Original Article

Serum Kisspeptinin Iraqi Men with Beta-Thalassemia Major: A Cross-Sectional Study

Bushra Mussad Kadim and Ekhlas Abdallah Hassan

ABSTRACT

Objective: To evaluate serum concentrations of kisspeptin in Iraqi men diagnosed with beta-thalassemia major and to identify factors that may influence circulating kisspeptin levels

Study Design: Cross sectional study

Place and Duration of Study: This study was conducted at the National Centre for Thalassemia Patients in Baqubah, Iraq from 1st September 2024 to 30th June 2025.

Methods: Eighty individuals with beta-thalassemia major and 40 healthy controls were included. The parameters measured included kisspeptin, luteinizing hormone, follicle-stimulating hormone, total testosterone, fasting blood glucose as well as hematological assessments and oxidative stress markers (malondialdehyde and ischemia-modified albumin).

R**esults:** Men with hypogonadism due to beta-thalassemia major exhibited significantly higher kisspeptin levels compared to controls (P=0.001). Conversely, levels of testosterone, luteinizing hormone and follicle-stimulating hormone were notably lower in the beta-thalassemia major group than in the control group. Within the patient group, we observed negative correlations between kisspeptin levels and luteinizing hormone and testosterone (P<0.05). Additionally, positive correlations were found for kisspeptin versus inhibin B (r=0.782, P=0.001) and kisspeptin versus ferritin (r=0.286, P=0.010). Lower levels of luteinizing hormone (OR=2.95, P=0.0319), testosterone (OR=0.86, P=0.04) and increasing age (OR=0.927, P=0.014) were associated with elevated kisspeptin levels among Iraqi males with beta-thalassemia major.

Conclusion: Elevated kisspeptin with suppressed luteinizing hormone and testosterone in male thalassemia patients is a hallmark of complex hypothalamic-gonadal-pituitary axis disruption due to iron overload.

Key Words: Kisspeptin, Hypogonadism, Beta-thalassemia, Testosterone

Citation of article: Kadim BM, Hassan EA. Serum Kisspeptinin Iraqi Men with Beta-Thalassemia Major: A Cross-Sectional Study. Med Forum 2026;37(6):126-131. doi:10.60110/medforum.370624.

INTRODUCTION

Kisspeptin, a reproductive neuropeptide, has emerged as a crucial regulator of mammalian reproduction, primarily due to its role as an upstream modulator of gonadotropin-releasing hormone (GnRH) secretion.[1] The release of GnRH from GnRH neurons into the hypophyseal portal circulation prompts the stimulation of pituitary gonadotrophs, leading to the secretion of gonadotropins, specifically luteinizing hormone (LH) and follicle-stimulating hormone (FSH).

Department of Chemistry, College of Science, University of Diyala, Baquba, Diyala Iraq.

Correspondence: Bushra Mussad Kadim, Department of Chemistry, College of Science, University of Diyala, Baquba, Diyala Iraq.
Contact No: 07753754630
Email: bushramussad@gmail.com

Received: December, 2025
Reviewed: January-February, 2026
Accepted: March, 2026

In males, LH stimulates Leydig cells to produce testosterone, while FSH acts on Sertoli cells to promote spermatogenesis.[2] The first investigation into kisspeptin administration in humans was conducted in 2005 using kisspeptin-54. Healthy male volunteers demonstrated dose-dependent increases in circulating LH following a 90-minute infusion (0.25–12 pmol/kg/min). Although there were increases in FSH and testosterone levels, these changes were not dose-dependent.[3] The stimulatory effect of kisspeptin on LH release is significantly more pronounced than that on FSH concerning increases above baseline levels.[4]

The relationship between serum kisspeptin levels and primary hypogonadism is an important factor in understanding male patients with beta thalassemia major. Hypogonadism is the most common endocrine complication, affecting 70-80% of patients with thalassemia major. This condition is likely caused by hypertransfusion therapy, leading to iron accumulation in the gonads, pituitary gland, or both. However, hypogonadotropic hypogonadism (HH), resulting from iron deposition in the pituitary gonadotropes, is more frequently observed. Kisspeptin serves as a potent stimulant of GnRH neurons, suggesting its involvement in the etiology of HH.[5-7]

The purpose was to evaluate kisspeptin concentrations in Iraqi men with beta thalassemia major, examining their relationship with testosterone levels, LH, FSH and identifying other clinical factors that may influence kisspeptin levels.

