

PREVENTION AND CONTROL OF HEMOGLOBINOPATHIES IN INDIA - THALASSEMIAS, SICKLE CELL DISEASE AND OTHER VARIANT HEMOGLOBINS



2016



National Health Mission Guidelines on Hemoglobinopathies in India

Ministry of Health & Family Welfare
Government of India

RBSK
RASHTRIYA BAL SWASTHYA KARYAKRAM
राष्ट्रीय बाल स्वास्थ्य कार्यक्रम



Blood Cell



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FOREWORD

The hereditary haemoglobin disorders, in particular the β -thalassaemia syndromes and sickle cell disease are the most commonly encountered single gene disorder in India and impose a heavy economic burden on both the families and on the state resources.

Considering the significance of this genetic disorder Ministry of Health and Family Welfare, Government of India, with help of experts, has developed a comprehensive, "Guidelines on Haemoglobinopathies in India". The purpose of the Guidelines is to provide not only a better future for all patients affected by Thalassaemia or Sickle Cell Disease but also to prevent the birth of children with such disorders, thereby aiming to reduce the number of children affected by Thalassaemia Major and Sickle Cell disease in our country. This would also help in better understanding of Haemoglobinopathies in India. It also documents the best practices of other countries in this field.

These guidelines are major milestone in the history of Haemoglobinopathies in India, and will promote significantly in improving the quality of survival in patients with Haemoglobin disorders in India. Once these guidelines are fully implemented in every state in India, there will be only a very few children with these disorders and children with such disorders will be transformed into productive citizens and integrated into the Indian society as valuable members.


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PREFACE

Haemoglobinopathies especially thalassaemia and sickle cell disease are preventable genetic disorders that in their severe forms are associated with chronic, life-impairing and life-threatening diseases with inherent serious health sequelae that can lead to disability or death. Unfortunately a large number of children in our country continue to be born and suffer from such disorders mainly due to lack of awareness and lack of a comprehensive programme and systematic strategies to prevent them. Data on the prevalence of silent carrier's for various Haemoglobinopathies like β -thalassaemia ranges from 2.9-4.6%, and for sickle cell anaemia especially among the tribal population ranges from 5-40 %, while haemoglobin variants like HBE in eastern India can be as common as 3-50%. At times, there could be various permutation and combination among the various Haemoglobinopathies e.g. one parent could be a carrier of Sickle cell disease and the other of β Thalassaemia or one parent carrier of Sickle cell disease and the other haemoglobin variant. Hence the strategy is required for a unified approach.

Considering the magnitude of the problem and the cost implications of management, suitable control measures need to be undertaken urgently. This could be both primary and secondary prevention. Primary being identifying the carriers and avoidance of marriage of carrier couples and secondary by preventing the birth of affected child through prenatal diagnosis.

The comprehensive guidelines on prevention and control with regard to Haemoglobinopathies have been prepared against this backdrop. I am positive that these guidelines will assist and facilitate the states to address the issues concerning Haemoglobinopathies.


(A. K. Panda)

Healthy Village, Healthy Nation



एड्स - जानकारी ही बचाव है
Talking about AIDS is taking care of each other



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PRELUDE

Haemoglobin disorders or Hemoglobinopathies are the most common single gene disorder globally. Haemoglobinopathies like β -thalassemia, sickle cell haemoglobin and haemoglobin E related disorders are important causes of genetic morbidity and mortality. 7 out of every 100 person, on this globe carry an abnormal haemoglobin gene. Annually 300,000 to 500,000 children are born with clinically significant haemoglobin disorder, of which seventy percent of the share is contributed by Sickle Cell disease and the rest by Thalassemia syndromes.

In our country, apart from nutritional anaemia and infectious disease, genetic disorder like Haemoglobinopathy are also emerging as a major national health problem. It is estimated that approximately 30 million people are silent carrier for β -thalassemia in our country and live a normal life, but the marriage between two silent carriers, may result in their children having a serious disease, requiring regular blood transfusion for survival. There are about 65,000 to 67,000 β -thalassemia patients in our country with around 8,000 to 10,000 cases being added every year.

Since only a limited number of patients can be cured by bone marrow transplantation from HLA identical donors, prevention of the birth of affected child appears to be the most feasible and cost effective approach for control of this disease. This is feasible through mass screening for silent carriers at schools before marriage and during pregnancy. Similarly sickle cell anaemia (HbS) in our country is largely but not restricted to around 7.5 crores of the tribal population with a prevalence rate varying from 5-40%. HbE another Haemoglobinopathy is present in about 5% of the Bengali population, reaching up to 19-20% in some pockets of Assam.

The three major Haemoglobinopathies of our country such as β -thalassemia, sickle cell haemoglobin (HbS) and haemoglobin E (HbE) also interact among themselves, such as one parent being β -thalassemia carrier and the other carrier for HbE or one parent being β -thalassemia carrier and the other for sickle cell haemoglobin results in children manifesting with features of severe disease.

Hence the challenge to control Haemoglobinopathies is substantial and an integrated comprehensive approach is required for both screening and management. This guideline emphasises on an integrated comprehensive approach to Haemoglobinopathies with special emphasis on prevention and control and is expected to help both the administrators and clinicians in this endeavour.


(MANOJ JHALANI)

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PROLOGUE

Thalassemia is one of the commonest single gene disorders representing a major health burden in India and the world. According to March of Dimes Global survey on birth defects (2001), the five common major birth defects of genetic or partially genetic origin are Congenital heart disease, Neural tube defects, Haemoglobin disorders (Haemoglobinopathies) like Thalassemia and Sickle cell disease, Down syndrome and G6PD deficiency. It is estimated that more than 20 million people are silent carriers of the β -Thalassemia gene globally, of which 30 million are in India. Every year about 10,000 children are born with Thalassemia major in India accounting for 10% of Thalassemia major worldwide.

While bone marrow transplant is the only curative option, the available remedy is lifelong blood transfusion and removal of iron by chelation therapy. Both the options are not only cost intensive but also result in a constant agony to the child. It is therefore needed that emphasis be laid on prevention of birth defects of such children through targeted carrier detection among both the school children as well as antenatal mothers. Further, new-born screening, especially for Sickle cell disease, is crucial in early identification and timely management which can change the overall prognosis of the child.

In view of this, both carrier detection and management of Haemoglobinopathies should not only be comprehensive but also be integrated with screening of other birth defects, which is already being carried out under the Rashtriya Bal Swasthya Karyakram (RBSK).

The Guidelines on Haemoglobinopathies is the outcome of concerted efforts of the Ministry of Health & Family Welfare to collate the knowledge encompassing the best practices in Haemoglobinopathies and provide guidance to the health providers. The objective of these Guidelines is to address practical issues concerning prevention and patients care in a simplified manner. I am certain that administrators and physicians would also find these Guidelines of immense value. I congratulate the entire team and hope the book will add to existing body of knowledge in the field and enable the administrations and practioners to face the challenges of Haemoglobinopathies practice.


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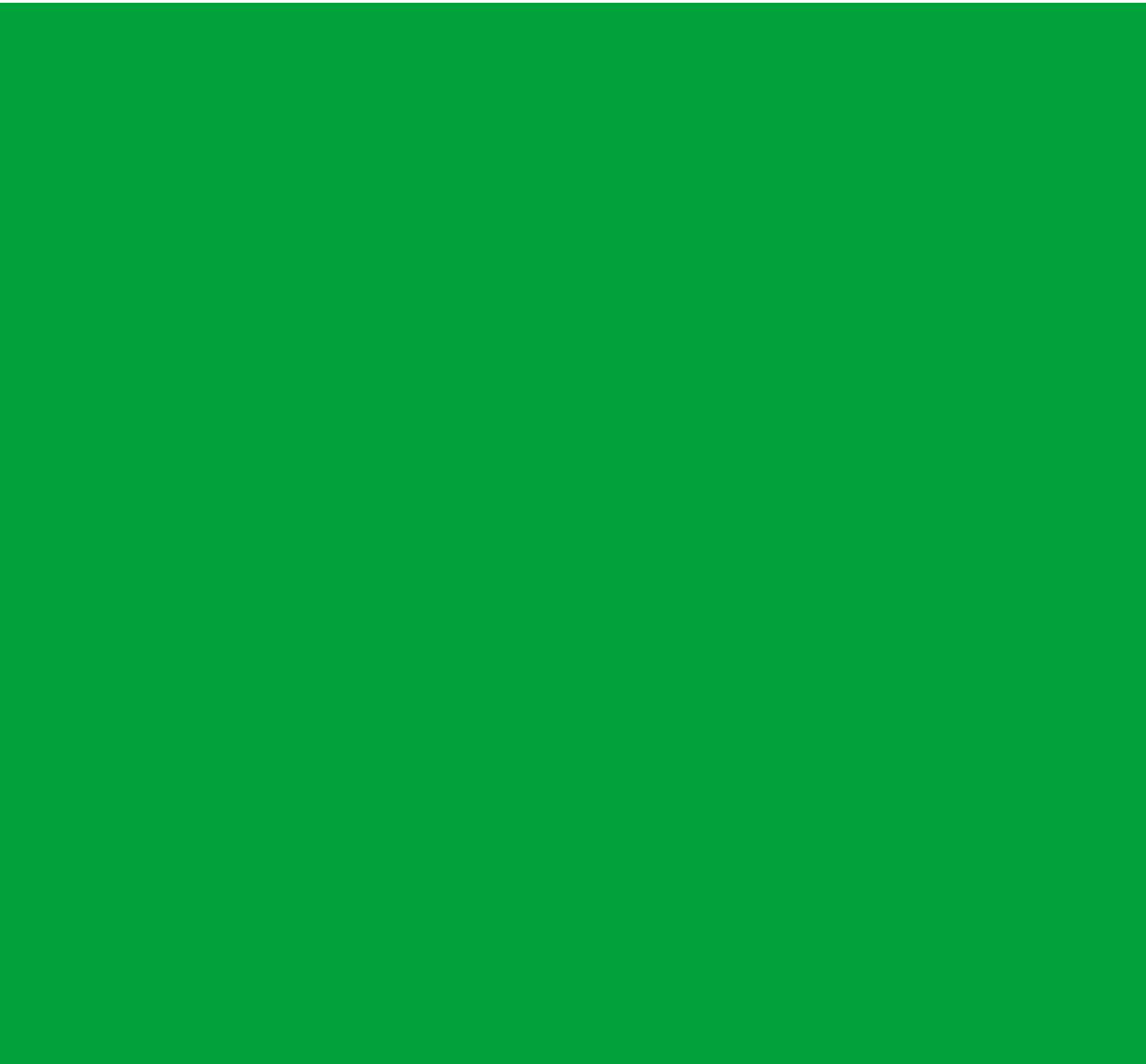
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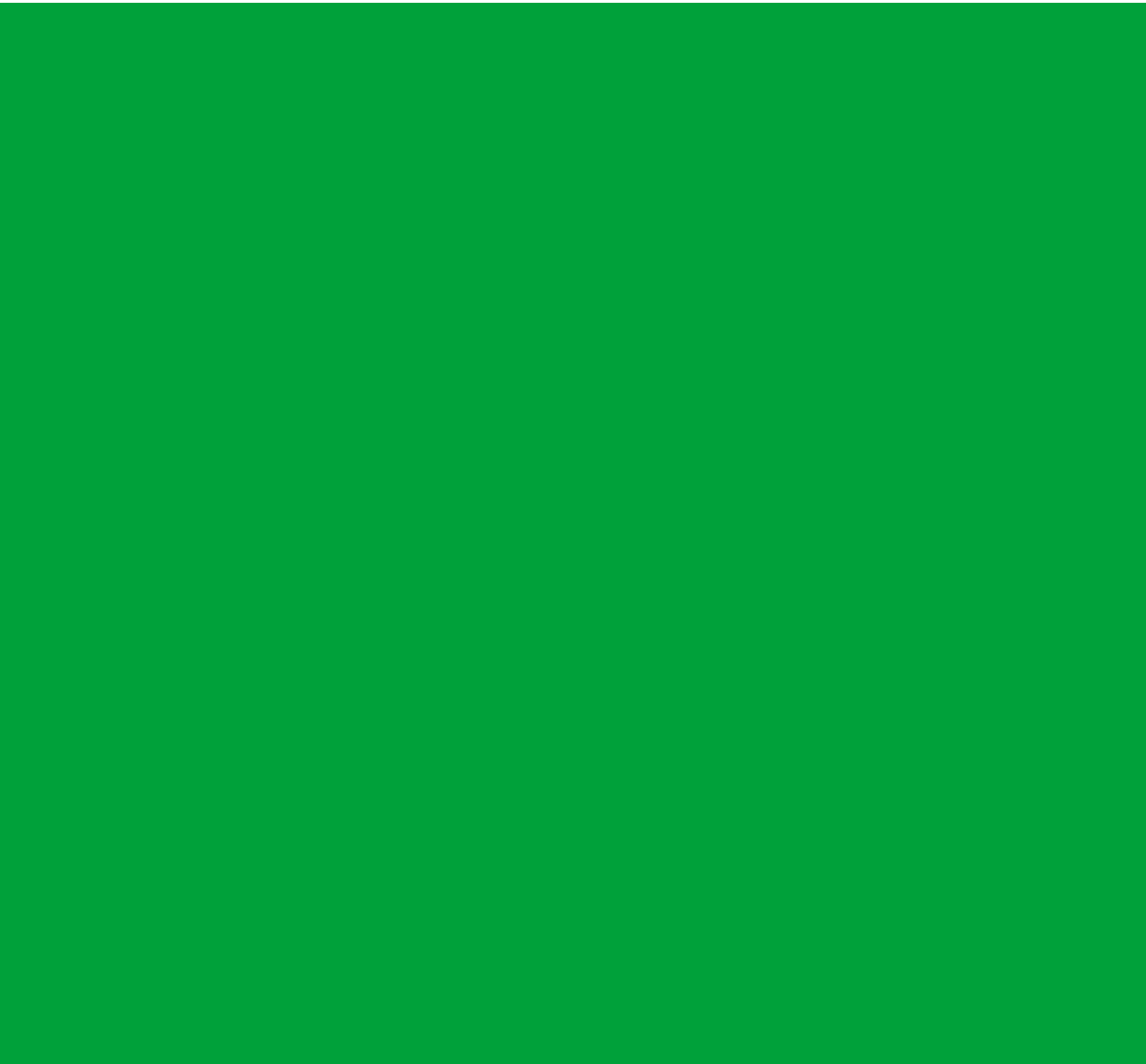
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SECTION A

POLICY FOR PREVENTION AND CONTROL OF HEMOGLOBINOPATHIES



SECTION A

NHM POLICY FOR PREVENTION AND CONTROL OF HEMOGLOBINOPATHIES IN INDIA

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NATIONAL HEALTH MISSION GUIDELINES FOR PREVENTION AND CONTROL OF HEMOGLOBINOPATHIES IN INDIA

EXECUTIVE SUMMARY

Hemoglobinopathies are inherited disorders of red blood cells. Being an important cause of morbidity and mortality, they impose a heavy burden on families and the health sector in our country. India has the largest number of children with Thalassemia major in the world – about 1 to 1.5 lakhs and almost 42 million carriers of β (beta) thalassemia trait. About 10,000 -15,000 babies with thalassemia major are born every year. Sickle cell disease affects many communities in certain regions, such as central India and States of Gujarat, Maharashtra and Kerala. The carrier frequency of the Sickle cell gene varies from 1 to 35 % and hence there are a huge number of people with Sickle cell disease.

In India, the technology, know-how and the means to adequately treat and control both thalassemia and sickle cell disease are available, but this has not yet been incorporated as a policy for various reasons. The new initiatives and vision under the National Health Mission provide a golden opportunity to provide a framework for prevention and management of hemoglobinopathies. This will include guidelines for adequate therapy for those affected helping them lead better lives and prevention through carrier screening, genetic counseling and prenatal diagnosis. A newborn screening program will be initiated for sickle cell disease with provision for appropriate management.

The World Health Organization has clearly outlined the goals for control of hemoglobinopathies - *provide affordable and adequate therapy for those affected, while at the same time reduce the number of births of children with the disease through strong political, administrative and financial support*^{1,2}. Keeping these guiding principles in mind, the vision of the National Health Mission is to provide optimal treatment to those affected and prevent the birth of children with disease through carrier screening, genetic counseling and prenatal diagnosis. Prevention and control will be achieved through the following strategies –

1) Carrying out awareness, education and screening programmes in the community and schools. 2) Establishing laboratories for carrier screening for hemoglobinopathies and newborn screening for sickle cell disease at the district level. 3) Screening pregnant women and their husbands to prevent the birth of children affected with thalassemia major or sickle cell disease; and 4) Establishing prenatal diagnostic centers in Medical Colleges in the States where required.

Adequate therapy will be provided by A) the development of treatment centres with the help of State health departments, B) providing facilities for bone marrow transplant and C) facilitate establishment of donor registries and public cord blood banks in major cities to provide a source of HLA matched stem cells through convergence with other programmes. The involvement of parent-patient organizations has to be ensured for success of the above mentioned goals.

BACKGROUND

Hemoglobinopathies are the commonest genetic disorders worldwide. They include thalassemias and abnormal variant hemoglobins such as Hemoglobin S, D, E etc. They constitute a major burden of disease, mainly in malaria endemic countries, but have now become global due to population migration. In 2006, World Health Assembly passed a resolution urging member states “to develop, implement and reinforce comprehensive national, integrated programmes for the prevention and management of hemoglobinopathies.”² The member states were also urged to develop and strengthen medical genetics services and community education and training.

An estimated 7% of the world population carry an abnormal hemoglobin gene, while about 300,000 -500,000 are born annually with significant hemoglobin disorders. They consist of two major groups – Thalassemias and Sickle cell syndromes. Sickle cell syndromes are more frequent and constitute 70% of affected births world-wide, the rest are due to thalassemias.¹

Thalassemias are clinically divided into Thalassemia Major (TM), Thalassemia Intermedia (TI) and Thalassemia Minor or Trait according to severity. Thalassemia Major (TM) and the severe form of Thalassemia Intermedia (TI) constitute the major burden of disease as management of both requires lifelong blood transfusions and iron chelation. While Thalassemia minor is the carrier state in which the person is clinically normal and is commonly referred to as β (beta)Thalassemia Trait (BTT). The thalassemia syndromes (TM, TI) are caused by inheritance of abnormal β thalassemia genes from both carrier parents, or abnormal β Thalassemia gene from one parent and an abnormal variant hemoglobin gene (HbE, HbS) from the other parent.

Sickle Cell Disease (SCD) is another hemoglobin disorder that requires lifelong management and contributes to infant and childhood morbidity and mortality. SCD is caused by inheritance of two abnormal HbS genes, one from each parent or Hb S gene from one parent and HbE or β thalassemia gene from the other. Sickle cell syndromes include Sickle Cell Disease (SCD, HbSS), also called Sickle Cell Anemia (SCA), as well as disorders due to sickle cell gene combined with another hemoglobinopathy such as Hb C, E, or β thalassemia.

Persons carrying only one of these genes are called ‘carriers’ as they do not suffer from any disease but carry the abnormal gene and transmit it to the next generation. Carriers cannot be recognized clinically but only by performing special blood tests. Where both mother and father are ‘carriers’, there is a chance that their children may inherit the abnormal gene from both parents and thus suffer from a severe thalassemia syndrome or a Sickle Cell syndrome (see figure 1) or may be normal without any abnormal gene or carriers like their parents.



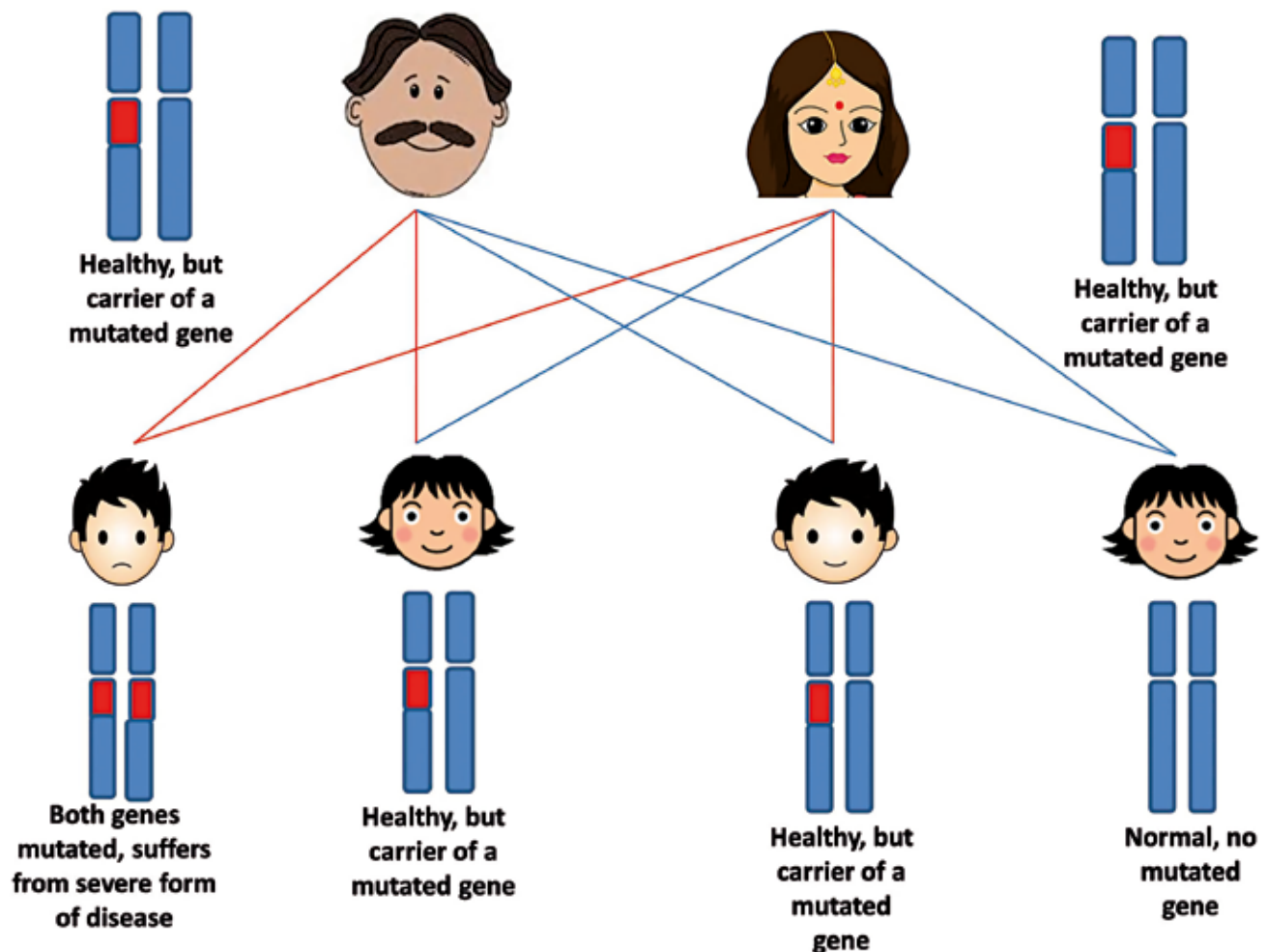
Fig 1.

Autosomal inheritance pattern seen in transmission of the disease β thalassemia major The figure depicts the case scenario in which both the parents have one mutated beta globin gene, they are asymptomatic carriers (beta thalassemia trait).

Each child has-

- 25% chance of inheriting two normal genes,
- 50 % of inheriting one altered gene and one normal gene (beta thalassemia trait),
- 25 % chance of inheriting two altered genes, in this case both the genes carry the mutation (Thalassemia major).

Autosomal recessive inheritance



Genetic disorders where both genes are required to be abnormal for the disease to manifest are called 'autosomal recessive' disorders. Genetic epidemiology of the disorders with recessive inheritance is such that the recessive gene, 'naturally selected' at some point of evolution due to survival advantage, continues to spread through asymptomatic healthy carriers, till it reaches an equilibrium with the disadvantage due to the severe disease manifesting in children having abnormality in both their genes. *It is the severity of these autosomal recessive disorders, manifested in children born to "healthy" carrier couples; that makes prevention and carrier detection an important public health issue.*³

BURDEN OF HEMOGLOBINOPATHIES IN INDIA

In India, β -Thalassemia is prevalent across the country, with an average frequency of carriers being 3-4%^{4,5,6}. A higher frequency has been observed in certain communities, such as Sindhis, Punjabis, Gujaratis, Bengalis, Mahars, Kolis, Saraswats, Lohanas and Gaur^{6,7}. HbS is highly prevalent in the tribal populations of Southern, Central and Western states reaching as high as 48% in some communities⁸. HbE is common in the North Eastern states, and has a carrier frequency as high as 50%, in some areas. It is found in lower frequencies in the Eastern states of West Bengal, Bihar and Uttar Pradesh, while HbD is present in about 2% of people in Punjab.

It is estimated that about 10000-15000 babies with Thalassemia Major (TM) are born every year¹⁰. 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 a regimen of regular blood transfusions followed by adequately monitored iron chelation therapy to remove the excessive iron overload-as a consequence of the multiple blood transfusions. Thus it is a transfusion dependent disorder and places a great burden on healthcare services.

In a cost / benefit analysis done in Israel, cost of treatment of one patient for average life expectancy in Northern Israel was calculated to be \$2,000,000 and cost of running a thalassemia control programme for one year was \$400,000¹². Prevention is thus extremely cost effective rather than treatment of those who are affected⁹.

In India, the cost of transfusing and chelating a 30 kg body weight child for one year was estimated at Rs. 200,000 for one year in 2008¹⁰. With an estimated birth of 10,000 children with Thalassemia Major every year, and survival for 50 years, the cost of managing 500,000 children (10,000 x 50) works out to Rs.10000 crores, and Rs.100 crores even if only 1% were to survive to 50 years of age¹⁰. Based on the experience of a pilot project funded and implemented under National Health Mission in Uttarakhand, the cost of screening one lakh adolescents was estimated at Rs.1 crore. Screening was based on estimation of Hemoglobin (Hb) by digital Hemoglobinometer and NESTROFT as the primary screening test, followed by Complete Blood Counts (CBC) and HPLC test, for the screen positive cases. Serum Ferritin was done in required cases to confirm concomitant iron deficiency anemia in suspected thalassemia carriers. Cost of DNA test for detection of causative mutation in HPLC confirmed cases was about Rs.1000-1500 per case. (The cost will be reduced further by about 30%, if screening for persons with mild and moderate anemias, are excluded).



IMPACT OF PREVENTION PROGRAMMES WORLDWIDE

In Cyprus, it was estimated that if no steps for prevention were taken then there would be an increase in prevalence of affected births from 1:1000 to 1:138 and 600 % increase in blood transfusion requirement estimated over a twenty year period⁹. The successful implementation of a prevention and control programme has brought down the birth rate of those affected with thalassemia to almost zero and an augmented care programme has enabled the affected to have fulfilling lives. In Sardinia, Italy, the introduction of the voluntary carrier screening programme was initiated in 1975 and it has reduced the incidence of β -thalassemia from 1:250 to 1:4000. In Latium, Italy a voluntary screening programme for secondary school children and young adults in place for more than three decades has brought down the incidence of affected births to zero¹⁵. In Montreal, Canada, a successful voluntary screening programme in high schools for Thalassemia was started in 1980 and has led to a 95% decrease in the incidence of β -thalassemia⁹.

It is evident from the above illustrated examples that an effectively implemented prevention and control programme can successfully bring down the birth of children affected with thalassemia major to almost zero over time. This reduces the burden of disease and enables better lifelong care of those already affected and surviving with the disease born before implementation of the programme.

WHO CRITERIA FOR SETTING UP SCREENING PROGRAMME FOR THALASSEMIA ¹

Generally when the Infant Mortality Rate (IMR) falls below 50, the burden of genetic disorders like thalassemia becomes apparent, due to the survival of children affected with thalassemia, who would otherwise have succumbed to the disease. Thus the time for formulation of a national policy for implementation of prevention and control of hemoglobinopathies in India is overdue.

The WHO has listed the components of a control programme as follows:

- A strong political will and support
- Adequate finances for staff, equipment and chemicals
- Optimal treatment of those affected
- Carrier screening
- Genetic counseling, premarital or antenatal
- Prenatal diagnosis in couples where both the partners are carriers
- Awareness programme in the community, starting from the schools
- Monitoring of the programme

STAGES IN DEVELOPMENT OF CONTROL PROGRAMME FOR HEMOGLOBINOPATHIES IN INDIA

The need for adopting and implementing a strategically planned prevention and control programme through the public health system across the country in India has been stressed upon by medical experts and patient- parent organizations for the past two decades. A brief record of these efforts and initiatives that have shaped the development of the current policy guidelines is provided below:

1997-The ICMR-DBT Brain Storming Session on Hemoglobinopathies was held in April 1997 at National Institute of Immunohaematology, Mumbai, comprising experts from major groups working on hemoglobinopathies in India. The recommendations envisaged a comprehensive programme including care and control components at each district extending to block level with screening of target population within the existing health system under the aegis of the national government. A pilot project in one state at district level was specifically recommended before extending the program to the block level and to the rest of the states.

1999-Patient-parent organizations, especially Delhi based Thalassemics India and National Thalassemia Welfare Society, supported by Thalassemia International Federation, became highly active, motivating parents to set up Thalassemia Societies across the country wherever required. They have been running public awareness campaigns, drawing attention of the government agencies towards the needs of persons with thalassemia and initiate prevention programmes by screening during pregnancy, prenatal diagnosis and family screening.

2000-Indian Council of Medical Research undertook an extensive multicenter study under the 'Jai Vigyan' project to create awareness in the population and to determine the prevalence of beta-thalassemia and other hemoglobinopathies in six States from different regions of the country.

The result of the study conducted between 2000 and 2005 were published in 2012. The *Jai Vigyan* project experience showed that NESTROFT missed an average of 13% of β thalassemia carriers. This was due to certain lacunae like non maintenance of water quality used at different centers, variations in preparation and dilution of the buffer due to frequent change of technicians who put up the test. When the NESTROFT buffer was prepared centrally and sent to the different centres; the results improved considerably. Red Blood Cell (RBC) indices also missed around 3 to 4 % of beta thalassemia carriers at different centres. However, if NESTROFT and RBC indices were taken together in the first step, less than 2% of beta thalassemia carriers were missed.

Around 25 to 40 % of HbS and HbE carriers were missed by both the methods. In a National programme we will also need to screen for HbE and sickle cell disorders.. Hence, addition of Solubility test for HbS carriers and DCIP test for HbE are required in regions of high prevalence.

In all cases a strict quality control for the hematology analyzers, reagents and training programmes is needed.

At NIIH, the recent experience with hemoglobin capillary zone electrophoresis has showed that in a few cases of homozygotes, the Hb variants were detected, but specific identification was not possible and the samples had to be re-run after mixing with normal samples for identification (communicated by Dr. Roshan Colah). Thus, at present HPLC is still the preferred method

2004-Indian Red Cross- Gujarat branch, implemented a programme from the year 2004 where they have screened more than 20 lakhs students. In addition to this, from 2009 till date through Antenatal Screening and Prenatal Diagnosis birth of 144 children with Thalassemia major has been prevented. The Society has been very active doing commendable service in this area.



2006-An Indo-US Symposium on Genetic Disorders with Focus on Hemoglobinopathies was held at Banaras Hindu University, Varanasi, sponsored by Indo-US Science and Technology Forum. The Varanasi Region Thalassemia Welfare Society brought together scientists and medical experts from major Indian groups working on thalassemias with experts from a USA Center of Excellence in hemoglobinopathies, Canada and UK to assess the prerequisites for a national programme including collation of data and mapping of resources. A follow up meeting was organized by Department of Hematology, PGIMER, Chandigarh in 2008.

2009-Publication of collated published data on β globin gene mutations in India and creation and publication of a web based informatics resource (registry)- ThalInd-for β Thalassemia and hemoglobinopathies, in collaboration with Centre for Comparative Genomics, Western Australia¹⁴.

2011-Publication of a comprehensive review assessing progress on all aspects of thalassemia care and control in India by Department of Medical Genetics, Sir Ganga Ram Hospital, New Delhi , one of the leading centres for hemoglobinopathies in India¹⁴.

2012-Initiation of a pilot project on thalassemia and other Birth Defects in the State of Uttarakhand (Action on Birth Defects Project) under NRHM and continued under *Rashtriya Bal Swasthya Karyakram (RBSK)*.

BASIS OF NHM GUIDELINES ON HEMOGLOBINOPATHIES

Hemoglobinopathies are the first among genetic disorders for which a national policy for prevention and control has been framed and is being put forth through this document. The elements of the policy are guided by WHO directives and guidelines on hemoglobinopathies including thalassemia and sickle cell disorders^{1,2} and WHO report on services for prevention and management of genetic disorders in developing countries¹³. The guidelines provide a framework based on strategies for prevention and management of hemoglobinopathies documented in publications of Thalassemia International Federation (TIF), various peer reviewed publications, reports of acknowledged groups and on experiences derived from implementing these strategies in public health set up under NHM.

The guiding elements of NHM Guidelines on Hemoglobinopathies are-

1. Hemoglobinopathies are genetic disorders with an autosomal recessive inheritance implying that
 - they are equally prevalent in males and females
 - have a 'carrier' and 'disease' state
 - the abnormal gene is passed on from one generation to another
2. The carrier state refers to a person carrying only one abnormal gene. Such individuals do not have any disease and clinically have no symptoms,
3. The disease state occurs when an individual's both genes are abnormal, one abnormal gene being inherited from each of the parents.
4. A couple where both the partners are carriers of an abnormal gene (mutated gene)

- have a 25% risk in each pregnancy of giving birth to a child with disease state.
- have 25% chance in every pregnancy of having a 'normal' child
- have a 50% chance in each pregnancy to give birth to a 'carrier' child

Thus, a carrier couple can have 'normal', 'carrier' or 'disease' affected children.

5. Thalassemia Major, Thalassemia Intermedia and Sickle Cell Disease are the major disorders that require lifelong management and are to be considered for prevention. Hematopoietic Stem Cell Transplant (HSCT), commonly known as Bone Marrow Transplant (BMT), is the only curative treatment but is possible in very few patients due to high costs and non-availability of matched donors.
6. Untreated Thalassemia Major is invariably fatal by 2-5 years of age. Commonly Thalassemia Major (TM) is managed by regular blood transfusions (Packed Red Blood Cells) and iron chelation therapy. Availability of leuko-depleted packed red blood cells (pRBC) and iron chelators are to be ensured for adequate management along with facilities for regular monitoring. Adequately treated patients can live a fulfilling life.
7. It is possible to know whether the child to be born will be affected by disease, or be a carrier or normal by detecting the mutations of both parents in the fetal tissue. The process is called Prenatal Diagnosis (PND). As Thalassemia Major is a severe and life threatening disease, termination of pregnancy is permitted under Indian laws.
8. Newborn screening can detect abnormal hemoglobin variants like HbS, both carriers as well as those with disease (HbSS) states. On the other hand, thalassemia major is difficult to detect by newborn screening and can be detected hematologically mostly after 3-6 months of age and confirmed at one year of age.
9. Carrier state is asymptomatic, but can be detected by relatively simple blood tests, opening up the possibility of controlling hemoglobinopathies by preventing birth of affected children by -
 - Avoiding marriage between two carriers
 - Prenatal diagnosis in pregnancies of couples where both partners are carriers, with the option of termination of pregnancy in case of an affected fetus.
10. Cost effective population screening programmes are possible for detection of carriers, as low cost screening tests with high negative predictive value are available for detection of carriers of β -thalassemia (also referred to as β Thalassemia Trait (BTT)), HbS Carriers (HbS Trait) and HbE carriers (HbE Trait).
11. Genetic counseling, community education and awareness play a very important role in successful implementation of prevention programmes. Services and screening programmes should be sensitive to cultural and social practices and religious beliefs. Awareness of ethical and legal issues is required to avoid misuse of legal provisions, and be culturally sensitive.
12. The time between initiation of implementation and visibility of impact is affected by the group that is chosen for carrier screening-adolescent, premarital, pre-conception or antenatal. Sustenance of preventive programmes for long periods of time extending to decades is required to achieve expected outcomes.



