# Proportion of Hypogonadism in Transfusion-Dependent Thalassemia Patients and Its Contributing Factors

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## ABSTRACT

***Background****: Beta thalassemia is a lifelong disease involving malformed red blood cells (RBC). One of the disease's complications is hypogonadism, in which adults tend to exhibit regression in sexual characteristics, experience sexual dysfunction, and therefore have a lower quality of life. Around 3-10% of the Indonesian population carries the beta-thalassemia gene. This study aimed to see the proportions of hypogonadism in transfusion-dependent thalassemia patients and its contributing factors.* ***Methods****: This is a cross-sectional study involving 60 male patients admitted to three Indonesian general hospitals from July 2022 to July 2023. All patients were diagnosed with beta-thalassemia via chromatography hemoglobin analysis. We performed a single-time physical examination and laboratory examinations to determine FSH, LH, and free testosterone levels. The correlation between Hb and sexual hormone levels was analyzed using Spearman's rank correlation coefficient. ROC curve analysis was conducted afterward. All statistical analysis was done in SPSS version 29.* ***Results****: 31 out of 60 thalassemia patients had hypogonadism. Pre-transfusion Hb count was found to be linearly correlated with FSH (r = 0.388, p = 0.049), LH (r = 0.338, p = 0.008), and free testosterone (r = 0.255, p = 0.049). ROC analysis indicated that pre-transfusion Hb was viable as a predictor for hypogonadism (AUC = 0.655, 65.5% sensitivity, 67.7% specificity).* ***Conclusion****: We confirmed the role of pre-transfusion Hb count as a potential predictor for hypogonadism due to the tissue hypoxia mechanism and transfusion-related iron overload in TDT patients. Decreased Hb is linearly correlated with FSH, LH, and testosterone levels. Decreased Hb also downregulates these factors.*

***Keywords*****:** *Pre-transfusion hemoglobin, hypogonadism, transfusion-dependent thalassemia.*

## INTRODUCTION

Beta thalassemia is a hematological disorder in which the body's erythrocytes become malformed. In Indonesia, approximately 27 million people are thalassemia gene carriers.[1] One of the common complications is hypogonadism, where the body's sex glands produce little to no hormones. Hypogonadism detection is important due to thalassemia often being diagnosed during adolescence.[2]

Based on demographic, community, or screening investigations, the incidence of hypogonadism ranges greatly, from 2.1 to 12.8%.[3] Nonetheless, the transfusion-dependent thalassemia (TDT) population has an incidence rate of hypogonadism that approaches 70%.[4,5] This is thought to be due to the accumulation of iron in endocrine organs such as the pituitary and testes which can lead to organ toxicity, and cause impaired hormone secretion in these organs.[6,7]

Several mechanisms are thought to play a role in the incidence of hypogonadism in thalassemia patients, not only due to reproductive hormones but also other mechanisms such as adipose tissue, leptin, body mass index (BMI), and pre-transfusion hemoglobin (Hb). TDT patients often require routine transfusion, and it is recommended that TDT maintain a hemoglobin range of 9–10.5 g/dL.[3] In addition, failure to maintain hemoglobin count is known to cause complications due to tissue hypoxia.[4]

However, there is little information available on the pathomechanism of hypogonadism in TDT patients. Serum ferritin is not necessarily linked to TDT, despite being considered to be the cause of hypogonadism. GnRH examination that cannot be examined at the periphery causes an unclear understanding of the pathway of hypogonadism. Our study aims to describe the proportions and risk factors of hypogonadism among TDT patients in Indonesia.

## METHODS

We performed a cross-sectional study among thalassemia patients aged 18 years and older who were admitted to the Thalassemia Clinic in either Cipto Mangunkusumo General Hospital in Jakarta, Fatmawati General Hospital in Jakarta, or Hasan Sadikin General Hospital in Bandung, Indonesia, between July 2022 and July 2023. All patients were reviewed by a hematologist to confirm their beta-thalassemia diagnosis via hemoglobin analysis and high-performance liquid column chromatography.

This study uses a cross-sectional design. Patients with transfusion-dependent thalassemia who are male, older than eighteen, diagnosed by pediatricians or internists, and who are willing to take part in the study are enrolled. Individuals with mental disorders, those using drugs that cause hyperprolactinemia, those who have had hypophyseal surgery in the past, those who have a hypophysis tumor, those who have had diabetes mellitus, testicular trauma, radiation, or enlargement, and those who have received testosterone therapy within the last two weeks were not included. Subjects included during July-August 2023 in Thalassemia Polyclinic in Cipto Mangunkusumo Hospital Jakarta, Thalassemia Polyclinic in Fatmawati Hospital Jakarta, and Hematology-Oncology Polyclinic in Hasan Sadikin Hospital Bandung.

