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Study the effect of hepcidin antimicrobial peptide gene polymorphism on fertility hormone in female with beta-thalassemia major

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Abstract:

BACKGROUND: Beta thalassemia major is a hereditary blood disorder characterized by defective hemoglobin production, leading to severe anemia and multiple complications. Among these, reproductive dysfunction is a significant concern for affected females, often linked to iron overload from frequent blood transfusions. The hepcidin antimicrobial peptide *(HAMP)* gene, a key regulator of iron homeostasis, may play a crucial role in this context. Variations in the *HAMP* gene could influence iron metabolism and, consequently, impact the secretion and regulation of fertility hormones. Understanding this relationship could provide insights into managing fertility issues and enhancing the quality of life for these patients.

OBJECTIVES: This study aims to investigate the effect of *HAMP* gene polymorphism on fertility hormones in female patients with beta-thalassemia major.

PATIENTS, MATERIALS AND METHODS: A cross-sectional case–control study was conducted over a period of 6 months from January to June 2024 involving 180 women (90 healthy control and 90 patients) aged 16–40 years. Patients were randomly selected according to the specific inclusion and exclusion criteria. The *HAMP* gene polymorphism was determined by polymerase chain reaction – restriction fragment length polymorphism, and the serum level of hepcidin hormone, ferritin, and fertility hormones were measured by Enzyme-Linked Immunosorbent Assay. SPSS Program was used to code, enter, and process the gathered data.

RESULTS: The genetic analysis of *HAMP* gene (c.582A>G, rs10421768) revealed significant differences in genotype polymorphisms between patients and controls. Patients with GG genotype had the lowest hepcidin levels (P = 0.001). AG genotype was associated with the highest ferritin levels. Fertility hormone levels were lower in GG versus AG and AA genotypes.

CONCLUSIONS: *HAMP* gene polymorphisms are associated with reduced hepcidin levels, increased iron overload, and altered fertility hormones in females with beta-thalassemia major, suggesting a genetic contribution to iron dysregulation and endocrine dysfunction in these patients.

Keywords:

Ferritin, fertility hormone, hepcidin antimicrobial peptide gene, hepcidin, polymorphism, thalassemia

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Introduction

Beta thalassemia is an inherited blood disorder affecting population in over than 60 countries, with an estimated 150 million people carrying the genetic trait.[1] Beta-thalassemia results from mutations in the βeta globin gene, leading to chronic hemolytic anemia and a need for frequent blood transfusions.[2] The life-saving blood transfusions that individuals with beta-thalassemia major rely on come with a devastating cost: The relentless buildup of iron in tissues and a high risk of endocrine

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disorders in patients.[3] Hypogonadism, menstrual irregularities, and infertility are often observed, largely attributed to iron deposition in the pituitary gland and gonads, which impair their function.[4] Recent studies have suggested that genetic factors may also play a role in the endocrine complications observed in beta-thalassemia major.[5] One such factor is the hepcidin antimicrobial peptide *(HAMP)* gene, which encodes hepcidin, a key regulator of iron metabolism.[6] Hepcidin's role in controlling iron absorption and distribution within the body suggests that variations or dysregulation of the *HAMP* gene could influence iron overload and its subsequent effects on endocrine function.[7] Deregulated hepcidin expression has been linked to altered iron homeostasis, potentially exacerbating pituitary iron deposition and impairing the secretion of fertility hormones such as luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol.[8] In the context of β-thalassemia, hepcidin's role in iron regulation is particularly crucial.[9] Patients with this condition often experience iron overload due to frequent blood transfusions, making hepcidin's regulatory function essential for maintaining healthy iron levels.[10] Despite the established link between iron overload and endocrine dysfunction in beta-thalassemia major, the specific impact of *HAMP* gene expression on fertility hormones remains underexplored.[11] This study aimed to investigate the effect of *HAMP* gene polymorphism on fertility hormones in female patients with beta-thalassemia major. Understanding this relationship could provide valuable insights into targeted therapeutic approaches to mitigate reproductive complications in these patients. This study is needed because previous research has not fully clarified the genetic mechanisms underlying iron overload and hormonal disturbances in beta-thalassemia major. While the role of iron metabolism in thalassemia is well recognized, the contribution of *HAMP gene* polymorphisms to hepcidin regulation, iron accumulation, and endocrine dysfunction, particularly in relation to fertility hormones in females, remains underexplored. This study addresses this gap by investigating the association between *HAMP* variants and both iron homeostasis and hormonal profiles, providing insight into potential genetic predispositions that could inform personalized management and therapeutic strategies in thalassemia care.

