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ORIGINAL ARTICLE

Mutation Analysis of Hemoglobin Subunit Beta (HBB) Gene in Beta-Thalassemia Patients in North Karnataka Population

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Abstract

Introduction: Beta-thalassemia is among the most frequent monogenic disorders around the globe. Over 100,000 children worldwide require regular transfusion due to beta-thalassemia, with India accounting for about 10% of cases ana a carrier rate of 5-17%. The disorder is caused by mutations in the beta-globin (HBB) gene, especially in exon 3, which affects beta-globin production and leads to sever anemia. Genetic testing is crucial for prenatal diagnosis, carrier screening, and counseling to manage and prevent the disease. **Material and Methods:** The study was conducted on 47 beta-thalassemia patients aged 6 months to 18 years, with ethical approval. After informed consent, 1ml blood sample were collected. DNA was extracted using the Kit, and Primers targeting exon 3 of the HBB gene were design. PCR amplification was performed with specific cycling conditions, and products verified by gel electrophoresis. **Results:** Sequencing identified a likely benign homozygous intronic variant g.5511G>C (rs107686883). Mutation analysis in 47 patients revealed heterozygous mutations g.5401G>A. **Conclusion:** Missense mutations, especially G>T transition, were common in HBB exon 3 of thalassemia major patients. Molecular screening and genetic counseling are vital to reduce disease impact. G>A mutation caused severe early disease, G>T moderate severity, and compound heterozygosity showed intermediate symptoms.

Key Words

HBB Gene, Beta-Thalassemia, Exon 3, Mutations

Introduction

Beta-thalassemia among the most frequent monogenic disorders around the globe[1]. Due to this disorder, more than 100,000 children rely upon regular blood transfusions to survive. India ranks among the top contributors for this issue, where nearly 8,000-10,000 thalassemic children are born annually and that accounts to about 10% of total world incidence[2]. In India, the carrier rate ranges from 5 to 17% varying with the geographic location. This disorder is common in countries of endemic malaria, such as the Mediterranean basin, some areas of Asia and Africa [3]

Beta-thalassemia results from mutations of beta-globin (HBB) gene. Hemoglobin consists of four protein chains — two alphas and two betas — that are produced by genes within the red blood cells, and themutations lead to less or no production of beta-globin. Currently more than 300 mutations exist in the HBB gene, a number of them produce abnormal beta-globin by various mechanisms,

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for example point missense significant mutatins acting on gene transcription or translation.[4] Exon 3 of the HBB gene is the most important part of this gene as it contains that portion of sequence which codes for functional beta-globin protein and its mutations are known to result in severe anemia[5]. Genetic testing for such mutations is the cornerstone of prenatal diagnosis, carrier detection, and genetic counselling as some of the essential components in management and prevention of beta-thalassemia.[6,7] Given the significant cansangunity and endemic incidence, our study supports public health measures for genetic screening by providing North Karnataka specific HBB exon 3 mutations profiles.

Objectives

Primary Objective:

- To purify and quatify high-quality genomic DNA from blood samples of beta-thalassemia major patients.

Secondary Objectives:

- To amplify the 3 exon region of the HBB gene using optimized Polymerase Chain Reaction (PCR).
- To identify specific mutations in exon 3 associated with beta-thalassemia using Sanger sequencing.

Material and Methods

Selection of Patient

The individuals in the Department of Pediatrics at BLDE (Deemed to be University) Shri B. M. Patil Medical College, Hospital, and Research Centre, Vijayapur, Karantaka, India, were the subjects of the study. The study duration was from January 2024 to June 2025, was accomplished at the Center for Advanced Medical Research (CAMR), and the Gentic Laboratory. The Ethical Committee of BLDE (Deemed to be University) gavethe Ethical Approval Certificate from the Institution (approval number: IEC/SBPMC/349/2024-25) on June 11, 2025. In order to ensure infomed consent, the patients and their families were fully told about the objectives of the study and methods prior to blood sample collection. A systematic questionnaire was used to choose 47 patients using convenience sampling techniquefrom North Karnataka's rural and urban areas who took part in the research.

Sample Size Calculation

The sample size calculation shows that if the expected proportion CODON 3 beta thalassemia casesin the population is about 19%, then to estimate this proportion accurately with a 95% confidencelevel and a margin of error of 11.2%, we would need to include 47 patients in the study. The formula utilized for calculating the sample size the equation is

$n = z^2 p^* q d^2$.

where P stands for proportion rate,

Z = Z statistic at significance level,

and α D2 = Absolute Error.

q = 100-p.

Blood Sample Collection

Written Consent was obtained from the participant's parent, who were registered in the study. Aftertaking consent, 1 ml peripheral blood sample were collected in the EDTA-coated vacutainer and kept at 4ºC until further use.

DNA Extraction & Quantification.

A DNA extraction kit (Qiagen kit, Germany)and about 200 ìl of blood samples were taken to extractDNA from the blood. A multimode reader (Tecon) was accustomed toevaluate the amount and quality of DNA samples also confirmed by agarose gel eletrophorosis.

