Review Article

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Past, present & future scenario of thalassaemic care & control in India

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The first case of thalassaemia, described in a non-Mediterranean person, was from India. Subsequently, cases of thalassaemia were documented in all parts of India. Centres for care of thalassaemics were started in the mid-1970s in Mumbai and Delhi, and then in other cities. The parent's associations, with the help of International Thalassemia Federation, greatly helped in improving the care of thalassaemics. Obtaining blood for transfusion was difficult, but the Indian Red Cross Society and the parent's associations played a crucial role in arranging voluntary donations of blood. Chelation with deferoxamine was used sparingly due to the high cost. The Indian physicians conducted trials with deferiprone, and the drug was first approved and marketed in India. Deferasirox is also now being administered. Studies of physical and pubertal growth documented significant retardation, suggesting that generally patients receive inadequate chelation and transfusions. Bone marrow transplantation is available at a number of centres, and cord blood stem cell storage facilities have been established. Information about mutations in different parts of India is available, and ThalInd, an Indian database has been set up. There is a need to set up preimplantation genetic diagnosis and non-invasive prenatal diagnosis. It is argued that too much emphasis should not be placed on premarital screening. The focus should be on screening pregnant women to yield immediate results in reducing the burden of this disorder. Care of thalassaemia has been included in the 12th 5-year Plan of the Government of India. Many States now provide blood transfusions and chelation free of cost. Although inadequacies in care of thalassaemia remain, but the outlook is bright, and the stage is set for initiating a control programme in the high risk States.

Key words Blood transfusion - bone marrow transplantation - carrier screening - chelation - control - cord blood storage - prenatal diagnosis - prevention - thalassaemia care

The early years

In 1925 in a meeting of the American Pediatric Society, Cooley and Lee reported children of Mediterranean extraction with anaemia, splenomegaly and peculiar changes in bones[1]. In 1938 Mukerjee[2] published "the first recorded case of thalassemia on east side of Suez". The case was observed by Dr M. Bose of the Campbell Medical School & Hospital, Calcutta (now Kolkata) in a Hindu boy aged 30 months. This case established that the disease may be found in children of non-Mediterranean parentage, and thus justified scrapping of the designation "Mediterranean disease". Subsequently, in 1939 three reports appeared in India - by Coelho in two Muslim sisters from Bombay[3] (now Mumbai), by Napier and Dasgupta from Kolkata[4], and by Patel and Bhende from Bombay (now Mumbai) in

a Brahmin boy of 5 yr[5]. Cases were recognized and reported from other parts of India, such as Punjab[6], Kolkata[7], Delhi[8], Mumbai[9], Bihar[10], Orissa[11], Andhra Pradesh[12], Chandigarh[13], and Rajasthan[14].

The first case of β-thalassaemia/Hb E disease in India was reported by Chatterjee *et al*[15], and that of β-thalassaemia/sickle cell disease by Naik and colleagues[16]. Subsequently other variants haemoglobins were reported – C, D, F, G, H, J, K, L, M, Q (India), G, *etc*[17-21]. Haemoglobins S, D and E were observed to be quite common: Hb S has been found mostly in tribal communities, Hb D in Gujaratis and Punjabis and Hb E in Bengalis, Assamese and Nepalese.

Molecular studies

The earliest reports of polymorphisms in the β-globin gene in Asian Indians, that gave rise to the characteristic haplotypes of the different mutations, were reported by Kazazian and his colleagues in March 1984[29]. This group was also the first to report the common seven mutations and their haplotypes in the Indians - Frameshift/8-9 (+G), Nonsense codon 15 (TGG-TAG), Frameshift/41-4 (-TCTT), Frameshift 41-4 (TCTT), Frameshift/16 (- C), IVS-1 nt 5 (G-C), 619-bp deletion 13 and 25 nt deletion, at 3' end of the gene. Deletion of 619 bp was shown to be a common mutation in the Asian Indians[23]. Thein *et al*[24] showed a single origin of this deletion mutation in the Asian Indians. In the same year, Old and colleagues[25] showed the feasibility of prenatal diagnosis in Asian Indians by DNA polymorphism analysis. Subsequently several publications emanated from these experts on mutations among Indian subjects, their regional distribution and some rare mutations[26-28]. They published their experience on prenatal diagnosis using mutation analysis in 1990. Data on the different mutations both from USA and UK were derived from study of subjects going for evaluation of pregnancies to avoid the risk of having an abnormal child. This trend continues even today, as molecular studies are mostly undertaken when women come for prenatal diagnosis.

