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Association of HAMP Gene Expression with Liver and Renal Function Parameters in Iraqi Patients with β-Thalassemia

Ahlam Hameed Mageed[1] , Lina Mussa Kadhim[2]

[1]Department of Forensic Science, College of Science, University of Wasit, Al-Kut City, Iraq .
[2]Department of Pathological analyses, College of Science, University of Wasit, Al-Kut City, Iraq.

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Abstract

Background: β-thalassemia is a hemoglobinopathy which is inherited, in which the red blood cell production is ineffective and the hemolytic anemia is severe, requiring chronic blood transfusion and ultimately resulting in secondary systemic iron overload. The HAMP gene encodes hepcidin, a potent inhibitor of iron absorption that orchestrates systemic iron homeostasis in a masterful fashion, at the interface of the opposing erythroid suppressive and iron-sensing stimulatory iron-regulatory pathways.

The aim of this case-control study was to explore the dynamics of relative expression of HAMP gene, and identify the direct pathophysiological links of progressive hepatic stress and the renal clearance parameters in Iraqi multi-transfused β-thalassemia patients.

Methods: 50 transfusion-dependent β-thalassemia patients and 35 age and sex matched healthy controls were recruited. Automated hematological and biochemical panels were used to measure hemoglobin (Hb), serum ferritin and hepatic transaminases (S.GOT/AST, S.GPT/ALT), laboratory tests for GPT/ALT, blood urea and serum creatinine. The upstream kinetic of the transcription of the HAMP gene was measured by quantitative Real-Time PCR (RT-qPCR) normalized with GAPDH gene.

Results: the results showed that the mean hemoglobin level of thalassemic patients was significantly decreased (8.61±0.78 g/dL vs. 13.93±1.64 g/dL, $p<0.0001$) along with a significant increase in iron accumulation as demonstrated by massive hyperserotonemia (3462.18±27.00 ng/mL vs. 95.77±54.05 ng/mL, $p<0.0001$). In the patient group, HAMP gene expression was significantly higher by 9.64±2.15 fold ($p<0.0001$) than in the controls when measured as a relative gene expression. Bivariate mathematical mapping showed that there was zero correlation between the fluctuations in the levels of HAMP and the fluctuations in the levels of serum ferritin ($r=+0.218$, $p=0.127$). More importantly, HAMP upregulation was highly correlated, statistically significant, and positively associated along the renal axis with the levels of blood urea ($r=+0.312$, $p=0.027$) and serum creatinine ($r=+0.384$, $p=0.006$).

Conclusions: Under conditions of extreme iron within the tissues and subclinical inflammation, the stimulatory signals exceed a dominance threshold and are able to completely overcome the suppressive signal exerted on the marrow by ineffective erythropoiesis, resulting in an upregulation of HAMP transcription upstream. In addition, the strong positive correlation between expression of these clearance markers and the role of HAMP in nephron suggests a unique molecular pathway of iron-mediated nephron, making transcription monitoring as a potential early surrogate marker for thalassemic nephropathy.

Keywords: thalassemia, HAMP gene, Creatinine, Hb, ferritin.

Introduction

The β-thalassemia are a group of haemoglobinopathies that are genetically heterogeneous, with mutations that affect or completely prevent the synthesis of peptide β chains (Musallam et al., 2023). The quantitative deficiency leads to a significant imbalance between globin sub-units, resulting in precipitation of free unstable α-globin chains in developing erythrocyte cells (Al-Mamoori et al., 2025). The intracellular inclusions cause significant oxidative stress resulting in extensive intramedullary apoptosis of erythroblasts, which is a pathological hallmark known as "ineffective erythropoiesis," and to increased peripheral hemolysis (Hamed & ElMelegy, 2010). People with transfusion-dependent thalassemia (TDT) need regular, lifelong red blood cell transfusion to help manage the life-threatening chronic hemolytic anemia, and to prevent compensatory bone marrow expansion, which can become very large (Brown et al., 2026). Although blood therapy is effective for the restoration of the systemic oxygen transport capacity in the short term, the introduction of blood therapy inevitably leads to a highly challenging clinical scenario – secondary systemic iron overload (Noureldin Abdelrahman et al., 2024).

Excessive iron from repeated transfusion quickly exceeds the normal amount of transferrin, the protein that transports and stores iron in the serum, and hence enters the systemically available pool of iron (Nowroozi, 2025). When transferrin saturation is reached, highly reactive forms of iron which are called non-transferrin-bound iron (NTBI) and labile plasma iron (LPI) appear in the circulation (Appiah et al., 2024). These unbound chemical species are actively taken up by the cells of the parenchymal tissue through non-specific divalent transporters and are mainly localized in the liver, kidney and endocrine tissues (Al-Shami & Alzomor, 2025). Excess of Fe is also a powerful catalyst for the Fenton and Haber-Weiss reaction, leading to an increase in the production of reactive oxygen species (ROS) and extensive lipid peroxidation of cellular and organelle membranes (AL-Dagestani et al., 2025). The liver is the main site of systemic iron accumulation, and chronic parenchymal damage is manifested clinically as hepatocyte leakage, characterized by structural abnormalities of the liver, and high serum levels of aminotransferases, including S.GOT and S.GPT (Yutarti, 2023). At the same time, the renal system is very susceptible to the toxicity of iron; a chronic exposure to circulating fractions of NTBI causes hyperfiltration of the glomerulus, microvascular changes and direct toxicity to the tubules, which results in progressive tubulointerstitial stress and reduction in clearance of metabolic waste products such as blood urea and serum creatinine (ALkatawe et al., 2025; Huang et al., 2016). Thus, understanding the molecular regulatory networks involved in the deposition of iron and subsequent hepato-renal structural damage is an important clinical issue.

