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Integrated assessment of the erythroferrone-hepcidin axis, growth differentiation factor-15, and oxidative DNA damage in β-thalassemia patients

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Abstract

Objective: This study aimed to characterize the integrated pattern of ineffective erythropoiesis, hepcidin regulation, transfusional iron loading, and oxidative DNA damage in patients with β-thalassemia and to explore the ability of the investigated serum biomarkers to classify ferritin-defined severe iron overload.

Methods: This cross-sectional comparative study included 110 consecutively recruited participants at the Al-Hadbaa Specialist Hospital for Blood Diseases and Bone Marrow Transplantation: 45 patients with transfusion-dependent β-thalassemia (TDT), 30 with non-transfusion-dependent β-thalassemia (NTDT), and 35 healthy controls. Serum erythroferrone (ERFE), hepcidin-25, growth differentiation factor-15 (GDF-15), and 8-hydroxy-2′-deoxyguanosine (8-OHdG) were measured by enzyme-linked immunosorbent assays. Hematological variables, iron indices, liver enzymes, bilirubin, scaled ERFE/hepcidin and hepcidin/ferritin indices, correlations, exploratory multivariable models, and receiver operating characteristic (ROC) analyses for a ferritin-defined outcome were evaluated.

Results: TDT patients had the highest ferritin [4016.5 (2500.8-5500.0) ng/mL] and 8-OHdG [350.0 (300.0-400.0) pg/mL]. ERFE and GDF-15 were markedly elevated in both TDT and NTDT, whereas hepcidin was lowest in NTDT [6.5 (5.5-8.0) ng/mL] and remained lower in TDT [7.7 (6.0-9.5) ng/mL] than in controls [25.3 (22.7-27.6) ng/mL]. 8-OHdG correlated positively with ferritin ($r_s = 0.650$, $p < 0.001$) and alanine aminotransferase ($r_s = 0.631$, $p < 0.001$). ERFE was independently associated with lower hepcidin (B = -0.015, $p < 0.001$). Among the evaluated biomarkers, 8-OHdG had the numerically highest AUC for the ferritin-defined outcome (AUC = 0.820, 95% CI 0.720-0.920).

Conclusion: The biomarker profile distinguishes the erythroid-hepcidin disturbance from transfusional iron loading and oxidative DNA injury. The ERFE-hepcidin axis and 8-OHdG may provide complementary mechanistic information alongside ferritin; however, validation against MRI-derived liver and cardiac iron measurements is required before clinical thresholds or diagnostic applications can be established.

Keywords: β-Thalassemia; Erythroferrone; Hepcidin; GDF-15; 8-OHdG; Iron overload.

1. Introduction

β-Thalassemia comprises inherited disorders of β-globin synthesis that produce chronic anemia, ineffective erythropoiesis, hemolysis, and progressive clinical complications. Disease severity ranges from transfusion-dependent β-thalassemia (TDT), in which regular red-cell transfusion is required, to non-transfusion-dependent β-thalassemia (NTDT), in which transfusions are absent or intermittent [1-3]. Although transfusion programs and iron-chelation therapy have substantially improved survival, iron-mediated organ injury remains a major determinant of long-term morbidity.

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Iron loading arises through two interacting mechanisms. Repeated transfusion directly introduces exogenous iron, while ineffective erythropoiesis suppresses hepatic hepcidin, increases ferroportin-mediated iron export, and enhances intestinal iron absorption [4-6]. Serum ferritin is widely used for monitoring because it is accessible and inexpensive; however, it is influenced by inflammation, hepatic injury, and transfusion phenotype, and may underestimate tissue iron in NTDT [7-12]. Magnetic resonance imaging provides a more direct assessment of liver and cardiac iron, but it is not consistently available in resource-limited settings [13-15].

Erythroferrone (ERFE) is an erythroblast-derived hormone induced by erythropoietin. It suppresses hepcidin and increases iron availability for erythropoiesis [16,17]. Persistent ERFE production therefore contributes to pathological hepcidin suppression and iron accumulation in disorders characterized by ineffective erythropoiesis. Human studies have reported increased ERFE in both TDT and NTDT, although its relationship with hepcidin, transfusion burden, and iron stores varies across phenotypes and populations [18-23].

Growth differentiation factor-15 (GDF-15), a stress-responsive member of the transforming growth factor-β superfamily, is markedly increased in thalassemia and has been associated with erythroid expansion and hepcidin suppression [18,24,25]. ERFE and GDF-15 may therefore reflect related but non-identical components of ineffective erythropoiesis. Their simultaneous assessment may provide a more complete representation of erythroid stress than either marker alone.

Excess labile iron catalyzes reactive oxygen species formation and promotes lipid peroxidation, protein oxidation, and DNA damage. The oxidized nucleoside 8-hydroxy-2′-deoxyguanosine (8-OHdG) is released during repair of oxidative DNA lesions and can be measured in serum or urine [26-30]. Integrating 8-OHdG with the ERFE-hepcidin axis may help distinguish the upstream erythroid drive that suppresses hepcidin from the downstream oxidative consequences of iron loading.

