Editorial

Can We Make Punjab Thalassemia Free?

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Abstract: Thalassemia is the single most common gene defect and poses a major health burden in Punjab especially in Khatris & Aroras. There are about one lakh children with β Thalassemia syndrome in India with over 10,000 new thalassemia major children born in India every year. There are about 68 β Thalassemia mutations described in India, the knowledge of which can help in prenatal diagnosis.CBC or NESTROFT can be used as a screening tool for differentiating Thalassemia trait from iron deficiency anaemia. HbA_2 estimation is the gold standard in carrier screening however final diagnosis is done by DNA studies. Evaluation for organ dysfunction from time to time is must for early detection of complications and their prompt treatment. Preventing the birth of thalassemia major child is essential.

Key Words: β -Thalassemia major, β-Thalassemia minor/ Thalassemia carrier/ trait, , Molecular genetics of β-Thalassemia, Prenatal diagnosis of thalassemia, Blood transfusion, Iron Chelation, Bone marrow hematopoietic stem cell transplantation, Stem cell registry, Hydroxyurea.

Introduction

The hereditary disorders of haemoglobin (Hb) may be classified into two broad groups, the haemogl-obinopathies and the thalassaemias. The haemogloinopathies are characterized by the production of structurally defective haemoglobin(Hb) due to abnormalities in the formation of the globin moiety of the molecule such as Hbs S, C, D, E, etc. The thalassemia is characterized by reduced rate of production of normal Hb due to absence or decrease in the synthesis of one or more types of globin polypeptide chains. Clinically, these disorders are known as the thalassemia syndromes, resulting from both underproduction of Hb and imbalanced globin chain synthesis, leading to a shortened red cell survival rate. The two principal types of thalassemias, alpha and beta, are due to a reduced rate of synthesis of the respective chains[1]. β Thalassemia is an autosomal recessive, single gene disorder seen all over the world caused by defect in the globin chain synthesis of Hb. In β Thalassemia, β chain is involved & if β chain is completely absent it is termed as β^0 Thalassemia and if practically absent then β^+ Thalassemia[2].

Classification of Thalassemia[2]

- **Homozygous & Heterozygous state**

A) Homozygous state – Homozygous states are of two types, the differentiation of whom is dependent on the clinical severity of the disease. **Thalassemia Major and Thalassemia Intermedia**. In both conditions Fetal Hemoglobin (HbF) is markedly elevated. **Thalassemia Major** Children are clinically severe type and are dependent on regular blood transfusion for the survival. Where as in Thalassemia Intermedia, symptoms are milder and are not dependent or blood transfusion for their survival. Symptom usually appear after the age of 2 years.

B) Heterozygous state: Thalassemia Minor has mild or no anemia and usually have no symptoms and practically lead a normal life. The mild anemia is usually refractory to hematinics. They are asymptomatic and the disease does not interfere with normal routine life. Elevated HbA_2 more than 3.4% characterize Thalassemia Minor. In addition they have microcytic hypochromic red cells.

Epidemiology

World over there are more than 200 million (1.5% of world population) carriers of β-Thalassemia gene, and in South East Asia 40 million carriers (50% in India alone i.e., 20 million). Despite availability of treatment, 50,000-100,000 children of β-Thalassemia die every year in low and middle income countries. It is the commonest inherited hemoglobin disorder in the Indian subcontinent with an uneven distribution among different populations. There are various pockets of the disease in India, mainly North West, North East and Central part of the country. Certain communities have been identified to have a higher carrier rate. It has been estimated that the risk communities could be responsible for 50% of the disease burden of Thalassemia. Mean prevalence in India is 3.3% (1-17%). If a line is drawn between Mumbai & Kolkata on the Indian Map, the region above the line, the incidence of Thalassemia minor is higher, (3-17%) where as in the region below the line, incidence is less than 3%. Incidence varies in different communities, religions and ethnic groups and is often seen more in communities like Sindhis, Punjabis, Khatris from north. It is estimated that about one lakh thalassemia major children are born all over the world. With the birth rate of 22.8 per 1000, it is estimated that about 10,000 children are born every year in our country alone[2].

Diagnosis

When to suspect ?