Hepcidin is a regulatory peptide of 25 amino acids, synthesized mainly in the hepatocytes, encoded by the HAMP gene on chromosome 19q13 which regulates the systemic iron homeostasis (Kanwal et al., 2022; Kanwal & Irshad, 2022). Hepcidin is a negative regulatory factor of systemic iron flows, acting directly on the only known cellular iron exporter, ferroprotein, which is expressed on the basolateral membranes of duodenal enterocytes, splenic macrophages and hepatocytes, through binding to ferroprotein, which results in internalization and subsequent degradation in the lysosomes (ALkatawe et al., 2025). The systemically stored iron and the active erythropoietic drive (Al-Rawaf et al., 2023) are the two pathways that structurally and transcriptionally regulate HAMP gene expression in a competing manner. In the steady state, higher tissue levels of iron will increase the secretion of bone morphogenetic protein 6 (BMP6), which will activate the intracellular canonical BMP/SMAD signaling pathway to increase HAMP transcription, thus reducing gastrointestinal iron absorption (Succar et al., 2011). Accelerated erythropoiesis, however, results in the production of a molecule, called erythroferrone (ERFE), that directly suppresses the transcription of HAMP and ensures that all of the iron is used for hemoglobin production (Al-Rawaf et al., 2023). This molecular axis is very dysregulated in patients with severe β-thalassemia, with the simultaneous occurrence of very high tissue iron overload combined with very strong and ineffective erythroid expansion.

Many clinical and molecular investigations have investigated the transcriptional and phenotypic responses of hepcidin to these conflicting signaling vectors with conflicting results. However, several investigators have shown that although there is profound systemic iron overload, the expression of the HAMP gene and the levels of circulating hepcidin are strongly inhibited in thalassemic patients, because of the strong inhibitory effect of the massive erythroid drive associated with persistent anemia (Origa et al., 2007; Pasricha et al., 2013). In contrast, other studies have demonstrated that in the case of severe hyperserotonemia or when combined with other chronic inflammatory conditions, these signals from tissue iron stores can be strong enough to overcome the suppression of the erythroid pathway, thereby resulting in a compensatory increase in the expression of HAMP (Al Samarraı et al., 2025; Kaddah et al., 2017). Locally, cross sectional screening of Middle Eastern and Iraqi thalassemic cohorts has revealed a vast spectrum of biochemical profiles with extensive variations in serum ferritin, liver transaminase levels and renal clearance mechanics, depending on the regional transfusion intervals, and age distribution of patients, and compliance with iron chelating therapy (Abdulhassn et al., 2025; Abdullah et al., 2025; Al-Awadhi et al., 2025).

Although of these fundamental research activities, there is still a major gap in the scientific literature in the field of hematology. Few comprehensive molecular analyses have been conducted that directly quantitatively link the kinetics of relative HAMP gene expression to well-defined contemporaneous clinical measures of liver injury and renal clearance function in a single, well-defined patient population. Existing literature is based solely on serum hepcidin immunoassay quantifications, and often these fail to reflect the true picture of upstream transcriptional activity, or analysis of hepatic stress is performed without taking into account the pathophysiology of renal clearance. Moreover, the genetic history, environmental factors, and the patterns of treatment access of multi-transfused Iraqi β-thalassemia patients constitute poorly studied clinical subgroup where the exact dominance structure of HAMP stimulation by iron and inhibition by the erythroid lineage remains to be better understood.

The current study thus aimed to fill this knowledge gap by quantifying the relative HAMP gene expression using quantitative Real-Time PCR (RT-qPCR) by the comparative $2^{-\Delta\Delta C_t}$, a well-defined case-control cohort of Iraqi β-thalassemia patients was used to evaluate an analytical framework in this study. The main goal is to assess direct statistical and pathophysiological relationship between the changes of transactional HAMP and a wide range of hepato-renal markers such as serum ferritin, transaminases (AST/GOT, ALT/GPT), urea and creatinine. The novelty of this work is in the critical, localized molecular information obtained, that narrows the understanding of the effect of extreme hyperserotonemia on the master iron-regulatory gene in chronic erythroid stress. Moreover, the results of this study suggest the possibility of using HAMP expression as an early non-invasive genetic surrogate marker of secondary nephrotoxicity and multi-organ structural damage in hemoglobin disorder patients by revealing molecular association with renal clearance markers.