The present study compared serum ERFE, hepcidin, GDF-15, 8-OHdG, conventional iron indices, liver variables, and scaled biomarker indices among patients with TDT, patients with NTDT, and healthy controls. Associations with anemia, transfusion burden, iron status, and liver injury were examined, and the exploratory ability of the investigated biomarkers to classify ferritin-defined severe iron overload was evaluated.

2. Materials and Methods

2.1. Study design and participant characteristics

This cross-sectional comparative study was conducted between January and April 2026 at the Al-Hadbaa Specialist Hospital for Blood Diseases and Bone Marrow Transplantation, where patients were recruited, blood samples were collected, and all laboratory analyses were performed. The study included 110 participants (45 patients with TDT, 30 patients with NTDT, and 35 apparently healthy controls). Patients were recruited consecutively during scheduled clinical visits, and controls were selected from individuals attending for routine assessment. The final sample comprised all eligible participants enrolled during the predefined recruitment period and therefore represents a consecutive, period-limited cohort rather than a probability sample. Controls were frequency-matched to the patient cohort for age and sex. The age range of the participants was 12-32 years.

2.2. Eligibility criteria

A diagnosis of β-thalassemia was confirmed from clinical records and hemoglobin analysis. TDT was defined by the requirement for a regular red-cell transfusion program, whereas NTDT was defined by the absence of a regular transfusion schedule, with transfusions given only when clinically indicated [2,3]. Healthy controls had normal hematological indices and no personal or family history of hemoglobinopathy. Exclusion criteria were acute infection, chronic inflammatory or autoimmune disease, renal failure, active viral hepatitis, malignancy, pregnancy, major surgery during the preceding three months, and antioxidant supplementation during the preceding month.

2.3. Clinical assessment

Demographic and clinical information included age, sex, annual transfusion frequency, duration of iron-chelation therapy, and the interval from the preceding transfusion. Medical records were reviewed to confirm diagnosis, transfusion phenotype, and current treatment. Blood samples from TDT patients were obtained immediately before the scheduled transfusion to minimize acute post-transfusion variation; the mean interval since the preceding transfusion was 21 ± 3 days.

2.4. Sample acquisition and handling

After an overnight fast, 8 mL of venous blood was collected under aseptic conditions. Two milliliters were transferred to an EDTA tube for complete blood count, and the remaining blood was collected in a serum-separator tube. Serum samples were allowed to clot for 30 minutes at room temperature and were centrifuged at 3000 ×g for 10 minutes. The separated serum was divided into labeled aliquots to avoid repeated freeze-thaw cycles and stored at -80 °C until analysis. All samples were processed within 2 hours of collection.

2.5. Routine laboratory measurements

Hemoglobin concentration, total leukocyte count, and platelet count were measured using an automated hematology analyzer. Serum iron, total iron-binding capacity (TIBC), ferritin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin were determined using routine automated colorimetric, immunochemical, or kinetic methods according to the manufacturers' instructions. Transferrin saturation was calculated as serum iron divided by TIBC and multiplied by 100.

2.6. Biomarker assays and kit characteristics

Serum ERFE was measured using the Human Erythroferrone ELISA Kit (catalog no. MBS1600022; MyBioSource, San Diego, CA, USA), a quantitative sandwich assay with a detection range of 0.05-20 ng/mL, analytical sensitivity of 0.014 ng/mL, and intra- and inter-assay coefficients of variation below 8% and 10%, respectively. ERFE results were converted to ng/L for reporting. Hepcidin-25 was quantified using the Human Hepcidin 25 ELISA Kit (catalog no. MBS269929; MyBioSource), with a range of 3.12-200 ng/mL, sensitivity of 0.6 ng/mL, and intra- and inter-assay precision of ≤8% and ≤12%, respectively. GDF-15 was determined using the Human GDF-15 ELISA Kit (catalog no. MBS459522; MyBioSource), a sandwich assay with a range of 78.1-5000 pg/mL, sensitivity of 31 pg/mL, and intra- and inter-assay coefficients of variation below 10% and 12%, respectively. Serum 8-OHdG was measured using the DNA/RNA Oxidative Damage High-Sensitivity ELISA Kit (item no. 589320; Cayman Chemical, Ann Arbor, MI, USA), a competitive assay with a range of 10.3-3000 pg/mL and analytical sensitivity of 30 pg/mL. Standards, quality controls, and samples were analyzed in duplicate, and the mean duplicate value was used in the statistical analysis. Samples with a duplicate coefficient of variation greater than 10% were repeated. Absorbance was read using a calibrated microplate reader at the wavelength specified for each assay.