METHODS

This cross-sectional study was conducted at National Centre for Thalassemia Patients in Baqubah, Iraq from 1st September 2024 to 30th June 2025 vide letter No. 3434/QM/Approval/JSDJNEHU dated 15th August 2024 and eighty patients with beta-thalassemia major were enrolled. Hypogonadism was diagnosed based on low levels of LH, FSH, free and total testosterone, along with patient history and physical examination findings that were consistent with the condition. The patient history included the onset of hypogonadism, marital status, presence of children, libido, and the adequacy of erection and ejaculation. Patients were evaluated using the Tanner classification.[8] The study group comprised newly diagnosed hypogonadism patients, who demonstrated onychoid features and were classified within Tanner stages 1-2. Transfusion-dependent patients with β-thalassemia major from various Iraqi populations, aged between 15 and 42 years, none of whom had undergone splenectomy or exhibited any obvious clinical infections, such as hepatitis were included. The data was collected their name, age, diagnosis, age at the onset of symptoms, splenectomy status, and any chronic illnesses. 5 ml of blood was drawn from all patients and controls. The blood samples were divided into two portions: 3 ml in tubes containing K3-EDTA and 2 ml in plain tubes with a gel separator. The tubes were then centrifuged at 300 rpm for five minutes to isolate the serum, which was subsequently transferred to another tube and stored at -20°C.

The determination of serum kisspeptin was conducted using an ELISA kit supplied by CUSABIO, China. Serum kisspeptin concentration was estimated through the sandwich technique of the ELISA method. The microplate being pre-coated with a specific antibody against kisspeptin and standards were introduced into the wells, allowing binding to occur when kisspeptin was present in the samples through the immobilized antibody. Following a washing process to eliminate any unbound enzyme reagents, horseradish peroxidase was introduced. After another washing step, the substrate solution was added to develop the end product color. The intensity of the color was measured after the color development was halted. Serum levels of T3, T4, TSH, FSH, LH, and testosterone were assessed using a Vidas kit provided by BioMeriux – France. The hormones were estimated through the Enzyme-Linked Fluorescent Assay (ELFA) method. The principle of the Vidas assay is based on a competitive immunoassay utilizing ELFA techniques. During the testing process, Solid Phase Receptors (SPR) served as the solid phase, alongside a device for pipetting. All steps of the assay were performed immediately using the VIDAS measuring device. The sample was introduced into wells containing hormone antigens labeled with alkaline phosphatase, which acts as the conjugate. The conjugated enzyme catalyzes the hydrolysis of this substrate, yielding a fluorescent product, 4-methyl-umbelliferone, which is measured at a wavelength of 450 nm. VIDAS automatically computes the results based on the calibration curve. Malondialdehyde was evaluated using the thiobarbituric acid reactive substance (TBARS) assay and ischemia-modified albumin levels were measured through the albumin cobalt binding (ACB) test. Serum glucose was determined using an enzymatic colorimetric method with a kit provided by Bio System, Spain. Serum urea levels were assessed through an enzymatic colorimetric reaction utilizing the Biomaghreb kit. Serum creatinine was measured using a kinetic test that does not require deproteinization, employing a kit from Biomaghreb-Maghreb, in conjunction with Spinreact, Spain. With a comprehensive menu of diagnostic tests and a high capacity for samples and reagents, the ARCHITECT c4000 is user-friendly and features advanced sample management capabilities, including a three-dimensional robotic sample handler.

The statistical analysis was using SPSS-24 The Student's t-test was employed to compare the diabetic and non-diabetic subjects. Spearman's product-moment correlation coefficient (rho) analysis was utilized. Additionally, multivariate analysis was performed to determine the independent contributions of various factors to the variance in kisspeptin.

RESULTS

The levels of iron, ferritin, glucose, urea, platelet count, ALT, AST, ALK, direct bilirubin, total serum bilirubin (TSB), TSH, Kisspeptin, IMA, and MDA were significantly higher ($P<0.05$) in BTM patients compared to the control group. In contrast, BMI, creatinine, Hb, HCT, T3, T4, FSH, LH, testosterone and inhibin-B levels were notably lower among the BTM patients compared to their controls (Table 1).

