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Original Article

BETA THALASSEMIA IN INDIA: CURRENT STATUS AND THE CHALLENGES AHEAD

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ABSTRACT

Hemoglobinopathies represent a significant national health burden in India. Among all the hemoglobinopathies beta-thalassemias are major health problems in India but have received little attention because of other health priorities, such as malnutrition and communicable diseases. To make possible prenatal diagnosis of beta thalassemia in India by direct detection of mutant beta globin genes, the spectrum of mutations producing the disorder in this part of the world must be known. So, the aim of this review was to analyze the spectrum of mutations causing beta thalassemia in India. It also includes data on mutation difference among the different ethnic/population groups in India. The IVS-1-5 mutation is the commonest mutation found in the Indian population and its prevalence (in homozygous state) varies from 22.8 to 81.4% in different regions of India, being the highest in Tamil Nadu in south-eastern India. In the north-western part of India the 619 bp deletion mutation is the commonest beta-thalassemia mutation observed in patients originating from Sindh, Gujarat or among the families migrated from Pakistan during partition of the country in 1947. Prognosis for individuals with beta-thalassemia has improved substantially in the last 20 years following recent medical advances in transfusion, iron chelation and bone marrow transplantation therapy. The prospect of curing such diseases holds great potential for alleviating human suffering in India. It is really a great challenge and needs an organized plan for action. The exact magnitude of the disease in India is still obscure. Only hospital-based data are available, which cannot be regarded as representative of the community or population. So, we collected all the reported data to know the exact spectrum of mutations causing beta thalassemia along with their ethnic diversity in India. This study would help in prenatal diagnosis by direct detection of mutant beta globin gene. The challenges highlighted in the study need to be sorted. The present study suggests the effective approaches to reduce the burden of disease.

INTRODUCTION

Beta-thalassemia is one of most common autosomal recessive disorders worldwide. Different molecular mechanisms, most of which are base substitutions or small deletions or insertions of one or two nucleotides in the β-globin gene are responsible for β-thalassemia [1-3]. Moreover, it has been found that β-thalassemia mutations are relatively population specific, *i.e.* each ethnic group has its own set of common mutants [4-7].

High prevalence is present in populations in the Mediterranean, Middle-East, Transcaucasus, Central Asia, Indian subcontinent, and Far East [8]. It is also relatively common in populations of African descent [9]. The highest incidences are reported in Cyprus (14%), Sardinia (12%), and South East Asia [10].

Clinical and Hematological Features

Thalassemia Major

Individuals with thalassemia major usually present within the first two years of life with severe anemia, requiring regular red blood cell [RBC] transfusions. Findings in untreated individuals with thalassemia major are growth retardation, pallor, jaundice, poor musculature, hepatosplenomegaly, leg ulcers, development of masses from extramedullary hematopoiesis, and skeletal changes that result from expansion of the bone marrow. Peripheral blood smear shows, in addition to microcytosis and hypochromia, anisocytosis, poikilocytosis [spiculated tear drop and elongated cells], and nucleated red blood cells [i.e., erythroblasts] (Figure 1). Hb pattern [by cellulose acetate electrophoresis or high performance liquid chromatography [HPLC]] varies according to the type of beta-thalassemia. In beta-thalassemia, characterized by the lack of beta globin chain synthesis, HbA is absent, HbF is 95–98%, and HbA2 is 2–5% (Table 1).

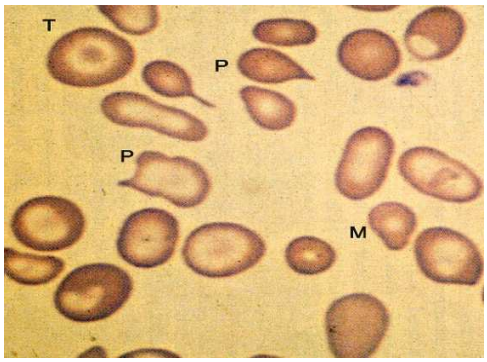

Fig. 1: Peripheral smear from a patient with beta-zero thalassemia major showing more marked microcytosis [M] and anisopoikilocytosis [P] than in thalassemia minor. Target cells [T] and hypochromia are prominent.

Thalassemia Intermedia

Patients have a moderate anemia and show a markedly heterogeneous hematological picture, ranging in severity from that of the beta-thalassemia carrier state to that of thalassemia major.

Thalassemia Minor

Carriers of beta-thalassemia are clinically asymptomatic. The characteristic hematological features are microcytosis (reduced red blood cell volume), hypochromia (reduced red blood cell Hb content) and increased HbA2 level (Figure 2).