The clinical manifestations of β Thalassemia varies widely. The serious homozygous form (Thalassemia Major) that presents in early infancy (6-18 months) with pallor, failure to thrive, irritability, intercurrent infections and untreated, more than 90% do not survive beyond 3 to 4 years of age. Untreated or irregulary treated children develop significant hemolytic facies including frontoparietal bossing with a hot-cross-bun appearance of the skull and "hair-on-end appearance" on X-ray skull, depressed bridge of nose, malar prominences and malocclusion of teeth with protrusion of maxillary teeth(Chipmunk facies). Radiological findings include widening of medulla due to bone marrow hyperplasia, thinning of cortex and trabeculation in the long bone. Usually these children are born normal and do not have any symptoms till the age of 2 to 6 months. Initially they grow well for initial few months and become irritable with gradually increasing pallor, not taking feeds well and develops mild hepatosplenomegaly. The heterozygous form (Thalassemia minor) in which the person leads practically a normal life except for mild persistent anemia not responding to hematenics and have normal life span. In between these two extremes, are forms with varying degrees of clinical manifestations of anemia, splenoheptomegaly and bony changes, who, maintain their life comfortably and are not dependent on blood transfusion for their survival and are called as Thalassemia Intermedia and are homozygous. These patients may need transfusion during periods of stress or accelerated growth as happens at puberty[2].

Laboratory Diagnosis

Complete blood cell (CBC) or the Naked eye single tube red cell osmotic fragility test (NESTROF) can be used as a screening tool for differentiating Iron deficiency anaemia (IDA) and Thalassemia trait. Estimation of HbA_2 remains the gold standard to diagnose the heterozygous Thalassemia state. The diagnosis of Thalassemia major is evident from the CBC, peripheral smear. High performance liquid chromatography (HPLC) is an excellent tool for making the diagnosis both in Thalassemia major and trait. Correlate clinical profile and ethnicity of the individual, with the blood count, blood film, and do DNA analysis to confirm the diagnosis[2].

Complete Blood Count (CBC) and Peripheral Blood Film (PBF) Examination:

CBC for primary screening, examination of PBF including presence of nucleated RBCs and reticulocyte count can help in identification and diagnosis of Hemoglobinopathies. **Thalassemia trait** is associated with high Red cell count relative to Hb concentration and hematocrit(Hct), marked reduction in mean cell volume (MCV) and a low Mean Cell Hemoglobin (MCH). The red cell distribution width (RDW) is within normal range. **Iron deficiency anemia (IDA)** is associated with a concomitant fall in the Red cell count, MCV and MCH relative to the fall in Hb. The RDW is

markedly increased. High RBC count relative to Hb & Hct, RDW-Normal. Low RBC relative to Hb & Hct, RDW-↑ . Peripheral Smear Examination in Thalassemia Major is diagnostic. **Naked-Eye Single-Tube Red-Cell Osmotic Fragility Test (NESTROFT):** Many investigators have studied NESTROFT as a screening modality in β–Thalassemia trait. The test has a high sensitivity of 95 % but its poor precision, inter technician variability and low specificity has precluded it from becoming a reliable test. **Hb Electrophoresis- HbF & HbA_2:** Fetal hemoglobin is increased in Thalassemia major child and HbA_2 is over 3.4% in both parents (Thalassemia Minor). The electrophoresis will be normal in IDA. Quantitation of HbA_2 remains the Gold standard for the diagnosis of thalassemia trait. Till recently electrophoresis was considered the gold standard for measurement of HbA_2. The technique is however, laborious, time consuming and unsuitable for mass screening. Hemoglobin Electrophoresis is accurate but suffers from the disadvantage that other Hb variant cannot be identified and their presence interferes with accurate measurement of HbA_2. **High Performance Liquid Chromatography (HPLC**): It accurately quantifies all normal & abnormal Hbs. Confirmation is done by DNA studies-Mutations and globin chains synthesis studies[2].