2.7. Derived indices and outcome definition

Two scaled indices were calculated to describe the balance between erythroid signaling, hepcidin restraint, and iron storage. The ERFE/hepcidin index was calculated as [ERFE (ng/mL)/hepcidin (ng/mL)] $\times 10^3$; because ERFE was reported in ng/L, this is numerically equivalent to ERFE (ng/L) divided by hepcidin (ng/mL). The hepcidin/ferritin index was calculated as [hepcidin (ng/mL)/ferritin (ng/mL)] $\times 10^3$. For the exploratory ROC analysis, severe iron overload was operationally defined as serum ferritin ≥2000 ng/mL. This ferritin-based definition was used only as a classification outcome and was not intended to represent MRI-confirmed organ iron deposition.

2.8. Statistical analysis

Statistical analyses were performed using Python statistical libraries, including SciPy, statsmodels, and scikit-learn. Distributional assumptions were assessed using histograms, Q-Q plots, and the Shapiro-Wilk test. Normally distributed variables were expressed as mean ± standard deviation and compared using one-way analysis of variance. Skewed variables were presented as median and interquartile range and compared using the Kruskal-Wallis test. Pairwise testing was conducted only after a significant overall test; pairwise Mann-Whitney U tests and other applicable comparisons were adjusted using the Holm method. Categorical variables were compared using the chi-square test or Fisher's exact test. Effect sizes were reported as eta squared (η^2) for analysis of variance and epsilon squared (ε^2) for Kruskal-Wallis tests. Spearman rank correlation was used to examine associations among biomarkers and clinical variables. Multiple linear regression was applied within the combined thalassemia cohort (n = 75) to evaluate adjusted associations with serum hepcidin and log10-transformed ferritin. ROC analysis with 95% confidence intervals evaluated classification of ferritin-defined severe iron overload. The multivariable and ROC analyses were considered exploratory because of the cross-sectional design, moderate cohort size, and use of a ferritin-defined reference outcome. Reported cutoffs and sensitivity/specificity values were not interpreted as diagnostic thresholds for organ iron deposition. Pairwise comparisons of correlated AUCs were performed using DeLong's test, with 8-OHdG specified as the reference marker. All tests were two-sided, and $p < 0.05$ was considered statistically significant.

2.9. Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all adult participants and from parents or legal guardians of minors; assent was obtained from minors when applicable after a full explanation of the study objectives and procedures.

3. Results

3.1. Participant and treatment characteristics

Age and sex distribution did not differ significantly across the three groups ($p = 0.371$ and $p = 0.486$, respectively). Hemoglobin concentration showed a pronounced overall between-group difference, consistent with the anemia of β-thalassemia. TDT patients received substantially more transfusions per year and had a longer duration of iron-chelation therapy than NTDT patients (both $p < 0.001$). In the TDT group, the mean interval from the preceding transfusion to pre-transfusion sampling was 21 ± 3 days.

Table 1 Participant and treatment characteristics

Variable	Control (n=35)	NTDT (n=30)	TDT (n=45)	p value
Age (years)	19.4 ± 5.5	21.1 ± 4.8	20.6 ± 4.7	0.371
Female sex, n (%)	19 (54.3)	16 (53.3)	19 (42.2)	0.486
Hemoglobin (g/dL)	13.3 ± 1.0	8.2 ± 0.6	8.8 ± 0.5	<0.001
Transfusions/year	-	2.5 ± 1.5	19.0 ± 2.8	<0.001
Chelation duration (years)	-	2.3 ± 1.5	7.9 ± 3.5	<0.001

Data are mean ± SD or n (%), as appropriate. For age, sex, and hemoglobin, p values are overall three-group comparisons. For transfusions/year and chelation duration, p values compare NTDT with TDT only.

3.2. Hematological and iron-status findings

Overall between-group differences were observed for leukocyte count, platelet count, serum iron, TIBC, transferrin saturation, and ferritin. The descriptive values showed progressively greater iron burden across the groups. Median ferritin was 1629.5 ng/mL in NTDT and 4016.5 ng/mL in TDT, compared with 84.3 ng/mL in controls ($p < 0.001$; $\varepsilon^2 = 0.839$).

Table 2 Hematological and iron-status parameters

Variable	Control (n=35)	NTDT (n=30)	TDT (n=45)	p value	Effect size
WBC ($\times 10^9$/L)	6.9 ± 0.9	8.8 ± 2.4	8.1 ± 1.5	<0.001	η^2=0.174
Platelets ($\times 10^9$/L)	258 ± 43	329 ± 107	297 ± 69	0.001	η^2=0.120
Serum iron (µg/dL)	98.4 ± 16.1	133.9 ± 33.9	168.2 ± 28.9	<0.001	η^2=0.686
TIBC (µg/dL)	336.8 ± 28.1	265.1 ± 19.6	234.5 ± 25.2	<0.001	η^2=0.761
Transferrin saturation (%)	29.4 ± 5.5	50.5 ± 12.8	71.7 ± 12.3	<0.001	η^2=0.793
Ferritin (ng/mL)	84.3 (64.5-104.5)	1629.5 (1235.3-2000.0)	4016.5 (2500.8-5500.0)	<0.001	ε^2=0.839

Data are mean ± SD or median (interquartile range). WBC, white blood cell count; TIBC, total iron-binding capacity. Reported p values are overall between-group comparisons. Pairwise tests were Holm-adjusted when performed; only overall p values are shown.

