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Original Article

Clinical and Biochemical Heterogeneity in Hemoglobin H Disease: A Comprehensive Analysis of α-Globin Mutations and Transfusion Requirements

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Abstract

Background: Hemoglobin H (Hb H) disease, a subtype of α-thalassemia, demonstrates marked clinical heterogeneity primarily driven by underlying genotypic differences. While non-deletional mutations are typically associated with more severe phenotypes, considerable variability is observed even among patients with similar mutation classes. This study aimed to examine genotype–phenotype correlations in Hb H disease by assessing the relationship between α-globin mutations, transfusion dependency, and a range of hematologic and biochemical markers.

Methods: Ninety patients with confirmed Hb H disease were evaluated. Genotyping was performed via multiplex gap-PCR, Sanger sequencing, and MLPA. Patients were classified by transfusion need into transfusion-dependent (TDT), occasionally transfused (OTDT), and non-transfusion-dependent (NTDT) groups. Genotypically, patients were categorized as non-deletional homozygotes (ND/ND), compound heterozygotes (ND/D), and deletional homozygotes (D/D). Complete blood count, hemoglobin fractions, iron profile, liver enzymes, and C-reactive protein (CRP) levels were measured and analyzed.

Results: Significant differences in hematologic and biochemical parameters were observed across genotypes. ND/ND patients had the highest hemoglobin (10.70 ± 1.83 g/dL), MCV (66.06 ± 8.43 fL), and HbA levels (93.72 ± 5.13%), and the lowest reticulocyte counts and Hb H percentages ($p < 0.01$). ND/D patients exhibited lower HbA, higher Hb H, and elevated ferritin (438.66 ± 840.60 ng/mL) and CRP (3.97 ± 4.75 mg/L) levels ($p < 0.05$), indicating greater erythropoietic stress. Transfusion dependence was most frequent in ND/D patients, though not statistically significant ($p = 0.34$).

Conclusion: This study highlights substantial phenotypic variability within genotypic groups, challenging the binary classification of deletional versus non-deletional mutations. Integrating molecular data with functional and inflammatory biomarkers may enhance risk stratification and support individualized management of Hb H disease.

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1. INTRODUCTION

Hemoglobin H (Hb H) disease is a clinically important subtype of α-thalassemia, arising from the deletion or dysfunction of three out of the four α-globin genes located on chromosome 16p13.3(1, 2). This condition typically arises from compound heterozygosity involving large gene deletions or point mutations, manifesting as either deletional Hb H (--/-α) or non-deletional Hb H (--/$\alpha^T\alpha$) genotypes (3). The fundamental pathophysiology of Hb H disease involves markedly reduced α-globin chain synthesis, resulting in an excess of unpaired β-globin chains. These surplus β-globin chains assemble into unstable β^4 tetramers, known as hemoglobin H (Hb H), which precipitate within erythrocytes. Their accumulation leads to membrane damage, increased oxidative stress, and chronic hemolysis, contributing to the clinical severity of the disease (4).

Although Hb H disease was historically perceived as a relatively benign condition, recent evidences has highlighted its clinical heterogeneity and potential significant morbidity (5, 6). The disease spectrum ranges from asymptomatic or mildly anemic individuals to those requiring intermittent or regular transfusions. Non-deletional variants are generally associated with more severe clinical phenotypes, including lower hemoglobin levels, higher reticulocyte counts, and increased transfusion dependency (3). Moreover, biochemical and hematologic indicators of disease manifestations, such as altered iron metabolism, hepatic dysfunction, and systemic inflammation, have been increasingly documented in pediatric and adult patients with Hb H disease (7).

The diagnosis of Hb H disease involves a combination of hematological indices and molecular testing. Patients typically present with microcytic hypochromic anemia, elevated red cell distribution width (RDW), and variable Hb H and Hb Bart's levels on hemoglobin electrophoresis (1, 8). Definitive diagnosis requires genotyping to identify specific α-globin mutations, which have important implications for risk stratification, genetic counseling, and treatment planning.

Despite prior studies on genotype–phenotype correlations in Hb H disease, the variability in hematologic and biochemical profiles across genotypic groups remains incompletely understood. Previous research in Iranian cohorts has demonstrated considerable phenotypic variation, even among patients with similar genotypes, and highlighted the absence of a strict genotype–phenotype correlation in Hb H disease, including among deletional variants (9). In particular, the relationship between mutation subtype and hematologic parameters, such as hemoglobin composition, iron status, hepatic enzymes, and inflammatory markers, has not been fully elucidated. This study aimed to assess the clinical relevance of genotypic diversity in Hb H disease by analyzing its association with transfusion requirement and key hematologic indicators, including hemoglobin composition, iron overload, and inflammatory markers.

2. MATERIALS AND METHODS

2.1. Study Population

A total of 90 patients with known Hb H disease conditions and presenting different percentages of hemoglobin H in their hemoglobin electrophoresis, were enrolled in this cross-sectional study. Patients were recruited from multiple major thalassemia centers across Iran, including Kariminejad-Najmabadi Pathology & Genetics Center, Mofid Children's Hospital (Tehran), Zafar Thalassemia Center (Tehran), and the Genetics Reference Laboratory of the Pasteur Institute of Iran. The study was conducted between December 2022 and September 2023.

Eligible individuals were identified through clinical records and laboratory data, including complete blood count and hemoglobin electrophoresis. Inclusion criteria were: patients of any age and sex with anemia and confirmed presence of Hb H (0.8%–40%) on hemoglobin analysis, normal or low HbA_2 levels, and normal fetal hemoglobin (Hb F) levels. Patients with β-thalassemia major or intermedia, silent α-thalassemia carrier status, or other unrelated causes of anemia (e.g., iron deficiency) were excluded.

Comprehensive demographic and clinical data were collected from all participants, including age, geographic location (city of residence), and disease characteristics. Clinical assessment included documentation of characteristic facial features, age at disease onset, and detailed transfusion history (age of first transfusion, frequency of transfusions per year, total number of transfusions, and date of most recent transfusion). Cardiovascular evaluation and hepatic status, including screening for hepatitis, were recorded. Splenic parameters were thoroughly documented, including spleen size, presence of splenomegaly, history of splenectomy (including age at splenectomy), and any post-splenectomy thromboembolic events. A complete medication history was obtained from each patient, including specific chelation therapies, doses, treatment duration, and other disease-related medications.

