CENTRE OF EXCELLENCE TO BE ESTABLISHED BY MINISTRY OF TRIBAL AFFAIRS
FOR NATIONAL SICKLE CELL ANAEMIA ELIMINATION MISSION

Introduction

Sickle Cell Disease (SCD) is a hemoglobin disorder that requires lifelong management and contributes to infant, childhood as well as adult morbidity and mortality. Prenatal diagnosis is an important option to screen women with genetic abnormalities and can prove to be a cost-effective preventive strategy. Prenatal testing is a choice to be exercised by families and healthcare providers. Based on the findings, if the fetus is affected, the family is given the option of pregnancy intervention (termination) for an affected child.

The guidelines on Haemoglobinopathies released by MoHFW in 2016 mention the setting up of a State lab/National Centres established in tertiary level institutions identified by the States. This guidance note elaborates on the role of the Centre of Excellence to be established at State level. The operational guidelines for National programme for Prevention and Management of Sickle Cell Disease released in 2023, reiterated about the Centres of Excellence. Being a tertiary care level facility, it has to be supported by Ministry of Tribal Affairs.

Center of Excellence (CoE) in Haemoglobinopathies

A Center of Excellence (CoE) in Haemoglobinopathies focuses on the prevention, management, and research of disorders related to hemoglobin, such as sickle cell disease and thalassemia. The specific functions of a CoE in Haemoglobinopathies can vary, but they typically include the following:

1. Diagnosis and Screening:

i. Providing state-of-the-art diagnostic services for patients suspected of having haemoglobinopathies.
ii. Pre-natal testing.
iii. Conducting newborn screening programs to identify affected infants early in life.
iv. Offering genetic counseling and carrier screening for families at risk.

2. Clinical Care and Management:

i. Offering comprehensive care for individuals with haemoglobinopathies, including sickle cell disease and thalassemia.
ii. Developing individualized treatment plans for patients, which may include transfusions, iron chelation therapy, and hematopoietic stem cell transplantation.
iii. Managing complications and comorbidities associated with these disorders, such as infections, pain crises, and organ damage.

3. Patient Education and Support:

i. Educating patients and their families about the nature of the disease, treatment options, and lifestyle management.
ii. Providing psychosocial support and resources to improve the quality of life for patients and their families.

4. Research and Clinical Trials:

i. Conducting research to advance understanding of haemoglobinopathies and develop new treatment modalities.
ii. Participating in clinical trials to test innovative therapies and interventions.
iii. Collaborating with other research institutions and organizations to share knowledge and resources.

5. Outreach and Public Awareness:

i. Support state in developing strategies for raising public awareness about haemoglobinopathies to reduce stigma and discrimination.
ii. Advocating for better policies, funding, and resources to support affected individuals and their families.

6. Healthcare Professional Training:

i. Offering training and continuing education for healthcare providers in the diagnosis and management of haemoglobinopathies.
ii. Promoting best practices and evidence-based care in the field.

7. Policy Development and Advocacy:

i. Engaging in advocacy efforts to influence healthcare policies and improve access to care for individuals with haemoglobinopathies.
ii. Participating in discussions related to newborn screening, insurance coverage under PM-JAY/ State programmes and other public health initiatives.

8. Genetic Counseling and Family Planning:

i. Providing genetic counseling services to families with a history of haemoglobinopathies to assess their risk and make informed family planning decisions.
ii. Assisting individuals and couples in making choices related to prenatal diagnosis and options for carrier status.

9. Quality Improvement and Research Translation:

i. Implementing quality improvement programs to enhance patient care and outcomes.
ii. Translating research findings into practical clinical applications and treatment strategies.

10. Multidisciplinary Collaboration:

i. Collaborating with Ministry of Tribal Affairs, institutions/ organisations other than those in the public sector who have been working in the area of Hemoglobinopathies.

A Center of Excellence in Haemoglobinopathies plays a crucial role in improving the lives of individuals affected by these disorders through accurate diagnosis, evidence-based care, research, and advocacy. It serves as a hub for expertise, patient support, and advancing knowledge in the field of Haemoglobinopathies. It is envisaged that one such centre is established in every State.

