappellini MD, Del Vecchio GC, et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Haematologica. 2004; 89:1187-93.
11. 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; 74:93-100.
12. Papadimas J, Mandala E, Pados G, Kokkas B, Georgiadis G, Tarlatzis B, et al. Pituitary–testicular axis in men with β-thalassaemia major. Hum Reprod. 1996; 11:1900-4.
13. Bronspiegel-Weintrob N, Olivieri NF, Tyler B, Andrews DF, Freedman MH, Holland FJ. Effect of age at the start of iron chelation therapy on gonadal function in β-thalassemia major. N Engl J Med. 1990; 323:713-9.
14. Jensen CE, Tuck SM, Old J, Morris RW, Yardumian A, De Sanctis V, et al. Incidence of endocrine complications and clinical disease severity related to genotype analysis and iron overload in patients with beta-thalassaemia. Eur J Haematol. 1997; 59:76-81.
15. Grinspon RP, Rey RA. Anti-Müllerian hormone and Sertoli cell function in paediatric male hypogonadism. Horm Res Paediatr. 2010; 73:81-92.
16. Grinspon RP, Loreti N, Braslavsky D, Bedecarras P, Ambao V, Gottlieb S, et al. Sertoli cell markers in

the diagnosis of paediatric male hypogonadism. J Pediatr Endocrinol Metab. 2012; 25:3.

17. Matuszczak E, Hermanowicz A, Debek W, Oksiuta M, Dzienis-Koronkiewicz E, Zelazowska-Rutkowska B. Serum AMH concentration as a marker evaluating gonadal function in boys operated on for unilateral cryptorchidism between 1st and 4th year of life. Endocrine. 2012; 41:334-7.
18. Laporte S, Couto-Silva AC, Trabado S, Lemaire P, Brailly-Tabard S, Esperou H, et al. Inhibin B and anti-Müllerian hormone as markers of gonadal function after hematopoietic cell transplantation during childhood. BMC Pediatr. 2011; 11:20.
19. Guibourdenche J, Lucidarme N, Chevenne D, Rigal O, Nicolas M, Luton D, et al. Anti-Müllerian hormone levels in serum from human foetuses and children: pattern and clinical interest. Mol Cell Endocrinol. 2003; 211:55-63.
20. Cuny A, Trivin C, Brailly-Tabard S, Adan L, Zerah M, Sainte-Rose C, et al. Inhibin B and antimüllerian hormone as markers of gonadal function after treatment for medulloblastoma or posterior fossa ependymoma during childhood. J Pediatr. 2011; 158:1016-22.e1.
21. Canale VC, Steinherz P, New M, Erlandson M. Endocrine function in thalassemia major. Ann N Y Acad Sci. 1974; 232:333-45.