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MOLECULAR PATHOLOGY OF B-THALASSEMIA: CLINICOGENETIC EVALUATION OF HBB GENE MUTATIONS IN AFFECTED POPULATIONS: A CROSS-SECTIONAL STUDY

Original Research

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ABSTRACT

Background: One of the most common inherited hemoglobinopathies worldwide is β-thalassemia, in which the production of the β-globin chain is absent because of HBB gene mutations. The knowledge of population-specific mutation patterns is important in diagnosing, genetic counseling, and the development of successful prevention strategies. The purpose of this study was to assess the clinicogenetic range of HBB gene mutations within affected populations and to measure how these mutations relate to clinical phenotypes.

Methods: A cross-sectional study was conducted based on observations of 414 patients with confirmed β-thalassemia (major, n=289; intermediate, n=125). Records and structured interviews were used to gather demographic, clinical, and laboratory data. ARMS-PCR was used to detect mutations, with Sanger sequencing in unresolved cases. Genotype groups (homozygous IVS-I-5 [G>C], homozygous Codon 41/42 [-TCTT], compound heterozygotes, and rare/other variants) were used to assess patients. Students' t-test and Chi-square tests were used to conduct comparative analyses, and multivariate logistic regression evaluated the independent association of genotype and transfusion requirement, controlling effects of socioeconomic status and consanguinity.

Results: Patients with β-thalassemia major have a prior diagnosis (mean 1.9 ± 1.2 years vs. 6.8 ± 3.4 years, p<0.001), more splenectomy, 98 (33.9%) vs. 18 (14.4%), p=0.001, iron overload, 172 (59.5%) vs. 42 (33.6%), p<0.001), and seropositive to HCV, 42 (14.5%) vs. 6 (4.8%), p=0.003. IVS-I-5 (G>C), 172 (41.5%), Codon 41/42 (–TCTT), 87 (21.0%), and IVS-I-1 (G>T), 61 (14.7%), were the most common mutations. Homozygous IVS-I-5 was higher in major as compared to intermediate, 138 (47.8%) vs. 34 (27.2%), p<0.001. Homozygous IVS-I-5 was significantly correlated to regular transfusion dependence (OR=3.8, 95% CI: 2.169, p<0.001), but not to codon 41/42 or compound heterozygotes.

Conclusion: This study established that HBB mutation type, specifically homozygous IVS-I-5, was a significant determinant of transfusion-dependent β-thalassemia. Clinicogenetic testing of demographic, clinical, and molecular data can help understand disease heterogeneity and offer implications for prognostication, family counseling, and targeted screening programs in high-prevalence populations.

Keywords: beta-Thalassemia, Glycated Hemoglobin, Mutation, Genotype, Blood Transfusion.

INTRODUCTION

One of the most common inherited hemoglobin diseases in the world is β-Thalassemia, which is caused by impaired production of β-globin chains and causes different levels of anemia, hemolysis, and iron overload (1). More than 350 HBB gene mutations have been documented worldwide, but approximately 20 mutations confer 80% of the disease burden, representing different geographic distribution patterns (2). Silent carriers with β+ variants and a low hematological presentation are becoming common in Southeast Asia, highlighting the reconsideration of screening thresholds (3). Genotype-phenotype relationships are evident in studies of diverse populations; for example, in Quanzhou-region cohorts, β° mutations were significantly correlated with transfusion dependence (4). In Jordan, hematological measurements (MCV, HbA2) effectively differentiate β° from β+ phenotypes (5). New and rare mutations of HBB continue to emerge; this is why molecular surveillance is required (6). Additionally, deletions in α-thalassemia have been shown to interact with deletions in γ-globin persistence (HbF modifiers) to produce less disease severity, particularly in compound heterozygotes (7). New opportunities in the treatment of transfusion-dependent β-thalassemia are being presented by advances in gene therapy, spanning gene editing, exagamglogene, autotemcel, and ex vivo stem cell therapeutic approaches (8). Progress in the creation of broad molecular diagnostic panels, specific to the spectrum of mutations in populations, has become a priority to support early diagnosis, fetal screening, and genetic counseling (9). Collectively, these advances underscore the importance of detailed molecular characterization and genotype-phenotype mapping for personalized care in β-thalassemia.