Molecular genetics of Beta Thalassemia:

Inheritance of β thalassemia is autosomal recessive. The gene for β-thalassemia is located on chromosome 16. Over 200 mutations are described in the β gene worldwide. Most of these are point mutations. It is well recognized that in a particular population, only few mutations are prevalent. Twenty eight different mutations have been identified in Indian thalassemics. Of these six account for more than 90% cases: IVS1-5 (G-C); IVS 1-1 (G-T); Codon 8/9 (+G); 619 bp deletion; Codon 41/42 (-CTTT); Codon 15 (G-A). The mutations can be identified by Polymerase Chain Reaction(PCR) by Amplification Refractory Mutation System(ARMS).[2]

The study of mutations helps in diagnosis of Thalassemia in following cases:

1. Who have received blood transfusion before undergoing electrophoresis studies, the results of electrophoresis may be misleading. In such cases molecular studies can identify mutations in the parents and in the individual case.
2. Confirmation of carrier status when HbA_2 is not elevated.
3. Prenatal diagnosis.

Prenatal Diagnosis[2]:

1. Fetal blood analysis: Fetal blood can be obtained by cordocentesis and can be studied for globin chain synthesis. Diagnosis of thalassemia is possible by estimating rate of synthesis of β chain relative to α and γ chain. The procedure can only be performed at 18-22 weeks of pregnancy and may be too late for intervention if the fetus is found affected.
2. Amniocentesis: Amniocentesis can be performed at 18-22 weeks of pregnancy. Fetal cells thus obtained are analyzed for the presence of thalassemia mutations.
3. Chorionic Villus Sampling (CVS): CVS is usually performed between 10-12 weeks of gestation. Transabdominal or transcervical routes are used for obtaining the tissue. Risk of fetal loss in experienced hands in <2%. The tissue is subjected to analysis to identify the mutations present in the parents or affected sibling.

In 1996 prenatal diagnosis was performed in All India Institute of Medical Sciences(AIIMS), New Delhi[3] followed by Christian Medical College(CMC), Vellore[4], and in Mumbai at the National Institute of Immunohaematology (NII) and Wadia Hospital for Children[5]. Currently prenatal diagnosis is available in the following centers in India: Delhi - Sir Ganga Ram Hospital, AIIMS, Mumbai – NII, Wadia Hospital for Children, Vellore – CMC, Lucknow - Sanjay Gandhi Postgraduate Institute of Medical Sciences(SGPGI), Hyderabad - Center for DNA Fingerprinting and Diagnostics, and Center for Cellular and Molecular Biology; Chandigarh - Postgraduate Institute of Medical Education and Research(PGIMER), and a few commercial laboratories.

The important steps for prenatal diagnosis are: (i) Be certain both parents are carriers. Even in families with an affected child who is on blood transfusions it is better

to establish that the parents are carriers by haematological studies. If there is no affected child, special care should be taken to ensure that parents are carriers. Electronic cell count should invariably be done to support the results of HbA2 by HPLC. Estimation of HbA2 by electrophoresis is not always reliable. (ii) When one of the partners is a carrier, take particular care that the other partner is not a carrier. This is because they may carry mild or 'silent' mutations of β-globin gene. In those with borderline values of red cell indices or HbA2, it is better to sequence their β-globin gene (iii) Know which combination of alleles is significant. For example, the combinations of the following alleles lead to clinical manifestations and require prenatal diagnosis: β-Thal/ β-Thal (Thalassemia major), β-Thal/HbS (Sickle/β- Thalassemia), β-Thal/HbE (E/β-Thalassemia), HbS/ HbS (sickle cell anaemia), HbS/HbD Pb/Hb C (SCD/ Symptomatic). On the other hand, the following combinations do not require prenatal diagnosis because these do not lead to any clinical problem: β-Thal/Hb D Punjab/Iran, β-Thal/Hb C/Hb Q, homozygous Hb E, Hb D Punjab/Hb D Iran. There are 64 β-globin mutations reported in Indians so far as recorded in the Thal Ind database[6].

Pre and Post test Counseling:

For the purpose of prenatal diagnosis, the family including the affected child if any and the parents who are carriers of thalassemia should undergo studies for identification of mutation responsible for disease in the concerned family. The family should be counseled before the prenatal test about various possible results. After results of prenatal testing are obtained the family is told about the fetus if normal, carrier or affected, that is homozygous for the mutations. In the later case the family is counseled about possible medical termination of pregnancy.