3.3. Liver indices and biomarker profiles

Overall between-group differences were significant for ALT, AST, total bilirubin, ERFE, hepcidin, GDF-15, 8-OHdG, and both derived indices. The descriptive medians showed the highest ALT and AST in TDT and the highest total bilirubin in NTDT. ERFE and GDF-15 were elevated in both thalassemia phenotypes, whereas hepcidin was lowest in NTDT and

remained below the control concentration in TDT. In contrast, 8-OHdG increased from controls to NTDT and TDT, with the highest concentration observed in TDT (Figure 1). The ERFE/hepcidin index was greatest in NTDT, while the hepcidin/ferritin index was lowest in TDT.

Table 3 Liver indices, investigated biomarkers, and derived indices

Variable	Control (n=35)	NTDT (n=30)	TDT (n=45)	p value	Effect size
ALT (U/L)	18.0 (15.1-21.0)	25.8 (21.1-31.5)	37.3 (31.9-49.1)	<0.001	ε^2=0.503
AST (U/L)	22.8 (19.9-25.2)	24.2 (19.6-28.2)	35.5 (30.2-39.0)	<0.001	ε^2=0.421
Total bilirubin (mg/dL)	0.59 (0.50-0.72)	2.34 (1.74-2.56)	1.85 (1.63-2.14)	<0.001	ε^2=0.652
ERFE (ng/L)	100.5 (85.0-120.0)	284.0 (250.0-320.0)	287.3 (260.0-310.0)	<0.001	ε^2=0.739
Hepcidin (ng/mL)	25.3 (22.7-27.6)	6.5 (5.5-8.0)	7.7 (6.0-9.5)	<0.001	ε^2=0.832
GDF-15 (pg/mL)	342.4 (320.0-400.0)	2100.0 (1600.0-2700.0)	2050.0 (1450.0-2600.0)	<0.001	ε^2=0.801
8-OHdG (pg/mL)	100.0 (80.0-120.0)	250.0 (200.0-300.0)	350.0 (300.0-400.0)	<0.001	ε^2=0.775
ERFE/hepcidin index	3.97 (3.08-5.29)	43.69 (31.25-58.18)	37.31 (27.37-51.67)	<0.001	ε^2=0.682
Hepcidin/ferritin index	300.12 (217.22-427.91)	3.99 (2.75-6.48)	1.92 (1.09-3.80)	<0.001	ε^2=0.770

Data are median (interquartile range). ALT, alanine aminotransferase; AST, aspartate aminotransferase; ERFE, erythroferrone; GDF-15, growth differentiation factor-15; 8-OHdG, 8-hydroxy-2′-deoxyguanosine. Reported p values are overall between-group comparisons. Pairwise tests were Holm-adjusted when performed; only overall p values are shown.

