International Journal of Community Medicine and Public Health
Sorathiya VP et al. Int J Community Med Public Health. 2020 May;7(5):1977-1981
http://www.ijcmph.com
pISSN 2394-6032 | eISSN 2394-6040

DOI: http://dx.doi.org/10.18203/2394-6040.ijcmph20202017

Original Research Article

Molecular analysis and prenatal diagnosis of β thalassemias in the Saurashtra region of Gujarat

Varsha P. Sorathiya[1]*, Roshan B. Colah[2], Nishith A. Vachhani[1], Sanjeev L. Nandani[1], Daya J. Vekariya[1], Heena N. Kashiyani[1]

[1]Department of Thalassemia, Indian Medical Scientific Research Foundation, Rajkot, Gujarat, India
[2]Department of Haematogenetics, National Institute of Immunohematology, KEM Hospital Campus, Parel, Mumbai, Maharashtra, India

Received: 19 March 2020
Revised: 15 April 2020
Accepted: 16 April 2020

***Correspondence:**
Dr. Varsha P. Sorathiya,
E-mail: lbc.bto@999life.org

ABSTRACT

Background: Hemoglobinopathies pose a significant health burden in the Saurashtra region in western India. Identifying couples at- risk of having a child with a severe hemoglobin disorder prenatally can help in counseling with the option of prenatal diagnosis.
Methods: All pregnant carriers of β thalassemia were advised to screen their husbands. If both were carriers, they were counselled to undergo prenatal diagnosis. Prenatal diagnosis was done in 174 couples. Chorionic villus sampling was done at 10 to 12 weeks gestation and the tissue sample was sent to the Genetic laboratory for DNA analysis along with blood samples of the parents. If the couple came after 14 weeks of pregnancy, amniocentesis was done and amniotic fluid was sent for DNA analysis. If the fetus was affected, the option of termination of the pregnancy was given.
Results: In 50.5% of couples, the fetus was a carrier of β thalassemia, in 1.7% the fetus had hemoglobin E trait and in 23.0% the fetus was normal. In 20.6% of couples, the fetus had β Thalassaemia major and after counseling, these couples opted for termination. 1.2% of couples had a fetus which was unaffected but remained in distinguished (normal/thalassemia carrier) while in 0.6% of cases the fetus had sickle-β thalassemia. In 1.2% of cases the result was inconclusive. In 1.2% of cases the results were not available for lack of follow up.
Conclusions: Screening antenatal women for identifying carriers and referring couples at-risk for prenatal diagnosis helped in preventing the birth of 36 thalassemia major children.

Keywords: β thalassaemia, Chorionic villus sampling, Amniocentesis, Prenatal diagnosis, Mutation analysis

INTRODUCTION

Hemoglobin disorders present a significant health problem in 71 % of 229 countries, and these 71 % of countries include 89% of all births worldwide.[1] β Thalassemia is one of the commonest inherited haemolytic anaemias seen in India and poses a significant health burden.[2] The other abnormal hemoglobins common in India are hemoglobin S (HbS) and hemoglobin E (HbE).[3] Co-inheritance of these hemoglobin variants with β thalassemia also results in a severe disorder. It is estimated that 10,000 to 12,000 babies with a severe form of thalassemia are born in India each year.[3] Screening, genetic counselling and prenatal diagnosis is the only way to reduce this burden. Few common mutations account for >95% of severe β thalassemias.[4] Around sixty-eight mutations have been characterized.[5-7] But this number keeps on increasing as

more studies are reported.[8] We run a screening program for detection of thalassaemia carriers. Antenatal women are also screened as well as husbands of carrier women. If both are thalassaemia carriers, the couple is counselled and referred for prenatal diagnosis. This paper describes our experience with prenatal diagnosis in the Saurashtra region of Gujarat in western India.

