Control of thalassemia in India

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Abstract

The β-thalassemias and sickle cell disorders pose a major health burden in the large and diverse Indian population. Education programs for awareness generation are being done by National Institutions, non-governmental organizations and Thalassemia Societies in different states. Several extensive epidemiological studies have shown that there are many non-tribal and tribal communities where the prevalence of β-thalassemia carriers is much higher (5.3 to 17.0%) than the average of 3 to 4% projected for the entire country. These variations have also been shown within small geographic regions in some states, emphasizing the need for micro mapping to estimate the true burden of disease. There are 10 to 12 centers where prenatal diagnosis for hemoglobinopathies is done and the Indian Council of Medical Research is establishing additional regional centers in states where they are most needed. Sixty-eight β-thalassemia mutations have been described so far among Indians and the knowledge on their prevalence and regional distribution has helped to undertake prenatal diagnosis in a cost effective way.

Introduction

The inherited disorders of hemoglobin (Hb), which include the thalassemias and sickle cell disorders, are the most common monogenic disorders with an autosomal recessive inheritance. Around 7% of the global population are carriers of these defective genes with an annual birth of more than 300,000 children with a severe hemoglobin disorder.[1]

India's 1.2 billion population accounts for 17.3% of the global population and is one of the world's most diverse with a large number of ethnic groups, regional languages and different religions. A large majority of people (68.8%) live in rural areas. 8.14% of people belong to different tribal groups and many of these socio-economically underprivileged groups still live in isolation in remote areas and do not mix with the mainstream populations (Census of India 2011).

The extent of the burden in India

The health burden of hemoglobinopathies is huge in most of the states of the country due to the size and genetic complexity of the population. Screening programs have shown that the average prevalence of β-thalassemia carriers is 3.0 to 4.0%[2] and based on this it has been estimated in different studies that there would be 35-45 million carriers and around 7500 to 12,000 affected babies with β-thalassemia would be born annually. In the absence of registries, the exact figures are not known.[3]

HbE is prevalent in north-eastern and eastern India with carrier rates varying from 3 to >50% in different communities, but is now seen in other regions also due to migration of people to other areas for work and more liberal views on inter-caste and inter-state marriages.[4,5] HbS is largely restricted to the tribal and scheduled caste population with the highest load in Madhya Pradesh in central India where the expected annual birth of babies with sickle cell disease is >3000.[6] The prevalence of carriers of HbS has ranged from 1 to 35% among these population groups from central, and parts of western, eastern and southern India.[7] These Hb variants are frequently co-inherited with the β-thalassemias resulting in disorders of variable severity.

The estimates of annual births of babies with these disorders have indicated that there would be at least 100,000 children with a β-thalassemia syndrome in India.

The need to strengthen prevention programs

Optimum management of a child with β-thalassemia major involves an expenditure of Rs 150,000/year to Rs 200,000/year.[3] As the child grows older a multidisciplinary approach is needed which further escalates the cost of management. A recent study from Kolkata in eastern India where both β-thalassemia major and HbE-β-thalassemia are common showed that when unrestricted funds were available through sponsored comprehensive care, there was a significant increase in the hemoglobin levels and a decrease in serum ferritin and the clinical parameters as well as the quality of life of the patients were significantly better than of those patients receiving routine care.[8] Unfortunately, only 5-10% of patients in India can afford optimum care and the majority of patients are not even regularly transfused or chelated.[9] Thus, there is a need to strengthen prevention programs.

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Thalassemia control programs

The remarkable success of education, screening, genetic counseling and prenatal diagnosis programs first introduced in the UK and Mediterranean countries like Cyprus, Italy and Greece in the 1970s[10-13] eventually led to the development of similar control programs in many other developing countries. A cost benefit analysis in Israel showed that the cost of treatment to prevention had a ratio of 4:1.[14] In Iran also the cost of treatment to prevention was 16:1.6.[15] In India too, prevention programs have been initiated almost 3 decades ago.

