International Journal of Health Sciences and Research
[Indexed, Peer-Reviewed Journal]
Volume 15; Issue: 3; March 2025
Website: www.ijhsr.org
Original Research Article *ISSN: 2249-9571*

Mapping the Mutation Spectrum in Prenatal Thalassemia: Insights from a Tertiary Care Center in Delhi

Komal Uppal[1], Jyotsna Gaur[1], Sunil Kumar Polipalli[1], Somesh Kumar[1], Sangeeta Gupta[2], Seema Kapoor[1]

[1]Division of Genetics and Metabolism, Department of Medical Genetics, Maulana Azad Medical College (Delhi University), Delhi 110002.
[2]Department of Gynecology and Obstetrics, Maulana Azad Medical College, Delhi, India

Corresponding Author: Sunil Kumar Polipalli

DOI: https://doi.org/10.52403/ijhsr.20250323

ABSTRACT

Background: Understanding ethnic and region-specific mutations helps in creating customized molecular testing panels, enabling accurate and timely prenatal diagnosis of thalassemia to prevent the birth of affected children.
Materials and Methods: A retrospective analysis was conducted on data from 338 high performance liquid chromatography (HPLC) screen positive beta thalassemia-positive individuals (162 couples) and 14 mothers who were referred to Lok Nayak Hospital, New Delhi, for thalassemia diagnosis between 2016 and 2022. Initially, ARMS PCR for targeted five prevalent mutations in the ß-globin gene, followed by whole gene molecular sequencing of HBB was done. Chorionic villus sampling was later performed for prenatal diagnosis of 144 fetuses from 144 couples.
Result: Among 482 tested individuals, the most common mutations in the cohort were IVS 1-5 {G>C} (53.11%), codon 41-42 {–CTTT} (11.20%), codon 8/9 {+G} (8.7%), Codon 26{G>A} (5.3%), and codon 16 {-C} (5.1%). Less frequent mutations included IVS 1:1{G>T}, 619 bp deletion, and Hb S (A>T), comprising 3.7%, 2.9%, and 1.8% respectively.
Conclusion: While PCR-based mutation-specific sequencing is reliable for screen-positive couples, whole HBB gene sequencing is more cost-effective and recommended for identifying all thalassemia mutations, including rare ones.

Keywords: Thalassemia screening, Prenatal diagnosis, Chorionic villus sampling, HBB gene sequencing.

Key message: Whole HBB gene sequencing should be conducted at government institutions to ensure identification of all thalassemia mutations, including less common ones.

INTRODUCTION

Prenatal screening and diagnostic tests keep their important place not only in determining and diagnosing the high-risk babies for specific and common genetic disorders especially for thalassemia but also imparts the significance of knowing population specific mutation spectrum in this genomic era. Thalassaemia a common haemolytic anemia is a major health burden

in 71% countries worldwide with carrier rate of about 3% of the populations around the world and of 1 to 11% in all Arab countries [1, 2, 3]. Estimates indicate that around 56,000 conceptions worldwide and around 100,000 patients in India have β thalassemia syndrome [4,5]. Hemoglobin S (HbS) 150,000 cases [5] and hemoglobin E (HbE) very common in the northeastern region with carrier rate of 20.0–64.0% and in West Bengal in the east 3.0 to 10.0% [6].

Depending upon homozygous and heterozygous (carrier) status, individual can have major, intermediate or minor form of thalassemia. Carrier parents are at risk of 25% of getting thalassemia major baby. Major form requires regular transfusion and iron-chelating agent or bone marrow transplantation which is very cumbersome and costly. So, the birth of the babies with thalassemia can be prevented by identifying the carrier status of high risk couple by complete haemogram and by HPLC (high performance liquid chromatography) and by HBB gene molecular testing. If both parents are found to be heterozygous, they can be further given the option of prenatal molecular diagnosis.

Literature shows that population specific common mutation (accounting for >95% of severe β thalassemias) prenatal diagnostic testing can be highly productive and cost effective, but it can miss the less common mutations [7]. Here, we are going to share our seven-year experience of prenatal beta-thalessemia mutational spectrum data in high-risk couples in New Delhi.