Continuum of Care System for Sickle Cell Disease

Centre of Excellence is primarily involved with Prenatal Diagnosis and Management of complications arising out of sickle cell disease or its treatment. The pool of referrals to the Centre of Excellence encompasses those referred from Sub-district Hospital, and District Hospital. At each of the facilities the in charge Medical Officers need to keep the list of PM-JAY empanelled healthcare facilities as a ready reference to identify an appropriate facility for referral. The patients being referred upward need to be informed adequately about the need for referral and provide ambulance support as required. The MO in charge shall ensure documentation of the referrals made. After the treatment, the patients shall be referred downward to the concerned primary health centre for continued monitoring at PHC-HWCs and subsequently for hydroxyurea medication dispensation (if initiated) by CHOs at SHC-HWCs. The table below represents a schema of upward and downward referrals for ensuring a continuum of care for people with Sickle cell disease.

Table 1. Referral chain

Level of care	Upward referral	Downward referral
SHC-HWC	The following will be referred to PHC or higher centre:- Individuals screened positive on the solubility test for confirmation of diagnosis Individuals screened positive by ICMR approved Point of care test for treatment initiation Patients on Hydroxyurea treatment for periodic monitoring Patients presenting with complications (pain crisis, severe anaemia) requiring specialist care. Screened positive pregnant mothers for prenatal diagnosis directly to the state level Centre of Excellence Children less than two years of age who are screened positive Patients requiring blood transfusion	All the patients from the Centre of Excellence (CoE) will be referred downward to the concerned PHC for continued monitoring as per the protocol. Once the patient is stabilised, the PHC MO shall in turn refer the patient to SHC-HWC for monthly medicine dispensation by CHO. ASHAs carry out home visits for reinforced treatment adherence and referral in case of any complications.
PHC-HWC	The following will be referred to CHC/Sub-divisional hospital/District Hospital: - Patients non-responsive to Hydroxyurea treatment Patients presenting with complications (Acute sequestration crisis, vaso-occulsive crisis, hyper haemolytic crisis) Screened positive pregnant mothers for prenatal diagnosis to state level Centre of Excellence. High risk ANC/delivery of pregnant mother with sickle cell disease Patients requiring blood transfusion Children less than two years of age who are screened positive	

CHC/Sub-divisional hospital/District Hospital	The following will be referred to Tertiary care/Centre of Excellence (CoE):- Screened positive pregnant mothers for prenatal diagnosis High risk ANC/delivery in SCD mother Patients presenting with complications (Acute sequestration crisis, vaso-occlusive crisis, hyper haemolytic crisis) Children less than two years of age who are screened positive if services are not available for management	

Prenatal diagnostic Technologies

The guidelines on Haemoglobinopathies released by MoHFW details 3 sampling methods – CVS, Amniocentesis and Fetal Blood Sampling. Conventional methods and Sanger sequencing is required to detect genetic mutations of thalassemia and other hemoglobinopathies. Key points on the same are reiterated here:

a. Chorionic villus sampling (CVS): Using ultrasound as a guide, the specialist removes a small sample of cells from the chorionic villi, i.e. cells that contain the same genetic information as the fetus and which will eventually form the placenta. The cells are removed either with a thin needle (21 Gauge needle) inserted through the mother's abdomen (trans- abdominal route) or via a thin catheter inserted through the vagina (trans-cervical). The cells are then analyzed, and a diagnosis is made through processing of fetal DNA. As with other prenatal diagnosis methods, information on potential risks and benefits of using this procedure must be provided to the couple by the specialist obstetrician. CVS is done in the first trimester of pregnancy between 10-12 weeks of gestation.
b. Amniocentesis: Using ultrasound as a guide, a trained obstetrician inserts a very thin needle through the mother's abdomen. A small amount of amniotic fluid, containing cells from the fetus, is withdrawn. This is then analysed in the laboratory to determine whether the fetus has β-thalassemia disease or sickle cell disease. Amniocentesis is conducted after 16 weeks of gestation in patients who come late for sampling or in those where the fetal position is such that it prevents the collection of chorionic villi. The cells (amniocytes) are separated by centrifugation and DNA analysis is conducted.
c. Fetal blood sampling (Cordocentesis): The fetal blood sample is collected in mid-trimester pregnancy at 18-20 weeks of gestation. The sampling is done by cordocentesis, cardiac puncture or from the hepatic vein. The sample is processed either by HPLC or by DNA analysis.
d. Future Direction **:** Newer NIPT (non-invasive prenatal testing) of Fetal cell free DNA from maternal plasma, will be available in these CoE in coming years and for this purpose, NGS would be required. Preimplantation genetic testing (PGT) may also be required for IVF centres in these CoE to choose the embryos without any chromosomal genetic abnormality before implanting them.