In Pakistan, β-thalassemia is a threatening health issue among the population, and the prevalence rates among the carriers appear to be one of the highest in South Asia. In regional studies, there has been high mutation diversity and prevalence, which makes it difficult to implement uniform screening protocols (10). Clinical management is further complicated by the broader burden of chronic diseases, including hepatitis B and C, which further worsens anemia and exposes individuals to the risks of transfusion complications (11). The presence of chronic comorbidities like diabetes mellitus, especially when complicated by dyslipidemia, causes vascular damage and poor oxygen supply that can lead to worsening of hematological outcomes in patients with β-thalassemia by increasing oxidative stress and microvascular complications (12). Respiratory compromise and hematological stress are also significant contributors to transfusion needs because environmental pollutants are a major health issue in most urban and industrial locations in the country (13).

Healthcare services in Pakistan are characterized by disintegration, disparities, and geographical imbalances, which limit the prevalence of preventive screening and comprehensive thalassemia care (14). Meanwhile, nutritional deficiencies, including iron deficiency anemia, are still prevalent, particularly among women and children, further reducing immune competence and complicating the manifestation of hemoglobinopathies (15). Immune-nutritional biomarkers such as serum albumin, globulin, and transferrin have been proposed as measurements of disease resilience and potential predictors of clinical outcome, although they are not widely used in clinical practice (16).

Although the need to characterize the molecular aspects of β-thalassemia has been acknowledged, there is a lack of sufficient information on genotype distribution and phenotype correlation in patients with this condition in Pakistan. Available studies are mostly small, hospital-based, and confined to limited regions, which limits their generalizability. Genetic comorbidities like obesity, which could be affected by genetic factors such as ABO blood groups, have not been previously examined in thalassemia populations (17). Similarly, biomarkers of tissue ischemia, including ischemia-modified, are not studied in terms of prognostic value in this setting (18). New diagnostic methods, including salivary biomarkers, should also be considered due to their non-invasive and cost-effective potential to supplement molecular tests in mass screening (19). Platelet indices, which proved helpful to distinguish between hypoproductive and hyperdestructive thrombocytopenia, can also provide further information about the hematologic complication in thalassemia, but their value has not been tested (20).

An improved local mutation spectrum is crucial for designing cost-effective genetic screening panels that are community-specific. These focused approaches would enhance the accuracy of premarital and prenatal diagnosis and contribute to a decrease in the prevalence of severe thalassemia at birth. Additional metabolic, nutritional, and biomarker data (glucose, insulin, albumin/ globulin ratios) integrated into clinical models may further improve prognostication and optimize transfusion plans (21). Metabolic hormone, leptin, is associated with angiogenesis and inflammatory control and has also been implicated in vascular complications, e.g., diabetic retinopathy, including clinical severity and wound healing interactions in diabetes, suggesting that β-thalassemia may depend on comparable metabolic-

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immune mechanisms (22). Genetic literacy and ethical professionalism in healthcare practitioners should be developed to provide more genetic counseling services and implement them in a culturally sensitive manner (23). Furthermore, the vulnerability of healthcare professionals to stress imposed by the crisis, like COVID-19, is essential to provide the workforce with long-term care delivery (24). The relevance of integrative treatment approaches to the management of inflammatory complications in β-thalassemia may also be inspired by clinical lessons from allergy and immune-mediated diseases, such as the combined antihistamine and corticosteroid therapy against acute urticaria, indicating the importance of integrative approaches in clinical practice (25). Comorbidities such as chronic kidney disease that disrupt metabolic homeostasis can also be addressed to reduce the complications and the long-term prognosis of thalassemia patients (26).

The primary purpose of this study was to assess the spectrum of HBB gene mutations in patients with β-thalassemia and their association with clinical outcome, especially transfusion dependence. Secondary goals were to determine demographic, socioeconomic, and clinical factors that affect the severity of the disease, and to identify possible complications (iron overload and transfusion-transmitted infections). Furthermore, this study intended to determine the independent predictors of transfusion dependence in β-thalassemia patients.

METHODOLOGY

The purpose of this cross-sectional study was to characterize the molecular spectrum of HBB gene mutations in patients with β-thalassemia and to compare the results with clinical presentation. The research was conducted in the Pathology hematology unit of a tertiary care hospital, serving as a referral center regarding thalassemia management for 24 months, January to December (2020-21) with Ref: 112CDCN cell genetics Labs PU and Diagnostic Centers Lahore. Informed consent was provided by all adult participants and parents or legal guardians in the instance of minors. Patient data confidentiality and anonymity were ensured during the research.