NHM GUIDELINES FOR PREVENTION & MANAGEMENT OF HEMOGLOBINOPATHIES.

Mission: To improve care of all Thalassemia and Sickle Cell Disease patients for their better future and to lower the prevalence of hemoglobinopathies through screening and awareness strategies.

GUIDELINES FOR PREVENTION

Based on public health goal of reduction in the prevalence of hemoglobinopathies

- Community education and awareness programmes to remove any myths regarding transmission of disease, gender bias, stigma related to disease and carrier states and informing the community about appropriate prevention options and their availability through public health facilities.
- Installation of sustainable and cost effective carrier screening programmes at school level for adolescents backed by adequate and effective prescreening educative programmes on genetic disorders in general and hemoglobinopathies in particular and post screening non directive genetic counseling ensuring confidentiality and generating trust to enable expected outcomes.
- Establishing services at the community level for pre-marital and pre-conception screening backed by genetic counseling services.
- Extended family screening of all known and detected carriers and patients

Implementation of strategies to achieve the public health goal of reduction in prevalence of these genetic disorders will be done in accordance with the guidelines laid down by WHO in its January 5-7,1999 Report on Genetic Disorders and Birth Defects emphasizing on preserving and respecting the social and cultural diversity and dignity and rights of the affected individuals and by voluntary genetic testing after informed consent.

Excerpts from the WHO document are given below, to inform those who run the programme:

- *These (public health) goals should never be set in ways to impose genetic tests or reproductive decisions on individuals.*
- *Accepted ethical guidelines of public health programs in genetics stipulate that genetic testing should always be voluntary, respecting the autonomous decisions of the patients, and should be preceded by proper information in the form of non-directive genetic counseling (WHO, 1998).*
- *Public health goals cannot override the cultural and personal values and beliefs of individuals and their reproductive rights, and oppose stigmatization and discrimination of affected persons (WHO, 1998).*
- *Governments should recognize that within any country there exists diversity of cultures and opinions about a number of issues relevant to genetics, such as human reproduction issues as well as about the significance of disabilities.*

Based on the rights of prospective parents at risk of having a child with a serious genetic disorder

- Establish carrier screening services for screening of pregnant women and their husbands, to prevent the birth of children with Thalassemia major or intermedia and Sickle Cell Disease.
- Create laboratory facilities for testing and confirmation of hemoglobinopathy carriers at district level in the District hospital laboratory or DEIC labs.
- Establishment of loco- regional centers in States with facilities for prenatal diagnosis and laboratory facilities for DNA analysis. Increase feasibility of antenatal screening by training of personnel in sampling techniques with help of tertiary centers.
- Train healthcare personnel for delivery of genetic counseling services for families at risk

GUIDELINES FOR MANAGEMENT OF AFFECTED CHILDREN

Based on rights of patients for access to care

- Provide optimal care to all patients of thalassemia and sickle cell disease by establishing day care facilities for transfusion and monitoring with the help of State health departments at the District Hospitals.
- Ensure availability of safe blood to children with thalassemia, strengthen existing Blood Banks to provide facilities for component separation and leuco-depletion, and help the States to set up new Blood Banks or blood storage facilities where there are none. Promote voluntary blood donation to fulfill the blood requirements.
- Provide financial support for obtaining medicines for iron chelation-an essential component of management without which blood transfusions given over the years will lead to iron-overload.
- Developing and implementing protocols for early diagnosis and intervention in cases of Sickle Cell Disease (SCD) and Thalassemia major (TM). Newborn screening for SCD – in order to provide timely intervention with prophylactic penicillin and vaccinations (see Section C on Management) and targeted screening of children with anemia, to identify those having thalassemia trait or disease. Referral for Hematopoietic stem cell transplant (HSCT), also termed Bone Marrow Transplant (BMT) and facilitate establishment of more HSCT centres,
- Inform the community about appropriate treatment and management options and making these available through public health facilities.

GOALS OF PUBLIC HEALTH STRATEGIES

Implementation strategies would be adopted to achieve the following public health goals:

1. Achieving maximum convergence with other health programmes for cost –effectiveness such as:
 - Management facilities for day care can be created at District Early Intervention Centres (DEICs) established under RBSK.
 - Newborn screening can be done by Dried Blood Spot samples used for screening other metabolic disorders



- Hemoglobinopathy carrier screening in adolescents may be combined with screening and treatment of anemia.
 - Antenatal screening for hemoglobinopathies can be integrated with testing for HIV, hepatitis B, VDRL diabetes mellitus, hypothyroidism etc.
2. Preventive strategies adopted are aimed at creation of an informed society that is willing to voluntarily participate in screening programmes to achieve the public health goal of reduction in prevalence of hemoglobinopathies. All preventive options available at each step should be clearly communicated and non-directive counseling provided to enable the individual to make an informed decision.
 3. A system of surveillance and registry will be established to track and evaluate outcomes such as number of patients registered under the care programme and their record, number of carrier couples opting for prenatal diagnosis and number of carriers detected in school or at premarital stage avoiding marriage with another carrier. Other statistics to be included are the number of couples opting for adoption or limiting family size, and those treated by BMT.
 4. Training of Doctors, Nurses, Laboratory Technicians, Social Workers and other healthcare personnel: to develop required skills for management of affected patients, clinical and laboratory diagnostic procedures including fetal DNA sampling by Chorionic Villus Sampling, community education and counseling and data recording and reporting procedures. This would be an important component for optimum prevention and management of thalassemia.
 5. Research and Analysis: Research, documentation, data analysis and evidence generation for future strategies.

POLITICAL WILL AND ADVOCACY

A strong political initiative and continued support is required for the success of a prevention programme for hemoglobinopathies. This has been repeatedly observed in the case of the successful preventive programmes for hemoglobinopathies from countries like Cyprus, Greece and Italy, which have been successful in preventing the birth of children with thalassemia major through committed government initiatives and support. There are also examples of successful control programmes for thalassemia even in developing countries, e.g. in Iran, Maldives, Sri Lanka and Pakistan. In India the Government has taken the decision to support the prevention and management of thalassemia and framing of this 0020ZZZdocument is the definite step initiated to this end.

INFORMATION, EDUCATION AND AWARENESS

It is important to make the community understand about hemoglobinopathies including the treatment and prevention modalities. The strategies that will be adopted to achieve this are:-

1. Mass communication and media – to incorporate with NHM- IEC at national and district state level.
2. Mid media activities – incorporation of the messages regarding thalassemia within the NHM Programme.

3. Including information and messages about hemoglobinopathies in school text books, as well as school and adolescent health programmes.

Organize Quiz programmes based on prescreening power point assisted educative talks and educational booklets distributed to students during school visits. These strategies were found to be very effective in class IX-XII students in Uttarakhand in assessing and reinforcing retention of information.

4. Include information on prevention of thalassemia in RBSK.
5. Inter Personal Communication and one to group communication- to be incorporated with Antenatal Care, ICDS program at Sub Centre, AWC and PHC level Institutionalizing counseling for thalassemia during ANC, PNC and in the Blood Banks.
6. Incorporation of a chapter at undergraduate MBBS level by putting in MCI curriculum.
7. Special awareness and education campaigns will be initiated for the following target groups.
 - Eligible couples- Increase awareness of the disease, and motivate for screening for carrier status.
 - Youth - Increase the awareness on the prevention and care of the disease.
 - Affected families- Encourage voluntary screening for thalassemia in the relatives (cascade screening).
 - Children who have thalassemia major- Inform about care and prevention of complications.
 - General community- Reduce myths and misconceptions.

BUDGET

Budgetary support from both the State and Central governments, is required to facilitate prevention and treatment of patients with thalassemia and sickle cell disease.

INVOLVEMENT OF STAKEHOLDERS

Non-government organizations (NGO), community based organizations (CBOs), support groups, Corporate and Private sectors will be involved in the prevention programme.

SURVEILLANCE

A national registry will be created which is an important tool for planning future patient services. Apart from numbers, the registry will collect other useful data- such as the location of patients to identify areas of high concentration, ethnicity or other characteristics of patients, age distribution, records of deaths and their cause. The registry will be computerized web- based and centrally controlled by health authorities. The quality of data will be assured and errors minimized. Data analysis will provide information both for planning services, for research and also for medical auditing and program evaluation. The registry will also be adequately funded to ensure sustainability and standards.

Identification of healthy carriers will be achieved through simple hematological tests, which are low cost and sensitive. The same tests will be used for epidemiological surveys designed to estimate the



proportion of carriers in a given population. The carrier rate will be measured from both surveys and screening programmes. It will give an overall indication on the magnitude of the problem in a given population and identify at-risk groups within a population. The service indicator for screening varies according to the population structure.

CONCLUSION

Hemoglobinopathies are one of the major public health problems in India. To achieve success in their prevention and control, an on-going holistic approach is required. It is expected that with optimal collaboration and support, effective prevention and control of thalassemia can be achieved. This will lead to a healthier new generation which enjoys a better overall quality of life.

References:

1. Management of Hemoglobin Disorders: Report of Joint WHO-TIF Meeting, Nicosia, Cyprus, 16-18 November 2007. (http://www.who.int/genomics/WHO-TIF_genetics_final.pdf)
2. WHO resolution on Sickle Cell Disease (WHA59.20) and Thalassemia (EB118.R1), 29 May 2006. (http://www.who.int/genomics/WHO-TIF_genetics_final.pdf)
3. Giordano PC, Hartveld CL, Bakker E. Genetic Epidemiology and Preventive healthcare in Multiethnic Societies: Hemoglobinopathies. *Int. J. Environ. Res. Public Health* 2014, 11(6), 6136-6146.
4. Madan N, Sharma S, Sood SK, Colah R, Bhatia HM. Frequency of β -thalassemia trait and other hemoglobinopathies in northern and western India. *Indian J Hum Genet.* 2010 Jan;16(1):16-25.
5. Sinha S, Black ML, Agarwal S, Colah R, Das R, Ryan K, Bellgard M, Bittles AH. 2009. Profiling β -thalassaemia mutations in India at state and regional levels: implications for genetic education, screening and counseling programmes. *HUGO J* 3:51-62
6. Mohanty D, Colah RB, Gorakshakar AC, Patel RZ, Master DC, Mahanta J, Sharma SK, Chaudhari U, Ghosh M, Das S, Britt RP, Singh S, Ross C, Jagannathan L, Kaul R, Shukla DK, Muthuswamy V. Prevalence of β -thalassemia and other hemoglobinopathies in six cities in India: a multicentre study. *J Community Genet* 2013;4:33-42
7. Balgir RS. Genetic epidemiology of the three predominant abnormal hemoglobins in India. *J Assoc. Physicians of India.* 1996, 44 (1) 25-28
8. Urade BP. Sickle Cell Gene Scenario in tribal India. *J Health Med Inform* 2012 3:114 doi:10.4172/2157-7420.1000114
9. Old J, Angastiniotis M, Eleftheriou A, Hartveld C, Petrou M, Traeger-Synodinos J. Prevention of Thalassaemias and other Haemoglobin Disorders Volume 1: Principles. 2nd Edition. 2013 Published by Thalassaemia International Federation, Cyprus
10. Chandy M. Developing a National Programme for India. In: Control and management of Thalassemia and other Hemoglobinopathies in the Indian Subcontinent_ Synoptic Views. Editor: Ghosh K, Colah R. Published by National institute of Immunohaematology, 2008.
11. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. *Bull World Health Organ* 2008;86(6):480-487.
12. Giordano, P.C, Rachmilewitz E, The price of mercy. Editorial to the paper entitled "Prevention of β -thalassemia in Northern Israel- A cost- benefit analysis. *Mediterr J Hematol Infect Dis.* 2014; 6(1): e2014022

13. Services for the Prevention and Management of Genetic Disorders and Birth Defects in Developing Countries. Report of a joint WHO/WAOBD meeting, January, 1999.
14. Verma IC, Saxena R , Kohli S. Past , present and future scenario of thalassemic care & control in India. Indian J Med Res. 2011 Oct;134(4): 507-521.
15. Amato A, Cappabianca MP, Lerone M, Colosimo A, Grisanti P, Ponzini D, Di Biagio P, Perri M, Gianni D, Rinaldi S, Piscitelli R. Carrier screening for inherited haemoglobin disorders among secondary school students and young adults in Latium, Italy. J Community Genet. 2014 Jul; 5(3):265-8.



SECTION B

GUIDELINES FOR PREVENTION OF HEMOGLOBINOPATHIES

**(THALASSEMIAS AND VARIANT
HEMOGLOBIN DISORDERS)**





SECTION B

GUIDELINES FOR PREVENTION OF HEMOGLOBINOPATHIES

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GUIDELINES FOR PREVENTION OF HEMOGLOBINOPATHIES

(THALASSEMIAS AND VARIANT HEMOGLOBIN DISORDERS)

A. INTRODUCTION

Hemoglobinopathies may be either qualitative or quantitative defects of hemoglobin. The major hemoglobinopathies consist of thalassemias (mainly α and β thalassemias) and variant hemoglobins (HbS, HbE, HbD Punjab etc). In India, the major symptomatic hemoglobinopathy disorders are β (beta) thalassemia and Sickle Cell Anemia. They result in clinical syndromes known as Thalassemia Major (TM), Thalassemia Intermedia (TI) and Sickle Cell Disease (SCD). These guidelines pertain to prevention of major hemoglobinopathy syndromes- Thalassemia and Sickle Cell Disease.

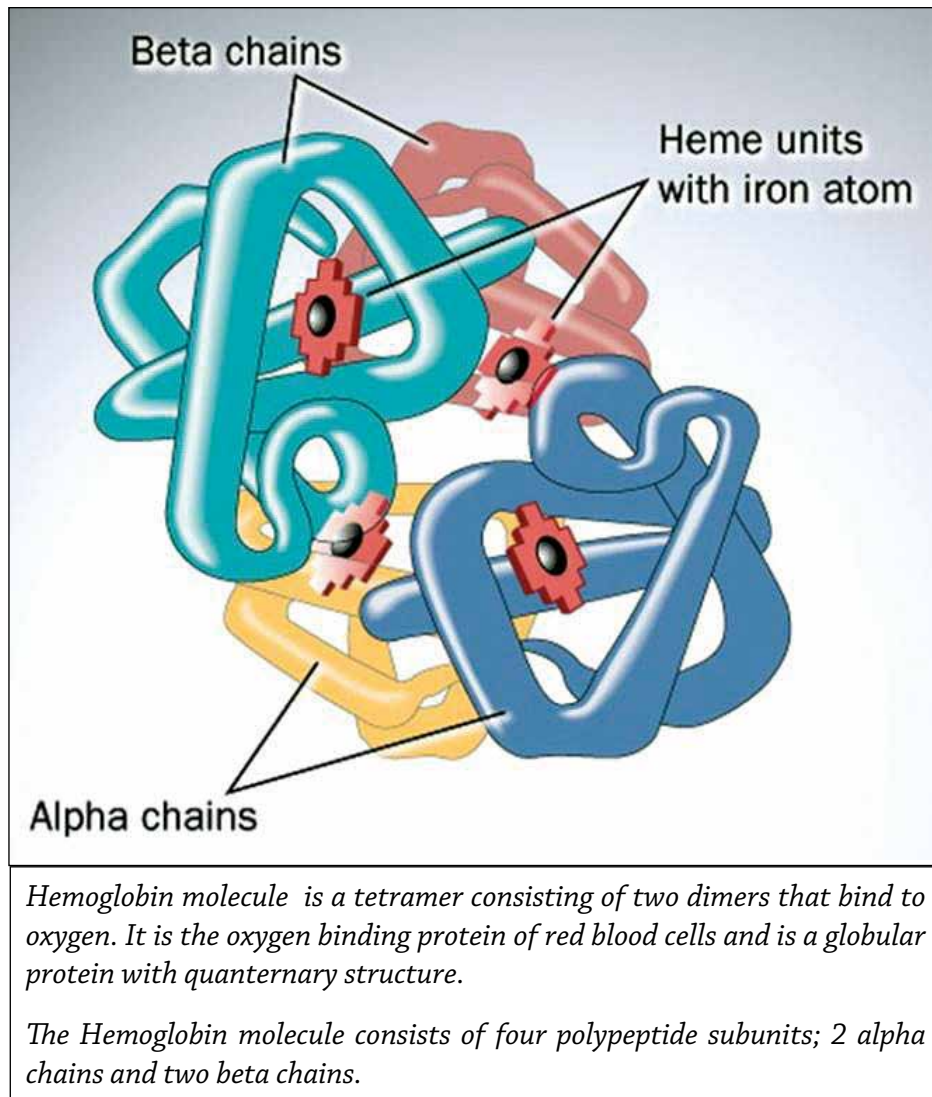
Table 1:
Common terms used in context of Hemoglobinopathies

Genotype	Genetic constitution of an individual
Phenotype	It is the detectable physical or clinical expression of genotype as a result of its interaction with environment
Mutation	It is a permanent inheritable change in a gene that definitely alters the genotype but may or may not alter phenotype
Allele(s)	The altered genes produced are called 'alleles.' Even normal genes are referred to as alleles. As genes exist in pairs, alleles are also in pairs
Heterozygous (Heterozygote)	Condition (individual) with one mutant and one normal allele
Homozygous (Homozygote)	Condition (individual) with similar mutant alleles on both chromosomes
Compound Heterozygous	Condition with different types of mutant alleles on both chromosomes.
Recessive allele	An allele that causes disease only in homozygous or compound heterozygous state
Dominant allele	An allele that causes disease in heterozygous state
Mutant allele	An allele carrying a mutation
Carrier / Trait	A heterozygote for a recessive allele is also referred to as 'carrier' or 'trait' e.g. ' β - Thalassemia Trait' (BTT) or 'Hb S Trait'. BTT is also referred to as Thalassemia Minor
β^0 thalassemia	Thalassemia syndrome in which there is complete absence of normal hemoglobin, HbA.
β^+ thalassemia	Thalassemia syndrome in which some amount of normal hemoglobin, HbA is present

A.1 HEMOGLOBIN STRUCTURE

Each hemoglobin molecule has a 3D structure composed of helical polypeptide chains. Hemoglobin consists of four polypeptide subunits; 2 alpha chains and 2 beta chains (see figure 1). Hemoglobin transports oxygen in the blood from the lungs to the rest of the body. These bind with iron ions to carry oxygen. Each heme molecule can carry four molecules of oxygen.

Figure 1: Structure of Hemoglobin molecule



A.2 GENETICS

At the genetic level the normal adult hemoglobin molecule is produced by two beta (β) and four alpha (α) globin genes. The normal adult hemoglobin molecule or HbA is composed of two alpha and two beta subunits ($\alpha_2\beta_2$), only then it can work or function normally (Figure 1, Table 2). If one of the two β globin genes is not working or functioning perfectly well, then the individual is called a carrier of β thalassemia. In case the mutant gene gives rise to a variant hemoglobin, the individual is a carrier of that variant Hemoglobin like HbS, HbE or HbD.

Table 2:
Normal Hemoglobin and its Genetics

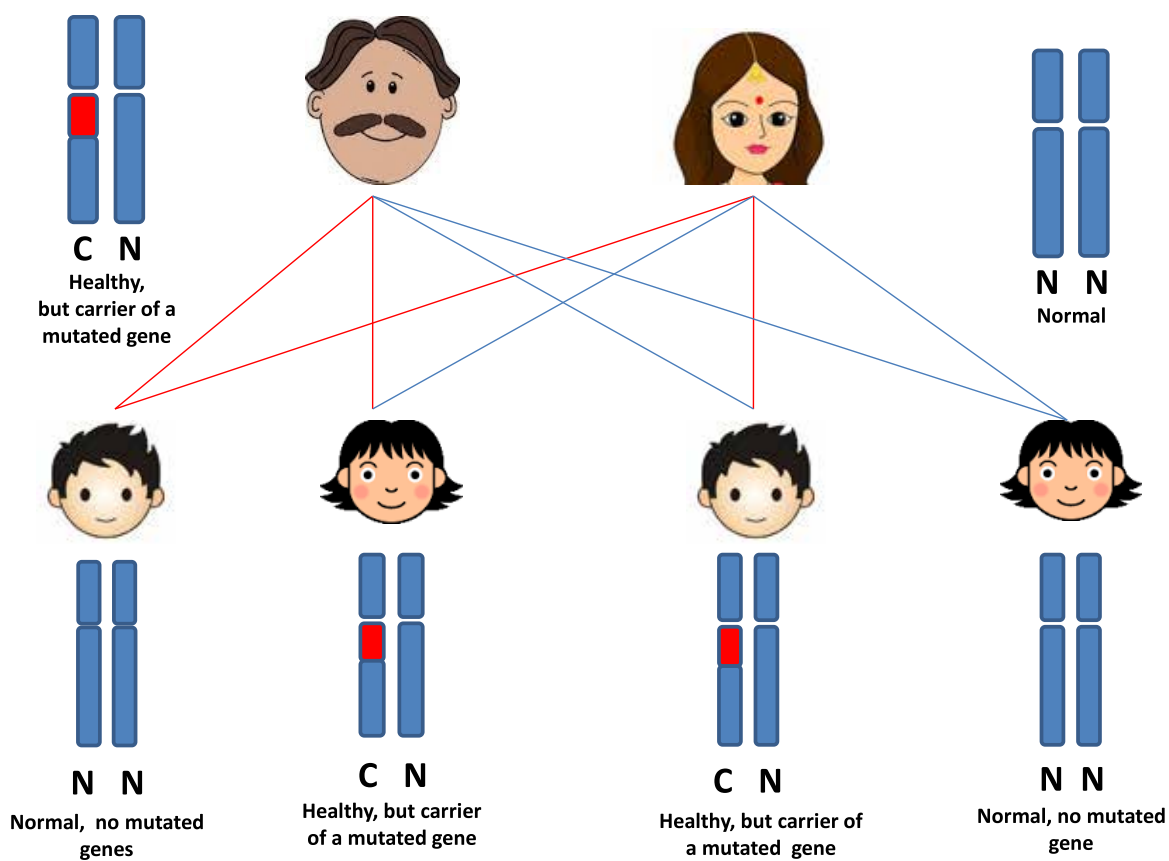
<p>When the two β- and the four α-globin genes that produce normal adult hemoglobin (HbA, $\alpha_2\beta_2$) work or function normally then the individual is normal.</p> <p>That is- they are neither asymptomatic carriers nor suffer from any of the hemoglobinopathies.</p> <p>Any alteration in a gene that leads to change in genetic composition, but may or may not alter its function, this alteration is called a 'mutation'.</p>	Normal Adult Hemoglobin: HbA ($\alpha_2\beta_2$)															
	alpha chain				alpha chain				beta chain				beta chain			
	There are 4 alpha genes: $\alpha\alpha/\alpha\alpha$ two on each chromosome								as well as 2 beta genes : β/β one on each chromosome							
	If one or two genes are absent/non-functional ($\alpha\alpha/\alpha\alpha^0$) or ($\alpha\alpha/\alpha^0\alpha^0$) then, he/she is a carrier								If one gene is absent/nonfunctional ($\beta\beta^0$) then he/she is a carrier							
	*Each parent contributes one beta gene and two alpha genes															
	Mother						Father									
	α	α	α	α	β	β	α	α	α	α	β	β				
	Child inherits from Mother						Child inherits from Father									
	α	α	β				α	α	β							
	Two α and any one β						Two α and any one β									

A.3 INHERITANCE

When one of the parents carries a mutated β -globin gene, i.e. when he/she is a β -thalassemia carrier (heterozygote) and the other parent carries two normal functional β -globin genes, then each child born to these parents (i.e. at every pregnancy) has a one-in-two (50%) chance of inheriting the mutated β -globin gene from the carrier parent and thus becoming a carrier (fig 2A). **Figures 2 (A-F). Shows inheritance patterns of different hemoglobinopathies and variant hemoglobins. Where N depicts the normal allele and C denotes the carrier mutant allele.** If both the parents have mutations in the beta-globin gene, the offspring has 25 % risk of inheriting two mutant alleles from his parents. When both genes are affected the child is homozygous for mutant genes and presents with severe disease -Thalassemia Major or Intermedia depending on the severity of the mutations and some other modifying factors(Fig 2B). If the mutation in the beta-globin gene causes production of a variant hemoglobin such as HbS, then the child will suffer from Sickle Cell Disease (Fig. 2C).

Fig 2A

The figure depicts the inheritance pattern outcome if only one parent is a carrier of the β thalassemia mutant allele and the other parent is normal. The blue colour depicts the normal allele, the red is for mutant β thalassemia allele.

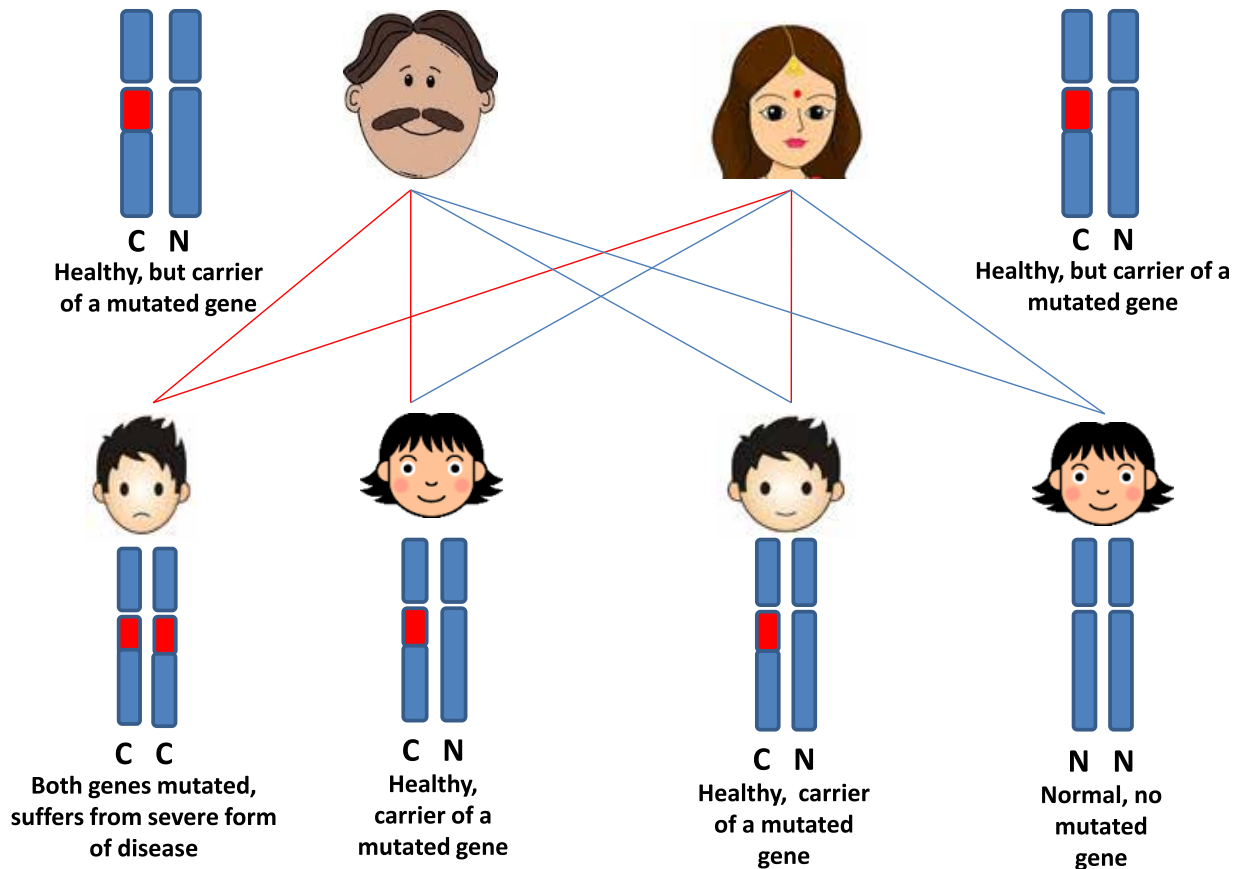


Each child has-

- 50% risk of inheriting one mutant allele (CN=Carrier).
- 50% chance of inheriting both normal alleles (NN=Normal).

Fig 2B

The inheritance and risk of having a child born with thalassemia major when both parents carry the mutant thalassemia allele (both parents are $\beta\beta$ thalassemia trait or carriers). The Blue colour depicts the normal allele, the red is for mutant β thalassemia allele.



The figure depicts the case scenario in which both the parents have one mutant allele for β Thalassemia, so they are asymptomatic carriers (beta thalassemia trait).

Each child has-

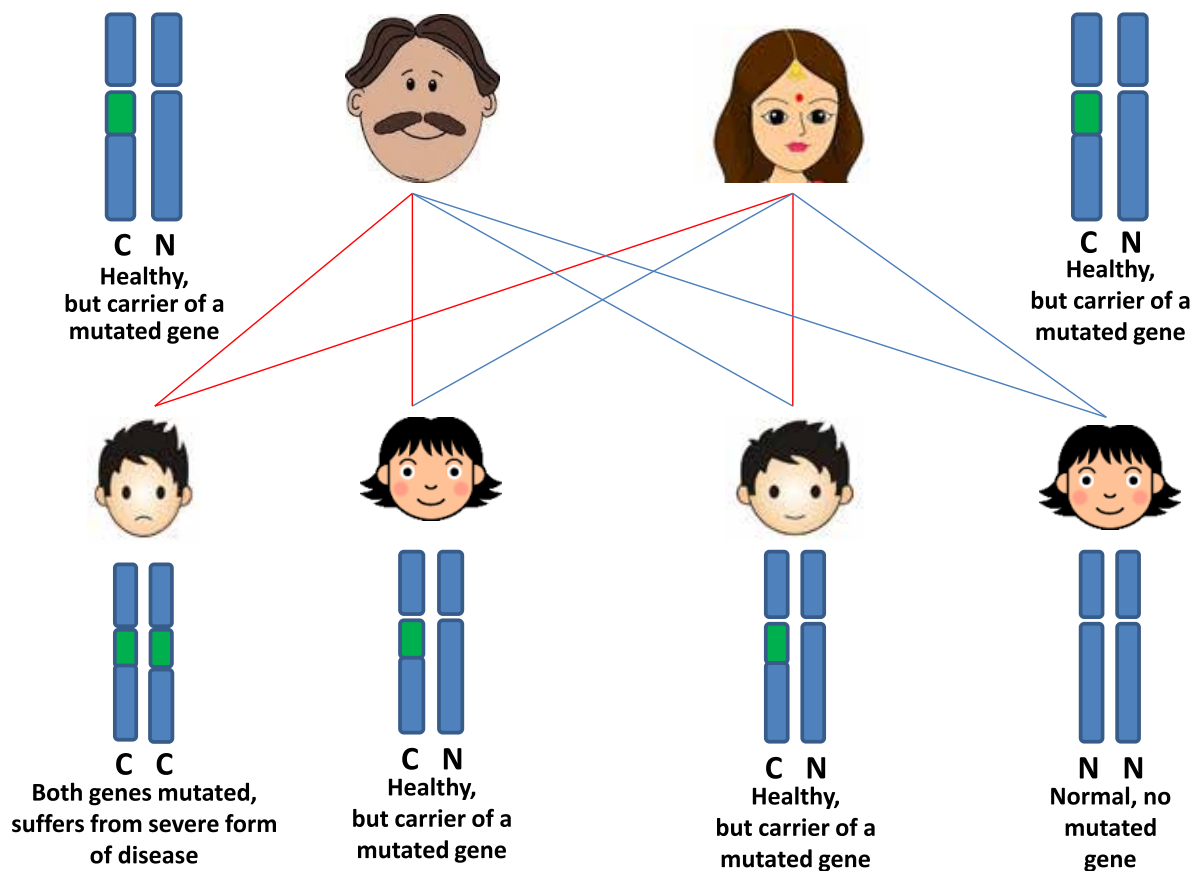
25 % chance of inheriting two altered alleles (CC=Thalassemia major).

50 % of inheriting one altered gene and one normal allele (CN=beta thalassemia trait),

25% chance of inheriting two normal alleles (NN=Normal).

Fig 2C

The inheritance and risk of having a child born with Sickle cell disease when both parents carry the mutant allele for sickle cell HbS. (both parents are sickle cell trait or carriers). The blue colour depicts normal allele, mutant allele is green in colour.

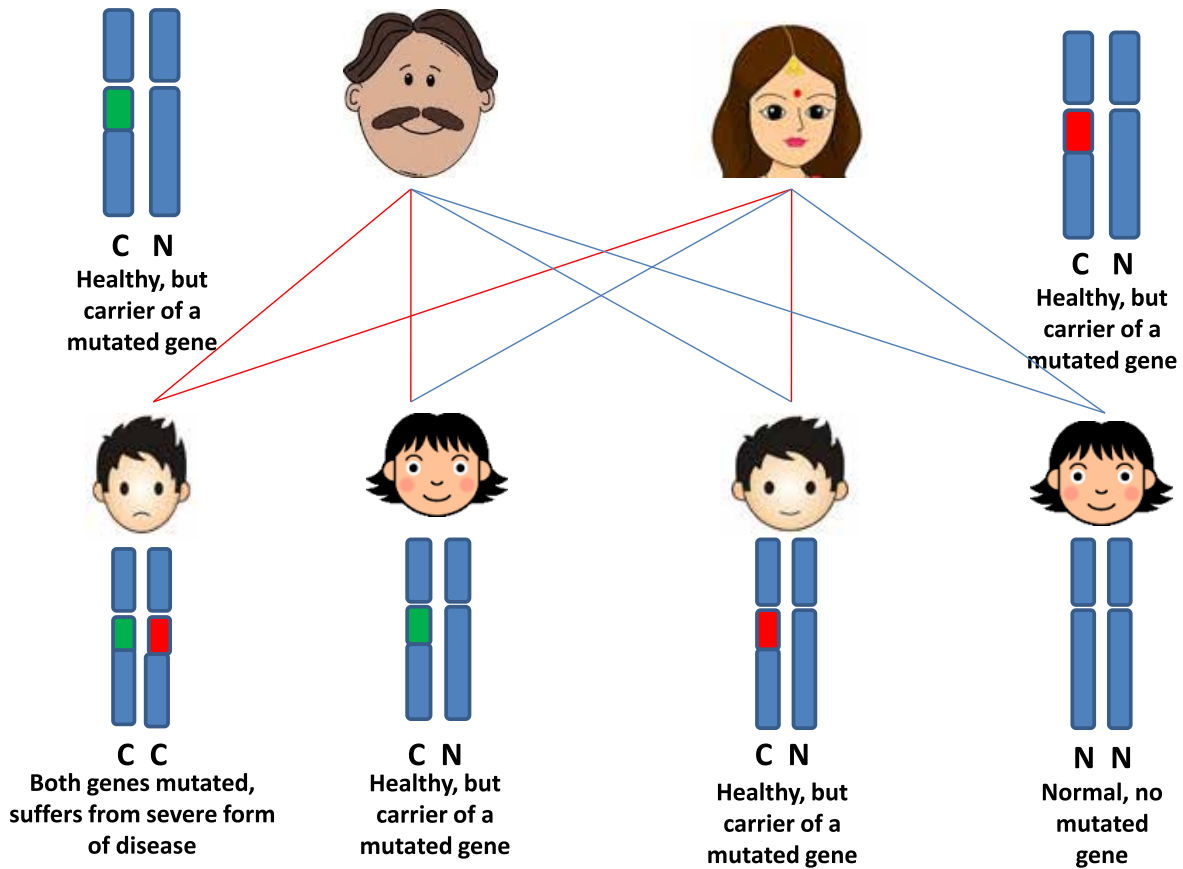


Each child has-

- 25% risk of inheriting both mutant alleles (CC=Sickle Cell Disease)
- 50% chance of inheriting the mutant allele (CN= Sickle cell trait or carrier)
- 25%chance of inheriting both normal alleles (NN=Normal)

Fig 2D

Inheritance pattern when both parents are carriers for two different hemoglobinopathies e.g. HbS and Beta thalassemia. The blue colour depicts the normal allele, the green is for mutant allele for sickle cell Hb, and the red is for the β thalassemia allele.