Management

(i) Blood transfusions: Marwah[7] has estimated that in India 2 million units of packed red cells are required for transfusion to thalassemic patients. The patient organizations were crucially important in ensuring supplies of blood by arranging donations. The Indian Red Cross Society (IRCS) also played a stellar role in collecting donations of blood.[8] In Delhi, IRCS, blood bank collects nearly 30,000 units a year, and supports nearly 50 per cent of thalassemic children with free supply of blood in the National Capital Region (NCR) territory. In the early years most of the blood donations were obtained from the relatives or professional donors. Therefore, ensuring safety was difficult, and some cases of transmission of HIV through blood donations were described. In 1987, the Government of India set up the National AIDS Control Organization (NACO) [8]**.** One of its main mandates was ensuring safe blood for transfusion by proper screening of blood and blood products throughout the country. In 2003, a National Blood Policy was enacted to ensure safe blood. In 1992-1999 NACO launched a scheme (NACP I) to modernize blood banks by providing government assistance to States to upgrade and provide minimum facilities to blood banks in the public sector, as well as those run by charitable organizations. The assistance facilitated purchase of equipment, consumables, test kits, blood bags, reagents, etc. Many reports appeared on the transmission of HIV, hepatitis B and C viruses in a high percentage of thalassemic patients who received multiple transfusions during 1990-2003[10,11,12] Now, all the blood banks supply blood that has been tested for malaria, syphilis, hepatitis B, HIV and hepatitis C. Currently, there are about 7000 blood banks in the country.[13] Of these 36.0 per cent are in the Government sector, 14.4 per cent are voluntary, 28.8 per cent are private, 20.7 per cent are private charitable.[13] Due to the stringent control of blood banks the transmission of infections through blood is now negligible.

(ii) Chelation: To remove the excess iron in the body resulting from repeated blood transfusions. In the early years desferal (deferoxamine, DFO) was the only chelator available. It was used for almost four decades, and proved its worth. However, its

use has a number of problems - it has to be given intravenously for many hours using a pump, and it is expensive. Therefore, compliance is poor. Oral iron chelator deferiprone [14] was finally approved for use in India in 1994. Deferasirox, once daily oral iron chelator, was the next big advance in this area.

(iii) Physical growth, endocrine and bone density changes: Growth failure is seen in nearly all patients, and is due to various factors viz endocrine dysfunction, anemia, hyperslenism, desferrioxamine and liver disease. Short stature is commonly noted at 10-11 years of age. Most of the complications of β–thalassemia re attributable to iron overload. Excess iron is toxic to the heart, liver, and various endocrine glands. In β–thalassemia, 70% of deaths are due to cardiac complications. Thus one must evaluate the functions of various organs particularly- endocrine glands, heart, and liver because of the impact of repeated blood transfusions leading to iron overload and transfusion transmitted infections (TTI). Clinically the organ dysfunction may not be evident initially, and hence, investigations are required for early detection, and should be done in all thalassemia children from time to time and appropriate action taken. Zoledronate therapy is helpful in Thalas-semia-induced osteoporosis.[15]

(v) Hydroxyurea: Hydroxyurea have been known to cause induction of foetal Hb and reduce ineffective erythropoiesis, and thus may alleviate the symptoms in thalassemia intermedia patients.

(vi) Bone-marrow haemopoietic stem cell transplantation (BMT): The first bone marrow transplant centre was established at Tata Memorial Hospital(TMH) in 1983 in Mumbai. However, they carry out only a limited number of transplants for thalassemia. The centre at CMCH, Vellore was started in 1986.[16] It has carried out the maximum number of bone marrow haemopoietic stem cell transplants for thalassemia in the country. At this centre from October 1986 to December 2006, 626 transplants were performed in 595 patients, with 28 patients having more than one transplant.[16] Thalassemia accounted for a third of these transplants. The average cost of allogenic BMT in India is around $15000 to 20000, which is considerably lower than the cost in the West. The other centers where BMT is being carried out are in Delhi (AIIMS, BL Kapur Hospital, Sir Ganga Ram Hospital, Army Hospital, R and R); Lucknow (SGPGI), Chennai (Apollo Hospital), Chandigarh (PGIMER), Pune (Sahayadri Hospital), Bangalore (Narayana Hrudayalaya Hospital, Manipal Hospital and Kidwai Memorial Hospital), Ahmedabad (Gujarat Cancer Research Institute), Thiruvananthapuram (Regional Cancer Center), Mumbai (Jaslok Hospital) and Ludhiana CMC Some of these centers carry out BMT for thalassemia in a limited number of patients.

