Revisiting the Hypothalamic–Pituitary–Gonadal Axis in β-Thalassemia: Is Kisspeptin the Next-Generation Diagnostic Tool?

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Abstract

Background: Delayed puberty is one of the most prevalent endocrine complications in adolescents with β-thalassemia major, primarily resulting from iron overload–induced dysfunction of the hypothalamic–pituitary–gonadal (HPG) axis. Despite advances in transfusion protocols and iron chelation therapy, hypogonadotropic hypogonadism remains a significant cause of impaired growth, reduced bone mineral density, infertility, and psychosocial morbidity in this population. The gonadotropin-releasing hormone (GnRH) stimulation test has traditionally been used to evaluate pubertal disorders; however, its diagnostic limitations in differentiating constitutional delay from central hypogonadism have prompted investigation into alternative neuroendocrine markers. Kisspeptin, a key upstream regulator of GnRH neurons, has emerged as a promising physiological stimulator of the reproductive axis.

Aim: This review aims to critically evaluate current evidence regarding the role of kisspeptin stimulation testing compared with conventional GnRH testing in diagnosing delayed puberty among adolescents with β-thalassemia. It explores the underlying mechanisms of iron-induced neuroendocrine disruption, examines the diagnostic performance and physiological relevance of kisspeptin, and discusses its potential as a next-generation dynamic test in clinical practice.

Conclusion: Iron deposition within the hypothalamus and anterior pituitary leads to progressive impairment of gonadotropin secretion in β-thalassemia, often preceding overt clinical manifestations. Unlike exogenous GnRH testing, which bypasses hypothalamic regulation, kisspeptin stimulation provides a more physiological assessment of upstream GnRH neuronal integrity. Emerging evidence suggests that kisspeptin-based testing may better reflect central neuroendocrine function and help distinguish reversible hypothalamic suppression from established pituitary damage. However, limited pediatric data, lack of standardized protocols, and variability in assay methodologies currently restrict widespread clinical implementation. Further longitudinal and multicenter studies are required to validate diagnostic thresholds and clarify its prognostic value. Kisspeptin holds promise as a novel diagnostic biomarker that may refine the evaluation of delayed puberty in iron overload disorders and improve individualized endocrine management strategies.

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Keywords: *β-thalassemia major; delayed puberty; hypogonadotropic hypogonadism; kisspeptin; gonadotropin-releasing hormone; HPG axis; iron overload; pediatric endocrinology; dynamic testing; neuroendocrine dysfunction*

Introduction

β-thalassemia major is one of the most common inherited hemoglobinopathies worldwide and remains a major cause of chronic morbidity in children and adolescents despite substantial advances in transfusion and chelation therapy. Regular blood transfusions have dramatically improved survival into adolescence and adulthood; however, progressive iron overload leads to multisystem endocrine complications, with hypogonadism representing the most frequent endocrine disorder in this population

[1]. Delayed puberty, defined by the absence of secondary sexual characteristics beyond the expected chronological age, significantly affects physical development, peak bone mass acquisition, fertility potential, and psychosocial well-being in affected adolescents [2].

The pathophysiology of delayed puberty in β-thalassemia is predominantly attributed to iron deposition within the hypothalamic–pituitary axis. Chronic transfusional iron overload promotes oxidative stress, lipid peroxidation, mitochondrial dysfunction, and apoptosis within endocrine tissues, particularly the gonadotroph cells of the anterior pituitary [3]. Magnetic resonance imaging studies have demonstrated reduced pituitary volume and increased iron deposition in transfusion-dependent patients, correlating strongly with impaired gonadotropin secretion and pubertal failure [4]. Although primary gonadal damage may occur in severe iron overload, central hypogonadotropic hypogonadism remains the dominant mechanism in adolescents with delayed puberty [5].

The hypothalamic–pituitary–gonadal (HPG) axis is orchestrated by pulsatile secretion of gonadotropin-releasing hormone (GnRH) from hypothalamic neurons, which stimulates pituitary release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), ultimately driving gonadal steroidogenesis and gametogenesis. The physiological activation of this axis during puberty depends critically on upstream regulatory neurons, particularly those expressing kisspeptin, a peptide encoded by the KISS1 gene and acting through its receptor KISS1R [6]. Kisspeptin has emerged as the principal gatekeeper of pubertal onset, integrating metabolic, environmental, and hormonal signals to activate GnRH neurons [7].