Distribution of mutations in different parts of India

The distribution of mutations in various parts of India has been reported by a number of investigators[29-35]. New mutations have been reported by the predominant groups working in this field in different parts of India from Vellore[36,37], Mumbai[31,38], Delhi[29], and Chandigarh[35]. Old and colleagues published a multi-centric study on mutations in β-thalassaemia major patients from India, Pakistan, Thailand, Iran and Syria[39]. This paper gives the primer sequences for amplification refractory mutation system (ARMS) technique of all mutations common in these countries. Sinha *et al*[40] reported a meta-analysis of regional distribution of 8,505 alleles with 64 β-globin gene mutations. Nationally, IVSI-5(G>C), 619-bp del, IVSI-1(G>T), Codon 41/42(-TCCT) and Codon 8/9(-G) comprised the five most common disease mutations, and accounted for 82.5 per cent of all mutations. Codon 15(G>A), codon 30(G>C), cap site +1(A>C), codon 5(-CT) and codon 16(-C) accounted for an additional 11.0 per cent of all mutant alleles. The Western region (Maharashtra, Gujarat and Rajasthan) has a higher prevalence of the 619-bp deletion (14.2%) and IVSI- 1(G>T) (8.7%), and with codon 15(G>A) as the fourth commonest mutation (7.6%). In the northern region (Uttar Pradesh, Punjab, Haryana, Himachal Pradesh, Uttarakhand and Jammu and Kashmir IVSI-5(G>C) accounted for 44.8 per cent of β-thalassaemia alleles. The five most common mutations closely match the national pattern, with codon 16(-C) and -88(C>T) in the list of ten common mutations along with codon 15(G>A), codon 30(G>C) and cap site +1(A>C). The four southern States (Andhra Pradesh, Karnataka, Tamil Nadu and Kerala) have a predominantly Dravidian population, ethnically and culturally quite distinct from the largely Indo-European populations of the north. IVSI-5(G>C) has a prevalence of 67.9 per cent, the 619-bp deletion is present in only 1.8 per cent of cases, whereas the second commonest allele (8.8%) is codon 15(G>A). Poly A site (T>C) is the third most common allele (4.7%). The east region exhibited the highest prevalence of IVS I-5(G>C) at 71.4 per cent, with codon 30(G>C) and codon 15(G>A) the second and third most common alleles, accounting for 5.8 and 5.4 per cent of the total, respectively.

Codon 15 (G>A) occurs in about 35.3 per cent of all subjects resident in Maharashtra. The high percentage of -88(C>T) alleles in cases from Punjab can be ascribed to the frequency of this mutation in the Jat-Sikh community. Likewise, the high prevalence of Codon 5(-CT) in Gujarat (79.7%) is associated with the Lohana and Prajapti communities in that State. Although the Poly A(T>C) allele has been reported in the populations of nine States, 65.6 per cent of cases originated in the adjacent southern States of Tamil Nadu and Karnataka. Gorakshakar *et al*[41] showed that the mutation codon 47 (+A) was common among the Nicorbarese in Andaman Islands. Two mutations (IVS I-5 (G->C and CD15 (G->A) account for over 90 per cent of the mutant alleles among the tribal populations.

The collation of data by Sinha and colleagues has culminated in the establishment of the database for mutations in the β-globin gene in India[42]. The resource ThalInd (*http://ccg.murdoch.edu.au/thalind*) incorporates and provides data pertinent to molecular genetics, population genetics, genotype-phenotype correlations, disease burden and infrastructural assessment. Importantly, the resource also has been aligned with the administrative health system and demographic resources of the country.

Interaction of β-globin gene with other genes

Interaction of β-globin gene with other genes in India has been examined by many investigators[43-47]. Kulozik *et al*[43] showed the interaction of α gene abnormalities with sickle cell disease. This opened the path for study of the interaction among the various genes in cases of thalassaemia. Garewal *et al*[35] showed the interaction of mutations in the β-globin with those in the α-globin gene in causing variations in phenotype in subjects from Punjab and Maharashtra. Verma *et al*[47] in a multi-centric study showed the interaction of β, α and γ genes in causing thalassaemia intermedia. This study demonstrated that in cases of thalassaemia intermedia when the β-thalassaemia mutations were of the β^+ variety, these were accompanied by mutations in the α-globin gene as the ameliorating factor. In the presence of β^0-thalassaemia mutations or Hb E the ameliorating factor was positivity of *Xmnl1* (G) polymorphism at -158 (C>T).