Patient Materials and Methods

A cross-sectional case–control study was conducted from January 2024 to June 2024. One hundred eighty women were enrolled in this study, including 90 healthy women and 90 patient women. The study participants' age range was between 16 and 40 years. Participants were randomly selected using a computer-generated randomization list to reduce selection bias. To minimize information bias, data collectors and laboratory personnel were blinded to the patients' clinical status and genetic results. The patients were selected randomly and sampled from the attendees of the thalassemia care centers in Ibn AL Baladi Hospital in Baghdad city. Controls were selected from the same geographic area to ensure homogeneity. The healthy control group was matched with the patient group in terms of age and socioeconomic status, and all women in the control group voluntarily consented to blood sampling after being informed of the study's objectives, following informed consent in accordance with research ethics.

Patient data were gathered through a comprehensive preprepared questionnaire, which included information on age, weight, height, marital status, number of children, menstrual history, and medical history, along with a review of their medical records.

Five milliliters of venous blood were aseptically collected from each patient before transfusion. Three milliliters of the anti-coagulated sample were utilized for biochemical analysis, including the measurement of hepcidin, ferritin, LH, FSH, estradiol, and prolactin levels. The remaining 2 ml of ethylenediaminetetraacetic acid-treated blood was stored at −20°C for genetic analysis.

Inclusion criteria

1. Female patients aged between 16 and 40 years with a confirmed diagnosis of Beta-thalassemia Major, verified by hemoglobin electrophoresis
2. Age-matched apparently healthy females with no history of hematological disorders, serving as the control group.

Exclusion criteria:

1. Women with recent infection or inflammation were excluded based on quantitative C-reactive protein (CRP) testing, which was performed immediately at the time of sample collection using standard laboratory procedures. Participants with CRP levels >6 mg/L were excluded from the study
2. Patients with other blood disorder or coexisting chronic diseases affecting hormone levels
3. Women with a history of previous ovarian surgery or those who are Pregnant.

Molecular analysis of hepcidin antimicrobial peptide gene polymorphism

The extension of genomic DNA from each blood sample for patients and control was done using the Genomic DNA mini kit Favorgen, following the manufacture instructions.[12] The purity and concentration of the isolated DNA were assessed spectrophotometrically

using a NanoDrop device (Thermo Scientific, USA). Absorbance readings at 260 nm and 280 nm were recorded to calculate the A260/A280 ratio, with values between 1.8 and 2.0 considered acceptable, indicating minimal protein contamination. The A260/A230 ratio was also measured, with values in the range of 2.0-2.2 considered indicative of low contamination from organic compounds or chaotropic salts. To evaluate DNA integrity, agarose gel electrophoresis was performed. A 1% agarose gel was prepared using TAE buffer, and DNA samples were loaded alongside a molecular weight ladder. Electrophoresis was carried out at 100 volts for approximately 30–45 min. Gels were stained with ethidium bromide and visualized under UV illumination. High molecular weight, intact DNA was identified by the presence of distinct, sharp bands with minimal smearing.

The extracted DNA samples were then amplified by a polymerase chain reaction (PCR) with specific published primers [Figure 1]. To amplify a 179 bp region of the HAMP gene, Forward Primer (F): 5′ GTGCTGGGCCATATTACTGCT 3′ and Reverse Primer (R): 5′ CACGTGCATAGGTTCTGGCA 3′ were used.[13]