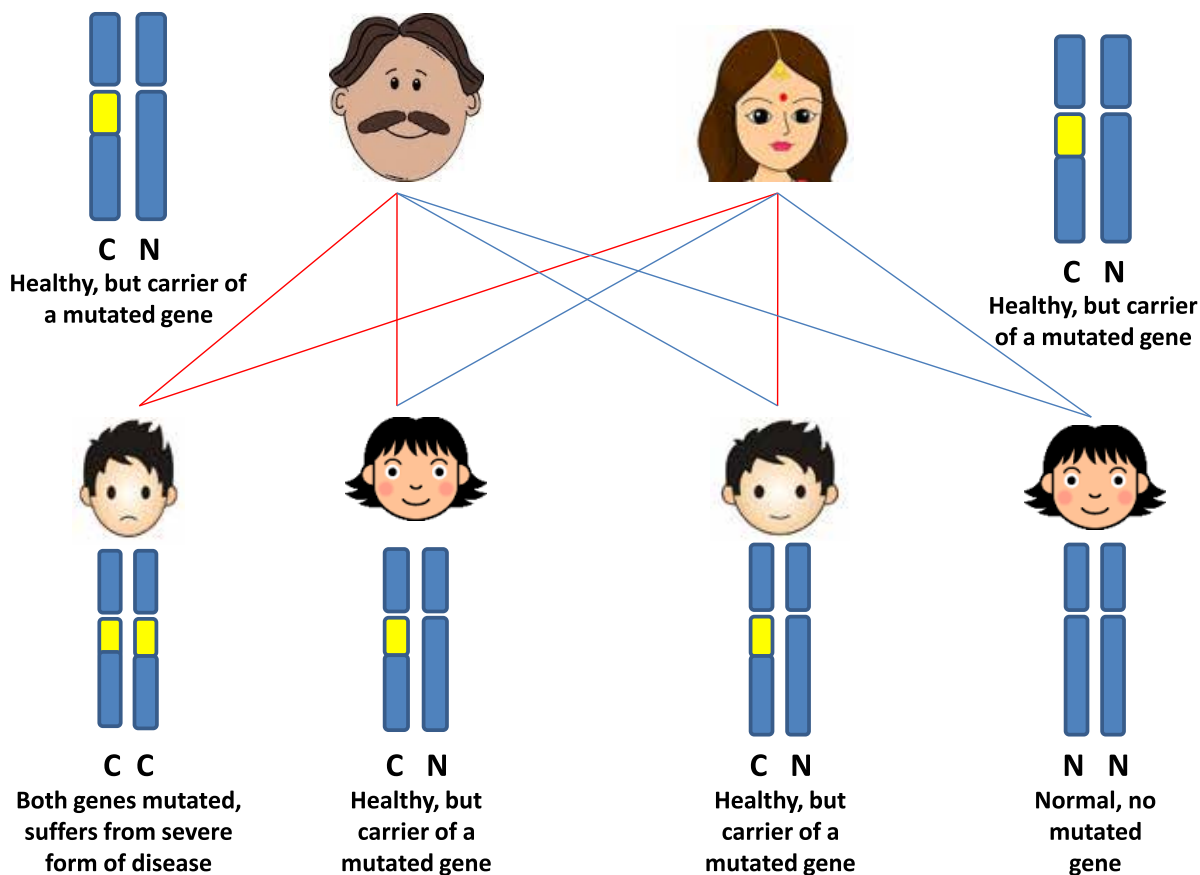
Each child has-

- 25% risk of inheriting two mutant alleles one HbS and one β (CC=two mutant alleles). This is a compound heterozygote state and the child suffers from HbS- β thalassemia
- 50% chance of inheriting one mutant allele (CN=Sickle cell trait or beta thalassemia trait).
- 25% chance of inheriting both normal alleles (NN=Normal)

Fig 2E

The inheritance and risk of having a child born with HbE disease when both parents carry the mutant allele causing HbE. (both parents are HbE trait or carriers).

The blue colour depicts the normal allele, the yellow is for mutant allele causing HbE.



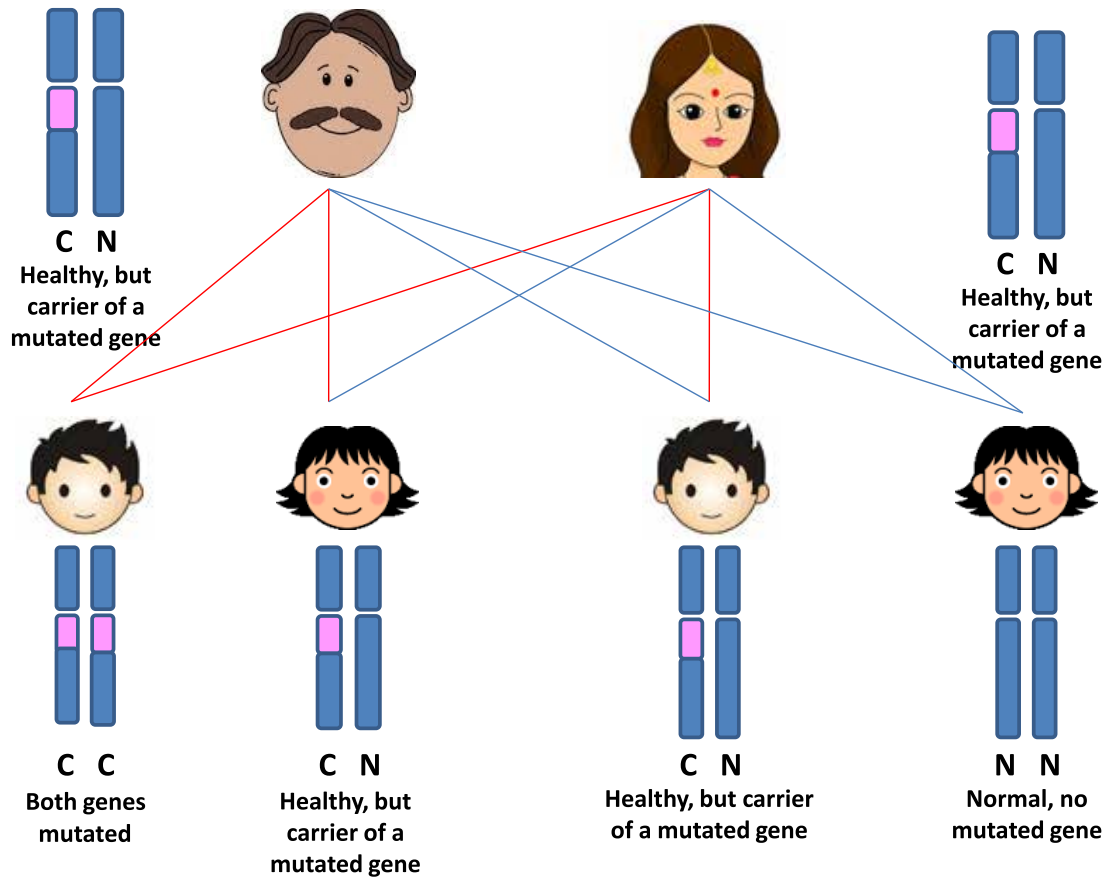
Each child has-

- 25% risk of inheriting both mutant alleles for HbE (CC= Homozygous HbE disease). *These patients may present as Thalassemia Intermedia.*
- 50% chance of inheriting one mutant allele for HbE (CN=HbE trait or carrier)
- 25% chance of inheriting both normal alleles (NN=Normal)

Fig 2F

The inheritance pattern when both parents are carriers for a mutant allele for HbD Punjab (both parents are HbD trait or carriers).

The Blue colour depicts the normal allele, light pink for mutation causing HbD.



Each child has-

- 25% risk of inheriting both mutant alleles for HbD (CC=Homozygous HbD)
This child presents usually only with mild anemia.
- 50% chance of inheriting the mutant allele for HbD (CN= HbD trait or carrier)
- 25% chance inheriting both normal alleles (NN=Normal)

Compound heterozygous states can also occur with other hemoglobin variants such as HbD Punjab/E disease, HbS/D Punjab disease and HbS / E disease etc. These can be found due to migration and inter marriages from previously identified hemoglobinopathy pockets across the country.

A.4 COMMON GENOTYPES ASSOCIATED WITH CLINICALLY SIGNIFICANT B-THALASSEMIA SYNDROMES AND SICKLE CELL SYNDROMES PREVALENT IN INDIA

Thalassemia Major and Thalassemia Intermedia may be caused by homozygous β^0 thalassemia or homozygous β^+ thalassemia alleles but may also be caused by various other compound heterozygous genotypes. HbE, HbD, Hb Lepore, $\delta\beta$ are some other mutant alleles associated with β thalassemias. Similarly Sickle Cell Disease may be caused by several other compound heterozygous genotypes. Inheritance pattern and clinical significance of some homozygous and compound heterozygous states, common in Indian populations are depicted in figures 2D,2E and 2F.

I. β -Thalassemia syndromes (TM and TI)

[β^T – indicating a β -thalassemia mutant allele]

1. β^T/β^T (includes β^0/β^0 , β^0/β^+ and β^+/β^+ genotypes)
2. $\beta^T/\text{HbLepore}$
3. $\beta^T/\delta\beta$
4. β^T/HPFH
5. β^T/β^E (HbE)
6. β^T/HbD

- All of the syndromes can be suspected on the basis of presence of increased proportion/ percentage of Fetal Hemoglobin (HbF) associated with moderate to severe anemia usually between 3-12 months of age and confirmed at 1 year of age.
- Homozygous β^0 and compound heterozygous E/β^0 genotype can be detected on newborn screening due to complete absence of HbA with confirmation of diagnosis at one year of age.
- Genotypes 5 and 6 can also be detected on newborn screening due to presence of variant Hemoglobins and confirmed at one year of age. Genotype 6 is usually asymptomatic or mild TI syndrome.

II. Sickle Cell Disease syndromes

[β^S -indicating the mutant allele for HbS]

1. β^S/β^S
2. β^S/β^T
3. β^S/β^D (HbD)

- These can be detected on newborn screening due to presence of variant Hemoglobins
- Screening for detection of asymptomatic heterozygotes or carriers is the basis of strategies for prevention of these β - thalassemia and sickle cell syndromes.



- III. Major asymptomatic carrier states of β -globin gene found in India (includes compound heterozygous states or homozygous states that are asymptomatic)
1. β -Thalassemia Trait (BTT);
 2. HbS Trait (Sickle Cell Trait) (mainly in Central, Southern and Western states)
 3. Hb E trait (mainly in North Eastern and Eastern India);
 4. Hb D trait (mainly in North India especially Punjab),
 5. $\delta\beta$ thalassemia trait,
 6. Hb Lepore trait
 7. HPFH Trait
 8. Compound heterozygote for Hb D and Hb E (β^D/β^E)
 9. Compound heterozygote for Hb D and β -Thalassemia (β^D/β^T)
 10. Compound heterozygote for Hb S and HbE (β^S/β^E)
 11. Compound heterozygote for Hb S and HPFH($\beta^S/HPFH$)
- Homozygous HbD (β^D/β^D) and HbE (β^E/β^E) genotypes are very mild syndromes presenting with mild or no anemia and may be detected only on screening for carriers.

B. DETECTION AND DIAGNOSIS OF HEMOGLOBINOPATHIES

Evaluation of patients and carriers of hemoglobinopathies is done by RBC indices and peripheral smear examination followed by examination of Hb pattern on HPLC or electrophoresis. Serum Ferritin values are taken into consideration where required. DNA analysis may be performed for confirmation of diagnosis. Three types of Hemoglobin are present in a normal individual-HbA, HbF (Fetal Hemoglobin), and HbA₂. At birth HbF is the predominant hemoglobin comprising approximately 80% of total hemoglobin and slowly reduces with rise in HbA. The HbA makes up the major component, comprising 96-98% of the total hemoglobin in an adult. Adult levels of all hemoglobins are reached by approximately one year of age and finally stabilize by two years of age.

B.1 TYPICAL LABORATORY FINDINGS IN THALASSEMIA (TM & TI) AND SICKLE CELL DISEASE

Clinical syndromes of β -thalassemias caused by reduction in HbA synthesis, show an increased proportion of HbF which forms the basis of their diagnosis as they manifest with reduction in total Hb levels causing anemia caused by decline in total HbA levels. In Sickle Cell Disease (SCD) presence of the variant Hemoglobin (HbS) along with varying amounts of HbF is the basis of diagnosis.

Table 3:
Typical findings in β^0 and β^+ Thalassemia (TM, Severe TI)
after 1 year of age and in Sickle Cell Disease

Tests	Findings in disease states	Normal values
Complete Blood Counts (CBC)	Severe anemia with microcytic hypochromic red cell indices (Hb<7g/dl; MCV:50-70fl; MCH: 12-20pg;)	Hb: 12-17 gm/dl MCV:80-100 fl MCH:27-32 pg
Peripheral blood smears	RBCs showing anisopoikilocytosis (tear drop cells, target cells), microcytosis hypochromia, and nucleated red cells markedly increased in relation to degree of anemia	Normocytic Normochromic
Hemoglobin HPLC	HPLC pattern in β -thalassemia	
	HbA : 0-30% HbF : 70-100% HbA2 : 2-5%	HbA: 96-98% HbF: <2% HbA2: 2.3-3.3%
	HPLC pattern in Sickle cell syndromes	
	HbA: 0-30% HbS: >50% HbF: <50% HbA2: <3.6% ((Only given here as typical finding of homozygous Hb SS. Details and differentiation in DEIC lab manual)	

Note: Hb is not reduced in all SCD. MCH and MCV are reduced in SCD due to Hb S/ β - thalassemia genotype. Peripheral smears may show irreversibly sickled cells

B.2 TYPICAL LABORATORY FINDINGS IN β -THALASSEMIA TRAIT (BTT) AND SICKLE CELL TRAIT

In carriers, the decrease in HbA is not enough to cause anemia but HbA2 has been consistently found to be increased in β - Thalassemia Trait and is the basis of detection of carriers of beta thalassemia. Those carriers who do not show an increase in HbA2 are referred to as 'silent carriers' and can be detected only by DNA analysis. These are missed by routine screening methods. Other carrier states can also be detected on the basis of HPLC pattern. Major criteria and cut -off values are provided in table 4.



Table 4:
Diagnostic criteria for Beta Thalassemia Trait (BTT) and other common carrier states

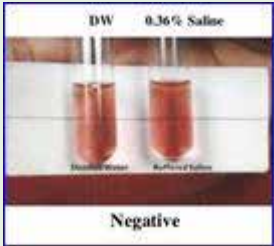
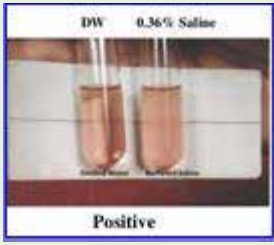


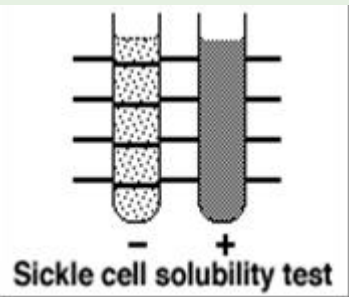


Genotype	HbA2	HbF	Variant Hb	MCV and MCH
Normal	2.3-3.5	<2.0		80-100fl and 27-32pg
β TT *	4.0-8.0	0.5-4.0%	-	Reduced
Hb Lepore Trait	<2%		5-15%	Reduced
$\delta\beta$ thalassemia trait	<3.0%	5-20%	-	Reduced
HPFH trait	Normal	15-30%	-	Normal
Hb S Trait	3- 4%		35-40%	Normal
Hb D Trait	Normal		40-45%	Normal
Hb E Trait	Normal		25-30%	Normal

*Values of HbA2 between 3.5 and 3.9% are considered equivocal and require detailed evaluation before a diagnosis of BTT can be made. Variant HbS may be as low as 20% and HbE as low as 15% when associated with α -gene deletions.

C. SCREENING FOR HEMOGLOBINOPATHIES IN PUBLIC HEALTH FACILITIES AND COMMUNITY SETTINGS

Carriers of beta thalassemia and other variant hemoglobinopathies are asymptomatic and hence have to be detected by the use of screening protocols using two or more tests. Cost effective testing protocols are used for detection of carriers in communities. Tests selected for initial screening are tests with low cost and high negative predictive value. Such tests have been evaluated and are recommended for screening for β -thalassemia, HbS and HbE. NESTROFT is the screening test used for detecting thalassemia trait (BTT), solubility test is used in screening for sickle cell (HbS) and DCIP test is used to screen HbE carriers (table 5). Complete Blood Counts provide highly valuable Red Blood Cell indices supporting the diagnosis of BTT related traits and for long have been used for screening of BTT. Hemoglobin fraction analysis by cation-exchange HPLC is the commonest test used worldwide for secondary screen or laboratory diagnosis of hemoglobinopathies. It provides percentage quantification of different Hemoglobin fractions. The DNA based tests identify the defect at the gene level and provide final confirmation of the defect.

Table 5:
Screening tests proposed for screening for carriers of hemoglobinopathies

NESTROFT Test (Naked Eye Single Tube Red cell Osmotic Fragility Test)	DCIP test (Di-Chlorophenol-Indo- Phenol)	Solubility Test	Hb test by Digital Hemoglobin meter
<p>For Beta thalassemia trait</p> <p>This test has a high specificity and sensitivity and is easy to perform. The positive test has to be followed by a confirmatory test</p> <p>Sensitivity of 91-100%, specificity of 85.47%.</p> <p>Positive predictive value of 66% and negative predictive value of 97-100%</p> <p>References: 3,4,6,14,15,</p>	<p>For hemoglobin E</p> <p>Carriers for hemoglobin E should not be missed in eastern states like Assam and Bengal. When married to a person with β Thalassemia trait, they can have a child who may require transfusion throughout his life.</p> <p>Screening test for hemoglobin E: 100% sensitivity, 98.7% specificity, positive predictive value of 98.6% and negative predictive value of 100%</p> <p>* Ref: Bull WHO, 2004; 82(5): 364-72</p>	<p>For Hemoglobin S,</p> <p>The solubility test is better for mass screening, because it is rapid (takes just about 5 min), reliable with minimal observer variation, does not need any microscope and requires very small blood sample.</p> <p>It is also a cost-effective test.</p> <p>The sensitivity is 100% while specificity is on an average 91.66%. Positive. Predictive value of 80% & Negative Predictive value of 100%.</p> <p>Ref: Journal of Research in Medical Education & Ethics 11/2012; 2(3):214-216</p>	<p>For Mild, moderate and severe anemia</p> <p>Treat Mild and moderate anemia as per guidelines.</p> <p>*Severe anemia needs to be referred whether picked clinically or through Hemoglobin meter.</p> <p>**The best predictor was a combination of definite pallor of the conjunctiva and pallor of the palms, with a sensitivity of 80% and a specificity of 85%</p>
<p>NESTROFT test : Negative</p>  <p>NESTROFT test Positive</p> 	 	 <p>Sickle cell solubility test</p>  <p>HbS</p>	<p>*Pallor has poor sensitivity for predicting mild anemia, but correlates well with severe anemia¹⁰.</p> 

Clinically it is very difficult to pick up mild and moderate anemia for which digital hemoglobinometer / WHO Hb scale is required. For the three hemoglobinopathy screening tests no special equipment is required and the total cost is nominal.

The screening protocol is a combination of tests used in a sequential manner to detect carriers.
Table 6 shows a general scheme of screening protocols in different public health settings.

Table 6:
Screening protocols for Hemoglobinopathies in community settings and public health facilities

Initial screening (1 and 2)	1) Test tube based Turbidity tests in Community settings (one or more tube tests may be included depending on prevalence)	NESTROFT (For BTT) SOLUBILITY TEST (For HbS) DCIP TEST (For HbE) & Hemoglobin estimation (*Hemoglobin estimation by Digital method/ WHO Hb scale etc)
	2) Based on RBC indices- MCV & MCH -in hospitals/ health centres where facility is available & Tube tests (one or more tube tests may be included depending on prevalence) Serum Iron and Ferritin studies to be done where required	Complete blood counts (CBC) (Samples positive for tube tests in field are also subjected to CBC in lab) NESTROFT (For BTT) SOLUBILITY TEST (For HbS) DCIP TEST (For HbE) Serum ferritin (reflects iron stores). <i>However in case of acute illness caution to be exercised as serum ferritin is an acute phase reactant</i>
Diagnostic test	Based on Hemoglobin fraction pattern by HPLC	Hemoglobin HPLC is used for diagnosis of thalassemia disease, variant Hemoglobin disease and thalassemia trait or variant Hemoglobin traits (e.g Sickle cell trait)
Confirmatory test	DNA Based tests	Reverse Dot Blot hybridization, ARMS, Gap PCR, DNA Sequencing -for unknown mutations <i>Specially to be used where HPLC is non contributory and for all prenatal diagnostic testing</i>

Following points are relevant in planning and implementation of screening strategies-

- Some of the homozygous and compound heterozygous states are also asymptomatic and may be detected only during screening. If detected they are to be reported as these mutant alleles may be transmitted to the offspring leading to a disease causing genotype. They may also lead to diagnostic errors.
- While β - thalassemia is prevalent almost across the country, variant hemoglobins-HbS, HbE and HbD are prevalent in certain populations and areas of some states.
- All population screening protocols have limitations and detection of all of the asymptomatic states is not possible.

- β - thalassemia carrier genotypes referred to as 'silent' carriers will not be detected on screening or by HPLC. Only carrier states with clear diagnostic cut off values are detectable. Some of the values will fall in equivocal range and may lead to missed detection. Similarly no primary or initial screening test is available for HbD.
- Presence of anemia and alpha thalassemia modifies the RBC indices and Hb fractions.

Clinically it is very difficult to pick up mild or moderate anemia. A digital hemoglobinometer or WHO Hb scale can be used. For the three screening tests for hemoglobinopathies no special equipment is required and the cost of these tests is nominal.

D. NEWBORN SCREENING

The objective of newborn screening is to detect infants at risk of sickle cell disease within the neonatal period, in order to allow early diagnosis and to improve outcomes through early treatment and care. It is essential that infants with these conditions are reliably diagnosed and that they are clearly reported as having a sickle cell disease so that the necessary clinical follow up is arranged. There is substantial evidence that early administration of prophylactic penicillin markedly reduces the incidence of pneumococcal sepsis in children with sickle cell anemia. There is also evidence that pneumococcal vaccines can increase immunity to pneumococcal infections in people with sickle cell disease. The analytical methods used will also detect some cases of β thalassemia major and related conditions. α and β thalassemia carriers will not be detected.

Newborn screening can be a part of the newborn dried blood spot screening. The service could be provided from Centralized Regional public laboratories in the District hospital lab or DEIC and could be integrated with those screening congenital hypothyroidism (CH), G6PD Deficiency and CAH. This may be co-ordinated with the local Pediatrics departments, or Hematology departments, if present.

D.1 REQUIREMENTS FOR NEWBORN SCREENING

Informed consent. An explanatory leaflet detailing the purpose, process and outcomes of newborn screening for sickle cell conditions must be provided to the parent(s) prior to screening. The purpose of screening should be explained by ANM or Staff Nurse during pregnancy and then again before taking the test. *In cases where the infant's parent(s) does/do not wish the child to be screened for sickle cell disease (or any of the other conditions), the decision to opt out of testing must be specifically documented.*

Newborn sampling. The same dried blood spot card is used for sickle screening as for the other newborn screening programmes. For the complete and proper processing of the specimen, four good quality spots are required. *Ideally, the sample should be dispatched to the newborn screening laboratory within 24 hours of collection.* In normal circumstances the delay should not affect analysis using the techniques described below. However, in occasional cases if it has been kept in unsuitable conditions, excessive oxidation may occur rendering the sample unsatisfactory for analysis. The dried blood spot sample card should have complete information regarding demographics of the infant and the mother, including the baby's MCTS number and the place of delivery if available, regarding blood transfusion prior to sampling to avoid the error of analyzing the hemoglobin of transfused red cells, gestational age of the infant and rank, if a multiple birth.



Information about the family origin of the parents and the mother's antenatal screening test results may be needed to help interpret the results and for quality assurance purposes.

D.2 TECHNIQUES USED FOR ANALYSIS OF NEWBORN SAMPLES

There are three techniques commonly used for analysis of Newborn dried blood spot sample as primary screen-

High performance liquid chromatography (HPLC)

Isoelectric focusing (IEF)

Capillary Electrophoresis (CE)

A second-line test on the same specimen using a different methodology is necessary to validate the initial findings. It is important to note that unequivocal identification of hemoglobin variants can only be achieved by either protein sequence analysis (e.g. using mass spectrometry) or analysis of DNA extracted from blood. It is also important to realize that occasionally the presumptive identification of a hemoglobin variant using screening methods is incorrect, since some variants give exactly the same results using current screening techniques. *Screening is not a Diagnostic service and no screening programmes have a diagnostic sensitivity and specificity of 100%.* However, with the techniques employed, Hemoglobin S, C, D (including D Punjab) and E should be detected reliably.

The basis of each of the techniques is briefly described below. (For details, the users may refer to DEIC Laboratory services manual)

High performance liquid chromatography (HPLC) utilizes an ion exchange resin, held in a column cartridge, in conjunction with a buffer gradient. The sample is injected in the cartridge and as the ionic strength and/or pH of the buffer changes different hemoglobins are eluted from the column, then are detected using a spectrophotometric technique and displayed as a chromatogram showing relative proportions of the different hemoglobins eluted. Quantification of the hemoglobin on the basis of relative proportions in the chromatogram help in differentiation of heterozygous, homozygous and compound heterozygous states. Hb F is eluted separately from HbA as are Hemoglobin S, C and D. HbE is eluted with HbA2 but is identified on the basis of a high proportion. However, different hemoglobins may elute at the same time requiring further testing by a second method.

Iso-Electric Focusing (IEF) gives good separation of Hb F from Hb A and variant hemoglobins S, C, D Punjab and E accomplished through application of a hemoglobin sample onto a precast agarose gel containing ampholytes at pH 6-8. Ampholytes are low molecular weight amphoteric molecules with varying isoelectric points (pIs). When an electric current is applied, these molecules migrate through the gel to their isoelectric points forming a stable pH gradient. The hemoglobin variants also migrate through the gel until they reach the point at which their pIs equals the corresponding pH of the gel. At this point, the net charges on the variants are zero and migration ceases. The electric field counteracts diffusion and the hemoglobin variants form discrete thin bands. IEF can be semi-automated, rendering the technique suitable for screening large numbers of samples.

Capillary electrophoresis (CE) utilizes a combination of ion migration and electro-osmotic flow to separate protein molecules. When a voltage is applied across the capillary tube filled with an electrolyte

solution, the solution begins to move towards one of the electrodes due to electro-osmotic flow. This drives the bulk flow of materials past the detector in the same way that a pump pushes the liquid in HPLC. The hemoglobin molecules move towards the detector at different speeds depending on their ionic charge and electrophoretic mobility. The combination of electro-osmotic flow and electrophoretic mobility (separate phenomenon) is exploited in CE for maximum separation of the hemoglobin fractions. Even so, different hemoglobins may migrate at the same rate and appear at the same position. HbF is separated from HbA. The Hemoglobin S, C, D, and E also have different mobility rates and characteristic profiles. In addition, the relative proportions of the different hemoglobin are recorded. It is therefore possible to detect the difference between carriers and affected infants.

HPLC and IEF are the preferred methods for newborn screening and may be chosen on the basis of cost-benefit analysis.

D.3 CLINICALLY SIGNIFICANT HEMOGLOBINOPATHIES THAT CAN BE DETECTED AT BIRTH

Large number of hemoglobin variants are detected using current screening methods. Those for which there is evidence that early intervention is likely to be beneficial can be specified as part of the national comprehensive Newborn Screening Programme.

**Table 7:
Hemoglobin patterns of clinically significant hemoglobinopathies at birth**

Homozygous and compound heterozygous genotypes causing SCD and TM that may be picked up in new-born screening are:	
Genotype (Phenotype)	Hb pattern on HPLC
Hb SS (SCD)	FS
Hb S/ β^{0*} thalassemia (SCD)	FS
Hb S/ β^+ thalassemia(SCD)	FSA
Hb S/HbD Punjab(SCD)	FSD
Hb S/E [#] (SCT)	FSE
Hb S/HPFH ^{**} (SCT)	FS
β^0/β^0 Thalassemia (TM)	F
Hb E/ β^0 thalassemia (TM)	FE

F=HbF; S=HbS; A=HbA; D=HbD; E=HbE.

SCD=Sickle Cell Disease; SCT=Sickle Cell Trait; TM= Thalassemia Major.

Hb fractions written from right to left starting from Hb fraction in highest amount]

HPLC is to be repeated at 1 year of age for confirmation of diagnosis

*It is not possible at birth to differentiate Hb S/ β^0 and Hb S/HPFH from Hb SS, as all of these conditions show similar pattern

In general Hb S/HPFH and HbS/HbE are regarded as milder conditions and behave as Sickle Cell Trait



Note: If the concentration of HbA on the bloodspot is abnormally high or comprises all of the hemoglobin present, then there is the possibility that a blood transfusion was given prior to taking the blood sample. Contamination of the bloodspot with adult blood as a result of poor practice should also be considered.

Additionally, in a small number of newborns other variants, mostly benign, may be detected which may not be immediately identifiable. As a policy, variants other than S, D and E should not be reported but the following specimens should still be sent for second line testing:

- Samples with 1.5% Hb A or less.
- Samples with variants (peaks) more positively charged than Hb A, i.e. eluting after Hb A by HPLC and located to the right of HbA on capillary electrophoresis.

This will ensure that the samples with little or no normal adult hemoglobin (Hb A) have the result confirmed before reporting and Hb S (or one of the designated hemoglobin) is not missed even if it falls outside the predefined analytical windows.

E. PRENATAL DIAGNOSIS-PREVENTING THE BIRTH OF AN AFFECTED CHILD OF “AT RISK COUPLE”.

Centre for Prenatal Diagnosis (PND): Hospitals with facility for obstetrical care, NICU and a genetic lab can serve as centres for PND. Testing can be done before a baby is born to find out if he or she has thalassemia. The biochemical and molecular methods to identify the particular phenotype/genotype is the key to PND. The first diagnosis of Hemoglobinopathies in utero was performed by using fetal blood samples by globin chain synthesis analysis. Since there are 22 common mutations and as well as other rare ones causing β -thalassemia in Asian Indians, the point mutation detection by reverse dot blot (RDB), allele-specific oligonucleotide hybridization for common mutations along with the amplification refractory mutation system (ARMS) technique was developed for PND.

Genetic recombination technique was used for the first time for diagnosing β thalassemia from amniotic fluid cell's DNA. Development of early and safe CVS has enabled PND to be undertaken in the first trimester of pregnancy. Though there is still a margin of error and precautions to prevent maternal contamination and other stringent care is necessary to not miss an affected child.

The pre-requisites of prenatal sampling include:

- Thalassemia carrier status of the couple under investigation.
- Blood group of the mother to prevent Rh incompatibility, if present.
- Pre test counseling and written informed consent of the couple undergoing the test.

There are three fetal sampling methods available for prenatal diagnosis:

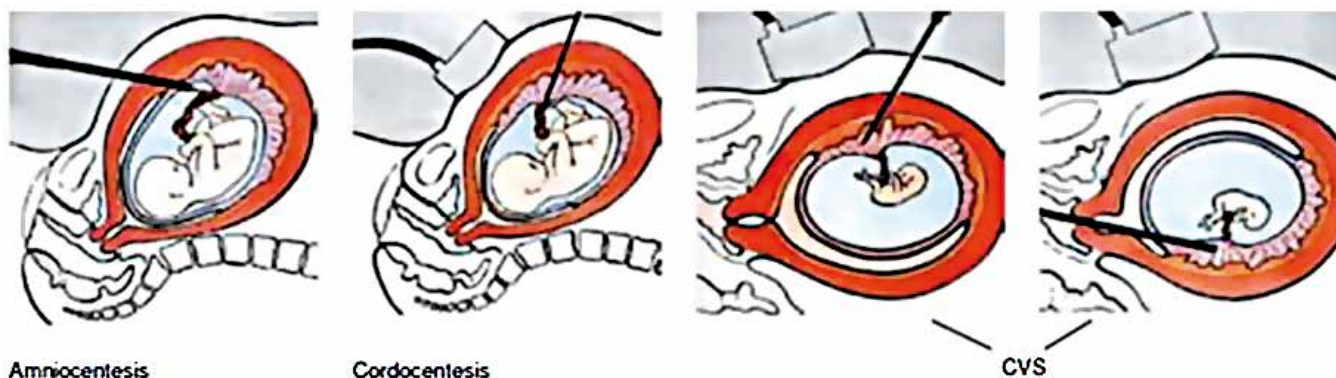
1. CVS
2. Amniocentesis
3. Fetal blood sampling.

All of three fetal sampling methods are conducted under ultrasound guidance.

1. **Chorionic villus sampling (CVS):** Using ultrasound as a guide, the specialist obstetrician 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.
2. **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.
3. **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.

Fig 3:

Diagram to show pictorial representation of the three different sampling techniques for obtaining sample for sending to the laboratory for prenatal testing (CVS, amniocentesis and fetal blood sampling).