In the patient group, significant negative correlations were identified between kisspeptin and LH, testosterone, ALT, AST, UIBC, urea, creatinine, T4, and TSH ($P<0.05$). Additionally, a positive correlation was observed between serum kisspeptin and INH-b ($r=0.782$, $P=0.001$), as well as between serum kisspeptin and ferritin ($r=0.286$, $P = 0.010$) within the beta-thalassemia major patient cohort (Table 2).

The analysis identified age (OR=0.927, P=0.014), LH (OR=2.95, P=0.0319), and testosterone (OR=0.86, P=0.04) as independent variables correlated with the development of low kisspeptin levels in patients with beta-thalassemia major (Table 3).

Table No. 1: Clinical and biochemical measurements of study participants

Characteristic	Patients (n=80)	Control (n=40)	P value
Age (years)	21.88±4.95	23.60±5.22	0.207
BMI (kg/m2)	19.69±3.68	24.96±2.54	0.001
Iron (µg\dl)	230.99±6.23	117.63±5.17	0.001
Ferritin (ng\ml)	1725.89±59.67	127.86±8.27	0.001
Glucose (mg/dl)	93.08±16.91	79.46±8.56	0.031
Urea (mg/dl)	28.49±4.76	22.67±4.68	0.001
Creatinine (mg/dl)	0.48±0.16	0.78±0.16	0.001
Hb	7.09±1.06	15.29±1.12	0.001
HCT	21.22±3.52	45.49±3.11	0.001
Platelet	487.5±57.2	265.8±42.4	0.001
TIBC (µg\dl)	284.08±10.35	295.90±7.05	0.501
UIBC (µg\dl)	99.28±7.76	178.10±6.00	0.001
ALT (U\L)	32.6±3.2	22.2±1.1	0.048
AST (U\L)	40.85±3.24	23.77±1.29	0.002
ALK (U\L)	164.56±10.7	97.86±4.53	0.001
Direct bilirubin (mmol\l)	0.97±0.05	0.26±0.02	0.001
TSB (mmol\l)	2.72±0.15	0.82±0.03	0.001
T3 (ng\mL)	0.71±0.18	1.82±0.43	0.001
T4 (ng\mL)	13.21±4.32	38.1±35.38	0.001
TSH (ng\mL)	0.68±0.13	0.23±0.05	0.001
FSH (ng\mL)	3.52±0.71	6.59±0.79	0.001
LH (ng\mL)	1.08±0.20	2.68±0.49	0.001
Testosterone (ng\mL)	2.19±0.36	4.77±0.97	0.001
Kisspeptin (pg/ml)	259.87±34.3	77.72±11.4	0.001
IMA (ng\mL)	5004.5±360.3	2191.6±254.3	0.001
Inhibin-b (INH-b) pg/mL	66.44±12.6	153.84±17.5	0.001
MDA (ng\mL)	394.14±45.8	198.8±30.9	0.001

P-value < 0.05 is statistically significant

Table No. 2: Associations between several correlates and the hypothalamic-pituitary-gonadal axis