Restriction fragment length polymorphism of DNA fragments

To facilitate enzymatic digestion of the PCR product, we prepared a reaction mixture consisting of 1 µL of enzyme (Fermentas, New England Biolab), 2 µL of buffer, and 10 µL of sample DNA. Distilled water was then added to bring the final volume to 20 µL. The enzyme Bstu1 was used to determine hepcidin polymorphism. The reaction mixture was incubated at the optimal temperature for enzyme (60°C) for 16 h to ensure complete digestion. Upon completion of the digestion process, BstUI was heat-inactivated by incubating the reaction mixture at 80°C for 20 min to prevent further cleavage activity, then the resulting products were analyzed by electrophoresis to assess the efficacy of enzymatic digestion. The digested amplified DNA fragments were then separated by electrophoresis on a 2.5% agarose gel, using 1X TBE buffer, and run at 100Voltage for 60 min. The gel was stained with ethidium bromide and visualized under UV light, revealing distinct bands. To estimate the size of the fragments, a fifty-base pair ladder was used as a size marker, providing a clear reference point for fragment size determination [Figure 2].

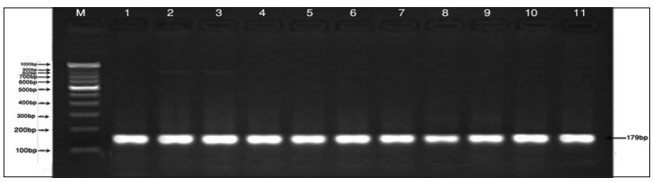

Figure 1: Gel electrophoresis of polymerase chain reaction product for Hepicidin gene

Enzyme-linked immunosorbent assay method for blood sample

Blood samples collected for Enzyme-linked immunosorbent assay (ELISA) analysis were centrifuged to separate the serum, which was then divided into smaller portion and stored at 80°C until further use to maintain the stability of the target biomarker. Before performing the ELISA, samples were thawed on ice and gently mixed to ensure uniformity. ELISA was then conducted according to the manufacturer's protocol to quantify the target biomarkers.[14] The ELISA kits used in this study included the Human Hepcidin ELISA Kit (Catalogue No. MBS269929, MyBioSource, USA), the Ferritin ELISA Kit (Catalogue No. ELA-1872, DRG, USA), the Human LH ELISA Kit (Catalogue No. MBS454235, MyBioSource, USA), the follicle-stimulating hormone (FSH) ELISA Kit (Catalogue No. EIA-1288, DRG, USA), the Human Estradiol ELISA Kit (Catalogue No. EIA-2693, DRG, USA), and the Human Prolactin ELISA Kit (Catalogue No. EIA-1291, DRG, USA).

The test procedures were performed strictly in accordance with the manufacturer's guidelines.

Ethical consideration

This study received approval from the Institutional Review Board of the College of Medicine at Al-Nahrain University (Approval Number 85). All participants provided written informed consent before taking part in the research. Data confidentiality was maintained in accordance with the principles outlined in the revised Declaration of Helsinki on bioethics.[15]

Statistical analysis

Data analysis was conducted using the Statistical Package for the Social Sciences (SPSS), version 26 (IBM Corp., Armonk, New York, USA). The Shapiro–Wilk test was first applied to assess the normality of the data distribution. statistical tests, including the independent *t*-test, Chi-square test, analysis of variance (ANOVA), and *post hoc* tests, were then performed as appropriate. Data are presented as mean ± standard deviation (SD).

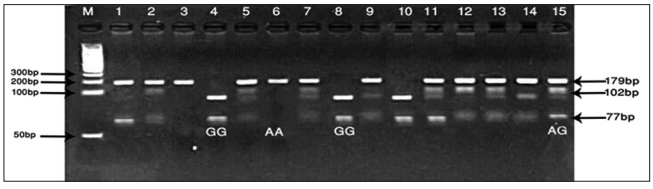

Figure 2: The electrophoresis pattern of the Hepcidin c.-582 A>G polymorphism, analyzed by polymerase chain reaction restriction fragment Length polymorphism using Hepcidin promoter primers and digestion with the BstuI restriction enzyme

A $P > 0.05$ was considered statistically nonsignificant, while a $P < 0.01$ was interpreted as highly significant.

Results

Demographic data

This study included 180 women (90 healthy women and 90 patient women), there was no significant difference between both groups concerning age (24.67 ± 7.16 vs. 26.01 ± 6.90) while the Thalassemia patients women group had a significantly lower body mass index (BMI) compared with controls (21.44 ± 2.62 vs. 24.93 ± 4.77, respectively; $P \leq 0.001$) as shown in Table 1.