Awareness and screening programs in India

There are several National Institutions, Medical Colleges, Central and State Government supported programs as well as Thalassemia Societies and non-governmental organizations groups involved in efforts for generating awareness on the hemoglobinopathies as well as conducting epidemiological studies. The major ones are summarized in Table 1.

Recent reports on large epidemiological studies done in different states have shown that the average prevalence of β-thalassemia carriers of 3-4% do not represent accurate figures for all states and communities and can be very variable even within small geographical distances (Table 2).[2,5,16-19] The castes and tribal communities showing much higher prevalence rates than the average (5.3 to 17.0%) are shown in Table 3.[2,5,17,18,20-22] This emphasizes the need for micro-mapping studies in all the

states as well as in different communities to know the true burden of disease.[2,5,16-22]

The most appropriate time to screen

There has been a continuous debate on who should be screened and when. Our earlier work showed that screening school going children might be too early. We attempted to assess the impact of screening and counseling high school children by following up β-thalassemia carriers after a period of 20 years and observed that majority of them did not recollect being carriers and none of the 41 individuals who were married had their partners tested before marriage.[23] On the other hand, the advantage of screening this group is that they are easily approachable even in rural areas. There have been several discussions on including some formal education on thalassemias in the school curriculum. Introducing formal education on thalassemias in secondary schools was crucial in providing knowledge on the thalassemias to family members in the Mediterranean region.[24-26]

University students in the age group of 17-25 years are more receptive and this group has been screened in many programs in India. However, only a small percentage of students would enter university and hence large population groups particularly in rural areas would not be covered. Premarital screening of partners of carriers is generally not acceptable as majority of marriage partners in India are still selected by the parents or other family members and there would be considerable social stigmatization if a planed marriage is called off for this reason.[3] On the other hand, Tamhankar *et al.* 2009 undertook premarital screening in adult college going students, extended family members of affected children and unmarried adults with anemia coming to their hospital outpatient department in Lucknow in Uttar Pradesh and showed that majority of prospective couples who were carriers did get married even after knowing they were at a high risk of having an affected child and then opted for prenatal diagnosis.[27] Our own studies also showed that cascade screening for extended family members of affected children was acceptable and cost effective as it identified 5 to 6 times higher number of carriers than screening the general population.[28] Ahmed *et al.* had also found that in Pakistan, cascade screening was useful and identified 31% of carriers within families.[29]

Screening pregnant women in antenatal clinics has been successful in some private hospitals in India, however experience in public hospitals where women from a low socio-economic status would go has shown that only 15-20% register for their first antenatal check-up in the first trimester of pregnancy.[30,31] Majority of the husbands do not accompany their wives and by the time couples at

Table 1. Major programs for awareness and screening for β-thalassemias.

Institutes of the Indian Council of Medical Research (ICMR)	i) National Institute of Immunohaematology, Mumbai ii) Regional Medical Research Centre for Tribals, Jabalpur, Madhya Pradesh iii) Regional Medical Research Centre, Dibrugarh, Assam iv) Regional Medical Research Centre, Bhubhaneshwar, Orissa
National Institutes	i) All India Institute of Medical Sciences, New Delhi ii) Postgraduate Institute for Medical Education and Research, Chandigarh iii) Sanjay Gandhi Postgraduate Institute of Medical Education and Research, Lucknow, Uttar Pradesh
Anthropological Survey of India	Centers in different states
State government supported programs	i) Districts of Gujarat ii) Districts of West Bengal iii) Districts of Maharashtra
Private hospitals	Different states
Non-government organizations and Thalassemia Societies	Different states

Table 2. Prevalence of β-thalassemia trait reported in large studies.