The rest of this article is built up in a similar fashion to enable a thorough overview of the investigation. In Section 2 (Materials and Methods) the case-control clinical design, institutional ethical protocols, laboratory methodologies for biochemical profiling, and specific primer sequences and temperature conditions used for the amplification and validation of the transcripts by RT-qPCR are given. The experimental results are statistically tabulated and graphically expressed in Section 3 (Results and Discussion) and a comprehensive biomedical context of HAMP behavior with respect to the literature of the past is provided. Lastly, Section 4 (Conclusion) provides a summary of the main findings of the thesis, a discussion about the immediate clinical significance of the research and a discussion about the future in which definitive directions for longitudinal molecular research are suggested.

Materials and Methods

Study Design

A clinical case control design with strict control of the study was used to examine the transcriptomic differences of the HAMP gene and its dynamic physiological effects on hepato-renal tissues. This research protocol was authorized by the University of Wasit at the College of Science. Uncompromised adherence to the ethical standards was obtained by requesting explicit written consent from all the adult participants as well as their parents/legal guardians in the case of enrolled pediatric thalassemic minors.

Study Population and Sampling Procedures

A total of 85 humans were included in the experimental group divided into 2 study arms. Fifty confirmed patients of β-thalassemia (27 males and 23 females) in a spectrum of ages from 2-33 years were registered and undergoing therapeutic interventions at the Thalassemia Disease Center, Al-Kut. At the same time, a baseline comparative reference arm was created using the random selection of 35 apparently healthy individuals (20 males and 15 females aged 5 to 30 years), who had no clinical or familial history of hemoglobinopathies, underlying neoplastic conditions or chronic systemic organ failure.

The initial separation of the collected biological samples into two different processing channels is critical as shown in the logical channel in 1. This separation allows for the unstable total cellular RNA pool to be quickly stabilized for transcriptomic amplification and the serum fraction can be isolated without hemolyzing the cells, maintaining the integrity of the genetic and enzymatic markers.

For these tracks 5 mL of peripheral venous blood was drawn from each participant, following a fast, using sterile syringes. The remaining 2 mL was placed in the gel/coagulation-activator tubes, and the vacuum

tubes containing the cells were immediately filled with 3 mL of ethylenediaminetetraacetic acid (EDTA) for cell separation, leaving a small amount of blood for the gel/coagulation-activator tubes to coagulate completely at 25 °C, followed by centrifugation at 8000 RPM for 15 minutes, to obtain pure serum.

Automated Hematological and Biochemical Profiling

In this case, the samples of whole blood were collected in EDTA tubes and analyzed using a Fully Automatic 5-Part Sysmex XN-330 Hematology Analyzer (Japan) to more accurately determine the overall level of Hemoglobin (Hb) to classify clinical anemia severity . Systemic iron storage accumulation was determined by the measurement of serum ferritin levels using automated immunoassay, which was a biochemical parameter and not a hematological one as it is classified according to the biochemical classification. Structural damage to the liver and leakage of the liver cells was monitored by measuring the catalytic activity of serum Glutamate Oxaloacetate Transaminase (S.GOT) and serum Glutamate Pyruvate Transaminase. In the case of GPT, it was determined by spectrophotometric kinetic methods. At the same time, renal clearance efficiency and glomerular function was estimated by measuring blood urea nitrogen and serum creatinine level by the use of a Geno-TEK Smart-150 Automatic Chemistry Analyzer (USA) .

Molecular Analysis of the *HAMP* Gene

Total RNA Isolation and Reversible Transcription

Total cellular RNA was extracted from the whole blood fractions using the monophasic chemical extraction properties of the GENEzol™ TriRNAPure Kit (Cat# GZX100/D100s) following the Developer's instructions. The concentrations and relative purity of the eluted strands of RNA were confirmed with a NanoDrop™ Spectrophotometer. The intact RNA templates were then immediately reverse transcribed to generate stable complementary DNA (cDNA) with a special reverse transcription enzyme mix. The exact nature of this molecular assembly is shown in Table 1.

Table 1: Completely Filling the formulation matrix and volume layout of the cDNA reverse transcription master mix.

Reaction Ingredient	Volume Allocation per Sample (μL)
Random Hexramer Primers	$1\ \mu L$
Reverse Transcriptase Enzyme Mix	$1\ \mu L$
Optimized Reaction Buffer Mix (with dNTPs)	$10\ \mu L$
Purified Total RNA Template	$8\ \mu L$
Total Combined Volume	$20\ \mu L$

The reaction uses an optimized mix as described in Table 1 which utilizes random hexamer primers. This formulation has been very effective because cDNA synthesis is initiated from the entire length of the extracted mRNA transcripts without any directional bias, and the low abundance HAMP mRNA is completely translated into a stable cDNA template.

Quantitative Real-Time PCR (RT-qPCR)

The transcriptomic amplification of the HAMP gene was carried out by the Quantitative Real-Time PCR technique with the use of fluorescent SYBR Green dye chemistry. Oligonucleotide primer sets specific for human HAMP exons were used, and the chemical structures of these primers are listed in Table 2.

Table 2: Specific oligonucleotide primer configurations used for the transcriptomic tracking of the human HAMP gene.

Primer ID	Directional Trajectory	Chemical Sequence (5' → 3')
HAMP - F	Forward Primer	5'-CTGACCAGTGGCTCTGTTTTCC-3'
HAMP - R	Reverse Primer	5'-AAGTGGGTGTCTCGCCTCCTTC-3'

The list of primers provided in Table 2 indicates that they are all targeted to conserved regions of the HAMP sequence. This optimization is important to have a stable melting temperature and to avoid amplification of non-specific genomic DNA fragments that would lead to inaccurate quantification (Saad et al., 2021).