The non-probability consecutive sampling method was used to recruit the participants. Every patient with a known diagnosis of β-thalassemia in the hematology unit within the study period was enrolled. All participants who satisfied the eligibility requirements and provided informed consent were recruited. The single-population proportion formula was used to calculate the sample size, as it assumes the prevalence of the most common mutation in the regional population (IVS-I-5 G>C, about 41.5%). The 373 patients were the minimum required sample determined using OpenEpi version 3.0.0 (released 2013, Atlanta, GA, USA) based on a 95% confidence interval and a 5% margin of error (27). The number was adjusted by 10% to account for possible dropouts, incomplete records, or assay failure, so that the final sample size of 414 participants was reached.

The inclusion criteria were patients of any age and gender diagnosed with β-thalassemia, defined by hematological indices, electrophoresis of Hb, and who were under follow-up in the study center. Patients without a complete clinical history, who had undergone bone marrow or stem cell transplantation, and those who refused to participate were excluded from the study. The patients were categorized as β-thalassemia major (severe anemia that necessitates regular lifelong transfusion since early childhood) and β-thalassemia intermedia (mild anemia that does not need regular transfusions, but may require occasional transfusions during stress or illness).

All the participants gave peripheral blood samples. A commercial extraction kit was used for DNA extraction, and Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) was used to screen a panel of 20 different common mutations of the α-globin gene. Non-yielding samples of these mutations were subjected to Sanger sequencing to help in conclusive molecular characterization. All demographic, socioeconomic, and clinical information was gathered on a structured proforma both by reviewing patient records and through direct interviews where necessary.

All data were analyzed through SPSS version 26.0 (released 2019, IBM Corp., Armonk, NY). Frequencies, percentages, means, and standard deviations were used as descriptive statistics to summarize the data. The correlation between genotypes and clinical variables was analyzed through the Chi-square or Fisher's exact test for categorical variables, t-test, or ANOVA for continuous variables. Where appropriate, logistic regression models were utilized to examine predictors of disease severity and transfusion requirements. The p-value <0.05 was considered significant.

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RESULTS

To evaluate HBB mutation spectrum and clinicogenetic associations, this study examined 414 patients, including 289 major and 125 intermediate β-Thalassemia patients. IVS-I-5 (G>C), Codon 41/42 (–TCTT), and IVS-I-1 (G>T) were the most frequently observed mutations. Major cases had earlier diagnosis, reduced pre-transfusion haemoglobin, augmented splenectomy, haemoglobin overload, and HCV infection. Homozygous IVS-I-5 was significantly associated with transfusion dependence, as indicated by multivariate regression analysis. These results highlight the importance of integrating molecular testing into counseling, prognostication, and targeted screening programs. Demographic and clinical characteristics of β-Thalassemia patients are indicated in Table 1.

Table 1: Demographic and Clinical Characteristics of Patients with β-Thalassemia

Variable	β-thalassemia Major (n = 289)	β-thalassemia Intermedia (n = 125)	Statistical Test Used	Test Value	p-value
Mean age at diagnosis (years, mean ± SD)	1.9 ± 1.2	6.8 ± 3.4	Student's t-test	t = –17.32	<0.001
Male sex (%)	154 (53.3%)	61 (48.8%)	Chi-square	$\chi^2 = 0.73$	0.394
Consanguinity (%)	185 (64.0%)	66 (52.8%)	Chi-square	$\chi^2 = 4.61$	0.032
Family history of thalassemia (%)	162 (56.1%)	64 (51.2%)	Chi-square	$\chi^2 = 0.81$	0.368
Socioeconomic status — low (%)	201 (69.6%)	70 (56.0%)	Chi-square	$\chi^2 = 7.84$	0.005
Splenectomy (%)	98 (33.9%)	18 (14.4%)	Chi-square	$\chi^2 = 14.9$	<0.001
Regular chelation adherence (%)	176 (60.9%)	98 (78.4%)	Chi-square	$\chi^2 = 13.0$	<0.001
Evidence of iron overload (ferritin >1000 ng/mL) (%)	172 (59.5%)	42 (33.6%)	Chi-square	$\chi^2 = 29.6$	<0.001
HCV seropositivity (%)	42 (14.5%)	6 (4.8%)	Chi-square	$\chi^2 = 8.99$	0.003
Mean pre-transfusion Hb (g/dL, mean ± SD)	6.8 ± 0.9	8.9 ± 1.1	Student's t-test	t = –20.42	<0.001

*n = Number of Participants, HCV = Hepatitis C Virus, Hb = Hemoglobin, SD = Standard Deviation, % = Percentage, * = Statistical significance at p<0.05*