The choice available to an 'at risk' couple: Today, parents who are aware that they are both carriers of β -thalassemia or Sickle Cell disease have a number of choices with regard to having a family. These should be discussed as early as possible with an expert health professional and/or a genetic counselor and include:

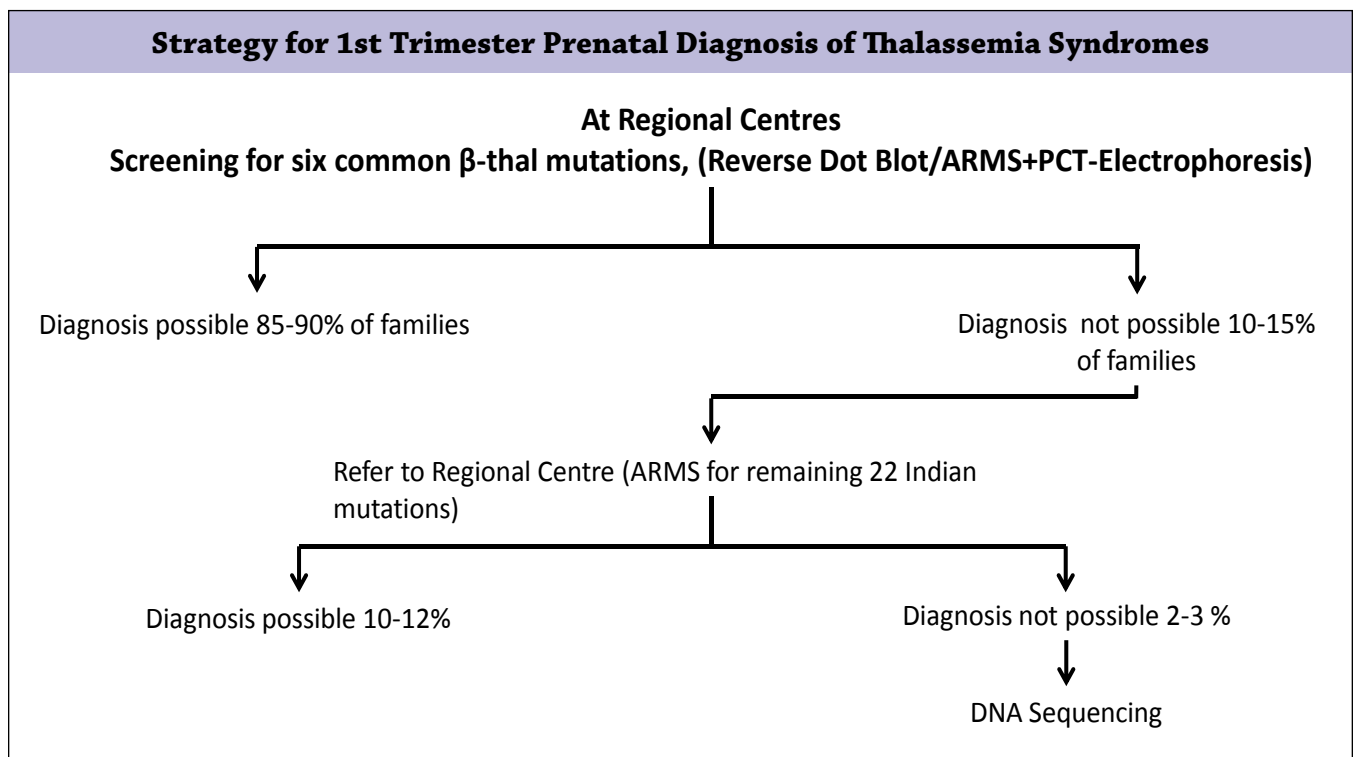
Prenatal testing is a choice to many families. The mutation studies are performed and then the doctor proceeds to find out whether the fetus is affected or not and then the family is given the option of pregnancy intervention (termination) for an affected child.

Where and if this is culturally, ethically and religiously accepted by the couple and the country;

- Not to have children;
- To adopt children;
- To proceed to in-vitro fertilization (e.g. PGD).

Limitations of prenatal diagnosis: No tests are absolute (2% errors reported). Termination of pregnancy in case of an affected child, which is difficult for the mother, and may be ethically unacceptable for many people. Post-test counseling is extremely important to help the couple cope with emotional stress due to attachment towards their yet unborn child.

Fig. 4.
Algorithm for Prenatal diagnosis



F. SELECTION OF TARGET POPULATION AND STRATEGIES FOR SCREENING

No single strategy can meet the needs of every population. Selection of target population and timing of screening are important determinants of outcomes of prevention and control strategies as they determine the options available for prevention and control and guide community education and counseling needs. They also identify issues related to compliance and participation of the target population in screening programmes. The table 6 illustrates advantages of different screening timings.

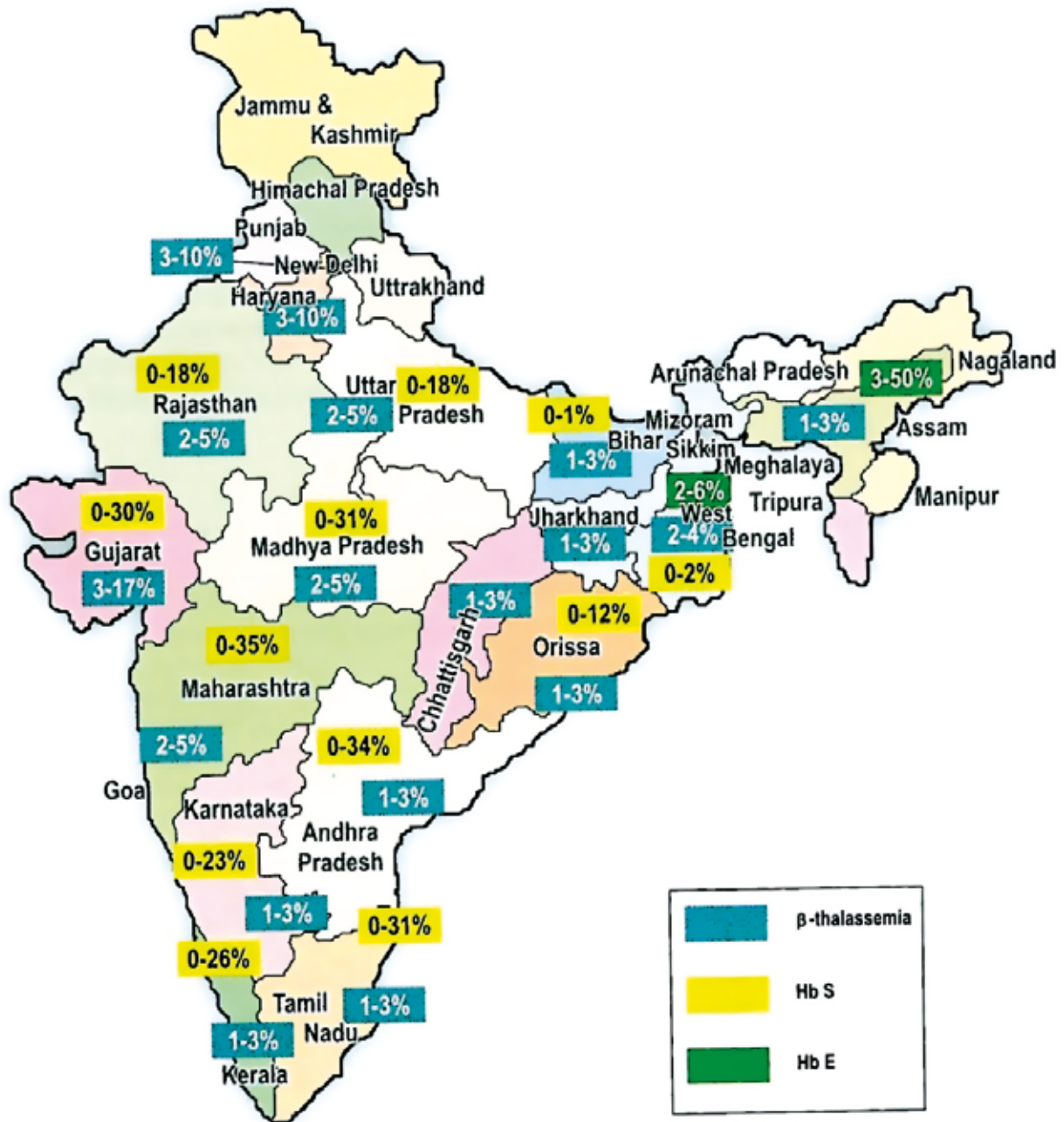
**Table 8:
Timing of screening for Hemoglobinopathies**

Newborn	Suitable for screening for Sickle Cell Disease and few cases of Thalassemia major
Adolescence	Most suitable for carrier screening, as a long term sustainable strategy.
Premarital	Carrier screening at this stage is effective in a well-informed community
Preconception	Carrier screening is effective in communities where termination of pregnancy in case of affected fetus is permitted. Married couples can also seek pre-implantation genetic diagnosis if available
Antenatal screening & Prenatal diagnosis (PND)	Serves as a net to screen those who have not been screened at earlier stages. If both parents are carriers i.e. “at-risk” couple : then the status of the fetus for Thalassemia disease or sickle cell disease can be ascertained through prenatal diagnosis

- Limitations of each strategy should be taken into consideration when planning and implementing a screening programme.
- Newborn screening provides an opportunity for early detection of Sickle Cell Disease and some severe forms of Thalassemia.
- Adolescent screening provides an opportunity for screening of carriers before they have selected partner for marriage.
- Premarital screening provides an opportunity to a carrier to make an informed decision before going into marriage.
- Pre-conceptual and Antenatal screening provides an opportunity to a carrier to avoid giving birth to an affected baby.



Fig 5.
Showing the usual reported prevalence of hemoglobinopathies from India⁶



G. SUMMARY GUIDELINES FOR IMPLEMENTATION OF PREVENTION AND CONTROL STRATEGIES

From the previous discussions it is obvious that all the screening approaches have their benefits and limitations. Broadly screening can be divided into two groups on the basis of expected outcomes-

- Screening for early detection of Thalassemia (TM and severe TI) and Sickle Cell Disease to achieve reduction in mortality and morbidity with improvement in quality of life of the affected.
- Screening for detection of carriers of β -Thalassemia Trait and Sickle Cell Trait to reduce birth of children affected with Thalassemia or Sickle Cell Disease.

In India all of the approaches need to be adopted and applied as below:

**Table 9:
Screening guidelines for implementation**

Timing of screening available for hemoglobinopathies	
Group I	Screening utility and strategy for early detection of disease - Thalassemia major/ intermedia and Sickle cell disease in newborn and children
Newborn	<p>Universal screening for genotypes with clinically significant SCD: β^S/β^S, β^S/β^0, β^S/β^+, β^S/β^D by Dried Blood Spot (DBS) sampling.</p> <p>Reporting of suspected cases of Thalassemia Major due to β^0/β^0, β^E/β^0 genotype manifesting as complete absence of HbA in the Hb pattern. Repeat sample for confirmation on follow up at 12 months of age</p> <p>Other conditions may be detected as a by product of newborn screening that require to be reported are carriers of variant hemoglobins : HbS Trait (β/β^S), HbD (Trait β/β^D), HbE Trait (β/β^E) and clinically insignificant compound heterozygotes - $\beta^S/HPFH$, β^S/β^E.</p>
Childhood 6 months to 6 years	<p>Universal screening of all children with severe anemia (Hb <7 gm/dl) for Thalassemia Major. This strategy will also identify many cases, though not all, of Thalassemia Intermedia.</p> <p>As all children with clinically significant thalassemia develop severe anemia, restricting screening to this subgroup can be cost effective without the risk of missing severe thalassemia syndromes.</p> <p>Children with Sickle cell disease having severe anemia will also be detected.</p> <p>These children comprise preschool age group and can be reached through <i>Anganwadis</i>.</p>
Group II	Screening for Carriers of β-thalassemia trait (BTT or βTT)

Adolescence	<p>Universal screening of adolescents through schools in class VIII. It is easy to reach out to adolescents through schools and there is a high acceptance rate with retention of information among students when backed by intense education programmes including lectures in school before screening. The approach helps in removing stigma due to applicability to all students. Growing into adulthood with knowledge of carrier status provides time to 'adapt' this information for choice of partner for marriage. Carriers unable to make a choice of avoiding marriage with another carrier still have the option of prenatal diagnosis and other options for carrier couples to be exercised later.</p> <p>The experience of the adolescent screening programme in government schools, implemented as part of a pilot project on Birth Defects in Uttarakhand; has also been positive with high acceptance rate and retention of information by the students.</p>
Premarital	<p>Screening to be offered to those individuals or couples who seek it as social norms vary from community to community, family to family, it might be welcomed by some but not by others.</p> <p>Though the approach presents both options to a carrier - that is of not going ahead with marriage or option for prenatal diagnosis later in each of the pregnancies. Choosing the first option becomes a delicate issue at this stage and will depend on the families and the couple's level of emotional bonding. Religious beliefs and customs may also influence.</p>
Pre-conceptional	<p>Screening to be offered to those individuals or couples who seek it with appropriate genetic counseling. Though most will opt for prenatal diagnosis with termination of pregnancy, some with religious restrictions might find the option of adopting a child better rather than having their own child withstanding the 25% risk factor. Couple can also seek Pre Implantation Genetic Diagnosis (PGD) if available.</p>
Antenatal	<p>Universal screening should be offered to all pregnant women during first trimester at all levels. As most pregnant women are likely to come in contact with health services, uptake is expected to be high. States providing it should back it up with prenatal diagnostic services either through referral network or by developing centres within their health system.</p>
Cascade screening	<p>Screening of siblings and extended family members of patients and carriers of β-thalassemia and variant hemoglobins. Cascade screening of families of detected carriers is an integral component of the screening strategy protocol. In the Uttarakhand project it was found to be more effective in adolescent screening where it was perceived as an extension of benefit of school programme to siblings and extended family. Some families with an affected child may be either unwilling to communicate this diagnosis to their extended family and occasionally misinformed members of the extended family may try to distance themselves from the family with affected child, hence counseling is necessary .</p>

Montreal Screening programme implemented in Canada in High School students (equivalent to Indian class 11, 12 students) has been considered as the gold standard for a population screening programme.

H. IMPLEMENTATION OF PREVENTIVE STRATEGIES IN INDIA

Different strategies are to be employed at primary level centres, secondary level centres (District Hospital or DEIC), State medical colleges and Tertiary level hospitals.

1. Primary level- PHC, CHC and in community settings

- Antenatal screening for carrier status (early 1st trimester) in all women by tube tests and hemoglobin estimation. Any woman with a positive tube test or severe anemia needs to be referred to District hospital by 104 / 108 service for further investigations including CBC and HPLC. If she is found to be a hemoglobinopathy carrier, then her husband is to be tested for his carrier status. ***If both are found to be carriers then referral to a higher centre is required for prenatal diagnosis before twenty weeks of pregnancy for an informed decision regarding continuation of pregnancy.*** Pre-conception identification of carriers can be done at sub-centres by ANMs.
- **Screening of adolescents for identification of carriers before marriage** through universal screening in schools coupled with extensive education, awareness and counseling programmes to avoid marriages between two carriers or avail prenatal diagnosis after marriage. Ensure community sensitization and participation for successful implementation of this strategy. The tube based screening tests and clinical detection of pallor may also be done at AWCs (for out of school children) by RBSK Team.
- **Newborn screening**-universal new born screening to be implemented in areas with high prevalence of Sickle cell hemoglobin for screening of sickle cell disease. This may also identify sickle cell carriers, other variants and few cases of thalassemia major. ***Dried Blood spot samples to be obtained and transported to District or State level lab for testing***
- **Cascade screening**- screening of extended family members of carriers and cases detected by any of the screening strategies.

2. Secondary Level -at the District Hospitals or DEIC Laboratory

Tests to be done:

- RBC indices through three - part cell counter
- Tube based screening tests (NESTROFT, Solubility test and DCIP test)
- Hemoglobin HPLC for diagnosis of beta thalassemia and common variants
- Iso-electric focusing (IEF) for newborn screening in districts with high prevalence of HbS
- Serum ferritin, if required
- Peripheral smear, if required (Serum iron, TIBC if available)

Instruments required: HPLC, IEF equipment. Three part cell counter, Microscope and Elisa Reader
Detection of carriers is based mainly on Hb pattern on HPLC. Carrier States to be specifically screened for on the basis of given criteria (Table 4) are -



Beta Thalassemia Trait

HbS Trait

HbE Trait

Note: Other traits and asymptomatic conditions mentioned in the list that may be picked up in the course of screening are to be reported. In cases of diagnostic difficulties where cut off values are ambiguous they may be referred to State level, tertiary or referral centres.

3. State / regional level centres

Tests to be done:

- HPLC for identification of rare variants
- DNA analysis for detection of 8 common mutations by Reverse Dot Blot (RDB) hybridization and other common mutations by ARMS (Amplification Refractory Mutation System).

Screening for 8 common mutations may cover 80% of mutations in a population.

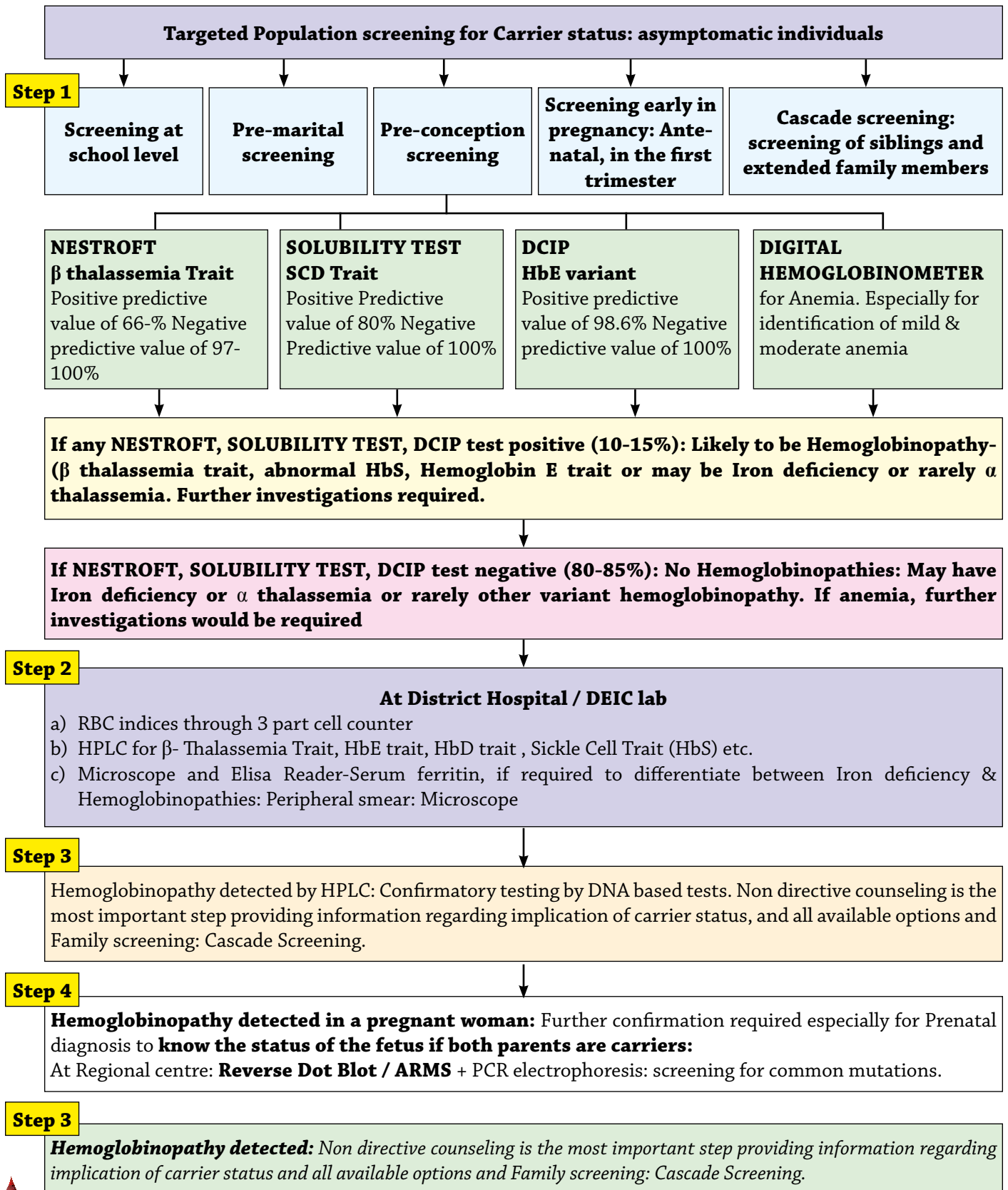
- Newborn screening for Sickle Cell Disease through Dried Blood Spot sampling
DBS samples may also be used for screening of Congenital Hypothyroidism, Congenital Adrenal Hyperplasia, and G6PD deficiency

4. Tertiary level / national referral centres:

Tests to be done:

- Identification of rare Hb variants not identified at State level labs, by capillary electrophoresis, HPLC or IEF
- DNA analysis by DNA sequencing or RFLP analysis for detection of rare mutations not detected at State level centres.

Fig 7. Algorithm for population screening for carriers of hemoglobinopathies



REFERENCES FOR GUIDELINES FOR PREVENTION OF HEMOGLOBINOPATHIES

- 1) Old J, Angastiniotis M, Eleftheriouthies A, Hartveld C, Petrou M, Traeger-Synodinos J. Prevention of Thalassaemias and other Haemoglobin Disorders Volume 1: Principles. 2nd Edition. 2013 Published by Thalassaemia International Federation, Cyprus.
- 2) Mehta BC. NESTROFT: A screening test for β thalassemia trait. *Indian J Med Sci* 2002;56:537-44.
- 3) Mamtani, M, Jawahirani, A et al., Bias-corrected diagnostic performance of the naked eye single tube red cell osmotic fragility test (NESTROFT): An effective screening tool for β -thalassemia. *Hematology* (2006) 11(4) 277-286.
- 4) Piplani S, Manan R, Lalit M, Manjari M, Bhasin T, Bawa J. NESTROFT- A valuable , Cost Effective Screening Test for Beta Thalassemia Trait in North Indian Punjabi Population. *J Clinical and Diagnostic Research* (2013) 7(12);2784-2787.
- 5) Giordano P. NEWBORN SCREENING FOR HEMOGLOBINOPATHIES. In : Angastiniotis M, Eleftheriou A, Galanello R, et al authors; Old J, editor Prevention of Thalassaemia and Other Haemoglobin Disorders: Volume 1: Principles [Internet]. 2nd edition. Nicosia, Cyprus: Thalassaemia international Federation; 2013. Chapter 10.
- 6) Ghosh, K Colah R. Editors: Control and management of Thalassemia and other Hemoglobinopathies in the Indian Subcontinent_ Synoptic Views. Published by National institute of Immunohaematology, 2008.
- 7) Bender MA, Douthitt SG. Sick Cell Disease. 2003 Sep 15 [Updated 2014 Oct 23] In: Gene Reviews [Internet] Seattle (WA) University of Washington, Seattle; 1993-2015.
- 8) Cao A, Galanello R, Oiga R. Beta Thalassemia. In: Pagon RA, Adam MP, Ardinger HH et al editors. Gene Reviews [Internet] Seattle (WA) University of Washington, Seattle; 1993-2015.
- 9) Mohanty D, Colah RB, Gorakshakar AC, Patel RZ, Master DC, Mahanta J, Sharma SK, Chaudhari U, Ghosh M, Das S, Britt RP, Singh S, Ross C, Jagannathan L, Kaul R, . Shukla DK, Muthuswamy V. Prevalence of β -thalassemia and other haemoglobinopathies in six cities in India: a multicentre study. *J Community Genet* (2013) 4:33-42.
- 10) Luby SP, Kazembe PN, Reddy SC, et al. Using clinical signs to diagnose anaemia in African children. *Bull World Health Organ*. 1995; 73(4):477-482.
- 11) Mitchell JJ, Capua A, Clow C et al: Twenty-year outcome analysis of genetic screening programs for Tay-Sachs and β -Thalassemia disease carriers in high schools. *Am J Med Genet* 1996; 59: 793-798.
- 12) Cousens NE, Gaff CL, Metcalfe SA, Delatycki MB. Carrier screening for Beta-Thalassaemia: a review of international practice. *Eur J Human Genetics*. 2010;18, 1077-83.
- 13) Raghavan K, Lokeshwar MR, Birewar N, Nigam V, Manglani MV, Raju N. Evaluation of naked eye single tube red cell osmotic fragility test in detecting beta Thalassemia trait. *Ind Paed*. 1991; 28(5): 469-72.
- 14) Thomas S, Srivastava A, Jeyaseelan L, Dennison D, Chandy M. NESTROFT as a screening test for the detection of thalassemia and common haemoglobinopathies - An evaluation against a high performance liquid chromatographic method. *Ind J Med Res*. 1996; 104: 194-7. [20].
- 15) Suri V, Sidhu P, Kanwal S, Chopra B. Evaluation of Naked Eye Single Tube Red Cell Osmotic Fragility Test NESTROFT as a screening test in detection of beta-thalassemia trait. *Int J Hematology and Blood transfusion*. 2001; (19):

ANNEXURE B-1

NAMES OF TESTS AND ASSOCIATED ACRONYMS USED IN DIAGNOSIS OF DISEASE AND CARRIER STATES OF HEMOGLOBINOPATHIES ALONG WITH THEIR BRIEF DESCRIPTION.

Test Name	Description
Solubility test	Estimation of Hemoglobin in gm % by digital Hemoglobinometer using a finger prick sample in field / screening point (school).
NESTROFT	Naked Eye Single Tube Red cell Osmotic Fragility Test in a single tube with a saline concentration of 0.36%. Can be done on finger -prick sample as screening test for selecting samples for Hb HPLC for detection of β Thalassemia Trait.
CBC	Complete Blood Counts are obtained by an automated Blood Cell Counter. Used for determination of Hb level and for RBC parameters (RBC, MCV, MCH, MCHC and RDW) for evaluation of type of anemia. MCV and MCH are the most important indices in diagnosis of thalassemia.
PS or GBP	Microscopic examination of a stained peripheral blood smear (PS) on a glass slide provides a General Blood Picture. Required to evaluate cases mainly of severe anemia and moderate anemia. GBP in thalassemia major and severe TI is quite characteristic and highly supportive of diagnosis.
Reticulocyte count	Reticulocytes (or Retics) are young RBCs identified by staining by supravital stains like New Methylene Blue. They are usually found to be increased in hemolytic anemias when there is destruction of normal population of RBCs. G6PD enzyme levels are normal in young RBCs even in G6PD deficiency thus a falsely normal or high level of G6PD enzyme may be obtained if test done after clinical symptoms have appeared



Solubility test	Used as a simple low cost screening test for sickle cell Hemoglobin (HbS) based on the property of insolubility of HbS in a high molarity phosphate buffer solution forming tactoids (water crystals) producing turbid solution. It does not distinguish between heterozygous or homozygous states. HbD and HbG showing similar mobility as HbS on electrophoresis are soluble. False positives are common due to polycythemia and other abnormal hemoglobins and high HbF may result in a 'false negative' test thus should be used only as a screening test. The test is unreliable up to 6 months of age due to high HbF and thus cannot be used for newborn screening
Sickling Test	It is a simple functional test for distinguishing Hb S disorders- HbSS; HbS/E; HbS / β^0 thal, HbS/ β^+ thal; HbS/HbD; from other variants having same mobility as HbS. The test is based on 'sickling' of RBCs in reduced oxygenation. There are some other rare variants other than HbS that also produce sickling.
DCIP Test	Di-Chloro-Indo-Phenol Test is a simple screening test for detection of HbE based on oxidation of the exposed -SH group by DCIP at neutral pH leading to precipitation of the variant hemoglobin leading to a particulate cloudy solution or precipitated HbE at the bottom of the tube observed by naked eye. The test is positive in other unstable hemoglobins also including HbH.
Serum Ferritin by ELISA	At some stage of the diagnostic protocol, it may become important to determine iron status to arrive at diagnosis. It may be necessary to exclude iron deficiency and in carriers of thalassemia and variant hemoglobins or to establish coexistent iron deficiency that may alter hematologic parameters. Normal or increased iron are found in thalassemia. Quantitative assay of serum Ferritin is a cost effective method for establishing iron deficiency.
Hb HPLC	The test based on automated High Performance Liquid Chromatography of Hemoglobin to separate different hemoglobin fractions is used for detection of Thalassemia and common hemoglobinopathies.
Newborn Hb-HPLC	Sickle Cell Disease and other hemoglobin variants. Hemoglobin fraction pattern at birth is very different from that at one year of age. Also for universal newborn screening Dried Blood Spot samples are used. Thus the HPLC equipment used for newborn screening for hemoglobinopathies is programmed for separation and analysis of Hb fractions from a dried blood spot sample of a newborn. Other than β^0
Newborn screening by IEF	Iso electric focusing (IEF) is used for detection of different hemoglobin variants. The test can be performed on Dried Blood Spot samples and thus is a suitable and cost effective alternative to Hb HPLC for newborn screening.
PCR based DNA Analysis	Detection of causative mutation is the confirmatory test for diagnosis of hemoglobinopathies. Even in abnormal hemoglobins like HbS, sometimes, a DNA analysis is required to identify the causative mutation as there are other variants that can cause sickling. Several PCR based methods most commonly Reverse Dot Blot Hybridization, and ARMS are used for detection of a limited number of known mutations, and DNA sequencing is used for unknown mutations.

ANNEXURE B-2

VARIOUS METHODS OF SAMPLING FOR NEWBORN SCREENING



1 Equipment: Heel warmer with tygon tubing, sterile alcohol prep, sterile gauze pads, and cloth. Wash collection heel, gloves.

2 Complete all information blood collection form page needs by allowing the site to be warm or contact with air prior to the blood collection or after blood collection keep "NEONATAL SCREENING" if applicable.

3 Wash heel (with alcohol prep) include site with heel warmer.

4 Warm site with cloth, soothed with warm water up to 37 C. for 10-15 min. (if available).

5 Cleanse site with alcohol prep. Wipe DRY with sterile gauze pad.

Neonatal Screening

Blood Specimen Collection and Handling Procedure

6 Prick heel. Wipe away first blood drop with sterile gauze pad. Allow another LARGE blood drop to form.

7 Lightly touch filter paper to LARGE blood drop. Allow blood to soak through and completely fill circle with SINGLE application to LARGE blood drop. (The enhanced blood flow, VERY GENTLE intermittent pressure may be applied for a one second duration pressure study. Apply blood to one side of filter paper only.

8 Fill remaining circles in the same manner as step 2, with successive blood drops. If blood flow is diminished, repeat steps 3 through 7. Care of skin puncture site should be consistent with your institution's practices.

9 Dry blood spots on a dry, clean, flat non-absorbent surface for a minimum of four hours.

10 Mail exemplified form to testing laboratory within 24 hours of collection.

Schleicher & Schuell

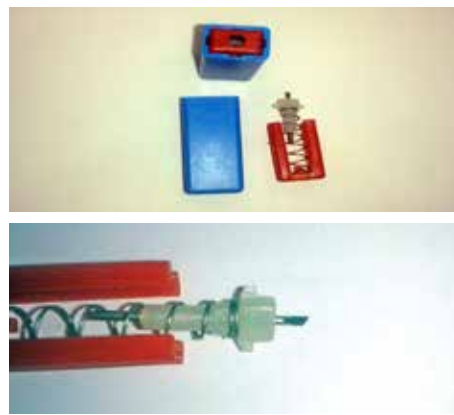
Information provided by The New York State Department of Health, Schreiber & Taylor, Inc. and submitted to the New York State Health Department, Department of Health.





Dried Blood Spot sample card made from Whatman Filter paper No.3.

Usually Guthrie cards are used for collecting DBS samples. They are essentially made of Whatman grade 3 paper (labeled as 901 in the catalogue) complete with a barcode and label for identification. DRIED BLOOD SPOT (DBS) Sample cards can also be prepared using Whatman filter paper no 3, as shown in the above photograph and print 1cm circles on it along with the sample card number.



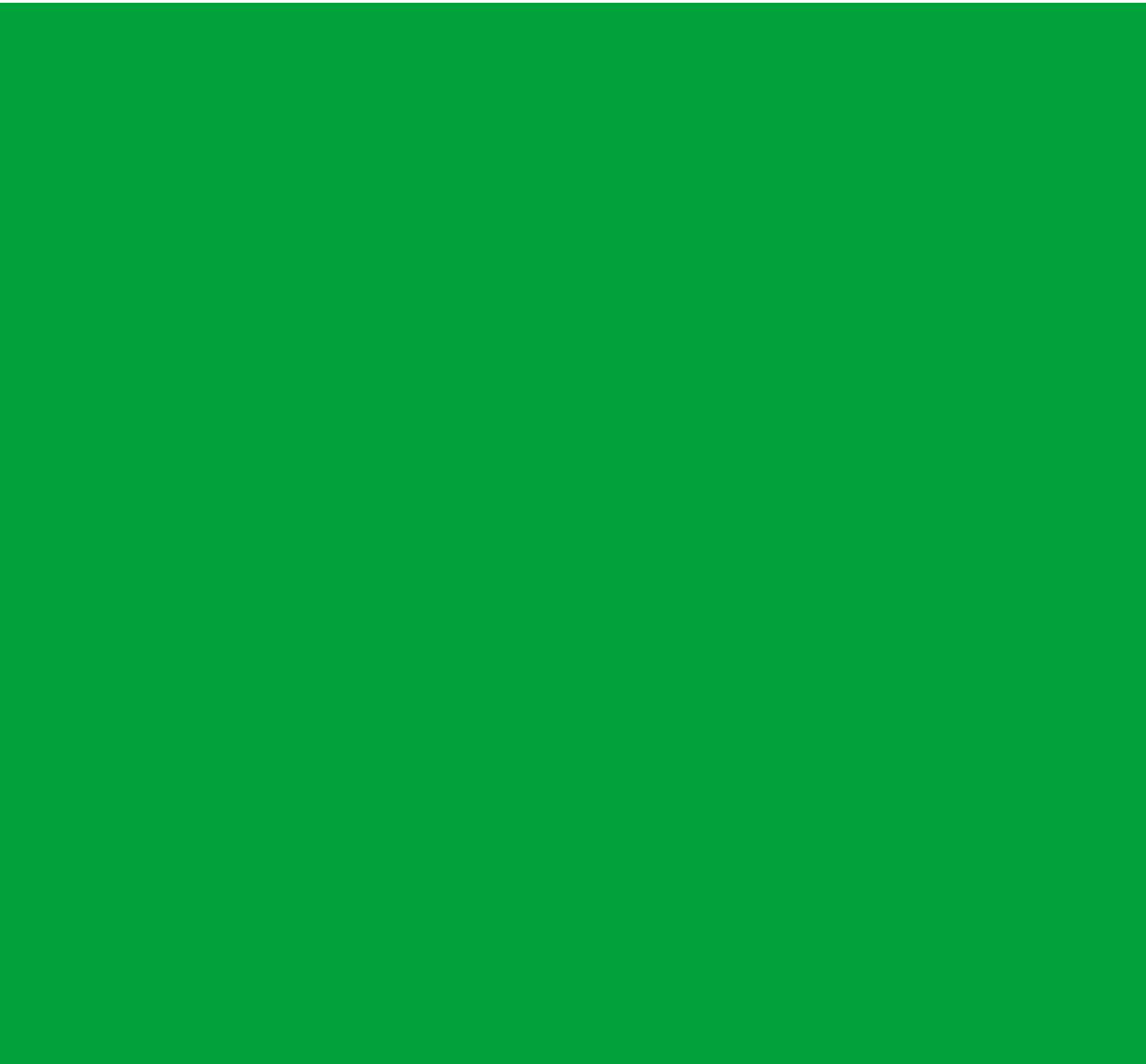
Spring controlled Lancet



Simple steel lancet

Both types of lancets are safe and can be used for obtaining newborn samples by heel prick depending on the cost.