To compare means between two independent groups (Control vs. Patients), the Independent Samples *t*-test is used. If the $P < 0.05$, it suggests a statistically significant difference.

Restriction fragment length polymorphism of hepcidin gene

The results of the hepcidin gene analysis by Restriction fragment length polymorphism using the BstuI restriction enzyme showed that the Homozygous AA pattern had one band (179 bp), Homozygous GG pattern had two bands (102 and 77 bp), and Heterozygous AG pattern had three bands (179, 102, and 77 bp). The results revealed that the AA (wild type) pattern was more frequent in the control group (81.1%) than in patients (53.3%), whereas the AG (hetrerozygotes) pattern was more frequent in patients (33.3%) than in the control group (16.7%). In addition, the GG (mutant homozygote) pattern was more common in patients (13.4%) compared to the control group (2.2%) as shown in Table 2.

AA wild type is most common among control while the AG and GG are most common in patient.

The statistical test used in this table is the Chi-square test of Independence. The *P* values (all < 0.05) suggest statistically significant differences in genotype distribution between patients and control.

Comparison of serum hepcidin and serum ferritin level among different genotype in patients and control

As listed in Table 3, the mean serum hepcidin level was lowest in patients with the homozygous GG genotype compared to those with the heterozygous AG genotype and the homozygous AA genotype, with a highly significant difference ($P \leq 0.001$), the Ferritin levels are significantly higher in patients compared to controls across all genotypes, [Figure 3].

One-Way ANOVA is used to compare the means of independent groups to see if there is a statistically significant difference between them then the *post hoc* test is used after ANOVA if a significant difference is found, to determine which specific groups differ from each other. If the $P < 0.05$, it suggests a statistically significant difference.

Table 4 shows that the AG genotype exhibited higher mean serum ferritin than AA and GG genotypes, particularly in patients [Figure 4].

One-Way ANOVA is used to compare the means of independent groups to see if there is a statistically significant difference between them then the *post hoc* test is used after ANOVA if a significant difference is found,

Table 1: Demographic characteristics in patients and control

Parameter	Control, mean±SD	Patients, mean±SD	*P*
Age (years)	26.01±6.90	24.67±7.16	0.2
BMI (kg/m^2)	24.93±4.77	21.44±2.62	<0.001

BMI=Body mass index, SD=Standard deviation

Table 2: Distribution of genotype in study group

Genotype	Patient (%)	Control (%)	OR	*P*
AA	53.3	81.1	3.76	0.02
AG	33.3	16.7	0.5	0.025
GG	13.4	2.2	0.4	0.008

OR=Odds ratio

Table 3: Comparison of serum hepcidin level among different genotype in patients and control

Groups	Genotype	%genotype	Hepcidin (ng/mL), mean±SD	*P*
Patients	AA	53.3	6.18±0.89	<0.001
	AG	33.3	4.23±0.43	
	GG	13.4	3.57±0.29	
Control	AA	81.1	21.10±2.32	<0.001
	AG	16.7	16.62±2.1	
	GG	2.2	14.46±0.23	
Between patients and control	AA			<0.001
	AG			<0.001
	GG			<0.001

SD=Standard deviation

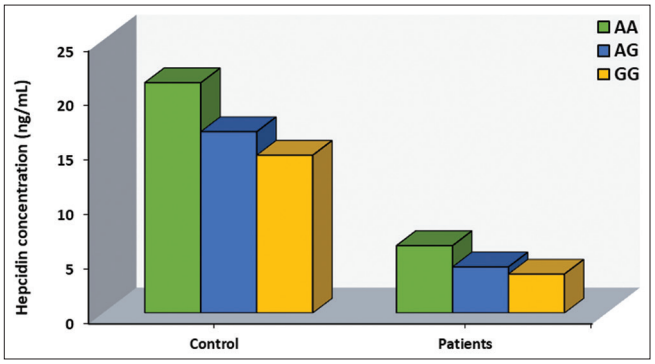

Figure 3: Hepcidin level in different genotypes of hepcidin gene polymorphism in patients and control

to determine which specific groups differ from each other. If the $P < 0.05$, it suggests a statistically significant difference.

