International Journal of Reproduction, Contraception, Obstetrics and Gynecology
Gupta A et al. Int J Reprod Contracept Obstet Gynecol. 2025 Jun;14(6):1851-1855
www.ijrcog.org
pISSN 2320-1770 | eISSN 2320-1789

DOI: https://dx.doi.org/10.18203/2320-1770.ijrcog20251573

Original Research Article

Prenatal invasive diagnostic testing for hemoglobinopathies: a retrospective cohort study at a tertiary care public hospital in central India

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Received: 01 April 2025
Revised: 04 May 2025
Accepted: 05 May 2025

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ABSTRACT

Background: Transfusion-dependent hemoglobinopathies such as sickle cell disease, thalassemia, etc. have a huge impact on the Indian economy due to the need for lifelong care and treatment of associated morbidities. Primary prevention strategies focus on awareness generation and pre-marital and pre-conceptional counseling to prevent the conception of a child with a homozygous genotype. Aim was to study the prevalence of carrier status of hemoglobinopathies, the couples at risk of carrying a fetus with major hemoglobinopathies, and the prevalence of fetal affection with major hemoglobinopathies in prenatal invasive testing.
Methods: It was a retrospective cohort study conducted at AIIMS, Nagpur. Study conducted from 24 months (June 2021-June 2023). All antenatal women screened for hemoglobinopathies with HPLC and diagnosed to have hemoglobinopathies.
Results: The 5,432 antenatal women were screened for hemoglobinopathy using high-performance liquid chromatography (HPLC). Out of these, 214 women were carriers of hemoglobinopathies. 53 couples were found to be at risk of carrying a fetus with major hemoglobinopathy. The 52 women underwent prenatal diagnosis (PND), 11 (21.1%) were found to be affected with major hemoglobinopathies and underwent medical termination of pregnancy (MTP). 24 babies were found to be carriers while 17 had no mutation.
Conclusions: Owing to the high prevalence of hemoglobinopathies and the continually increasing impact on global health each year, the benefit of carrier screening programs to control incidence of new cases is recognized worldwide.

Keywords: Hemoglobinopathy, Antenatal screening, Prenatal invasive testing for hemoglobinopathy, Sickle cell disease, Thalassemia

INTRODUCTION

Transfusion-dependent hemoglobinopathies such as sickle cell disease, Thalassemia, etc. have a huge impact on the Indian economy due to the need for lifelong care and treatment of associated morbidities.[1] It also adds to social and psychological problems and poor quality of life.[2] The hemoglobinopathies are characterized by abnormal synthesis of the globin chains either due to reduced synthesis of the affected globin chains like thalassemia syndromes or structural globin variants such as sickle-cell syndromes. Beta Thalassemia is a spectrum of a hereditary disease caused by the genetic deficiency in the synthesis of beta-globin chains of hemoglobin causing severe anemia in thalassemia requiring frequent blood transfusions and iron chelation therapy. Similarly, sickle cell disease is a result of genetically mutated hemoglobin, causing a vaso-occlusive crisis in multiple organs and requiring blood transfusion therapy to reverse disease process. Both these hemoglobinopathies are responsible

for poor quality of life because of the frequent requirement of blood transfusion and the complications related to both, disease and blood transfusions. Being autosomal recessive disorders, the risk in couples of having children with a hemoglobinopathy is 25% and affects 2.7 per 1000 conceptions worldwide.[3] The situation in India is worst where the prevalence of significant hemoglobinopathies is 1.2/1000 live births, with an annual birth of 32,400 babies with a hemoglobin disorder. India constitutes 10% of the total thalassemia burden in the world with about 10,000 children being born with thalassemia major each year.[4,5] The overall prevalence of beta-thalassemia trait has been reported to be 2.78% and varies from 1.48% to 3.64% in different states of the country.[6]

Mortality and morbidity associated with these hemoglobinopathies are liable for families' mental and physical distress, increasing a huge burden on health sector facilities and therefore country's economy. We can reduce the incidence of these hemoglobinopathies by carrier screening in whole population in areas where prevalence is high/at least testing the antenatal women. Once couples at risk of inheriting hemoglobinopathies are identified, still we can avoid the birth of an affected fetus by invasive PND like amniocentesis and chorionic villi sampling.