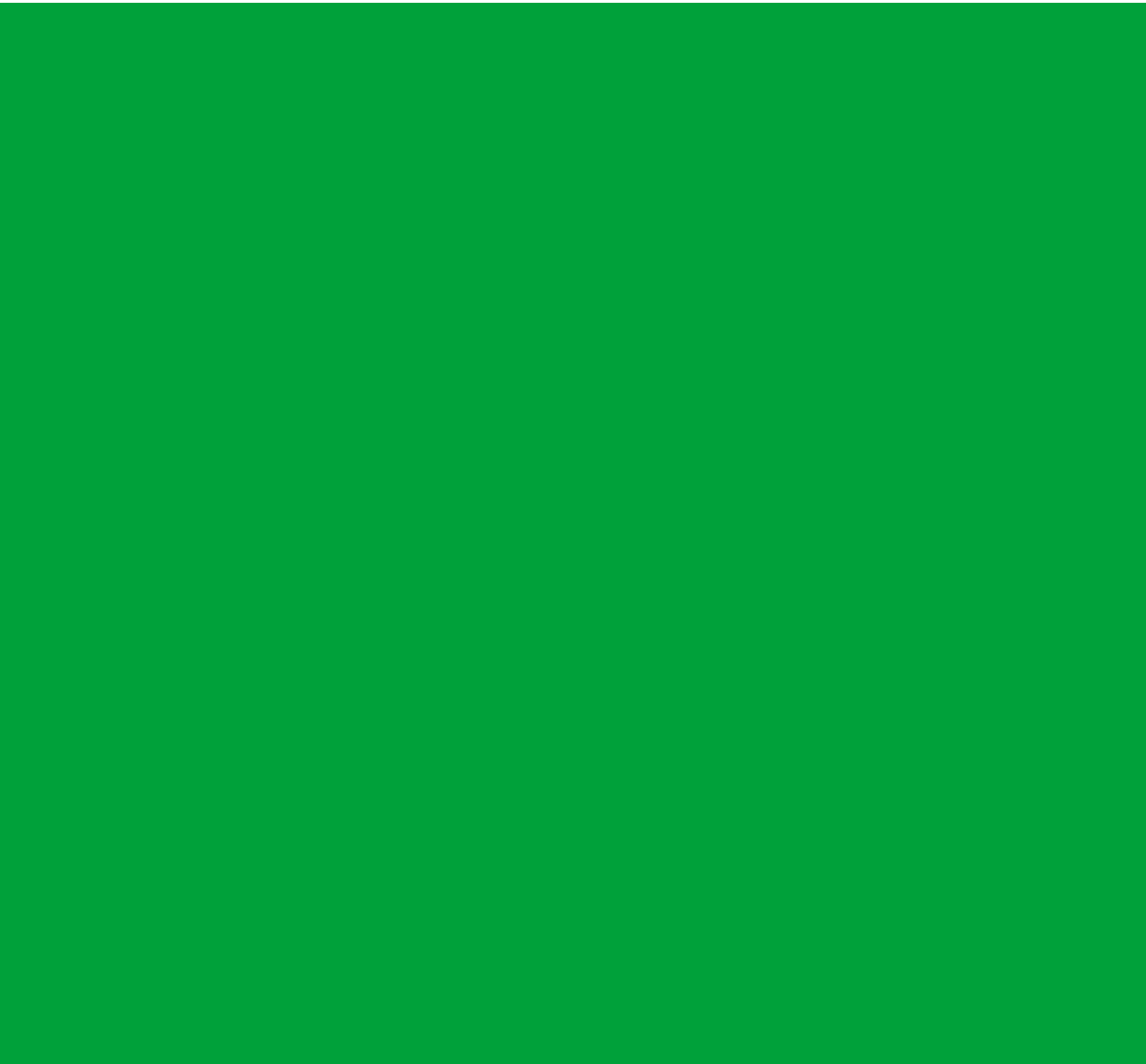
The objective is to obtain at least two full circles of samples with minimum pain without causing any injury to the newborn.



SECTION C

MANAGEMENT OF THALASSEMIA AND SICKLE CELL DISEASE





SECTION C

GUIDELINES FOR MANAGEMENT OF THALASSEMIA AND SICKLE CELL DISEASE

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MANAGEMENT OF THALASSEMIA AND SICKLE CELL DISEASE

This section will discuss the management guidelines for

1. Thalassemia major
2. Non transfusion dependent thalassemias
3. Sickle cell disease

A. MANAGEMENT OF THALASSEMIA MAJOR

The optimal current management of thalassemia major children is lifelong transfusion and iron chelation and needs to be meticulous in order to reduce complications. The only cure for thalassemia major is a bone marrow transplant, more appropriately called Hematopoietic stem cell transplantation (HSCT). While for carriers of β -thalassemia trait, it is important to reassure them that they are not ill and will not develop problems due to their carrier status. They need to be aware, so that they can if possible avoid marriage with another carrier. Or at least know their risk of having a thalassemia major child if the partner is also a carrier, and optimally utilizing available prenatal testing modalities.

The management outline:

- I. Blood transfusion therapy with packed red blood cells (pRBCs)
- II. Iron chelation for iron overload,
- III. Monitoring of complications due to the disease and their treatment
- IV. Management of complications (endocrine, cardiac, skeletal etc.),
- V. Bone marrow transplantation (BMT)/ Hematopoietic stem cell transplant (HSCT),
- VI. Psychological support.

I. BLOOD TRANSFUSION THERAPY:

It is important to strengthen the blood banks and ensure component therapy, this is mandatory.

Without component therapy, packed red blood cells (pRBCs) are not available, and only transfusion of this component is able to maintain the required hemoglobin (Hb) levels necessary for growth and normal activity. Because of lack of this facility many patients travel long distances for transfusion therapy. The frequency of transfusions varies from every 2-4 weeks depending on the age, weight of child and other factors. All blood products should be transfused with the availability of a trained physician.

Following investigations should be undertaken prior to therapy:

- i. Red cell typing of ABO & Rh-D (forward and reverse).
- ii. In newly diagnosed patients, ideally prior to transfusion therapy, patients should have extended red cell antigen typing that includes at least C, c, E, e, and Kell, in order to provide phenotype

matched blood where possible and to help identify and characterize antibodies in case of later development of allo-immunization.

- iii. Periodically a Direct Coombs test (DCT) and antibody screening followed by compatibility testing should be performed for all patients. Those positive for antibodies should be given phenotype matched blood. Patients requiring antigen negative RBCs may be referred to a center where this is available.
- iv. Regular screening for patients for hepatitis B, hepatitis C and HIV.
- v. Initiation of Hepatitis B vaccination for the patient and family members (if not vaccinated earlier). Routine vaccinations should continue as per the recommended schedule. In addition, all patients with thalassemia should receive hepatitis A, chickenpox and typhoid vaccinations.

Transfusion Regimen: Pre-transfusion Hemoglobin (Hb) should be kept between 9- 10.5 g/dl.

Type of blood to be transfused: Packed red blood cells (pRBC) are the component of choice and whole blood should not be given. All the pRBCs should be leuco-depleted and preferably pre-storage leuco-depletion is recommended. Where it is not possible, bed side filtration may be done. Packed red blood cells (preferably not more than 2 weeks old) should be transfused. Mandatory screening of blood for HIV, hepatitis B, hepatitis C, malaria and syphilis is to be ensured. Nucleic acid testing (NAT) is optional, but desirable to reduce the chance of transfusion transmitted infections

Amount of blood to be transfused: Packed red blood cells 15ml/kg body weight, should be administered at the rate of 5ml/kg/hr. The patient may require 1-2 units of pRBCs, or even more depending upon their body weight and pre-transfusion hemoglobin. To raise Hb by 1gm/dl we need to transfuse 3.5ml/kg of pRBCs (with at least HCT 60%). In the presence of congestive cardiac failure or Hb less than 5g/dl, the patient should be given a small volume of transfusion the total volume not exceeding 5ml/kg or less of packed red cells at the rate of 2ml/kg per hour, with close monitoring.

Storage and transport of blood: Blood units should preferably be transported in monitored insulated boxes which maintain a temperature of between 2-8°C. Blood units need to reach the transfusion centres as soon as possible.

Evaluation of transfusion treatment and clinical record-

The following data should be regularly recorded at each transfusion:

- Date of transfusion
- Time of initiation and time of completion of transfusion.
- Bag number of the blood unit transfused
- Weight/ volume of packed cells transfused
- Patient demographics (height, weight, pre-transfusion Hb, blood group and other details)
- Clinically assess the size of liver and spleen.



- Transfusion details of each patient to be entered into their transfusion card, to ensure proper data base maintenance and traceability.

The blood transfusions are usually needed at 2-4 week intervals. When more frequent blood transfusions are needed, or the required level of hemoglobin is not maintained as with previous transfusion regimen of the patient, then further assessment is needed. Check the weight of child and volume of blood transfused is adequate, as children grow they will require an increase of their packed red cell volume. If this is adequate then further tests are needed, evaluation of the presence of red blood cell allo-immunization by the blood bank is needed, these allo- antibodies lead to more rapid destruction of the transfused blood. If these are negative, then the child should be evaluated for hypersplenism.

A child being admitted with very low Hb should receive an additional transfusion, as the hemoglobin (Hb) will again drop in the 2-4 week period. This will enable the pre-transfusion Hb to be maintained at a level of 9 g/dl.

Blood Bank Facilities

Thalassemia patients are dependent on blood transfusion therapy to maintain their optimum hemoglobin levels. In order to provide standard of care of transfusion support to this special category of patients, advanced blood banking facilities are required.

These include-

- Component preparation facilities.
- Leuco-reduced packed RBCs (Ideally pre-storage leuco-depleted in the blood bank is recommended, if not available then use of leucocyte filters at the time of pRBC transfusion is needed.)
- Advanced immune-hematology facilities which include regular antibody screening and antibody identification in cases of positive screening tests, referral needs to be facilitated to ensure timely assessment and management.
- Semi-automated/automated serological /molecular typing would be preferable

Hemovigilance

- i. If a transfusion reaction is suspected, it should be reported to the blood bank immediately and work up done. (See details in Annexure 1)
- ii. Transfusion reaction reporting form will be filled up by blood bank (Annexure 2)
- iii. Monitoring and reporting of any adverse reaction and near miss cases to the blood bank is essential. For reporting to Hemovigilance Programme of India see website- <http://nib.gov.in/haemovigilance.html>

II) IRON OVERLOAD

Each milliliter of pRBCs contains 1.16 mg of iron. On an average each unit of packed cells contains 200 to 250 mg of iron. A patient, who receives 15-30 units of pRBC units per year, receives an excess of 3-6 grams of elemental iron. Hence, iron overload occurs and is a serious problem amongst multi transfused patients.

In the condition of thalassemia major and thalassemia intermedia, additionally iron absorption from the intestine increases to as much as 3 to 5 mg per day, depending upon severity of anemia, resulting in an additional 1-2 gm of iron loading per year. The iron absorption may increase up to even 10 mg per day if iron supplements are given, and hence they are contraindicated.

Evaluation of Iron Overload

1. Serum Ferritin: Serum ferritin reflects the overall iron stores in the body tissues and thus is a useful indicator of iron storage status. Serum ferritin is an acute phase reactant, so its value varies with the presence of any infection or inflammation in the body. Thus a single value of this test is of no practical utility for monitoring iron overload. The trend of serum ferritin values should be monitored for assessing iron overload. This test needs to be performed once in six months.
2. MRI of liver and heart: The serum ferritin test may not be able to give information regarding organ specific iron overload. The T2* MRI though available in a few centers in the country, is a good non-invasive method of estimating quantitative iron overload in both the heart and liver. Iron overload in both these organs is independent of each other, and hence both should be tested separately. Having a T2* MRI facility with validation in each region of India will allow referral and evaluation of the patients in a streamlined manner.
3. Liver Biopsy: Liver biopsy though highly reliable, is an invasive method which entails increased risk to the patient. It should be reserved only for special indications such as assessment prior to bone marrow transplant or if indicated by the treating doctor.

Iron Chelation

When to start chelation? The serum ferritin levels should be assessed after 10 to 15 transfusions and chelation therapy should be initiated when the serum ferritin value is more than 1000 μ g/L.

Chelation Drugs:

a. Desferrioxamine:

The recommended dose is 25-50mg/kg/day, subcutaneously with the help of an infusion pump, over 8-12 hours, or more. A ten percent (10%) desferrioxamine solution is prepared in water for injection i.e. one vial of desferrioxamine (500 mg) is dissolved in 5ml water for injection. If more than one vial is to be administered, then 2.5 to 5 ml of water for injection can be added per vial of desferrioxamine. The re-constituted desferrioxamine solution should not be stored for more than 24 hours. Intravenous desferrioxamine is required in the presence of severe iron overload. The desferrioxamine should never be added directly into the blood bag. Oral Vitamin C (50-200 mg) should be given after starting the infusion.

Toxicity: Frequent pain, swelling, induration, erythema, burning, pruritus, and rashes at site of injection/ infusion may occur occasionally accompanied by fever, chills and malaise. High doses of desferrioxamine, especially in patients with a low serum ferritin may lead to visual and auditory side effects. Desferrioxamine increases the susceptibility to *Yersinia enterocolitica* and *Yersinia pseudo tuberculosis* infections.

Required Monitoring: Hemoglobin levels, Serum ferritin and sitting height.



b. Deferiprone:

This was the first oral iron chelator, introduced in 1995. The standard dose is 50-100 mg/kg/day in two or three divided oral doses. It is available as 250 & 500 mg capsules. Due to its low molecular weight, it is more efficient in removing iron from the heart, compared to desferrioxamine.

Toxicity: agranulocytosis, cartilage damage leading to pain

Required Monitoring: Hb, TLC, DLC, and platelet count every 2-4 weeks and during every febrile episode and Serum ferritin monitoring is necessary.

c. Deferasirox:

This is a new oral iron chelator introduced in India in 2008. This has been proven to be effective and safe. Not recommended below two years of age. It is administered at a dose of 20-40 mg / kg / day. It is to be given dispersed in water or juice. Use a glass or non metal container for dissolving medicine.

Toxicity: Diarrhea, skin rash (usually disappears within 2 weeks even on continuation of medication). Non- progressive increase of serum creatinine or rise in the level of AST and ALT. Avoid before the age of two years,

Required Monitoring: BUN, Serum creatinine, AST/ALT, urine routine, should be monitored before starting the medicine and every month after the initiation of the medicine. Serum ferritin monitoring is mandatory as with all chelation therapy.

d. Combination Therapy:

Combination of desferrioxamine and deferiprone is advisable in patients not responding to maximum dosages of monotherapy. Addition combinations can be done under the supervision of hematologists.

III) MONITORING OF PATIENTS WITH THALASSEMIA IN A DAYCARE CENTRE:

The iron chelation therapy is an important component and needs regular monitoring to see for efficacy of chelation and necessary modifications in the drug dosage. The patients need to be evaluated for toxicities and other complications which may develop. Even blood transfusion therapy needs an accurate record to decide when patient needs evaluation for increased requirement and any patient may develop transfusion reactions or other complications.

A day care centre dedicated to thalassemia facilitates good care of these patients and fosters a team spirit among the health care providers. It may be attached to a Pediatrics department, District hospital or DEIC.

Such a facility is an important component of thalassemia care, and has several advantages:

- It is convenient, economical and provides a supportive environment friendly area for children with a chronic illness.
- It helps in developing a good rapport with staff and better compliance and efficient monitoring of patients.

Requirements for setting up a day care centre for management of patients with thalassemia and sickle cell disease have been detailed in section D of this document.

Table 1: Monitoring at each transfusion

Name, age, address, hospital number			
	date		
Pre transfusion hemoglobin			
Liver size (by clinical examination)			
Spleen size (by clinical examination)			
Transfusion reaction			

Table 2: Monthly monitoring for patients

	date	
Amount of blood transfused		
Pre- transfusion Hb		
If on deferasirox-SGPT, SGOT, BUN, s.creatinine, Urine R/E		
If on deferiprone- Complete blood counts		
Next date of transfusion		
Note :- Routine post-transfusion Hb checking is not recommended.		

Table 3:Monitoring every 6 months for all patients

	Date	
S. ferritin (ng/ml)		
SGPT		
SGOT		
BUN		
S.creatinine		
Calcium		
Phosphorus		
Height		
Weight		

Table 4: Monitoring every year

	Date	
Anti HBs antibody		
HCV IgG antibody		
HIV 1 & 2		
The following tests to be performed annually after the age of 10 years		
Blood sugar (fasting) or GTT		
TSH		
ECG		
Echocardiography		
MRI T2* Heart/ liver		
DEXA Scan		

Table 5 :Tests to be performed when indicated by physician

	Date	
Holter monitoring		
PTH		
FSH		
LH		
Estradiol		
Testosterone		
Cortisol		

IV) MANAGEMENT OF COMPLICATIONS OF IRON OVERLOAD AND INDICATIONS OF SPLENECTOMY

Even with adequate iron chelation patients may go on to develop complications. Iron overload results in toxicity to the heart, liver, and harms the endocrine system- affecting growth and development. It can even result in skeletal and bone mineralization problems. The patients may be affected by transfusion transmitted diseases like hepatitis B, C or HIV. Toxicity from iron chelation medicines, if occurs may also need to be managed.

Therefore, a multi-specialist team including a pediatrician, cardiologist, gastroenterologist, and endocrinologist are necessary. Psychological counseling and support are needed to deal with the consequences of a chronic disease.

Splenectomy is needed only in few cases where hypersplenism is symptomatic. Splenectomy causes

many late complications and may increase the risk of infections and allo- immunization hence should not be performed routinely.

V) BONE MARROW TRANSPLANTATION (BMT) OR HEMATOPOIETIC STEM CELL TRANSPLANT (HSCT):

Hematopoietic stem cell transplant is the only curative therapy for Thalassemia major and HLA matched sibling transplants is a well-established and accepted therapy, available in India. The outcomes of this procedure depend on patient characteristics. The Lucarelli classification divides patients into groups based on pre transplant morbidity and this predicts the risk of serious complications and morbidity with transplant. Younger children, with adequate iron chelation, no hepatomegaly or hepatic fibrosis do best with this procedure. The older children with organomegaly, iron overload, cardiac or hepatic compromise require additional medications and extra therapy, even then risk of mortality and complications is high. There is a risk of bone marrow graft rejection, graft versus host disease, and veno-occlusive disease (VOD). VOD of the liver may occur, more likely if heavy iron overload, irregular chelation, hepatitis or fibrosis of liver. VOD is a serious complication of transplant and can lead to multi-organ failure and death. Indian data has identified a subset of patients who are more 7 years old and had a liver size more 5 cm, these are at very high-risk of transplant related complication compared to even the conventional Class III group (Class III high-risk (HR)⁵). These patients of high risk group may be better managed with conventional treatment. Unrelated transplant and other newer kinds of transplant like Haplo are not standard therapy at present.

HLA typing of siblings leads to the commonest source of transplants - the HLA matched brother or sister. The sibling can be normal or a carrier for thalassemia, both are acceptable. Unrelated HLA matched transplants are both more expensive and more difficult, but may be needed if no sibling donor is available. Cord blood transplant can be done from a sibling or another person but is not usually preferred for thalassemia patients, due to risk of rejection, but may be tried if no bone marrow matched donor is available. Even cord blood transplant needs HLA matching. At present a national stem cell donor registry is lacking and is the need of the hour.

VI) PSYCHOLOGICAL SUPPORT AND COUNSELING

Thalassemia is a chronic disease and the need for continuity of care and psychological support for chronic diseases is widely accepted). The patient has the right to long survival and good quality of life, with the opportunity to fulfill normal life expectations including, work and marriage. This means full integration into the community as a productive member without stigmatization.

Also counseling is essential when introducing antenatal screening in an at-risk population. The patients need counseling to ensure lifelong adherence to chelation therapy and to help them deal with inevitable complications and deal with issues like employment, marriage and other problems.

References for Management of Thalassemia

1. Cappellini MD, Cohen A, Porter J, Taher A, Viprakasit V. guidelines for the management of transfusion dependent thalassaemia (TDT). publishers thalassaemia international federation if publication no. 20. <http://www.thalassaemia.org.cy/wp-content/uploads/eBook-publications/guidelines-for-the-management-of-transfusion-dependent-thalassaemia/>



2. Ghosh K, Colah R, Manglani M, Choudhry VP, Verma I, Madan N, Saxena R, Jain D, Marwaha N, Das R, Mohanty D, Choudhary R, Agarwal S, Ghosh M, Ross C. Guidelines for screening, diagnosis and management of hemoglobinopathies. *Indian J Hum Genet* 2014;20:101-19.
3. Falvo DR. In: *Medical and psychosocial aspects of chronic illness and disability* (5th ed). Jones & Bartlett Learning: Burlington, Mass, 2014.
4. Lubkin IM, Larsen PD. In: *Chronic illness: impact and interventions* (8th ed). Jones & Bartlett Learning: Burlington, Mass, 2013
5. V. Mathews and BN Savani. Conditioning regimens in allo-SCT for thalassemia major. *Bone Marrow Transplantation* (2014) 49, 607–610.

B. MANAGEMENT OF NON TRANSFUSION DEPENDENT THALASSEMIA (NTDT)

Current attention is now being given to a group of thalassemia states together called non-transfusion dependent thalassemia (NTDT). They are responsible for serious complications and often the diagnosis is missed or unsuspected due to their unusual or late presentation¹. A common error is to diagnose them to have iron deficiency and empirically treat them with iron supplementation which in these cases can have significant adverse effects.

The pathology and clinical features of NTDT are linked

1. The chronic anemia and ineffective erythropoiesis, results in growth retardation, skeletal abnormalities, extra-medullary hematopoiesis (EMH). Anemia is compounded by hemolysis, which results in gall stones.
2. Signs and symptoms related to iron overload. These patients have increased intestinal absorption of iron and are predisposed to iron overload and all its complications even though they are not transfused or have been transfused only occasionally.
3. These patients have a tendency to hyper coagulable states and can present with features of thromboembolism. The pathophysiology of this is complex and involves abnormalities in both red cells and platelets, this hyper coagulable state is significantly worsened post splenectomy.¹
4. These patients can present with pulmonary hypertension, chronic leg ulcers, hypogonadism and other endocrine abnormalities. These patients also frequently have osteopenia and fractures.

Confirmation of diagnosis of NTDT:

Following exclusion of the possibility of iron deficiency the next step would be to confirm the subtype of NTDT (after excluding transfusion dependent thalassemia's). The diagnosis is made as for other hemoglobinopathies by HPLC or capillary hemoglobin electrophoresis to evaluate the abnormal hemoglobin.²

Molecular tests for thalassemias

Often the HPLC analysis of the patient and their parents are needed to identify the disease. Many times further evidence may be required by molecular testing. This is needed to diagnose alpha thalassemias, when one parent is a silent carrier or a compound heterozygote state.³

Management of Non Transfusion Dependent Thalassemia (NTDT) –

1. Monitoring of iron overload- which can occur even if transfusions are not given. Iron chelation recommended when serum ferritin $>750\mu\text{g/L}$.
2. Monitoring of growth and endocrine and bone problems, including extra-medullary hematopoiesis.
3. Surveillance for gall stones, liver, cardiac disease.
4. Trial of hydroxyurea, with appropriate monitoring for side effects may be done to attempt to reduce the need for blood transfusions and increase the hemoglobin level in the patients. In studies including NTDT patients, the primary hematological outcome was improvement in total hemoglobin level. Mean increase ranged between 0.5 and 2.5 g/dl with an average of around 1.5 g/l. ^{4,5}

Most physicians start with a hydroxyurea dose of 10 mg/kg/day and escalate the dose according to response and toxicity (maximal tolerated dose) up to a maximum of 20 mg/kg/day. Response should be evaluated after 3 and 6 months of therapy and should be defined as a total hemoglobin level increase of >1 g/dl at 6 months.

When prescribing hydroxyurea the following safety measures should be evaluated and treatment discontinued or tailored accordingly. Monitor complete blood counts, initially every two weeks for the first three months then monthly. Hepatic and renal function studies, every two weeks for the first three months then monthly.

5. If patients are given transfusion support during growth spurt or to maintain hemoglobin, safe blood banking and transfusion guidelines as per transfusion dependent patient guidelines must be followed.

REFERENCES-

1. Khaled M. Musallam, Stefano Rivella, Elliott Vichinsky, and Eliezer A. Rachmilewitz. Non-transfusion-dependent thalassemias. *Haematologica*.2013; 98(6) 833-844.
2. Gwendolyn M. Clarke and Trefor N. Higgins .Laboratory Investigation of Hemoglobinopathies and Thalassemias: Review and Update. *Clinical Chemistry* 46:8(B) 1284–1290 (2000)
3. Globin Chain Disorder Working Party of the BCSH General Haematology Task Force. Guidelines for the fetal diagnosis of globin genes disorders. *J Clin Pathol* 1994;47:199–204.
4. Guidelines for the management of non transfusion dependent thalassemia (NTDT) Publishers Thalassaemia International Federation, TIF publication No. 19. Editors Ali Taher, Elliott Vichinsky, Khaled Musallam, Maria Domenica Cappellini, Vip Viprakasit.
5. Musallam KM, Taher AT, Cappellini MD, Sankaran VG. Clinical experience with fetal hemoglobin induction therapy in patients with beta-thalassemia. *Blood* 2013;121(12):2199-2212

C. SICKLE CELL DISEASE (SCD)**General principles of Management.**

India has also a very huge populations of tribal communities about 18 crore and expected to have 1.80 crore sickle cell trait and 14 lakhs of sickle cell disease¹. The basic principles of preventive care for children with sickle cell disease include prevention of infections by encapsulated organisms due to the functional asplenia present in these children. Though these children may be asymptomatic in the newborn period,



early diagnosis may be the only measure to save children from life threatening infections. These patients benefit from pneumococcal immunization and penicillin prophylaxis². Prevention of other complications can be achieved by prescribing Hydroxyurea and judicious use of blood transfusions. Hydroxyurea benefits children who suffer from painful crisis, helps to prevent organ damage, reduce transfusion requirement and improves overall survival. The other need is appropriate management of complications and crises. These children can have pain crisis, acute chest syndrome, splenic sequestration crisis and rarely even aplastic crises which is due to Parvo B19.

By regular health maintenance and parental counseling, the early high mortality seen in these children has gone down. Physicians need to be aware of fever, jaundice, pallor and should monitor the spleen size on each health visit. Basic tests like complete blood count, reticulocyte count, routine biochemistry tests like LFT, RFT are useful to monitor the patients. An estimation of HbF is necessary. Other tests such as Transcranial Doppler ultrasonography (TCD), magnetic resonance imaging (MRI) with or without angiography, and neuro-psychometric (NPM) studies may be done if provision is available, or child can be referred to a centre where they can be performed. Educational material should be given to the caregiver and older children, so they understand about the disease, and especially about fever. Sickle cell carriers, usually have mild disease, but may need follow up for regular health maintenance, some will need intervention for fever, pain etc. Genetic counseling should be made available to all carriers.

Fever

Mandatory routine Pneumococcal vaccination and penicillin prophylaxis have reduced the risk of mortality for SCD children. All children with SCD who have fever ($>38.5^{\circ}\text{C}$ or 101°F) or /and other signs of infection (chills, lethargy, irritability, poor feeding, vomiting) should be assessed promptly. A minimum evaluation should include a blood culture, complete blood count, reticulocyte count, and chest x- ray (if younger than 3 years of age). Immediately after the blood culture is taken, the child should always be given broad-spectrum antibiotics, preferably intravenously. Prophylaxis: Newborn to 3 years: Penicillin VK, 100- 125 mg orally twice daily (PO BID), 3 to 5 years: Penicillin VK, 200-250 mg PO BID.

Pain

This is common in all patients with SCD, it may manifest as dactylitis (“hand-foot syndrome”), vaso-occlusive pain may involve the limbs, abdominal viscera, ribs, sternum, vertebrae etc. Pain relief needs to be appropriately done, and includes good hydration along with NSAIDS and even opioids may be needed. The initial medicines usually prescribed are acetaminophen and non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen and mild opioids, such as codeine, for young children. Oral morphine can safely be used if needed, under adequate supervision.

Hydroxyurea

This has been proven to decrease complications in children, such as- pain crisis, acute chest syndrome and strokes, it does so by several mechanisms including increasing levels of HbF. The starting dose of Hydroxyurea is 10-15 mg/kg/day in a single daily dose, it is started after 2 years of age. It is available as a 500mg capsule; follow the CBC every 2 weeks; if possible can monitor the HbF every 6-8 weeks; serum chemistries every 2-4 weeks. If no major toxicity, try to escalate dose every 6-8 weeks until the desired endpoint is reached.

Treatment Endpoints: Decrease in pain, increase in HbF to 15-20%, increase hemoglobin level if severely anemic, improved well-being, acceptable myelotoxicity. Failure of HbF (or MCV), then check for compliance. We can increase the dose slowly up to a maximum of 35 mg/kg/day.

Acute chest syndrome (ACS)

This is an acute illness characterized by fever and respiratory symptoms, accompanied with a new pulmonary infiltrate on a chest x ray. Even though the ACS usually is self-limited, it can present with or result in respiratory failure. The cause is thought to be pulmonary fat embolization (PFE), as defined by the finding of lipid-laden macrophages, which are seen in 59% of broncho-alveolar lavage specimens, or infection, which is seen in one third of patients.

Oxygen is to be given to moderately hypoxemic patients ($\text{PaO}_2 = 70\text{-}80$ mmHg, O_2 saturation = 92-95 %) nasally at a rate of 2 liters/minute. Assessment of blood oxygenation is needed and a baseline arterial blood gases (ABG), and estimation of the alveolar-arterial (A-a) oxygen gradient and the $\text{PaO}_2/\text{FiO}_2$ ratio, is useful for appropriate management. Simple transfusions (or rarely exchange transfusions,) decrease the proportion of sickle red cells. Intravenous broad-spectrum antibiotics should be given if febrile or severely ill ACS as it is difficult to exclude bacterial pneumonia or super added infection of lung infarct. The guidelines suggest using erythromycin and cephalosporin. The rationale for a macrolide or quinolone antibiotic is because atypical pneumonia may be the causative organism. Pain control and incentive spirometry can prevent chest atelectasis. The subsequent frequency of ACS can be reduced with Hydroxyurea- by 50%, if the patient is compliant.

Transfusions

This is needed in only special indications, not all patients will require blood transfusion, most patients with Arab- Indian haplotype, only rarely needed. If transfusions needed, then a pre transfusion extended red cell typing is required [Rh Sub group (Cc, Ee), Kell, Kidd, S/s], as these patients frequently develop Delayed Hemolytic Transfusion Reaction (30% cases) and allo-immunization. Children receiving regular transfusions will need to have serum ferritin monitoring and chelation therapy.

Strokes and transient ischemic attacks (TIAs)

Though rare in the Indian phenotype, children who develop these complications will benefit from hydroxyurea. These patients may benefit from blood transfusions to decrease HbS levels, and post stroke may need anticoagulation, along with required monitoring for anticoagulation medicines. Though unusual, this is a serious condition and such patients should be referred to a higher center to receive evaluation and required management. Patients who have suffered strokes, TIAs etc. will need transcranial Doppler (TCD), computerized axial tomography, MRI, or MRI with angiography. Comprehensive management of SCD requires a multi-specialty team, especially for young children with these complications.



Table 6 : Daycare center sheet for patients of sickle cell disease, (additional information needed for their care in a daycare unit)

	date	
Age of child		
Weight/height		
Episodes of fever		
Pain crises		
Name of pain medicine , dose		
Hemoglobin level		
Need for transfusion		
Patient taking penicillin prophylaxis		
Date and dose of pneumococcal vaccine		
Patient on hydroxyurea, dose		
Other complications		

Other complications.

Rare complications include leg ulcers, pulmonary hypertension, avascular necrosis head of femur, psycho social issues etc. At least an annual review by a hematologist will be necessary for these children, they will need to transit to adult care for further management as they grow older. Some patients may benefit from allogeneic hematopoietic stem cell transplant. Sickle cell disease transplant indications are very selective, due to the risks of morbidity associated with the transplant procedure.

Indications for allogeneic Hematopoietic stem cell transplant (HSCT) for sickle cell disease as suggested by Walters et al (3).

1. Stroke or central nervous system event lasting longer than 24 hours, acute chest syndrome with recurrent hospitalizations or previous exchange transfusions.
2. Recurrent vaso-occlusive pain (more than 2 episodes per year over several years) or recurrent priapism.
3. Impaired neuropsychological function with abnormal cerebral MRI scan
4. Stage I or II sickle lung disease
5. Sickle nephropathy (moderate or severe proteinuria or a glomerular filtration rate 30 to 50% of the predicted normal value)
6. Bilateral proliferative retinopathy with major visual impairment in at least one eye
7. Osteonecrosis of multiple joint
8. Red-cell allo-immunization during long-term transfusion therapy

Evidence supported management strategies in Sickle cell disease.

- Penicillin prophylaxis prevents pneumococcal sepsis in children [evidence from Prophylactic Penicillin Studies I and II (PROPS I & II)].
- Pneumococcal vaccine prevents pneumococcal infection in children.
- Transfusions to reduce Hb S levels to below 30 % prevent strokes in children with high central nervous system blood flow [evidence from the Stroke Prevention Trial in Sickle Cell Anemia (STOP I)].
- Hydroxyurea decreases crises in patients with severe sickle cell disease [evidence from the Multicenter Study of Hydroxyurea in Sickle Cell Anemia (MSH) trial]

REFERENCES

1. <http://nrhm.gujarat.gov.in/Images/pdf/SickleCellAnemiaManual.pdf>
2. The management of sickle cell disease-.https://www.nhlbi.nih.gov/files/docs/guidelines/sc_mngt.pdf
3. Mark C. Walters, Melinda Patience, Wendy Leisenring et al. NEJM 1996;335:369-76. Bone Marrow Transplantation For Sickle Cell Disease

Annexure C-1 Transfusion Reaction work-up

Annexure C-2 Transfusion reaction reporting form

Annexure C-3 Requirements for daycare centers



ANNEXURE C-1 TRANSFUSION REACTIONS WORK-UP

Acute transfusion reactions occur within 24 hours of transfusion.

S.No.	Type of Reaction	Clinical Signs and symptoms
1.	Hemolytic Transfusion Reaction	Fever/chills, hypotension/tachycardia, cola coloured urine, nausea, vomiting, pain in flanks/back/abdomen/ chest
2	Bacterial Contamination	Fever/chills, hypotension, nausea, vomiting, dyspnoea, diarrhoea.
3	Transfusion related Acute Lung Injury	Dyspnoea or cyanosis, fever, tachycardia, hypotension
4	Febrile non hemolytic transfusion reaction	Fever, chills, rigors, cold, headache, nausea, vomiting
5	Allergic/Anaphylactic reaction	Pruritis, urticaria, flushing, angioedema, hoarseness, stridor, wheezing, chest tightness, dyspnoea, cyanosis, anxiety, nausea, abdominal cramps and diarrhoea

The signs and symptoms of acute transfusion reactions often overlap and diagnosis may not be possible without a complete workup

During blood component transfusion closely monitor the patient for the signs and symptoms of a transfusion reaction.

Action to be taken in case a reaction is suspected-

- Stop the transfusion immediately and keep the IV line open with normal saline.
- Institute immediate resuscitative care as per the nature of the transfusion reaction
- Send the following to the Blood Bank.
 - Blood bag and transfusion set
 - Post-transfusion blood sample-2ml EDTA and 3ml plain
- Fill the Reaction form with details of the nature of reaction.
- The blood bank staff will re-check all records and do a Direct Coombs test and repeat all pre-transfusion tests to confirm compatibility of the implicated unit.

Investigations to be sent if hemolytic/septic reaction is suspected:

- Blood Culture-from patient and from component bag
- Complete hemogram
- Plasma hemoglobin
- Urine hemoglobin
- Coagulation profile
- Bilirubin (conjugated/unconjugated)
- Urea
- Creatinine and Serum electrolyte.

ANNEXURE C-2

TRANSFUSION REACTION REPORTING FORM

Patient Details-

Name

Age/sex

Ward no. / Bed no.

CR no (or HID of hospital patient)

Diagnosis

Indication for transfusion

blood group of patient

Date and time of transfusion

Details of Transfused Unit-

Transfused product (PRBC/leuco-depleted PRBC/RDP/FFP/cryo ppt /SDP/ other specify below)

Unit no.

Date of collection

Date of expiry

Blood group of unit

Date and time of issuing unit from blood bank

Date and time of starting transfusion

Duration of transfusion

Patient monitoring-

Time after starting transfusion	Hypo tension	Chills	Rigors	Fever	Urticaria	Difficulty Breathing	Pain abdomen	Headache	Myalgia

Post transfusion blood sample collected
(Note: should be within one hour of reaction).