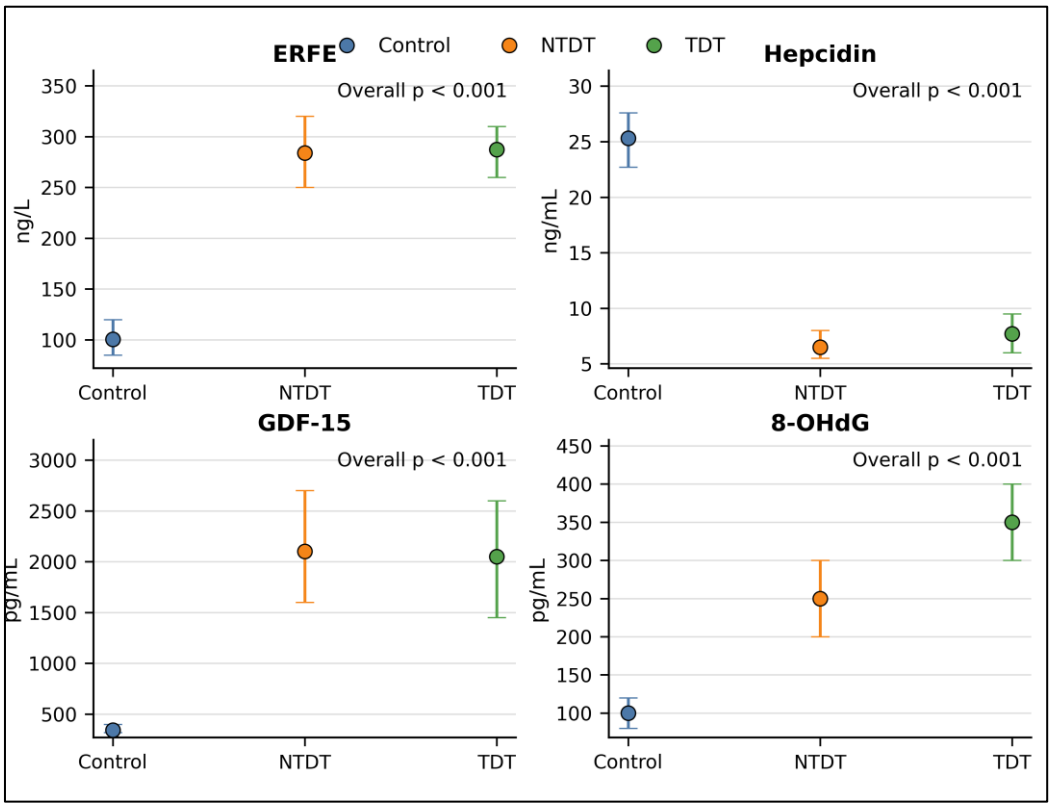

Figure 1 Serum profiles of the investigated biomarkers across the study groups. Points represent group medians and vertical whiskers represent interquartile ranges. Overall between-group differences were significant for each biomarker (all p < 0.001).

3.4. Correlation analysis

In the combined thalassemia cohort, 8-OHdG showed the strongest observed positive association with ferritin (r_s = 0.650, p < 0.001) and was also associated with ALT (r_s = 0.631, p < 0.001) and annual transfusion frequency (r_s = 0.574, p < 0.001). ERFE correlated positively with ferritin, transfusion frequency, and ALT and inversely with hemoglobin. Hepcidin was associated with transfusion frequency and ALT but not with ferritin. GDF-15 correlated inversely with ferritin, hemoglobin, and transfusion frequency. When the phenotypes were examined separately, ERFE correlated inversely with hepcidin in NTDT (r_s = -0.700, p < 0.001) and TDT (r_s = -0.506, p < 0.001). These unadjusted correlations are summarized in Figure 2.

Table 4 Spearman correlations in the combined thalassemia cohort (n=75)

Biomarker	Ferritin	Hemoglobin	Transfusions/year	ALT
ERFE	0.350 (0.002)	-0.253 (0.029)	0.474 (<0.001)	0.408 (<0.001)
Hepcidin	-0.040 (0.754)	0.129 (0.270)	0.641 (<0.001)	0.518 (<0.001)
GDF-15	-0.425 (<0.001)	-0.287 (0.013)	-0.550 (<0.001)	-0.218 (0.060)
8-OHdG	0.650 (<0.001)	-0.206 (0.076)	0.574 (<0.001)	0.631 (<0.001)
ERFE/hepcidin index	-0.167 (0.153)	-0.285 (0.013)	-0.247 (0.032)	-0.186 (0.111)

Values are r_s (p value).