The level of hormones in different genotypes of hepcidin gene polymorphism in patients

It can be seen from Table 5 that there is a statistically significant difference in the allele frequency AA, AG, and GG in Female patients groups in LH, FSH, Estradiol, and Prolactin. The levels of all hormones Significantly decreased in patients with the GG (mutant homozygote) genotype compared to patients with AA (wild type) and AG (heterozygotes) genotypes, on the other hand, GG and AG exhibit significantly decreased hormones level compared with patients with AA (wild type) genotypes as shown in Figure 5.

Statistical test used in the table is ANOVA, ANOVA is used to compare the means of three or more independent groups to determine if at least one group mean is significantly different from the others. If the $P < 0.05$, it suggests a statistically significant difference exists among the group means.

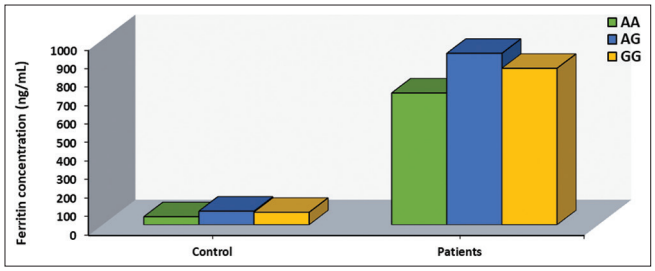

Figure 4: Ferritin level in different genotypes of hepcidin gene polymorphism in patients and control

Table 4: Comparison of serum ferritin level among different genotype in patients and control

Groups	Genotype	%genotype	Ferritin (ng/mL), mean±SD	*P*
Patients	AA	53.3	712.12±170.13	<0.001
	AG	33.3	962.10±86.80	
	GG	13.3	845.33±122.3	
Control	AA	81.1	43.22±30.55	<0.001
	AG	16.7	73.53±15.09	
	GG	2.2	67.50±2.12	
Between patients and control	AA			<0.001
	AG			<0.001
	GG			<0.001

SD=Standard deviation

Discussion

Hepcidin is a significant regulator of iron hemostasis, particularly in thalassemia, and is implicated in a number of iron metabolic pathways.[16] The impact of single nucleotide polymorphisms (SNPs) situated in the promoter region of HAMP on hepcidin expression has been observed in multiple investigations.[17] This study investigated the association between hepcidin gene polymorphism and its effects on iron metabolism and fertility hormonal levels in female patients with Thalassemia.

The finding of current results indicated that Thalassemia patients had a significantly lower BMI compared to healthy controls, the frequency of hepcidin gene variant (C.-582A>G), The AA genotype of the hepcidin gene was more common in the control group, while the AG and GG genotypes were more prevalent among patients. Our results were comparable with a study's findings that was conducted by Zarghamian *et al*.[18] who discovered that the frequency of (C.-582A>G) GG, AG genotypes were higher frequency in thalassemia patient compared to control group, while AA genotype showed a significant lower frequency in thalassemia patient compared to the control group. It is also agree with a study in Kirkuk city by Ismail and Muhammad[19] who found that polymorphism of the hepcidin gene *(HAMP)* in patients that the AA allele was 68 (68%), the AG allele 21 (21%) and the GG allele was 11 (11%) in 100 patients, in While in the group of healthy people, the percentage of the AA allele was 37 (76%), the percentage of the AG allele was 12 (24%), and the percentage of the GG allele was 1 (2%).

A study was done in by Parajes *et al*.[20] found that the (C.-582A>G) variant reduces the expression of *HAMP*, and this lead to iron overload, causing multiple organ damage, especially in the liver and heart such as cirrhosis, diabetes, and heart failure, also causing endocrinal disturbances, bone and skin abnormalities.