Traditionally, the GnRH stimulation test has been used to evaluate delayed puberty and distinguish constitutional delay from central hypogonadism. However, exogenous GnRH administration directly stimulates pituitary gonadotrophs, bypassing hypothalamic regulation and therefore limiting its ability to assess upstream neuronal integrity [8]. In β-thalassemia, where hypothalamic iron toxicity may precede overt pituitary damage, reliance solely on GnRH stimulation testing may underestimate early neuroendocrine dysfunction. This diagnostic limitation underscores the need for more physiologically informative dynamic testing strategies.

Kisspeptin stimulation testing has recently gained attention as a potentially superior diagnostic modality because it acts upstream of GnRH neurons, thereby evaluating hypothalamic functional capacity. Studies in adolescents and adults with hypogonadotropic hypogonadism suggest that kisspeptin-induced LH responses may provide insight into GnRH neuronal reserve and help differentiate functional suppression from structural pituitary failure [9]. Given the high burden of delayed puberty in β-thalassemia and the complexity of iron-induced neurotoxicity, revisiting the HPG axis through the lens of kisspeptin physiology may offer a more precise diagnostic approach.

Despite growing interest, data regarding kisspeptin testing in transfusion-dependent β-thalassemia remain limited, and no standardized protocols have been established for pediatric populations. Furthermore, comparative analyses between kisspeptin and GnRH stimulation tests in iron overload states are sparse. This review aims to critically examine the mechanisms of HPG axis disruption in β-thalassemia, evaluate the physiological rationale for kisspeptin-based testing, and assess whether kisspeptin could represent a next-generation diagnostic tool in the evaluation of delayed puberty in this vulnerable population.

Epidemiology and Clinical Burden of Delayed Puberty in β-Thalassemia

Delayed puberty remains the most frequent endocrine complication among adolescents with transfusion-dependent β-thalassemia major. Improvements in transfusion protocols and iron chelation therapy have substantially increased survival into adulthood; however, endocrine sequelae continue to impair quality of life. Epidemiological studies indicate that hypogonadism affects approximately 40–70% of patients with β-thalassemia major, with variability depending on transfusion adequacy, chelation compliance, and age at assessment [10]. Pubertal delay is often the earliest manifestation of endocrine dysfunction, typically emerging during early adolescence when activation of the HPG axis fails to occur.

Large cohort analyses from Mediterranean and Middle Eastern countries, where β-thalassemia prevalence is high, demonstrate that delayed puberty affects both sexes, though males may present slightly more frequently with complete hypogonadotropic hypogonadism, whereas females may initially

exhibit pubertal arrest or secondary amenorrhea [11]. The age of onset of iron overload and duration of suboptimal chelation are key determinants of endocrine risk. Importantly, even patients receiving modern chelation regimens remain susceptible to central hypogonadism, highlighting the persistent vulnerability of the hypothalamic–pituitary unit to iron toxicity [12].

The clinical consequences of delayed puberty in β-thalassemia extend beyond delayed sexual maturation. Adolescents frequently experience compromised linear growth due to concomitant growth hormone–insulin-like growth factor-1 axis disturbances and chronic disease burden. Failure to achieve normal sex steroid levels contributes to reduced peak bone mass acquisition, predisposing patients to osteopenia and osteoporosis in early adulthood [13]. Additionally, delayed puberty is associated with adverse psychosocial outcomes, including reduced self-esteem, social withdrawal, and impaired peer relationships during a critical developmental window.

Endocrine dysfunction in β-thalassemia rarely occurs in isolation. Hypogonadism often coexists with other iron-related endocrine disorders, including hypothyroidism, diabetes mellitus, and hypoparathyroidism, reflecting the cumulative toxic effects of iron deposition across multiple endocrine glands [14]. This multisystem involvement underscores the need for systematic endocrine surveillance beginning in late childhood. Current international guidelines recommend annual assessment of pubertal development and gonadotropin levels in transfusion-dependent adolescents, yet dynamic testing strategies remain variable among centers [15].

