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GOOD CLINICAL
PRACTICE
RECOMMENDATIONS

THALASSEMIA

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GOOD CLINICAL PRACTICE RECOMMENDATIONS (GCPR) FOR UNIVERSAL ANTENATAL THALASSEMIA SCREENING, PREVENTION, AND DIAGNOSIS IN INDIA

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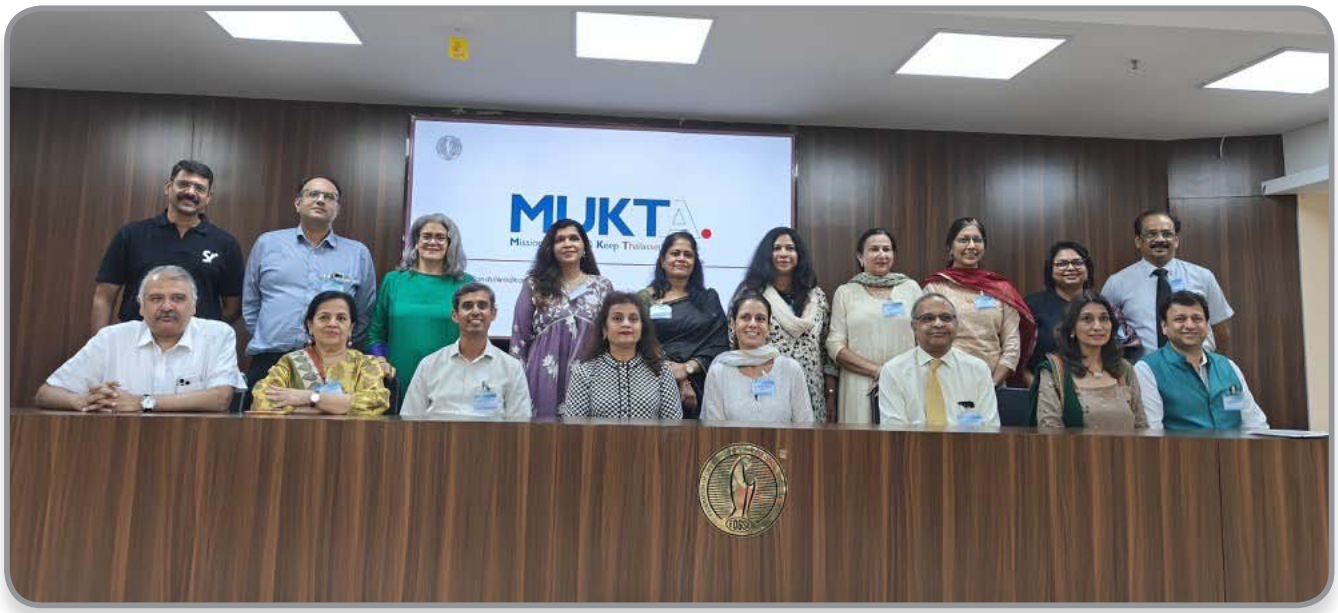

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FOGSI GOOD CLINICAL PRACTICE RECOMMENDATIONS

Background

Thalassemia is a prevalent hereditary blood disorder that poses a significant public health concern.[1,2] It is classified based on defects in the synthesis of alpha (α) or beta (β) globin chains. Beta-thalassemia includes three clinical types: thalassemia major, characterized by severe anemia managed with regular lifelong transfusions; thalassemia intermedia, presenting with moderate anemia and variable transfusion requirements depending on clinical status; and thalassemia minor, a mild or asymptomatic carrier state that generally does not require transfusion but requires genetic counseling. Alpha-thalassemia presents with a wide clinical range, from silent carriers and mild anemia to more severe forms such as Hb Bart's hydrops fetalis, which is associated with high perinatal morbidity and mortality. Additionally, hemoglobinopathies like Hb E/β-thalassemia, involving both structural and quantitative defects in globin chain production, show variable clinical severity. Children may survive only 1–2 years, but with regular transfusions, they can live 15–20 years. This classification helps doctors decide how to manage and care for patients. [1,3]

Consanguineous marriages and large-scale global migrations have amplified the prevalence of thalassemia in recent decades.[2,4] Globally, approximately 5.2% of the population, including more than 7% of pregnant women, carry a clinically significant hemoglobin variant.[2] India has been identified as the world capital for thalassemia,[4,5] and estimates suggest that one in every eight thalassemia carriers worldwide reside in India.[1]