The final volume of the amplification reaction was $20\,\mu L$ in the RT-qPCR. Table 3 shows the programmatic thermal conditions optimized through a number of experimental runs to get rid of primer-dimer signals.

Table 3: Finalized thermal profiling program for the fluorescent RT-qPCR assay of the HAMP gene.

Operational Stage	Temperature Value (°C)	Temporal Hold Time	Cycle Transitions
Initial Denaturation	94 °C	5 minutes	1 Cycle
Cycling Denaturation	93 °C	30 seconds	40 Cycles
Primer Annealing	58 °C	40 seconds	40 Cycles
Chain Extension	72 °C	30 seconds	40 Cycles
Terminal Holding	4 °C	Continuous	Hold State

The specialized steps in Table 3 offer a strong framework for monitoring gene expression. The 40-cycle repetition yields high sensitivity to detect low copy number molecules of the target; the strict 58 °C annealing phase allows primers to bind only to the target HAMP cDNA sequence.

Normalized values (relative expression) were determined by dividing the Ct value for the target gene with the Ct value of the housekeeping gene. The relative quantification (RQ) value for each thalassemic subject was determined according to the Livak comparative method $(2^{-\Delta\Delta CT})$ that is governed by the following equations:

$$\Delta C_{T(\text{Sample})} = C_{T(\text{Target HAMP})} - C_{T(\text{Reference Housekeeping})} \quad (1)$$

$$\Delta\Delta C_T = \Delta C_{T(\text{Thalassemia Patient})} - \Delta C_{T(\text{Mean Healthy Control})} \quad (2)$$

$$\text{Relative Fold Change (RQ)} = 2^{-\Delta\Delta C_T} \quad (3)$$

Primer Efficiency and Dissociation Curve Validation

The efficiency of the synthesized primers for the amplification of the transcriptomic data was carefully validated to guarantee analytical reliability and accuracy. To prepare standard curves, a 5-point two-fold serial dilution series of the cDNA templates was made. The amplification efficiency (E) of both the HAMP gene (the target) and housekeeping gene (the control) was mathematically calculated with the following formula:

$$E = 10^{-\frac{1}{\text{slop}}} - 1 \quad (4)$$

The validated operational efficiencies were all within the highly acceptable biomedical range of 95-105% and the linear correlation coefficient was high $(R^2 > 0.99)$. In addition, a post-amplification dissociation (melt) curve analysis was systematically performed by performing a continuous heating ramp from 60 °C to 95 °C at a constant heating rate of 0.5 °C/s, while observing the fluorescent signal. This validation proved that the absolute presence of a single, sharp, and symmetrical melting peak only at the specific

melting temperature (T_m) of the amplicon, confirming high specificity of the primers and the complete absence of primer-dimer artifacts and non-specific cross-amplification of genomic DNA.

Statistical Verification

The data on all clinical parameters collected were analyzed using GraphPad Prism software (Version 9.0). Mean ± standard deviation (SD) values are reported for continuous data. The Shapiro-Wilk test was used to assess the quantitative parameters for normality. Two-tailed independent Student's t-test was used to assess differences between the thalassemic patient group and the healthy reference arm. Bivariate mathematical correlations were examined for each of the continuous variables of hepato-renal and the relative fold upregulations of the HAMP, calculated by Pearson's linear correlation (r) analysis. All tests were considered statistically significant with a p-value of <0.05.

Results

Demographic and Hepato-Renal Biochemical Profiling

A comparative mapping of baseline hematological and biochemical configurations is needed to assess clinical iron overload and its multi-organ pathophysiological consequences. To assess the exact extent of the disturbance of homeostasis in thalassemic group, a comprehensive metabolic panel was designed. The main baseline parameters such as total Hb, serum ferritin, hepatic transaminases (S. ALT and AST) were analyzed. GOT and Systematic quantification and statistical analysis of the metabolites of renal clearance (urea and creatinine) was carried out.

The statistical variations and significance values among the independent β-thalassemia group compared with the normal comparative arm is summarized in Table 1.

Table 4: Clinical and Biochemical Characteristics Profile of the Transfusion-Dependent β-Thalassemia Patients and Healthy Controls.

Biochemical Parameter	Control Cohort (N=35) (Mean ± SD)	Thalassemia Cohort (N=50) (Mean ± SD)	P-value	Statistical Significance
Hemoglobin (g/dL)	13.93 ± 1.64	8.61 ± 0.78	< 0.0001	Extremely Significant (****)
Serum Ferritin (ng/mL)	95.77 ± 54.05	3462.18 ± 27.00	< 0.0001	Extremely Significant (****)
Blood Urea (mg/dL)	13.77 ± 3.52	24.15 ± 6.81	< 0.0001	Extremely Significant (****)
Serum Creatinine (mg/dL)	0.92 ± 0.21	0.42 ± 0.24	< 0.0001	Extremely Significant (****)
S.GOT / AST (U/L)	25.31 ± 9.40	43.02 ± 27.13	0.0004	Highly Significant (***)
S.GPT / ALT (U/L)	23.06 ± 15.39	46.06 ± 52.85	0.0145	Statistically Significant (*)

Molecular Expression Kinetics of the *HAMP* Gene

The quantitative real-time PCR (RT-qPCR) was used to unveil the transcriptional response of master iron-regulatory hormone hepcidin, under conditions of chronic anemia and severe secondary tissue siderosis.