The MoHFW tribal health expert committee report has listed sickle cell disease as one of the 10 special problems in tribal health that affect tribal people disproportionately, thus making this an important intervention. The MoHFW has kept a goal to eliminate sickle cell disease as a public health problem in India before 2047. Primary prevention strategies focus on awareness generation and pre-marital and pre-conceptional counseling to prevent the conception of a child with a homozygous genotype. Prevention requires setting up genetic counseling and testing interventions in high-prevalence districts to prevent sickle cell disease in the offspring.

The present study aims to study the prevalence of carrier status of hemoglobinopathies, couples at risk of carrying fetuses with major hemoglobinopathies and the prevalence of fetal affection with major hemoglobinopathies in prenatal invasive testing.

METHODS

Study design

It was a retrospective cohort study conducted at AIIMS, Nagpur.

Study duration

Study conducted from 24 months (June 2021-June 2023).

Inclusion criteria

All antenatal women screened for hemoglobinopathies with HPLC and diagnosed to have hemoglobinopathies.

Sample size

Keeping 95% confidence, 5% error, and 8.1% prevalence of sickle cell disease in prenatal testing, the sample size was calculated as 115.[7] 115 women who were screened for hemoglobinopathies will be needed for the study.

The study was conducted after approval from the Institute Ethics Committee. Consent of participation in the study was waived off as it is a retrospective observational study, however, throughout the study period, confidentiality of the patient's identity was maintained by proper de-identification of data. According to our hospital protocol, we routinely screen all antenatal women for hemoglobinopathies with an HPLC test, if not done previously. If the woman is found to be either affected or a carrier of a hemoglobinopathy, the partner's testing is also done using HPLC, if the partner is found to be the carrier of the hemoglobinopathy, prenatal testing is offered in the form of either chorionic villus sampling or amniocentesis depending on gestational age after genetic counselling in collaboration with a clinical haematologist. The sample is then sent for mutation analysis to detect major hemoglobinopathy in the fetus. In case, the fetus is found to be affected by major hemoglobinopathy, MTP is offered, otherwise patient receives routine antenatal care for the rest of her pregnancy. Relevant data was collected in a predesigned proforma from the patient's case records which included: Age and order of pregnancy.

Clinical details

Time of detection of hemoglobinopathy status, type of hemoglobinopathy in self and in partner, previous pregnancy testing was done or not, any previous MTP for affected foetus, period of gestation of testing, type of test done, any complication of testing, the result of mutation analysis, a post-test decision for MTP taken or not. The data was analysed with the help of SPSS statistical tool.

RESULTS

A total of 5,432 antenatal women were screened for hemoglobinopathy using HPLC as primary test from June 2021-June 2023 (Table 1).

Table 1: Demographic details.

Demographic details	Values
Mean age (in years)	29.3
Parity	
Primigravida	48.5%
Multigravida	51.5%
Urban	57.2%
Rural	42.8%
Educated	63.1%
Uneducated	36.9%

Out of 5432, 214 women were found to be carriers of hemoglobinopathies. Four women were excluded as they

were carriers of minor hemoglobinopathy namely, Singapore haemoglobin, Meerut J haemoglobin, and persistent HbF. Ten women with sickle cell disease were referred from peripheral hospitals for PND. The partners of all these 220 women were tested with HPLC and the results of the reports are given in Table 2.

Table 2: Hemoglobinopathy status of antenatal women and their partners.