METHODS

Study design

We referred 174 couples for prenatal diagnosis. Among them, 133 pregnant women were sent in the first trimester and 41 were in the second trimester. 2 couples at-risk did not come for prenatal diagnosis after counselling. The study was approved by the Institutional Review Board. Chorionic villus sampling was done between 10-12 weeks gestation by an experienced gynaecologist under ultrasound guidance after an informed consent from the couple and the tissue sample, along with the parents' blood samples collected in EDTA were sent to the Genetic laboratory for DNA analysis. If the fetal DNA report showed that the baby would have thalassaemia major, the option of termination of the pregnancy was given. If the DNA report showed that the baby was thalassaemia minor or normal, the couples were advised to continue the pregnancies. If the woman came for screening after 14 weeks of pregnancy, amniocentesis was done and amniotic fluid was sent for molecular analysis. Known β gene mutations were analyzed by Reverse dot blot hybridization, Amplification refractory mutation system (ARMS) or PCR- Restriction enzyme analysis.[9,10] In cases with unknown mutations, direct sequencing of amplified DNA was done.

Study place and period

The study was undertaken by Department of Thalassemia, Indian Medical Scientific Research Foundation, Life Blood Centre, Rajkot, Gujarat during the period from February to December 2019.

Selection criteria

Only couples at - risk of having a child with a severe hemoglobin disorder were included in the study. Couples referred in the late second and third trimesters of pregnancy were excluded and did not undergo prenatal testing.

Statistical analysis

No statistical analysis was done as this was not necessary for this paper, hence this is not given.

RESULTS

Out of 174 couples who underwent prenatal diagnosis, 88 (50.5%) had fetuses with β thalassaemia trait, 40 (23%) had normal fetuses, and 36 (20.6%) had a fetus with a β thalassaemia major child. The latter group of couples were counselled and given the advice/option to terminate the pregnancy. The report of 2 (1.2%) couples were inconclusive, in 2 (1.2%) the fetus was unaffected but could not be conclusively identified as normal or thalassemia trait. 3 (1.7%) fetuses had Hb E trait while 1(0.6%) had sickle - β thalassemia. In 2 couples (1.2%) the results were not available (Table 1).

Table 1: Diagnosis of the fetuses.

Diagnosis	No. of fetuses	Percentage (%)
β thalassaemia trait	88	50.5
Normal	40	23
β thalassaemia major	36	20.6
Normal/thalassaemia trait	2	1.2
Hb E trait	3	1.7
Sickle-β thalassaemia	1	0.6
Inconclusive result (thalassaemia trait/ thalassaemia major)	2	1.2
Not available	2	1.2
Total	174	100.00

Table 2: Distribution of mutations in the parents.

S. no.	Mutations	Number (%)
1	IVS 1-5 (G→C)	167 (48.0)
2	619 bp deletion	43 (12.4)
3	Codon 41-42 (-TCTT)	28 (8.0)
4	Codon 15 (G→A)	22 (6.3)
5	IVS 1-1 (G→T)	20 (5.8)
6	Codons 8/9 (+G)	16 (4.6)
7	Codon 5 (-CT)	15 (4.3)
8	Codon 16 (-C)	6 (1.7)
9	IVS 1-1 (G→A)	6 (1.7)
10	Codon 30 (G→C)	5 (1.4)
11	CAP+1 (A→C)	2 (0.6)
12	IVS 1-130 (G→A)	2 (0.6)
13	Other mutations	11 (3.2)
14	Unidentified mutations	5 (1.4)
15	Total	348 (100)

The five common mutations identified were IVS 1-5(G→C) (48.0%), 619 bp deletion (12.4%), Codons 41-42 (-TCTT) (8.0%), Codon 15 (G→A) (6.3%) and IVS 1-1(G→T) (5.8%). The prevalence of 7 other mutations varied from 0.6% to 4.6% (Table 2).

We also studied the caste or community wise prevalence and distribution of mutations. Of the 174 couples, 74 were Patels and 56 were Lohanas comprising two major groups. 26 were Vankar, 24 were Muslim, 22 were Brahmin, 20 were Sindhi, 16 were Darbar, 14 were Kumbhar and 11 were Vania. Few couples belonged to

other castes like Chamar, Sagar, Harijan, Rajput, Devipujak, Aahir, Bavaji, Bharvad, Jain, Koli, Vanand, Suthar, Luhar, Sathvara, Kadia, Charan, Ghanchi, Mer, Nayak, Prajapati, Soni and Sumra. As the number of individuals of these castes were very few, they all are included in a group 'Others'. Our study revealed that IVS 1-5(G→C) and 619 bp deletion were the most common mutations. Among the caste/community wise mutation analysis IVS 1-5(G→C) was the most common mutation seen in Patel, Vankar, Muslim, Brahmin, Vania and other castes, whereas the 619 bp deletion was the most common mutation in Lohana and Sindhi castes/communities. Codons 8/9 (+G) mutation was most common in Darbar and Codon 5(-CT) in Kumbhar (Table 3).