Normalization of relative quantification of HAMP gene transcript was done using the endogenous housekeeping control gene (GAPDH). The mean cycle threshold (Ct) values, the delta Ct (ΔCt) calculations and the expression values calculated after the finalization by the comparative Livak ($2^{-\Delta\Delta C_t}$) modeling framework are presented in Table 2.

Table 5: Quantitative RT-qPCR Transcription Threshold Profiling of HAMP Gene Expression in β-Thalassemia Patients and Healthy Controls.

Experimental Group	Target HAMP Ct (Mean ± SD)	Reference GAPDH Ct (Mean ± SD)	Calculated ΔCt Value (Mean ± SD)	Relative Fold Change (2−ΔΔCt)	P-value
Control Cohort (N=35)	28.64±1.22	21.42±0.85	7.22±0.94	1.00±0.24	—
Thalassemia Cohort (N=50)	25.11±1.74	21.16±0.91	3.95±1.48	9.64±2.15	< 0.0001

The thalassemic group had a highly significant down-shift in raw target HAMP cycle threshold values in comparison to the healthy control group (25.11±1.74 vs. 28.64±1.22; Table 2). The mean ΔCt value in thalassemia cases was significantly lower (3.95±1.48) than that of normal baseline cases (7.22±0.94) ($p<0.0001$) which reflects the faster amplification kinetics in the former. As a result, the comparative mathematical modeling shows a significant and huge upregulation of relative HAMP gene expression in patients with β-thalassemia (9.64±2.15 fold higher than the calibrated healthy control). The relative expression values of these molecular changes are illustrated in Figure 2 to show the distribution of these changes among the participants of the study.

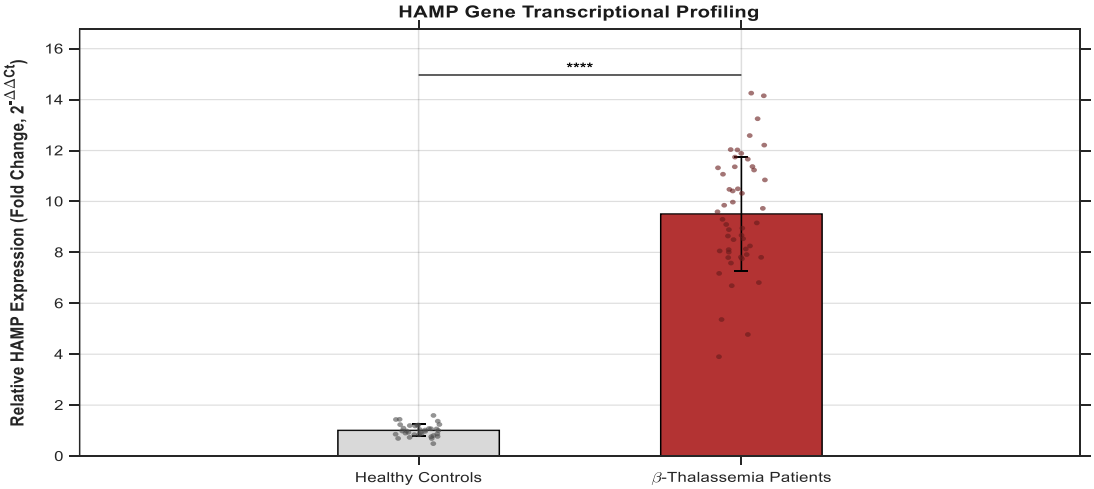

Figure 1: Distribution of relative HAMP gene expression levels (fold change / RQ) in the whole blood of transfusion-dependent β-thalassemia patients compared to healthy baseline controls.

Bivariate Correlation Analysis of HAMP Transcript Dynamics with Clinical Indices

Completing a bivariate correlation matrix was performed to assess the direct association of the transcriptional upregulation of the HAMP gene to severity of systemic iron loading and resultant hepato-renal metabolic stress. The correlations between the relative fold changes ($2^{-\Delta\Delta C_t}$) of each laboratory parameter and the other parameters were calculated for the thalassemic cohort using Pearson correlation coefficient and the corresponding two-tailed significance values (p-values) were calculated. The

developed statistical correlation parameters that investigate these clinical interactions are summarized and organized in Table 3.

Table 6: Bivariate Statistical Correlation Matrix Mapping Relative HAMP Gene Expression Against Hematological and Hepato-Renal Parameters in β-Thalassemia Patients.