Variables	Sickle cell trait	Beta thalassemia trait	HbD trait	HbE trait	Sickle cell disease
Normal	143	13	1	2	7
Sickle cell trait	39	2	1	0	3
Beta thalassemia trait	4	3	0	0	0
HbD trait	0	0	0	0	0
HbE trait	0	1	0	0	0
Delta-beta heterogenous status	0	1	0	0	0
Total	186	20	2	2	10

The 53 couples were found to be at risk of carrying fetus with major hemoglobinopathy. After genetic counseling, all these couples underwent prenatal diagnostic testing except one couple who presented at 26 weeks where the wife was beta thalassemia trait and the husband had heterozygous delta-beta thalassemia which was never detected previously as he did not have transfusion-dependent anaemia. So, total 52 prenatal diagnostic procedures including amniocentesis and chorionic villus sampling were performed over one year.

Out of a total of 52 procedures carried out for prenatal invasive testing, 16 (30.7%) were CVS done at 11-12 weeks and 36 (69.23%) were amniocentesis done after 15 weeks (Figure 1).

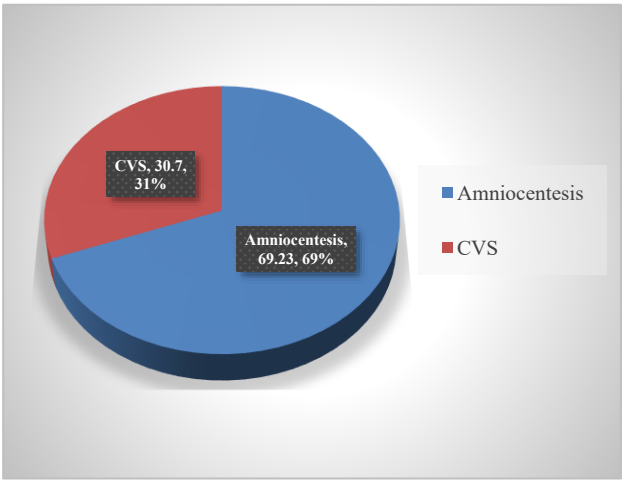

Figure 1: Types of prenatal procedures performed.

The 21 (40.38%) couples presented in their first pregnancy for PND and the remaining 31 couples presented for prenatal diagnosis for subsequent pregnancies. Out of these 31 couples, 9 had done PND in their previous pregnancies. Surprisingly, during our prenatal counselling session, we got the HPLC of previous offspring for the couple who missed PND in previous pregnancy and 3 were found to be affected with major hemoglobinopathy.

Fetal genomic DNA was extracted from the chorionic villus after dissection and removal of maternal tissue under a microscope, or from amniotic fluid containing fetal cells.

For the detection of b-thalassemia mutations, genomic DNA was isolated using a standard protocol and the common five β-thalassemia mutations [IVS1-5(G-C), IVS 1-1 (G-T), 619 bp deletion (619 bpd), CD 8-9 (+G) and CD 41-42 (-TTCT)] and other hemoglobinopathies, HbS (CD 6 A-T), HbD (CD 121 G-C) and HbE (CD 26 G-A) was detected using ARMS-PCR. Unidentified mutations in the above panel are detected using automated DNA Sanger sequencing.

Out of 52 reports of PND, 11 (21.1%) were found to be affected with major hemoglobinopathies and underwent MTP. 24 babies were found to be carriers while 17 had no mutation. Out of the 11 affected foetuses with major hemoglobinopathies, 9 were sickle cell disease and 2 were beta thalassemia. Sickle cell disease results from a single base A>T mutation in the triplet encoding the sixth residue of the β-globin chain, leading to a substitution of valine for glutamic acid and the abnormal hemoglobin S (HbS). The molecular result for b-thalassemia showed the IVS 1-5 G →C and IVS 1 -1 G→T mutation.

There was no complication of the amniocentesis procedure while 1 missed abortion occurred 10 days after CVS.

