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Prevalence of Beta Thalassemia in Antenatal Diagnosis in North India

Jyotsana Gaur[1], Dr. Rashmi Arnold[2], Dr. Sunil K Pollipalli[3], Dr. Somesh Kumar[4], Dr. Seema Kapoor[5], Dr. Komal Uppal[6]

Abstract: ***Beta thalassemia major, caused by mutations in HBB gene, poses a significant public health challenge in India, with 10, 000-12, 000 affected children born annually. This study aimed to determine the prevalence of beta-thalassemia among 253 antenatal screening couples in North India. Key findings revealed IVS 1: 5 (G>C) as the most common mutation, highlighting genetic diversity in the population. Employing targeted Sanger sequencing, the study offers a cost-effective solution for identifying HBB mutations. The findings underscore the importance of early screening and preventive programs to manage thalassemia in developing nations.***

Keywords: beta thalassemia, HBB mutations, antenatal screening, North India, genetic diversity.

1. Introduction

Thalassemia is hereditary, meaning that only one parent is required to carry the disease (Bajwa & Basit, 2022). It is caused by either a genetic mutation or a deletion of certain key gene fragments. Thalassemia patients have fewer than normal hemoglobin molecules. Red blood cells can carry oxygen because of hemoglobin. Anemia and fatigue are major symptoms of thalassemia, so patients of thalassemia in its severe variants may necessitate frequent blood transfusions. However, unlike thalassemia, in anemic condition, the body does not have enough normal and healthy red blood cells.

Thalassemia was once believed to be limited to tropical regions, but migration has made it a worldwide issue (De Sanctis et al., 2017). Thalassemia is an inherited blood disorder characterized by abnormal hemoglobin production. There are two forms: Alpha and beta-thalassemia, with beta-thalassemia caused by mutations in the beta-globin gene. DNA polymorphism in the beta-globin gene results in various haplotypes, with different frequencies among Asian Indians. The seven common mutations and haplotypes in Indian individuals, shedding light on the genetic diversity of thalassemia in this population. The β-globin gene in Asian Indians has diverse DNA polymorphisms that create unique haplotypes with varying frequencies. (Source: Kazazian et al., 1984).

For the 1st time, the common seven mutations and their haplotypes in the Indians – Frameshift β 8-9 (+G), Nonsense Codon 15 (TGG-TAG), Frameshift β 41-42 (- TCTT), Frameshift β 16 (-C), IVS-1-5 (G-C), 619 bp deletion, and 13 and 25 nucleotide deletion, at 3' end The Kazazian et al. team discovered a common mutation of a 619 bp deletion in Asian Indians in 1984. Recent studies show that between 300, 000 and 400, 000 babies are born with serious hemoglobin disorders annually in low-or middle-income countries.23, 000 of these cases are β-thalassemia major, with 90% of them resulting in newborns (De Sanctis et al., 2017). Carrier frequencies of β-thalassemia range from 1-20% worldwide, with some regions experiencing even higher rates (Black et al., 2010).

India has around 30 million β-thalassemia patients, with 10% of the world's total cases being born in the country annually. (Bashyam et al., 2004). In India, communities like Sindhis (Colah et al., 2010), Guajarati's (Bhukhanvala et al., 2013), Punjabis (Grow et al., 2014), and Bengalis (De et al., 1997), are commonly affected by β-thalassemia with incidence ranging from 1% to 17%. Research highlights the prevalence of the disease (Gupta et al., 2003).

Over 150-200 mutations causing β-thalassemia reported worldwide in various studies conducted across different regions (Cao & Galanello, 2010). Studies in India have identified around 28 mutations (Old et al., 2001), with five to six common mutations including IVS 1-5 (G-C), 619 bp deletion, IVS 1-1 (G-T), Frameshift 8/9, 41/42, and Codon 15 S (Bandyopadhyay et al., 2004). Different ethnic groups show varying mutation types, with IVS 1-5 (G-C) being the most common mutation (Bashyam et al., 2004). The frequency of mutations varies by region, reflecting the genetic diversity of Indian populations (Agarwal et al., 2000).

Research on thalassemia in the Indian subcontinent has been conducted (Grow et al., 2014) across various regions, including Pakistan, Sindh (Usman et al., 2009), Punjab (Garewal & Das, 2003), Gujarat (Bhukhanvala et al., 2013), Tamil Nadu (Colah et al., 2009), Maharashtra (Ambekar et al., 2001), and Kerala (Edison et al., 2008). Understanding the genetic diversity of beta-thalassemia mutations in North India is crucial for developing effective screening programs and reducing the burden of this hereditary disorder. So, this study was postulated to identify the prevalence of beta thalassemia in antenatal screening couples from north India.