(vii) Bone marrow and cord blood stem cell registries:

A major limitation in haematopoietic stem cell transplantation (HSCT) is the availability of a unrelated HLA matched donor. Kanga et al[17] reported that of the 688 patients requiring HSCT, only 39.3% had an HLA matched sibling. Families with sibship size of = 4 had a higher probability (68.8%) compared with those with sibship size of = 3 (29.7%). The difficulties in finding an unrelated suitable donor in India are due to the extensive allele and haplotype diversity, and the presence of unique haplotypes in India. These limitations are being overcome by establishing unrelated volunteer marrow donor registries. The first one was set up in AIIMS, New Delhi, as the Asian Indian Donor Marrow Registry in 1994. By 2008, 3830 donors have been enrolled. This has been enlarged to a multi-centric project to enroll donors from different cities in India by initiating new registries (Mumbai, Chandigarh, Lucknow, Vellore, Vadodra). Another registry has been started in Mumbai at the Tata Cancer Hospital - Marrow Donor Registry India (MDRI) in

2008. The total number of samples processed for HLA typing till October 2009 was about 300084. This registry has received a boost as the Salman Khan Foundation has joined as a partner in order to increase the enrolment. DATRI Blood Stem Cell Donors Registry based in Chennai is another important repository.[18] It has 12,398 donors on its rolls and is listed in the Bone Marrow Donors Worldwide (BMDW). The Cord Stem Cell Registry India (SCRI) is based at Army Hospital (R & R), Delhi, it networks with Rotary blood banks in Bangalore, Chennai and Delhi, Prathama Blood bank in Ahmedabad and Association of Voluntary Blood Donors in Chennai. There are at least ten cord blood storage centers in India. Reliance Life Sciences cord blood repository Mumbai (Relicord) probably has the largest collection. It supplies enriched cord blood stem cells.[19] Some registries abroad are a source of finding appropriate donors for Indians, e.g., the Asian American Bone Marrow Foundation (AABMF) in USA. In the International Bone Marrow Donor registries about 20,000 Indians are registered. The Bone Marrow Donors Worldwide has 66 stem cell donor registries from 47 countries, and 47 cord blood banks from 28 countries.[20] The current number of donors and cord blood units in the BMDW database is 18,195,087 (17,707,159 donors and 487,928 CBUs).[21]

Prevention of thalassemia major

The need for prevention of thalassemia is important due to high frequency of the condition, the great expense and difficulties in providing optimal treatment for patients, and the innumerable fatalities from untreated β-thalassemia. Prevention would not only be a good public health practice, but it would also be cost-effective, as the ratio of the cost of treatment to prevention is 4:1.[22] It would help tremendously in reducing the burden of the disease for patients, families and the health services.

The chief elements of a control program were developed in 1970s by a team of experts at the World Health Organization(WHO).[23] These are (i) Political and financial support, (ii) Improving curative services; (iii) Prenatal diagnosis in couples who have given birth to an affected child , as well as those identified to be at risk, (iv) Prospective antenatal screening, (v) Community carrier screening, (vi) Counseling and prenatal diagnosis, and (vii) Network of centers, and National/Regional working groups.[24,25]

i. Financial support is required for training and employing the manpower required for execution of the control program (social workers, technicians, doctors, counselors); purchase of equipment (electronic cell counters, HPLC machines, DNA diagnostic laboratories, or link with a laboratory); and record keeping and information system in a confidential manner.