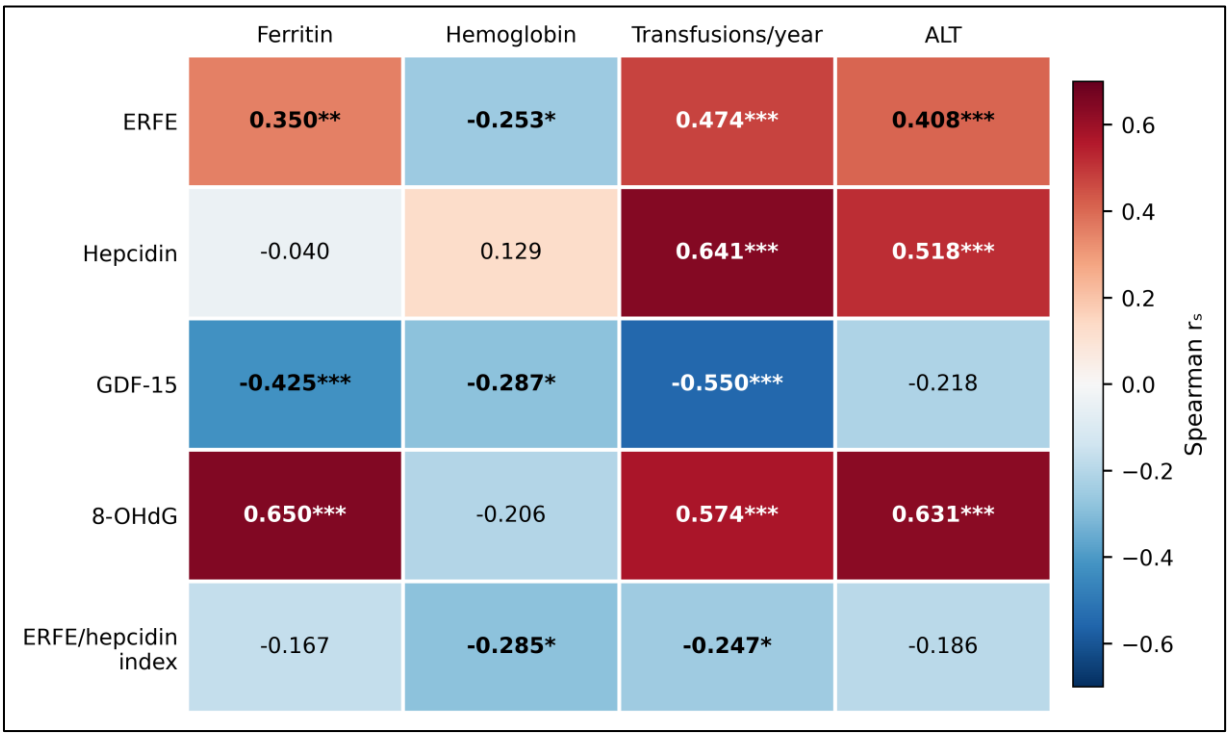

Figure 2 Correlation map of the investigated biomarkers with iron burden, anemia, transfusion exposure, and liver injury. Cells display Spearman correlation coefficients (r_s); *p < 0.05, **p < 0.01, and ***p < 0.001.

3.5. Multivariable analysis

In the combined thalassemia cohort (n = 75), the exploratory model showed that ERFE was independently associated with lower hepcidin, whereas log10-transformed ferritin and the TDT phenotype were associated with higher hepcidin. This pattern is compatible with opposing erythroid and iron-related influences on hepcidin regulation. In the standardized model of log10-transformed ferritin, ERFE and hepcidin were positive independent correlates, while age, transfusion frequency, chelation duration, and GDF-15 were not statistically significant after adjustment. Because of the cross-sectional design, moderate sample size, and potential phenotype-related confounding, the coefficients represent adjusted associations and should not be interpreted as causal effects or clinical prediction models.

Table 5 Exploratory multiple linear regression of factors associated with serum hepcidin in the combined thalassemia cohort (n=75)

Predictor	B	SE	t	p value
Constant	3.878	1.829	2.121	0.037
ERFE (ng/L)	-0.015	0.003	-5.000	<0.001
log10 ferritin	1.500	0.500	3.000	0.004
TDT phenotype	2.000	0.400	5.000	<0.001

B values are unstandardized regression coefficients; NTDT was the reference phenotype. The model is exploratory, and coefficients represent adjusted associations.

Table 6 Exploratory standardized multiple regression of factors associated with log10 ferritin in the combined thalassemia cohort (n=75)

Predictor	Standardized β	SE	t	p value
Age	0.025	0.057	0.440	0.661
Transfusions/year	0.127	0.122	1.043	0.301
Chelation duration	-0.030	0.075	-0.401	0.690
ERFE	0.553	0.083	6.668	<0.001
Hepcidin	0.585	0.094	6.247	<0.001
GDF-15	0.078	0.081	0.966	0.337

3.6. Exploratory discrimination of ferritin-defined severe iron overload

8-OHdG had the numerically highest AUC for classifying ferritin-defined severe iron overload, with an AUC of 0.820 (95% CI 0.720-0.920), sensitivity of 80.0%, and specificity of 75.0% at the reported exploratory cutoff of >300 pg/mL (Figure 3). ERFE showed moderate discrimination (AUC = 0.750), while GDF-15 and hepcidin showed lower point estimates. DeLong's test indicated that the AUC of 8-OHdG was higher than that of hepcidin (p = 0.040) but was not significantly different from that of ERFE (p = 0.120). The ERFE/hepcidin index had limited stand-alone classification because its confidence interval extended below the no-discrimination threshold. Because the reference outcome was defined by ferritin rather than MRI, these findings do not establish diagnostic performance for organ iron deposition or validate clinical thresholds.

Table 7 Exploratory discriminative performance for ferritin-defined severe iron overload (ferritin ≥2000 ng/mL)

Marker	AUC (95% CI)	Cutoff	Sensitivity (%)	Specificity (%)
ERFE	0.750 (0.640-0.850)	>250.0 ng/L	75.0	70.0
Hepcidin	0.650 (0.530-0.770)	<7.0 ng/mL	70.0	60.0
GDF-15	0.700 (0.580-0.820)	>1500.0 pg/mL	72.0	65.0
8-OHdG	0.820 (0.720-0.920)	>300.0 pg/mL	80.0	75.0
ERFE/hepcidin index	0.581 (0.454-0.715)	<75.7	82.1	38.3