Characteristic	Kisspeptin		LH		FSH		Testosterone	
	r	P	r	P	r	P	r	P
Kisspeptin	1							
LH	-0.279	0.012*	1					
FSH	0.174	0.123	0.895	0.001*	1			
Testosterone	-0.232	0.038*	0.771	0.001*	0.739	0.001*	1	
IMA	-0.126	0.267	-0.254	0.023*	-0.323	0.004*	-0.204	0.068
INH-b	0.782	0.001*	0.018	0.876	0.084	0.461	-0.083	0.462
MDA	-0.063	0.587	0.081	0.477	0.104	0.358	-0.131	0.446
ALT	-0.226	0.043*	0.063	0.576	0.110	0.331	-0.128	0.256
AST	-0.235	0.036*	0.114	0.315	0.134	0.238	-0.148	0.191
ALK	-0.081	0.477	0.194	0.084	0.286	0.010*	-0.211	0.060
TIBC	-0.206	0.066	0.082	0.469	0.035	0.760	0.016	0.890
UIBC	-0.307	0.006*	-0.176	0.118	-0.114	0.316	-0.054	0.633
Ferritin	0.286	0.010*	0.111	0.329	0.111	0.326	-0.041	0.718
Iron	-0.004	0.972	-0.660	0.001*	-0.599	0.001*	-0.651	0.001*
Hb	0.117	0.303	-0.295	0.008*	-0.263	0.018*	-0.107	0.334
HCT	0.034	0.767	-0.214	0.057	-0.210	0.062	-0.098	0.385
Platelet	0.008	0.944	0.057	0.618	0.093	0.410	0.011	0.924
Urea	-0.279	0.012*	-0.192	0.089	-0.079	0.487	-0.098	0.389
Creatinine	-0.232	0.038*	-0.157	0.163	-0.118	0.298	-0.151	0.182
T3	0.174	0.123	-0.083	0.465	-0.127	0.261	0.012	0.915
T4	-0.279	0.012*	-0.074	0.517	-0.113	0.318	-0.013	0.909
TSH	-0.232	0.038*	-0.113	0.320	0.100	0.376	-0.038	0.735

r = Correlation coefficient P* = <0.05 is significant

Table No. 3: Risk assessment of serum kisspeptin

Risk factors	OR	95% CI	P
Age	0.927	0.46-1.83	0.014
LH	2.95	0.02-33.8	0.0319
FSH	0.403	0.04-3.87	0.416
Testosterone	0.86	0.39-1.57	0.04*
BMI	0.459	0.08-2.36	0. 341

*Significance at $P < 0.05$

DISCUSSION

The kisspeptin concentrations were elevated in men with beta thalassemia major (BTM), while levels of FSH, LH, and testosterone were significantly lower compared to the control group in the present study. This aligns with the study of Öztin et al[9], who examined 30 male patients with hypogonadotropic hypogonadism (HH) over the age of 30 and found significantly higher kisspeptin levels in this patient group, coupled with notably lower averages of FSH, LH, and testosterone. Similarly, De Sanctis et al[10] studied 11 adult men with thalassemia major, ranging in age from 26 to 54 years (34.3±8.8 years), compared to 12 age- and sex-matched patients with thalassemia major who exhibited normal pubertal development as a control group. Al-Zuhairy et al[11] also conducted research on 50 children with β-thalassemia major, aged 11 to 16 years, against a control group of 50 age- and sex-matched healthy adolescents. The results indicated that male patients had significantly lower serum levels of FSH, LH, testosterone, and estradiol compared to controls, suggesting an altered function of the hypothalamic cells that secrete luteinizing hormone-releasing hormone (LHRH).[12]

rashid

In transfusion-dependent thalassemia (TDT), chronic blood transfusions result in iron overload within the pituitary and hypothalamus, this accumulation of iron catalyzes the production of reactive oxygen species (ROS), which can damage gonadotropes and GnRH neurons, disrupting the pulsatility of GnRH. Approximately 50% of men with TDT develop hypogonadotropic hypogonadism (HH), characterized by impaired secretion of GnRH and gonadotropins, leading to low testosterone levels and compromised spermatogenesis. Ali et al[21] have shown significantly elevated serum kisspeptin levels in thalassemic men with HH, which were inversely correlated with testosterone and gonadotropin levels. Clark et al[14] also elevated kisspeptin may act as a biomarker for disruption of the hypothalamic-pituitary-gonadal (HPG) axis. According to Skorupskaite et al[15], the rise in kisspeptin levels is likely attributable to reduced testosterone feedback, resulting in unopposed kisspeptin secretion that cannot effectively stimulate GnRH due to the damage occurring downstream.