India has the highest number of children affected by thalassemia major globally, with an estimated 100,000 to 150,000 cases, and around 42 million people in the country carry the β-thalassemia trait. Each year, approximately 10,000 to 15,000 babies are born with thalassemia major.[6]

Bone marrow transplantation (BMT) is currently the only curative treatment for thalassemia major. However, BMT is unaffordable for the majority of population. Additionally, the limited availability of suitable human leukocyte antigen (HLA)-matched donors and the scarcity of specialized BMT centers pose significant challenges. The current standard treatment, consisting of regular blood transfusions followed by iron chelation therapy to manage the excessive iron load, places a significant burden on patients due to its high cost, nonadherence, and limited accessibility.[6] The average annual cost of managing a person living with thalassemia in India is INR 74,948 (ranging from INR 41,514 to INR 151,800). An analysis estimated that the actual treatment cost per year at current rates without subsidies, to be INR 167,750 per patient.[7]

Given these challenges, prevention through early identification of carriers and at-risk couples is the most effective strategy to reduce disease incidence. This includes raising public awareness, implementing population-based and cascade screening, offering premarital and antenatal screening, providing genetic counseling for at-risk couples and prenatal diagnosis when required.[7]

The most effective and feasible approach to reduce the incidence of thalassemia major is implementation of a carrier antenatal screening program in early pregnancy.[8] Universal antenatal screening enables women to plan their pregnancy and prevent the birth of offspring affected by thalassemia.[1,9]

Screening methods such as Complete Blood Counts (CBC), hematological indices (Mean Corpuscular Volume [MCV], Mean Corpuscular Hemoglobin [MCH], Total Red Blood Cells [TRBC]), the Mentzer index, and high-performance liquid chromatography (HPLC) are valuable tools for effective diagnosis of thalassemia.[7] Screening using a reliable index, followed by confirmatory tests using HPLC, can provide a reliable diagnostic framework for hemoglobinopathies.[10]

HPLC is considered the gold standard for diagnosing thalassemia and other hemoglobinopathies.[9] It allows for the precise quantification of hemoglobin fractions and identification of hemoglobin variants, making it an invaluable tool in distinguishing thalassemia from iron deficiency anemia and ensuring timely intervention.[5] Both the Indian Academy of Pediatrics (IAP) and the Federation of Obstetric and Gynaecological Societies of India (FOGSI), recommend HPLC as the preferred method for antenatal screening of hemoglobinopathies.[7,11]

Scope and methodology

These recommendations have been developed by a dedicated task force to address the need of universal antenatal screening for Thalassemia in India. It highlights the limitations of conventional screening tools and underscores the importance of HPLC as the preferred first-line diagnostic method. The focus is on offering context-specific, practical recommendations for implementation within preconception and antenatal care, especially in gynaecology and obstetrics settings.

The recommendations were formulated through a robust review process to ensure scientific accuracy, clinical relevance, and alignment with national healthcare priorities. The final structure has been designed to serve as an evidence-based guidance document for clinicians, with appropriate references and actionable points for day-to-day practice.

A standardized grading framework was used to critically appraise the quality of available evidence and determine the strength of each recommendation.

Table 1. Grading based on the level of evidence and class of recommendations

Level of evidence	**Description**
Level A	Data from multiple randomized trials, meta-analyses, or evidence-based clinical practice guidelines
Level B	Data from a single randomized or large non-randomized trial
Level C	Consensus of opinion of experts or small studies, retrospective studies or registries or narrative/literature reviews
Level D	Data from clinical experience
Class of recommendations	
Class I	Strong evidence and or general agreement that the treatment or procedure is beneficial, useful or effective. It is recommended
Class IIa	Evidence is in favor of efficacy/usefulness and should be considered
Class IIb	Efficacy/usefulness is less well established and recommendation may be considered
Class III	Evidence and/or general agreement that an intervention is not beneficial, useful or effective and in some cases may cause harm. Not recommended

Universal antenatal screening for thalassemia and hemoglobinopathies

PRACTICE POINTS

- Hemoglobinopathy carrier screening must be offered to all previously untested women, irrespective of identifiable risk factors. (Level A, Class I)
- If pre-conceptional screening has been not performed, testing should be conducted early in pregnancy, preferably at the first antenatal visit and before 8 weeks of gestation, to facilitate timely fetal diagnosis and informed reproductive choices. (Level A, Class I)
- Both partners should preferably be screened simultaneously to ensure optimal reproductive decision-making. (Level A, Class I)
- The expert panel strongly recommends a strategy combining universal screening, accurate carrier identification, genetic counseling, and antenatal diagnosis to be an effective strategy to reduce thalassemia-related morbidity and disease burden. (Level A, Class I)
- HPLC—the definitive technique of choice—should be strongly recommended, provided the screening has not been done earlier, for detecting carriers of β-thalassemia and other hemoglobin variants (HbS, HbE, HbD). (Level A, Class IIa)