Demographic parameters included patients' age, thalassemia type, history of splenectomy, and history of iron chelator consumption. Laboratory examinations for hypogonadism include FSH, LH, and free testosterone levels. Patients were considered hypogonadal if the free testosterone level was < 5pg/mL. We exclusively selected male participants to eliminate any hormone fluctuations caused by the menstrual cycles of female patients. Hemoglobin data were assessed as the patient visited the hospital and before they underwent transfusion.

The association between Hb count and FSH, LH, and free testosterone levels was analyzed with Spearman's correlation analysis. Additionally, the ROC curve was used to identify the diagnostic efficacy of pre-transfusion Hb count for hypogonadism and its cutoff value. A p-value of less than 0.05 was considered statistically significant. All data processing was performed using SPSS 29.0.

This study does not include interventions for the patient that would otherwise be regulated by the Declaration of Helsinki. The study's aim

and goals were explained clearly to the patients and their families; afterward, the patient or their families signed a consent form. This study was approved by the Faculty of Medicine Universitas Indonesia's ethical board on May 9, 2022, with registration number KET-435/UN2.F1/ETIK/PPM.00.02/2022.

## RESULTS

Our study involved 65 patients in total, of whom 5 were excluded for various reasons (Fig 1), resulting in 291 subjects being included in the final analysis. All participants had given their consent to be part of this study and were confirmed to have thalassemia. The characteristics of these subjects are presented in **Table 1**. Subjects had a median age of 22 (18 - 42) years old. The proportion of hypogonadism was 51.1%.

To determine hypogonadism, which has various signs and symptoms, we assessed both clinical manifestation and laboratory examination to accurately represent the hypogonadism found in our patients.

Overall, we have established that among this study's participants, signs of hypogonadism were prominent in at least half of the patients based on their clinical features alone. In addition, Tanner staging to objectively determine sexual growth delays.

To confirm the presence of hypogonadism in patients, our study also conducted laboratory examinations on the participants to determine their hormone levels. Our results are described in **Table 2**.

Subsequently, we performed ROC curve analysis and found that pre-transfusion hemoglobin count could be used to verify clinical diagnosis of hypogonadism with a cutoff value < 8.75 g/dL (AUC = 0.655, 65.5% sensitivity, 67.7% specificity).

## DISCUSSION

We found that 51.1% of the subjects experienced hypogonadism compared to similar studies in other countries.[5] Moreover, low pre-transfusion Hb, high FSH, and high leptin were associated with an increased risk of hypogonadism.

The distribution of pubic hair in this study appeared to be sufficient at the tanner stage IV-V level, with a distribution as high as 53.4%. Meanwhile, the other 46.6% of subjects had less or no pubic hair growth. A popular physical test for determining puberty is the Tanner stage. This examination assesses a person's secondary sex characteristics. Tanner stage and testosterone levels have a significant positive association, according to research by Balzer et al. Several factors, including pubic hair, testicular length, and testicular volume, are evaluated during the Tanner stage itself. At least at stages IV and V, the Tanner stage is believed to have grown