Our study's association reveals significant dysregulation within the hypothalamic-pituitary-gonadal (HPG) axis, marked by distinct correlations among kisspeptin, gonadotropins, testosterone, and various metabolic and hematological parameters. We found a strong positive correlation between kisspeptin and inhibin, suggesting that kisspeptin may play a compensatory role in regulating Sertoli cell function, potentially offsetting impaired spermatogenesis associated with thalassemia.[16,17] Additionally, there were notable inverse correlations with LH (r=−0.279, p=0.012) and testosterone (r=−0.232, p=0.038), indicating disrupted feedback mechanisms, likely caused by chronic iron overload that suppresses hypothalamic kisspeptin release.[18] We also observed negative correlations with ALT (r=−0.226, p=0.043), AST (r=−0.235, p=0.036), and TSH (r=−0.232, p=0.038), emphasizing the multisystem involvement in the suppression of the HPG axis. These findings highlight the multifactorial disruption of the HPG axis in thalassemia, driven by factors such as iron overload, hepatic dysfunction, and anemia. Kisspeptin's dual role-stimulating inhibin B while inversely correlating with LH and testosterone-positions it as a potential biomarker for gonadal health.[11,15] While iron chelation and hormonal replacement remain critical, targeted therapies involving kisspeptin merit further investigation.[19]

This study indicated a potential interplay between kisspeptin regulation and gonadal function, LH and Testosterone levels, along with an age-dependent effect .This triad (↑Kisspeptin, ↓LH, ↓Testosterone) suggests a combination of primary and central hypogonadism due to iron overload, which is a hallmark complication of transfusion-dependent thalassemia. There are three primary mechanisms involved: First, iron deposition directly damages Leydig cells in the testes, impairing testosterone synthesis. Srisukh et al[20] documented the presence of iron deposition and fibrosis in Leydig cells, which was directly correlated with the severity of hypogonadism. The consequence of this is that low testosterone results in reduced negative feedback to both the hypothalamus and the pituitary gland. Second, iron accumulation occurs in the pituitary gland, particularly affecting gonadotrophs-cells responsible for producing LH and FSH. Bozdağ et al[21] demonstrated that MRI hypointensity in the pituitary, indicative of iron deposition, correlates with low LH/FSH levels in thalassemic males. Consequently, despite low testosterone levels (which would normally stimulate LH release through diminished negative feedback), the damaged pituitary gland fails to adequately respond by increasing LH secretion. This result in secondary (hypogonadotropic) hypogonadism superimposed upon primary failure. There is a compensatory increase in Kisspeptin (↑Kisspeptin). Kisspeptin neurons in the hypothalamus exhibit relative resistance to iron toxicity when compared to GnRH neurons or pituitary gonadotrophs. The hypothalamus senses the chronically low testosterone levels resulting from the combined

testicular and pituitary failure. Yeo et al[22] suggested that kisspeptin neurons integrate metabolic and endocrine signals, driving GnRH release in response to low sex steroid levels. However, this compensatory mechanism fails in the long term due to damage to the pituitary gonadotropins and their inability to respond effectively to GnRH. Furthermore, the GnRH cells themselves may be partially damaged by chronic iron exposure or inflammation. Also Oxidative stresslead to chronic diseases.[23]

CONCLUSION

Elevated kisspeptin levels have been observed in Iraqi males diagnosed with beta thalassemia major. Additionally, lower concentrations of LH and testosterone, along with older age, serve as independent predictors of increased kisspeptin levels in men. The importance of kisspeptin in the regulation of the gonadal-pituitary axis and may inform the management of hypogonadism in men.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Bushra Mussad Kadim, Ekhlas Abdallah Hassan
Drafting or Revising Critically:	Bushra Mussad Kadim, Ekhlas Abdallah Hassan
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No. 3434/QM/Approval/ JSDJNEHU Dated 15.08.2024