MATERIALS & METHODS

Retrospective data from 2016–2022 were analyzed for 338 prenatal screening-positive individuals at the genetic laboratory of Lok Nayak Hospital, New Delhi, for thalassemia diagnosis. History and clinical examination details were recorded using a standardized format. Couples confirmed as carriers by HPLC initially underwent ARMS PCR to identify five common ß-globin gene mutations prevalent in the region: IVS 1-5G>C [c.92+5G>C], Codon 30G>C [c.92G>C], Codon 15G>A [c.48G>A], frameshift 41–42(-TTCT) [c.126_129delCTTT], and frameshift 8–9(+G) [c.27dupG]. Samples negative for these mutations underwent HBB DNA Sanger sequencing. Testing for hemoglobin variants like HbE and HbS was also done when indicated. Whole HBB gene sequencing was later adopted for cost-effectiveness and to identify less common mutations. PCR and Sanger Sequencing Methodology, Primer sequences for internal controls, common primers, and Sanger sequencing for the HBB gene are provided in the Supplementary Material (Supplementary Material). After the identification of ß-mutations in the parents, chorionic villous sampling (CVS) was done to identify whether the fetus was affected or not. Ethical clearance from institute's ethical committee and informed consent after explaining the risk of the procedure was taken from every participant.

RESULT

Out of 338 cases studied, 162 were males and 176 were females, with a mean age of 27.30 years (range 18–48). HBB molecular diagnosis was conducted for 162 couples and 14 mothers. Prenatal diagnostic testing via CVS was performed on 144 couples. Eighteen couples did not undergo prenatal testing: eight due to the COVID pandemic, six declined invasive testing, and four sought testing at a late gestational age. Demographics and distribution of mutations in female, male and fetus over the years (2016-2022) shown in table 1.

Table 1: Distribution Of Mutations in Female, Male and Fetus Over the Years (2016-2022)

Year	Female (mother)	Mutation percentage in female	Male (father)	Mutation percentage in male	Fetus	Mutation percentage in fetus	Total number of mutations
2016	20	33.3	20	33.3	20	33.3	60
2017	26	33.3	26	33.3	26	33.3	78

2018	26	33.3	26	33.3	26	33.3	78
2019	25	42.4	17	28.8	17	28.8	59
2020	12	41.37	10	34.48	7	24.13	29
2021	24	36.92	23	35.38	18	27.69	65
2022	43	38.05	40	35.39	30	26.54	113
TOTAL	**176**		**162**		**144**		**482**

ARMS PCR and HBB gene sequencing data for 482 individuals including 176 mothers, 162 fathers and 144 fetuses. Table 2 and figure 1.

Table 2: Distribution of Mutation Spectrum in 482 Individuals Over the Years (2016-2022)

	MUTATION	NUMBER OF MUTATIONS FOUND (N=482)	MUTATION PERCENTAGE
1	IVS 1:5 (G>C)	256	53.11
2	Cd 41/42(-CTTT)	54	11.20
3	Cd 8/9 (+G)	42	8.7
4	Cd 26(G>A)	26	5.3
5	Cd 16(-C)	25	5.1
6	IVS 1:1 (G>T)	18	3.7
7	619 bp deletion	14	2.9
8	Cd 15(G>A)	10	2.0
9	Cd 30 (G>C)	9	1.8
10	CAP+1 (A>C)	8	1.6
11	Hb S (A>T)	8	1.6
12	IVS 1 (-C)	3	0.6
13	Cd 88 (- CT)	3	0.6
14	Cd 19 (A/G)	2	0.4
15	Cd 15-19 del (ATCTT)	2	0.4
16	IVS 1:1 (G>A)	1	0.2
17	Cd 5 (-CT)	1	0.2