DISCUSSION

The prevalence of hemoglobinopathies ranges from 12.5% in North India to around 25% in West Bengal to 35% in some parts of Central India.[8-11] HPLC is a rapid, accurate, and reproducible tool for the early detection and management of hemoglobinopathies and variants. This is especially important given the high incidence of beta thalassemia trait and sickle cell disease in the Indian subcontinent. Thus, antenatal screening and prenatal testing should be mandatory to prevent the birth of offspring with major hemoglobinopathies. Moreover, knowledge of common Hb patterns in a particular region helps to formulate appropriate preventive and therapeutic

strategies. Sickle cell disease is the commonest hemoglobin disorder followed by serious forms of thalassemia syndromes.

It results from a single base A>T mutation in the triplet encoding the sixth residue of the β-globin chain, leading to a substitution of valine for glutamic acid and the abnormal HbS. The primary pathophysiology is based on the polymerization of deoxy-HbS with the formation of long fibers within the RBCs causing a distorted sickle shape which eventually leads to increased hemolysis and vaso-occlusion of sickle red cells. In Maharashtra, the sickle gene is widespread in all the eastern districts, also known as the Vidarbha region, in the Satpura ranges in the north, and some parts of Marathawada.[12] The prevalence of sickle cell carriers in different tribes varies from 0-40%.[13] Tribal groups with a high prevalence of HbS (20-35%) include the Bhils, Madias, Pawaras, Pardhans and Otkars.[14] It has also been estimated that Gadchiroli, Chandrapur, Nagpur, Bhandara, Yavatmal and Nandurbar districts would have more than 5000 cases of sickle cell anemia.[12] Thus, prenatal diagnosis remains an important option for couples at risk of having a child with homozygous sickle cell anemia, sickle-β-thalassemia or HbSD disease even though it is impossible to predict the severity of the disease and many individuals may have a milder clinical presentation. With increasing awareness in the community, more couples are opting for prenatal diagnosis. Screening in antenatal clinics is the best way to identify couples at immediate risk of having an affected child. However, experiences in India have shown that only 15-20 percent of pregnant women come to antenatal clinics in public hospitals in the first trimester of pregnancy when prenatal diagnosis should ideally be done. This emphasizes the need for generating awareness in the population for early registration in antenatal clinics as well as among obstetricians to ask for screening for thalassemia and other hemoglobinopathies along with other investigations that are done routinely.

In a study done by Ghosh et al total of 657 CVS samplings were done out of which 163 (24.8%) fetuses were affected (homozygous or compound heterozygous for ß-thalassemia), 166 (25.2%) fetuses were ß-thalassemia/hemoglobinopathy carriers and 328 were unaffected (49.9%).[15]

Couples have more time to know their risk factors, they can receive counseling about genetic risks, discuss their reproductive options like preimplantation genetic testing and prenatal diagnosis; and make informed decisions. The acceptance rate for prenatal invasive testing varies depending on the financial status of the couple as the test is expensive.[16] In our study, we found that the acceptance rate among the couple was almost 100%. To make it more affordable and feasible, PCR-based diagnosis facilities should be available in medical colleges for the detection of β-thalassemia mutations and other hemoglobinopathies.[17]

The risk of maternal complications is low with these procedures and fetal loss can occur up to <0.5%.[18-20]

Similar results were found in our study. Also, the procedure can be performed on an outpatient basis. All these factors contribute to fewer expenses rather than having a fetus suffering from major hemoglobinopathy.

CONCLUSION

Owing to the high prevalence of hemoglobinopathies and the continually increasing impact on global health each year, the benefit of carrier screening programs to control the incidence of new cases is recognized worldwide.

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

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Cite this article as: Gupta A, Gangane N, Dhanvij M, Davile M, Mundle S, Yadav A. Prenatal invasive diagnostic testing for hemoglobinopathies: a retrospective cohort study at a tertiary care public hospital in central India. Int J Reprod Contracept Obstet Gynecol 2025;14:1851-5.