Patients were stratified into three clinical subgroups according to transfusion requirements: transfusion-dependent Hb H, occasionally transfused HB H, and non-

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transfusion-dependent Hb H. Classification was determined by reviewing transfusion history and medical records, with all cases confirmed by an expert hematologist before inclusion. Peripheral blood samples were collected in EDTA tubes for hematologic and genetic analyses. Written informed consent was obtained from all participants or their legal guardians. The study protocol was approved by the Ethics Committee of Pasteur Institute of Iran (IR.PII.REC.1403.027) and adheres to the 1964 Helsinki Declaration and its later amendments.

2.2. Hematologic Parameters and Iron Status

A comprehensive hematologic evaluation was performed for all patients. Complete blood count parameters (Hb, MCV, MCH) and reticulocyte count were assessed using a Sysmex XN-2500 automated analyzer (Japan). Hemoglobin electrophoresis was conducted using Sebia Capillarys systems (models 2 or 3, France) to determine the proportions of Hb A, Hb A_2, Hb F, Hb H, and Hb Barts.

Iron status evaluation included serum ferritin measurement using either Abbott Architect i1000 or Roche Cobas e411 analyzers, while serum iron and TIBC were determined using a Mindray BS-200 system. Liver function tests (ALT, AST) were performed using the Siemens Advia clinical chemistry system. C-reactive protein (CRP) levels were quantified via immunoturbidimetric assay (Mindray BS2000), and Total bilirubin was measured spectrophotometrically. All analyses followed manufacturers' protocols and standard laboratory procedures.

2.3. Molecular Analysis

After genetic counseling, genomic DNA was extracted from peripheral blood leukocytes by the salting-out method (10). Initial screening for Common α-globin gene deletions $-\alpha^{3.7}$, $-\alpha^{4.2}$, $-(\alpha)^{20.5}$, $\sim^{Med}$ was performed using multiplex gap-PCR. PCR products were electrophoresed on a 1% agarose gel and analyzed against normal and positive controls (11).

In patients where no hemoglobin H-related common deletions were identified during the previous step, PCR amplification followed by Sanger sequencing of the HBA1 and HBA2 genes was performed using primers listed in Table 1 (ABI 3130xl Genetic Analyzer) was performed to detect non-deletional mutations (point mutations and small indels).

Resulting sequences were aligned with NCBI RefSeq transcripts (NM_000558.5 for HBA1 and NM_000517.6 for HBA2) (13), using Codon Code Aligner Software for mutation identification. In cases where no causative mutations for Hb H disease were identified by sequencing and multiplex gap-PCR, multiplex ligation-dependent probe amplification (MLPA) was conducted (SALSA® MLPA® Probemix P140 HBA) to identify uncommon deletions or duplications. MLPA products were analyzed on the ABI 3500 Genetic Analyzer and interpreted using Gene Marker V2.6.3 Software.

2.4. Statistical Analysis

All statistical analyses were performed using SPSS Statistical Software (version 23.0). Means and standard deviations (SD) were calculated for continuous variables, and frequencies and proportions were reported for categorical variables. The distribution of quantitative variables was assessed using the Kolmogorov–Smirnov test. Depending on whether the data were normally distributed, either parametric or nonparametric tests were applied. The Kruskal–Wallis test and one-way ANOVA were used to evaluate the relationship between transfusion dependence, Hb H genotypes, and hematological parameters. Categorical variables were analyzed using the Chi-square test or Fisher's exact test, as appropriate. Given the influence of age and gender on ferritin levels, an analysis of covariance (ANCOVA) was performed to compare ferritin levels among the patient groups while adjusting for potential confounders. A p-value of <0.05 was considered statistically significant.

3. RESULTS

In this study, 90 patients with Hb H disease were categorized into three clinical subgroups based on transfusion history (Table 2): transfusion-dependent Hb H (25.6%), occasionally transfused Hb H (16.7%), and non-transfusion-dependent Hb H (57.8%). Mean age showed no significant difference among groups ($p = 0.24$), but sex distribution varied significantly, with females comprising 74% of the transfusion-dependent Hb H group compared to only 33% of the non-transfusion-dependent Hb H group ($p = 0.003$).

Hematologic assessment revealed non-transfusion-dependent Hb H patients had significantly higher hemoglobin levels (10.35 ± 1.67 g/dL) than transfusion-dependent Hb H (9.09 ± 1.22 g/dL) and occasionally transfused HB H (8.65 ± 1.31 g/dL) patients ($p < 0.001$). Reticulocyte counts were elevated in transfusion-dependent Hb H (5.26 ± 4.25%) and occasionally transfused Hb H (5.92 ± 3.91%) compared to non-transfusion-dependent Hb H (3.92 ± 3.48%), though this difference was not statistically significant ($p = 0.12$).

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Table 1. Primers used for PCR amplification of the HBA1 and HBA2 genes prior to Sanger sequencing

Gene	Primer Name	Primer Sequence	Product Length	Type of PCR
HBA1	Alpha Seq1-F	GCT CCG CGC CAG CCA ATG AG	1001 bp	Sequencing-PCR
	Alpha Seq1-R	CAT GTGTGT CCC AGC TGC TGT C	1001 bp	Sequencing-PCR
HBA2	Alpha 2-F	GCT CCG CGC CAG CCA ATG AG	999 bp	Sequencing-PCR
	Alpha 2-R	GAG AGG TCC TTG GTCTGA GAC AG	999 bp	Sequencing-PCR

Table 2. Demographic Characteristics, Complete Blood Count, Hemoglobin Electrophoresis Parameters, Iron Status, Liver Function Tests, and Inflammatory Marker Levels Among Patients with Hb H, Stratified by Transfusion Requirement