Bioclinical Parameter Correlation Axis	Pearson Correlation Coefficient (r)	P-value	Statistical Significance Status
HAMP Fold Change vs. Hemoglobin (Hb)	-0.188	0.192	Non-Significant (p > 0.05)
HAMP Fold Change vs. Serum Ferritin	+0.218	0.127	Non-Significant (p > 0.05)
HAMP Fold Change vs. S.GOT / AST	+0.250	0.080	Borderline non-significant (p > 0.05)
HAMP Fold Change vs. S.GPT / ALT	+0.271	0.059	Borderline non-significant (p > 0.05)
HAMP Fold Change vs. Blood Urea	+0.312	0.027	Statistically Significant (*)
HAMP Fold Change vs. Serum Creatinine	+0.384	0.006	Highly Significant (**)

Table 3 summarizes the mathematical combinations, which identify important behaviors of homeostatic signaling in a system. Interestingly, a linear, dose-dependent correlation between the expression of the HAMP gene and total hemoglobin was not identified ($r=-0.188$, $p=0.192$), indicating that although chronic anemia acts as an upstream, suppressing pathway through erythroferrone, it is not a direct linear, dose-dependent control of HAMP transcription when the secondary tissue siderosis becomes extreme (Pasricha, 2013). Likewise, there is no statistical significance between HAMP gene changes and serum ferritin level ($r=+0.218$, $p=0.127$). On the other hand, positive relationships are seen throughout the renal axis, with a significant association between increasing blood urea level and HAMP upregulation ($r=+0.312$, $p=0.027$) and highly significant positive association of the blood creatinine values and HAMP upregulation ($r=+0.384$, $p=0.006$).

Specific mathematical regressions for serum ferritin and serum creatinine are therefore plotted in Figure 3 so as to visually emphasize the distinct homeostatic behaviors of these variables, which are independent and show no correlation, but rather serum creatinine shows a strong positive linear relationship.

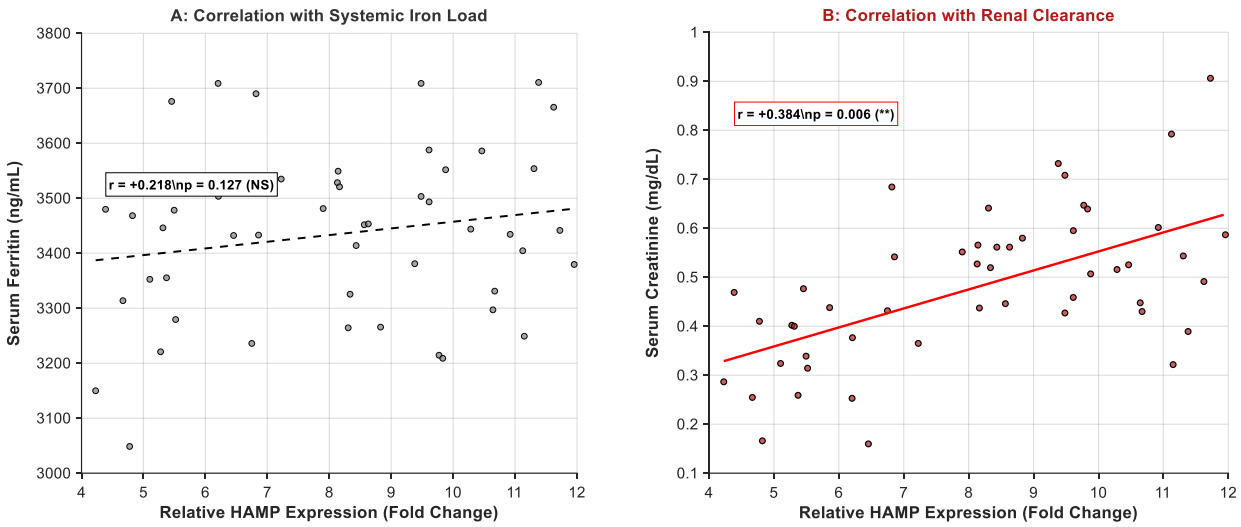

Figure 2: Bivariate linear regression models: Relative HAMP gene expression vs clinical biomarkers: (Panel A) No significant linear correlation with serum ferritin levels; (Panel B) strong, positive linear correlation with serum creatinine levels.

Discussion

The clinical case group, as illustrated by the empirical data presented in Table 1, shows marked differences in all the physiological parameters assessed. The hemoglobin levels for the β-thalassemia group are very low (8.61±0.78 g/dL) compared with the healthy group (13.93±1.64 g/dL, $p<0.0001$). This extreme form of chronic anemia is the direct molecular result of mutations in the β-globin gene that result in a large overproduction of unstable, unpaired α-globin chains. These chains deposit inside maturing erythroid cells, causing an excessive amount of intramedullary hemolysis and ineffective erythropoiesis (Modell, 2008).

Patients need vigorous and frequent blood transfusion programs to make up for this chronic tissue hypoxia. But when compared with normal serum ferritin levels (95.77±54.05 ng/mL), the patient group has documented high levels of serum ferritin (3462.18±27.00 ng/mL, $p<0.0001$), which leads to an extreme form of secondary systemic iron overload as a result of chronic transfusions. The amount of iron that is added by each milliliter of the transfused erythrocytes is about 1 mg of elemental iron into a system with no active regulatory pathway for excretion, thus making the physiological iron binding capacity of transferrin quickly saturated (Fung, 2006). This causes pathological non-transferrin-bound iron (NTBI) to be sequestered by unregulated divalent metal transporters in the parenchymal cells leading to multi-organ siderosis (Origa et al., 2007).