Yes

No

Post transfusion urine sample collected
(Maybe collected within 6 hours of occurrence of reaction, visual observation too, report to blood bank)

Yes

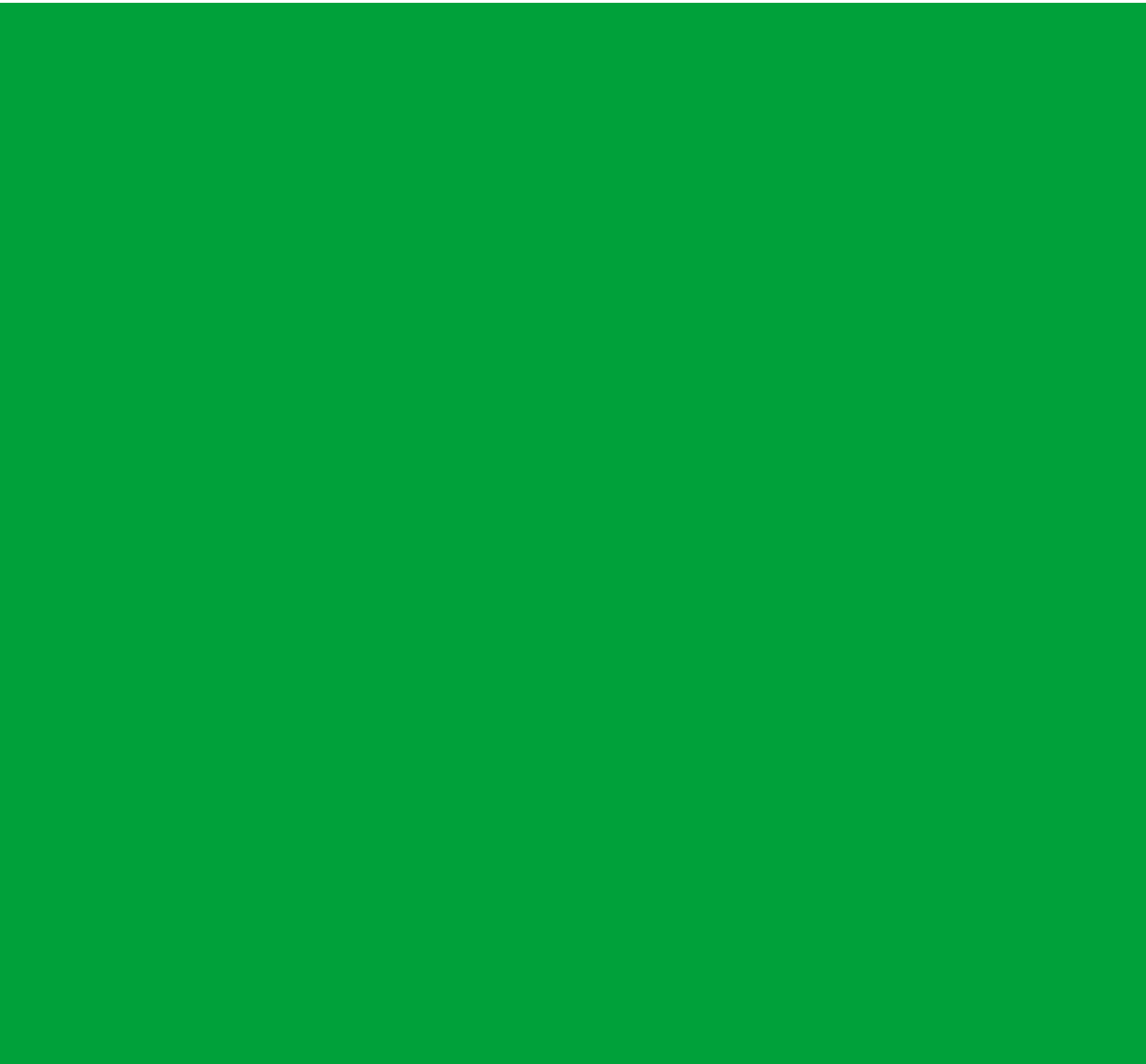
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ANNEXURE C-3

Plan for a 10 bedded day care centre : where daily 10-15 patients can be accommodated. 300 patients per month can be managed for thalassemia and sickle cell disorders, in such a center.

S.No.	Description
1.	Recurring salaries Staff nurses (2) Counselor (1) Attendant (1) Doctor (1) Lab Technician-1
2	Recurring Medicines Blood bags and other blood bank disposables *NAT tested bag will cost extra Rs 600/bag ** Extended red cell pheno-typing per bag will cost extra Rs1200/bag *** leuco-depleted filter will cost extra Rs 650/bag Iron Chelation medicines Multivitamins and minerals
3.	Recurring essentials Bed sheets Cleaning Dustbins Bio waste Stationary (case files, prescription pads, pens, follow up sheets, measuring tape, height charts, A4 papers, record books)
4.	Non recurring equipment Beds with IV stands Electronic weighing scales Plastic kidney trays Needle destroyer BP instruments Stethoscopes Examination table with step stool with mattress Almirah Refrigerator 220 L Telephone

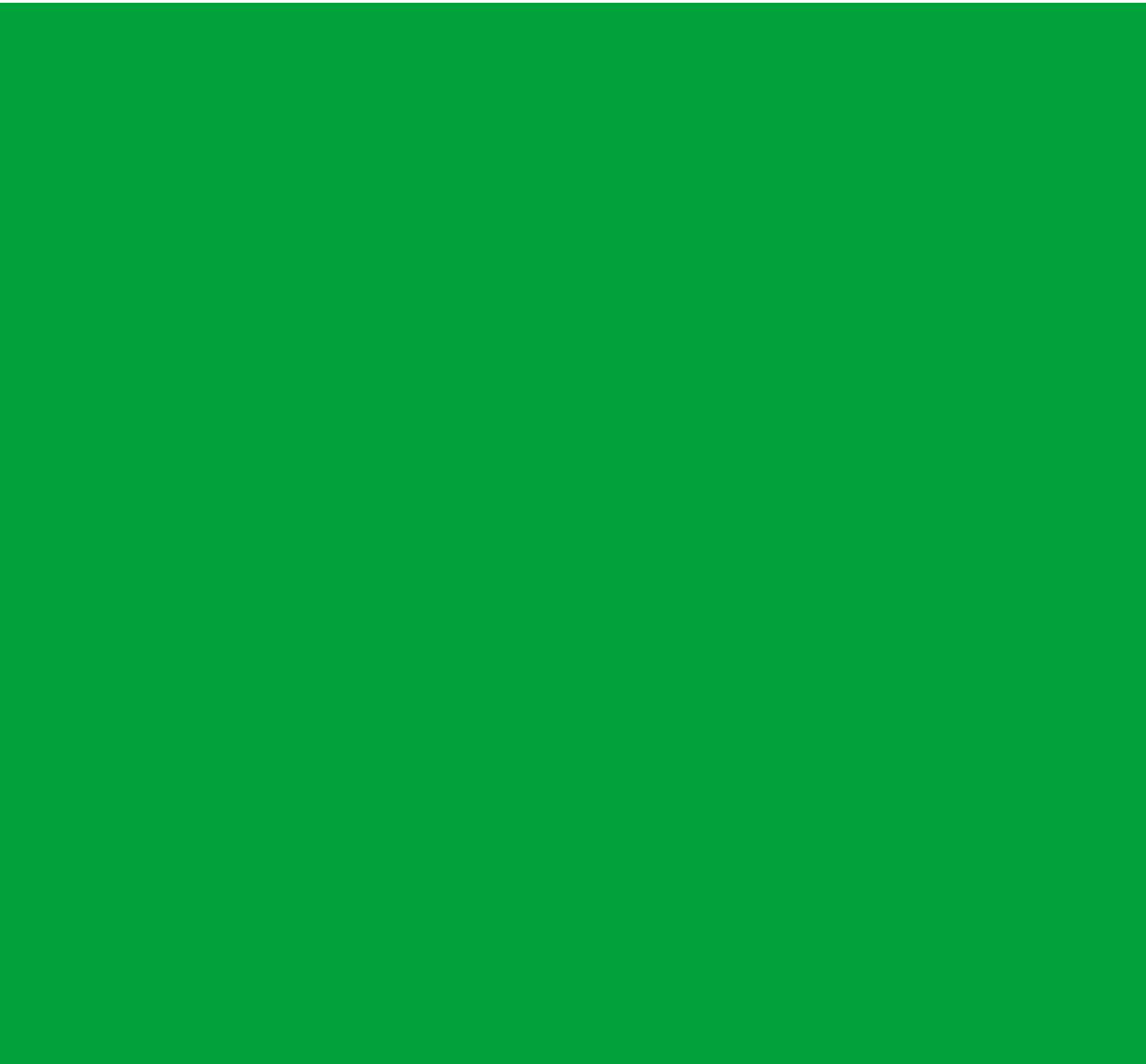


SECTION D

OPERATIONAL GUIDELINES

IMPLEMENTATION FRAMEWORK, HUMAN RESOURCE REQUIREMENTS AND BUDGET ESTIMATES





SECTION D

OPERATIONAL GUIDELINES

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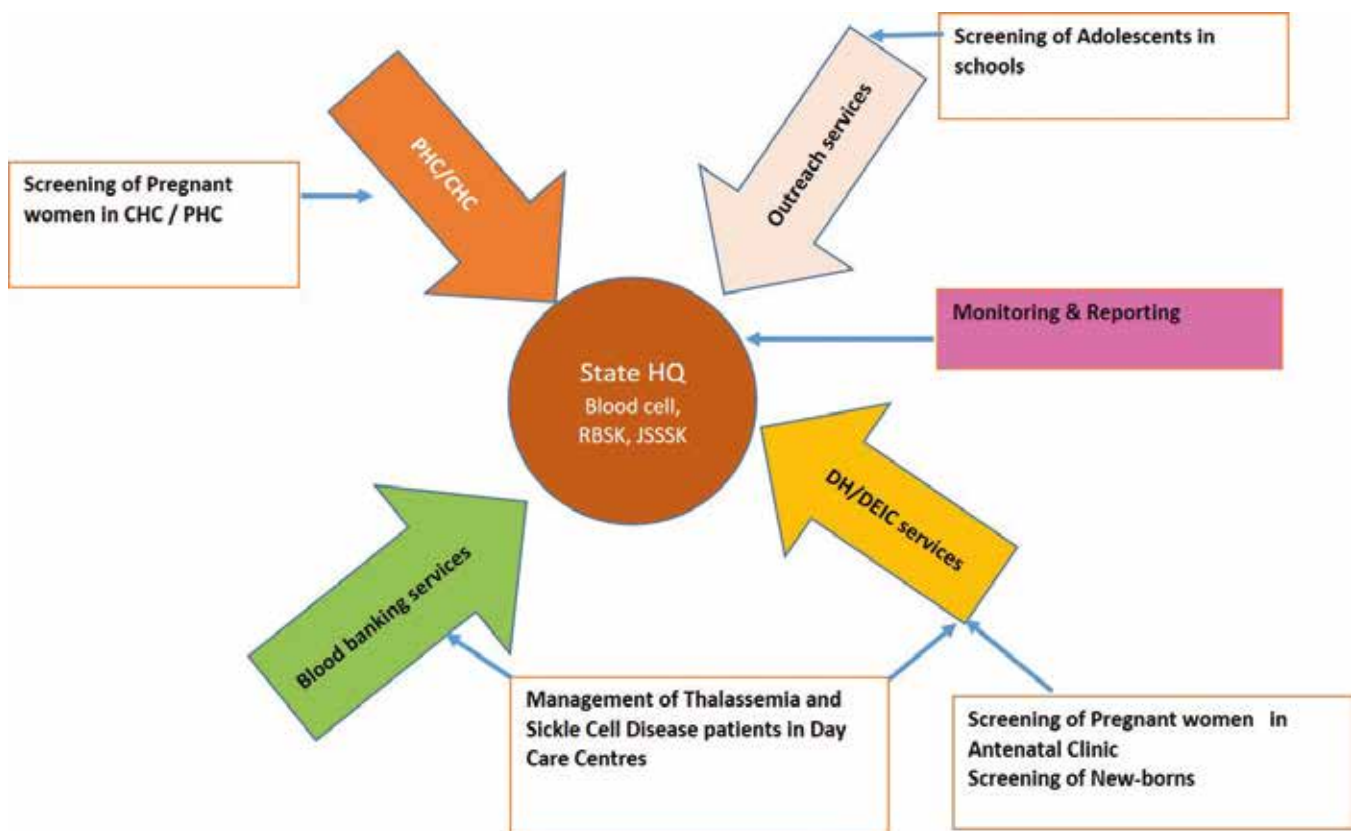
OPERATIONAL GUIDELINES

Implementation framework, human resource requirements and budget guidelines

1. FRAMEWORK FOR IMPLEMENTATION

In accordance with the earlier stated goals of public health, implementation of screening and management strategies has to be undertaken largely within the framework of RBSK and in coordination with other existing programmes like JSSK to achieve maximum convergence. Screening for hemoglobinopathies can easily be conformed to the screening and referral process undertaken for other conditions under RBSK with changes in schedule and strengthening wherever required. Screening of pregnant women will be undertaken at DH, SDH, CHC and PHC under JSSK in coordination with RBSK. District Hospitals or DEIC will be strengthened and upgraded to provide Day Care Centre facilities for providing care and monitoring of patients affected with thalassemia and sickle cell disease.

Figure 1. Showing schematic framework for programme implementation



Screening of different target groups- newborns, young children and adolescents -for prevention of hemoglobinopathies and detection for early intervention and management is to be undertaken under RBSK through coordination between DEICs and the Mobile Health Teams at AWCs and schools from district to block level. Figure 2 depicts the screening module for hemoglobinopathies under RBSK.

Figure 2. Hemoglobinopathies Screening module under RBSK

0-6 weeks	6 weeks -6 years	6- 18 years	>18 years
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Population to be screened

<p>Newborns (Day 2-7 DBS) born at: Govt. Hospitals or at home</p>	<p>Children with severe anemia (6 months to 6 years) referred to DH or DEIC</p>	<p>Adolescents At school: Screening of all class VIII students of govt. and aided schools and non school going at AWCs backed with continued IEC for Class IX to XII students</p>	<p>Premarital At Sub centre / PHC/ CHC Pre conceptional At Sub centre / PHC/ CHC</p>	<p>Pregnant Women (10-12 weeks) at Antenatal Clinic at PHC/ CHC/ DH</p>
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Conditions to be screened

<p>Sickle Cell Disease (SS, SD, Sβ⁰, SE)</p> <p>β⁰Thalassemia (β⁰/ β⁰, β^E/β⁰)</p> <p>Hb Variant traits Hb S Trait (β/ β^S) Hb E Trait (β/ β^E) Hb D Trait (β/ β^D)</p>	<p>Thal Major and severe Thal Intermedia β-Thalassemia (β⁰/ β⁰, β⁰/ β⁺, β⁺/ β⁺, β⁰/δβ, β⁰/ HPFH)</p> <p>Hb S- β thalassemia (β⁰/β^S, β⁺/β^S) Hb E - β thalassemia(β⁰/β^E, β⁺/β^E) Hb D- β thalassemia (β⁰/β^D, β⁺/β^D)</p>	<p>β-Thalassemia Trait and variant Hb Traits and mild Thal Intermedia (β⁰/ β, β⁺/ β)</p> <p>δβ-Thal Trait (β/δβ)</p> <p>Hb Lepore (δβ) Trait (β/ (δβ)⁰, β/ (δβ)⁺),</p> <p>Hb S Trait (β/ β^S) Hb E Trait (β/ β^E) Hb D Trait (β/ β^D)</p>
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<p>Hb HPLC by NBS Variant Follow up with Hb- HPLC at 6 months and 12 months</p>	<p>Complete Blood Count Peripheral smear Hb HPLC S. Ferritin or sTfR</p>	<p>NESTROFT + DCIP + SOLUBILITY TEST Complete Blood Counts Hemoglobin HPLC Serum Ferritin, if required</p>
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DNA based tests for detection of mutation are essential for prenatal diagnosis and thus all carrier couples should be further tested for detection of causative mutation at the earliest

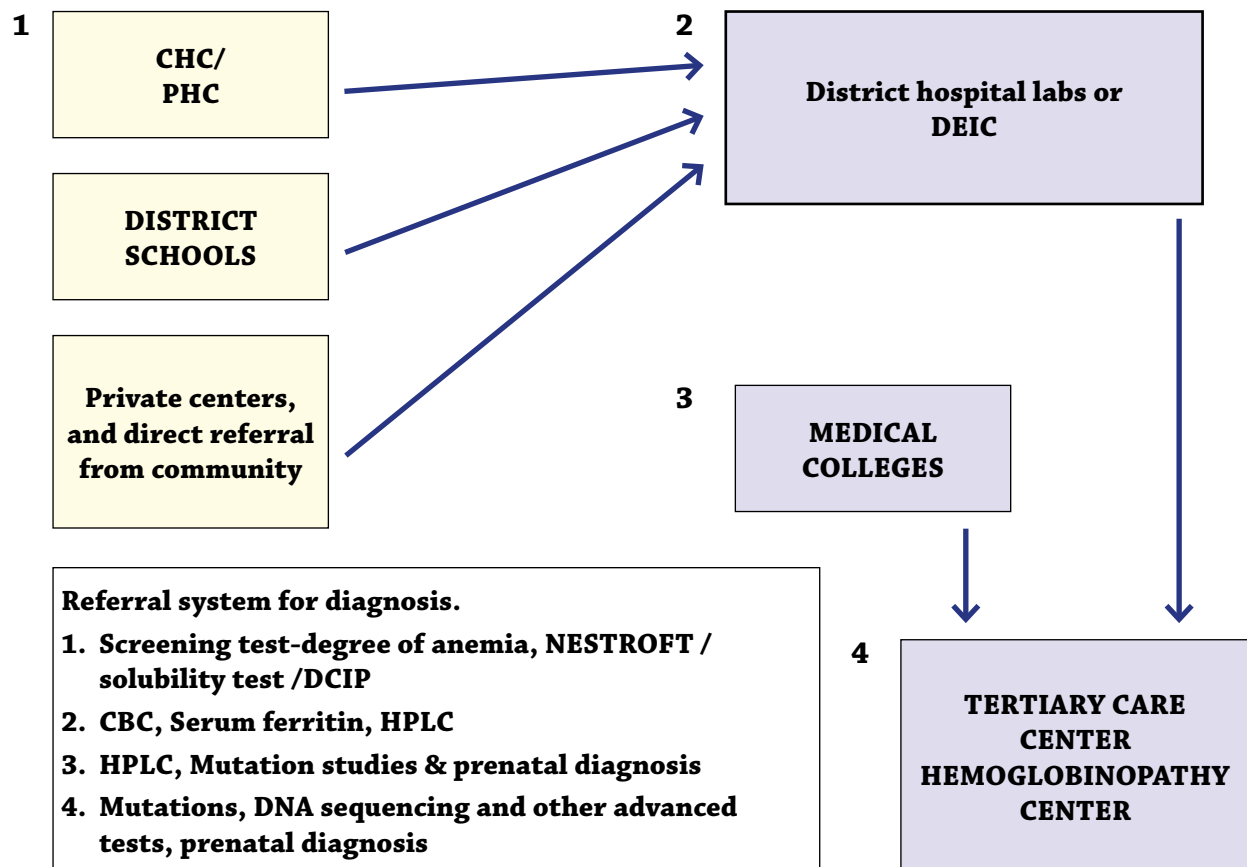
All patients should also be tested for causative mutations.
In adolescents, detection of the causative mutation can be undertaken at a later stage but should be clearly mentioned in the report and during counseling.



2. ESTABLISHING LABORATORY SERVICES AT DIFFERENT LEVELS

The laboratory services have to be established and strengthened at all levels in accordance with requirements of recommended tests to be done at that level as per screening and management guidelines.

Figure 3. Availability of tests at various centres



2.1 LABORATORY SERVICES AT PRIMARY LEVEL –CHC, PHC, AWC AND IN SCHOOLS

Only the three single tube based screening tests and hemoglobin estimation are to be performed. These tests are meant only for initial screening and are non-diagnostic. ANMs, Staff Nurses working at CHCs, PHCs and those with Mobile Health Teams, Lab Technicians at CHCs and Field Officer and Field Assistants based at DEICs are to be trained to perform these tests.

Tests required to be performed

- NESTROFT
- Solubility test
- DCIP test
- Hemoglobin

Instruments required- Digital hemoglobinometer / Hemoglobin card for Hb estimation

Incubator for conduction of DCIP test, refrigerator for stock solutions, Glassware and Disposables- test tubes, syringes, racks, vials etc - all these are available at CHCs and PHCs.

Prepared stock solutions of reagents for conduction of tube tests are to be periodically supplied by District Hospital or DEIC labs (where available) to maintain quality of solutions

- Pregnant women showing a positive result for any of the tube based screening tests and those showing severe anemia are to be referred to District Hospital or DEIC by appropriate transport services (104/ 108), for further investigation and treatment.
- Samples of students showing a positive screening test to be collected in appropriate vials and transported to DEIC labs. (For details refer to DEIC lab manual).

2.2 LABORATORY SERVICES AT SECONDARY LEVEL-DISTRICT HOSPITAL OR DEIC

The DEIC lab established under RBSK or District Hospital labs can be upgraded, to serve as secondary level diagnostic centres for the hemoglobinopathies programme. The lab technicians should be trained to conduct all the tests required.

Tests required to be performed:

- RBC indices through blood cell counter
- Tube based screening tests (NESTROFT, DCIP test and Solubility test)
- Hemoglobin HPLC
- IEF
- Serum ferritin, if required
- Peripheral smear, if required (Serum iron, TIBC if available)

Instruments required: HPLC equipment, IEF equipment, three part Blood Cell Counter, Microscope and Elisa Reader

Detection of carriers is based mainly on Hemoglobin pattern on HPLC

2.3 STATE / REGIONAL LEVEL LAB

A lab of a good District Hospital or Medical College can be selected to function as a State level lab or a Regional level lab in big States for the Hemoglobinopathies programme.

Tests required to be performed-

- HPLC Variant
- RBC indices through blood cell counter
- Serum ferritin, if required
- Peripheral smear, if required (Serum Iron & TIBC, if available)
- Mutation studies and prenatal diagnostic procedures. (Instruments required are mentioned in table 1).



2.4 TERTIARY LEVEL REFERRAL LAB / NATIONAL CENTRES

The States to identify institutions that can be strengthened to function as national referral centres. These institutions will function as national referral centers for hemoglobinopathies with facility for Hemoglobin HPLC system / Isoelectric Focusing system and Capillary Zone Electrophoresis system for identification of unknown rare hemoglobin variants picked up during screening and facility for DNA sequencing to detect unknown rare mutations.

Table 1 is a checklist of all the equipment required for conduction of these tests. Of these many of the equipment are likely to be present in the lab or granted under DEIC lab requirements as shown in the budget section of this document. The equipment specific for Hemoglobinopathies will be provided through this programme to strengthen the DEIC / District Hospital labs, State level and tertiary referral lab.

Equipment required for DNA analysis have not been included in the list as in the first phase DNA analysis will be conducted in existing molecular labs.

**Table 1:
Equipment required at District / DEIC level, Regional / State level
and tertiary / national referral labs
Check List of Lab Equipments**

S. No.	Name / Description	Specifications/ Purpose
1	Binocular Microscope	For peripheral blood examination
2	Automated Blood Cell Counter (3 part differential)	3 part differential automated blood cell counter for complete blood counts of samples for anemia and thalassemia or hemoglobinopathies
3	Hemoglobin HPLC Equipment	For district hospital or DEIC labs. Equipment capable of loading a minimum of 10 test at one time for Hb fraction estimation - HbA0, HbA1c, HbF, HbA2 and common Hb for analysis of samples for thalassemia and hemoglobinopathies
4	ELISA Reader with washer	ELISA of Dried Blood Spot Samples of newborns, Serum Ferritin in case of anemia to establish or rule out iron deficiency
5a	Hemoglobin HPLC* equipment for Newborn screening by DBS	HPLC equipment that can process Dried Blood Samples for separation of Hb fractions to enable diagnosis of Sickle Cell Syndromes and Hb D, HbE and HPPFH syndromes and traits *for Regional / State level labs.

S. No.	Name / Description	Specifications/ Purpose
5 b	Isoelectric focusing (IEF) equipment for newborn screening*	The equipment is used to isolate hemoglobin forms by means of isoelectric focusing (IEF). The IEF technique combined with the specially formulated gels enables good separation between hemoglobin bands differing by only 0.02 pH units, resulting in highly resolved, identifiable results from whole blood, cord blood or blood spot samples . It is an end point method with high throughput capability and thus can be used for newborn screening <i>*for State or District / DEIC labs</i>
6	Hemoglobin HPLC Variant system #. This equipment capable of differentiating different hemoglobin variants and also performing cord blood HPLC to diagnose thalassemia major prenatally	The equipment is capable of differentiating different hemoglobin subtypes and also performing cord blood HPLC to diagnose prenatal thalassemia major – <i># for Tertiary level centres (Hematology departments) / Medical colleges</i>
7	Capillary zone Electrophoresis System	The equipment is based on electro osmotic flow provides high resolution electrophoretic separation with identification of most of the hemoglobin variants. For national level tertiary referral centres only. To enable identification of unknown variants picked up during the course of screening.
8.	Refrigerator (two)	180-310 L, (Domestic) one for storage of samples, kits reagents in use and one for stock kits and reagents.
9.	Laboratory centrifuge	For separation of serum, plasma
10.	Incubator	For conduction of DCIP test (a portable incubator may be required if test to be conducted in outreach community settings like schools and AWCs as the test requires incubation of tubes at 37°C for 1 hour)
11	Rotor sample mixer	For mixing samples
12	Syringe Needle destroyer	For safe disposal of needles and syringes
13	Air Conditioner	Must for maintenance and working of equipment

S. No.	Name / Description	Specifications/ Purpose
14	Micropipettes- Variable volume	100 to 1000 microlitre- two & 5 to 20 microlitre –two
15	Micropipettes- Fixed volume	Two for use in lab and 4 /block for use by Block teams for conducting NESTROFT and other tube tests
16	Deep Freezer one	For long term storage of samples
17	10 KVA UPS	To ensure continuous power supply for equipments
18	Desktop computer with internet connection	For DH or DEIC based Field Officer to enter and store and mail the screening data for follow up and counseling purposes.

**As the cost of the equipment and cost per test is considerably less than HPLC equipment for newborn screening (5a). Thus it may be provided even at District level labs in States with high prevalence of Sickle Cell Anemia*

3. SCREENING PROTOCOLS FOR DETECTION OF CARRIERS

Objective of screening for carriers is identification of carriers before marriage so as to avoid marriage between two carriers to eliminate possibility of birth of an affected child. The identification of carrier status of both parents during early pregnancy will enable prenatal diagnosis of an affected fetus so as to provide an opportunity for termination of pregnancy avoiding birth of an affected child.

Carrier states to be screened for are-

- β -thalassemia trait (BTT)
- HbE trait
- HbS trait

Other carrier states and asymptomatic conditions detected during the course of screening are also to be reported. Compound or homozygous states if detected during the course of screening are to be reported.

Note: A carrier rate of 1% or more may be taken as cut off for implementation of universal screening programmes in a defined population such as pregnant women, adolescents in a given geographically or administratively defined area such as a district or a state

3.1 PRIMARY LEVEL SCREENING-AT CHCS, PHCS, AWCS AND SCHOOLS.

- **Initial screening** of all individuals is done by NESTROFT, DCIP test and Solubility Test respectively for BTT, HbE trait and HbS trait performed on a finger prick blood sample. *Turbidity of the solution with a drop of blood is indicative of a positive screening test. Hemoglobin test by digital Hemoglobinometer or Hb card is also done on the same finger prick sample.*
- **Samples of those with a positive screening test and a Hb of 8 gm/ dl or more are** collected and transported to the DEIC (where available) or District Hospital lab for further tests- Complete Blood Count (CBC) and HPLC.
- Results of CBC and HPLC are to be interpreted as per Screening guidelines (Table 4, Section B). *Diagnosis is based on the results of Hb HPLC.*

Note: Severe anemia defined as an Hb of <8 gm/ dl (as per National Iron Plus Initiative guidelines) itself can cause a positive tube test. Those with severe anemia, are to be referred directly for investigation and treatment to Pediatrics Department of District Hospital or DEIC. If nutritional anemia is found then screening of these individuals for hemoglobinopathies is to be repeated after correction of anemia.

Those with mild or moderate anemia are to be treated with the aim of –

- a) Replenishing iron stores in IDA to well within normal limits (corresponding to serum levels of around 100 ng/ml) so as to decrease prevalence of anemia, especially maternal anemia;
- b) Avoid unnecessary iron therapy in the absence of iron deficiency as reflected by serum ferritin levels;
- c) Enable cause appropriate treatment / management of anemia.

Precautions

- For an effective screening, minimizing false negative tests is necessary and it is recommended that calibrated digital hemoglobinometers be used for determination of Hemoglobin on a finger prick sample as pallor has a poor sensitivity for mild and moderate anemia making it difficult to accurately distinguish severe anemia from moderate and mild anemia.
- It is essential to maintain quality of NESTROFT solution possibly by preparing this solution centrally and calibration of Hemoglobinometer.
- Samples collected for CBC and HPLC should be transported to DH or DEIC properly and timely; as given in DEIC lab manual.

3.2 SECONDARY LEVEL SCREENING- AT DISTRICT HOSPITALS / DEICs

District Hospital or DEIC is expected to be the most common point for individuals or couples seeking premarital or pre- conceptional and antenatal screening for carrier status as a result of awareness created by IEC campaigns.

Initial screening for β -thalassemia carrier state is done by collection of blood by venepuncture and conduction of CBC as the DEIC lab or a District Hospital lab is equipped with an automated Blood Cell Counter.

- DCIP test and Solubility test are also to be added if there is any significant prevalence as they are simple and very low cost tests. (HbS and HbE carriers usually have a normal MCV and MCH and thus will be missed by CBC).
- CBC report showing an MCV of <80 and MCH <27 in a sample with Hb > 8 gm/dl is regarded as a positive screening test for BTT, and the sample is taken up for diagnostic testing by HPLC

(Note: It is recommended that NESTROFT be also conducted on the samples screened at DEIC or District level labs and if a sample with normal MCV and MCH and Hb >8 shows a positive NESTROFT, it should also be taken up for further testing by HPLC as combined screening with CBC and NESTROFT has been shown to be more effective as mentioned in Section A)



Results of HPLC are to be interpreted as per screening guidelines (Section B, Table 4). *Diagnosis is based on the results of Hb HPLC.*

All the tests reports with ambiguous or equivocal cut off values and unknown variants should be referred to a State level or national level tertiary centre. An incorrect diagnosis should be avoided at all costs

Issuing of report of HPLC analysis must be accompanied by counseling and collection of sample for confirmatory test (DNA analysis); after informed consent is taken.

- Pregnant women screened before 16 weeks, if found to be carriers should be followed by screening of husband and if husband is also found to be a carrier, **offer counseling to the “at risk couple”**

The choice available to an ‘at risk’ couple: *Today, parents who are aware that they are both carriers of β -thalassemia or Sickle Cell disease have a number of choices with regard to having a family. These should be discussed as early as possible with an expert health professional and/or a genetic counselor. Prenatal testing is a choice to many families. The mutation studies are performed and then the doctor proceeds to find out whether the fetus is affected or not and then the family is given the option of pregnancy intervention (termination) for an affected child.*

- If the couple opts for prenatal diagnosis (PND) they should be referred to a tertiary centre with facilities for PND. Ideal time for Chorionic Villus Sampling (CVS) for PND is 10-12 weeks. However, women coming after 12 weeks can be provided PND by amniocentesis, providing an opportunity for termination of pregnancy before 20 weeks if required.

3.3 IMPLEMENTATION OF UNIVERSAL ANTENATAL SCREENING AND PRENATAL DIAGNOSIS

Under this provision all pregnant women attending public healthcare facilities at all the levels are to be screened for carrier status as per screening guidelines and are to be followed up with prenatal diagnosis where required.

Facilities for provision of prenatal diagnosis (PND) are to be established in two phases

Phase-I- Establishing a referral system for couples in need of PND to existing centres with facilities for prenatal diagnosis.

Phase- II-Establish facility for prenatal diagnosis at one or more district level government hospitals

Phase-I- Establishing a referral system for couples in need of PND to existing centres with facilities for prenatal diagnosis.

During antenatal screening, pregnant women whose husbands are also detected to be hemoglobinopathy carriers, are to be referred for prenatal diagnosis to the nearest centre. *The required financial support for test cost and permissible fare for two persons (mother and father or accompanying person) should be budgeted by the State as per budget guidelines.*

Step 1

Micro-planning for antenatal screening and referral for prenatal diagnosis. Screening of all pregnant women on first visit (1st trimester) to CHC/ PHC by tube tests (NESTROFT, DCIP test, Solubility test) along with routine Hb, Urine test for protein, Blood Group, HIV, and Blood sugar.

Step 2	If Screening test positive, refer the patient along with husband by 104 / 108 service to District Hospital/ DEIC for further testing
Step 3	The pregnant woman to be admitted at District Women Hospital under JSSK for conduction of CBC and Hemoglobin HPLC test of both wife and husband
Step 4	If wife and husband both are detected to be hemoglobinopathy carriers on Hb- HPLC testing, an Ultrasound test (USG) of the wife (the pregnant woman) to be done to determine the gestational age of the fetus and provision of counseling the couple
Step 5	One of the following steps to be followed depending on gestational age- a) If gestational age 12 weeks or less, couple to be referred to nearest tertiary centre for prenatal diagnosis by CVS through coordination with the DH / DEIC b) If gestational age >12 weeks but < 20 weeks, consultation for possible PND by amniocentesis at the tertiary centre c) If gestational age >20 weeks, counseling for possible outcomes of pregnancy and follow up of pregnancy. If the baby born is an affected child determined by DNA based tests, then after family counselling, early intervention by registration in DH/ DEIC for management and care is required.

Note: Effort should be to ensure maximal referral before 10 to 12 weeks of gestation

Table 2: List of some government centres with facility for prenatal diagnosis

S. No.	Name of centre / institution
1.	All India Institute of Medical Sciences, New Delhi
2.	Sanjay Gandhi Post Graduate Institute of Medical sciences, Lucknow
3.	Post graduate institute of medical education and research, Chandhigarh
4.	IHTM, Calcutta Medical College, Kolkata
5.	Maulana Azad Medical College, Delhi
6.	Centre for DFD Hyderabad
7.	ICMR National Institute of Immunohaematology, Mumbai
8.	SAT Trivandrum Thalassemia Control Unit
9.	NRS Medical College, Kolkata

Phase-II-Establish facilities for prenatal diagnosis at one or more district level government hospitals in each State

Requirements for establishing prenatal diagnosis facility at State level government hospitals / State medical colleges

- Training of obstetricians and / or radiologists to build capacity for CVS, amniocentesis or Fetal blood sampling and training of pathologists and District Hospital and DEIC Lab technicians in genetic testing
- To frame guidelines for setting up DNA analysis lab facility at select State level labs



Important to take note while setting up a prenatal diagnosis centre, that it must follow the strict operational framework per the PCPNDT Act, to avoid misuse of the facility.

3.4 IMPLEMENTATION OF UNIVERSAL SCREENING OF ADOLESCENTS IN SCHOOLS

Under this provision one- time screening of all students studying in government and government aided schools is to be done followed by providing screening report and counseling to students detected to be carriers, in presence of parents/ guardians in schools or at nearest PHCs/ CHCs.