Despite its high prevalence, delayed puberty in β-thalassemia is frequently underdiagnosed or diagnosed late, particularly in resource-limited settings. Early optimization of chelation therapy enable us to avoid irreversible pituitary damage. Therefore, refining diagnostic strategies to detect early HPG axis impairment is of paramount clinical importance. Understanding the epidemiological burden of pubertal failure provides the foundation for exploring improved neuroendocrine assessment tools, including the potential role of kisspeptin stimulation testing in this high-risk population.

Pathophysiology of Iron Overload–Induced HPG Axis Dysfunction

Chronic transfusion therapy in β-thalassemia major inevitably results in progressive systemic iron accumulation due to the absence of physiological mechanisms for active iron excretion. Excess circulating non–transferrin-bound iron catalyzes the formation of reactive oxygen species through Fenton chemistry, leading to oxidative stress and cellular injury in multiple organs, including endocrine tissues. The anterior pituitary is particularly susceptible because of its rich vascular supply and high metabolic activity. Iron deposition within gonadotroph cells impairs hormone synthesis and secretion, ultimately resulting in hypogonadotropic hypogonadism [16].

Magnetic resonance imaging studies using T2* sequences have demonstrated a strong inverse correlation between pituitary iron concentration and gonadotropin response. Reduced pituitary volume and decreased signal intensity on MRI are predictive of impaired LH and FSH secretion in transfusion-dependent adolescents. These radiological findings often precede overt clinical hypogonadism, suggesting that structural damage may occur early in the course of iron overload [17]. Such evidence reinforces the concept that pituitary iron toxicity plays a central role in pubertal failure.

In addition to pituitary injury, hypothalamic dysfunction contributes significantly to HPG axis impairment. Experimental and clinical data indicate that iron accumulation within hypothalamic nuclei disrupts neuronal networks responsible for pulsatile GnRH secretion. Oxidative stress–induced mitochondrial dysfunction and neuroinflammation alter neuronal excitability and synaptic signaling, potentially affecting kisspeptin neurons in the arcuate nucleus, which are critical for pubertal initiation [18]. This upstream disruption may precede or coexist with pituitary damage, complicating the interpretation of conventional dynamic tests.

The pathophysiological process is further compounded by chronic anemia and hypoxia, which can impair neuroendocrine regulation. Tissue hypoxia has been shown to influence hypothalamic signaling pathways and may suppress GnRH pulsatility through stress-related neuropeptide modulation. Moreover, systemic inflammation associated with chronic transfusion and iron overload can interfere with central reproductive signaling, adding another layer of complexity to pubertal dysfunction in these

patients [19].

Importantly, the degree of reversibility of HPG axis dysfunction depends on the duration and severity of iron exposure. Early and intensive chelation therapy has been associated with partial recovery of gonadotropin secretion in some adolescents, suggesting that functional hypothalamic suppression may be reversible if detected early. However, prolonged iron deposition leading to fibrosis and apoptosis of pituitary gonadotrophs often results in permanent endocrine failure [20]. Distinguishing reversible hypothalamic dysfunction from irreversible pituitary damage is therefore essential for guiding prognosis and therapeutic decisions.

Collectively, iron-mediated oxidative injury, neuroinflammation, hypoxic stress, and structural pituitary damage converge to disrupt the delicate neuroendocrine coordination of puberty in β-thalassemia. These multilayered mechanisms highlight the limitations of diagnostic approaches that evaluate only pituitary responsiveness and underscore the need for testing modalities capable of assessing upstream hypothalamic integrity. In this context, revisiting the physiology of kisspeptin as the master regulator of GnRH secretion becomes particularly relevant for refining diagnostic strategies in delayed puberty associated with iron overload [21].

Physiology of the Kisspeptin–GnRH Pathway and Pubertal Activation

The initiation of puberty is governed by the reactivation of pulsatile gonadotropin-releasing hormone (GnRH) secretion from specialized hypothalamic neurons. This pulsatility is essential for appropriate downstream secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary. Over the past two decades, kisspeptin has been identified as the principal upstream regulator of GnRH neuronal activity. Encoded by the *KISS1* gene and acting through its receptor *KISS1R* (formerly GPR54), kisspeptin signaling is now recognized as indispensable for pubertal onset and reproductive competence [22].