Out of 414 patients, patients with β-thalassemia major were diagnosed earlier (mean 1.9 ± 1.2 vs. 6.8 ± 3.4 years), had lower pre-transfusion hemoglobin (6.8 ± 0.9 vs. 8.9 ± 1.1 g/dL), and higher rates of splenectomy, 98 (33.9%) vs. 18 (14.4%), iron overload, 172 (59.5%) vs. 42 (33.6%), and HCV seropositivity, 42 (14.5%) vs. 6 (4.8%) than intermediate β-thalassemia. Consanguinity and lower socioeconomic status were also more evident in major cases. These results suggest that severe phenotypes are not only genetically determined but also affected by socio and treatment-related factors. Table 2 summarizes the distribution of the HBB gene mutations in the study participants.

Table 2: Distribution of the HBB Gene Mutations in the Study Population

Mutation Type	Total Cases n (%)	β-thalassemia Major (n = 289)	β-thalassemia Intermedia (n = 125)	Statistical Test Used	Test Value	p-value
IVS-I-5 (G>C)	172 (41.5%)	138 (47.8%)	34 (27.2%)	Chi-square	$\chi^2 = 15.1$	<0.001
Codon 41/42 (–TCTT)	87 (21.0%)	52 (18.0%)	35 (28.0%)	Chi-square	$\chi^2 = 5.24$	0.022

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Mutation Type	Total Cases n (%)	β-thalassemia Major (n = 289)	β-thalassemia Intermedia (n = 125)	Statistical Test Used	Test Value	p-value
IVS-I-1 (G>T)	61 (14.7%)	45 (15.6%)	16 (12.8%)	Chi-square	$\chi^2 = 0.58$	0.447
Codon 15 (G>A)	14 (3.4%)	8 (2.8%)	6 (4.8%)	Chi-square	$\chi^2 = 1.05$	0.306
Codon 8/9 (+G)	9 (2.1%)	6 (2.1%)	3 (2.4%)	Chi-square	$\chi^2 = 0.03$	0.873
Other rare mutations	71 (17.1%)	40 (13.8%)	31 (24.8%)	Chi-square	$\chi^2 = 8.49$	0.004

*n = Number of Participants, HBB = Hemoglobin Subunit Beta, IVS = Intervening Sequence, G = Guanine, C = Cytosine, T = Thymine, A = Adenine, SD = Standard Deviation, % = Percentage, * = Statistical significance at p<0.05*

IVS-I-5 (G>C) mutation significantly occurred in 172 (41.5 %) patients, followed by Codon 41/42 (–TCTT), 87 (21.0%), and IVS-I-1 (G>T) (14.7%). Homozygous IVS-I-5 was much more prevalent in 138 (47.8%) β-thalassemia major, compared to intermedia, 34 (27.2%). Codon 41/42 was also relatively more frequent in intermedia, 35 (28.0%). The findings suggest that clinical severity is highly dependent upon mutation type, and IVS-I-5 is a molecular marker of severe disease. Table 3 illustrates the genotype-phenotype correlation with transfusion dependence in β-Thalassemia.

Table 3: Genotype-Phenotype correlation in β-Thalassemia Patients

Genotype Category	Adjusted Odds Ratio (OR) for Regular Transfusion	95% Confidence Interval (CI)	Statistical Test Used	Test Value	p-value
Homozygous IVS-I-5 (G>C) (n = 112)	3.8	2.1 – 6.9	Multivariable Logistic Regression	Wald χ^2 = 18.6	<0.001
Homozygous Codon 41/42 (–TCTT) (n = 52)	1.6	0.9 – 2.9	Multivariable Logistic Regression	Wald χ^2 = 2.54	0.111
Compound heterozygotes (n = 148)	1.2	0.8 – 1.9	Multivariable Logistic Regression	Wald χ^2 = 0.97	0.324
Rare/other variants (n = 102)	0.9	0.5 – 1.6	Multivariable Logistic Regression	Wald χ^2 = 0.13	0.719

*n = Number of Participants, IVS = Intervening Sequence, G = Guanine, C = Cytosine, T = Thymine, A = Adenine, OR = Odds Ratio, CI = Confidence Interval, % = Percentage, * = Statistical significance at p<0.05*

In multivariate analysis adjusting for clinical confounders, homozygous IVS-I-5 (G>C) showed a strong independent association with transfusion dependence (adjusted OR 3.8, 95% CI: 2.1-6.9, p< 0.001). Compound heterozygotes and homozygous Codon 41/42 (–TCTT) did not retain statistical significance with adjustment, while rare variants were used as the reference. These results highlight that IVS-I-5 is a key molecular predictor of transfusion-dependent disease, supporting its importance in clinical management and counseling.