The type of instrument and technique used could have a significant impact on the outcome of the procedure. If we take the example of CVS, the sample is submitted to the laboratory and DNA is extracted using a suitable technique (glass bead disruption of

cells, conventional collagenase or chelex-based extraction method) developed specifically for these samples. This is then sent for the CVS DNA sequencing; later data analysis allows the analysis of all positive reactions chosen to be sequenced.

Bone Marrow Transplantation

The only cure available for these children with thalassemia major is bone marrow transplantation (BMT) more appropriately called hematopoietic stem cell transplant (HSCT). However, this can help only a few patients because of cost, paucity of BMT centres, or non-availability of a suitable HLA matched donor. Therefore, the mainstay of treatment is not HSCT, but enhanced availability of HSCT centres at the proposed CoE, which will go a long way in the management.

Patients likely to benefit from HSCT are to be identified by the pediatrician at the DEIC, or the Pediatrics departments of District hospital/ Medical colleges. They may be referred to Centre of Excellence with facilities for HSCT. Here the transplant team will assess the patient and counsel the family about the procedure, risks and take up the case after adequate assessment of the patient and donor. The cost of BMT/HSCT is not currently supported under NHM. The same is envisaged to be supported by the Ministry of Tribal Affairs.

Areas of Support

The support is extended to the provision of equipment for sequencing and recurring costs for reagents. The currently used technology is Sanger sequencer, while the newer one is NGS (Next Generation Sequencing). In principle, the concepts behind Sanger vs. next-generation sequencing (NGS) technologies are similar. In both NGS and Sanger sequencing (also known as dideoxy or capillary electrophoresis sequencing), DNA polymerase adds fluorescent nucleotides one by one onto a growing DNA template strand. Each incorporated nucleotide is identified by its fluorescent tag. The comparison of Sanger Sequencing and NGS techniques is given below.

Table 2: Comparison of Sanger Sequencing and NGS

	Sanger Sequencing	Targeted NGS
Benefits	• Fast, cost-effective sequencing for low numbers of targets (1–20 targets) • Familiar workflow	• Higher sequencing depth enables higher sensitivity (down to 1%) • Higher discovery power* • Higher mutation resolution† • More data produced with the same amount of input DNA‡ • Higher sample throughput
Challenges	• Low sensitivity (limit of detection ~15–20%) • Low discovery power • Not as cost-effective for high numbers of targets (> 20 targets)	• Less cost-effective for sequencing low numbers of targets (1–20 targets) • Time-consuming for sequencing low numbers of targets (1–20 targets)

	• Low scalability due to increasing sample input requirements	
Experimentation **and procedure**	• DNA isolation step is same for sanger and NGS both • Complete process to put up the sanger sequencing experiment is less complicated and less time consuming • Experiment run time is short. (1-2 hours for beta globin gene)	• DNA isolation step is similar as in sanger sequencing • Experimental procedures (library preparations, purifications, template preparations) is more complex and time consuming • Experiment run time on NGS is higher
Data Analysis	• Sequencing data analysis is much simpler than NGS	• As big amount of data generated, bioinformatics analysis is needed.

** Discovery power is the ability to identify novel variants.*
† Mutation resolution is the size of the mutation identified. NGS can identify large chromosomal rearrangements down to single nucleotide variants.‡ 10 ng DNA will produce ~1 kb with Sanger sequencing or ~300 kb with targeted resequencing (250 bp amplicon length × 1536 amplicons with an AmpliSeq for Illumina workflow).

In view of the comparison of Sanger sequencing with Targeted NGS it is quite evident that the Sanger sequencing is most cost effective and very well-established method of mutation/variant detection (known, unknown and novel) in hemoglobinopathies. The technique has relatively low sensitivity than NGS but capable of detecting all the genetic variants as mutations being germline in nature in the thalassemia and other Hemoglobinopathies. Also, Sanger sequencing is a faster method for carrier screening as sequencing run time is approximately one hour as beta globin gene is very short having only three exons. Data analysis is also relatively simple and less time consuming as compared to NGS.