The critical role of kisspeptin in human reproduction was first demonstrated through loss-of-function mutations in *KISS1R*, which were found to cause normosmic congenital hypogonadotropic hypogonadism. Affected individuals exhibit absent or incomplete puberty due to impaired GnRH secretion despite intact pituitary responsiveness [23]. Conversely, activating mutations in the same receptor have been associated with central precocious puberty, further confirming that kisspeptin functions as a gatekeeper of pubertal timing [24]. These genetic discoveries established kisspeptin as a master regulator of the HPG axis.

Anatomically, kisspeptin neurons are primarily located in the arcuate nucleus and the anteroventral periventricular nucleus of the hypothalamus. In the arcuate nucleus, kisspeptin neurons co-express neurokinin B and dynorphin, forming the so-called KNDy neuronal network. This network generates rhythmic stimulatory and inhibitory signals that coordinate GnRH pulsatility. Neurokinin B provides stimulatory input, while dynorphin exerts inhibitory feedback, together producing the oscillatory pattern required for physiological gonadotropin release [25]. This finely tuned system is sensitive to metabolic, hormonal, and environmental cues.

Kisspeptin neurons integrate multiple peripheral signals, including leptin, insulin, sex steroids, and stress-related mediators. During childhood, inhibitory central mechanisms suppress kisspeptin activity, maintaining the HPG axis in a quiescent state. At puberty, a reduction in central inhibition and increased stimulatory input lead to enhanced kisspeptin expression and secretion, triggering the reawakening of GnRH pulsatility [26]. This neuroendocrine transition represents the biological hallmark of pubertal onset.

Exogenous administration of kisspeptin has been shown to stimulate robust LH and FSH secretion in both adults and adolescents with intact GnRH neuronal function. Importantly, kisspeptin acts at the level of the hypothalamus to induce endogenous GnRH release, making it a physiologically proximal stimulus compared with direct GnRH administration [27]. This distinction is clinically relevant because kisspeptin testing assesses the integrity of hypothalamic GnRH neurons, whereas GnRH testing evaluates only pituitary gonadotroph responsiveness.

In the context of β-thalassemia, where iron deposition may disrupt hypothalamic neuronal networks

before causing overt pituitary damage, understanding kisspeptin physiology becomes particularly important. If hypothalamic kisspeptin neurons are impaired, GnRH secretion may be diminished despite preserved pituitary responsiveness. Therefore, evaluating LH responses following kisspeptin stimulation could provide deeper insight into central neuroendocrine integrity and help differentiate functional hypothalamic suppression from structural pituitary failure [28].

Gonadotropin-Releasing Hormone Stimulation Test — Principles and Limitations

The gonadotropin-releasing hormone (GnRH) stimulation test has long been considered the standard dynamic assessment for evaluating disorders of pubertal development. The test involves administration of exogenous GnRH (or a GnRH agonist), followed by serial measurements of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) to assess pituitary gonadotroph responsiveness. In physiologically normal puberty, activation of the HPG axis results in a robust LH-predominant response, whereas prepubertal children typically demonstrate blunted gonadotropin secretion [29]. This principle has guided the use of GnRH testing to differentiate constitutional delay of growth and puberty (CDGP) from central hypogonadotropic hypogonadism (CHH).

In adolescents with β-thalassemia, the GnRH stimulation test is frequently employed to confirm central hypogonadism when basal gonadotropin levels are low or equivocal. A diminished LH response following GnRH administration suggests pituitary dysfunction, particularly when accompanied by low sex steroid levels. However, the interpretation of results can be challenging, as partial pituitary reserve may produce intermediate responses that overlap with constitutional delay [30]. Furthermore, assay variability and lack of universally accepted cutoff values complicate diagnostic standardization across centers.