Region	Number of individuals screened	Target groups	Technology used	Prevalence of β-thal trait (range)	Reference
Mumbai and Delhi	11,090	Secondary school students	CBC, Hb electrophoresis	4.05% (2.68-5.47%)	2
All Districts of Maharashtra	10,647	School and college students, pregnant women	CBC, HPLC	2.7% (1.0-6.0%)	16
All Districts of Gujarat	8004	School and college students, pregnant women	CBC, HPLC	3.5% (0-9.5%)	16
Bangalore, Kolkata, Ludhiana, Dibrugarh, Vadodara, Mumbai	56,780	College students, pregnant women	CBC, HPLC	2.78% (0.48-3.96%)	5
Districts of Gujarat	317,539	Tribal and non-tribal communities	CBC, HPLC	1.95% (1.74-2.18%)	17
South Gujarat	32,857	School and college students, community members	CBC and Hb electrophoresis, HPLC	4.4% (2.7-4.7%)	18
Districts of Rural West Bengal	35,413	School and college students, married couples, pregnant women	CBC, HPLC	10.38% (8.96-11.21%)	19

CBC, complete blood count; Hb, hemoglobin; HPLC, high performance liquid chromatography.

risk are identified, it is too late for a prenatal diagnosis.

Thus, it is felt that due to the complexity of the Indian population, multiple target groups would have to be screened in different regions.

It has been emphasized that a pre-requisite for a screening program would be the organization of adequate facilities to meet the demand both for screening and prenatal diagnosis.[32] This is very important for a vast country like India. In one of the surveys in Uttar Pradesh in north India, some families could not get themselves tested due to non-availability of screening facilities in a nearby town as well as due to the cost involved.[33]

The spectrum of β-thalassemia mutations

The spectrum of β-thalassemia mutations is known from various studies in different regions in India. Sixty-eight mutations have been characterized,[34-36] but this number keeps increasing as more studies are reported. The regional distribution of mutations is also known.[37] Based on this data, the distribution of the most common mutations in different states is shown in Figure 1. A meta-analysis on 17 selected studies was done covering 8505 alleles and near national coverage which showed that 52 mutations accounted for 97.5% of all β-thalassemia alleles. The prevalence of IVS 1-5(G>C), the commonest mutation ranged from 44.8 % in the north to 71.4 % in the east.[38] The following rare mutations were found to be common in some communities:[35,37,39,40]

CD5 (-CT)	Prajapatis in Gujarat
IVS II-837(T>G)	Gaud Saraswat Brahmins in Goa and Karnataka
CD 110 (T>C)	Agris in Maharashtra
CD 47 (+A)	Nicobarese in Car Nicobar, Andaman and Nicobar Islands
CD 30 (G>A)	Kachiya Patels in South Gujrat

There are many communities particularly in rural areas, which have not been studied, and this data needs to be generated.

Identification of carriers

Majority of population screening programs in India now use red cell indices in the first phase followed by automated high performance liquid chromatography (HPLC) for quantitation of Hb A_2, Hb F and other Hb variants when the mean cell volume and mean cell hemoglobin levels are reduced. In few studies both complete blood count and HPLC have been done in all individuals. Osmotic fragility (NESTROFT) as a first line screen has also been used by some groups. The approach used has varied depending on the availability of the machines and the cost involved.

Borderline Hb A_2 levels and/or near normal red cell indices have been a major problem for interpretation of the carrier status of β-thalassemia. In a report from north India, screening for the β-thalassemia mutations was done in 25 individuals with borderline Hb A_2 levels (3.0 to 4.0%). A β globin gene mutation was detected in 8 cases (32%) where the Hb A_2 levels were between 3.5 and 3.9%. This included 6 cases with common severe β^+ or β° thalassemia mutations [IVS1-5 (G>C), 619 del, CD41/42 (CTTT)] and 2 cases with the capsite mutation.[41] Borderline Hb A_2 levels have also been seen in β-thalassemia heterozygotes with associated δ gene mutations, in particular the δpromoter region substitution (-68C>T), associated α-thalassemia and also occasionally with common β-thalassemia mutations like the IVS1-5(G>C) and CD30(G>C) mutations.[42]