NGS is a high throughput technique but technically and analytically very challenging. It is not cost effective for a smaller number of samples. The cost effectiveness cannot be achieved if load of sample is not very high. It is quite high with a limited number of samples, which also depends on the NGS model. Equipment run cost, reagent and consumable costs are also very high coming with short expiries. One key benefit can be detection of alpha and beta globin gene both simultaneously on NGS. However, very big deletions of alpha globin gene are not properly detected on NGS. MLPA is a better choice for detecting big deletions of alpha gene. MLPA can be performed on the same Sanger sequencing platform with fragment analysis option. Moreover, alpha globin gene analysis is not required in all the individuals, suspected cases on CBC and HPLC can be subjected for alpha analysis.

In view of all the advantages and disadvantages associated with both the techniques, it is advisable to have at least one Sanger sequencer (16 or 24 capillaries) with its ease of use, shorter run time and unbeatable cost. Scalability can be increased with regular back-to-back runs can be put up on single

equipment for optimal utilization of equipment. The labs already having Sanger sequencing facility can subsequently opt for addition of one NGS.

Table 3. Cost of Sequencer

Equipment	Capital cost (INR)	Recurring cost (INR)	
	Approx. cost of device	Average Cost per test (single variant test, including consumables)	Annualized cost (unit cost*annualization factor)
Sanger sequencing device	45 lakh – 1.8 Cr	2,031	4,95,000 – 19,80,000
NGS Device	Upto 3 Cr	5,675	13,20,000 - 99,00,000

*Cost per test calculated considering approximately 100 tests per month

Note:

- **All CoE where Sanger sequencer is already established** – support can be given for recurring cost for recurring for reagent supply for Sanger based on details in table 3. Based on the load, States can submit proposals to MoTA. **Subsequently** - NGS equipment can be added as and when the role of CoE expands, and supported on an actual basis at a maximum capital cost of INR 3 Crores and recurring for reagent supply as per Table 3. Based on the load, State Govt./Institutions can submit proposals to MoTA.
- **All CoE where NGS is already established** – Support to be provided for recurring cost for NGS based on details in Table 3. Based on the load, State Govt./Institutions can submit proposals to MoTA.
- **CoE where neither Sanger sequencer nor NGS is established** – The labs where none of the facility is there, Sanger Sequencing facility is the primary requirement for variant detections in Hemoglobinopathies. Capital as well as recurring Cost of Sanger sequencer to be provided. **Subsequently** – As and when the scope of CoE expands and newer techniques are available, NGS equipment can be added and supported for capital as well as recurring cost as per Table 3. Based on the load, State Govt./Institutions can submit proposals to MoTA.

- {During Covid 19 pandemic, the Indian SARS CoV-2 genomics consortium was jointly established by MoHFW, DBT, CSR & ICMR. The purpose was to correlate whole genomics sequencing data with clinical/epidemiological data for advance preparedness for public health interventions. As on 23rd March 2022, NGS facilities were established in 24 States and 3 UTs in 84 institutions. There were other sources of funding and based on specifications, there would be variations in cost. For the purpose of funding support, the estimate can be made around 3 Crores for NGS, which is recommended if there is a sample load of more than 20 per cycle.}

Additional Mandatory Equipment (along with Sanger sequencer or NGS):

Few mandatory equipment is required in the molecular lab to perform the experiment on

NGS or Sanger Sequencer e.g. Thermal Cycler, spectrophotometer (Qubit/Nanodrop), Gel electrophoresis, Power supply, Gel documentation, etc. Remaining ancillary equipment depends on Lab setup already existed.

Table 4: Cost of mandatory equipment:

Equipment	**Approx. Capital cost of device (INR)**	**Annualized cost (unit cost*annualization factor) (INR)**	**Average Cost per test** (single variant test, including consumables) **(INR)**
DNA extraction equipment	4 lakh-10 lakh	46,800 - 1,17,000	568
Thermal Cycler	*3 Lakh - 6 Lakh*	35,000 – 70,000	NA
Spectrophotometer	3.5 Lakh - 5 Lakh	38,000 – 55,000	200
Gel Electrophoresis	2 Lakh - 5 Lakh	22,000 – 55,000	100 - 500

Further, States */Institutions* are advised to form a technical committee to make an action plan, roadmap in consonance with the Sickle Cell Elimination Mission and the Hemoglobinopathy guidelines.