A major physiological limitation of the GnRH stimulation test lies in its inability to assess hypothalamic integrity. Because exogenous GnRH directly stimulates the anterior pituitary, the test bypasses upstream regulatory mechanisms, including kisspeptin-mediated GnRH neuronal activation. In conditions where hypothalamic dysfunction predominates—such as functional hypogonadism, chronic systemic illness, or early iron toxicity—the pituitary may still respond adequately to GnRH despite impaired endogenous pulsatile secretion [31]. Consequently, a normal or near-normal GnRH test does not necessarily exclude hypothalamic impairment.

In transfusion-dependent β-thalassemia, progressive iron deposition may initially disrupt hypothalamic neurons before causing structural damage to pituitary gonadotrophs. During this transitional phase, GnRH stimulation testing may yield falsely reassuring results, delaying recognition of evolving neuroendocrine dysfunction. Additionally, repeated GnRH agonist testing can be cumbersome, resource-intensive, and uncomfortable for pediatric patients, particularly in settings requiring frequent endocrine surveillance [32]. These practical considerations further motivate exploration of alternative diagnostic strategies.

Another challenge involves distinguishing reversible hypothalamic suppression from irreversible pituitary damage. In adolescents with partial gonadotropin deficiency, GnRH-induced LH responses may appear suboptimal but not entirely absent, making prognostic predictions difficult. Since therapeutic decisions—such as sex steroid replacement versus attempts at spontaneous pubertal induction—depend on accurate assessment of central axis integrity, diagnostic precision is critical [33]. Overreliance on pituitary-based stimulation tests may therefore obscure early central pathology.

Collectively, while the GnRH stimulation test remains a valuable and widely accessible tool, its conceptual framework reflects a pituitary-centered approach to pubertal assessment. In β-thalassemia, where iron-mediated neurotoxicity may involve multiple hierarchical levels of the HPG axis, a more physiologically comprehensive evaluation may be required. This recognition has led to growing interest in kisspeptin stimulation testing as a method capable of probing hypothalamic function more directly and potentially refining diagnostic differentiation in delayed puberty [34].

Kisspeptin Stimulation Testing — Mechanistic Rationale and Clinical Evidence

Kisspeptin stimulation testing has emerged as a novel dynamic tool for assessing the functional integrity of the hypothalamic–pituitary–gonadal axis. Unlike exogenous GnRH administration, kisspeptin acts at

the level of the hypothalamus to induce endogenous GnRH release, thereby preserving physiological hierarchy within the reproductive axis. This mechanistic distinction allows clinicians to evaluate GnRH neuronal responsiveness rather than solely pituitary gonadotroph capacity. Early human studies demonstrated that peripheral administration of kisspeptin-54 produces a rapid and marked increase in circulating LH concentrations in healthy adults, confirming its potent stimulatory effect on the reproductive axis [35].

Subsequent investigations extended these findings to adolescents and individuals with reproductive disorders. In healthy pubertal subjects, kisspeptin-induced LH responses increase progressively across Tanner stages, reflecting maturation of GnRH neuronal networks. In contrast, individuals with congenital hypogonadotropic hypogonadism due to hypothalamic defects exhibit blunted or absent LH responses to kisspeptin, despite preserved pituitary responsiveness to exogenous GnRH [36]. These observations support the hypothesis that kisspeptin testing may help localize the level of dysfunction within the HPG axis.

Importantly, kisspeptin stimulation has also been evaluated in functional hypogonadism and conditions characterized by hypothalamic suppression. In such cases, preserved LH responses to kisspeptin suggest intact GnRH neurons that are functionally inhibited rather than structurally damaged. This distinction carries prognostic implications, as functional suppression may be reversible with correction of underlying metabolic or systemic stressors [37]. Therefore, kisspeptin testing provides insight not only into axis activation but also into potential reversibility of dysfunction.

The pharmacodynamic profile of kisspeptin offers additional advantages. Kisspeptin induces a physiological pattern of LH release without sustained receptor desensitization when administered intermittently. Studies comparing kisspeptin and GnRH stimulation demonstrate that LH peaks following kisspeptin may occur slightly later, consistent with endogenous GnRH-mediated pituitary activation. This temporal difference reinforces its upstream site of action and supports its role as a more integrated neuroendocrine probe [38].