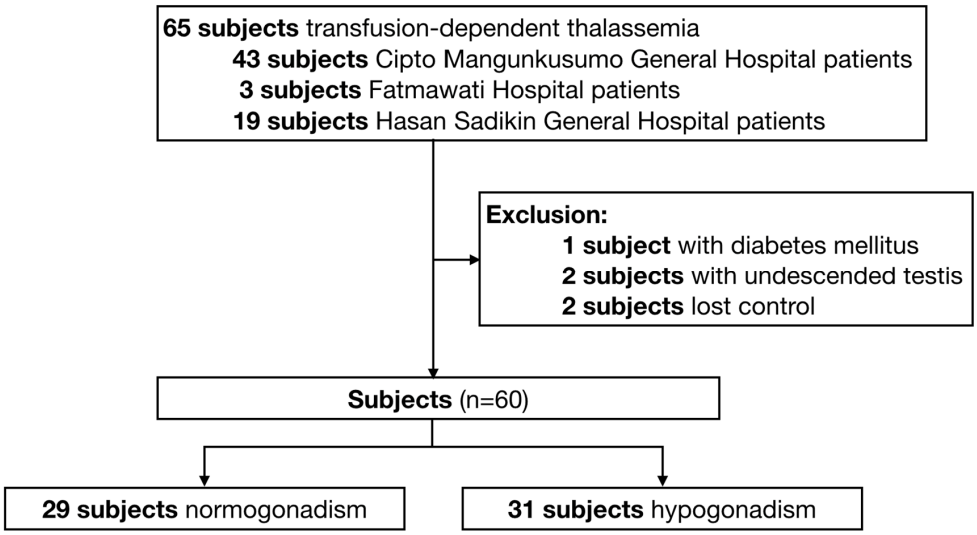


**Figure 1.** Flow diagram summarizing the subject recruitment process.

**Table 1.** Clinical characteristics.

| Characteristics | Total (n = 60) |
|---|---|
| Age (year), median (min-max) | 22 (18-42) |
| **Clinical manifestation, n (%)** | |
| Family history of delayed sexual development | |
| Yes | 0 (0) |
| No | 60 (100) |
| Erectile dysfunction | |
| Yes | 51 (85) |
| No | 9 (15) |
| Decreased libido | |
| Yes | 3 (5) |
| No | 57 (95) |
| **Physical examination, n (%)** | |
| Body Mass Index (kg/m$^2$), mean (SD) | 18,63 + 2,45 |
| Underweight (BMI < 18.5 kg/m$^2$) | 32 (53) |
| Normoweight (BMI 18.5 – 22.9 kg/m$^2$) | 24 (40) |
| Overweight (BMI 23 – 24.9 kg/m$^2$) | 3 (5) |
| Obese (BMI >25 kg/m$^2$) | 0 (0) |
| Abnormally high-pitched sound | |
| Yes | 24 (40) |
| No | 36 (60) |
| Presence of pimples | |
| Extensive | 30 (50) |
| Minimal | 30 (50) |
| Presence of facial hair (mustache and beard) | |
| Extensive | 35 (59) |
| Minimal | 25 (41) |
| Presence of armpit hair | |
| Extensive | 37 (61) |
| Minimal | 23 (38) |
| Spleen size | |
| Not palpable | 3 (5) |
| Schuffner 1 – 4 | 43 (71) |
| Schuffner 5 – 8 | 8 (13) |
| Post-splenectomy | 6 (10) |
| Flaccid penile length (cm), median (min-max) | 7 (4 – 8) |
| Orchidometer testicle size (ml), mean (SD) | 11 ± 6 |
| Tanner stage, n (%) | |
| I | 13 (21.7) |
| II | 6 (10) |
| III | 9 (15) |
| IV | 25 (41.7) |
| V | 7 (11.7) |

SD: standard deviation, BMI: body mass index.

**Table 2.** Laboratory examination.

| Parameters | Median (min-max) Total (n = 60) |
|---|---|
| Pre-transfusion Hb (g/dL) | 8.7 (5.1 - 14.2) |
| Leptin (pg/mL) | 1,942 (242-22,270) |
| FSH (mIU/mL) | 8.3 (0.1 - 46.1) |
| LH (mIU/mL) | 18.1 (0.5 – 80.8) |
| Free testosterone (pg/mL) | 4.7 (0.1 - 35.78) |
| Hypogonadism | 31 (51.6%) |

Hb: hemoglobin, FSH: follicle stimulating hormone, LH: luteinizing hormone.

**Table 3.** Bivariate analysis of association between independent variables and hypogonadism.

| Variables | Normogonadism (n=19) | Hypogonadism (n=31) | p |
|---|---|---|---|
| BMI (kg/m$^{2)}$ | 17.7 ± 1.9 | 19.3 ± 2.6 | 0.122 |
| Pre-transfusion Hb (g/dL) | 8.9 (6-12.6) | 7.2 (5.1-14.2) | 0.569 |
| FSH (mIU/mL) | 8.09 (3.65-24.3) | 12.4 (0.1-46.1) | 0.040 |
| LH (mIU/mL) | 18.1 (6.95) | 27.8 (0.5-90.8) | 0.005 |
| Leptin (pg/mL) | 1,405 (411-5,089) | 3,616 (242-22,270) | 0.053 |

BMI: body mass index, Hb: hemoglobin, FSH: follicle stimulating hormone, LH: luteinizing hormone.

**Table 4.** Multivariate analysis of association between independent variables and hypogonadism.

| Variables | OR | p-value |
|---|---|---|
| Model 1 | | |
| BMI | 1.112 | 0.606 |
| Pre-transfusion Hb | 0.571 | 0.033* |
| FSH | 1.086 | 0.146 |
| LH | 1.017 | 0.568 |
| Leptin | 1.001 | 0.013* |
| Model 2 | | |
| Hb | 0.575 | 0.035* |
| FSH | 1.084 | 0.154 |
| LH | 1.018 | 0.540 |
| Leptin | 1.001 | 0.003* |
| Model 3 | | |
| Hb | 0.573 | 0.033* |
| FSH | 1.110 | 0.019* |
| Leptin | 1.001 | 0.003* |