In addition to above, the equipment list for tertiary centres (Hemoglobin HPLC equipment for newborn screening by DBS, Isoelectric focusing for newborn screening, given in the Guidelines on Haemoglobinopathies (page 99-100), Hemoglobin HPLC variant System, Capillary Zone Electrophoresis) 2016 may also be considered for support, the details of which are given in Annexure. The CoEs not having any of these equipment, may be supported by MoTA as per the proposal submitted.

Human resources for Centre of Excellence[1]

The identified Centre of Excellence institutions are expected to have the requisite human resources for identification/diagnosis and treatment, including complications of Haemoglobinopathies. Nevertheless, key specialists required are enlisted below for clarity:

(a) Haemato-pathologist: MBBS and MD (Pathology) with advanced degree (DM/DNM) / training in Haemato-pathology and trained in gene sequencing and analysis

Responsibilities:

- Diagnose haemoglobinopathies using CBC, PBS, HPLC / Hb Electrophoresis and molecular methods.
- Perform mutational analysis and diagnostics for hemoglobinopathies.
- Research haemoglobinopathies and contribute to developing new diagnostic and treatment strategies.
- Collaborate with other specialists in the centre.

b) General Medicine Physician: MBBS with a postgraduate degree in Internal Medicine or relevant field.

Responsibilities:

- Manage adult patients with complications of haemoglobinopathies (e.g., sickle cell disease, thalassemia).
- Provide pre- and post-natal counselling for couples with a risk of haemoglobinopathy.
- Collaborate with other specialists for comprehensive patient care.

c) Gynecologist: MBBS with a postgraduate degree in Obstetrics and Gynecology.
Responsibilities:

- Perform chorionic villus sampling (CVS), amniocentesis, and fetal blood sampling for prenatal diagnosis of haemoglobinopathies.
- Provide counselling to pregnant women with the risk of haemoglobinopathy.
- Collaborate with the geneticist and other specialists for patient management.

d) Paediatrician: MBBS with a postgraduate degree in Pediatrics.
Responsibilities:

- Diagnose and manage haemoglobinopathies in children.
- Provide follow-up care and education for children with haemoglobinopathies.
- Collaborate with other specialists for comprehensive care.

e) Clinical Haematologist: Qualifications: MBBS with a MD / DNB in Internal Medicine and DM / training in Clinical Hematology.
Responsibilities:

- Provide supportive care for patients with haemoglobinopathies (e.g., blood transfusions, pain management).
- Diagnose and manage co-morbidities associated with haemoglobinopathies.
- Contribute to research on haemoglobinopathies and treatment optimisation.

f) Molecular Scientist: PhD in Medical / Biological Sciences, with expertise in PCR and gene sequencing analysis (including interpretation).
Responsibilities:

- Diagnose haemoglobinopathies using molecular methods.
- Perform gene sequencing and provide genetic counselling.
- Research haemoglobinopathies and contribute to developing new diagnostic and treatment strategies.
- Collaborate with other specialists in the centre.

g) Lab Technician: Diploma or Bachelor's degree in laboratory technology with experience in molecular biology techniques.
Responsibilities:

- Perform PCR and gene sequencing under the supervision of the geneticist or bio-informatics specialist.
- Maintain and manage laboratory equipment and reagents.
- Document and report laboratory results.

h) Counselor: Providing pre-conception and prenatal counselling to individuals and

couples at risk of having children with haemoglobinopathies.

Apart from this, the following additional personnel are desirable and may also be considered:

i) Clinical Psychologist: Providing emotional support and counselling for patients and their families, coping with the psychological impact of haemoglobinopathies and chronic illness.
j) Social Worker: Facilitating access to social services, resources, and advocacy for patients and families, navigating healthcare systems and financial assistance.
k) Public Health Specialist: Developing and implementing community education programs about haemoglobinopathies to raise awareness, promote carrier screening, and encourage early diagnosis.

Note: The cost of HR will be borne by concerned institution only and no financial support will be provided.

Annexure

Table 5. Additional Equipment list as per Haemoglobinopathy guidelines

Sl. N	Equipment for Haemoglobinopathies	Capital	Recurring
1	Hemoglobin HPLC equipment for newborn screening by DBS	40 lakhs	150/test
2	Isoelectric focusing for newborn screening	15 lakhs	
3	Hemoglobin HPLC variant System	43 lakhs	250/test
4	Capillary Zone Electrophoresis	37 lakhs	
	Total*	135 lakhs	Based on load estimated by State

**estimating 1 equipment each for every CoE*

- **[1]** indicative. HRH is not budget supported.