In India, the carrier rate for β-thalassemia ranges from 3% to 17%, depending on the region and population groups. Carrier rates for other variants, such as HbE and HbS, may reach up to 40% in certain regions.[11]

Despite the high carrier frequency, awareness and intervention often occur only after the birth of a child with homozygous or compound heterozygous β-thalassemia (thalassemia major or intermedia), reflecting a predominantly secondary prevention strategy. The identification of β-thalassemia carriers through primary prevention strategies, such as extended family screening or preconception testing, remains uncommon in India. In contrast, several Mediterranean countries have demonstrated significant success in reducing disease incidence through structured screening programs.[11] The high disease burden and carrier frequency of β-thalassemia highlight the urgent need for population-based screening to identify carriers.[12]

Thalassemia major is a preventable disorder through screening, either prior to marriage or early in pregnancy. A combination of universal antenatal screening, partner screening, genetic counseling, prenatal diagnosis, and when appropriate the option of medical termination of affected pregnancies can significantly reduce the national disease burden.[7] From an economic standpoint, the cost of prevention is estimated to be only one-hundredth of the treatment costs.[12] Hence, universal thalassemia screening is not only clinically justified but also economically essential in the Indian context.

Guidelines and evidence on antenatal screening for thalassemia and hemoglobinopathies

The National Health Mission (NHM) of India strongly advocates for the establishment of carrier screening services for pregnant women and their husbands, to prevent the birth of children affected by thalassemia major or thalassemia intermedia, and sickle cell disease. Furthermore, NHM recommends integrating antenatal screening for hemoglobinopathies into the existing panel of routine antenatal tests, including those for Human Immunodeficiency Virus, hepatitis B, diabetes and thyroid dysfunction.[6]

As of 26th March 2025, data updated by the States on National Portal show that out of 15,87,903 individuals screened for thalassemia, 5,037 were diagnosed with thalassemia and 50,462 identified as carriers.[14]

According to the British Society for Hematology (BSH) Guideline, the availability of fully automated systems and reagents for HPLC has replaced cellulose acetate electrophoresis (CAE) as a first-line screening method.[15]

- Laboratories conducting antenatal screening should use methods capable of detecting clinically significant variants and must be equipped to measure HbA_2 and HbF at the action values required by the national antenatal screening programme.[15]

Similarly, the FOGSI emphasizes that carrier screening for hemoglobinopathy should be offered to all pregnant women, given the high carrier prevalence in the Indian population. If pre-conceptional screening is missed, early antenatal testing becomes essential to facilitate timely fetal diagnosis and informed reproductive choices.[16]

In a study, researcher reported 3.15% women to be carriers of β-thalassemia trait and other hemoglobinopathies.[17] A two-year study conducted at a single center in Madhya Pradesh screened 1,006 pregnant women and found a β-thalassemia trait prevalence of 2.78%, with an additional 0.69% carrying other minor hemoglobin variants. The authors recommended that carrier screening be offered to all pregnant women, ideally during the preconception phase or as early as possible in pregnancy. The study concluded that routine screening during pregnancy is both feasible and effective in the Indian healthcare setting.[18] Similar findings have been reported across multiple clinical studies, which consistently emphasize the following:

- Early and universal antenatal screening is critical for identifying thalassemia carriers.[18]
- Carrier screening during pregnancy is both practical and feasible within the Indian context.[18]
- Routine antenatal screening effectively facilitates early detection of carriers.[19]
- A comprehensive approach comprising universal screening, accurate identification of carriers, partner screening, genetic counseling, and antenatal diagnosis forms an effective strategy to reduce thalassemia-related morbidity.[19]
- Early antenatal screening, along with partner testing and comprehensive counseling of screen-positive individuals regarding available treatment and reproductive options, is essential.[20]

In India, all healthcare facilities should be equipped to provide access to essential tests for the screening and diagnosis of thalassemia.[21] District hospitals should have the capability to perform screening tests, including HPLC analysis. Tertiary care centers must be equipped to conduct both HPLC and molecular analysis, while referral centers should have advanced facilities for DNA sequencing and capillary electrophoresis. Adequate referral systems should be available.[21]

Screening and timing of the test for thalassemia and hemoglobinopathies

PRACTICE POINTS

- Screening for thalassemia and other hemoglobinopathies should be conducted during preconception counseling or at the first antenatal visit, preferably in the first trimester.
- At-risk couples identified through antenatal screening should be provided access to prenatal diagnostic testing and genetic counseling early in pregnancy.