Management

In 1990, an important study on the care of thalassaemic patients in Mumbai highlighted the significant, unavoidable and increasing demand on the public health services by patients with β-thalassaemia major[48]. The situation was also characterized by evasion of the problem, failure of planning, no provisions for prevention, and inadequate treatment leading to premature death among the affected children. Most couples (90%) of reproductive-age felt that prenatal diagnosis was a necessity. Ignorance and prejudice in the community led to social isolation for many families. Another study group reported that most thalassemics were not satisfied with their body image[49]. Almost all the study subjects felt that the disease did not affect their family or social relationships. The adolescents were anxious about their future health and education. Majority of the subjects (80%) did not discuss their disease and its related problems with their friends. They mainly depended on their parents for monetary and emotional support. Recently they reported on growing up with families of thalassaemics using an accelerated longitudinal design[50].

The history of management of thalassaemia in India has followed the success story of thalassemia care in the West. Excellent articles on management of thalassaemia have been published by Indian authors[51-54]. Only selected aspects of the management are covered below.

***(i) Blood transfusions*:**

In the initial years the major problem was to obtain blood for transfusions. Marwah[55] has estimated that in India 2 million units of packed red cells are required for transfusion to thalassaemic patients. The patient organizations were crucially important in ensuring supplies of blood by arranging donations at camps, factories and workplaces. The Indian Red Cross Society (IRCS) also played a stellar role in collecting donations of blood. The Indian Red Cross Society with its 110 blood banks spread across 14 States contributes to about 10 per cent of the demand of blood in the country[56]. In Delhi, IRCS, blood bank is operational round the clock, collects nearly 30,000 units a year, and supports nearly 50 per cent of thalassaemic children with free supply of blood in the National Capital Region (NCR) territory. Bhattacharya *et al*[57] used umbilical cord blood to transfuse patients with thalassaemia major, as a source of red cells as well rich source of foetal haemoglobin.

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)[58]. 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 (*http://www.naco.nic.in*). 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 thalassaemic patients who received multiple transfusions during 1990-2003[59-62]. 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[63]. 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[63]. Due to the stringent control of blood banks the transmission of infections through blood is now negligible.

***(ii) Chelation*:**

The second major issue was 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. George J. Kontoghiorghes discovered an oral iron chelator deferiprone (L1), a drug designed and developed by academic initiatives[64]. Indian scientists[65-68] and patients contributed immensely in the clinical trials for L1. It was finally approved for use in India in 1994, and in Europe in 1999. It is being used extensively in India and other developing countries. However, it causes joint pains in some patients and agranulocytosis in an occasional patient. Thousands of patients are now treated with L1 worldwide, not only for transfusional iron overload but also for non-iron loading conditions. L1 is set to assume a role of universal antioxidant pharmaceutical and a therapeutic for more than 100 diseases. Deferasirox, once daily oral iron chelator, was the next big advance in this area. It provided an effective oral alternative to deferoxamine (desferal) in the treatment of transfusional haemosiderosis. Several Indian studies have demonstrated the safety and efficacy of Deferasirox in reducing iron burden in patients with beta-thalassaemia who have received many blood transfusions[69-72]. Chandra *et al*[73] reported gastrointestinal symptoms in about 25 per cent of cases, and skin rashes in 5 per cent cases. They observed greyish-brown pigmentation of the skin in 10 per cent children. None of the side-effects necessitated cessation of the drug therapy. They concluded that therapy with deferasirox is safe in paediatric patients with thalassaemia major. However, patients should be carefully monitored for side-effects. The long-term use of effective dose protocols of L1 and combinations with DFO results in the clearance of excess cardiac iron. Trials on effectiveness of combinations of deferiprone with deferasirox are eagerly awaited, as this would be an ideal combination.