ii. Education of the professionals and the public should be done using all components of mass media - newspapers, TV and films. An important component is informing the policy makers. The Parent Organizations have a very important role to play in this. Any educational program on thalassemia has three main messages - carrier state has no disadvantage, homozygous state is associated with a very severe disorder, that is eventually fatal with no curative therapy, and foetal diagnosis is available and safe. Experience around the world clearly indicates that carrier testing must be voluntary, and mandatory measures should be discouraged. In an analysis of the reasons for the successful control programs in Cyprus and Greece it was concluded that one of the important steps was the introduction of formal education on thalassemia in the school curriculum.[26,27]

iii. **Preventive programs** carried out based on heterozygous detection and counseling to avoid marriage between carriers, without prenatal diagnosis, have not been very effective around the world. In contrast, preventive programs based on heterozygote detection, counseling and foetal diagnosis have been very effective in reducing birth of thalassemia major infants in Sardinia, Cyprus, Greece and Italy [28]. The strategy of identifying carriers and asking carriers not to marry carriers (without providing foetal diagnosis) led to no significant difference in the proportion of marriages between hetero-zygote's as compared with random mating, and no change in the incidence of thalassemia

major in some studies.[29,30]

iv. Premarital screening has been advocated by many investigators and haematologists in India as a preventive strategy. In countries where prenatal diagnosis is available, most of the people prefer prenatal diagnosis rather than using the information about carrier status for choosing a marriage partner. This is because marriage is a complex social phenomenon, and marriage partners are selected for a strong personal preference, family or traditional reasons. When a planned marriage is called off, it results in social embarrassment, stigma to the young people involved and their families.

The Cyprus program is often used to justify premarital counseling. Initially the model in Cyprus was based on screening and counseling of subjects, and marriage between carriers was discouraged. This approach proved unacceptable to the general public, and was soon abandoned, because of evasions. Once prenatal diagnosis became available within Cyprus health service, confidential premarital screening was mandated by the Church, but there was no compulsion that carriers should not marry carriers. The objective was that people should know their carrier status and after marriage, get the foetus tested in those cases where both the husband and wife were carriers. A study showed that 98 per cent of at risk couples detected prior to marriage proceeded to marry, but opted for prenatal diagnosis during pregnancy. It has been reported that less than 5 per cent of the fall in thalassemia major births is due to separation of engaged couples, while about 80 per cent is due to prenatal diagnosis and selective abortion.[26,27]

Which strategy is likely to succeed in India↑

If the policy of premarital screening were to be successful, control of thalassemia in India should have been achieved a long time ago, because this course of action has been available for decades. For the reasons given above the policy of identifying carriers and advising carriers not to marry carriers is not likely to be successful, given the current state of knowledge of the general public about science and genetics. Chattopadhya[31] observed The risk of non-marriage affects women disproportionately, and parents are not inclined to test their daughters because of the possibility of not being able to marry them off to eligible suitors. The stigma associated with having thalassemia minor is a deterrent to the disclosure of thalassemia status as well as to testing. Tamhankar et al[32] carried out premarital testing for thalassemia carrier state in three groups: extended family members of diagnosed cases of thalassemia/ haemoglobinopathies, unmarried adult cases of anaemia attending the hospitals' outpatient department and adult college students. As much as 99 per cent of prospective carrier couples married even after knowing their high-risk status and opted for prenatal diagnosis. This reinforces the view that over-emphasis on premarital screening should be avoided in India. The policy should determine the carrier status of subjects before reproduction, or during early pregnancy, so that prenatal diagnosis can be obtained in the at risk couples. Strategy for screening programs for β-thalassemia should be to sensitize the community to the problem, establish haematological technologies for screening, and molecular and obstetric techniques for prenatal diagnosis.

How should the carriers be identified?

The technique to be used should be affordable, applicable, and accurate. Should one use naked eye single tube red cell osmotic fragility test (NESTROFT), or electronic cell count, or HbA_2 estimation using HPLC↑ No doubt HPLC is the best, but in view of large numbers of subjects requiring screening in India, the better option would be use electronic cell counters for measuring red cell indices. If only one partner has to be screened the husband should be preferred because it avoids the anaemia of pregnancy disturbing the red cell indices. One of the two partners must clearly be shown to be negative for thalassemia carrier status. In case of any doubtful result HPLC analysis should be done. In rural areas where electronic cell counters are not available, initial analysis may be by NESTROFT. This should work out well because most investigators have pointed out that NESTROFT has a sensitivity of over 90-95%, and a negative result picks out the normal very well.[33,34,35,36]

The other important observation made in India is that concomitant iron deficiency is

present in many β-thalassemia carriers.[37,38,39] It was shown that once iron deficiency is established, therapy with iron leads to a significant rise in Hb. The authors demonstrated that in β-thalassemia carriers HbA_2 decreased in the presence of iron deficiency, but the fall was slight, and it never reached the normal range.