Table 3: Caste wise distribution of the mutations in the parents.

Castes mutations	Patel	Lohana	Vankar	Muslim	Brahmin	Sindhi	Darbar	Kumbhar	Vania	Others
IVS 1-5(G→C)	59	7	24	8	9	3	2	2	8	45
619 bp deletion		25		4		10		1		4
Codon 41-42 (-TCTT)		2	1	3	8		5		1	8
Codon 15 (G→A)	12	1	1		1		1	4		2
IVS 1-1(G→T)		16				3				2
Codon 8/9 (+G)		4				3	7	1		1
Codon 5 (-CT)	1	1		2	2			5		4
IVS 1-1(G→A)	1						1		1	3
Codon 16 (-C)					2					4
Unidentified mutations	1					1			1	2
Codon 30 (G→C)				1				1		3
IVS 1-130 (G→A)				2						0
CAP + 1 (A→C)										2
Codon 26(G→A)-HbE				3						0
IVS II-613(C>T)				1						0
-88 (C>T)										1
Asain Indian INV/DEL Gythal										1
Condon 6 (A>T) HbS										1
IVS1- 5 (G→A)										1
IVS1-1 (G→C)										1
Total	74	56	26	24	22	20	16	14	11	85

In 100 couples (57.5%), both husband and wife had the same mutation, while in the remaining 74 couples (42.5%) both parents had different mutations. However, in both groups, IVS 1-5(G→C) was the commonest molecular defect seen.

Among the 36 fetuses who were affected with thalassaemia major, the most accountable mutation was also IVS 1-5 (G→C) in 15 (41.7%) of them.

DISCUSSION

The Indian population comprises of numerous castes and communities, each revealing different genetic traits.[11] The highest frequency of β thalassaemia trait is reported in Gujarat (10-15%), followed by Sindh (10%), Punjab (6.5%), Tamil Nadu (8.4%) and Maharashtra.[12,13]

Our present study showed that antenatal carrier screening for β thalassemia followed by counselling and prenatal diagnosis was acceptable in the Saurashtra region and helped to prevent the birth of thalassemia major children, thus reducing the burden of the disease in the country as well as psychological and financial burden to the family and to society. 76.4% of pregnant women could be screened early in pregnancy and could get their husbands screened soon after and opt for prenatal diagnosis in the first trimester of pregnancy. Early prenatal diagnosis of thalassaemia has great relevance as it is safer both for the mother and the unborn baby in a high prevalence area like Gujarat.

The five common mutations - IVS 1-5(G→C), 619 bp deletion, Codons 41-42 (-TCTT), Codon 8/9(+G) and IVS 1 -1 (G→T) account for over 90% of the mutations in β - thalassaemia patients.[14,15]

IVS I-5 (G→C) substitution which is a severe β^+ thalassemia mutation was found to be the most frequent mutation in the Saurashtra region in our study with a prevalence of 48%. This is in agreement with previous studies, reported from India and the Indian sub-continent region.[16] The incidence of this mutation reported from various regions from the Indian sub-continent is 12% from Sindh region, 38% from Punjab, 41% from Gujarat,

60% from West Bengal and 81% from Tamilnadu.[17] The 619 bp deletion was the second most common mutation found in our study. This mutation was seen in Asian-Indians originally coming from Gujarat and Punjab.[18]

Table 4: Prenatal diagnosis programmes in different regions in India.

S. no.	Study	City/State	Total no of pregnancies tested
1	Colah et al[20]	Mumbai/Maharashtra	131
2	Saxena et al[10]	New Delhi	415
3	Thakur et al[21]	Mumbai/Maharashtra	787
4	Gajra et al[22]	Kolkata/West Bengal	26
5	Agarwal et al[23]	Lucknow/Uttar Pradesh	53
6	Verma et al[24]	New Delhi	1033
7	Garewal et al[25]	Chandigarh	112
8	Colah et al[26]	Mumbai/Maharashtra	15
9	Tamhankar et al[27]	Lucknow/Uttar Pradesh	133
10	Colah et al[5]	Mumbai/Maharashtra	2221
11	Bhukhanvala et al[28]	Surat/Gujarat	11
12	Patel et al[29]	Ahmedabad/Gujarat	282
13	Gupta et al [30]	Jodhpur/Rajasthan	17
14	Mahey et al[31]	New Delhi	636
15	Bandyopadhyay et al[32]	Kolkata/West Bengal	235
16	Present study 2019	Rajkot/Gujarat	174