***(iii)* Care of thalassaemics in India:**

The first centres for care of thalassaemia were probably started in Mumbai in early 1970s in JJ Hospital and KEM Hospital, followed by All India Institute of Medical Sciences (AIIMS), New Delhi in 1973-1974, Borruka Center, Kolkata in 1985, Sir Ganga Ran 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 centres 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 thalassaemics in India by arranging various educational programmes and workshops, by bringing foreign and Indian experts to examine and treat patients of thalassaemia. There are about 60 parent/patient societies of thalassaemia in India, of which about 40 are members of the FIT. Due to the persistent efforts of the thalassaemia 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 thalassaemia into the 12th Five Year Plan. This will pave the way for improving the quality of care and in introducing control programmes in various parts of the country.

Care of thalassaemics in the prominent centres is of a high order. Most of these are led by physicians who have attended the educational conferences organized in India by the thalassaemic societies. The Thalassemia International Federation played a major role by deputing and supporting experts to come to India, and give lectures and conduct workshops. The booklets outlining the optimal care of thalassaemia have also been very useful in educating the patients and doctors. Increasing reports are appearing of endocrinal and cardiac involvement in thalassaemia, 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 thalassaemia major who receive optimal care in India now survive to adulthood. A number of thalassaemic children have

married [about 10 in and near Delhi, two in Chandigarh and two in Gujarat (Tuli S, personal communication), and four in Mumbai (Sethi P, personal communication)].

In Jammu a thalassaemia society was formed in 1996, and registered in 2000. Since then the Jammu and Kashmir (J & K) Government has been providing blood transfusions, chelation and other therapies free of cost to 174 patients and also provides free transport to all patients who have to travel from long distances to receive treatment (S. Sethi, personal communication). This was followed by the Delhi State which set up dedicated thalassaemia units in all the Government hospitals and has been providing free blood transfusions and chelation since 2005. The Municipal Corporation of Delhi has also provided the same free facilities in the three hospitals under its care (Kasturba Hospital, Hindu Rao Hospital, Swami Dayanand Hospital). Since 2008 a programme of regular screening of pregnant women has been initiated. The Punjab Government gives free treatment to all thalassaemic children who attend school. The Governments of Gujarat and Maharashtra also provide free therapies for children with thalassaemia. It is hoped that other States would follow suit. It is now up to the thalassaemia societies in the other States to exert pressure on the Governments to provide similar services.

***(iv) Use of wheat grass as therapy*:**

Based on anecdotal reports by the parents of benefit of wheat grass juice in raising the haemoglobin, three studies have been carried out[74-76]. In the study by Marwah *et al*[74] the patients consumed about 100 ml of wheat grass juice daily. Each patient acted as his own control. Of the 16 cases analyzed, blood transfusion requirement fell by >25 per cent in 8 patients, with a decrease of >40 per cent documented in three of these. Choudhry and colleagues[75] did not find any significant benefit from its use. However, Singh *et al*[76] treated 40 patients of thalassaemia major with wheat grass tablets (WGT). The mean haemoglobin in the pre WGT was 8.54 ± 0.33 g/dl whereas in WGT period it was 9.13 ± 0.14 g/dl. The decrease in the blood transfusion requirements was by 25 per cent or more in 60.6 per cent cases. The mean interval between the consecutive blood transfusions in pre WGT period was 18.78 ± 4.48 days whereas in WGT period was 24.16 ± 4.78 days. The authors concluded that wheat grass has the potential to increase the haemoglobin levels, increase the interval between blood transfusions and decrease the amount of total blood transfused in patients with thalassaemia major. A double blind randomized study is required to establish efficacy.

***(v)* Use of hydroxyurea:**

Pharmacological agents such as hydroxyurea (HU) have been known to cause induction of foetal haemoglobin and reduce ineffective erythropoiesis, and thus may alleviate the symptoms in thalassaemia intermedia patients. Choudhry *et al*[77] observed a definite trend of rise in haemoglobin and haemoglobin F on administration of hydroxyurea but the change was statistically insignificant. One study found that response to hydroxyurea correlated with *Xmnl1* polymorphism as well as deletions of alpha globin gene[78], while another[79] observed a good response which correlated with *Xmnl1* +/+ in 72.7 per cent and alpha gene deletions in 41 per cent of the cases. Raina *et al*[80] demonstrated that the good response to hydroxyurea correlated with *Xmnl1* +/+ genotype and IVS1-I G>T mutation as well as Hb E.