Table 5: Hormones level in different genotypes of hepcidin gene polymorphism hepcidin antimicrobial peptide in patients

Hormones	Genotyping AA (53.3%) concentration (mean±SD)	Genotyping AG (33.3%) concentration (mean±SD)	Genotyping GG (13.4%) concentration (mean±SD)	*P*
LH (µ/mL)	1.52±0.78	1.49±0.81	0.69±0.35	0.003
FSH (µ/mL)	2.81±1.17	1.91±0.84	1.27±0.75	<0.001
Estradiol (pg/mL)	12.15±0.63	10.94±0.69	10.36±1.00	<0.001
Prolactin (ng/mL)	3.17±0.68	2.06±0.81	0.68±0.15	<0.001

SD=Standard deviation, LH=Luteinizing hormone, FSH=Follicle-stimulating hormone

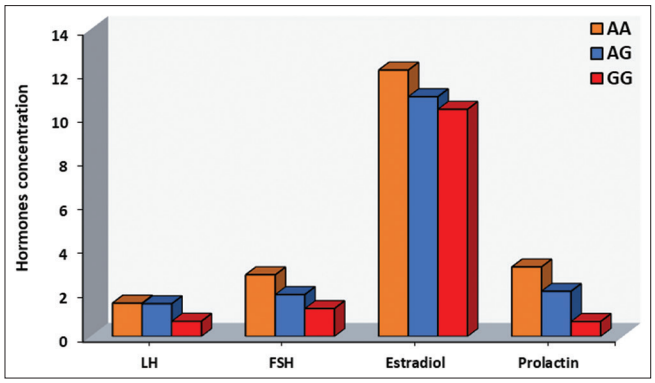

Figure 5: Hormones level in different genotypes of hepcidin gene polymorphism in patients

In the current study, serum hepcidin levels were significantly lower in thalassemia patients, especially those with the GG genotype (P = 0.001). This results indicate a significant association between serum hepcidin level and (C.-582A>G) polymorphism. Our results were comparable with studies on *HAMP* function suggesting that due to the major role of hepcidin in iron metabolism, different incidences of the hepcidin gene caused phenotypic differencing among the patients.[21] Another study by Sokkar *et al*. demonstrated[22] that Significant down regulation of hepcidin expression was found in thalassemia patients compared to healthy controls.

Hepcidin expression is often notably downregulated in thalassemia due to ineffective erythropoiesis or specific gene polymorphisms, significantly worsening iron overload, which is a key factor in the morbidity and mortality of affected individuals. Genetic mutations in the *HAMP* gene are critical in determining the severity of iron overload among these patients. Studies indicate that such variations can influence hepcidin levels and functionality, impacting clinical management strategies for iron overload.[23] The complex relationship between hepcidin and iron metabolism necessitates a thorough understanding of these genetic factors to optimize treatment approaches. A comprehensive strategy, including genetic screening and personalized therapies, could improve patient outcomes. Future research should aim to clarify how these genetic changes impact hepcidin dynamics and iron homeostasis, leading to targeted treatments that address the specific needs of thalassemia patients.

In the current study, Serum ferritin levels were significantly higher in patients compared to controls, with the AG genotype showing the highest mean levels, and also significantly influenced by the SNP rs 10421768 of *HAMP* gene. This is consistent with a study by Coates TD *et al*.[24] In contrast to our study, a study was done by Tuo *et al*.[25] who showed no significant variations noted in the ferritin, iron, and transferrin levels between the AA and AG in c.-582A > G GG genotypes. Nonetheless, the reason for this disparity may be traced back to the role that polymorphism plays in determining the degree of gene expression, resulting in varying levels. Variations in *HAMP*, particularly SNPs, are closely tied to impaired iron homeostasis, a key feature in thalassemia patients, especially those needing regular transfusions. These genetic changes can disrupt iron metabolism, worsening the issues faced by these individuals. Dysregulated iron levels not only heighten the symptoms of thalassemia but also increase the risk of organ dysfunction from iron overload. Understanding the genetics of *HAMP* is crucial for developing targeted therapies.[26] Regarding the effect *HAMP* gene polymorphism on the levels of fertility hormones (LH, FSH, E2, and prolactein) in patients. The current study shown a significant difference (P < 0.05) in the mean levels of LH, FSH, E2, and prolactin hormones in different genotypes of hepcidin gene polymorphism (*HAMP*) in female patients. The current study found the level of all hormones significantly decreased in patients with the GG (mutant homozygote) genotype compared to patients with AA (wild type) and AG (heterozygotes) genotypes; on the other hand, GG and AG exhibit significantly decreased hormone levels compared with patients with AA (wild type) genotypes, from these result, the current study suggest that *HAMP* gene polymorphism associated with significant alterations in fertility hormones concentrations in females with beta-thalassemia major. The reduction of LH levels in thalassemia patients with GG genotypes of the *HAMP* gene, compared to AG and AA genotypes, necessitates examination concerning iron overload and endocrine function. The *HAMP* gene is vital for hepcidin synthesis, influencing iron homeostasis. GG variants disrupt iron metabolism, leading to increased iron in key endocrine regions, notably the hypothalamic-pituitary axis.[27]