Variable	Total (n=90)	TD (n=23)	OTD (n=15)	NTD (n=52)	P-value
Age (years)a	26.23±15.79	30.56±18.47	27.26±17.48	24.01±13.80	0.24
Sex (Female)b	43 (48%)	17 (74%)	9 (60%)	17 (33%)	0.003
Complete Blood Count[a]					
MCV (fL)	62.27±9.53	63.95±8.86	64.18±6.24	60.97±10.48	0.32
MCH (pg)	18.95±4.88	18.60±3.05	18.33±2.55	10.29±5.95	0.74
Hb (g/dL)	9.75±1.67	9.09±1.22	8.65±1.31	10.35±1.67	<0.001
Reticulocyte Count	4.60±3.81	5.26±4.25	5.92±3.91	3.92±3.48	0.12
Hemoglobin Electrophoresis[a]					
Hb A2 (%)	1.39±0.77	1.10±0.39	1.36±1.26	1.50±0.68	0.15
Hb F (%)	2.37±11.28	1.41±1.90	1.02±1.34	3.04±14.21	0.84
Hb A (%)	90.68±6.99	88.40±6.75	86.95±8.58	92.63±5.99	0.005
Hb H (%)	9.67±7.35	8.62±6.25	11.14±9.01	9.76±7.40	0.62
Hb Barts (%)	3.81±12.86	1.88±1.46	10.85±25.20	0.99±0.51	0.24
Iron Profile Tests[a]					
TIBC (µg/dL)	301.63±84.03	287.09±96.27	281.96±61.50	313.46±83.50	0.28
Ferritin (µg/dL)	279.30±510.33	519.27±923.39	197.16±136.95	195.24±205.53	0.04
Fe (µg/dL)	109.77±61.57	125.36±95.74	108.60±60.17	103.51±40.36	0.38
Liver Function Tests[a]					
ALT (U/L)	24.00±16.43	24.17±16.15	20.73±9.96	24.86±18.09	0.69
AST (U/L)	25.56±10.84	26.52±11.33	24.26±8.94	25.51±11.26	0.82
Inflammatory Marker[a]					
Total Bilirubin (mg/dL)	5.42±31.55	2.83±2.37	2.39±1.81	7.44±41.53	0.78
CRP (mg/dL)	2.45±2.93	2.86±4.26	2.38±1.76	2.29±2.51	0.74

a=mean± standard deviation, b=number (percent), Abbreviations: TD=Transfusion Dependent, OTD=Occasional Transfusion Dependent, NTD=Non-Transfusion Dependent

Regarding iron status, serum ferritin was significantly higher in the transfusion-dependent Hb H group (519.27 ± 923.39 ng/mL) than in occasionally transfused Hb H (197.16 ± 136.95 ng/mL) and non-transfusion-dependent Hb H (195.24 ± 205.53 ng/mL) groups (p = 0.04). Serum iron and total iron-binding capacity (TIBC) showed no significant differences across subgroups.

Hemoglobin electrophoresis demonstrated that non-transfusion-dependent Hb H patients had significantly higher HbA levels (92.63 ± 5.99%) compared to transfusion-dependent Hb H (88.40 ± 6.75%) and occasionally transfused Hb H (86.95 ± 8.58%) patients (p = 0.005). Other hemoglobin components (HbA_2, Hb F, Hb H, and Hb Bart's) showed no significant differences among the transfusion groups. Liver enzymes (ALT, AST), CRP, and Total bilirubin exhibited no statistically significant differences across groups, although bilirubin levels displayed wide variability within the non-transfusion-dependent Hb H group.

Genotypic analysis revealed three distinct groups: non-deletional homozygotes (ND/ND, n = 25), compound heterozygotes (ND/D, n = 27), and deletional homozygotes (D/D, n = 38) (Table 3). Age (p = 0.76) and sex distribution (p = 0.17) were comparable across these groups.

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Table 3. Demographic Characteristics, Complete Blood Count, Hemoglobin Electrophoresis Parameters, Iron Status, Liver Function Tests, and Inflammatory Marker Levels Among Patients with Hb H, Stratified by Type of Mutations

Variable	Type of mutations			P-value
	ND/ND (n=25)	ND/D (n=27)	D/D (n=38)	
Age (years)a	26.24±14.00	27.89±18.78	24.97±14.75	0.76
Sex (Female)b	8 (32%)	15 (54%)	20 (54%)	0.17
Complete Blood Count[a]				
MCV (fL)	66.06±8.43	62.07±7.15	59.86±11.07	0.04
MCH (pg)	20.10±3.59	18.27±2.65	18.70±6.60	0.36
Hb (g/dL)	10.70±1.83	9.36±1.54	9.40±1.40	0.003
Reticulocyte Count (%)	3.00±3.43	5.95±4.19	4.65±3.41	0.01
Hemoglobin Electrophoresis[a]				
Hb A2 (%)	1.78±0.59	1.03±0.65	1.38±0.85	0.002
Hb F (%)	0.64±1.07	0.78±0.61	4.81±17.58	0.39
Hb A (%)	93.72±5.13	85.18±8.29	92.82±4.09	<0.001
Hb H (%)	7.58±4.25	15.03±8.27	5.74±3.81	<0.001
Hb Barts (%)	1.26±0.57	1.47±0.93	6.79±19.31	0.57
Iron Profile Tests[a]				
TIBC (µg/dL)	311.04±119.11	305.48±79.06	292.47±55.97	0.67
Ferritin (µg/L)	271.48±282.17	438.66±840.60	160.80±142.56	0.04
Fe (µg/dL)	134.11±99.39	104.92±33.02	96.86±35.97	0.05
Liver Function Tests[a]				
ALT (U/L)	24.44±11.65	25.46±24.02	22.59±11.84	0.77
AST (U/L)	24.28±11.04	29.57±11.67	23.40±9.42	0.05
Inflammatory Marker[a]				
Total Bilirubin (mg/dL)	2.27±2.27	2.17±2.05	10.01±49.18	0.52
CRP (mg/L)	1.62±1.25	3.97±4.75	1.94±1.29	0.005
Transfusion Dependency[b]				
TD	4 (16%)	10 (37.1%)	9 (23.7%)	0.34
OTD	3 (12%)	5 (18.5%)	7 (18.4%)	
NTD	18 (72%)	12 (44.4%)	22 (57.9%)	

a=mean± standard deviation, b=number (percent), Abbreviations: TD=Transfusion Dependent, OTD=Occasional Transfusion Dependent, NTD=Non-Transfusion Dependent

The ND/ND group exhibited significantly higher hemoglobin levels (10.70 ± 1.83 g/dL) compared to ND/D (9.36 ± 1.54 g/dL) and D/D (9.40 ± 1.40 g/dL) groups ($p = 0.003$). MCV values differed significantly ($p = 0.04$), with ND/ND showing the highest mean (66.06 ± 8.43). Reticulocyte counts were highest in ND/D patients (5.95 ± 4.19%) and lowest in ND/ND patients (3.00 ± 3.43%) ($p = 0.01$).

Hb A levels were lower in ND/D (85.18 ± 8.29%) compared to ND/ND (93.72 ± 5.13%) and D/D (92.82 ± 4.09%) groups ($p < 0.001$), while Hb H was highest in ND/D (15.03 ± 8.27%) and lowest in D/D (5.74 ± 3.81%) ($p < 0.001$). HbA_2 also varied significantly between groups ($p = 0.002$).