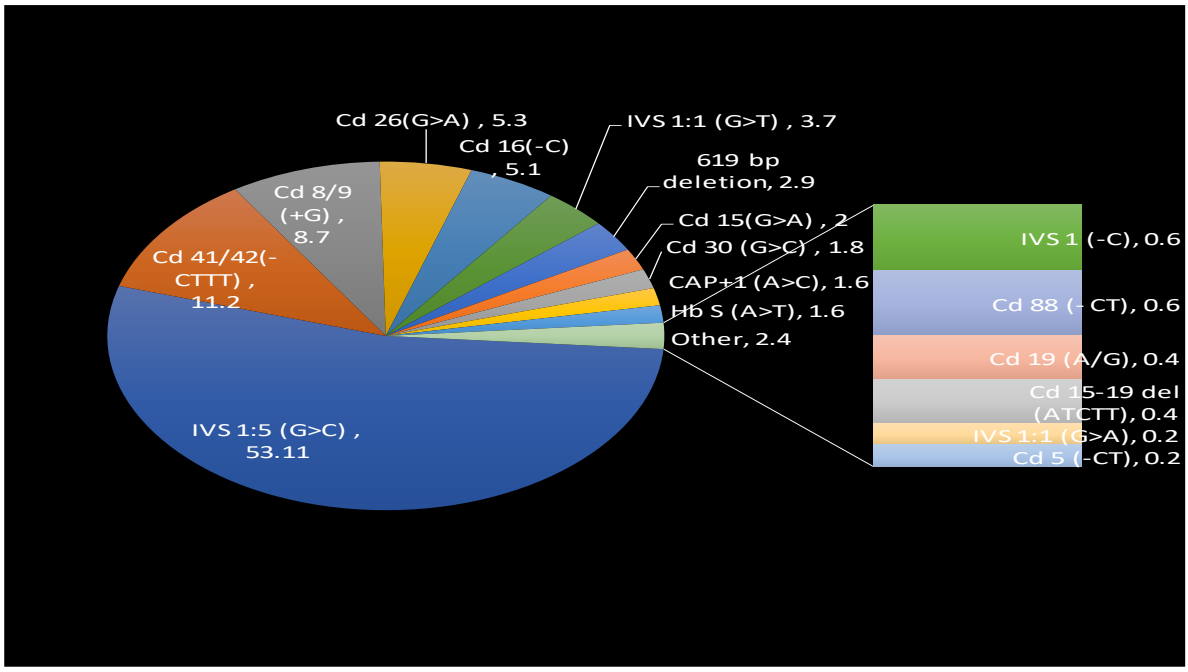

Figure 1: Distribution of mutation Spectrum in 482 individuals over the years (2016-2022). In our cohort, five common mutations made up 83.41% of total mutations in decreasing order: IVS 1-5 {G>C} (53.11%), codon 41-42 {–CTTT} (11.20%), codon 8/9 {+G} (8.7%), codon 26 {G>A} (5.3%), and codon 16 {-C} (5.1%). IVS 1:1 {G>T}, 619 bp deletion, and Hb S {A>T} mutations were found in 3.7%, 2.9%, and 1.8% of cases, respectively. Other less common mutations accounted for 7.8% of cases.

DISCUSSION

Thalassemia creates a significant public health problem and financial strain in India, highlighting the urgency for an effective and time bound pre-conceptional or prenatal screening and confirmatory testing. While screening initiatives are being considered, the expensive confirmatory tests remain a hurdle. Literature indicates the cost-effectiveness of these tests, particularly in India, for preventing thalassemia disorders, provided they are tailored to the population's specific mutation spectrum [8].

Inusha P et al. (2007) identified several common beta-thalassemia mutations in India, including IVS 1-5 (G>C), 619 bp deletion, Codon 8/9 (+G), IVS-1 nt 1 (G>T), and Codons 41-42 (-TTCT), which constitute 90% of cases [9]. Shouriyo Ghosh et al. reported the most frequent mutations as IVS 1-5 (G>C), Codon 26 (G>A)/HbE, Codon 30 (G>C), Codon 15 (G>A), and FS 41-42 (-CTTT) [10]. Keti Keler et al. (2020) identified common pathogenic / likely pathogenic variants, with IVS-I-5 (c.92+5G>C) being the most prevalent, followed by the 619-bp deletion, c.92+1G>T, c.27_28insG, c.47G>A, and c.126_129delCTTT [11]. They emphasized the need for a cost-effective, specific, and sensitive indigenous targeted assay to detect HBB gene mutations for thalassemia prevention.