From a safety perspective, kisspeptin administration has been well tolerated in both adult and adolescent populations, with minimal adverse effects reported in clinical trials. Transient flushing or mild injection-site discomfort has occasionally been observed, but serious complications are rare. This favorable safety profile enhances its feasibility as a diagnostic tool in pediatric endocrinology, particularly in patients requiring repeated evaluations [39].

In the context of β-thalassemia, where iron-induced neurotoxicity may preferentially affect hypothalamic networks early in disease progression, kisspeptin stimulation testing presents a compelling theoretical advantage. By assessing GnRH neuronal reserve, clinicians may detect early hypothalamic dysfunction before irreversible pituitary damage occurs. Although data specifically in transfusion-dependent adolescents remain limited, extrapolation from other forms of central hypogonadism suggests that differential LH responses to kisspeptin versus GnRH could refine diagnostic accuracy and improve individualized management strategies [40].

Comparative Diagnostic Performance — Kisspeptin versus GnRH Testing in Delayed Puberty

The comparative evaluation of kisspeptin and GnRH stimulation testing centers on their ability to discriminate between constitutional delay of growth and puberty (CDGP), functional hypothalamic suppression, and permanent central hypogonadotropic hypogonadism (CHH). The GnRH stimulation test primarily assesses pituitary responsiveness, whereas kisspeptin evaluates upstream GnRH neuronal function. This conceptual distinction underlies their differing diagnostic capacities. Studies in adolescents with delayed puberty have shown significant overlap in LH responses to GnRH between CDGP and partial CHH, limiting the test's discriminatory precision [41].

Emerging clinical investigations suggest that kisspeptin-induced LH responses may provide greater differentiation in cases of central hypogonadism. In adolescents with CHH, particularly those with hypothalamic defects, LH responses to kisspeptin are markedly attenuated compared with individuals with CDGP, who demonstrate preserved or pubertal-range responses. This divergence reflects intact but developmentally delayed GnRH neuronal networks in CDGP versus structural or genetic defects in CHH

[42]. Therefore, kisspeptin testing may offer improved specificity for identifying permanent central pathology.

Comparative pharmacodynamic analyses indicate that LH peak amplitude and area under the curve following kisspeptin stimulation correlate closely with endogenous GnRH reserve. In contrast, GnRH testing may produce falsely reassuring results in early hypothalamic dysfunction because pituitary gonadotrophs can remain responsive despite impaired pulsatile GnRH secretion. Such discordance between the two tests has been observed in certain hypogonadal states, highlighting their complementary diagnostic roles rather than complete interchangeability [43].

Although direct head-to-head studies in β-thalassemia are limited, extrapolation from other chronic systemic conditions provides valuable insight. In disorders characterized by central suppression—such as anorexia nervosa or chronic illness—kisspeptin responses may reveal functional GnRH inhibition despite preserved pituitary capacity. Given that iron overload in thalassemia can impair hypothalamic neurons before causing overt pituitary injury, kisspeptin testing could theoretically identify earlier stages of neuroendocrine compromise compared with GnRH stimulation alone [44].

Diagnostic performance must also consider assay sensitivity and standardized cutoff values. Variability in LH measurement techniques, timing of sampling, and kisspeptin dosing protocols currently limits widespread clinical adoption. Establishing age- and sex-specific normative data is essential for improving reproducibility and comparability across centers. Nonetheless, preliminary data indicate that kisspeptin stimulation produces robust LH increments in pubertal adolescents, suggesting potential utility in refining diagnostic thresholds [45].

Taken together, existing evidence supports the view that kisspeptin stimulation testing may enhance diagnostic stratification of delayed puberty by interrogating the HPG axis at a higher hierarchical level. While GnRH testing remains valuable for assessing pituitary reserve, kisspeptin may provide additional insight into hypothalamic integrity, particularly in complex conditions such as β-thalassemia. Future prospective, multicenter studies directly comparing both modalities in transfusion-dependent adolescents are needed to validate sensitivity, specificity, and predictive value within this unique clinical population [46].