Among biochemical parameters, ferritin levels were significantly higher in ND/D (438.66 ± 840.60 ng/mL) than in ND/ND (271.48 ± 282.17 ng/mL) or D/D (160.80 ± 142.56 ng/mL) groups ($p = 0.04$). CRP levels followed a similar pattern, with the highest values in ND/D patients (3.97 ± 4.75 mg/L; $p = 0.005$). AST levels approached statistical significance ($p = 0.05$), while other liver enzyme parameters showed no group differences. The distribution of transfusion dependency across genotype categories did not differ significantly ($p = 0.34$).

Table 4 presents the genotypic distribution across transfusion categories. The most frequent genotype was med/3.7 (24.4%), followed by $\alpha^{PolyA1}\alpha/\ \alpha^{PolyA1}\alpha$ (7.8%),

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Table 4. Frequency of Genotypes in 90 Patients with Hb H, Stratified by Transfusion Requirement.

Types of mutation	Total (n=90)	TD (n=23)	OTD (n=15)	NTD (n=52)
ND/ND				
$\alpha^{codon19}\alpha$ / $\alpha^{codon19}\alpha$	3 (3.3%)	0	0	3 (5.8%)
$\alpha^{codon90}\alpha$ / $\alpha^{codon19}\alpha$	1 (1.1%)	0	0	1 (1.9%)
α^{CS} α / α^{CS} α	5 (5.6%)	1 (4.3%)	1 (6.7%)	3 (5.8%)
Hb Dartmouth/Hb Dartmouth	1 (1.1%)	0	1 (6.7%)	0
Hb Adana/Hb Adana	1 (1.1%)	1 (4.3%)	0	0
IVS donor site/IVS donor site	1 (1.1%)	0	0	1 (1.9%)
$\alpha^{polyA1}\alpha$/ $\alpha^{polyA1}\alpha$	7 (7.8%)	2 (8.7%)	1 (6.7%)	4 (7.7%)
$\alpha^{PolyA2}\alpha$ /IVS donor site	2 (2.2%)	0	0	2 (3.8)
$\alpha^{PolyA2}\alpha$ / $\alpha^{PolyA2}\alpha$	4 (4.4%)	0	0	4 (7.7%)
D/ND				
$-(\alpha)^{20.5}$/ $\alpha^{-5nt}\alpha$	5 (5.6%)	2 (8.7%)	0	3 (5.8%)
$-(\alpha)^{20.5}$/ $\alpha^{PolyA2}\alpha$	3 (3.3%)	1 (4.3%)	1 (6.7%)	1 (1.9%)
$-\alpha^{3.7}$/ α^{-5nt} α	1 (1.1%)	0	0	1 (1.9%)
$-\alpha^{3.7}$/ α^{CS} α	1 (1.1%)	0	0	1 (1.9%)
$-(\alpha)^{20.5}$/ α^{CS} α	2 (2.2%)	2 (8.7%)	0	0
$-\alpha^{unknown\ deletion}$ /α^{CS} α	2 (2.2%)	1 (4.3%)	1 (6.7%)	0
~^{Med}/ $\alpha^{-5nt}\alpha$	1 (1.1%)	0	0	1 (1.9%)
~^{Med} /$\alpha^{codon\ 19}\alpha$	2 (2.2%)	1 (4.3%)	1 (6.7%)	0
~^{Med} / $\alpha^{codon\ 90}\alpha$	2 (2.2%)	1 (4.3%)	1 (6.7%)	0
~^{Med} / α^{CS} α	3 (3.3%)	2 (8.7%)	0	1 (1.9%)
~^{Med} / $\alpha^{PolyA2}\alpha$	5 (5.6%)	0	1 (6.7%)	4 (7.7%)
D/D				
$-\alpha^{3.7}$/$-(\alpha)^{20.5}$	6 (6.7%)	1 (4.3%)	1 (6.7%)	4 (7.7%)
$-\alpha^{4.2}$/$-(\alpha)^{20.5}$	1 (1.1%)	1 (4.3%)	0	0
$-\alpha^{3.7}$/$-\alpha^{unknown\ deletion}$	1 (1.1%)	0	0	1 (1.9%)
$\alpha^{65\ bp\ indel}\alpha$, $-\alpha^{3.7}$/$-\alpha^{3.7}$	1 (1.1%)	0	0	1 (1.9%)
~^{Med}/$\alpha^{21\ bp\ dup}\alpha$	1 (1.1%)	1 (4.3%)	0	0
~^{Med} /$-\alpha^{3.7}$	22 (24.4%)	5 (21.7%)	4 (26.7%)	13 (25%)
~^{Med} /$-\alpha^{4.2}$	6 (6.7%)	1 (4.3%)	2 (13.3%)	3 (5.8%)
TOTAL	90 (100%)	23 (25.6%)	15 (16.7%)	52 (57.8%)

Abbreviations: TD=Transfusion Dependent, OTD=Occasional Transfusion Dependent, NTDT=Non-Transfusion Dependent, ND=Non Deletional, D=Deletional.

med/4.2 (6.7%), and $-(\alpha)^{20.5}$ /$-\alpha^{3.7}$ (6.7%). Among patients with med/3.7 genotype, 21.7% were transfusion-dependent, 26.7% occasionally transfused, and 25% non-transfusion-dependent. No statistically significant association was observed between genotype and transfusion status (p = 0.34).

Hematologic profiles varied across genotypes (Table 5). Among more prevalent genotypes, $\alpha^{PolyA2}\alpha$/ $\alpha^{PolyA2}\alpha$ had the highest mean hemoglobin level (11.05 ± 1.72 g/dL), while -~^{Med}/$\alpha^{4.2}$ showed lower levels (8.55 ± 0.26 g/dL). MCV was highest in $\alpha^{CS}\alpha$/ $\alpha^{CS}\alpha$ (77.52 ± 2.20 fL) and lowest in ~^{Med} / $\alpha^{PolyA2}\alpha$ (56.00 ± 9.07 fL). Reticulocyte counts ranged from 2.87 ± 1.95% in $\alpha^{PolyA2}\alpha$/ $\alpha^{PolyA2}\alpha$ to 6.14 ± 2.14% in ~Med/ $\alpha^{PolyA2}\alpha$ for hemoglobin fractions, and Hb A was highest in $\alpha^{PolyA2}\alpha$ /IVS donor site and $\alpha^{codon19}\alpha$/ $\alpha^{codon19}\alpha$ (>97%) and lowest in ~^{Med} / $\alpha^{codon90}\alpha$ (70.20 ± 9.47%). Hb H levels were notably elevated in ~^{Med} / $\alpha^{codon90}\alpha$ (25.25 ± 14.49%) and ~^{Med}/ $\alpha^{PolyA2}\alpha$ (14.65 ± 4.10%), compared to values below 7% in $\alpha^{PolyA2}\alpha$/ $\alpha^{PolyA2}\alpha$ and $\alpha^{codon19}\alpha$/ $\alpha^{codon19}\alpha$.