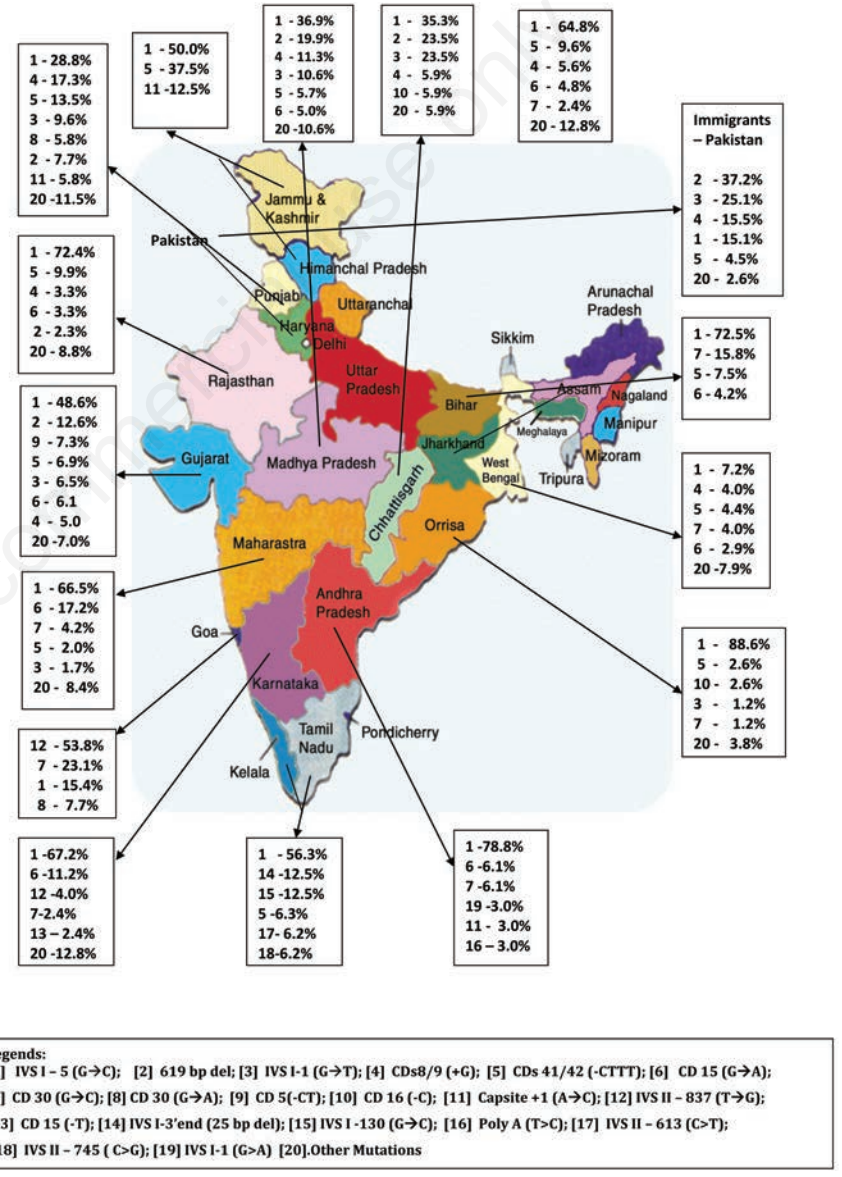

Figure 1. Map showing the distribution of the most common mutations in different states.

Such cases may be missed in population screening programs but one has to be very careful in accurate diagnosis of these individuals particularly in a couple when the other partner is a carries of β-thalassemia or another Hb variant like Hb E or Hb S.

Genetic counseling

Most of the genetic counselors in prevention programs in India are Medical Social Workers with in-house training in centers where screening and prenatal diagnosis in done. There is only one formal course for genetic counselors being conducted in Lucknow in Uttar Pradesh. Non-directive counseling is given but majority of the couples at-risk opt for prenatal diagnosis.

Prenatal diagnosis

Prenatal diagnosis facilities are available at 10 to 12 centers in India.[34] Recently, the Indian Council of Medical Research (ICMR) has established 5 more regional centers mainly in medical colleges. Fetal DNA analysis from chorionic villus tissue or amniotic fluid cells is generally done. Fetal blood sampling and analysis of fetal blood by HPLC or molecular methods is also used when the couple at risk is identified late as often happens in India. Amplification refractory mutation system and reverse dot-blot hybridization are the 2 techniques used for identification of mutations followed by DNA sequencing if required.