The optimal time to screen couples for thalassemia and other hemoglobinopathies is either during pre-conception counseling or at the first antenatal visit, preferably in the first trimester. This timing coincides with heightened parental concern for fetal well-being, making couples more receptive to screening and timely intervention.[12]

This early timeline is essential for several reasons:

- If a pregnant woman is a carrier, her partner should also be screened to assess the risk of having a child with thalassemia major.[6]
- In cases where both partners are carriers, confirmatory fetal testing through chorionic villus sampling (CVS) should be offered between 10 and 14 weeks of gestation.[21]
- This timeline allows at-risk couples to receive necessary information well before the 24-week legal limit for medical terminations under the medical termination of pregnancy (MTP, Amendment) Act, 2021. For those detected beyond 24 weeks, MTP requires medical board approval.[6,22]

Table 2. Optimal timing for screening of Hemoglobinopathies[6]

Stage	Screening purpose and utility
Newborn	Ideal for early detection of sickle cell disease.
Infants	Thalassemia major is typically identified in infancy, after 3–6 months of age.
Adolescence	Optimal time for identifying carriers as part of a sustainable long-term screening strategy.
Young adults	Effective for carrier identification in communities that are well-informed about hemoglobin disorders.
Preconception	Enables carrier screening in populations where prenatal interventions, such as termination or preimplantation genetic diagnosis (PGD), are accessible and acceptable.
Antenatal and prenatal diagnosis	Functions as a safety net for unscreened individuals. If both parents are identified as carriers, prenatal testing can determine whether the fetus is affected by thalassemia or sickle cell disease.

- Access to safe abortion services under the MTP Act framework is crucial to enable timely, informed reproductive choices.
- Consideration of the limitations of each screening method is crucial for the successful planning and implementation of a screening program.[6]
- Infants screening enables early detection of sickle cell disease and some of the more severe forms of Thalassemia.[6]
- Adolescent screening allows for the identification of carriers prior to partner selection for marriage.
- Screening of young adults enables carriers to make informed decisions prior to marriage.[6]
- Pre-conceptional and antenatal screening allows carriers to take steps to prevent the birth of an affected child.[6]

Early antenatal screening and genetic counseling empower couples to make fully informed reproductive decisions—whether that means preparing for a child with special healthcare needs or considering medical termination of pregnancy in cases of diagnosed thalassemia major. By completing the screening, diagnosis, and decision-making process within the first trimester, we not only respect reproductive autonomy but also prevent the avoidable suffering associated with the birth of a child with a lifelong, resource-intensive condition.[6]

A 5-year study screened 93,871 individuals to prevent the birth of children with thalassemia major through a comprehensive antenatal and spouse screening program using Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT), CBC, and HPLC. Among the 10,983 individuals tested by HPLC, 7.07% were diagnosed with hemoglobinopathies, of which 5.84% had β-thalassemia trait. Specifically, the prevalence of β-thalassemia trait among antenatal women was 5.02%. The program identified 42 at-risk couples, of whom 16 underwent prenatal diagnosis, leading to the termination of three fetuses diagnosed with thalassemia major.[23]

The study concluded that routine antenatal screening, coupled with accessible prenatal diagnostic services, is essential for the prevention of thalassemia births. However, key barriers included lack of awareness, delayed screening, and limited access to prenatal diagnostic facilities.[23]

In a separate study conducted in Surat, South Gujarat, 3,009 pregnant women were screened to identify couples at risk for severe hemoglobinopathies. Both women and their spouses were tested using blood indices, hemoglobin electrophoresis, and HPLC. The study reported that 3.38% of women had β-thalassemia trait, 1.5% had sickle cell trait, and 0.86% carried other variants. Among the 14 identified at-risk couples, 11 underwent prenatal diagnosis, which detected three fetuses affected by severe hemoglobinopathies, all of which were medically terminated. Follow-up confirmed the accuracy of prenatal diagnosis.[24]

The study emphasized that late antenatal registration, partner non-cooperation, and refusal of prenatal testing were major barriers to effective prevention. It concluded that increasing community awareness and promoting early antenatal registration are critical to improving program outcomes.[24]

Rational for choosing HPLC over other methods

PRACTICE POINTS

- Red cell indices (such as MCV, MCH) and Mentzer Index alone are insufficient for definitive thalassemia diagnosis due to low specificity, especially in populations with overlapping iron deficiency anemia.
- HPLC should be used as the confirmatory test for thalassemia screening, given its superior ability to detect hemoglobin variants and quantify HbA_2 and HbF, as recommended by national and international guidelines.