In our cohort, five common mutations constituted 83.41% of total mutations in decreasing order: IVS 1-5 {G>C} (53.11%), codon 41-42 {–CTTT} (11.20%), codon 8/9 {+G} (8.7%), codon 26 {G>A} (5.3%), and codon 16 {-C} (5.1%). IVS 1:1 {G>T}, 619 bp deletion, and Hb S {A>T} were found in 3.7%, 2.9%, and 1.8% of cases, respectively. Other less common mutations accounted for 7.8%. While ARMS-PCR can detect the common mutations, the remaining 16.2% of mutations are significant and should not be missed. Therefore, direct HBB gene sequencing is a reliable and cost-effective approach [10].

Previous studies suggest that for developing countries, targeted panels for beta thalassemia mutations provide timely and significant phenotype information, are less expensive, and are often considered the gold standard for diagnosing hemoglobinopathies [9,10,12]. However, whole HBB gene sequencing is important due to the limitations of targeted panels in detecting rare mutations. Colah R et al. (2018) identified several rare mutations in India, such as -90 (C>T), -88 (C>T), codon 15 (-T), IVS1-129 (A>C), IVS1-130 (G>C), IVSII-1 (G>A), IVSII-837 (C>T), and IVSII-848 (C>A) by DNA sequencing 14. Choudhuri S et al. (2015) highlighted sequencing's role in identifying ~7% of rare mutations responsible for thalassemia [13]. Colah R et al. (2009) also emphasized whole HBB gene sequencing for uncharacterized samples [14]. A cost-benefit analysis is essential before establishing sequencing infrastructure, considering its high cost, although the cost of having a thalassaemic child is much higher. Alternatives like HPLC on fetal RBCs, non-invasive sequencing of fetal cell-free DNA, MALDI-TOF, and APEX have been explored in various studies, but high costs and limited validation on large populations are barriers to their adoption as diagnostic tests [15, 16, 17].

In our study, factors such as late arrival for testing, lack of consent for invasive testing, follow-up difficulties, counselling of spouses of carrier women, social stigma, and the COVID-19 pandemic in 2020 led to fewer couples undergoing invasive testing [18]. Couples identified with homozygous thalassemia mutations through invasive testing were counselled for informed reproductive decisions. Counselling and raising awareness about prenatal diagnostic tests are essential strategies for helping couples make informed reproductive choices. Mohanty et al. (2008) also highlight the importance of addressing social, cultural, and religious issues and promoting educational and awareness programs, particularly through mass media, to reach the population [19].

In India, few centres have trained obstetricians, sonologists for prenatal invasive procedures, and adequate sequencing infrastructure for prenatal thalassemia diagnosis. Although the government has considered an antenatal β-thalassemia carrier screening program, there is a pressing need to establish more centres offering confirmatory HBB gene sequencing. Expanding these services with support from international agencies, public-private partnerships, and NGOs will alleviate the burden of thalassemia and reduce referrals to distant centres.

Strength

This study contributes in providing better mutation spectrum of thalassemia in specific regional population. It also highlights how HBB gene sequencing not only surpasses ARMS-PCR in terms of coverage and precision but also contributes significantly to the advancement of prenatal thalassemia diagnostics and provides better understanding for counselling at-risk families, especially in prenatal settings.

Limitations

A small sample size in our study not only limits the generalization of its findings to the broader population or other geographical regions but it also limits the likelihood of identifying rare mutations. Being conducted in a tertiary care center, the study may miss the full mutational spectrum of cases seen in primary or secondary healthcare settings which can disproportionately reflect the demographics or socioeconomic backgrounds of patients attending the tertiary care center. Addressing these limitations such as by increasing sample size or incorporating multicenter data, more studies should be conducted in future to enhance the study's impact and applicability.

CONCLUSION

Despite small size of our study, it significantly contributes in providing mutation spectrum of thalassemia in specific regional population. Though the panel target approach being a cost effective and gold standard method to reduce the burden of thalassemia, but, due to the limitation of missing out a considerable number of less common and rare mutations, our study highlights the need of government support for whole HBB gene sequencing in future. Public awareness and agreeing in getting confirmatory tests can significantly impact thalassemia public indicators outcomes.

Declaration by Authors

Ethical Approval: Approved

Acknowledgement: Gratitude to the Division of Genetics, Lok Nayak Hospital, New Delhi & patient's family.