BMI: body mass index, Hb: hemoglobin, FSH: follicle stimulating hormone, LH: luteinizing hormone. *Statistically significant.

to adult size. This study found a significant correlation between pre-transfusion Hb count and its downregulating effect on sexual hormones associated with hypogonadism.[6]

Among our participants, FSH levels tended to be within the normal range of 1.5–12.4 mIU/mL. Conversely, LH levels were usually elevated, with the highest value being recorded at 80.8 mIU/mL. Furthermore, free testosterone levels tended to be very low across all ages. The elevated LH and decreased testosterone levels suggested that most of the participants had primary hypogonadism. In a similar study regarding the sexual characteristics of female beta thalassemia major patients, fully developed adults tended to exhibit sexual dysfunction and a regression,[7] a finding that supports our physical examination results.

Our study shows that pre-transfusion Hb count is directly correlated to FSH, LH, and free testosterone levels, suggesting that lower Hb counts impair sex hormone levels. Similar studies have associated endocrinopathies with post-transfusion iron overload and its subsequent deposition in visceral organs.[8,9] Several studies were in agreement with our study and proposed that the occurrence of hypogonadism is likelier in TDT patients with lower pre-transfusion Hb counts (OR 0.38, 95% CI [0.145 – 0.994]).[10,11] One study supported this by elaborating that thalassemia complications could also be caused by chronic tissue hypoxia due to low baseline Hb count. Low testosterone impairs erythropoiesis via inadequate stimulation to erythropoietin (EPO) secretion and decreased erythroid progenitor cell formation.[12,13] Therefore, EPO also exacerbates thalassemia in a positive feedback loop manner, which is consistent with our findings that the patients had low testosterone levels and low Hb counts.

We also establish the potential of pre-transfusion Hb count as a reliable predictor for hypogonadism clinical diagnosis with a resulting AUC of 0.655. The cutoff of 8.75 g/dL is also reasonably low without compromising tissue perfusion, which usually falls below 7-8 g/dL.[14,15] Another study found that a Hb countless to or equal to 6.81g/dL could predict hypogonadism in TDT patients (AUC = 0.708, 66.7% sensitivity, 63.4% specificity) with findings that are quite similar to our results.[10] Furthermore, since 8.75 g/dL is categorized as moderate anemia, it is possible that consistently impaired perfusion may slowly damage gonadal tissues over time. Furthermore, it is known that lower Hb counts in thalassemia patients indicate ineffective erythropoiesis and increased hemolysis.[10] Several studies provide support for these results; it has been found that thalassemia increases oxidative damage by inducing a hypercoagulable state, coupled with excessive tissue iron and chronic hypoxia,[2,16] which in turn causes impaired steroidogenesis in primary hypogonadism and gonadal insufficiency in secondary hypogonadism.[7]

Multivariate studies have agreed that pre-transfusion Hb count is an important parameter for determining quality of life for both TDT and NTDT due to its correlation with various complications, including hypogonadism.[10,11] However, to the best of our knowledge, this is one of the first studies to explore the potential of Hb count as a predictor for hypogonadism as a thalassemia complication. By knowing pretransfusion Hb as a predictor of hypogonadism in transfusion-dependent thalassemia patients, it is anticipated that patients can achieve the recommended Hb target, either with an adequate number of transfusion packs, or an adequate transfusion frequency.

Our investigation showed an association between leptin and an increased risk of hypogonadism (OR 1.001, p = 0.003). This finding was consistent with a study by Lima et al.[17] Leptin seems to control testicular cells, which in turn controls male reproduction, independent of the GnRH central regulatory route. Leptin can affect steroidogenic processes because it is known to be able to penetrate the blood testis barrier. Leydig cells appear to be the only place where leptin receptor (LPR) expression occurs in the gonads of males. The levels of LPR are negatively connected with testosterone levels as well as anomalies in sperm quality and production. Testicular tissue is primarily affected by leptin through the activation of the signal transducer and activator of the transcription 3 (STAT3)/Janus kinase 2 (JAK2) pathway.[18]

The main limitation of this study lies in its cross-sectional design, which does not represent a causal effect. This study design may have confounding variables that we did not analyze with a multivariate analysis. On the other hand, the study's main strength is that it is among the first to correlate and discover the potential of pre-transfusion Hb count potential as a predictor for hypogonadism using ROC curve analysis. Our results are in agreement with the previous study.

In conclusion, we identified the measurement of pre-transfusion Hb as a potential diagnostic approach that may be widely used to predict hypogonadism in thalassemia patients due to its cheap cost and high practicality. This is especially true in Indonesia, where primary health facilities can conduct peripheral blood counts.

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## COMPETING INTERESTS

The authors declare that there is no conflict of interest.

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