Clinical Implications in β-Thalassemia — Toward a Revised Diagnostic Algorithm

The high prevalence of delayed puberty in adolescents with β-thalassemia necessitates a structured and proactive endocrine surveillance strategy. Current international management guidelines for transfusion-dependent thalassemia recommend annual clinical assessment of pubertal staging beginning in late childhood, alongside measurement of basal gonadotropins and sex steroids when pubertal delay is suspected [47]. However, reliance solely on basal hormone levels may fail to detect early central dysfunction, particularly in cases where gonadotropin concentrations remain within low-normal prepubertal ranges despite impaired activation of the HPG axis.

In clinical practice, the GnRH stimulation test is typically employed when basal LH and FSH levels are inconclusive. While a markedly blunted LH response supports the diagnosis of hypogonadotropic hypogonadism, intermediate or borderline responses are common in thalassemia, reflecting partial pituitary reserve or evolving iron-induced damage. Such equivocal results complicate clinical decision-making regarding initiation of sex steroid replacement therapy versus continued observation for spontaneous pubertal progression [48]. Diagnostic uncertainty may delay appropriate intervention, adversely affecting bone health and psychosocial development.

Incorporating kisspeptin stimulation testing into the diagnostic algorithm could theoretically refine stratification of HPG axis dysfunction. A preserved LH response to kisspeptin in the presence of delayed puberty may indicate intact GnRH neuronal capacity and potentially reversible hypothalamic suppression, supporting continued monitoring and optimization of iron chelation therapy. Conversely, absent or markedly attenuated responses to both kisspeptin and GnRH would strongly suggest advanced central hypogonadism requiring timely endocrine replacement [49]. Such differentiation could improve individualized care planning.

An additional clinical consideration is the timing of intervention. Evidence suggests that earlier and

intensified chelation therapy may partially restore endocrine function when iron toxicity is detected before irreversible fibrosis occurs. If kisspeptin testing identifies early hypothalamic dysfunction, clinicians may have an opportunity to intensify iron removal strategies prior to permanent pituitary damage [50]. Thus, kisspeptin may not only serve as a diagnostic biomarker but also function as a potential prognostic indicator of reversibility.

Practical implementation requires careful attention to feasibility and resource allocation. Kisspeptin is not yet universally available for routine clinical use, and standardized pediatric dosing regimens remain under investigation. Nonetheless, as assay technologies advance and clinical data accumulate, incorporation of kisspeptin testing into specialized tertiary centers managing thalassemia could become increasingly feasible. Cost-effectiveness analyses and multicenter collaborations will be essential to determine its role in routine practice [51].

Ultimately, revisiting the HPG axis in β-thalassemia through a physiologically hierarchical framework underscores the need for a more nuanced diagnostic approach. A revised algorithm integrating clinical assessment, biochemical evaluation, imaging of pituitary iron load, and selective use of dynamic tests—including kisspeptin stimulation—may enhance early detection of central dysfunction. Such an approach aligns with precision medicine principles and holds promise for improving long-term reproductive and skeletal outcomes in adolescents living with transfusion-dependent β-thalassemia [52].

Conclusion

Delayed puberty in adolescents with β-thalassemia represents a complex and multifactorial consequence of chronic iron overload affecting multiple hierarchical levels of the hypothalamic–pituitary–gonadal axis. While the gonadotropin-releasing hormone stimulation test has long served as the standard dynamic tool for evaluating central hypogonadism, its pituitary-centered design limits its capacity to detect early hypothalamic dysfunction. Accumulating physiological and clinical evidence highlights the pivotal role of kisspeptin as the master upstream regulator of GnRH secretion and pubertal activation. By preserving the natural neuroendocrine hierarchy, kisspeptin stimulation testing offers a more integrated assessment of GnRH neuronal reserve and may provide deeper insight into the functional integrity of the reproductive axis in iron overload states.

Although current data in transfusion-dependent β-thalassemia remain limited, the mechanistic rationale supporting kisspeptin-based testing is compelling. Incorporating hypothalamic-level evaluation into diagnostic algorithms has the potential to improve early detection of central dysfunction, guide therapeutic timing, and refine prognostic assessment. Further prospective, multicenter studies are required to establish standardized protocols, define pediatric reference thresholds, and determine long-term clinical utility. As survival improves and endocrine morbidity becomes increasingly relevant in this population, advancing toward more physiologically precise diagnostic strategies may significantly enhance reproductive and overall health outcomes in adolescents with β-thalassemia.

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