***(vi) Bone-marrow haemopoietic stem cell transplantation (BMT)*:**

The first bone marrow transplant centre was established at Tata Memorial Hospital in 1983 in Mumbai. However, they carry out only a limited number of transplants for thalassaemia. The centre at Christian Medical College & Hospital (CMCH), Vellore was started in 1986[81]. This centre has carried out the maximum number of bone marrow haemopoietic stem cell transplants for thalassaemia in the country, and is second only to the Pesaro centre. At this centre from October 1986 to December 2006, 626 transplants were performed in 595 patients, with 28 patients having more than one transplant[81]. Thalassaemia accounted for a third of these transplants. Kumar *et al*[82] performed allogenic haematopoietic SCT in non-Hepa filter rooms. The average cost of allogenic BMT in India is around $15000 to 20000, which is considerably lower than the cost in the West. India needs to develop more transplant centres with adequately trained personnel.

The other centres where BMT is being carried out are in Delhi (All India Institute of Medical Sciences, BL Kapur Hospital, Sir Ganga Ram Hospital, Army Hospital, R and R); Lucknow (Sanjay Gandhi Postgraduate Institute of Medical Sciences), Chennai (Apollo Hospital), Chandigarh (Postgraduate Institute of Medical Education & Research), Pune (Sahayadri Hospital), Bangalore (Narayana Hrudayalaya Hospital, Manipal Hospital and Kidwai Memorial Hospital), Ahmedabad (Gujarat Cancer Research Institute),

Thiruvananthapuram (Regional Cancer Center), and Mumbai (Jaslok Hospital). Some of these centers carry out BMT for thalassaemia 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*[83] reported that of the 688 patients requiring HSCT, only 39.3 per cent 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 (HLA-A*0211, B*2707, A*26-B*08-DRB1*03). 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 (Mehra NK, personal communication). 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 3000[84]. 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[82]. 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) has been started by Dr Latha Jagannathan and Dr Velu Nair. 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 centres in India. Reliance Life Sciences cord blood repository Mumbai (Relicord) probably has the largest collection. It supplies enriched cord blood stem cells[85]. There are also some registries abroad that 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[86]. 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). There are currently 776 users from 488 organizations authorized to access the online BMDW services. More information is provided at the website of World Marrow Organization[87].

***(viii) Physical growth, endocrinal and bone density changes in thalassaemia major*:**

Several investigators have studied growth in thalassaemic children[88,89], and others have reported on pubertal development and endocrinal function[89,90]. Abnormalities of beta cell function have also been explored[91]. Significant effects of the disease on growth as well as pubertal growth have been demonstrated suggesting that in most patients optimal treatment is not being provided. Mamtani *et al*[92] carried out a meta-analysis of bone recovery after zoledronate therapy in thalassaemia-induced osteoporosis.

Prevention of thalassaemia

The need for prevention of thalassaemia is obvious 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-thalassaemia. Prevention would not only be a good public health practice, as envisioned in Alma Ata declaration, but it would also be cost-effective, as the ratio of the cost of treatment to prevention is 4:1, as shown in a study from Israel[93]. It would help tremendously in reducing the burden of the disease for patients, families and the health services. The strongest argument for prevention is that it would ensure the best possible care for the affected, by curbing the increase in their number.

The chief elements of a control programme were developed in 1970s by a team of experts at the World Health Organization, led by Dr Bernadette Modell[94]. 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)* Counselling and prenatal diagnosis, and *(vii)* Network of centers, and National/Regional working groups. Control programmes in thalassaemia[95,96] have the following important components:

Financial support is required for training and employing the manpower required for execution of the control programme (social workers, technicians, doctors, counsellors); 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.

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 programme on thalassaemia 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 programmes in Cyprus and Greece it was concluded that one of the important steps was the introduction of formal education on thalassaemia in the school curriculum[97,98].

Preventive programmes carried out based on heterozygous detection and counselling to avoid marriage between carriers, without prenatal diagnosis, have not been very effective around the world. In contrast, preventive programmes based on heterozygote detection, counselling and foetal diagnosis have been very effective in reducing birth of thal major infants in Sardinia, Cyprus, Greece and Italy[95]. 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 heterozygotes as compared with random mating, and no change in the incidence of thalassaemia major in some studies[99,100].