Our experience on prenatal diagnosis in over 3000 couples has shown that only around 10% of couples come prospectively for prenatal

Table 3. Caste and tribal communities with a high prevalence of β-thalassemia carriers.

Group	Region	Prevalence of β-thalassemia carriers	Reference
Caste populations			
Sub castes of Sindhis	Nagpur, Maharashtra	8.0-17.0%	20
Bhatias	Mumbai, Maharashtra	5.9%	2
Khatri	Mumbai, Maharashtra	6.9%	2
Lohana	Mumbai, Maharashtra	5.6%	2
Brahmin	Delhi	5.3%	2
Jath	Delhi	6.9%	2
Kayastha	Delhi	5.3%	2
Kachhia Patels	Surat, Gujarat	6.0%	21
Modh Banias	Surat, Gujarat	6.2%	21
Muslim Fakirs	Surat, Gujarat	5.6%	21
Muslim Memons	Surat, Gujarat	7.2%	21
Prajapati	Surat, South Gujarat	6.2%	18
Ganchi	Surat, South Gujarat	6.2%	18
Mayavanshi	Surat, South Gujarat	6.9%	18
Lohana	Surat, South Gujarat	10.8%	18
Sindhi	Surat, South Gujarat	10.2%	18
Rajput	Bangalore, Karnataka	6.3%	5
Jain	Bangalore, Karnataka	9.6%	5
Muslim Shiya	Bangalore, Karnataka	6.3%	5
Mondal	Kolkata, West Bengal	8.6%	5
Brahmin	Dibrugarh, Assam	6.0%	5
Arora	Ludhiana, Punjab	9.8%	5
Vellala	Mumbai, Maharashtra	10.5%	5
Lohana	Vadodara, Gujarat	7.4%	5
Bhanushali	Gujarat	8.1%	17
Bhakta	Gujarat	7.9%	17
Lohana	Gujarat	6.5%	17
Tribal populations			
Chaudhry	Surat, South Gujarat	12.6%	18
Gamit	Surat, South Gujarat	15.9%	18
Rohit	Surat, South Gujarat	6.3%	18
Vasava	Surat, South Gujarat	13.6%	18
Kokana	Surat, South Gujarat	14.7%	18
Bhuyan	Sundargarh district, Orissa	6.5%	22
Paik	Sundargharh district, Orissa	7.8%	22
Paraja	Sundargharh district, Orissa	12.7%	22
Dudh Kharia	Sundargharh district, Orissa	8.1%	22

diagnosis of β-thalassemias while more than 30% of couples come prospectively for prenatal diagnosis of sickle cell disorders.[34] However, termination of pregnancies is acceptable in all the communities. This again emphasizes the need for greater awareness in the community to enable couples at-risk to come prospectively for fetal diagnosis.

Pre-implantation genetic diagnosis

Although globally there are many centers where pre-implantation genetic diagnosis (PGD) of the β thalassemias is done,[43] in India PGD is still in it's infancy with only one report of PGD in a single family so far.[44] However, increasingly there are requests from couples wanting to opt for PGD in urban areas.

Non-invasive procedures for prenatal diagnosis

There is very limited data on experience with non-invasive prenatal diagnosis from India. We were able to achieve an accuracy of 84 to 90% by isolating fetal nucleated cells from maternal blood[45] and 80% by looking for the presence or absence of paternal mutations in cell free fetal DNA from maternal plasma.[46]

Conclusions

Over the years many education and awareness programs on the thalassemias and sickle cell disorders have been undertaken followed by screening of different target population groups but micro-epidemiological studies are still needed to know the true burden of the disease in different regions of this vast country. These programs have gradually allowed couples at-risk to take informed decisions. Majority of them have opted for prenatal diagnosis and termination of affected pregnancies.

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