Iron-related and biochemical parameters by genotype are presented in Table 6. The common genotypes, $\alpha^{PolyA1}\alpha$/ $\alpha^{PolyA1}\alpha$ and $\alpha^{CS}\alpha$/$\alpha^{CS}\alpha$ showed moderately elevated ferritin levels (320.70 ± 282.02 and 340.65 ± 351.50 ng/mL, respectively), while the highest ferritin concentration appeared in $-(\alpha)^{20.5}$/ $\alpha^{-5nt}\alpha$ (1114.04 ± 1912.09 ng/mL). Patients with $\alpha^{codon19}\alpha$/ $\alpha^{codon19}\alpha$ and $\alpha^{IVS\ donor\ site}$/$\alpha^{IVS\ donor\ site}$

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Table 5. Mean of Complete Blood Count, and Hemoglobin Electrophoresis Parameters [± standard deviation (SD)] for Each Genotype in 90 Patients with Hb.

Genotype	Total (n=90)	MCV (fL)	MCH (pg)	Hb (g/dL)	RETIC (%)	Hb A2(%)	Hb F (%)	Hb A (%)	Hb H (%)	Hb Barts (%)
$\sim^{Med}/-\alpha^{3.7}$	22 (24.4%)	56.77±11.86	18.43±8.26	9.39±1.38	4.17±3.25	1.29±0.43	0.74±0.72	94.39±3.20	4.50±3.28	0.88±0.61
$\alpha^{polyA1}\alpha/\ \alpha^{polyA1}\alpha$	7 (7.8%)	65.18±9.59	19.41±4.69	10.10±2.10	4.08±4.99	1.48±0.71	0.38±0.36	90.68±7.56	12.17±4.24	1.26±0.57
$-\alpha^{3.7}/-(\alpha)^{20.5}$	6 (6.7%)	62.28±8.37	18.00±1.56	9.81±0.70	6.15±4.80	1.50±0.37	0.16±0.05	90.68±5.67	9.30±4.74	0.63±0.49
$\sim^{Med}/-\alpha^{4.2}$	6 (6.7%)	61.42±2.83	17.73±1.53	8.55±0.26	5.21±2.49	1.75±1.82	1.66±1.15	89.08±6.43	9.54±6.85	23.66±38.39
$\alpha^{CS}\ \alpha/\ \alpha^{CS}\ \alpha$	5 (5.6%)	77.52±2.20	24.25±1.91	10.58±1.08	4.66±4.22	1.36±0.23	1.26±1.44	91.72±2.29	5.32±0.64	-
$-(\alpha)^{20.5}/\ \alpha^{-5nt}\alpha$	5 (5.6%)	61.04±7.21	17.52±1.73	9.26±0.40	3.67±2.47	0.82±0.20	1.00±0.43	82.40±6.30	22.78±4.53	-
$\sim^{Med}/\ \alpha^{PolyA2}\alpha$	5 (5.6%)	56.00±9.07	16.42±2.39	9.60±1.77	6.14±2.14	1.44±1.06	0.30±0.28	87.18±6.42	14.65±4.10	1.03±0.45
$\alpha^{PolyA2}\alpha/\ \alpha^{PolyA2}\alpha$	4 (4.4%)	60.92±5.05	18.40±2.63	11.05±1.72	2.87±1.95	1.85±0.59	0.27±0.35	94.52±3.70	6.85±1.06	-
$\alpha^{codon19}\alpha/\ \alpha^{codon19}\alpha$	3 (3.3%)	61.46±2.71	19.46±1.17	11.96±1.47	1.060.11	2.30±0.20	0.10±0.00	97.70±0.20	-	-
$-(\alpha)^{20.5}/\ \alpha^{PolyA2}\alpha$	3 (3.3%)	58.85±1.22	17.54±2.23	9.30±0.95	5.23±4.44	1.06±0.25	0.50±0.00	84.46±10.02	13.10±9.88	1.56±1.22
$\sim^{Med}/\ \alpha^{CS}\ \alpha$	3 (3.3%)	68.38±5.85	23.05±2.92	8.93±2.20	4.33±1.52	0.70±0.00	-	90.40±3.14	6.40±2.35	2.15±1.76
$-(\alpha)^{20.5}/\ \alpha^{CS}\ \alpha$	2 (2.2%)	65.20±3.11	17.90±0.70	8.95±1.34	4.70±0.14	0.60±0.00	0.20±0.00	81.80±0.56	12.30±1.69	1.95±0.49
$-\alpha^{unknown\ deletion}/\alpha^{CS}\ \alpha$	2 (2.2%)	73.17±1.08	20.22±0.38	8.25±1.06	17.00±4.24	1.50±0.42	0.85±0.21	95.05±0.07	2.60±0.28	-
$\alpha^{PolyA2}\alpha$ /IVS donor site	2 (2.2%)	64.30±5.09	19.70±1.41	12.00±0.98	1.00±0.28	2.35±0.21	0.10±0.00	97.65±0.21	-	-
$\sim^{Med}/\ \alpha^{codon19}\alpha$	2 (2.2%)	65.45±2.33	18.55±0.63	9.25±3.88	4.45±1.34	0.80±0.28	0.80±0.98	80.55±0.77	17.20±0.42	-
$\sim^{Med}/\ \alpha^{codon90}\alpha$	2 (2.2%)	58.45±5.30	16.55±0.77	9.75±1.20	8.25±2.47	0.50±0.14	0.65±0.77	70.20±9.47	25.25±14.49	-
$\alpha^{codon90}\alpha\ /\ \alpha^{codon19}\alpha$	1 (1.1%)	65.50	20.30	10.30	1.50	2.20	0.10	97.80	-	-
Hb Dartmouth/Hb Dartmouth	1 (1.1%)	64.00	18.00	7.10	0.80	2.50	3.70	93.80	-	-
Hb Adana/Hb Adana	1 (1.1%)	53.00	14.70	9.00	3.00	-	-	-	2.00	-
IVS donor site/IVS donor site	1 (1.1%)	68.50	21.10	13.60	1.20	2.00	-	98.00	-	-
$-\alpha^{3.7}/\ \alpha^{-5nt}\alpha$	1 (1.1%)	69.00	22.00	13.30	1.90	2.50	-	97.50	-	-
$-\alpha^{3.7}/\ \alpha^{CS}\ \alpha$	1 (1.1%)	58.56	16.10	9.40	1.20	1.50	2.10	96.40	8.80	-
$\sim^{Med}/\ \alpha^{-5nt}\alpha$	1 (1.1%)	60.00	16.60	9.50	11.00	0.60	-	83.90	14.90	0.60
$-(\alpha)^{20.5}/-\alpha^{4.2}$	1 (1.1%)	63.00	22.00	8.00	5.00	-	-	-	3.90	-
$-\alpha^{3.7}/-\alpha^{unknown\ deletion}$	1 (1.1%)	78.69	25.98	13.90	1.00	2.60	4.90	92.50	-	-
$\alpha^{65\ bp\ indel}\alpha, -\alpha^{3.7}/-\alpha^{3.7}$	1 (1.1%)	60.00	18.00	8.90	8.80	0.50	0.87	87.00	11.10	-
$\sim^{Med}/\ \alpha^{21\ bp\ dup}\alpha$	1 (1.1%)	81.00	25.00	8.70	3.50	1.20	6.90	85.50	2.30	4.40