2. Materials & Methods

Sample collection

A total of 253 male and female from the north India, suspected to be the carriers either for beta thalassemia and referred to the diagnostic laboratory from Delhi, NCR region as routine services were investigated. Sample collected were confirmed as beta-thalassemia carriers. A written informed consent was obtained from all the individuals for participating in this study duly IEC approval from the Institute.

DNA Extraction and Amplification

Three ml blood sample was taken in EDTA vial for molecular genetic testing. The genomic DNA isolation was performed according to the salting out method (Miller et al., 1988). The β-globin gene mutations were first distinguished using two sets of allele-specific ARMS-PCR to identify common mutation in north India Two sets of primers were used to amplify the betaglobin gene (HBB) Six regions around and within the beta-gene cluster amplification by polymerase chain reaction (PCR), using primers. Primer sequences those given by Old [Old et al., 1996] were used with minor modifications. Briefly, beta-globin gene amplified by dNTP, Taq DNA polymerase Taq buffer, each primer, MgCl2 and DNA was added and the volume was adjusted to 25 µl with water. Amplifying fragment initially denatured at 95 °C for one minute, followed by 35 cycles of denaturation at 95 °C for 60 s, annealing for 60 s, and extension at 72 °C for 60 s and a final extension at 72 °C for 5 min. The samples then subjected to a final extension step at 72 °C for 5 min. A 5-6-µl aliquot of each PCR product were run on a 2% agarose gel to check for positive amplification [Samavat A et al., 2004].

Unknown β-thalassemia genes were further characterized by direct DNA sanger sequencing using 3500 Genetic Analyzer Applied Biosystems (ABI) for all coding regions and exon–intron boundaries to detect uncommon point mutations and small rearrangements in the β-globin gene. The c.92+5G>C mutation was detected by Sanger sequencing and PCR-ARMS, and deletion 619 bp was done by end point PCR (gel electrophoresis). Other mutations were analyzed only by Sanger sequencer. Direct Sanger sequencing is the method of choice for detecting rare and novel mutations. The sequencing method was performed with the help of 3500 Genetic Analyser. The human beta-globin gene was sequenced to identify causative mutations which would not be detected by the ARMS PCR protocol. DNA sequence analysis of the HBB region was performed with three sets of overlapping primers which was designed to amplify the complete DNA sequence. Thus, three PCR was performed for each sample. Primer design was performed online by using primer 3 software. Three sets of overlapping primers covering the entire HBB region was prepared (Table-1).

The HBB gene region was amplified using PCR, with three tubes processed for each sample. Three primers was used with Q5®HotStart High-Fidelity 2X Master mix, template DNA, and adjusted volume. The PCR mix was added to a plate and cycled in a thermal cycler. A distinct 600 bp PCR amplicon band was observed on gel. Enzymatic purification of amplified product with the help of ExoSap-IT Reagent, sequenced using forward or reverse primers, and analyse for mutations using NCBI Blast online tool. Short nucleotide variations and Single nucleotide polymorphism was identified using sequence BLAST. Variants would be identified by Human Genome Variation Society. SNP accession numbers was indicated for DNA sequence variations. Overall, the process will involve amplification, sequencing, mutation identification, and statistical analysis of DNA samples from the target region.

Table 1: Control and Common Primers

1	Common A	5’ ACC TCA CCC TGT GGA GCC AC
2	Common B	5’ CCC CTT CCT ATG ACA TGA ACT TAA
3	Control Forward	5’ GAG TCA AGG CTG AGA GAT GCA GGA
4	Control Reverse	5’ CAA TGT ATC ATG CCT CTT TGC ACC

3. Result

The study was conducted in accordance with the guidelines set by the Indian Council of Medical Research with the patient’s informed consent prior to sampling (J Hum Genet et al., 2004). This study included patients who visited from 2019 to 2022. Two Hundred and fifty-three patients from different regions of North India were enrolled in this study. Our demographic variables provide insight into the genetic landscape of beta thalassemia and related disorders in the sampled population, highlighting the mutations most responsible for the conditions and their prevalence. In the current study, we have performed capillary sequencing technique to detect HBB gene mutation. This method demonstrates effectiveness as a practical and affordable tool for single-gene disorder analysis. The different mutations observed in patients with beta-thalassemia. The mutation IVS 1: 5 (G>C) is the most common, accounting for more than half (52.57%) of all mutations found. Other mutations like Cd 41/42 (-CTTT) and Cd 26 (G>A) are less frequent, making up 13.04% and 9.88% respectively. Some mutations, such as Cd 8/9 (+G), are relatively rare, making up only 6.7%, Cd 30 (G>C) and IVS 1: 1 (G>T) each found in 12 cases (4.74%). The table no.1 depicts the distribution of genetic mutations found in beta-thalassemia patients. It lists nine specific mutations with their corresponding HGVS nomenclature observed, and their percentage relative to the total number of mutations in the study population.