Source of Funding: None

Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

1. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ. 2008; 86:480-7.
2. Mahdavi MR, Karami H, Akbari MT, Jalali H, Roshan P. Detection of rare beta globin gene mutation [+22 5UTR(G>A)] in an infant, despite prenatal screening. Case Rep Hematol. 2013; 2013:906292.
3. Hamamy HA, Al-Allawi NA. Epidemiological profile of common haemoglobinopathies in Arab countries. J Community Genet. 2013; 4:147-67.
4. Cao A, Kan YW. The prevention of thalassemia. Cold Spring Harb Perspect Med. 2013;3: a011775.
5. Madan N, Sharma S, Sood SK, Colah R, Bhatia HM. Frequency of β thalassemia trait and other hemoglobinopathies in northern and western India. Indian J Hum Genet. 2010; 16:16-25.
6. Verma IC, Saxena R, Kohli S. Past, present and future scenario of thalassemic care and control in India. Indian J Med Res. 2011; 134:507-21.
7. Colah R, Italia K, Gorakshakar A. Burden of thalassemia in India: The road map for control. Pediatr Hematol Oncol J. 2017; 2:79-84.
8. Mishra KK, Patel P, Bhukhanvala DS, Shah A, Ghosh K. A multiplex ARMS PCR

approach to detection of common β-globin gene mutations. Anal Biochem. 2017; 537:93-8.
9. Inusha P, Marwaha R. Mutational spectrum of thalassemias in India. Indian J Hum Genet. 2007;13(1):36-7.
10. Ghosh S, Chakrabarti S, Bhattacharyya M. Prenatal Screening and Diagnosis of ß-Thalassemia in India: Is ARMS-PCR Enough? Indian J Hematol Blood Transfus. 2021;37(3):448-52.
11. Kelkar K, Ramanan V, Anand S, Majethia P, Ranade S, Patil K, et al. HBB gene mutation spectrum in an Indian cohort of 1530 cases using an in-house targeted next-generation sequencing assay. Hemoglobin. 2020; 13:239-48.
12. Sinha S, Black ML, Agarwal S, Colah R, Das R, Ryan K, et al. Profiling β-thalassaemia mutations in India at state and regional levels: implications for genetic education, screening and counselling programmes. Hugo J. 2009;3(1):51-62.
13. Choudhuri S, Bhattacharyya M, Sen A, Bhattacharyya D, Ray SS. Importance of additional mutation analysis in chorionic villous sample for prenatal diagnosis of thalassemia. Blood. 2015;126(23):4572.
14. Colah R, Gorakshakar A, Nadkarni A, Phanasgaonkar S, Surve R, Sawant P, et al. Regional heterogeneity of β-thalassemia mutations in the multi ethnic Indian population. Blood Cells Mol Dis. 2009; 42:241-6.
15. Colah R, Nadkarni A, Gorakshakar A, Sawant P, Italia K, Upadhye D, et al. Prenatal diagnosis of HbE-β-thalassemia: experience of a center in Western India. Indian J Hematol Blood Transfus. 2018;34(3):474-9.
16. Rosatelli MC, Saba L. Prenatal diagnosis of beta-thalassemias and hemoglobinopathies. Mediterr. 2009;1(1): e2009011.
17. Li Y, Di Naro E, Vitucci A, et al. Size fractionation of cell-free DNA in maternal plasma improves the detection of a paternally inherited beta-thalassemia point mutation by MALDI-TOF mass spectrometry. Fetal Diagn Ther. 2009; 25:246-9.
18. Colah R, Gorakshakar A. Control of thalassemia in India. Thalass Rep. 2014; 4:84-9.
19. Mohanty D, Colah R, Gorakshakar A, editors. Report of the Jai Vigyan S & T Mission Project on community control of thalassaemia syndromes - awareness, screening, genetic counselling and prevention. A national multicentric task force study of Indian Council of Medical Research. New Delhi: ICMR; 2008.

How to cite this article: Komal Uppal, Jyotsna Gaur, Sunil Kumar Polipalli, Somesh Kumar, Sangeeta Gupta, Seema Kapoor. Mapping the mutation spectrum in prenatal thalassemia: insights from a tertiary care center in Delhi. *Int J Health Sci Res.* 2025; 15(3):157-162. DOI: *https://doi.org/10.52403/ijhsr.20250323*

******