Knowledge of prevalent mutations in different castes helps in developing cost effective approaches for prenatal molecular diagnosis. Overall, implementation of this knowledge has helped to successfully establish a programme for genetic counselling and prenatal diagnosis of beta-thalassemia in this highly prevalent area of Saurashtra region.[19]

There have been very few prenatal diagnosis programmes in different regions for a large country like India. These are summarized in (Table 4). It is difficult for couples to travel long distances and hence many more facilities are required to provide these services. Trained gynaecologists are needed for chorionic villus sampling in the region and samples can then be sent to genetic laboratories for molecular diagnosis even if this is not locally available. Ours is an example of one such programme which has been successful in Saurashtra where the burden of thalassemia is high.

CONCLUSION

Screening antenatal women for identifying carriers and referring couples at-risk for prenatal diagnosis helped in preventing the birth of 36 thalassemia major children.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bulletin of the World Health Organization. 2008;86:480-7.
2. Sukumaran PK. Abnormal hemoglobins in India. In 'Trends in Hematology'. Eds. Sen, N. N. and Basu, A. K. J. B. Chatterjee Memorial Committee, Calcutta; 1975:247-56.
3. Colah R, Italia K, Gorakshakar A. Burden of thalassemia in India: The road map for control. Pediatric Hematol Oncol J. 2017;2:79-84.
4. Inusha P, Marwaha R. Mutational spectrum of thalassemias in India. Indian J Hum Genet. 2007;13(1):36-7.
5. Colah R, Gorakshakar A, Nadkarni A. Invasive and non-invasive approaches for prenatal diagnosis of haemoglobinopathies: experiences from India. Indian J Med Res. 2011;134:552-60.
6. Gorakshakar A, Das M, Phanasgaokar S, Nadkarni A, Colah R, Mohanty D. Origin of the codon 47 (+ A) β-thalassaemia mutation among the Nicobarese of the Andaman and Nicobar Islands in India. British J Haematology. 2007;139(2):345-6.
7. Edison E, Venkatesan R, Govindanattar S, George B, Shaji R. A novel 26 bp deletion [HBB: c. 20_45del26bp] in exon 1 of the β-globin gene causing β-thalassemia major. Hemoglobin. 2012;36(1):98-102.
8. Colah R, Gorakshakar A. Control of thalassemia in India: Thalassemia Reports. 2014;4:1955.
9. Colah R, Gorakshakar A, Nadkarni A, Phanasgaonkar S, Surve R, Sawant P, et al. Regional heterogeneity of beta-thalassemia mutations in the multi ethnic Indian population. Blood Cells Mol Dis. 2009;42(3):241-6.
10. Saxena R, Jain P, Thomas E, Verma I. Prenatal diagnosis of beta-thalassaemia: experience in a developing country. Prenat Diagn. 1998;18(1):1-7.