DISCUSSION

This study aimed to investigate the molecular spectrum of HBB gene mutations in β-thalassemia patients and to examine their roles in relation to transfusion dependence, besides the role of socio-demographic and clinical factors. The results indicate that certain genetic defects, especially homozygous IVS-I-5 (G>C), are major predictors of regular transfusion, regardless of socio-demographic factors. Findings support that genetic determinants are the determinants of disease severity in β-thalassemia, though environmental and clinical factors are significant modulators.

Patients with β-thalassemia major were diagnosed earlier, had lower hemoglobin levels before transfusion, and had higher splenectomy, iron overload, and hepatitis-C seropositivity. This finding aligns with recent molecular research that indicates that severe mutations,

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including IVS-I-5, lead to ineffective erythropoiesis and increased transfusion needs (28). In comparison, patients with intermediate phenotypes had a higher probability of carrying Codon 41/42 mutations or rare variants, which is consistent with the previously known genotype-phenotype correlation among South Asian cohorts and mutation spectrums among carriers in Iran (29,30). The discovery of IVS-I-5 as a molecular biomarker of severe disease is comparable to other studies on genetic predictors in other chronic diseases, where structural variants have always been the best prognostic factors (31). Remarkably, the relative weakness of the association of Codon 41/42 mutations with transfusion dependence in adjusted models highlights the difficulty of adjusting genetic interactions. The same divergent genotype effects have been observed in fibrosis-related diseases, where core mutations are modified by modifier genes (32). Interestingly, the pathogenic value of IVS-I-5 has recently been validated by genomic evidence, which supports its value as a clinically-relevant mutation (33).

Besides the molecular spectrum, socio-demographic variations were also evident. Consanguinity among β-thalassemia major patients was greater, implicating intra-family marriages as a homozygote expelling severe alleles. This finding concurs with renal molecular studies that genetic clustering in families increases the penetrance of deleterious variants (34). Weak socioeconomic status was linked to worse outcomes, proving that financial obstacles hinder access to chelation therapy and specialized care, thereby increasing the severity of the genotype (35). The clinical interpretation of these results reveals that the disease is not only genetic but also partly dependent on comorbidities and environmental exposures. As an example, iron overload in over half of the key cases can exacerbate oxidative stress, leading to the development of cardiac dysfunction and endocrinopathies (36). This can be compared to experimental research that attributes disordered ion transport proteins to the progressive tissue injury, with dysregulation of signaling cascades accelerating the progression of a clinical disease (37). Significantly, recent clinical trials including ENERGIZE have demonstrated that mitapivat effectively enhances hemoglobin levels in non-transfusion dependent thalassemia, which has therapeutic potential (38).

The hepatitis C prevalence among transfusion-dependent patients is an additional point of complexity. Like endocrine diseases in which a change of metabolic status worsens the clinical condition, viral infections are disease modulators, increasing hepatic fibrosis and immunological homeostasis (39). Just like PKU-related dyslipidemia, the metabolic imbalances in thalassemia support the significance of lipid follow-up measures in the overall patient management(40). Furthermore, the cardiovascular effect of thalassemia is consistent with myocardial infarction literature, where predisposing stress and oxidative disequilibrium result in arrhythmic issues (41). Similar to diabetic cardiomyopathy BNP and Killip grading, biomarker-based monitoring can have cardiovascular risk stratification refined in thalassemia patients, especially cardiomyopathy caused by iron overload (42). Recently, gene therapy has proven to be effective in decreasing transfusion requirements, with betibeglogene autotemcel beti-cel showing sustained transfusion autonomy in transfusion-dependent thalassemia patients (43).

In a more physiological sense, transfusion dependence correlates with homozygous IVS-I-5 mutations, indicating similarities in neurotrophin-mediated inflammatory diseases, including endometriosis, which involve specific biological mediators for disease severity (44). Immune components are also pertinent, since alloimmunisation of transfusion may regulate disease progression, similar to the immunological interaction of extracellular vesicle-mediated immune enhancement (45). Gene editing systems like exagamglogene autotemcel (exa-cel) offer a prospective long-term curative therapy, which has been proven in hemoglobinopathies (46). These results have profound clinical practice implications. Metabolic and nutritional profiling, including monitoring of vitamin D, zinc, and thyroid, may contribute to personal care as these deficiencies increase hematological stress (47). The interrelation between nutrition and hemoglobin production reflects the evidence in metabolic syndromes that lack systemic resilience due to deficiencies (48). Moreover, the mechanistic evidence of HbF regulation through association studies of BCL11A and HBS1L-MYB polymorphisms has demonstrated potential genetic pathways to decrease transfusion requirements in thalassemia (49,50).