Hemoglobinopathy carrier screening in schools to be done in class VIII or above students

by DH /DEIC based team of one Field Officer and one Field Assistant who will join the Mobile Health Teams deployed under RBSK at respective Blocks for screening in school.

IEC activities in schools to be conducted for all classes from VIII to XII

Estimation of screening target

As about 160 working days are available for conducting screening in school and a three member team of Field Officer, Field Assistant and Staff Nurse of MHT, can screen 150 students during a school visit, one DEIC or if not available the District hospital based team can screen 24000 students in an academic year in coordination with Mobile Health Teams of RBSK.

Two such teams in a district accompanying each of the two Block MHTs can cover a target population of 50000 students / district by conducting screening of 300 students/day for an average of 20 days/ month on 4-6 days/week spread over 32 weeks in 8 months in a year.

In the remaining days of the year, the DH/DEIC based team can schedule 40-80 follow up visits required for providing screening reports and counseling of students detected to be carriers at nearest PHCs/CHCs or in schools in presence of their parents/guardians. Collection of samples for confirmatory testing by DNA analysis with consent of parents can also be done during these visits

Requirements for screening and follow up visit:

For a district, one or two DH / DEIC based teams depending on the screening target and one vehicle hired on a daily/ monthly basis are required and to be budgeted for by the State

Visit to schools in blocks distant from DH/ DEIC will require overnight stay of the team. DA will be permissible to team members.

Pathologists are required to accompany the team in follow up visits and at least 10% of screening visits in school.

Micro planning for screening of adolescents in schools

Preparation of screening schedule:

Step 1

DH/ DEIC based Field Officer to prepare quarterly visit schedule for all government and government aided schools for one time screening of class VIII students in the district and communicate and coordinate with respective Block MHTs so that Staff Nurse of Mobile Health Team can join the Field team for screening of the blood disorders, anemia and Hemoglobinopathies.

The schedule will be prepared taking into account the number of students to be screened in each school and on the capacity of one team to screen 150 students / visit.



Schedule for school visit by DH/ DEIC based field team for adolescent screening

Date	School Name	School code	Block	No. of students

Schedule of follow up visits for counseling to be prepared by Field Officer based on screening reports indicating the number of students required to be followed up

Step 2 In school, the Field Officers give an awareness and education talks for class VIII-XII students prior to screening.

- As students of class VIII and IX are relatively young to understand and retain the information regarding carrier status, it is of vital importance that the mandatory educational talk on anemia, hemoglobinopathies and other inherited disorders prior to screening in the school be conducted for all students from Classes VIII to XII each year.

Step 3 Particulars of each student of class VIII to be recorded in screening sheet and screening tests – NESTROFT and Hb estimation- to be conducted and results recorded. (Solubility test and DCIP test to be added in districts with high prevalence)

- All adolescents with mild or moderate anemia should be referred to PHC or CHC or should be provided therapeutic iron and folic acid (IFA) by the RBSK team in school.
- All students showing severe anemia (Hb <8 gm/dl) to be referred to DH / DEIC. Collected samples are subjected to CBC and HPLC at the DH/ DEIC lab and results recorded and interpreted as per screening guidelines (Section B, table 4).

Step 4 Collection of samples in EDTA and plain vial of those students showing a positive NESTROFT and an Hb > 8 gm/dl by Field Assistant and Staff Nurse and carried to District hospital or DEIC lab by the team

- If required, serum ferritin may be done specially in those which show unequivocal result of Hb A2 between 3.5 to 3.9% and those with HbA2 values diagnostic of BTT but Hb <12 gm/dl to detect concomitant iron deficiency.

Step 5 Follow up visit to be scheduled for nearest PHC / CHC where students detected to be carriers should be called along with parents/ guardians for counseling and collection of samples for confirmatory testing after written consents on sample collection forms.

- Counseling is the most important component of adolescent screening in schools. While pre screening educational talk primes the students about the purpose of screening. At the time of individual counseling, it is important to clearly reinforce the two options available to a carrier to avoid birth of an affected child later in his/her family by 1) avoiding marriage with a carrier and 2) opting for antenatal screening after marriage.

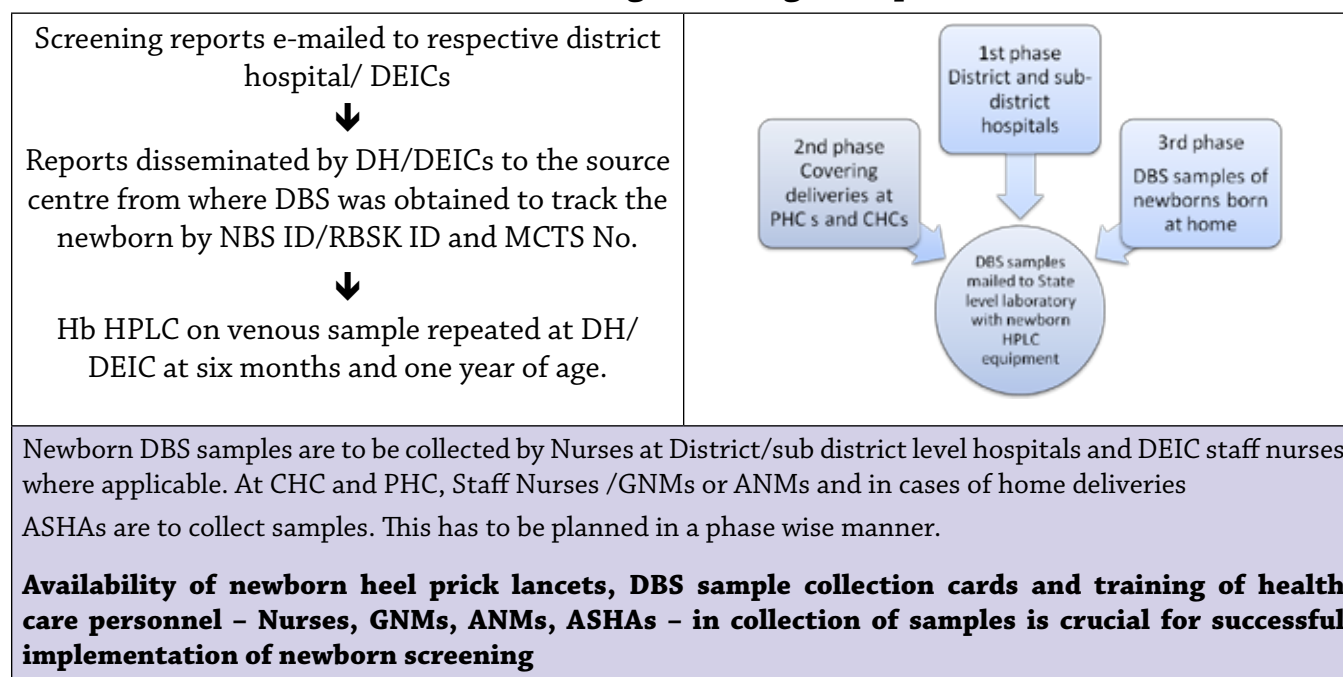


3.5 NEWBORN SCREENING FOR HEMOGLOBINOPATHIES

The screening is to be done by Dried Blood Spot samples collected by heel prick of newborns between 24 and 48 hours of age. The DBS samples can be used for screening of other metabolic disorders like Congenital Hypothyroidism, G6PD deficiency, CAH and other IEMs.

- For screening of sickle cell disease and other hemoglobinopathies the samples will be mailed to the State level lab equipped with Newborn Hb HPLC system or to District Hospital / DEIC lab equipped with IEF system.
- The sickle homozygotes and heterozygotes should be recorded separately under the confirmatory testing column. The SCD syndromes are to be screened on the basis of Hb pattern found at birth (Section B, table 7)
- In addition complete absence of Hb A in β^0/β^0 , E/ β^0 with presence almost 100% HbF or HbE and F at birth indicates thalassemia syndromes which need to be reported for follow up and confirmation at a later stage.
- A diagnostic test by HPLC of venous blood to be done at 6 months of age for timely institution of therapy and repeated at 1 year of age for final diagnosis.

Figure 4.
Newborn screening for hemoglobinopathies



3.6 SCREENING FOR EARLY DETECTION OF CHILDREN AFFECTED WITH THALASSEMIA DISEASE

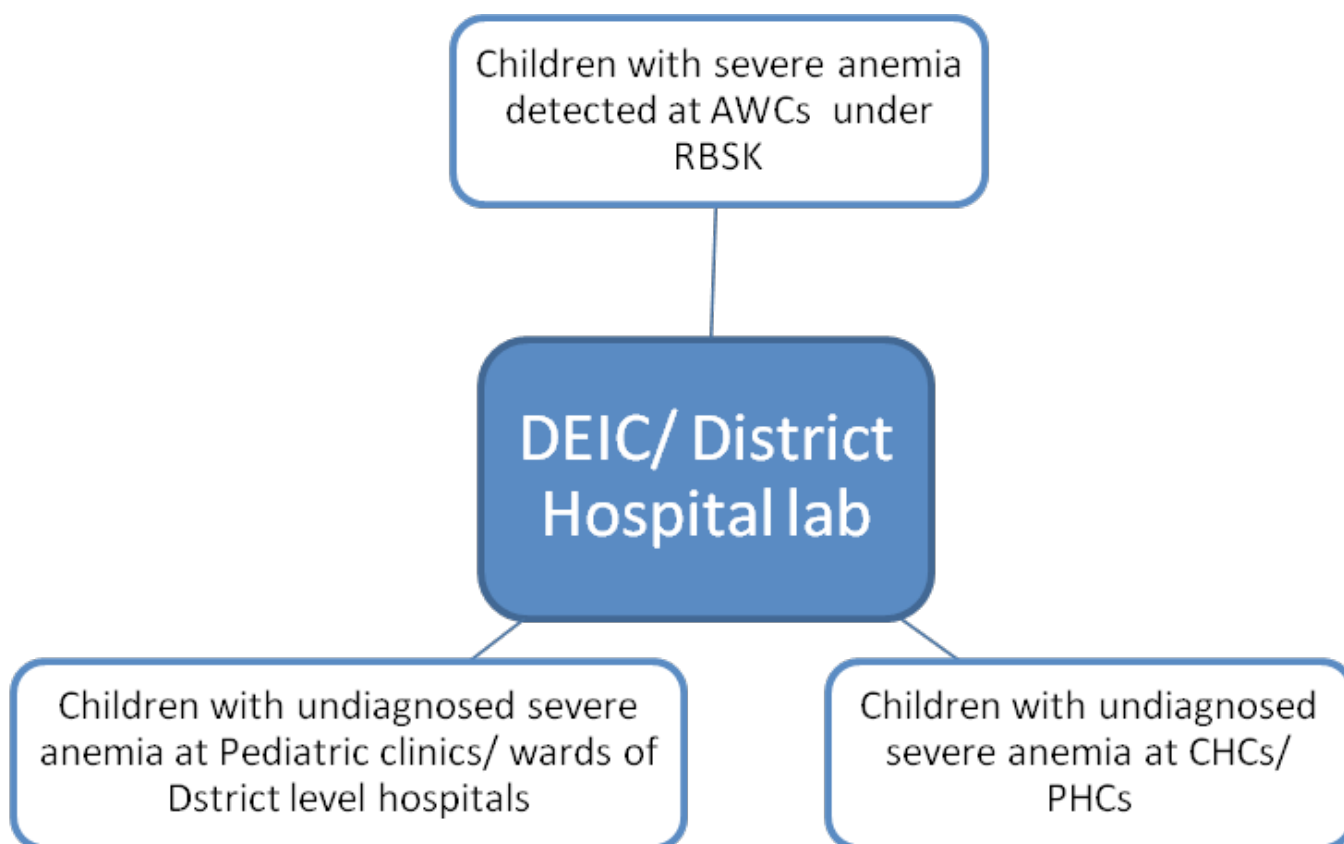
Children affected with clinically severe thalassemia disease manifest with severe anemia usually after 6 months of age when Fetal Hb production starts reducing and in normal cases is replaced by adult hemoglobin HbA, by the age of one year. In children affected with thalassemia, there is deficient synthesis of HbA due to lack of β -globin chains hence development of severe anemia is the earliest

manifestation of the disease. This anemia is accompanied by normal or increased levels of iron. Later on other complications develop. Most of the severe cases of thalassemia that require management by regular blood transfusions manifest by 5-6 years of age.

Thus, selective screening of children between 6 months and 6 years of age presenting with severe anemia for thalassemia disease is a cost- effective approach for early detection of thalassemia.

Figure 5.

Schema for screening of children with severe anemia, for the early detection of thalassemia disease at the District hospitals or DEIC centres.



Under RBSK all children between 6 weeks to 6 years of age are screened for anemia by clinical examination at AWCs. Severe anemia, defined as $Hb < 7\text{gm/dl}$ in children, is usually detectable by clinical examination. These children are referred to DEIC for further investigation and treatment.

Similarly, children with severe anemia without an obvious disorder attending pediatric clinics at District level hospitals or at CHC and PHC should be referred to DEIC for further screening and intervention.

4. MANAGEMENT OF PATIENTS WITH THALASSEMIA AND SICKLE CELL DISEASE

Regular transfusion and iron chelation is the mainstay of management of patients with thalassemia disease and regular monitoring and care is required for SCD patients. HSCT is the only curative therapy currently available for these disorders.

4.1 DAY CARE CENTRES AT DISTRICT HOSPITAL / DEIC

Management of thalassemia and SCD patients through a day care facility is convenient, economical and provides a supportive environment friendly area for children with a chronic illness. It helps in developing a good rapport with staff and better compliance and efficient monitoring of patients.

Requirements for a day care centre-

1. A transfusion facility / ward with 5- 10 beds as per requirement, providing transfusion services on daily basis in multiple shifts to accommodate more patients with provision for night shift for working or school going patients.
2. Trained nursing staff for conducting regular transfusions, monitoring of growth and development and maintenance of records, during the period of blood transfusions
3. Staff turnover should be as low as possible to provide continuity of care.
4. Should have management protocols for transfusion therapy, transfusion reactions and chelation therapy to serve as guidelines for staff.
5. Must have a well-trained doctor to handle complications related to transfusion reactions in a hospital setting.
6. Transfusions in the domiciliary setting, is highly discouraged.
7. It is desirable to have recreational facilities to keep children entertained during transfusion.
8. Provision of oral iron chelator drugs (medicines)
9. Supporting laboratory for conduction of regular tests for evaluation and monitoring purposes
10. For the care of thalassemia patients the following requirements are mandatory to ensure regular and safe blood transfusion-
 - Supporting Blood Bank to provide component therapy in the form of PRBC.
 - Establishment of leuco-depletion facilities and extended red cell phenotyping
 - Blood transfusion sets and availability of leuco-depleting filters, if blood bank does not provide pre-storage leuco-depletion.
 - Transfusion charts for monitoring
 - Emergency kit for managing blood transfusion reactions
 - Care of transfusion transmitted infections (TTI) of positive patients.

Thus trained dedicated staff are necessary for treating children with defects and disabilities that includes a Pediatrician and / or Medical Officer, Staff Nurses, Laboratory technician, Counselor and a Psychologist with, laboratory and recreational facility to provide an ideal setting for providing day care management facility for thalassemia and SCD patients. These centres may be set up within or co-ordinated by the pediatrics departments of the hospital.

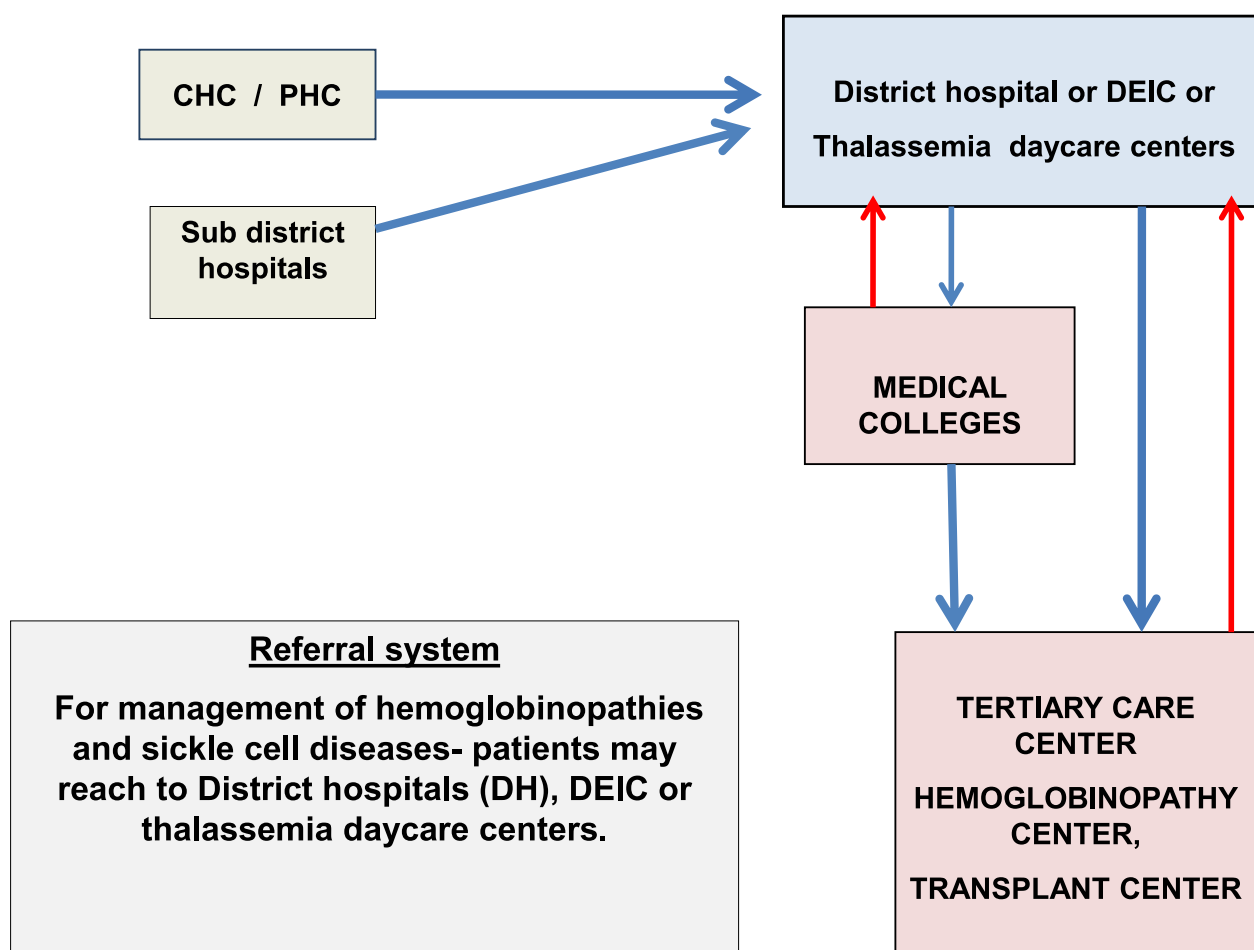
In a 10 bedded day care centre, daily 10-15 patients can be accommodated. 300 patients per month could be managed for thalassemia and sickle cell disorder.

Monitoring of patients registered at Day care centres of District hospitals or DEICs

Thalassemia Daycare Patient Monitoring Sheets have been formatted and need to be filled on every visit (Section C, Annexure C-1). This ensures adequate management and serves as a baseline of minimum monitoring that is needed for care.

Each patient’s record file is maintained along with a computerized record file by the staff nurse in the DH/ DEIC computer. The monthly reporting of number of patients registered under care is to be done as indicated in the Monthly Progress Report format provided later in this section

Fig 6. Showing the entry points to the District or DEIC daycare or blood transfusion centers.



4.2 HEMATOPOIETIC STEM CELL TRANSPLANT (HSCT) /BONE MARROW TRANSPLANT (BMT)

Hematopoietic Stem Cell transplant (HSCT), commonly called Bone Marrow Transplantation (BMT) is the only curative therapy for thalassemia major and is well established. However older children, with inadequate chelation, liver enlargement, fibrosis, splenomegaly are at high risk of adverse outcomes and complications to this procedure. Sickle cell disease transplant indications are very selective, due to the risks of morbidity associated with the transplant procedure.

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 centres 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. Few government centers have provision for HSCT, but many private centers offer this procedure. Below is a list of some of the larger transplant centers in India

List of some centres with facilities for Hematopoietic Stem Cell Transplant

1. CMC Hospital Vellore
2. Tata Memorial Hospital, Mumbai
3. Hematology department, AIIMS, New Delhi
4. Apollo Specialty Hospital, Chennai
5. R and R hospital, New Delhi
6. Tata Medical Centre, Kolkata, W Bengal
7. PGI Chandigarh, Punjab
8. Sahayadri Hospital, Pune, Maharashtra
9. Rajiv Gandhi Cancer Centre, New Delhi
10. Narayana Hirudalaya, Bengaluru, Karnataka
11. Manipal Hospital, Bengaluru, Karnataka
12. Apollo Hospital, Ahmedabad Gujarat
13. Sterling Hospital, Ahmedabad, Gujarat
14. Deenanath Mangeshkar Hospital, Pune, Maharashtra
15. CMC Ludhiana, Punjab
16. Sterling Hospital, Vadodara, Gujarat
17. Malabar Cancer Centre, Thalassery
18. Meenakshi Mission Hospital, Madurai, TN
19. Kovai Medical Centre, Coimbatore, TN
20. GKNM Hopsital, Coimbatore, TN
21. SRMC, Chennai, TN
22. SGPGI, Lucknow, UP
23. BL Kapur Memorial Hospital, New Delhi

- 24. Artemis Hospital, New Delhi
- 25. Medanta medicity, Gurgaon, Haryana
- 26. Sir Gangaram hospital New Delhi
- 27. Apollo Hospital, New Delhi
- 28. Max Superspecialty hospital Saket, New Delhi

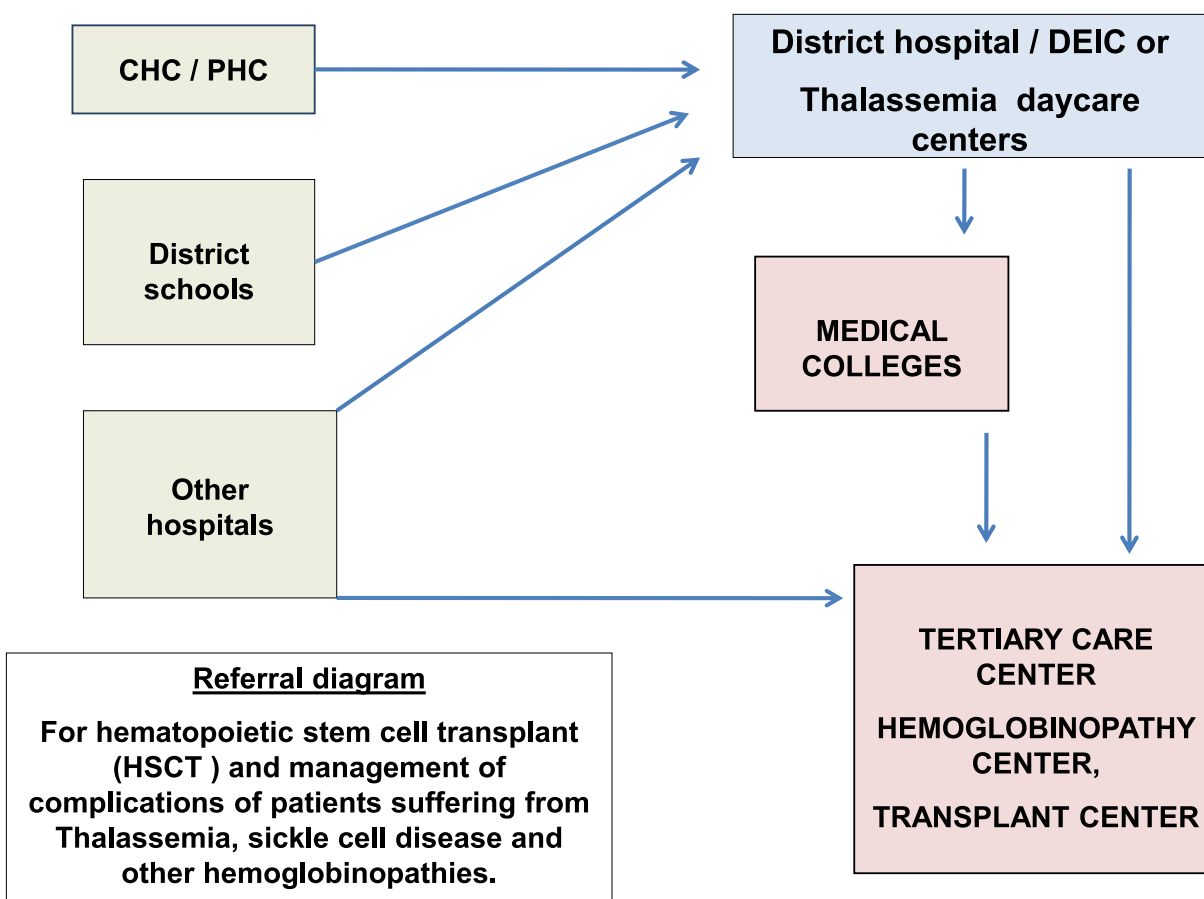
This is an expanding list as other centers are being added frequently

Budget estimates for the procedure have been provided later in the section

However, as of now, the financial support for the procedure of BMT / HSCT is not being provided through this programme under NHM.

In patients who are eligible for the procedure and fulfill the given criteria, financial support for the procedure may be identified from individuals, parent –patient organizations, charity institutions, CSR and other resources available with the State.

Fig 7. Referral schema for children needing Hematopoietic stem cell transplant (HSCT).



5. IEC STRATEGIES AND MODULES

It is the most important component of a hemoglobinopathies prevention and control programme. Hemoglobinopathies are the first group of single gene disorders with a Mendelian recessive inheritance pattern to be addressed through public health strategies. The strategies for educating the community about hemoglobinopathies, their treatment and prevention modalities will require to educate about inheritance pattern of the disease, carrier states and their role in prevention, the basis of transmission of the disease, variation in severity of the disease in the same family, factors in making choices for prevention, dissemination of information within the family and community. In the case of recessive genetic disorders, detection of carriers plays a key role in preventive strategies. The aim of IEC strategy is creation of an informed society willing to participate voluntarily in screening programmes and take steps for preventing births of children affected with the disease and access care for those affected with the disease.

The strategies to be adopted to achieve this are:-

Mass communication and media – to incorporate with NHM- IEC at national, state and district level. The messages should aim to remove any stigma and gender biases by promoting knowledge of genetics and inheritance by general and targeted campaigns and awareness about prevalence of disease and that it is preventable. People should be encouraged to acquire complete information about these disorders and should be made aware of specific initiatives of the government.

Mid media activities – IEC material and campaigns developed by the States should also focus on promotion of voluntary blood donation to fulfill requirement of blood and to improve access to care services to all affected by promoting knowledge of the treatment modalities available through the public health facilities. The display of posters at all health facilities and identified community places should be ensured. Non-government organizations (NGO) and community based organizations should be involved in

Educational curriculum – States should work with education department for inclusion of information about hemoglobinopathies in the school text books and school health programs

IPC and one to group communication – These are very effective IEC tools with well trained counselors and informed healthcare personnel. Some specific points of application are listed below below:

1. Adolescent screening in schools: An organized IEC module to ensure communication and retention of information is vital for success of carrier screening programme for adolescents. It should comprise.
 - A pre-screening power point assisted 30 minute educational talk by Field IEC officer.
 - Distribution of booklets on hemoglobinopathies and anemia urging the students to read and keep the booklet and organizing a quiz session based on booklet and talk. Encouraging better performing students by their participation in interschool quiz programmes events that may include other adolescent health issues.
 - One to one communication at the time of collection of venous blood sample of those with positive screening test

- One to one counseling at the time of follow up visit at school or PHC/CHC for collection of sample for confirmatory testing of those with single positive diagnostic test.
- Genetic counseling at the time of providing final report.

Repeated group and interpersonal communications make the screening process very effective.

2. At AWCs and AFHCs during screening of out of school adolescents: One to one counseling in at least two to three sessions and reinforcement of information by healthcare workers – ASHAs.
3. At SC, PHC, CHC and DH during antenatal screening of pregnant women.
4. During Blood donation camps and at Blood banks offering screening and counseling of voluntary donors.
5. At DEICs inform children who have thalassemia major about care and prevention of complications and affected families about the importance of family (cascade) screening.

The IEC material- posters, booklets and PowerPoint presentations with the centre will be provided to the States and States can develop their own material based on guidelines.

6. REPORTING AND MONITORING

6.1 RECORDING OF SCREENING DATA WITH LABORATORY RESULTS

A. Hemoglobinopathies Screening Datasheet:

Software formats in Microsoft excel for recording screening data of adolescents and pregnant women of different tests of each screened individual have been provided in the DEIC laboratory services manual. The hard copies of the same can be maintained in registers at DH/ DEICs. The initial part of the adolescent screening data will be entered by the Field Officers and transmitted to DH / DEIC where further laboratory data is filled in with diagnosis and outcomes. An ID is assigned to each of the students screened for the first time and it serves to follow up the student through the subsequent years and their reports will be retrievable at the DEICs using this ID at any time. The number of NESTROFT / Solubility / DCIP test positive samples should be recorded in 3 separate columns or else it will be difficult to know the false negatives found under each. Similar formats will be provided to PHCs, CHCs and DH for entering screening data of pregnant women using the MCTS ID.

B. Newborn Screening Datasheet:

Newborn screening for hemoglobinopathies is to be converged with screening for other disorders such as Congenital Hypothyroidism, G6PD Deficiency and other inherited errors of metabolism using the DBS sample. Hence the Microsoft excel newborn screening datasheet format comprises a section on the newborn's identity and is linked to the MCTS number and the DBS sample number with record of timing of sampling. Screening tests and confirmatory tests are recorded and outcomes recorded on their basis from a drop down menu. The number of NESTROFT / Solubility / DCIP test positive samples should be recorded in 3 separate columns or else it will be difficult to know the false negatives found under each. Formats of different reporting and recording forms that require to be printed have been provided in the laboratory services guidelines



2. MPR format for Antenatal Screening

Antenatal screening MPR		State name (code):					District name (code):					
Months	No. screened by CBC and NESTROFT	No. with NESTROFT/Solubility test/DCIP test positive	Screening positive by with CBC	Total no with screening positive	Severe Anemia Hb <8 gm/dl	No. tested by HPLC	No. with positive HPLC	No. of women whose husband tested	No. referred for prenatal diagnosis	No. of women undergone PND	No. of women with positive PND	No. of women with positive PND undertaken MTP
Jan	0	0	0	0	0	0	0	0	0	0	0	0
Feb	0	0	0	0	0	0	0	0	0	0	0	0
March	0	0	0	0	0	0	0	0	0	0	0	0
April	0	0	0	0	0	0	0	0	0	0	0	0
May	0	0	0	0	0	0	0	0	0	0	0	0
June	0	0	0	0	0	0	0	0	0	0	0	0
July	0	0	0	0	0	0	0	0	0	0	0	0
Aug	0	0	0	0	0	0	0	0	0	0	0	0
Sep	0	0	0	0	0	0	0	0	0	0	0	0
Oct	0	0	0	0	0	0	0	0	0	0	0	0
Nov	0	0	0	0	0	0	0	0	0	0	0	0
Dec	0	0	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	0	0	0	0	0	0	0	0	0

3.MPR format for newborn screening for hemoglobinopathies

State name (code) :		District name (code):									
Month	Hemoglobinopathies										
	Number of Newborns Screened			Screening Test (newborn DBS- HPLC) Positive			Number of confirmatory tests	Confirmatory Test (Venous blood Hb-HPLC at 1 year age) Positive			
	M	F	T	M	F	T		T	M	F	T
Apr	0	0	0	0	0	0	0	0	0	0	0
May	0	0	0	0	0	0	0	0	0	0	0
Jun	0	0	0	0	0	0	0	0	0	0	0
Jul	0	0	0	0	0	0	0	0	0	0	0
Aug	0	0	0	0	0	0	0	0	0	0	0
Sep	0	0	0	0	0	0	0	0	0	0	0
Oct	0	0	0	0	0	0	0	0	0	0	0
Nov	0	0	0	0	0	0	0	0	0	0	0
Dec	0	0	0	0	0	0	0	0	0	0	0
Jan	0	0	0	0	0	0	0	0	0	0	0
Feb	0	0	0	0	0	0	0	0	0	0	0
Mar	0	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	0	0	0	0	0	0	0	0

6.3 NATIONAL WEB BASED REGISTRY OF HEMOGLOBINOPATHIES

A national web based registry or database is an important tool for planning future patient services. Apart from number of carriers and cases identified it collects other useful data, such as the geographical and ethnic origin, to identify areas and populations with high prevalence, types of mutations, genotypic



and phenotypic data and out-comes of patients and other data which helps to evaluate the success and status of the control program, records of deaths and their cause which is a basic source of information directing the treatment choice.

ThalInd, is a web based database of beta thalassemia and abnormal hemoglobins created to serve as an informatics resource for hemoglobinopathies in India. The resource uploaded with collated data available in 2009 was created using the LOVD system, an open source platform independent system, promoted by the Human Genome Variation Society, the international consortium for genetic variants causing diseases. The resource aligned with the administrative health system and census based demographic resources accommodates data on mutations and their characteristics (molecular genetics), frequency of different mutations and their geographic and ethnic origin (population genetics), correlation of mutation with clinical data on gender and age distribution, disease type and severity (genotype- phenotype correlation) , mortality and morbidity (disease burden) and registration of patients with thalassemia centres and support groups (infrastructural services). The database designed with multiple access level transferred to a server under the ministry, upgraded and updated and modified to accommodate more types of data elements will serve as tool of surveillance of the programme.

7 HUMAN RESOURCE

The additional staff requirements have been proposed to strengthen the RBSK cell at State HQ and at the DEICs. Success of the programme depends heavily on the quality of laboratory services provided, the conduction of population screening and the IEC activities to back up the screening programmes.

Table 3.

The following table shows the existing and additional proposed staff under RBSK at State, District (DEIC) and Block (Mobile Health Team) level required for implementation of the hemoglobinopathies programme.

Professional	State HQ		DEIC/		Existing staff at Block level-Mobile Health Team
	Existing	Additional Proposed	Existing	Additional Proposed	
Technical Consultant (Pathologist/ Pediatrician)	-	1			
Child Health Consultant	1	-			
IEC Co-ordinator	-	1			
Accountant/ Ex. Asstt.	-	1			
Pediatrician			1	-	
Pathologist			-	1	

Professional	State HQ		DEIC/		Existing staff at Block level-Mobile Health Team
	Existing	Additional Proposed	Existing	Additional Proposed	
Medical Officer			1	-	
Psychologist			1		
Counselor			1	-	
Nursing Staff			1	-	
Lab Technician			2		
Data Entry Operator			2	-	
Manager			1	-	
Field (IEC) Officer cum Counselor			-	2	
Field Assistant (Lab attendant)			-	2	
Nursing Staff					1

7.1 IMPLEMENTATION OF SCREENING: ROLES AND RESPONSIBILITIES

- The Pathologist at the DH or DEIC lab is responsible for verification of screening datasheet and MPR ensuring preparation of correct reports and communication of correct data to the State HQ, besides supervising the staff and laboratory work
- Field Officer is the key person in implementation of the adolescent screening programme under the supervision of Pathologist at DH / DEIC lab right from preparation of screening schedules to communication of screening results and genetic counseling. He coordinates with the Mobile Health Team Nurse, conducts screening as per protocol and assures delivery of samples collected by the Nurse and Field Assistant to the LT at DEIC. Results when entered into the Hemoglobinopathies datasheet are verified by the Pathologist. The Field Officer prepares the list of detected carriers and communicates to respective schools or AWCs for follow up at PHC/ CHC for genetic counseling and collection of samples for confirmatory testing.
- Nursing Staff at DH / DEIC is the key person in implementation of Newborn screening for sickle cell disease and screening of children with severe anemia for thalassemia disease. The DBS samples are collected by her and delivered to LT and babies with positive screening test are recalled for confirmatory testing. Children with severe anemia are presented to the Medical Officer by the Nursing Staff and after clinical examination, their blood samples are collected by her and submitted for investigation as per protocol. If on investigation child is diagnosed to be suffering



from Thalassemia disease (TM, TI), she registers the child under care programme and provides genetic counseling to the family.