REFERENCES

1. Anderson RA, Millar RP. The roles of kisspeptin and neurokinin B in GnRH pulse generation in humans, and their potential clinical application. J Neuroendocrinol 2022;34(5):e13081.
2. 2, Koysombat K, Tsoutsouki J, Patel AH, Comninos AN, Dhillo WS, Abbara A. Kisspeptin and Neurokinin B: roles in reproductive health. Physiol Rev 2025;105(2):707-64.
3. Dhillo WS, Chaudhri OB, Patterson M, Thompson EL, Murphy KG, Badman MK, et al. Kisspeptin-54 stimulates the hypothalamic-pituitary gonadal axis in human males. J Clin Endocrinol Metab 2021;90(12):6609-15.
4. Thompson EL, Patterson M, Murphy KG, Smith KL, Dhillo WS, Todd JF, et al. Central and peripheral administration of kisspeptin-10 stimulates the hypothalamic-pituitary-gonadal axis. J Neuroendocrinol 2004;16(10):850-8.
5. Gomes RR. Hypogonadotropic Hypogonadism in a Female Patient with Thalassemia Major. Int J Blood Res Disord 2021;8:066
6. Kotani M, Katagiri F, Hirai T, Kagawa J. Plasma kisspeptin levels in male cases with hypogonadism. Endocrine J 2014;61(11):1137-40.
7. Cao Y, Li Z, Jiang W, Ling Y, Kuang H. Reproductive functions of kisspeptin/KISS1R systems in the periphery. Reprod Biol Endocrinol 2019; 17(1):65.
8. Selvaraj S, Ohga H, Nyuji M, Kitano H, Nagano N, Yamaguchi A, et al. Subcutaneous administration of Kiss1 pentadecapeptide accelerates spermatogenesis in prepubertal male chub mackerel. Comp Biochem Physiol Mol Integr Physiol 2013;166(2):228-36.
9. Öztin H, Çağıltay E, Çağlayan S, Kaplan M, Akpak YK, Karaca N, Tığlıoğlu M. Kisspeptin levels in idiopathic hypogonadotropic hypogonadism diagnosed male patients and its relation with glucose-insulin dynamic. Gynecol Endocrinol 2016; 32(12):991-4.
10. De Sanctis V, Elsedfy H, Soliman AT, Elhakim IZ, Pepe A, Kattamis C, et al. Acquired hypogonadotropic hypogonadism in thalassemia major patients: an underdiagnosed condition. Mediterr J Hematol Infect Dis 2016; 8:e2016001.
11. Al-Zuhairy TNAHS, Al-Ali AJR. Evaluation of reproductive hormones in patients with β-thalassemia major in Misan Province, Iraq. Med Legal Update 2020; 20:274–279.
12. Safarinejad MR. Evaluation of semen quality, endocrine profile and hypothalamus pituitary testis axis in male patients with homozygous β-thalassemia major. J Urol 2008; 179:2327-32.
13. Ali S, Ullah H, Jahan S. Changes in kisspeptin, growth and thyroid hormones in thalassemic patients of pubertal age group. Pak J Zool 2017;49(3).
14. Clarke H, Dhillo WS, Jayasena CN. Comprehensive Review on Kisspeptin and Its Role in Reproductive Disorders. Endocrinol Metab (Seoul) 2015; 30(2):124-41.
15. Skorupskaite K, George JT, Anderson RA. The kisspeptin-GnRH pathway in human reproductive health and disease. Hum Reprod Update 2014;20(4):485-500.
16. Peng YJ, Tang XT, Shu HS, Dong W, Shao H, Zhou BO. Sertoli cells are the source of stem cell factor for spermatogenesis. Development 2023;150(6): dev200706.
17. Wang X, Liu X, Qu M, Li H. Sertoli cell-only syndrome: advances, challenges, and perspectives in genetics and mechanisms. Cell Mol Life Sci 2023; 80(3):67.
18. Santomauro F, Lorini C, Tanini T, Indiani L, Lastrucci V, Comodo N. Adherence to

Mediterranean diet in a sample of Tuscan adolescents. Nutrition 2014; 30)11-12): 1379-83.
19. Uenoyama Y, Inoue N, Maeda KI, Tsukamura H. The roles of kisspeptin in the mechanism underlying reproductive functions in mammals. J Reprod Develop 2018;64(6):469-76.
20. Srisukh S, Ongphiphadhanakul B, Bunnag P. Hypogonadism in thalassemia major patients. J Clin Transl Endocrinol 2016;5:42-45.
21. Bozdağ M, Bayraktaroğlu S, Aydınok Y, Çallı MC. MRI assessment of pituitary iron accumulation by using pituitary-R2 in β-thalassemia patients. Acta Radiologica 2017;59(6):732-9.
22. Yeo SH, Colledge WH. The Role of Kiss1 Neurons As Integrators of Endocrine, Metabolic, and Environmental Factors in the Hypothalamic-Pituitary-Gonadal Axis. Front Endocrinol (Lausanne) 2018;9:188.
23. Hassan EA. The Relation between homocysteine, oxidative stress and atherosclerosis disease. IJPHRD 2019;10(7):537.