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Table 6. Mean of Iron Status, Liver Function Tests, and Inflammatory Marker Levels [± standard deviation (SD)] for Each Genotype in 90 Patients with Hb H.

Genotype	Total (n=90)	TIBC (µg/dL)	Ferritin (µg/dL)	Fe (µg/dL)	ALT (N/L)	AST (U/L)	Total Bilirubin (mg/dL)	CRP (mg/dL)
$\sim^{Med}$ /-$\alpha^{3.7}$	22 (24.4%)	305.79±58.47	129.72±153.40	91.18±32.01	24.63±14.11	24.00±9.84	2.03±1.49	2.21±1.54
$\alpha^{polyA1}\alpha$/ $\alpha^{polyA1}\alpha$	7 (7.8%)	334.57±203.98	320.70±282.02	95.00±86.10	25.00±10.93	24.57±11.17	3.22±3.58	1.08±0.53
-$\alpha^{3.7}$/-$(\alpha)^{20.5}$	6 (6.7%)	258.16±34.95	247.71±80.93	104.33±51.86	19.50±5.75	20.00±7.66	1.74.0.62	1.43±0.70
$\sim^{Med}$ /-$\alpha^{4.2}$	6 (6.7%)	279.33±62.24	119.50±45.86	101.66±36.76	18.66±6.43	22.66±9.45	51.45±12.25	1.56±0.62
$\alpha^{CS}\alpha$/ $\alpha^{CS}\alpha$	5 (5.6%)	309.00±30.24	340.65±351.50	162.20±56.84	19.40±10.69	24.80±11.23	3.30±1.67	1.14±0.61
-$(\alpha)^{20.5}$/ $\alpha^{-5nt}\alpha$	5 (5.6%)	268.80±38.87	1114.04±1912.09	113.40±43.10	20.00±5.70	30.20±7.04	1.55±0.71	2.08±0.43
$\sim^{Med}$ / $\alpha^{PolyA2}\alpha$	5 (5.6%)	350.20±42.71	231.36±245.36	85.80±33.97	42.20±52.66	40.00±21.57	1.29±0.45	5.96±5.53
$\alpha^{PolyA2}\alpha$/ $\alpha^{PolyA2}\alpha$	4 (4.4%)	304.25±63.87	163.26±141.02	159.00±39.40	29.75±12.68	33.00±13.39	1.10±0.82	2.20±1.73
$\alpha^{codon19}\alpha$/ $\alpha^{codon19}\alpha$	3 (3.3%)	368.00±22.51	96.53±35.51	84.95±12.16	30.00±15.62	20.66±6.50	0.53±0.11	2.00±2.34
-$(\alpha)^{20.5}$/ $\alpha^{PolyA2}\alpha$	3 (3.3%)	298.00±60.30	199.80±176.35	88.66±29.19	18.33±4.61	24.00±1.00	1.90±1.22	4.70±4.60
$\sim^{Med}$ / $\alpha^{CS}\alpha$	3 (3.3%)	366.00±147.40	323.66±224.81	132.33±49.66	39.00±21.16	33.33±10.59	5.73±5.03	1.00±0.00
-$(\alpha)^{20.5}$/ $\alpha^{CS}\alpha$	2 (2.2%)	214.00±0	867.75±605.63	-	16.00±0.00	19.00±7.07	2.25±1.48	21.00±0.00
-$\alpha^{unknown\ deletion}$ /$\alpha^{CS}\alpha$	2 (2.2%)	278.00±74.95	216.00±55.15	89.00±15.55	33.50±6.36	34.50±6.36	2.15±1.62	4.50±2.12
$\alpha^{PolyA2}\alpha$ /IVS donor site	2 (2.2%)	314.00±4.24	79.10±36.39	73.00±35.35	16.00±1.41	21.00±5.65	1.75±1.06	2.30±2.12
$\sim^{Med}$ / $\alpha^{codon19}\alpha$	2 (2.2%)	226.50±21.92	216.14±99.47	102.50±31.81	12.50±0.70	26.00±5.65	2.00±0.28	0.90±0.14
$\sim^{Med}$ / $\alpha^{codon90}\alpha$	2 (2.2%)	380.50±171.82	121.85±11.52	104.00±31.11	13.00±1.41	18.50±2.12	3.15±1.34	4.30±0.00
$\alpha^{codon90}\alpha$ / $\alpha^{codon19}\alpha$	1 (1.1%)	338.00	78.56	86.00	17.00	11.00	1.40	3.00
Hb Dartmouth/Hb Dartmouth	1 (1.1%)	231.00	450.30	170.00	45.00	38.00	1.80	2.50
Hb Adana/Hb Adana	1 (1.1%)	82.00	1000.00	502.00	12.00	17.00	4.10	1.80
IVS donor site/IVS donor site	1 (1.1%)	289.00	146.11	82.00	24.00	9.00	1.00	0.50
-$\alpha^{3.7}$/ $\alpha^{-5nt}\alpha$	1 (1.1%)	281.00	370.60	131.00	21.00	26.00	0.80	2.50
-$\alpha^{3.7}$/ $\alpha^{CS}\alpha$	1 (1.1%)	296.00	183.00	134.00	24.00	30.00	0.90	1.00
$\sim^{Med}$ / $\alpha^{-5nt}\alpha$	1 (1.1%)	317.00	500.00	96.00	21.00	24.00	1.80	5.10
-$(\alpha)^{20.5}$/-$\alpha^{4.2}$	1 (1.1%)	269.00	229.00	79.00	22.00	26.00	2.50	1.50
-$\alpha^{3.7}$/-$\alpha^{unknown\ deletion}$	1 (1.1%)	339.00	-	114.00	33.00	41.00	1.00	1.00
$\alpha^{65\ bp\ indel}\alpha$, -$\alpha^{3.7}$/-$\alpha^{3.7}$	1 (1.1%)	276.00	120.00	105.00	12.00	17.00	1.50	1.50
$\sim^{Med}$ / $\alpha^{21\ bp\ dup}\alpha$	1 (1.1%)	268.00	470.80	153.00	12.00	27.00	2.86	2.40