Limitation of red cell indices and Mentzer index in isolation

Although initial screening with CBC and indices such as MCV, MCH, and the MI can offer preliminary clues, they lack specificity in distinguishing thalassemia traits from iron deficiency anemia—particularly in populations with overlapping burdens of both conditions. This diagnostic overlap underscores the need for a more definitive approach.[25]

Table 3. Available test and their limitation: Rational for using HPLC over other[21]

Available tests	Limitations
NESTROFT	• Have limited sensitivity and specificity, especially in antenatal care • False negative results seen in a small proportion of β-thalassemia carriers, hence not recommended
CBC-based red cell indices	• Atypical β-thalassemia carriers may show normal MCV and/or MCH sometimes (Individuals may be missed while screening). • Carriers of variant Hbs like Hb E and Hb S may also have normal indices in 20%–30% of cases.
Capillary electrophoresis	• Not extensively evaluated in India
Cellulose acetate electrophoresis	• Laboratories should have adequate experience with this technique. • Time consuming and cumbersome and is much less used where automated HPLC is available.

HPLC: High performance liquid chromatography, NESTROFT: Naked eye single tube red cell osmotic fragility test, CBC: Complete blood count, MCH: Mean corpuscular hemoglobin

HPLC provides an accurate and reliable quantification of HbA_2 levels, making it the method of choice for screening hemoglobinopathies. To achieve a meaningful national impact, universal antenatal screening for thalassemia using HPLC should be mandated and systematically integrated into routine reproductive healthcare services. This approach is supported by current evidence and constitutes an ethical, clinical, and economic imperative for reducing the burden of preventable hemoglobinopathies in India.

Importance of accurate early diagnosis for clinical decision-making

Timely and accurate differentiation between thalassemia and other microcytic anemias is essential to guide genetic counseling, prenatal diagnostic interventions, and informed reproductive choices. Early screening must therefore be supplemented by a reliable confirmatory modality.[12]

Gaps in detection using hematology alone

Several studies from India and other high-prevalence regions have demonstrated that CBC-based approaches may miss up to 14% of hemoglobinopathy carriers, especially those with rare or compound variants. This highlights the necessity of using a more comprehensive tool for conclusive identification.[27]

Emerging consensus on the role of HPLC

Given its superior analytical precision and capacity to quantify HbA_2 and HbF alongside identifying a wide spectrum of hemoglobin variants, cation-exchange HPLC is increasingly recognized as the gold standard for thalassemia screening—particularly in antenatal settings.[15, 16]

Alignment with national and international recommendations

Both Indian (for example, NHM, FOGSI) and international guidelines (for example, British Society of Hematology) now advocate for confirmatory testing using HPLC as an essential step in any thalassemia screening algorithm, especially in high-risk or high-prevalence populations.[14,16,17]

Role of HPLC in thalassemia screening and diagnosis

PRACTICE POINTS

- High-Performance Liquid Chromatography (HPLC) plays a central role in the screening and diagnosis of thalassemia and other hemoglobinopathies. It gives an advantage of accurate quantification of HbA_2 and HbF, as well as for reliable detection of common and rare hemoglobin variants. (Level A, Class I)
- HbA_2 values must be interpreted in the context of iron status, as iron deficiency can lower HbA_2 levels and mask the diagnosis of β-thalassemia trait. Additionally, recent blood transfusions can alter the results of HPLC; therefore, clinical correlation and iron studies are strongly recommended. (Level A, Class I)
- Clinicians should maintain a high index of suspicion for compound variants—such as HbE/β-thalassemia or HbD/β-thalassemia—when atypical peaks are observed in conjunction with elevated HbA_2 or HbF levels. These cases require confirmatory evaluation. (Level B, Class IIa)
- HPLC is strongly recommended for routine use in young adults, preconception, antenatal, and infants (after 3 months) screening programs, having demonstrated consistent effectiveness in identifying carriers and clinically significant hemoglobinopathies. (Level A, Class I)
- Borderline HbA_2 values (3.3–3.9%) must not be dismissed. Such findings should prompt molecular testing and clinical correlation, in all patients especially in high-prevalence regions or in couples at genetic risk. (Level B, Class IIa)