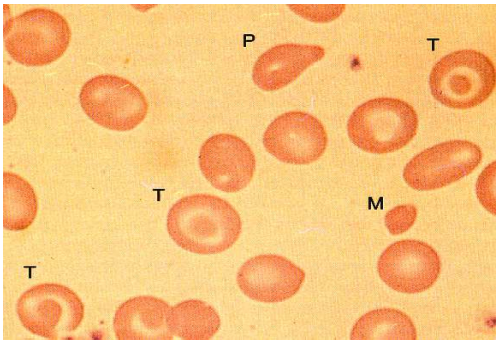

Fig. 2: Peripheral smear in beta-zero thalassemia minor showing microcytes [M], target cells [T], and poikilocytes.

Diagnosis

Can be done by Hematological Testing and Molecular Genetic Testing

Hematological Testing can be done by

1. *Measuring Red blood cell indices*
2. *Peripheral blood smear*

- Affected individuals demonstrate the red blood cell [RBC] morphologic changes of microcytosis, hypochromia, anisocytosis, poikilocytosis [spiculated tear-drop and elongated cells], and nucleated red blood cells [i.e., erythroblasts].

3. Qualitative and quantitative hemoglobin analysis [by cellulose acetate electrophoresis and DE-52 microchromatography or HPLC] identifies the amount and type of hemoglobin present.

Molecular Genetic Testing

- *Targeted mutation analysis*. The β-thalassemias can be caused by more than 200 different *HBB* gene mutations [14]; however, the prevalent molecular defects are limited in each at-risk population. The most commonly used methods are reverse dot blot analysis or primer-specific amplification ARMS PCR, real-time PCR or microarray technology [15-17].

- *Sequence analysis* detects mutations in the *HBB* coding region and associated flanking regions. Sensitivity is 99%.

- *Deletion/duplication analysis*. Deletions of variable extent of the *HBB* gene or of the beta-globin gene cluster that result in β-thalassemia or in the complex β-thalassemias called γδβ-thalassemia and δβ-thalassemia are rare causes of β-thalassemia. Some *HBB* alleles with deletion mutations can be common in certain ethnic groups [e.g., the 619-bp deletion in Asian Indians].

Table 1: Hemoglobin Patterns in Beta-Thalassemia [Age >12 Months] [11].

Hemoglobin Type	Normal [1]	Affected		Carrier
		β^0-Thal Homozygotes	β^+-Thal Homozygotes or β^+/β^0 Compound Heterozygotes	β-Thal Minor
HbA	96%-98%	0	10%-30%	92%-95%
HbF	<1%	95%-98%	70%-90%	0.5%-4%
HbA_2	2%-3%	2%-5%	2%-5%	>3.5%

Table 2: Hematological profiles of Normal and Beta-Thalassemia patients [12,13].

Red Blood Cell Index	Normal	Affected		Carrier
		β-Thal Major	β-Thal Intermedia	β-Thal Minor
Mean corpuscular volume [MCV fl]	80-100	<50<70	50-80	<79
Mean corpuscular hemoglobin [MCH pg]	27-31	12-20	16-24	<27
Hemoglobin [Hb g/dl]	Males: 13.8 -18.0 Females: 12.1- 15.1	<7	7-10	Males: 11.5-15.3 Females: 9.1-14

Beta Thalassemia in India

β-thalassemia is the commonest single-gene disorder in the Indian population [18]. Ten percent of the total world thalassemics are born in India every year [19]. Certain communities in India, like Sindhis, Gujratis, Punjabis, and Bengalis, are more commonly affected with beta thalassemia, the incidence varying from 1 to 17% [20] (Gupta et al., 2003). It has been estimated that the prevalence of pathological hemoglobinopathies in India is 1.2/1,000 live births [21], and with approximately 27 million births per year [22] this would suggest the annual birth of 32,400 babies with a serious hemoglobin disorder. Within this overall disease classification a 1989 WHO Working Group on guidelines for the control of hemoglobin disorders estimated a 3.9% carrier frequency for b-thalassemia in India, encompassing all types of b-thalassemia trait [23]. A WHO update on b-thalassemia in India indicated a similar overall carrier frequency of 3–4%, which given the current national population would translate to between 35.6 and 47.5 million carriers of the disorder nationwide [22, 24]. This health burden emphasizes the need for prenatal diagnosis and carrier status detection to contain the disease and reduce the load of the mutant alleles in the gene pool.