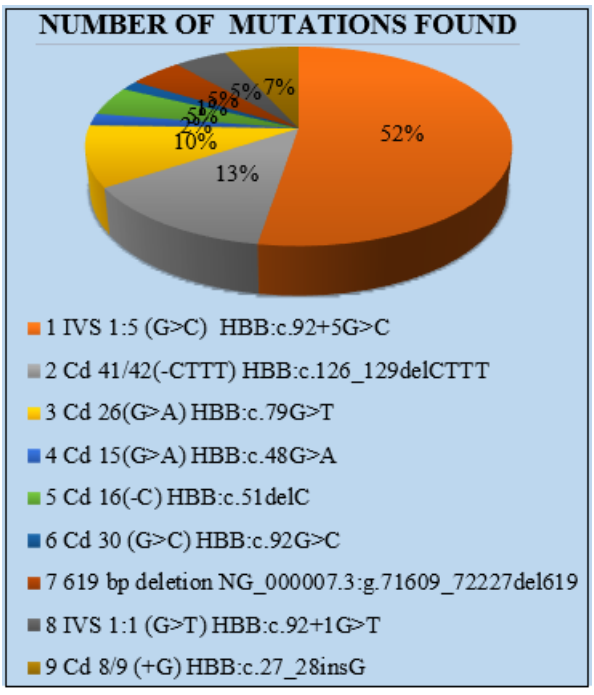

Table 1: Distribution of genetic mutations found in beta-thalassemia patients

S. No.	Mutation	HGVS Nomenclature	Number of Mutations Found	Mutation Percentage (N=253)
1	IVS 1: 5 (G>C)	HBB: c.92+5G>C	133	52.57
2	Cd 41/42 (- CTTT)	HBB: c.126_129delCTTT	33	13.05
3	Cd 26 (G>A)	HBB: c.79G>T	25	9.89
4	Cd 15 (G>A)	HBB: c.48G>A	5	1.98
5	Cd 16 (- C)	HBB: c.51delC	12	4.75
6	Cd 30 (G>C)	HBB: c.92G>C	4	1.59
7	619 bp deletion	NG_000007.3: g.71609_72227del619	12	4.75
8	IVS 1: 1 (G>T)	HBB: c.92+1G>T	12	4.75
9	Cd 8/9 (+G)	HBB: c.27_28insG	17	6.72

Table 2: Frequency Distribution of Mutations among β-thalassemia Carriers

Mutation Name	Mutation No	% of Abnormal Hemoglobinopathies	% of βthalassemia carriers	HGVS Name	Name Genotype	Male (N) %	Female (N) %
IVS1: 5 (G>C)	133	52.3	55.3	HBB.92+5G>C	homo	51	82
Cd 41/42 (-CTTT)	33	12.9	13.7	HBB.126_129delCTTT	hetro	19	13
Cd 26 (G>A)	25	9.8	10.4	HBB.79G>T	hetro	16	11
Cd 15 (G>A)	5	1.96	2	HBB.48G>A	homo	3	2
Cd 16 (-C)	5	1.96	1.4	HBB.51delC	homo	3	2
Cd 30 (G>C)	12	4.7	5	HBB.92G>C	homo	8	4
619 bp deletion	12	4.7	5	NG_000007.3.71609_ 72227del619	homo	5	7
IVS 1: 1 (G>T)	12	4.7	5	HBB.92+1G>T		6	6
Cd 8/9 (+G)	17	6.6	2.2	7	7	7	7
Total	254	100	240 (254)				

The frequency of different mutations associated with beta thalassemia in the studied population were depicted in table 2 which shows various mutations, their occurrences, the percentage of cases where these mutations cause abnormal hemoglobin or beta thalassemia. The HGVS column provides the genetic codes for each mutation. The genotype of affected individuals (homozygous or heterozygous) is recorded, along with the distribution between males and females for each mutation. In our study we identified the key mutations that include IVS1-5 (G-C), which appears in 55.3 % of beta thalassemia carriers, and Cd 41/42 (-CTTT), appearing in 13.7% of cases.