HPLC is the primary screening and diagnosis tool for thalassemia and other hemoglobinopathies. Globally endorsed as the method of choice, HPLC enables precise quantification of HbA_2 and HbF, and reliable identification of both common and rare hemoglobin variants, including HbS, HbE, and HbD-Punjab. In clinical settings where iron deficiency or atypical presentations coexist with thalassemia traits, reliance on red cell indices alone results in missed or inconclusive diagnosis. HPLC overcomes these diagnostic challenges through a standardized, automated approach, making it an essential diagnostic tool in both individual case evaluation and population-level screening programs.[21]

In a recent study, 2,698 anemic patients were evaluated for various hemoglobinopathies using HPLC and found a high prevalence of hemoglobinopathies, with β-thalassemia trait being the most common. The study concluded that HPLC is a rapid, sensitive, and reproducible method for detecting different hemoglobinopathies.[27]

Principle of HPLC

HPLC differentiates hemoglobin variants which enables precise identification of both normal hemoglobin fractions such as HbA, HbA_2, HbF as well as variant forms, including HbS, HbE, HbD, and HbC.[28]

Advantages of HPLC in screening and diagnosis

HPLC is widely recognized as the preferred method in both clinical and population-based screening programs due to the following advantages:

- High analytical precision and reproducibility: HPLC consistently demonstrates a low coefficient of variation (CV < 5%) for hemoglobin fraction quantification, ensuring reliable and consistent results. [29]
- Automation and scalability: HPLC facilitates semi-automated or automated analysis, making it suitable for high-throughput settings such as infants and antenatal screening initiatives.[28]
- Detection of hemoglobin variants: HPLC reliably identifies common variants including HbS, HbE, HbD-Punjab, HbC, and Hb Lepore, as well as HbH and Hb Bart's.[29]
- Accurate quantification of diagnostic fractions: The method enables precise measurement of HbA_2 and HbF, which are essential for the detection and classification of β-thalassemia traits and major forms.[28]

Interpretation of HPLC results

HPLC is a highly accurate and reproducible technique used in the diagnosis and characterization of thalassemia syndromes and hemoglobinopathies. It enables identification and quantification of abnormal hemoglobin fractions, which can then be correlated with clinico-hematological profiles for comprehensive patient evaluation.[21]

HPLC chromatograms assist in the identification of hemoglobin disorders based on characteristic hemoglobin fractions:

- Samples with hemoglobin levels >8 g/dL and red cell indices showing MCV <80 fL and MCH <27 pg are considered screen-positive for β-thalassemia trait and are taken up for confirmatory testing by HPLC.[6]
- β-thalassemia trait (carrier state): A hallmark finding is elevated HbA_2 levels (>3.5%). However, iron deficiency anemia may lower HbA_2 levels and potentially mask carrier status. Therefore, iron parameters such as serum ferritin must be evaluated in such cases. Despite this, HbA_2 levels >4% remain highly suggestive of true carriers, even in individuals with iron deficiency. [4,12,13,29,]
- β-thalassemia major: HPLC typically shows absent or markedly reduced HbA and a significantly elevated HbF fraction, with mean HbF levels often exceeding 60%. This finding is especially indicative of β-thalassemia major, where HbA production is completely suppressed.[29]

- Compound hemoglobinopathies: Co-inheritance of structural variants such as HbE or HbD with β-thalassemia produces overlapping red cell indices and complex HPLC patterns, necessitating careful chromatogram interpretation and, in some cases, molecular confirmation.[12]
- HbH Disease (α-thalassemia): HPLC may reveal fast-moving abnormal peaks, including HbH or Hb Bart's, which are diagnostic of HbH disease associated with three-gene α-globin deletions.[28]
- Borderline HbA_2 levels (3.3%–3.9%): These findings are diagnostically ambiguous and may require confirmation with molecular testing.[30]
- Hereditary Persistence of Fetal Hemoglobin (HPFH), δβ-thalassemia, and Hb Lepore syndromes may present with disproportionately high HbF levels (5%–30%) in the absence of significant HbA_2 elevation, aiding in differential diagnosis.[14]

Table 4. Normal and abnormal hemoglobin levels[15]