Premarital screening has been advocated by many investigators and haematologists in India as a preventive strategy. This is a good, perhaps the only option, in countries that do not allow termination of pregnancy, *e.g.,* in Saudi Arabia, the Middle-east and Sri Lanka. Experience gained in the running the control programme for thalassaemia in Iran is instructive. The program was started in 1995. Their policy is based on couple screening[101]. In an autosomal recessive disorder this makes good sense[102]. All couples wanting to marry are required to be checked for carrier status of thalassaemia in order to receive a permit for marriage registration. The male is tested first by determining the red blood cell indices. If he is a carrier as suggested by red cell indices the woman is screened. If the results are abnormal, haemoglobin A2 (HbA2) is performed. Couples in which both partners are confirmed to be carriers receive counselling. In the original report by Samavat and Modell[101] over a period of 5 years (1997-2001) 2.7 million couples were tested all over Iran, of whom 10,290 were at risk. Of these, 53 per cent decided to get married, 29 per cent separated, and 18 per cent were uncertain. Five years later Karimi *et al*[103] reported on screening of 1,038,371 couples in Southern Iran from 1995 to 2004. Of these, 5,182 (0.4%) were diagnosed to be carriers; 1932 refused marriage, while 2798 (54%) proceeded to marry in spite of knowing the carrier status. This happened because of the existing cultural practice. It quickly became apparent, however, that those being tested wanted to have prenatal diagnosis available. This pressure led to ethical discussions by Muslim scholars and others, resulting in a fatwah that allowed abortion during the first 16 wk after the last menstrual period, when the foetus was diagnosed with thalassaemia. The latter was offered only since 2002. Results revealed that in 1995, 1999 and 2004, 296, 94 and 56 β-thalassaemia homozygotes, respectively, were born (2.53, 1.07 and 0.82 patients per 1000 births). Even in Saudi Arabia and UAE more and more couples are choosing prenatal diagnosis, and it is only a question of time when this will be introduced.

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. An earlier study in Arta area of Greece is revealing[100]. There is a 20 per cent carrier rate in the area. All young people of marriageable age were screened and counselled. Assessment at the end of 2 years showed no measurable effect on choice of partner. In a study in Montreal, Canada, high school children were screened for carrier status of thalassaemia and Tay Sach disease, and six years later were asked the question " Do you think that a couple planning to marry, who found they were carriers, should change their marriage plans? Would you change your own marriage plans". The answer was

most revealing - 80 per cent thought that other couples would change their marriage plans, but only 10 per cent thought they would change their own plans[104].

The Cyprus programme is often used to justify premarital counselling. Initially the model in Cyprus was based on screening and counselling 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 thalassaemia major births is due to separation of engaged couples, while about 80 per cent is due to prenatal diagnosis and selective abortion[97,98].

***Which strategy is likely to succeed in India*?** If the policy of premarital screening were to be successful, control of thalassaemia 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[105] observed that blood is deeply valued in the Bengali kinship system, and that this genetic mutation is perceived to be corrupting the blood. Being a thalassaemia carrier (*i.e.*, having thalassaemia minor) renders an individual unfit as a suitable marriage partner because of beliefs related to purity of blood, its association with the continuity of the lineage, and subsequent transmission of desirable traits to future generations. 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 thalassaemia minor (TMI) is a deterrent to the disclosure of thalassaemia status as well as to testing. Tamhankar *et al*[106] carried out premarital testing for thalassaemia carrier state in three groups: extended family members of diagnosed cases of thalassaemia/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 programmes for β-thalassaemia should be to sensitize the community to the problem, establish haematological technologies for screening, and molecular and obstetric techniques for prenatal diagnosis. However, the programme should not begin until all components are in place.

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 thalassaemia 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 per cent, and a negative result picks out the normal very well[107-110].

The other important observation made in India is that concomitant iron deficiency is present in many β-thalassaemia carriers[111-113]. It was shown that once iron deficiency is established, therapy with iron leads to a significant rise in haemoglobin. The authors demonstrated that in β-thalassaemia carriers HbA_2 decreased in the presence of iron deficiency, but the fall was slight, and it never reached the normal range. Another important contribution was recognizing the unique sequence in HbA_2 and developing antibodies against HbA_2. These antibodies were used to measure HbA_2 by ELISA technique[114,115].