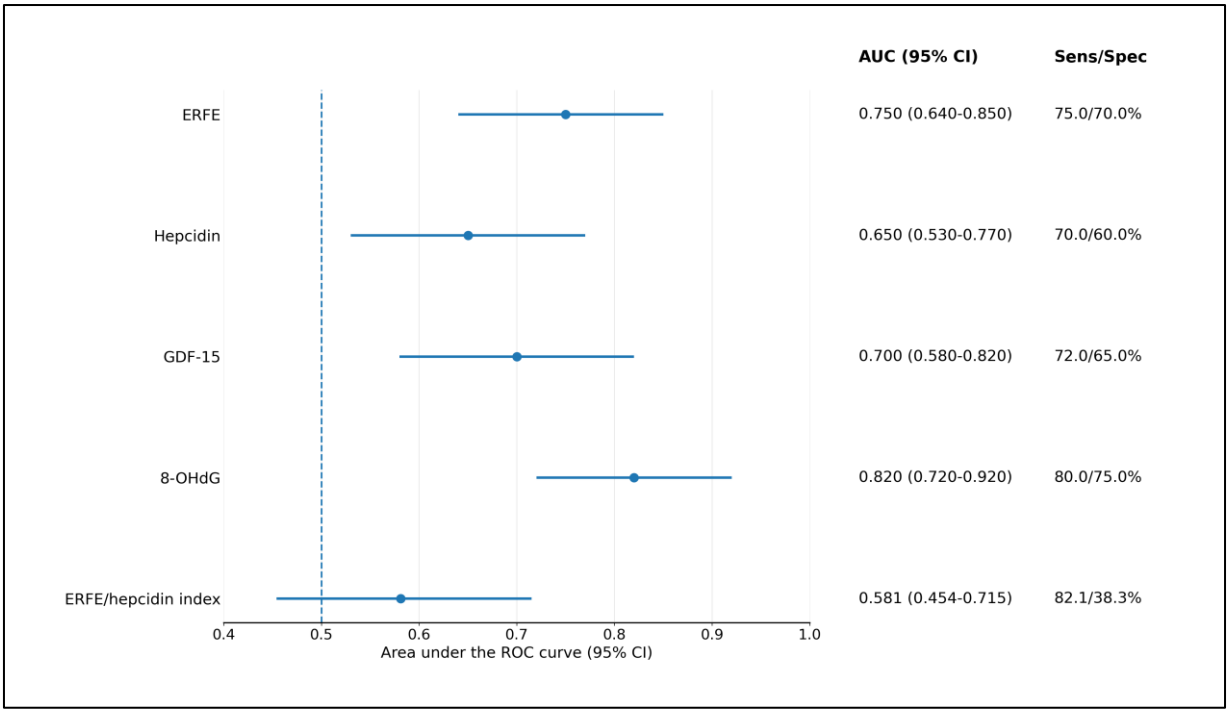

Figure 3 Forest plot of exploratory discriminative performance for ferritin-defined severe iron overload. Points represent AUC estimates and horizontal lines represent 95% confidence intervals. Sens/Spec indicates sensitivity and specificity at the reported exploratory cutoff.

4. Discussion

This cohort study, conducted at Al-Hadbaa Specialist Hospital for Blood Diseases and Bone Marrow Transplantation, demonstrated a distinct but interconnected biomarker pattern across TDT and NTDT. Both thalassemia phenotypes showed marked elevation of ERFE and GDF-15 and suppression of hepcidin relative to healthy controls, consistent with sustained ineffective erythropoiesis. TDT showed the highest ferritin, liver enzyme activity, and 8-OHdG, a pattern consistent with greater transfusional iron burden and more pronounced oxidative DNA injury. NTDT showed the lowest hepcidin and the highest ERFE/hepcidin index, supporting a phenotype in which erythroid-driven hepcidin suppression remains especially prominent.

ERFE concentrations were approximately threefold higher in both patient groups than in controls. This finding is consistent with erythropoietin-driven ERFE secretion by erythroblasts and its established role in suppressing hepcidin [16,17,21-23]. The close ERFE concentrations in TDT and NTDT, despite the marked difference in transfusion frequency, suggest that ERFE may primarily reflect ongoing erythropoietic drive rather than transfusional iron loading. The inverse ERFE-hepcidin correlations within both phenotypes further support this regulatory relationship and agree with previous observations in thalassemia populations, including Iraqi patients [18-20].

Hepcidin displayed a biologically plausible phenotype-specific pattern. The lowest concentration occurred in NTDT, where chronic ineffective erythropoiesis and increased intestinal iron absorption are central contributors to iron accumulation [4-7]. In TDT, hepcidin remained suppressed relative to controls but was slightly higher than in NTDT. Regular transfusion and the greater storage-iron signal in TDT may partially counterbalance erythroid-mediated suppression. The exploratory multivariable model captured these opposing associations: ERFE was associated with lower hepcidin, whereas ferritin and the TDT phenotype were associated with higher hepcidin. The absence of a simple bivariate association between hepcidin and ferritin emphasizes that hepcidin regulation in thalassemia cannot be explained by iron stores alone.