Who should be screened? There are a number of options - pregnant women, relatives of the affected, high school or college students, or the community at large. There is little doubt that the primary focus of screening in developing countries, including India, should be pregnant women. This yields results immediately, in terms of reducing the burden. Screening of high school or college students is good to create awareness, but by the time they are to marry many tend to forget their carrier status. Screening of school students is unlikely to make a significant reduction in the burden of thalassemia.

Cascade or inductive screening identifies many more carriers than general population screening; the percentage of β-thalassemia carriers identified was 5-6 times higher using this cascade screening approach.

Current Scenario of Care of thalassaemics in India[40]

The first centers for care of thalassemia were probably started in Mumbai in early 1970s in JJ Hospital and KEM Hospital, followed by AIIMS, New Delhi in 1973-1974, Borruka Center, Kolkata in 1985, Sir Ganga Ram Hospital in 1986, and Wadia Hospital for Children in Mumbai in 1987. The Preeti Tuli Thalassemia Center at Sir Ganga Ram Hospital in Delhi was set up in 1994. It was the first dedicated centre which started a free Day Care Clinic with multiple specialists and routine use of filters during transfusions. Subsequently similar centers were opened in many cities in India. Shobha Tuli of Thalassemics India and JS Arora of the Federation of Indian Thalassemics (FIT) have played an important role in improving the care of thalassemics in India by arranging various educational programs and workshops, by bringing foreign and Indian experts to treat patients of thalassemia. There are about 60 parent/patient societies of thalassemia in India, of which about 40 are members of the FIT. Due to the persistent efforts of the thalassemia societies and the health professionals working in this field, a most significant step has been taken by the Government of India by including the care and management of thalassemia into the 12th Five Year Plan. This has paved the way for improving the quality of care and in introducing control programs in various parts of the country. The Thalassemia International Federation played a major role by deputing experts to come to India, and give lectures and conduct workshops. Increasing reports are appearing of endocrinal and cardiac involvement in thalassemia, which are commonly observed in the second decade, as it takes some time for iron to accumulate in these organs. Adequate chelation therapy delays the onset of these complications. Indeed subjects with thalassemia major who receive optimal care in India now survive to adulthood. A number of thalassemic children have married. In Jammu a thalassemia society was formed in 1996. Since then the Jammu and Kashmir Government has been providing blood transfusions, chelation and other therapies free of cost to patients and also provides free transport to all patients who have to travel from long distances to receive treatment. This was followed by the Delhi State which set up dedicated thalassemia units in all the Government hospitals and has been providing free blood transfusions and chelation since 2005. Since 2008 a program of regular screening of pregnant women has been initiated. The Punjab Government gives free treatment to all thalassemic children who attend school. The Governments of Gujarat and Maharashtra also provide free therapies for children with thalassemia.

The largest program of screening for thalassemia in the population has been carried out in Gujarat by the IRCS in Ahmedabad and other cities.[41] They screened 370,117 subjects for carrier status among whom there were 173,112 students, 45,000 youths and 8,377 pregnant women. Carrier rate has varied from 4.3 to 5.0 per cent. Testing was done using the HPLC system.