Hemoglobin type	Normal range	Abnormal levels
Hb A ($\alpha_2\beta_2$)	20–30% at term (in fetus) Predominant by 3 months of age	• Low/Absent in β-thalassemia major (TDT), sickle cell anemia
Hb A_2 ($\alpha_2\delta_2$)	3.5% in adults	• ≥3.5% + MCH <27 pg → β-thalassemia trait • >4% → possible mild β-thalassemia • >8% → consider Hb Lepore • >15% → may indicate Hb E trait
Hb F ($\alpha_2\gamma_2$)	<2–3% by 6 months of age	• ≥5% + MCH <27 pg → δβ-thalassemia • ≥10% + normal MCH → HPFH • Very high (e.g., Hb F only) → β-thalassemia major
Hb S, C, D-Punjab, E, H, Lepore, O-Arab	Absent in normal individuals	• Presence indicates carrier or disease states (e.g., SCD, Hb E/β-thalassemia)
Hb Bart's (γ_4)	May be detected in normal term infants	• Presence indicates α-thalassemia (e.g., Hb H disease, hydrops fetalis)

Clinical significance of HPLC in thalassemia and hemoglobinopathy screening and diagnosis

Timely and accurate detection

Hosseeini et al, conducted a five-year study on 3,780 patients to evaluate the effectiveness of HPLC in detecting thalassemia and hemoglobinopathies. Using CBC, HPLC, electrophoresis, and ferritin tests, the study identified β-thalassemia trait in 20.66%, β-thalassemia major/intermedia in 12.84%, and iron deficiency anemia in 8.67% of patients. Rare variants like HbE, HbD, and HbC were also detected. The authors concluded that HPLC is a fast, reliable method, especially valuable in premarital and neonatal screening (where feasible).[31]

- HPLC allows for early and precise identification of β-thalassemia trait and other clinically significant hemoglobin variants—essential for implementing reproductive counseling.

Integration into young adults and antenatal screening

The prevalence of thalassemia and other hemoglobinopathies was assessed in 90,210 individuals using HPLC. Findings showed identification of abnormal hemoglobin patterns in 11.43% of participants. The most common findings were β-thalassemia trait, HbE trait, Eβ-thalassemia, and β-thalassemia major/intermedia. The authors highlighted the importance of HPLC in antenatal screening and considered it a rapid and reliable tool for diagnosing hemoglobin disorders.[33]

Hemoglobinopathy screening using HPLC was carried out in 119,336 individuals, including antenatal, hematological, and transfusion-dependent cases. Abnormal variants were detected in 12.17%, with β-thalassemia trait, HbE trait, β-thalassemia major/intermedia, and Eβ-thalassemia being the most

common, while rare variants like sickle cell disease and HbD-Punjab were also identified. The authors confirmed the utility of HPLC in early detection and highlighted the importance of mandatory premarital and antenatal screening in high-burden regions.[34]

A study involving 105,211 individuals compared solubility testing and electrophoresis with HPLC for hemoglobinopathy screening. While initial tests detected 12.33% with sickle cell trait and 2.91% with sickle cell disease, further HPLC analysis identified β-thalassemia, HbS-β-thalassemia compound heterozygotes, and rare forms such as HbE, HbD, HPFH, δβ-thalassemia, and HbQ-India. The researchers concluded that HPLC was essential for precise diagnosis of β-thalassemia and rare hemoglobin variants.[35]

Hemoglobinopathies were evaluated using HPLC in 65,779 individuals undergoing premarital testing, hemogram evaluation, or with a family history of thalassemia. Abnormal variants were found in 18.44%, with β-thalassemia trait being most common, followed by sickle cell trait and disease. Less frequent findings included β-thalassemia syndromes, HbE trait, and borderline HbA_2, while rare variants like Hb Beth Israel and Hb Hofu were identified via molecular testing. The authors confirmed HPLC as a reliable and effective tool, particularly for β-thalassemia screening.[36]

In another study that screened 1,000 unrelated individuals using NESTROFT, CBC, and HPLC, 7.8% were diagnosed as β-thalassemia trait using HPLC as the gold standard. Hence, HPLC is essential for accurate detection of β-thalassemia trait and other hemoglobinopathies.[26]

Multiple large-scale studies have confirmed the superior diagnostic performance of HPLC over conventional methods such as CBC, solubility testing, and electrophoresis. HPLC is widely accepted as a cost-effective, time-efficient tool that reliably detects β-thalassemia carriers and common hemoglobin variants such as HbE, HbD, and sickle cell disorders.[26, 32-36]

Genetic counseling

Objectives of genetic counseling

Genetic counseling aims to educate patients and families about the hereditary thalassemia and other hemoglobinopathies, assess carrier status, provide reproductive options, and support informed decision-making. It also addresses psychosocial concerns and reduces stigma associated with hemoglobinopathies.[22,38]

In cases where HPLC reports are inconclusive or show atypical/complex results, referral to a clinical counselor is strongly advised.