Polymerase Chain Reaction (PCR)

Primers covering the entire length of exon 3 were created based on the gene ofinterest. (NG_059281.1 is the reference sequence). Several bioinformatics tools, including UCSC Virtual Polymerase Chain Reaction (PCR) and Genome Build 36, were used in the primer design. Oligonucleotide Synthesis Laboratory (Eurofins, India) produced the designed primers. Table 1 lists the primer sequences and annealing temperature for the HBB gene. The PCR reaction mixture included 7.2µl molecular grade water, 10 µl Takara Master Mix, 0.4 ìleach of forward and reverseprimers, and 1µl template DNA. Amplification wasdone using Master cycler with Initial denaturation at 95 for 5 seconds 1 cycle, followed by 35 cycles at 95 for 30 seconds, annealing at a temprature specific to the primers for 30 seconds, and extension at 72 for 45 seconds, concluding with a final extension of 10 minutes at 72. PCR products were checked using a100 bp ladder and gel electrophoresis. The PCR product outputs were visualized using UV gel documentation system (Cleaver Scientific, UK).

Sequencing

Capillary sequencing using the ABI3730 Sanger sequencing platform (thermoscientific) was used to subject PCR products. Before sequencing, the PCR products obtained processed with cycle sequencing and plate processing using Big-Dye Terminator v5.4 method.

Statistical Analysis

This study was a descriptive molecular screening of HBB gene mutations without group comparisons or quantitative clinical co-relations, hence no statistical testing were conducted.

Results

A total of 47 beta-thalassemia patients, aged between 6 months and 18 years, were included in the mutational analysis of the Haemoglobin Subunit Beta (HBB) gene. The study cohort demonstrated a male predominance, with 29 males (58%) and 21 females (42%), and the majority of children (46%) belonged to the 7-9 year age group, with a mean age of 6.6 ± 2.4 years.

The HBB gene variant g.5511G>C (c.315-16 G>C, rs10768683), an intronic variant located in intron 2, was identified in a homozygous state. According to ClinVar, this variant is classified as likely benign, with multiple submissions confirming no pathogenic impact on beta-globin function or splicing. It appears in population databases without any association with beta-thalassemia major or severe phenotypes. Homozygosity indicates biallelic inheritance but does not cause disease, as functional studies demonstrate minimal disruption. No clinical recommendations for intervention are warranted;

Table 1: Primer sequences and annealing temperatures used in exon 3 in the HBB gene.

Primer Name	Primer Details	Melting temperature	Sizeoftheexon
Th3Forward	GCTCACCTGGACAACCTC A	65°C	232 bp
Th3Reverse	AACGATCCTGAGACTTCC ACA		

Table 2: Variant Details

Sample no	gDNA position	cDNA position	Status	Variant type	Condition	Phenotype/ Disease
2,8,12,38	g.5511G>C	c.315-16G>C	Rs10768683	Intronvariant	Homozygous	LikelyBenign

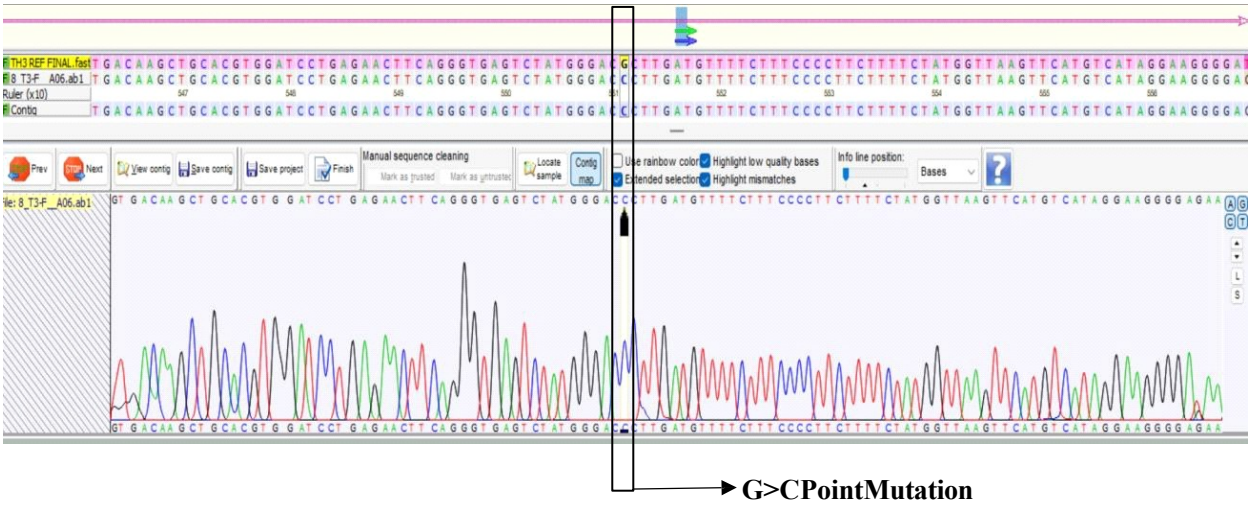

Figure 1: Electropherogram showing G>C Homozygous, Point Mutation.

routine monitoring suffices in thalassemia screening contexts (Table 2).