An important project was carried out under the **Prime Minister's Jai Vigyan Thalassemia Control Program,** in six cities in India with a high prevalence of haemoglobinopathies - Mumbai (Maharashtra), Vadodora

(Gujarat), Dibrugarh (Assam), Kolkata (West Bengal), Ludhiana (Punjab) and Bangalore (Karnataka).[42,43] Screening was done in 29,898 college students, and 26,916 pregnant women. The prevalence of β-thalassemia trait varied from 1.5 to 3.4 per cent among college students, and 1.3 to 4.2 per cent among pregnant women. A high frequency of carriers was observed among certain communities - Vellalas, Sindhis Aroras, Lohanas, Mandls, Pillais, Jains, Khatirs and Baidyas. Essential recommendations of this extensive study were as follows: Multimedia awareness programs should be a continuous process; adequate number of centers should be established so that people do not have to travel long distances for the tests; Verma et al said NESTROFT test, though cheap, cannot be recommended as the only test for preliminary screening, it should be combined with RBC indices measured on a cell counter; the blood count should be done within 48 hr after collection; MCH was more stable parameter than MCV; HbA_2 estimation by HPLC was the most accurate method for diagnosis of β-thalassemia heterozygote. Considering the cost involved in using HbA_2, assay it was recommended that all samples that are NESTROFT +ve or have MCV less than 80 fl, or MCH less than 27 pg/cell should be screened further for HbA_2; high MCV values were observed in 1.7 per cent of carriers suggesting concomitant vitamin B12 or folic acid deficiency; and need for ensuring screening early in pregnancy.

Verma IC et al 2011[40] carried out a pilot study on prevention of β-thalassemia in a district hospital in Delhi. Calculation of cost of the care of two affected children for a period of ten years covered the entire cost of screening and prenatal diagnosis in the study subjects, highlighting that the preventive screening and prenatal diagnosis are cost effective.

Government of India has included thalassemia in the list of disability conditions and also in the list of chronic disease. **Government of Punjab** has taken an initiative for thalassemia prevention and care. Govt. is already providing free treatment to the thalassemics at secondary & tertiary care centers by providing free blood transfusion, inline filters, iron chelation, free transportation for the school going children. It is also going to start mandatory screening of all antenatal mother's for carrier status with provision of diagnostic facilities at tertiary care centers of the state and select District hospitals viz. Ludhiana & Jallandhar by HPLC with final diagnosis of the carrier status at PGIMER, Chandigarh by doing DNA analysis. Prenatal screening of the fetus for carrier parents will also to be provided at PGIMER Chandigarh & CMC Ludhiana followed by counseling. Govt. also intends to sensitization of the youth & population by IEC hence encouraging them to know their carrier status before marriage.

Future of Thalassemia care in India

The international situation changed in 2006 with the recognition by the Executive Board of the World Health Organization that thalassemia and sickle cell anaemia were major global health problems which needed to be urgently addressed, a move reinforced by their inclusion in the current Global Burden of Disease Study.[44] This was accompanied by adoption of the resolution WHA63.17 in May 2010, to redress the limited focus to date on preventing and managing birth defects, especially in low- and middle-income countries.[45]

The Government of India is providing sufficient funds to the care and control of thalassemia in India. Currently in most cities thalassemia care centers have been established, both in the private sector as well the Government hospitals. Paediatricians and paediatric haematologists looking after these centers have sufficient training and skills to manage these cases. Supply free or subsidized blood transfusions, chelating agents and filters in 14 States in India, like Jammu and Kashmir, Delhi, Maharashtra, Gujarat, Punjab etc.

The program of prevention through carrier screening and prenatal diagnosis should receive the highest priority in the future, in order to reduce drastically the birth of affected children. Talks have been ongoing for initiating control programs in the cities and States that have a high prevalence of carriers of β -thalassemia, yet no concrete programs have been started. The 6-centre study on control.[43] has been extremely useful in providing the basics of organizing and running a control program in India. Even without a control program in place we must motivate the

obstetricians to screen every woman at first visit for carrier status of thalassemia. As far as feasible, husband should be screened at the same time.

There is a need to increase the number of centers in India able to perform prenatal diagnosis, and provide this facility at a subsidized cost, or free for the poor, and introduce quality control programs. An important challenge is to develop pre-implantation genetic diagnosis as many couples are distressed by having affected children in consecutive pregnancies. Investment in non invasive techniques for prenatal diagnosis would be worthwhile, as this would help to provide prenatal diagnosis in peripheral areas also. The facilities for voluntary cord stem cell storage should be established in the Government sector, as currently most of these exist in the private sector at a huge cost. Centers for bone marrow transplantation need to be expanded and facility subsidized. The need of the hour is to introduce control programs in the high risk States.

Conflict of Interest : None

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