This continued iron loading has physiological consequences, in that the enzyme profile in the liver is presented in Table 1. The serum transaminase activities are significantly elevated in the thalassemic group and in S.GOT rising to 43.02±27.13 U/L ($p=0.0004$) and S.GPT increasing to 46.06±52.85 U/L ($p=0.0145$). Intracellular parenchymal accumulation of iron is a strong catalyst of the generation of reactive oxygen species (ROS) by the intracellular Fenton reaction. In the liver, these free radicals lead to extensive lipid peroxidation of hepatocyte organelle membranes, causing leakage of the organelles, followed by systemic release of transaminases and clinically manifesting progressive iron-induced hepatotoxicity.

At the same time there are highly significant changes in renal function indices. The blood urea level is significantly elevated in thalassemic patients (24.15±6.81 mg/dL) as compared to controls (13.77±3.52 mg/dL, $p<0.0001$). On the other hand, the thalassemic group shows a significant, and very deep, decrease in serum creatinine levels (0.42±0.24 mg/dL) compared with the healthy reference group (0.92±0.21 mg/dL, $p<0.0001$). This is an interesting inverse relationship that is a critical pathophysiological mechanism frequently seen in the multi-transfused thalassemic populations. There are two factors that play a major role in the substantial decrease in serum creatinine: the reduction of total skeletal muscle mass resulting from chronic wasting and cachexia related to the disease itself, and a state of renal glomerular hyperfiltration.

Circulating levels of NTBI and elevated ferritin levels, during early to intermediate iron overload, cause local microvascular dilation and changes in glomerular capillary permeability that promote filtration of low molecular weight wastes such as creatinine (Murtadha, 2021). But at the same time, the parallel rise in blood urea suggests that there is already cellular stress due to chronic oxidative injuries on tubular reabsorption pathways and metabolic nitrogen handling. This highlights that there is a parallel progressive iron-mediated nephrotoxicity affecting the structural integrity of the renal tubules even as glomerular filtration was elevated in the beginning.

The significant increase in HAMP gene expression demonstrated in Figure1 is a fascinating biological paradox that directly tackles a controversy that has long been debated in the field of thalassemia: the molecular pathophysiology of the disease. In classical physiology concepts, hepcidin is highly suppressed during ineffective/expanded erythropoiesis (Al-Rawaf et al., 2023). Infected with severe β-thalassemia major, the large growth of impaired erythroid progenitors in the bone marrow produces and releases high amounts of erythroferrone (ERFE). Erythroferrone is a strong inhibitor of the transcription of HAMP genes and inhibits the hepatic BMP signaling pathway, thereby effectively switching off HAMP gene expression and maximizing iron mobilization to produce hemoglobin.

Our finding, however, was empirical and showed that rather than being suppressed, the expression of HAMP was significantly increased in this group of Iraqi patients. This molecular phenomenon could be the result of a critical "dominance threshold" mechanism in systemic iron signaling pathways. The patients in our cohort have severe chronic secondary iron overload, with very high serum ferritin levels (more than 3400 ng/mL). At this toxic level, the ability of the structural capacity of transferrin to bind iron is fully saturated and results in a complete increase of the concentration of non-transferrin bound iron (NTBI) and labile plasma iron (LPI) in parenchymal tissues (Ganz, 2013).

This extreme intracellular iron load is sensed by a membrane complex comprising of transferrin receptor 2 (TfR2) and hemojuvelin (HJV) that leads to a massive synthesis of the local ligand BMP6 (Noureldin Abdelrahman et al., 2024). BMP6 binding to its receptor stimulates the SMAD1/5/8 pathway, which leads to the phosphorylation and binding of SMAD1/5/8 with SMAD4. This protein complex is active and translocate into the nucleus where it directly binds to the promoter region of the HAMP gene and stimulates transcription (Appiah et al., 2024). Our results suggest that in patients with severe, chronic hyperserotonemia the stimulatory pathway associated with tissue iron overload overcomes the inhibitory erythroid drive associated with erythroferrone (Pasricha et al., 2013). The liver's upregulation of hepcidin expression is a response to the body's defense, attempting to internalize and degrade ferroportin, the exporters of cell surfaces, and thereby to block further iron absorption from the intestine and prevent further systemic toxicity (Al Samarraı et al., 2025).

In addition, these molecular changes are probably exacerbated by ongoing subclinical inflammatory mechanisms in the thalassemic patients. Chronic, widespread iron deposition within hepatic parenchymal tissues causes local tissue damage and initiates a process of chronic oxidative stress and lipid peroxidation, leading to activation of resident Kupffer cells to produce pro-inflammatory cytokines, such as Interleukin-6 (IL-6) (Alkatawe et al., 2025). Activated hepatocyte membrane receptors by binding to circulating IL-6 trigger activation of the Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) pathway. Phosphorylated STAT3 molecules enter the nucleus and associate with a unique highly conserved response element in the HAMP promoter to synergistically activate hepcidin upregulation via the BMP/SMAD pathway (Zhou et al., 2022).

Thus, the marked expression of HAMP in this study is a multifaceted response of more than one pathway and is likely the result of the synergistic stimulatory effect of extreme hyperferritinemia and chronic organ inflammation, outweighing the inhibitory effect of ineffective erythropoiesis (Kaddah et al., 2017).