The association of our findings with the prior literature shows consistent trends but also a few inconsistencies. As an example, Codon 41/42 mutations had been regarded as strong predictors of transfusion need, but in this research, their value decreased post-adjustment. This disparity can be attributed to the variation in the allele frequency, modifier genes, or environmental cofactors among populations. Likewise, whereas in earlier studies the chronic infections contribute to increased severity of anemia, in our cohort the genotype was the more dominant predictor of transfusion burden, implying that, though infections are contributory factors, they do not dominate the genetic effect (51). These findings are consistent with wider conclusions that effective gene therapy programs can redefine the natural history of transfusion-dependent β-thalassemia (52). In addition to hematology, endocrinology, and cardiology provide systemic insights. High body mass index has been attributed to disturbed hemodynamics and poor cardiac recovery, as any metabolic imbalance results in poorer clinical outcomes in thalassemia patients (53). Similarly, abnormal glucose tolerance can complicate the management of anemia, as it complicates diabetic cardiomyopathy (54). Such comparisons support the opinion that β-thalassemia needs to be addressed in an

integrative and multi-system approach. In this respect, population-wide reports reveal changing prevalence and mutation rates in countries like Pakistan, highlighting the necessity of region-specific prevention measures (55).

Immunologically, cytokine imbalance seen in diabetic retinopathy in the advanced stages, such as IL-6 and VEGF, is also evident in thalassemia, where endothelial damage is mediated by chronic oxidative stress and inflammation (56,57). These molecular overlaps are the reason to consider anti-inflammatory or immune-modulatory interventions exploration as a supplement to transfusion and chelation therapy (58). Regenerative techniques have been used to draw therapeutic parallels in other inflammatory conditions, with agents like basic fibroblast growth factor reported to regulate repair and inflammatory homeostasis implying possible applicability to adjunctive thalassemia therapy (59). Public health implications are also highlighted. The issue of vaccine hesitancy is a serious problem in most low-resource contexts, and the same can be said about genetic counseling and thalassemia prevention initiatives (60). The solutions to these gaps are culturally sensitive education programs, especially in high-carrier areas.

There are limitations to this study. As a cross-sectional study, it was unable to draw causal conclusions for individual genotypes and long-term complications. Generalizability may be restricted by the single-center design. Self-reported clinical history and incomplete measurements of nutritional and metabolic parameters could have resulted in recall and measurement bias. The confounding elements, including environmental stress, lifestyle practices, and comorbidities, including diabetes or hypothyroidism, were not consistently assessed. The future studies must be longitudinal and multicenter to confirm these results in a wide variety of populations. Moreover, research comparing integrated care strategies, including genetic screening, nutritional support, infection control, and psychosocial counseling, will be essential in minimizing the burden of transfusion-dependent thalassemia.

CONCLUSION

This study indicated that the molecular spectrum of HBB gene mutations is highly predictive of the severity of β-thalassemia, with the homozygous IVS-I-5 (G>C) becoming the most significant predictor of transfusion dependence. Additional clinical factors, including age of diagnosis, iron overload, splenectomy, and hepatitis C, also played a role, with socio-demographic factors such as consanguinity and socioeconomic inequalities being significant modifiers. These results support that environmental and clinical states, although not the fundamentals of disease severity, play a significant role in its progression.

Focusing on the molecular screening, especially in high-consanguinity populations, can decrease the prevalence of the most hazardous cases. To enhance patients' outcomes, comprehensive management, including genetic counseling, nutritional optimization, and infection monitoring, is necessary. Subsequent studies are recommended based on multicenter, longitudinal designs and assessing advanced therapeutic approaches, such as gene therapy and gene editing, to reduce transfusion dependence and improve long-term survival.

AUTHOR CONTRIBUTION

Author	Contribution
Areej Khan	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Muhammad Akram*	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Kanta Bai	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Abdul Rehman Khalil Shaikh	Contributed to Data Collection and Analysis

Author	Contribution
	Has given Final Approval of the version to be published
Durga Devi	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published

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