11. Verma P, Ghildiyal A, Verma D, Krishna A, Singh S, Kumar A, et al. Prevalence of hemoglobinopathies in different regions and castes of Uttar Pradesh, India-A hospital-based study. Asian J Med Sci. 2015;6(5):21-5.
12. Patel J, Patel A, Patel J, Kaur A and Patel V. Prevalence of Haemoglobinopathies in Gujarat, India: A Cross-Sectional Study. Int J Hematol. 2009;5:1.
13. Patel J, Shrivastav A, Joshi J, Agnihotri A, Kaur A, Thakkar B. Detection of hemoglobinopathies and thalasemias in population of Gujarat State using HPLC: Analysis of 2022 cases. Pathol Lab Med. 2012;4(2):80-4.
14. Thein S, Hesketh C, Wallace R, Weatherall D. The molecular basis of thalassaemia major and thalassaemia intermedia in Asian Indians: Application to prenatal diagnosis. Br J Haematol. 1988;70:225-31.
15. Varawalla N, Old J, Sarkar R, Venkatesan R, Weatherall D. The spectrum of beta-thalassaemia mutations on the Indian subcontinent: The basis for prenatal diagnosis. Br J Haematol. 1991;78:242-7.
16. Agarwal S, Hattori Y, Agarwal SS. Rare beta thalassemia mutation in Asian Indians. Am J Hematol. 2000;65(4):322-3.
17. Satpute S, Bankar M, Momin A. The prevalence of β-thalassemia mutations in south western Maharashtra. Indian J Clin Biochem. 2012;27(4):389-93.
18. Nigam N, Munshi N, Patel M, Soni A. Distribution of betathalassemia mutation and its correlation with alpha thalassemia inGujarati families. Int J Hum Genet. 2003;3(4):221-4.
19. Verma I, Saxena R, Thomas E, Jain P. Regional distribution of beta-thalassemia mutations in India. Hum genet. 1997;100(1):109-13.
20. Colah R, Dastur A, Aras A, Pawar A, Mudera V, Gorakshakar A. Prenatal diagnosis of Thallassemia in India: The first experience. J Obstetr Gynaecol India. 1991;4(6):733-8.
21. Thakur C, Vaz F, Banerjee M, Kapadia C, Natrajan P, Yagnik H, et al. Prenatal diagnosis of β-thalassaemia and other haemoglobinopathies in India. Prenatal Diagnosis. Int Society Prenatal Diagnosis. 2000;20(3):194-201.
22. Gajra B, Chakraborti S, Sengupta B. Prenatal diagnosis of thalassaemias. Int J Human Genetics. 2002;2(3):173-8.
23. Agarwal S, Gupta A, Gupta U, Sarwai S, Phadke S, Agarwal SS. Prenatal diagnosis in beta-thalassemia: an Indian experience. Fetal Diagn Therapy. 2003;18(5):328-32.
24. Verma I, Saxena R, Lall M, Bijarnia S, Sharma R. Genetic counselling and prenatal diagnosis in India-experience at Sir Ganga Ram Hospital. Indian J Pediatr. 2003;70(4):293-7.
25. Garewal G, Das R, Jaur J, Marwaha R, Gupta I. Establishment of prenatal diagnosis for b thalassemia: a step towards its control in a developing country. Ann Hum Biol. 2005;32(2):138-44.
26. Colah R, Surve R, Wadia M, Solanki P, Mayekar P, Thomas M, et al. Carrier screening for β-thalassemia during pregnancy in India: A 7 years evaluation. Genet Test. 2008;12:181-5.
27. Tamhankar P, Agarwal S, Arya V, Kumar R, Gupta U, Agarwal SS. Prevention of homozygous beta thalassemia by premarital screening and prenatal diagnosis in India. Prenal Diagn. 2009;29(1):83-8.
28. Bhukhanvala D, Sorathiya S, Sawant P, Colah R, Ghosh K, Gupte S. Antenatal screening for identification of couples for prenatal diagnosis for severe hemoglobinopathies in Surat, south Gujarat. J Obstet Gynaecol India. 2013;63(2):123-7.
29. Patel A, Patel R, Patel S, Vaniawala S, Patel D, Shrivastava N, et al. βthalassemia mutations in western India: outcome of prenatal diagnosis in a hemoglobinopathies project. Hemoglobin. 2014;38(5):329-34.
30. Gupta V, Sharma P, Jora R, Amandeep M, Kumar A. Screening for thalassemia carrier status in pregnancy and pre-natal diagnosis. Indian Pediatr. 2015;52:808-9.
31. Mahay BS, Arora V, Puri R, Lall M, Saxena R, Verma J, et al. The changing scenario in prenatal diagnosis of genetic disorders: Genetics to genomics. Curr Med Res Pract. 2018;8:203-8.
32. Bandyopadhyay R, Bandyopadhyay U, Basu K. Prenatal Diagnosis of Thalassaemia by Chorionic Villus Sampling: A Clinico-Hematological Study in a Government Medical College of Eastern India. IOSR J Dental Med Sci. 2019;18(8):15-20.

Cite this article as: Sorathiya VP, Colah RB, Vachhani NA, Nandani SL, Vekariya DJ, Kashiyani HN. Molecular analysis and prenatal diagnosis of β thalassemias in the Saurashtra region of Gujarat. Int J Community Med Public Health 2020;7:1977-81.