The first case of Thalassemia, described in a non-Mediterranean person, was from India. Subsequently, cases of thalassemia were documented in all parts of India [25]. More than 200 thalassemia mutations have been identified all over the world [26] (Firkin et al. 1989) and of these about 28 mutations have been documented in Indian patients [27-45]; Six mutations; 619 bp deletion at 3' end of beta-globin gene, IVS-1 nt 5 (G-C), IVS-1 nt 1 (G-T), frameshift mutations FS 8/9 (+G), codon 41/42 (-CTTT) and nonsense codon 15 account for 90–94% of the beta-thalassemia mutations in India [34,37]. There are mutations which are less frequent but have been observed in the Indian people: codon 15 [G-A), codon 16 (-C), codon 30 (G-C), IVS-1-110 (G-A), -88 (C-T), CAP+1 (A-C), codon 5 (-CT), FS 41/42 (-CTTT), codon 88 (+T), 25 bp deletion, IVS-2 nt 1 (G-A) and IVS-1 minus 1 (G-A). These mutations have been observed in patients in homozygous, heterozygous or in combination with other mutations (in double heterozygote form). There are few rare mutations which have

either been detected in India or among the Indian people abroad but are yet uncharacterized.

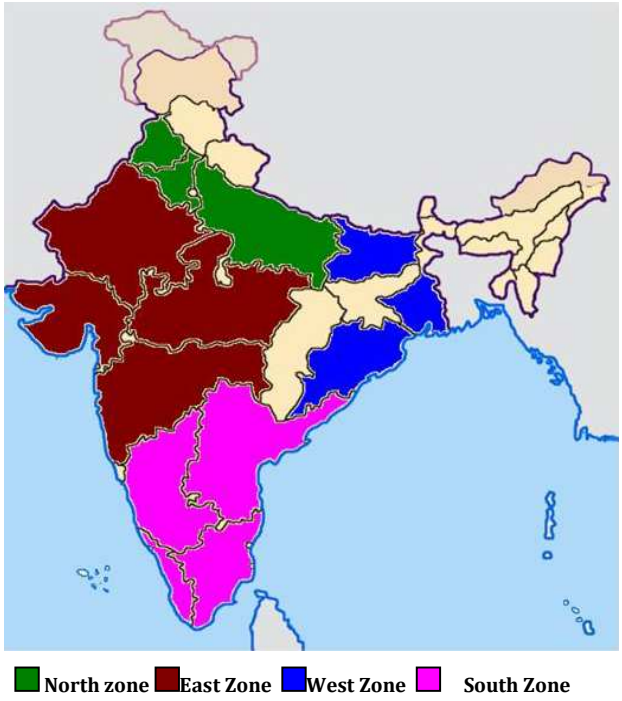

Fig. 3: Map showing distribution of beta thalassemia in India.

The prevalence of different mutations varies significantly in different regions of India. The IVS-1-5 mutation is the commonest mutation found in the Indian populations and its prevalence (in homozygous state) varies from 22.8 to 81.4% in different regions of India, being the highest in Tamil Nadu in south-eastern India. In the north-western part of India (including the states of Punjab, Haryana, Uttar Pradesh and Rajasthan, adjoining Delhi), the 619 bp deletion mutation is the commonest beta-thalassemia mutation observed in patients originating from Sindh, Gujarat or among the families migrated from Pakistan during partition of the country in 1947. Data have been reported from Punjab, Haryana and Rajasthan Madhya Pradesh, Bihar, Orissa, West Bengal and South India [46], Maharashtra and Gujrat [47], Uttar Pradesh [48] and Sindh (migrants) [49] (Table 3). The difference in prevalence of DNA mutations in beta-thalassemia from different regions of India reflects the ethnic and genetic diversity of populations in India. The heterogeneous populations belonging to the Indian subcontinent origin (Pakistan, Sindh, Punjab, Gujarat, Tamil Nadu, Maharashtra, West Bengal, Andhra Pradesh, and Kerala) were studied abroad by a number of investigators [38, 39, 50, 51].

Challenges

Thalassemias are a major health problem, and approximately 1 in 14 of the population are carriers for one of the sub types. Over the past three decades, regular blood transfusions and iron chelation have dramatically improved the quality of life and transformed thalassemia from a rapidly fatal disease in early childhood to a chronic disease compatible with prolonged life [52,53]. Today life expectancy varies between 25-55 years, depending on the compliance with medical treatment [54,55]. Despite increased life expectancy, complications keep arising.