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had some of the lowest ferritin values (<150 ng/mL). CRP levels were highest in $\sim^{Med}$ / $\alpha^{PolyA2}\alpha$ (5.96 ± 5.53 mg/L), $\alpha^{CS}\alpha$ /-$(\alpha)^{20.5}$ (21.00 mg/L), and $\sim^{Med}$ / α^{-5nt} α (5.10 mg/L), while most other genotypes showed values below 3 mg/L. AST and ALT levels remained comparable across most genotypes, although occasional elevations were observed in less common combinations such as Hb Dartmouth/Hb Dartmouth (AST: 38.00 U/L) and $\sim^{Med}$ / $\alpha^{CS}\alpha$ (ALT: 39.00 ± 21.16 U/L). Bilirubin levels were generally low, except in $\sim^{Med}$ /-$\alpha^{4.2}$ (51.45 ± 12.25 µmol/L), which showed the highest recorded value among all genotypes.

4. DISCUSSION

Hemoglobin H (Hb H) disease represents a clinically diverse spectrum of α-thalassemia syndromes, influenced by both the type and combination of underlying genetic mutations. Understanding the genotype-phenotype relationships in these patients is crucial for accurate prognostication and tailored management. In this study, we evaluated 90 patients with Hb H disease to explore how molecular subtypes correlate with clinical severity, transfusion dependency, and laboratory parameters. The findings highlight key patterns and unexpected variabilities in hematologic and biochemical profiles that provide new insights into the disease's complexity.

In line with previous studies (1, 3), patients with non-deletional homozygous (ND/ND) genotypes displayed relatively mild hematologic manifestations. This group demonstrated the highest hemoglobin levels, elevated MCV, and reduced reticulocyte counts, reflecting more stable erythropoiesis. Notably, specific genotypes, including $\alpha^{PolyA2}\alpha$ / $\alpha^{PolyA2}\alpha$ and $\alpha^{codon19}\alpha$/ $\alpha^{codon19}\alpha$, showed near-normal Hb A percentages and minimal Hb H, suggesting efficient α-globin synthesis and diminished globin chain imbalance. These findings are consistent with previous studies reporting that polyadenylation site mutations, such as $\alpha^{PolyA2}\alpha$ lead to reduced but not abolished mRNA stability, resulting in residual α-globin production and a milder clinical phenotype (1).

Despite the traditional assumption that non-deletional mutations result in more severe clinical manifestations, our study observed milder hematologic profiles in patients with homozygous non-deletional (ND/ND) genotypes when compared to compound heterozygous (ND/D) or homozygous deletional (D/D) genotypes. This unexpected finding may reflect the relatively benign nature of specific non-deletional mutations, which appear to preserve a degree of functional α-globin synthesis or the specific mutation type detected in this study.

In contrast, compound heterozygotes (ND/D) displayed more severe hematologic impairment, with significantly elevated reticulocyte counts and higher Hb H levels. Genotypes such as $\sim^{Med}$ / $\alpha^{PolyA2}\alpha$ and $\alpha^{CS}\alpha$ /-$(\alpha)^{20.5}$ were particularly associated with low Hb A, high Hb H, and increased inflammatory markers such as CRP. These profiles point toward ineffective erythropoiesis and increased hemolytic burden, hallmarks of more clinically severe disease (5, 6). The presence of both structural defects (e.g., Hb Constant Spring) and deletional mutations in these patients may synergistically exacerbate α/β-globin chain imbalance, contributing to erythroid stress and splenic hyperactivity.

Deletional homozygotes (D/D) exhibited intermediate hematologic features between the ND/ND and ND/D groups. Their hemoglobin levels and MCV values were lower than those of ND/ND patients but higher than ND/D. Similarly, their reticulocyte and Hb H levels were elevated compared to ND/ND, though not as pronounced as in ND/D individuals. These patterns reflect partial loss of α-globin synthesis capacity without the added burden of dysfunctional chain production typical of non-deletional mutations.

Transfusion requirement, a key clinical endpoint, varied significantly between genotype groups. The ND/D group showed the highest proportion of transfusion-dependent Hb H, while ND/ND genotypes were predominantly non-transfusion-dependent Hb H. The absence of statistically significant associations between specific genotypes and transfusion dependence may reflect limited statistical power due to small sample sizes in subgroup analyses.

Although no statistically significant association was detected between genotype and transfusion dependency, certain ND/D genotypes were more frequently represented among patients requiring regular transfusions. These combinations, such as $\sim^{Med}/\alpha^{codon90}\alpha$, $\sim^{Med}$ /$\alpha^{PolyA2}\alpha$ or $\alpha^{CS}\alpha$ /-$(\alpha)^{20.5}$, were often linked to indicators of severe erythroid dysfunction, including heightened reticulocyte response and elevated hemoglobin H formation. This trend aligns with previous research reporting that patients harboring both deletional and structurally unstable non-deletional mutations often experience chronic hemolysis and ineffective erythropoiesis, increasing their reliance on transfusion support. Such genotypic profiles may identify candidates for novel therapeutic interventions targeting erythroid maturation and stress response pathways.

Our data showed that patients with ND/D genotypes had the highest serum ferritin levels (438.66 µg/L) compared to the D/D (160.80 µg/L) group, with ND/ND patients showing intermediate values (271.48 µg/L). This

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observation raises the possibility of intrinsic dysregulation of iron metabolism in more severe genotypes, particularly since the distribution of transfusion dependencies did not differ significantly across these genotype groups. Studies in β-thalassemia intermedia have demonstrated similar findings, where increased ineffective erythropoiesis and hepcidin suppression led to excessive gastrointestinal iron absorption even without transfusion (9). A comparable mechanism may be at play in α-thalassemia syndromes, suggesting that certain genetic combinations might influence iron metabolism independently of transfusion history (14, 15).