Our results were comparable with a study by Kim A *et al*. found[28] that patients with the GG genotype had significantly lower estradiol levels, possibly due to increased iron toxicity in the hypothalamic–pituitary–gonadal axis.

Comparatively, other studies have suggested that variations in the *HAMP* gene contribute to diverse clinical presentations in thalassemia. For instance, Ghassemi *et al*.[29] reported that AG and AA genotypes might confer some protective effect against severe endocrine dysfunction, including hypogonadism, which aligns with their less pronounced estradiol decline.

A study by Farmaki *et al*.[30] highlighted that GG genotypes exhibit a stronger suppression of hepcidin expression, leading to more severe iron overload and oxidative stress. This may result in greater disruption of FSH secretion compared to AG and AA genotypes.

Consistent with this, a study by De P *et al*.[31] reported lower gonadotropin levels in thalassemia major patients with higher serum ferritin, especially among those with specific *HAMP* polymorphisms.

In contrast to our findings, a study conducted by AL-Zuhairy and AL-Ali[32] evaluated the levels of selected pituitary gonadal hormones, including follicle FSH and prolactin in patients with beta-thalassemia major. The study involved 50 male patients aged between 16 and 23 years, who were compared with age-matched healthy controls. The results demonstrated a statistically significant elevation in serum levels of FSH and prolactin among thalassemia patients. The possible reasons for these contradictory results may be related to the variation in study design, such as sample size, sample selection criteria, and also the presence of gene-gene and gene-environment interactions in the various studied populations.

Contrasting evidence emerges in investigations that overlooked stratification according to *HAMP* genotypes, revealing gonadotropin suppression predominantly attributed to overarching iron overload. This observation indicates that the *HAMP* GG genotype may intensify preexisting pathophysiological conditions, resonating with the notion that genetic susceptibilities serve to magnify endocrine disorders within thalassemia patients. The current study findings align with previous research indicating dysregulated iron metabolism in Thalassemia patients. However, the study provides new insights into the genetic basis of this dysregulation, highlighting the role of *HAMP* gene polymorphism.

Conclusion

The polymorphism of hepcidin gene *(HAMP)* distribution differed significantly between patients and controls, with the AG and GG genotypes more frequent in thalassemia patients.

Hepcidin and ferritin levels varied significantly across different *HAMP* genotypes, indicating a potential genetic influence on iron regulation. There is a possible association between hepcidin gene polymorphism and reduced hepcidin level and excessive iron in patients with beta-thalassemia major. The *HAMP* Polymorphism appear to significantly influence fertility hormone level in females with beta-thalassemia major; a genetic predisposition to altered hepcidin levels, influencing iron overload severity and hormonal disturbances in Thalassemia patients may be partly attributable to genetic variations in the hepcidin pathway.

Recommendations

1. Conduct large-scale, multi-center studies to verify the association between hepcidin gene polymorphisms and clinical characteristics across diverse populations. This will enhance the understanding of genetic variations and their impact on Thalassemia progression
2. Implement long-term follow-up studies to investigate the influence of hepcidin polymorphisms on iron overload progression over time, providing deeper insights into its role in disease development
3. Explore the molecular mechanisms linking hepcidin gene variations to hormonal imbalances, which may help clarify their contribution to disease pathophysiology and clinical manifestations
4. Assess the potential of genetic screening for hepcidin polymorphisms as an early diagnostic tool in Thalassemia management, facilitating improved risk assessment and personalized treatment strategies
5. Evaluate the feasibility of developing targeted therapies based on hepcidin levels as a novel approach for managing iron overload and enhancing patient outcomes, whether through pharmacological interventions or gene-based therapies.

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