- Lab Technician has to receive all samples and verify the samples with the Field Officers and Nursing Staff and conduct all tests as per laboratory protocols.

The one very important step is entry of all the test results correctly in the screening datasheet. It is encouraged that all values are entered in the datasheet by the LT himself or he should be present at the time of entry with the Data Entry Operator to avoid errors. Any results that are doubtful or equivocal should be flagged and brought to the notice of the Pathologist.

- Data Entry Operator is responsible for filling up of the screening datasheet on a regular basis. He should be in regular communication with the Field Officer, LT and the nursing staff, the people who provide him the patient details and test results and outcomes. He is also responsible for making reports to be issued by the DH or DEIC. The MPR is to be prepared by him and verified by the DH/ DEIC manager and the Pathologist and the Pediatrician.
- DH or DEIC Manager is responsible for ensuring availability of all consumables and maintenance of equipment. He has to prepare the monthly Statement of Expenditure and keep track of the budget estimates and allocations under the supervision of the doctors.

7.2 ROLES AND RESPONSIBILITIES OF DAY CARE CENTRE STAFF AT DISTRICT HOSPITAL (DH) / DEIC

The staff nurse at the Day care centre at DH/ DEIC is the key person in running the management programme under the supervision and advice of the Pediatrician and / or the Medical Officer. Routinely two nurses, should be appointed. If the number of patients registered under care programme exceeds 60, deployment of additional 1-2 staff nurses will be required.

The main components of a day care management programme are:

1. Registration of a patient under the care programme with creation of a record file for long term management with baseline clinical examination and investigations
2. Preparing transfusion schedule for each patient
3. Coordination with Blood Bank and Blood Bank Officer for ensuring timely availability of pRBC units by keeping a tabular record of number of patients, blood groups with extended immunophenotyping (if facility available) and monthly transfusion schedule
4. Regular maintenance of growth charts and investigations
5. Regular maintenance and monitoring of chelation therapy
6. Arranging periodic detailed examination of each patient by the pediatrician
7. Looking for alert signs for development of any complications
8. Arranging sessions with counselor and psychologist
9. Maintaining an updated database of patients and family pedigrees
10. Identifying and arranging for tertiary referral of cases with diagnostic difficulties or complications like serious cardiac problems, allo- immunization etc.

8. TRAINING

Training module:

1) Compulsory basic training (Orientation Programme- 2 day)

This will be for all levels of staff- physicians, nurses, technicians. It will be designed to cover the aims and objectives of the programme, explain the programme arms, roles of each staff and how to refer and connect patients for smooth movement and continuity of care. Explain the roles of the teams- screening and awareness, lab, management etc. This will focus on orientation and knowledge to ensure success of the programme.

2) Advanced level training (Induction Training- 3 day)

This will be focused training with specialized training in accordance with work requirement.

- A) Screening and awareness team
- B) Laboratory screening and diagnostic techniques
- C) Hemoglobinopathies patient management training for doctors and nurses

Training Plan:

A cascade training plan will be adopted.

1) Training of Trainers:

National level training will be provided to doctors- Pathologists, Pediatricians and Medical Officers nominated by the State.

Aim will be to provide orientation and induction training to all Pediatricians and Pathologists at national level State IEC Coordinators will be trained at national level

- 2) A 2 day Orientation and Induction training of all the other staff involved in implementation; in batches at State level with trainers from national team
- 3) Medical Officers, Nursing Staff and Field Officers are the key persons and their 3 day Induction training will be organized at State level in batches with Technical Experts, Pathologists and Pediatricians as trainers.
- 4) State level induction training will be organized for Lab Technicians at a District hospital lab or DEIC lab.
- 5) Field Assistants, Nursing Staff of MHTs and DEOs will be trained at DIEC
- 6) Hands-on training for gynecologists and ultra-sonologist for the intervention procedures may be included for prenatal testing, if needed.

Training curriculum:

1) Compulsory basic training:

Outline of programme, aims and objectives

- Basic knowledge regarding thalassemia and sickle cell disease and other hemoglobinopathies
- Basic genetics



- Assessment of anemia, role of various tests
- Basics of care
- Options for cure
- Correct blood transfusion techniques
- Data base management, data entry
- Documentation
- Rules for referral

2) Advanced level training: training specific for role/team

I) Awareness and screening – (Mobile teams and staff)

- Genetics
- Counseling skills
- Prevention strategies
- IEC presentation
- Understanding of programme guidelines (refresher)

II) Laboratory techniques (refer to DEIC laboratory manual)- Screening tests (technician, doctors at CHC, PHC))

- How to conduct tests, fallacies and chances of error- when repeat testing needed.
- Reporting and documentation.
- Referral for diagnostic testing.

III) Laboratory techniques (refer to DEIC laboratory manual)- diagnostic tests

(technician, doctors at Medical colleges and DH/ DEIC centers). These will be conducted at medical colleges, tertiary centers (and hopefully later at DEIC centers)

How to conduct tests, fallacies and chances of error, when repeat testing needed.

Quality control

- Reporting and documentation.
- Referral for management
- Molecular testing and pre natal diagnosis

IV) Management course doctors and nurses

(Doctors of district hospitals, DEIC centers, transfusion daycare centers, others)

(Nurses of district hospitals, DEIC centers, transfusion daycare centers, others)

- Genetics of Hemoglobinopathies

- Disease spectrum
- Diagnosis
- Management principles and guidelines (emphasis on blood transfusion and iron chelation)
- Blood banking practises
- Management monitoring
- Monitoring for iron overload and its complications
- Referral for complications e.g. allo-immunization, cardiac, endocrine problems and HSCT Guidelines for selection of patients for HSCT.
- Basics of HSCT (to better guide families)

V) Blood Banks

(Blood Bank Officers)

Upgradation of blood banks- component therapy and NAT screening (optional but desirable).

Correct use of leuko-depletion.

Follow blood bank guidelines for management of chronically transfused patients, monitor and investigate for blood transfusion reactions.

Assess and monitor chronically transfused patients

When to suspect for allo- immunization?

Referral to other larger blood bank for testing and management.

Training modules:

The training programmes will be from 3-5 days for each group.

Requirements for conducting training program-

- For training a large and quiet room with adequate seating is needed. LCD projector arrangements needed, if conducted in a very large hall, may need a microphone.
- Adequate area for demonstration- tables for demonstration of tests, devices, etc.
- Power back up if high chances of power failure.
- Group photo and certificates of attendance

These programmes will be needed at both baseline and refresher courses annually in the initial phases to ensure understanding of the programme and compliance.

Training programme curriculum is prepared and will be circulated.

Each training module will have-

- 1) Lectures and discussion
- 2) Skill demonstration- hands on for the lab tests, visit to clinic or daycare center or blood bank as needed for group. Field visits for field team for real life training on organization and conducting of camps.
- 3) Counseling skills will be developed by role play



9. BUDGET GUIDELINES

The programme is to be implemented in convergence with existing programmes for child health mainly the RBSK and some components with JSSK in coordination with Blood Banks under Blood Cell. The facilities and services provided at DEIC and by the Mobile Health Team under RBSK are utilized.

Budgetary guidelines are provided under the following heads

1. Establishment cost: for setting up screening and management facilities at DH or DEIC Lab and clinic and office equipment.
2. Consumables: Reagents, kits and drugs for screening (primary level (adolescents), secondary level (pregnant women), antenatal with PND, severe anemic children for Thalassemia disease, newborn screening for SCD) and for management of patients registered with DEIC
3. Hematopoietic Stem Cell Transplant
4. Human Resource cost for additional HR Proposed to strengthen DEIC and RBSK cell State HQ
5. Mobility

For IEC activities and training States are expected to submit budgetary proposals as per established norms assessing the need on the basis of guidelines

9.1 ESTABLISHMENT COST: LABORATORY AND OFFICE EQUIPMENT FOR DH/ DEIC/STATE/ TERTIARY REFERRAL LABS AND FOR UP GRADATION OF DH/ DEICS TO CREATE DAY CARE CENTRE FACILITIES

S. No.	Budget Head	Description/purpose of equipment	Unit cost (in Rs.)	Quantity			Remarks
				CHC / PHC Lab/ MHT	DH or DEIC lab	State level / tertiary lab	
1.	Binocular Microscope	For examination of peripheral blood film	40000	1	1	1	Available at DH/DEIC lab
2.	Digital Hemoglobinometer (optional)	For determining Hb in finger prick samples	30000	2	0	0	To be provided by State
3.	Automated 3 part Blood Cell Counter	3 part differential automated blood cell counter for complete blood counts of samples for anemia and thalassemia or hemoglobinopathies	500000	0	1	0	Available at DH / DEIC lab
4.	Fully automated five part hematology analyzer	5 part differential automated blood cell counter for complete blood counts of samples for anemia and thalassemia or hemoglobinopathies,	1000000	0	0	1	for State and Regional level

S. No.	Budget Head	Description/purpose of equipment	Unit cost (in Rs.)	Quantity			Remarks
				CHC / PHC Lab/ MHT	DH or DEIC lab	State level / tertiary lab	
5.	ELISA Reader with washer	For ferritin estimation	300000	0	1	1	Available at DH / DEIC lab
6.	Hemoglobin HPLC Equipment	Equipment capable of loading a minimum of 10 test at one for Hb fraction estimation - HbA0,HbA1c, HbF, HbA2 and common Hb for analysis of samples for thalassemia and hemoglobinopathies	1500000	0	1	1	For district hospitals
7.	Hemoglobin HPLC Equipment capable of differentiating different hemoglobin subtypes and also performing cord blood HPLC to diagnose prenatal thalassemia Major	Equipment capable of loading a minimum of 100 test at one for Hb fraction estimation - HbA0,HbA1c, HbF, HbA2 and common Hb for analysis of samples for thalassemia and Hemoglobinopathies. To detect variant hemoglobin's including the rare ones	4300000	0	0	1	Available at some State Medical colleges
8a.	Hemoglobin HPLC equipment for Newborn Screening*	HPLC equipment that can process Dried Blood Samples for separation of Hb fractions to enable diagnosis of Sickle Cell Syndromes and Hb D, HbE and HPFH syndromes and traits	4000000	0	0	1	At Medical colleges
8b.	Isoelectric focusing (IEF) equipment for newborn screening*	The equipment uses the IEF technique for separation of different from whole blood, cord blood or blood spot samples. It is an end point method with high-throughput capability and thus can be used for newborn screening	1500000		1	1	For DEIC or District Hospital labs
9.	Hemoglobin Capillary zone electrophoresis system	To detect different variant hemoglobins including the rare ones	3700000	0	0	1	For national level tertiary referral labs

S. No.	Budget Head	Description/purpose of equipment	Unit cost (in Rs.)	Quantity			Remarks
				CHC / PHC Lab/ MHT	DH or DEIC lab	State level / tertiary lab	
10.	Refrigerator (two)	180-310 L. Domestic one for storage of samples , reagents in use kits, one for stock kits and reagents	20000	1	2	2	
11.	Incubator	For conduction of DCIP tests (Portable incubator may be required for outreach settings such as schools and AWCS	10000	1	1	1	Available at District hospital or DEIC lab
12.	Laboratory centrifuge	For separation of serum , plasma	20000	1	2	2	Available at DH or DEIC lab
13.	Rotor sample mixer	For mixing samples	20000	0	1	1	Available at DH or DEIC lab
14.	Syringe Needle destroyer	100 to 1000ul-two and 5 to 20ul two	3000	1	1	1	Available at DH or DEIC lab
15.	Air Conditioner	Essential for proper function of equipment	40000	1	1	2	
16.	Micropipettes-Fixed volume	2 for use in lab and 4/ block for use by Block teams for conducting NESTROFT	3000	4	2	2	
17.	Micropipettes-Variable volume	100 to 1000ul-two and 5 to 20ul two	5000	0	2	4	
18.	Desktop computer	1 for data entry and data collection	30000		1	1	
19.	Laptop computer with data card	1 for each Field IEC officers	40000		2		
20.	Up gradation of DEIC/ DH for creating a day care centre with transfusion facility	Essential for appropriate management of hemoglobinopathies	500000		1		5.00

* One of the two equipments can be used for newborn screening.

9.2 BUDGET GUIDELINES FOR REAGENTS AND CONSUMABLES FOR SCREENING AND MANAGEMENT OF PATIENTS REGISTERED WITH DAY CARE CENTRE AT DH OR DEICS.

The cost of screening is calculated as cost per individual by calculating cost of initial screening test per person that is done in the entire target population. Subsequent diagnostic test is done only in those with positive screening test and confirmatory test is done only in those with a positive diagnostic test. The total costs of screening, diagnostic and confirmatory tests are averaged against the total number comprising the target population to estimate screening cost per person.

(For budgeting, the target population is to be estimated).

S. No	Budget Head	Details of costing	Unit of measure	Unit cost in Rs.	Quantity/ target	Budget in lakhs
1	Primary level Hemoglobinopathies carrier screening	For estimation purposes the cost is calculated for a target population of 100000 where about 25% population is anemic and 15% show a positive NESTROFT and 1% carrier prevalence rate	Screening cost / person	(a+b+e+f+g+h) / 100000= 97.25	100000	97.25
1.a	Hemoglobin cuvettes for digital hemoglobinometer (with disposable lancet and swab)	Estimated cost/ test=Rs.25.00 No.of tests= Total target population +25% (Mild & Mod anemia) in follow up =125000 tests	cost / test	31.25	125000	31.25
1.b	Reagents for NESTROFT (NaCl, Na ₂ HPO ₄ , NaH ₂ PO ₄ , deionized water and tubes)	Estimated cost/test = Rs.3.00 No.of tests=Total target population =100000 tests	cost / test	3	100000	3
1.c	Reagents for Solubility test for HbS (KH ₂ PO ₄ , K ₂ HPO ₄ , Sodium dithionite, saponin and tubes)	estimated cost/test = Rs.3.00* No. of tests=Total target population =100000 tests [*The cost has not been added in calculating screening cost / person. Can be added in regions where the test is used]	Cost/ test	2	100000	3
1.d	Reagents for DCIP test for HbE(DCIP, EDTA, TrisHCl, saponin and tube)	Estimated cost/test = Rs.3.00* No. of tests=Total target population =100000 tests [*The cost has not been added in calculating screening cost / person. Can be added in regions where the test is used]	cost/test	2.5	100000	3

S. No	Budget Head	Details of costing	Unit of measure	Unit cost in Rs.	Quantity/target	Budget in lakhs
1.e	Blood Cell Counter Reagent for CBC	Estimated cost/test= Rs.30.00. No.of tests=15000 tests (15% of screened population)	cost / test	30	15000	4.5
1.f	Reagent for S. Ferritin by ELISA (Microwell ELISA kit)	Estimated cost/test= Rs.110.00. No.of tests=10000 tests (10% of screened population)	cost / test	110	10000	11
1.g	Reagent for Hb HPLC	Estimated cost/test= Rs.250.00. No. of tests=15100 tests (15% of screened population)	Cost per test	250	15000	37.5
1.h	Genetic test for mutation	Estimated cost /test= Rs.2000/test. For 1000 carriers	cost /test	2000	2000	20
2	Secondary level screening for hemoglobinopathies (premarital, pre-conceptual and antenatal)	For estimation purpose cost of Screening at secondary level is Estimated for 2000 pregnant women/ lakh population/ year	Screening cost / person	(a+b+c+d) 100000= 104.50	2000	2.09
2.a	Screening test (CBC+3 tube tests+ (Syringe +EDTA vial +Tip + micro-centrifuge tube)	Estimated cost/test (Rs. 30 +3.0+3.0+3.0+5) =Rs.44.00. No. of tests=2000tests (total target population)	Cost/ person	44	2000	0.88
2.b	Reagent for Hb HPLC	Estimated cost/test= Rs.250.00. No. of tests=300 tests (15% of screened population)	Cost/ test/ person	250	300	0.75
2.c	Reagent for S. Ferritin by ELISA (Microwell ELISA kit)	Estimated cost/test= Rs.110.00 No. of tests=200 tests (10% of screened population)	cost / test	110	200	0.22
2.d	Genetic test for mutation	Estimated cost /test= Rs.2000/test No. of tests=20 tests (1% of target population)	cost /test	2000	10	0.2

S. No	Budget Head	Details of costing	Unit of measure	Unit cost in Rs.	Quantity/ target	Budget in lakhs
3	Antenatal screening with Prenatal Diagnosis	Screening cost is estimated for 20 couples - 1% of a target population of 2000 pregnant women and their husbands	Cost/couple	632	20	0.1269
3.a	Screening of pregnant women	Estimated cost / pregnant woman as per above (2)=104.5 No. of tests=2000 tests (total target population)	Cost / woman	104	20	0.0209
3.b	Screening of husbands of pregnant women with (CBC+HPLC)	Estimated cost/test (Rs. 30 +Rs.250)= Rs.280.00. No. of tests=20tests (1% of screened population)	Cost/ husband	280	20	0.056
3.c	Referral for Prenatal diagnosis test to tertiary centres (cost of test + cost of transport for two people)	Estimated cost/couple= Rs. 4000 + cost of transport @ Rs1000.00)=5000 No. of PND tests=1test (1%)	Cost/ couple	5000	10	0.05
4	Newborn screening for hemoglobinopathies	Cost of screening of 2000 newborns (expected births in a year/ lakh population) for a population with estimated carrier prevalence of 10%	Screening cost/ newborn	(4a+4b+4c)/ 20000= 175.00	2000	3.5
4.a	Newborn DBS sample card	Estimated cost /card=Rs. 10(cards made from Whatman Filter paper No.3) No. of cards= 2000 (total target population)	Cost / card	10	2000	0.2
4.b	Reagents for HPLC of newborn DBS sample	Estimated cost/test= Rs. 150 No. of tests=2000tests (Total screened population)	Cost / newborn	150	2000	3
4.c	Reagents for Hb HPLC of venous sample at 6 months- one year of age	Estimated cost/test= Rs.250.00. No. of tests=200 tests (10% of screened population)	Cost/ test	250	200	0.5



S. No	Budget Head	Details of costing	Unit of measure	Unit cost in Rs.	Quantity/ target	Budget in lakhs
5	Screening of children with severe anemia for Thalassemia disease	Cost estimated for 1000 children with severe anemia referred to DEIC/ year	Screening cost per child	(a+b+c)/ 1000= 175	1000	1.75
5.1	Blood Cell Counter Reagent for CBC	Estimated cost/ test= Rs.30.00. No. of tests=1000 tests (total target population)	cost / test	30	1000	0.3
5.2	Reagent for S. Ferritin by ELISA (Microwell ELISA kit)	Estimated cost/test= Rs.110.00. No. of tests=1000 tests (total target population)	cost / test	220	1000	1.1
5.3	Reagent for Hb HPLC	Estimated cost/test= Rs.250.00. No. of tests=100 tests (expecting up to10% of screened population to show increased ferritin)	Cost / test	250	100	0.25
5.4	Genetic test for mutation	Estimated cost /test= Rs.2000/test. No. of tests=10 tests (1% of target population)	cost /test	2000	10	0.2
6	Lab glassware and plasticware	Test tubes, slides, beakers, racks, flasks, funnels etc	Cost /DEIC/ year	5000	1	5000
7	Lab disposables and miscellaneous chemicals- stains, acid, pH paper,	Micro centrifuge tubes, disposable syringes, tips, EDTA and plain vials etc	Cost / DH or DEIC/ year	20000	1	20000
8	Management cost of patients registered with DH or DEIC	Estimated Cost of management of 100 patients / year/ DH or DEIC	Cost/ patient/ year	35000	100	35
8.1	Leukocyte filter	500-1000/ filter(1-2 units required / mth)=12000/ year	Cost / year	12000	100	12
8.2	Iron chelating medicines	1000-1500/ patient/ month=18000/patient/ year	Cost/ year	18000	100	18
8.3	Monitoring investigations	HIV, HCV, HBsAg, etc=5000/ year	Cost/ year	4000	100	5

9.3 BUDGET ESTIMATES FOR HEMATOPOIETIC STEM CELL TRANSPLANT

As of now, the financial support for the procedure of BMT / HSCT is not being provided through this programme under NHM.

In patients who are eligible for the procedure and fulfill the criteria as given in section C, the financial support for the procedure may be identified from individuals, parent –patient organizations, charity institutions, CSR and other resources available with the States.

S. No	Item/ procedure	Cost in Rupees.
1	Total cost of HSCT/ Bone marrow transplant procedure [exact cost will vary due to weight of child, disease class, and occurrence of transplant related complications]	14 lakhs
1.1	HSCT isolation room charges for 4 -6 weeks	120,000
1.2	Drugs (including antibiotics, anti-fungals, growth factors etc.)	280,000
1.3	Blood and Components (stem cell collection, Packed cells, platelet concentrates kits etc.)	175,000
1.4	Investigations: (HLA Typing, Pre transplant work up, virology surveillance, chimerism, Blood counts, microbiology Cultures, LFTs, Electrolytes, cyclosporine and other drug monitoring, radiology)	2,75,000
1.5	Disposables : (Hickman Catheter, syringes, three ways, Leukocyte filters, TPN Bags,)	150,000
1.6	Immunosuppressive drugs, conditioning meds	250,000
1.7	Total Parental Nutrition/ nutritional supplement (if needed)	50000
1.8	Misc costs- (other drugs, special investigations, therapy for complications etc)	100,000

Note: These figures are approximate and depend on:

- 1. The disease*
- 2. The age and weight of the patient*
- 3. The post transplant complications*
- 4. An uncomplicated transplant in a 12 kg child may cost Rs. 9-10 Lakhs while serious complications (infections, VOD or graft versus host disease) after transplant can increase the cost to as much as Rs. 25 Lakhs or more, mainly because of costs related to prolonged hospitalization, additional immunosuppressive drugs (ATG/ ALG), antibiotics, transfusions and parenteral nutrition.*
- 5. Un-utilized funds will be returned to the funding agency.*



Under the CGHS no costing has yet been done with the reimbursement being made as per expenditure bills of individual cases with verification from enlisted centres. The above mentioned costs are approximate and are provided as guideline for verification of bills by appropriate authority.

9.4 BUDGET ESTIMATES FOR PROPOSED STAFF

Professionals	Nos.	Brief job profile
Technical Consultant (lab services)* (State HQ) @ Rs. 90000-100000.00 / month (*The responsibility can be entrusted to other technical consultants such as India Newborn Action Plan Consultant or Child Health Consultant or Adolescent Health (RKSK) consultant at State level with appropriate qualifications, experience and training) or Nodal officer of RCH or Nodal officer of Blood Cell	1	Required for a maximum period of 3 years to provide technical support to Regional and DEIC labs and coordinate with the State HQ to develop capacity. He/she can be deputed from the State Medical College or any other Medical College or a term appointment for 3 years as per TOR <i>For details refer to TOR</i>
IEC Co-ordinator (State HQ Blood cell-NHM) @Rs.42000.00/ month	1	The post is proposed for a period of 3 years (which may be extended further) in States undertaking Hemoglobinopathies programme with Adolescent Carrier Screening programme for running an intensive event based campaign. The success of Adolescent carrier screening is dependent on a highly effective IEC. <i>For qualifications and detailed job responsibilities refer to annexure-TORs</i>
Pathologist (Regional EIC / Path lab) @Rs.100,000.00 for MD Pathology	1	Required for EIC labs a Path labs at regional level with up scaled lab services for conduction and monitoring of diagnostic and screening tests and verification of test results all data generated at district level and for coordinating with State HQ for compilation of data and with tertiary referral labs. <i>For qualifications and detailed job responsibilities refer to annexure-TORs</i>
Field Officer cum Counselor (DEIC) Rs.20000.00/month	2*	Required in Districts undertaking Adolescent Hemoglobinopathies carrier screening to conduct population screening covering a population of 40000-60000 students enrolled in classes VIII or above of government and government aided schools in convergence with Mobile Health Teams deployed under RBSK at block level. <i>For qualifications and detailed job responsibilities refer to annexure-TORs.</i>
Field Assistant(DEIC) Rs.10000.00/ month	2*	

9.5 BUDGET ESTIMATES FOR MOBILITY COSTS

Mobility budget will be required for

- 1) DEIC based Field Teams to visit schools in all blocks and CHCs PHCs for screening visits not exceeding Rs. 35000/ month with higher rates of 40 000 applicable in hilly districts. For distant blocks overnight stay is recommended.
- 2) Mobility budget should be provided for the Technical Expert for monitoring visits As per applicable norms.
- 3) The State IEC coordinator should be provided with mobility budget to make visits to all districts expecting field work on about 20 days / month and TA/ DA to be provided as per applicable norms.

Budget estimates for State and district level training need to be prepared as per RCH norms by the States. National level training programmes will be organized by the Centre.



ANNEXURE

TERMS OF REFERENCE OF ADDITIONAL HR PROPOSED

Job Title: Technical Consultant (DEIC / DH lab services) will report to RBSK as well as to Blood Cell.

Place of posting: State HQ

Duration: one year contractual assignment extendable to 3 years for any further extension proposal to be made to GOI with justification by the state

QUALIFICATIONS AND SKILLS:

Essential:

MD (Pathology) with three years' experience post MD preferably in hematology and clinical pathology / genetics by way of experience as independent consultant in- public / private sector lab / teaching hospital

Desirable:

Experience in clinical/ community genetics with relevant (1st/2nd author) publications in peer reviewed journals

Consolidated remuneration @ Rs.900000.00 /month;

For candidates with added desirable experience proposed remuneration is @ Rs.100000.00 / month

Job description: Provision of this post has been made to provide technical expert support to States undertaking screening and intervention for Thalassemia, Sickle cell Anemia and other disorders that might be included for newborn screening such as Congenital Hypothyroidism, G6PD Deficiency and other disorders that may be added to the list under RBSK and others.

Job responsibilities:

In 3 years the incumbent is expected to

- help establish lab and lab procedures for regional and District level/ DEIC labs
- train personnel (Pathologist, Field Officers cum counselors, LTs and Staff Nurses and DEOs) for carrying out screening protocols.
- establish quality assurance procedures for lab services
- establish procedures for data collection. verification and compilation at all levels in the State as per provided formats

After 3 years, implementation to be done by Consultant (Child Health / Blood Cell / Adolescent Health) at State level and Pathologists at Regional and District level/ DEIC lab.

JOB TITLE: IEC CO-ORDINATOR

Duration: 3 years (one year extendable to 3 years as per yearly evaluation)

(State may consider further extension depending on impact evaluation of the campaign)

Place of Posting: Blood cell-NHM (State HQ)

Qualifications and Skills

Essential:

Graduation [BA (Sociology) /B.Sc (Biology group)/ BA (Mass Comm.)] from a recognized university; should have excellent communication skills both written and verbal both in Hindi and English; Should have done a 6 months certificate course in Computer application. Should have minimum 5 year documented or certified experience in governmental / public or registered non-profit organizations of standing of executing and running awareness campaigns aiming for voluntary community participation for example screening for thalassemia carrier status, testing for HIV infection, voluntary blood donation, family planning initiatives or any similar health related campaign where an individual from the community is motivated to voluntarily participate or take actions leading to related health benefit

Desirable:

Post graduate degree in Social Work / Sociology/Public Health/Mass Communication

Job Description:

To improve impact of Anemia – Haemoglobinopathies programme implemented under the Project through mainly event based campaign in all districts of the State and through coordinating and monitoring activities of Field teams. Awareness among the student about voluntary blood donation.

Job Responsibilities:

The incumbent will work as a member of the Blood cell based at the State Headquarter He/She will be responsible for conducting Statewide effective event based IEC activities specifically directed to achieve three main objectives:-

- Retention of thalassemia carrier status information by adolescents who are detected to be carriers of thalassemia trait during anemia- thalassemia carrier screening programme in government and government aided schools so as to make use of the information in preventing births of children with thalassemia major in their families and community at large.
- To understand the importance of complete treatment of even mild and moderate anemia during adolescence leading to improved compliance to iron therapy in Iron Deficiency Anemia or any other therapy as per cause of anemia
- To stress upon the need of increased voluntary blood donation to fulfill the transfusion requirements of children affected with thalassemia.



The event based activities will be mainly conducted in government and government. aided schools where screening anemia- thalassemia carrier screening programme is being conducted.

- Reaching out to non school going adolescents and college students and young adults through innovative initiatives.
- All of the districts in the State are to be covered with Field visits to all districts of state of about 20 days / month including outstation visits
- The IEC Field officer will responsible for monitoring and evaluation of DEIC based Field teams and for upgrading the communication skills required for the screening programme and counseling of Field Officers through on spot training and assistance
- Convergence with adolescent directed activities under RKSK and other such institutions and organizations involved in youth based activities directed towards control of thalassemia – anemia will need to be undertaken

JOB TITLE: PATHOLOGIST, STATE / REGIONAL LAB

Place of posting: State level or Regional level lab

Qualifications and Skills:

Essential:

MD (Pathology) with experience in laboratory hematology in a general or specialized hematology lab of 3 years after acquiring MD degree.

Desirable:

2 yrs. experience post MD. As most of the disorders and tests that require skills and interpretation by a pathologist are related to hematology, training and skills in hemato-pathology are desired for the person heading the Regional EIC lab / Regional Path Lab.

A candidate with Diploma in Clinical Pathology may be recruited for the regional DEIC / DH lab if the above mentioned requirements are not available and if required may be sent for training for in a hematology laboratory of an academic institution.

Job description: The incumbent, to function as technical-in-charge of the Regional EIC lab that apart from carrying out all functions of a DEIC lab, functions as a referral lab for all DEIC labs in the region by:

- providing them logistic and technical support as and when required
- monitoring and ensuring proper functioning of the other District DEIC labs in the region by making at least bimonthly visits or more if and as and when required.

For other job responsibilities refer to TOR for Pathologist, DEIC lab

*** DH stands for District Hospital**



JOB TITLE: PATHOLOGIST, DH/DEIC LAB

Place of posting: DH/ DEIC Lab

Qualifications and Skills:

Essential:

Diploma in Clinical Pathology

Desirable:

3 yrs experience post DCP

Job Responsibilities

1. Will function as a team with Pediatrician and Medical Officer, DH/DEIC forming medical professional support system of the DEIC and collaborate with other clinical and laboratory disciplines to determine accurate diagnosis and appropriate line of management and follow up, and timely intervention for complete prevention of disease and its sequelae in newborns and children screened for specific diseases covered under RBSK and other as well.
2. Will function as technical in-charge of laboratory work of DEIC / and work related to Hemoglobinopathy.
 - directly conducting special tests especially peripheral smear examinations in all cases of severe anemia and other cases where diagnosis is not possible by way of given algorithms.
 - Will accompany the Field team to a minimum of 10% of screening visits to school for anemia thalassemia carrier screening and possibly all 2nd and 3rd follow up visits arranged at PHC/ CHC
 - All lab reports of positive cases detected under screening for various disorders will be duly verified and signed by the Pathologist.
 - will be responsible for monitoring and training of Lab Technicians, Staff nurses for screening of newborns by Dried Blood sampling, Field Officers and Field Assistants for Anemia-Thalassemia carrier screening.
 - Verification of all datasheets and records and technical reports submitted by the DEIC / DH labs and Concerned to Hemoglobino Path Regional EIC labs before submission to the State office.

The State office will only compile the data received from different Districts and submit to GOI .

- Pathologists of Regional EIC labs / DH labs will regularly check and verify the data from DH/ DEIC labs in their region-Pathologists, DEIC lab will regularly coordinate with Regional EIC Lab.
3. Monitoring of budget and financial expenditure and statements prepared by DEIC manger as per guidelines obtained from the State office and keeping track with State RBSK cell Blood Cell and prepare PIP for the DEICs lab unit and its incorporation within the DEICs PIP.
 4. Will report to CMS and coordinate with Nodal Officer (Pathologist and Pediatrician) nominated by the CMS to carry out all related administrative work



JOB TITLE: FIELD OFFICER CUM COUNSELOR (DEIC)

Place of posting: DH/DEIC Lab

Qualifications and Skills:

Essential:

MSW from any recognised institution with two year experience in health sector and 6 months certificate course in Computers.

Desired experience and skills:

Should be able to carry out work independently in the field and should have good communication skills and affable disposition to carry out communication with officials, staff and community representatives and have good counseling skills and ability to deliver talks to groups/ gatherings with or without the aid of power point presentation and generate confidence among the target population. Ability to work on computer and internet with proficiency in MS Office package, required.

Job Responsibilities

Will be posted at a District level hospital at a District Early Intervention Centre or at District will be and will be conducting screening DIEC Lab / District Hospital Lab for diseases by finger prick based blood tests in field- mostly schools and will work as a member of the entire DEIC team.

Will be entirely responsible to plan, carry out and report Anemia - Thalassemia screening in schools in Class VIII students and if required out of school adolescents by mobilizing them through ASHAs and Anganwadi workers as per provided guidelines under the supervision of a Pathologist/ Pediatrician.

Along with a Field Assistant (a trained lab attendant) and Staff Nurse/ ANM of Mobile Health Team will comprise a team to carry out the above work that entails -

- Preparation of detailed visit plan and screening schedule as per provided guideline
- Deliver talks to students mostly aided with power point presentation as part of counseling and informing process prior to screening and provide counseling to individual students when required. Ensure delivery of IFA tablets to anemic students by SN/ ANM and ensure compliance through motivation and monitoring during follow up visits at CHC/ PHC.
- conduct screening tests on finger prick samples along with Field Assistant and SNs and ensure blood sample collection and their proper transport back to DEIC lab.
- Ensure that the required tests on samples delivered to the lab are conducted timely and report data is entered into datasheets to carry out follow up work in those found to be anemic and those found to be thalassemia trait carriers as per guidelines
- Maintain updated screening records in hard copy (screening formats) and soft copy and keep them coordinated with the records in the main computer in the DEIC. Provide records timely to the DEO and monitor and verify MPR before it is sent to the State.

- Maintain inventory of consumables involved in field testing, IFA tablets and IEC material (booklets, posters).

Coordinate with other Field Officers, DEIC teams, Pathologists, and State Office Blood cell NHM

JOB TITLE: FIELD ASSISTANT (DH / DEIC)

Place of posting: DH /DEIC Lab

Qualifications and Skills:

Essential :

Intermediate or equivalent examination passed from recognized institution

1 year work experience as lab attendant, ability to take blood samples

Job responsibility:

As part of the DEIC based field team for anemia- thalassemia carrier screening will be required to accompany Field Officer for screening visits to field –schools, CHC/ PHC.

- Will carry out lab attendant's work at DH / DEIC lab when not on field visit including minor tests as per guidelines.
- Will work as a team member of DEIC staff
- Will carry out specific Field Assistant duties directly under the supervision of Field Officer and Lab Technician as per directions of the Pathologist / Pediatrician
- Will be required to prepare required reagent solutions and other preparations for field visit and conduct screening tests on finger prick samples collect blood samples in those indicated and transport back to DEIC/ hospital lab.



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**Ministry of Health & Family Welfare
Government of India
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