Inflammatory markers such as CRP were also significantly elevated in the ND/D group (3.97 mg/L) compared to ND/ND (1.62 mg/L) and D/D (1.94 mg/L) groups, notably in those with $-^{Med}$ / $\alpha^{PolyA2}\alpha$ and $\alpha^{CS}\alpha$ /-$(\alpha)^{20.5}$ genotypes. This points to a potential association between certain genotypes and inflammatory status. In thalassemia, chronic inflammation has been linked to hemolysis, oxidative stress, and iron overload, all of which contribute to organ damage and disease progression. The molecular mechanisms underlying this association could involve differences in ineffective erythropoiesis, oxidative stress, or immune system activation across genotypes (16). The interplay between genotype, erythroid stress, and inflammation in Hb H disease remains underexplored but could represent a novel axis for therapeutic targeting.

Hemoglobin electrophoresis data provided valuable insight into genotype-phenotype relationships in this study. The ND/D genotypes exhibited a significant reduction in Hb A levels alongside increased Hb H and, occasionally, Hb Bart's. This is consistent with the known effects of non-deletional α-globin mutations, which often generate dysfunctional α-globin variants that impair hemoglobin tetramer formation (4). Patients with ND/D mutations typically exhibited reduced hemoglobin A and elevated hemoglobin H fractions, contrasting with the near-normal profiles observed in ND/ND individuals. These electrophoretic features may serve as a practical surrogate for genotype prediction in settings where molecular diagnostics are unavailable. The detection of markedly elevated hemoglobin H can help identify individuals with severe or compound genotypes and inform early clinical decision-making, underscoring the continued relevance of hemoglobin electrophoresis as a cost-effective diagnostic and prognostic adjunct.

The $-^{Med}/\alpha^{codon90}\alpha$ genotype was associated with particularly high Hb H levels, reflecting severe imbalance in α/β-globin chain synthesis. The Mediterranean deletion ($-^{Med}$) removes both α-globin genes on the affected chromosome, while the $\alpha^{codon90}\alpha$ non-deletional mutation severely impairs the function of the remaining α-globin gene, resulting in a profound deficiency of α-globin chains and consequently extensive Hb H formation (17). This finding exemplifies how specific combinations of deletional and non-deletional mutations can produce distinctive hematological profiles.

Interestingly, patients with the $-^{Med}$ /-$\alpha^{3.7}$ genotype, despite constituting the largest group in this study, exhibited relatively moderate clinical and hematological manifestations. This genotype results in the loss of three α-globin genes (two from the Mediterranean deletion and one from the -$\alpha^{3.7}$ deletion), theoretically leaving just one functional α-globin gene (17). However, the wide range of hemoglobin levels and transfusion requirements observed in this group suggests that additional genetic or environmental factors likely influence the clinical expression of this genotype (18). This variability likely reflects the influence of genetic modifiers, such as co-inherited β-thalassemia traits, triplicated α-globin loci, or variants affecting erythropoietin signaling and hepcidin regulation (19).

Patients with the $\alpha^{codon19}\alpha$/ $\alpha^{codon19}\alpha$ exhibited the highest hemoglobin levels (11.96 g/dL) and the lowest reticulocyte counts (1.06%), suggesting a very mild phenotype. Conversely, the $-^{Med}$ / $\alpha^{codon90}\alpha$ genotype had the highest Hb H levels (25.25%), indicative of severe α-globin chain deficiency. The coexistence of a null deletional mutation ($-^{Med}$) with a highly deleterious non-deletional allele ($\alpha^{codon90}\alpha$) exemplifies how compound genotypes modulate disease severity through synergistic effects on globin chain imbalance.

The Hb Dartmouth/Hb Dartmouth genotype displayed unexpectedly low hemoglobin levels (7.10 g/dL) but also low reticulocyte counts (0.80%), pointing toward impaired globin synthesis rather than hemolysis. This contrasts with other severe genotypes, such as $-^{Med}$ / $\alpha^{CS}\alpha$ or $\alpha^{CS}\alpha$ /-$(\alpha)^{20.5}$, where elevated reticulocyte counts suggest compensatory erythropoietic drive in response to hemolysis.

Interestingly, liver enzymes (ALT, AST) did not differ significantly across most genotype categories, except in a few isolated genotypes (e.g., $-^{Med}$/ $\alpha^{codon90}\alpha$, $-^{Med}/\alpha^{4.2}$) where AST and Total bilirubin levels were mildly elevated. While these may reflect iron-related hepatocellular injury or subclinical hemolysis, the absence of consistent patterns limits their interpretability. Future longitudinal studies incorporating liver MRI and hepcidin assays may help clarify these associations.

Our findings have significant implications for personalized management in α-thalassemia. The observation that 72% of ND/ND patients were classified as non-transfusion-dependent Hb H contradicts the common belief that non-deletional mutations universally result in more severe

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disease. This reinforces the need for molecular-level diagnosis rather than broad categorizations based on mutation type. Comprehensive genotyping can refine prognosis and guide treatment strategies such as transfusion thresholds, chelation intensity, and monitoring frequency.

Elevated ferritin and CRP in ND/D patients suggest that these individuals may benefit from earlier initiation of iron chelation therapy and monitoring of inflammatory markers, even in the absence of regular transfusions. Furthermore, the inflammatory profile observed raises the possibility of novel therapeutic targets focused on reducing erythroid stress and oxidative burden.

The heterogeneity observed within single genotypes, particularly $-^{Med}/-\alpha^{3.7}$, illustrates the limitations of genotype-only approaches to disease classification. Epigenetic factors, post-transcriptional modifications, and environmental influences (e.g., splenectomy, infections, nutritional status) likely contribute to phenotype diversity. These insights argue for integrative models incorporating genetic, biochemical, and clinical data to better stratify risk and tailor interventions.

5. LIMITATION

Several limitations must be considered when interpreting these findings. First, the cross-sectional design and relatively small sample sizes within certain genotype subgroups may have limited the statistical power to detect genotype-specific associations with clinical outcomes. Moreover, the absence of longitudinal follow-up data precluded assessment of disease progression or transfusion burden over time. Lastly, potential contributions from genetic modifiers, epigenetic mechanisms, or environmental influences, such as co-inherited traits or access to care, were not systematically evaluated and may have contributed to phenotype variability.

6. CONCLUSION

This study emphasizes the complex and heterogeneous nature of Hb H disease, shaped by diverse α-globin genotypes and their impact on hematologic and biochemical profiles. While certain mutation patterns were associated with more severe laboratory findings, the considerable variability within genotypic groups underscores the limitations of current binary classification models. These results support the need for integrated diagnostic approaches that combine molecular, hematologic, and inflammatory data to stratify clinical risk more accurately. A personalized framework based on comprehensive genotype–phenotype assessment may guide more effective and individualized management strategies in α-thalassemia care.

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Conflict of interest

the authors declare that they have no conflict of interest.

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Ethical statement

The patient's information was confidential, and consent was obtained during the investigation.

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