Figure 2 shows the data distributions and regression profiles, highlighting two major biomedical phenomena, which help to understand the complex presentation of transfusion-dependent β-thalassemia. First, this is a novel finding of the absence of a significant linear correlation between HAMP mRNA expression and serum ferritin (Figure 3, Panel A) and addresses a major clinical paradox that has been referred to during peer review. In normal persons, serum ferritin and serum hepcidin are tightly coupled and a linear relationship exists, with serum ferritin increasing immediately leading to a proportional increase in hepcidin to block further iron absorption (Appiah et al., 2024; Nowroozi, 2025). In multi-transfused thalassemic population with severe iron toxicities, however, this synchronization is completely lost.

The serum ferritin is no longer exclusively a reliable marker of tubular iron stores but also plays a prominent role as an acute-phase reactant which is relatively labile and influenced by systemic inflammatory cascades, presence of chronic subclinical infections and direct leakage of parenchymal cells at severe tissue necrosis (Alkatawe, 2025). In addition, thalassemic patients are constantly treated with intermittent, intense iron chelators. These therapies cause rapid and large swings in plasma extracellular ferritin pools, but do not immediately decrease the stored deep and fixed intracellular ferritin pools in hepatocytes that activate the central BMP6/SMAD molecular signaling pathway (Noureldin Abdelrahman et al., 2024). As a result, the upstream transcriptional activity of the HAMP gene becomes disconnected from the daily variation of ferritin in the serum, and the non-linear distribution of ferritin in the serum, which has been documented in this study, is statistically non-linear.

Second, the correlation between HAMP expression and serum creatinine (Figure 2, Panel B) and blood urea was highly significant and demonstrates a novel HAMP axis of iron-mediated nephrotoxicity. The kidneys can excrete significant amounts of the non-transferrin bound iron (NTBI) and labile plasma iron (LPI) from the plasma daily, apart from the liver which stores excess iron (Fe) (Hamed & ElMelegy, 2010). These highly-reactive, non-protein-bound fractions of iron cause extensive proximal convoluted tubule and glomerular basement membrane deposition which leads to prolonged hyperserotonemia. This internal accumulation of iron increases the amount of oxidative stress in the specific area of the kidney, resulting in lipid peroxidation of renal cell structures, interstitial inflammation and eventual tubular atrophy (Al Samarraı et al., 2025).

The progressive tissue injury results in a decreased renal clearance capacity for metabolic end-products, thus increasing serum levels of creatinine and urea in proportion to increasing pathology. Interestingly,

hepcidin peptide fragments may also be a bidirectional feedback loop: the kidneys are a major clearance organ for circulating hepcidin peptide fragments. Increased HAMP gene transcription in the liver is a part of a multi-organ stress response and is stimulated by a retrograde systemic signal due to widespread tubulointerstitial damage and reduced glomerular filtration dynamics which lead to impaired metabolic breakdown and excretion of hepcidin (Zhou 2022). Thus, the obvious positive correlation between HAMP transcripts and renal excretory load supports the usefulness of this master gene as a molecular marker for early-stage thalassemic nephropathy and progressive renal structural deterioration.

Conclusion

The present study is mechanism of HAMP gene expression in a well-phenotyped population of Iraqi β-thalassemia patients with a high rate of transfusion. The empirical results confirm a physiological switch: at very high levels of iron, the suppressive signals from the erythroferrone mediated by increased bone marrow erythropoiesis get overwhelmed by the competing regulatory signals in the form of BMP6/SMAD signaling pathway. This shift in dominance causes a major, compensatory increase in the transcription of upstream HAMP. Also, the fact that HAMP transcripts are not directly correlated with circulating serum ferritin suggests that serum ferritin is not a direct indicator of steady-state iron-sensing mechanisms in the genome, and that it is more likely to be an unstable acute-phase reactant in late stages of clinical disease, related to chronic tissue inflammation and cellular necrosis.

This study shows not only another systemically-regulatory role for HAMP, but also a very interesting and clinically-relevant association between HAMP expression kinetics and progressive renal impairment. The excellent positive linear correlation seen between serum creatinine and blood urea is reflective of a different pathway of iron induced nephrotoxicity. This association suggests that chronic exposure of the renal parenchyma to toxic non-transferrin-bound iron fractions results in chronic oxidative damage and subsequent tubulointerstitial stress and dysfunction of glomerular clearance dynamics. At the same time, the impaired filtering ability of the damaged kidneys may lead to a backflow of circulating hepcidin peptide fractions, which will simply further disrupt the master homeostatic regulatory loop.

Such molecular and biochemical relationships indicate that a genetic surrogate marker to monitor relative expression changes in HAMP could be used as an important non-invasive tool to identify subclinical nephropathy at an early stage and to make timely therapeutic decisions in hemoglobin disorders. Further longitudinal multi-center studies are needed to confirm the clinical relevance of these results. Further studies are needed to combine high throughput mass spectrometry to measure the quantities of bioactive hepcidin-25 peptide isoforms in circulation with the evaluation of the compliance of patients on specific iron chelation schemes to progress towards personalized thalassemia treatment.

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