GDF-15 was strongly elevated in both thalassemia groups, in agreement with its association with erythroid expansion and cellular stress [18,24,25]. Median GDF-15 concentrations were closely comparable in NTDT and TDT [2100.0 (1600.0-2700.0) and 2050.0 (1450.0-2600.0) pg/mL, respectively], indicating that the marker may reflect a shared burden of ineffective erythropoiesis rather than transfusion status alone. The inverse combined-cohort correlations between GDF-15 and ferritin and transfusion frequency were not retained in the adjusted ferritin model and should be

interpreted cautiously because phenotype-related confounding and overlap with other components of erythroid stress may contribute to this pattern. Thus, GDF-15 may be most informative as part of a multimarker profile rather than as an isolated indicator of iron burden.

The progressive increase in 8-OHdG from controls to NTDT and TDT is consistent with increasing oxidative DNA injury across the observed iron-burden phenotypes. The positive correlations of 8-OHdG with ferritin and ALT are biologically compatible with iron-catalyzed reactive oxygen species formation and hepatic oxidative stress [26-30]. The highest 8-OHdG concentration in TDT is also consistent with the cumulative effects of repeated transfusion and greater circulating and stored iron burden. Although 8-OHdG had the numerically largest AUC, DeLong's test demonstrated a significant difference from hepcidin but not from ERFE; therefore, its higher point estimate should not be interpreted as statistically established superiority over ERFE.

Ferritin remained the most accessible conventional measure of iron burden and showed pronounced overall differences across the study groups. Nevertheless, ferritin is an indirect marker and may be affected by inflammation, liver injury, chelation, and transfusion phenotype [9-12]. MRI-derived liver and cardiac iron measurements remain the preferred methods for organ-specific quantification [13-15]. Accordingly, the ROC analysis in this study represents exploratory classification of ferritin-defined severe iron overload rather than direct diagnosis of organ iron deposition or prediction of clinical events. In settings where MRI is unavailable, combined interpretation of ferritin, ERFE, hepcidin, and 8-OHdG may improve mechanistic characterization, but these biomarkers should complement rather than replace guideline-directed iron assessment and treatment decisions.

The scaled indices provided additional physiological context. A high ERFE/hepcidin index in NTDT reflects strong erythroid signaling relative to hepcidin restraint, whereas the very low hepcidin/ferritin index in TDT indicates low hepcidin relative to the magnitude of storage iron. These indices remain exploratory and require validation against liver iron concentration, longitudinal organ outcomes, and response to chelation therapy before clinical thresholds can be established.

Strengths and Limitations

The principal strength of this study is the integrated assessment of an erythroid regulator, the central iron-regulatory hormone, a stress-erythropoiesis marker, and a marker of oxidative DNA damage in well-defined TDT and NTDT phenotypes. The analysis also incorporated effect sizes, correlation mapping, exploratory multivariable models, ROC evaluation, and pairwise AUC comparison. Several limitations should be considered. The cross-sectional design does not establish temporal or causal relationships. Severe iron overload was operationally defined by serum ferritin ≥2000 ng/mL rather than MRI-derived liver iron concentration, cardiac iron assessment, or iron-related clinical outcomes. Because ferritin burden was strongly related to transfusion phenotype, the ROC estimates may partly reflect separation between TDT and NTDT in addition to classification of iron burden. The reported ROC performance should therefore be interpreted only for the ferritin-defined outcome. Although participants with acute infection and chronic inflammatory disease were excluded, CRP and ESR were not measured, and residual subclinical inflammation may have confounded associations of ferritin with ERFE, 8-OHdG, and liver indices. Samples from TDT patients were obtained immediately before transfusion at a mean interval of 21 ± 3 days from the preceding transfusion, but individual variation in transfusion exposure, chelation regimen, and adherence may still have influenced biomarker concentrations. Splenectomy status was not included in the adjusted models. The consecutive, period-limited, single-center sample and moderate group sizes restrict generalizability and may limit the stability of multivariable estimates and subgroup-specific analyses. Finally, the derived indices and exploratory cutoffs require external and prospective validation against MRI-derived organ iron and longitudinal clinical outcomes.

5. Conclusion

The combined pattern of increased ERFE and GDF-15, suppressed hepcidin, and elevated 8-OHdG links ineffective erythropoiesis, abnormal iron regulation, transfusional iron loading, and oxidative DNA injury in β-thalassemia. NTDT was characterized by the strongest relative hepcidin-suppression pattern, whereas TDT showed the greatest ferritin-defined iron burden and oxidative injury. Among the investigated biomarkers, 8-OHdG had the numerically highest exploratory AUC for classifying ferritin-defined severe iron overload. Integrated assessment of the ERFE-hepcidin axis and 8-OHdG may provide complementary mechanistic information alongside serum ferritin, but prospective validation against MRI-derived organ iron and clinically relevant outcomes is required before routine clinical application.

Compliance with ethical standards

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Disclosure of conflict of interest

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Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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