Sanger sequencing of samples 2, 8, 12, and 38 confirmed the G>C substitution at the rs10768683 locus. The electropherograms exhibited clear, distinct peak patterns, confirming the presence of this intronic variant. This mutation was consistently identified in the intronic region c.315-16 G>C and classified as likely benign. The high-quality sequencing data validated the accuracy of mutation detection in these samples (Figure 1).

Discussion

The genetic analysis identified a predominantly benign intronic variant (g.5511GC c.315- 16GC, rs10768683) occurring in homozygous form in several patients. This variant resides 16 base pairs downstream of exon 3, in the intronic region, and is classified as likely benign, indicating it does not disrupt beta-globin gene functionality or contribute to disease pathology. This finding resonates with existing literature emphasizing extensive genetic heterogeneity in beta-thalassemia, where many polymorphisms— although prevalent— do not have pathogenic consequences. These benign intronic polymorphisms are critical to recognize to avoid misclassification of variants as disease-causing. Their identification enriches populationspecific genetic databases and supports precise genetic counseling efforts by distinguishing disease-causing mutations from innocuous variants[8].

The sequencing also revealed heterozygous missense and synonymous mutations in multiple samples, though some results were constrained by low electropherogramquality[9]. Missense mutations, typically involving nucleotide substitutions resulting in amino acid changes, are clinically impactful as they alter beta-globin structure and stability and thus influence the severity of beta-thalassemia symptoms[10].

Synonymous mutations, while not altering protein sequences, may affect splicing or gene expression regulation. The prevalence of missense mutations in exon 3 underscores the importance of this exon in encoding critical beta-globin subunit regions essential to hemoglobin function[11]. These results align with extensive mutation catalogs that document the spectrum and effects of HBB mutations in various populations, including India[11]. Clinically, the study reaffirmed hallmarks of beta-thalassemia major: early onset severe anemia, microcytic hypochromic red cells, hepatosplenomegaly, skeletal deformities, and growth retardation[12]. The phenotypic presentation reflects the underlying molecular defect that defective or insufficient beta-globin chains cause ineffective erythropoiesis and hemolysis. Iron chelation and routine blood transfusions remains standard to control anemia and overconsumption of iron complications affecting cardiac, hepatic, and endocrine systems. The high rate of consanguinity observed supports epidemiological data linking genetic risk to sociocultural practices within this region[13]. The clinical severity reported corresponds with genotype-phenotype associations wherein certain mutations drive earlier symptom onset and more frequent transfusion dependency[14]. Methodologically, the study emphasizes the critical role of high-quality molecular diagnostics, including optimized PCR and Sanger sequencing protocols, to accurately detect mutations. The partial ambiguity in some sequencing results underscores the technological challenges and the need for more sensitive or next-generation sequencing approaches to capture complex mutations or low-frequency variants[14].

The practical implications highlight the importance of mutation screening for carrier detection, genetic counseling, and prenatal diagnosis to reduce disease prevalence and enable early intervention. Such strategies have proven effective in hemoglobinopathy-endemic regions worldwide and should be prioritized in public health policies within North Karnataka. The detailed mutation profiling presented can inform tailored community screening programs and raise awareness of beta-thalassemia's genetic basis. Recent gene therapy advances (CRISPR/Cas9, beti-cel) underscore the need for region-specific HBB profiles to guide patient selection[14].

Conclusion

Information analysis of HBB exon 3 in patients with thalassemia major showed mssense mutations to be the most common HBB carrying and G>T transitions were most ftequently found codon transforming to influence on protein synthesis. These results emphasize the importance of primary prevention by molecular screening,

carrier testing, genetic counseling, and public education in order to lower disease rates and serve the load for families/society. The authors point out consanguinity, male preponderance, early need for transfusions, severe anemia and organomegaly as well as the importance of iron chelation. Genotype-phenotype correlations showed that G>A was associated with severe early disease onset, G>T to moderate and compound heterozygosity had intermediary clinical features probably did to it having the highest number of transfusions highlighting individual risk stratification.

Limitations

It is a one-center study and has a limited sample size (n=47), which limits generalizability, and makes it less suitable for comparison. Furthermore, without long-term follow-up information, we are unable to obtain knowledge about diseases progression and effects like socioeconomic status or diet, which remain unconsidered are the main sources of bias on outcomes. More over, the reliance on the currently existing medical records also causes a risk of bias to inaccuracy of the documentations.

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Authors' Contribution

SM, DS, GKconducted the experiment and drafted the data; SP, DS, PP and GK has helped in making methodology standardize and execution of the work; SP, PP, GK in the correction and SM,DS,GK has completed the drafting with corrections and made upload.

Ethics

The ongoing research study received ethical review and approval from BLDE (Deemed to be University), along with the Institute Ethical Committee. The institutional review board granted ethical approval (IEC/ SBPMC/349/2024-25).

Conflict of Interest

The authors state that they have no financial interests or personal relationship that could affect the results of this research study. This study was not funded by any research funding agency.

Data Availability

The datasets utilized and/or examined in this study can beobtained from the corresponding author upon reasonable request.

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