Table 3: Distribution of most common mutations for beta-thalassemia among the northern zone of Indian population

State	Sample Size	IVS1: 5 (G>C)	IVS 1: 1 (G>T)	619 bp deletion	Cd 41/42 (-CTTT)	Cd 26 (G>A)
Punjab, Haryana	60	53.3	3.3	20	8	6
Uttar Pradesh	85	62	0	27.0	0	0
Delhi	37	21.6	2.70	28	13.7	8.10
Jammu, Kashmir, and Himachal Pradesh	20	44	23	0	0	0
Other North Indian States	38	15	0	57	0	0

The table no.3 is showing the geographical variation in beta thalassemia mutations across different northern Indian states, with some mutations being more prevalent in certain regions than others.

4. Discussion

Despite significant advancements in the past decade, thalassemia remains a national concern in India, where the World Health Organization reports that it accounts for 80-90% of cases, highlighting its prevalence as a common hemoglobinopathy. β-thalassemia causes significant health issues and economic challenges for affected communities (Piplani et al., 2013). Studies worldwide focus on thalassemia, with India facing challenges due to lack of awareness, funding, resources, and social stigma hindering pre-marital screening efforts (Mohanty et al., 2013; Verma et al., 1997). According to WHO records, β-thalassemia is prevalent in India, prompting extensive research on its mutation distribution among the diverse Indian population. Due to India's ethnic heterogeneity and certain communities' high inbreeding frequencies, thalassemia prevalence varies significantly.

A study in western India found that 22.7% of women diagnosed with anemia were carriers of thalassemia, highlighting the issue's severity in specific communities (Mulchandani et al., 2008). The table no.1 depicted the distribution of the most common mutations for beta thalassemia in different states within the northern zone of India. It shows how frequently certain mutations appear in various regional population. IVS1: 5 (G>C) shows the percentage from each state who have this mutation. It is particularly common in Uttar Pradesh (62%) and Punjab & Haryana (53.3%), IVS1: 1 (G>T) mutation is less frequent, seen in 3.3% of individuals from Punjab and Haryana and 2.7% in Delhi, 619 bp deletion indicates the frequency of this mutation. It is most common in Uttar Pradesh (27%) and Delhi (28%). Cd 41/42 (-CTTT) mutation appears in some populations, with Uttar Pradesh showing 8%, Delhi showing 13.7%. Cd 26 (G>A) mutation is most prevalent in Delhi (8.1%) and Jammu, Kashmir, and Himachal Pradesh (6%).

Overall, our study assessment towards thalassemia among the north region is consistent with previous studies by Moghaddam et al. (2014) in the population of southeast Iran and the studies of Chatterjee et al. (2016) and Basu (2015) in Bengali. The assessment of the attitude towards a thalassemic child in North India is also similar and corresponds to the results of Pauisri et al. (2011) in one of the hospital studies in Thailand and Srivastava et al. (2011) among Bengali populations.

A community-based study is necessary to accurately assess the disease burden and carrier status of thalassemia in India. Mass awareness campaigns and preventive measures, tailored to cultural practices and can help curb thalassemia prevalence. Knowledge and awareness are crucial in combating widespread diseases, and a large-scale premarital screening and awareness program, along with genetic counseling, is needed to reduce the burden of hemoglobinopathies in the community. Many previous studies have highlighted the need for proper education and public awareness program on thalassemia, as also reported by Seyam and Assemi (2010) and Basu (2015). Politis et al. (1991) also studied among Greek populations and reported that education had the strongest influence on people's attitudes towards thalassemia.

The limitation of the study was small sample size for the beta-globin gene polymorphisms in the North India. Using polymorphism with high heterozygosity along with the known markers is recommended. Larger sample size is required for haplotype investigations of a normal population can clarify the high incidence of haplotype in our population.

5. Conclusion

This study highlights the prevalence of beta-thalassemia mutations in North India, with IVS 1: 5 (G>C) being the most common. The findings reinforce the need for cost-effective screening methods and public health initiatives, including genetic counseling, to address the burden of beta-thalassemia in India. Therefore, enlightenment of hygienic practices is important in combating diseases like thalassemia, which is linked to family marriages and poses risks to children. Awareness and guidelines for prevention should be promoted at all levels of society.

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