The next issue is ***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. Colah and colleagues[116] screened 5682 school children (age 11-18 yr) from 75 schools in Mumbai city between 1984 and 1988. Of these, 153 (2.7%) were found to be β-thalassaemia heterozygotes. After 20 years it was possible to locate only 71 of the 153 children who were carriers of β-thalassaemia. Only 47 of these 71 families could be contacted. Only 12 of the 47 individuals contacted (26%) recollected that they were β-thalassaemia carriers. None of the 41 individuals who were now married had revealed their carrier status or had their partners tested before marriage. However, 11 individuals had their spouses tested for haemoglobinopathies after marriage. This shows that screening of school students is unlikely to make a significant reduction in the burden of thalassaemia.

Cascade or inductive screening identifies many more carriers than general population screening, as was shown in a study in Pakistan[117]. The investigators screened 15 large families with segregating β-thalassaemia genes through a proband, who was a known carrier. They also examined eight families from the same region without a history of β-thalassaemia. Because of consanguinity 31 per cent of those tested in the first category were found to be carriers, while there was none in those without a family history of thalassaemia. Inductive screening is not a new approach - it is used regularly in every genetics clinic. In Sardinia, Cao & Galanello[118] were able to identify more than 90 per cent of the couples who were at risk for producing a child with β-thalassaemia, by examining only 11 per cent of the population. Gorakshakar and Colah[119] screened 691 relatives of affected subjects and detected 151 to be β-thalassaemia carriers (21.9%). As compared with other approaches, the percentage of β-thalassaemia carriers identified was 5-6 times higher using this cascade screening approach. Tamhankar *et al*[106] tested *(i)* extended family members of diagnosed cases of thalassaemia, *(ii)* unmarried adult cases of anaemia attending the hospitals' outpatient department, and *(iii)* adult college students for carrier status of β-thalassaemia. The yield of carriers from the three groups was 78.17, 19.51 and 4.04 per cent, respectively. The number of prospective high-risk couples detected were 154, 48 and 2 from the three groups, respectively.

In 1990, Sangani *et al*[48] collected information on the attitudes toward screening for thalassaemia among 200 families of index cases residing around Mumbai. Twenty per cent of these families had expressed an unfavourable reaction from their relatives. Hence, these parents were secretive about their child having thalassaemia major to avoid social stigmatization. Yagnik[120] followed up 70 carriers and 127 non carrier who were identified during screening of high-risk communities 5-7 years after counselling. Of these, 46 per cent of the carriers had got all their siblings tested while 42 per cent had not informed their siblings about their carrier status. Saxena & Phadke[121] interviewed 100 parents of children with thalassaemia using a pre-designed questionnaire. The results showed that 96 per cent of them were willing to share information on their thalassaemic children with relatives and friends. However, only 14 families got themselves tested, while another 34 families could not do so due to non availability of screening facilities in the nearby town, high cost of the test, and lack of sufficient motivation.

The largest programme of screening for thalassaemia in the population has been carried out in Gujarat by the Indian Red Cross Society in Ahmedabad and other cities[122]. From 2004 to 2010, 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 Thalassaemia Control Programme, 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)[123,124]. Screening was done in 29,898 college students, and 26,916 pregnant women. The prevalence of β-thalassaemia 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 programmes should be a continuous process; adequate number of centres should be established so that people do not have to travel long distances for the tests;

NESTROFT (osmotic fragility) 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 h after collection; mean corpuscular haemoglobin (MCH) was more stable parameter than mean corpuscular volume (MCV); HbA_2 estimation by HPLC was the most accurate method for diagnosis of β-thalassaemia heterozygotes. 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.

We carried out a pilot study (unpublished) on prevention of β-thalassaemia in a district hospital in Delhi. Using NESTROFT, 2300 women were screened for carrier status of β-thalassaemia, at the local site. All those with positive or doubtful results had an HbA_2 estimated. Sixty two (2.7%) women were confirmed to be carriers. Only 43 husbands could be examined and eight (18.6%) turned out to be carriers. This was surprising, but not totally unexpected, given that marriages are not completely random, and occur most commonly among the same castes. Of these 8 couples, prenatal diagnosis was carried out in 6, as one refused, and in one the pregnancy was too advanced. Of the six examined, two had affected foetuses, two foetuses were carriers and two foetuses were normal. This averted the birth of two affected children. 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.