Table 5: Timing of genetic counseling (India-specific)

Timing	Target group	Rationale and evidence
Young adults	All adolescents especially in high-risk communities	Early carrier identification can prevent at-risk marriages. Premarital counseling in extended families has been shown to be effective in controlling thalassemia.
Preconception	All couples planning pregnancy	Allows time for reproductive decision-making. International guidelines recommend counseling for at-risk individuals.[40]
Antenatal	All pregnant women and their partners (1st trimester)	Critical to detect carrier couples early. Indian national guidelines recommend antenatal screening between 10–12 weeks.[40]
Postnatal	Parents of affected newborns	Educates on disease management and future pregnancy planning. Recommended in NHM guidelines.[39]
Post-diagnosis	Families with a child diagnosed with thalassemia	Supports cascade screening and reproductive guidance for future pregnancies.

Core components of genetic counseling[22, 38-40]

- **Detailed pedigree charting** (minimum three generations)[21]
- **Carrier screening** via HPLC and molecular confirmation
- **Inheritance risk communication** based on autosomal recessive pattern[21]
- **Discussion of reproductive options**: natural conception with prenatal diagnosis, IVF with PGD, donor gametes, adoption
- **Informed consent** before invasive diagnostic procedures
- **Psychological and social support** during and after diagnosis[21]
- **Referral to support groups and specialized centers** for ongoing care

Practical strategies to roll-out universal antenatal screening in India

- Awareness and advocacy of Universal Screening is recommended, rolling out universal screening in low-resource or rural areas is particularly needed
- Training needs for all community and facility-based healthcare workers (e.g., ASHAs, ANMs, Anganwadi workers, Multipurpose health workers, lab technicians, and medical officers).
- Explore widespread use of digital tools or registries for tracking carriers and documenting prevalence.

Pathways for the screening/diagnosis of thalassemia in adolescents/ young women/preconception, pregnancy and neonatal populations[5,19-20]

Adolescents and young adults – especially in school-based or community programs. (Boys and girls)

Any women visiting Gynecological clinic – including those undergoing routine health checks.

Preconception
– Couple planning pregnancy or undergoing premarital counseling.
Preconception screening
- Essential for all couples coming to infertility clinics for assisted conception

Perform HPLC (High-Performance Liquid Chromatography)
HPLC quantifies various hemoglobin fractions:

- **HbA_2 > 3.5%** → diagnostic for b-thalassemia trait
- **Elevated HbF** → suggestive of β-thalassemia or hereditary persistence of fetal hemoglobin
- **HbE, HbD, HbS peaks** → identify other hemoglobinopathies
- **If HPLC is normal** → routine documentation and education

Young women or adolescents with HMB

Child/parent counseling
- Record results in antenatal/health record
- Educate on long-term implications and carrier status for future pregnancies
- Encourage family screening where applicable (cascade screening)

Genetic counseling

- Cascade screening whenever possible
- Confirmation by DNA testing

Genetic counseling

Complete blood count (CBC) and analyse red blood cell indices:
- MCV < 80 fL (microcytosis)
- MCH < 27 pg (hypochromia)
- Mentzer Index < 13 → suggests thalassemia trait (vs. iron deficiency)
- If any values are abnormal or there is a family history, proceed to HPLC

Pre-conception/ART clinic

If the woman is a carrier of a significant hemoglobinopathy
- Screen her partner or sperm donor
- Ovum donor, the donor should also be screened
- Counseling for reproductive choices

Genetic counseling

- Counsel for further evaluation
- In high-risk individual confirmation by DNA testing

Genetic counseling

Pregnancy – screened during the first antenatal visit

If couple is a carrier for hemoglobinopathy

Couple gene mutation analysis

Gene identified

Offer prenatal diagnosis

- 11-14 weeks of gestation → Chorionic Villus Sampling (CVS) under ultrasound guidance → Educate and counsel the couple for continuation and discontinuation of pregnancy
- >16 weeks of gestation → Amniocentesis → Amniotic fluid can be analyzed directly by HPLC after ensuring that there is no maternal contamination

Appropriate intervention. follow-up counseling
Genetic counseling

Neonatal Screening- For sickle cell diseases
For thalassemia- testing to be done after 6 months

New born screening

1 and 7 days after birth or cord blood sample

Dried blood spots analyzed

Sickle cell anemia, sickle β-thalassemia or Hb SD disease

Confirmatory molecular analysis

- Register for comprehensive care
- Counsel/educate parents

Genetic counseling

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