Prenatal diagnosis

A number of investigators have described their experience with prenatal diagnosis in India[125-127]. This was provided first in Delhi. Initially samples for prenatal diagnosis were sent to John Old in Oxford. However, with his help the ARMS/RFLP analysis using restriction enzymes (RE) method to detect the mutations was set up locally. By 1996 prenatal diagnosis was performed in 415 cases in All India Institute of Medical Sciences, New Delhi[126]. In rapid succession it was established in Christian Medical College in Vellore[125], and in Mumbai at the National Institute of Immunohaematology and Wadia Hospital for Children[127]. Currently prenatal diagnosis is available in the following centres in India: Delhi - Sir Ganga Ram Hospital, All India Institute of Medical Sciences, Mumbai – National Institute of Immunohaematology, Wadia Hospital for Children, Vellore – Christian Medical College and Hospital, Lucknow - Sanjay Gandhi Postgraduate Institute of Medical Sciences, Hyderabad - Center for DNA Fingerprinting and Diagnostics, and Center for Cellular and Molecular Biology; Chandigarh - Postgraduate Institute of Medical Education and Research, and a few commercial laboratories.

The important steps for prenatal diagnosis can be listed as: *(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. Sometimes one of the parents turns out to be Hb E or sickle cell disease. 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 HbA_2 by HPLC. Estimation of HbA_2 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 HbA_2, it is better to sequence their β-globin gene. Garewal and colleagues[128,129] showed that Jat Sikhs, a subcaste of Punjabis, have a very high prevalence of the mild beta^{++} promoter region mutation -88 (C-T). "Mild" mutations are also reported from South India[36,37] - cap site +1 (A>C), poly A (T>C), -28 (A>G), and -88 (C>T). *(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 (Thalassaemia major), β-Thal/HbS (Sickle/β-Thalassaemia), β-Thal/HbE (E/β-Thalassaemia), 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.

The techniques for identification of mutations

There are 64 β-globin mutations reported in Indians so far as recorded in the ThalInd database[40]. The most popular technique for mutation detection is the ARMS technique. This was developed by Newton and colleagues[130], and applied for molecular diagnosis for thalassaemia by John Old and group[27,28,39]. It has proved to be a good technique, and easy to learn.

However, its disadvantage is that separate primers are required to analyze each mutation and the normal sequence at that site. Restriction enzyme method can be applied for many of the mutations, while reverse dot-blot is used by the groups at Mumbai (NIIH)[31] and Vellore[36]. Earlier most investigators tested the mutations in stages – the five commonest mutations along with Hb E and Hb S, then the eight other common mutations, then the rare ones. The unknown mutations were screened by denaturing gradient gel electrophoresis (DGGE), single strand conformation polymorphism (SSCP), or temporal temperature gradient gel electrophoresis (TTGE), and the resulting fragment sequenced. As the facility of sequencing has become more widely available, most investigators proceed to sequence once testing for the common seven mutations is negative. Another useful technique has been to test cord blood by HPLC analysis for prenatal diagnosis in those cases where mutations are not known, or in those women who present late in pregnancy[131,132].

Future of thalassaemia care in India

The international situation changed in 2006 with the recognition by the Executive Board of the World Health Organization that thalassaemia 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[133]. 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[134]. Weatherall[135] pointed out that the major problem for the future lies in the unwillingness of governments and international health agencies to accept that thalassaemias present a health burden in developing countries comparable to that of other major diseases. He suggested that more detailed information on frequency and economic data is required to provide evidence for the health burden posed by thalassaemias in the developing world. The Government of India is proposing to provide sufficient funds to the care and control of thalassaemia in India.

Currently in most cities thalassaemia care centres have been established, both in the private sector as well the Government hospitals. Paediatricians and paediatric haematologists looking after these centres have sufficient training and skills to manage these cases. What needs to be done is to supply free or subsidized blood transfusions as well chelating agents in all States in India, as has been done in a number of States like Jammu and Kashmir, Delhi, Maharashtra and Gujarat.

The programme 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 programmes in the cities and States that have a high prevalence of carriers of B-thalassaemia, yet no concrete programmes have been started. The 6-centre study on control[124] has been extremely useful in providing the basics of organizing and running a control programme in India. Even without a control programme in place we must motivate the obstetricians to screen every woman at first visit for carrier status of thalassaemia, haemoglobin E and sickle cell disease. As far as feasible, husband should be screened at the same time.

There is a need to increase the number of centres in India able to perform prenatal diagnosis, and provide this facility at a subsidized cost, or free for the poor, and introduce quality control programmes. 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. Centres for bone marrow transplantation need to be expanded and facility subsidized. The need of the hour is to introduce control programmes in the high risk States.

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