The majority of mutations causing the thalassemias have now been characterized and a small number of common mutations cause the bulk of disease in each particular population-base. With little more than 6 to 8 common mutations probes over 90% of thalassemic patients can now be characterized but challenges remain in the 10% where the mutations are rare, or have not yet been determined [55]. Newer developments in micro array technology in combination with current PCR based systems will lead to further characterisation of this group and aid proper genetic potential identification and control of the disorder.

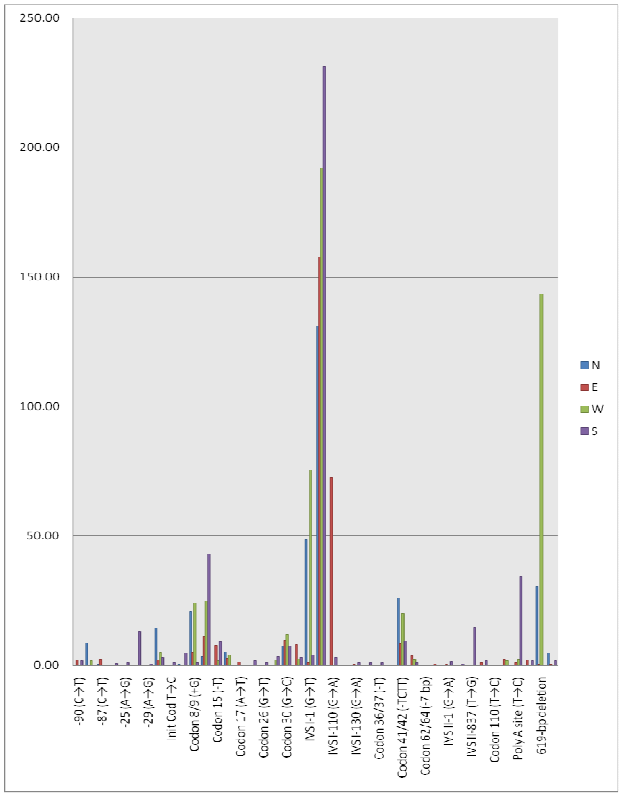

Fig. 4: Regional distribution of most common beta Thalassemia alleles in India.

Noninvasive prenatal diagnostic (NIPD) assay for β-thalassemia is developed recently. It is based on the detection of paternally inherited single nucleotide polymorphisms (SNPs) using the arrayed primer extension (APEX) method [47,56]. Mutation detection by microarray technology along with the low reagent costs and short processing time indicate a potential use of this technology for screening programs. For human gene therapy beta thalassemia represent more formidable problem because hemoglobin is the product of more than one gene, and its expression must be limited to a small sub-fraction of bone marrow cells, called the stem cells, which are the progenitors not only of erythrocytes but of granulocytes, macrophages and platelets. The prospect of curing such diseases holds great potential for alleviating human suffering in India. It is really a great challenge and needs an organized plan for action.

There is an urgent need for making the people aware of this lethal malady. Health education is an important component of the preventive genetic programs. This requires proper health education and adequate sensitization to the individual, family or community to accept these preventive remedial measures. High cost of treatment, repeated blood

transfusion and chelation therapy, and economic burden on family resources, all suggest that prevention is better than cure. Thus a joint venture of antenatal and inductive screening seems to be the most fruitful strategy for beta thalassemia in India. With improving environmental and socio-economic conditions, better public health care and medical facilities, effective malarial prophylaxis and better nutrition, children suffering from thalassemia and hemoglobinopathies can be better managed and rehabilitated in India [57-60].

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Table 3: Distribution of mutations causing beta thalassemia in India.

State	Andhra Pradesh	Karnataka	Kerala	Tamil Nadu	Bihar	Gujarat	Haryana	Madhya Pradesh	Maharashtra	Orrisa	Punjab	Rajasthan	West Bengal	Sindh (migrants)	Uttar Pradesh	Others	Total
N	136	118	67	212	111	586	24	65	748	47	26	34	529	322	626	42	3693
Mutation																	
-90 (C→T)	-	-	1.49	-	-	-	-	-	-	-	-	-	2.08	-	-	2.38	0.35
-88 (C→T)	-	-	-	-	-	-	4.17	1.54	-		3.85	-	-	-	0.5	-	0.08
-87 (C→T)	-	-	-	-	-	-	-	-	-	2.13	-	-	-	-	0.3	-	0.08
-80 (C→T)	0.74	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.02
-25 (A→G)	-	0.85	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.02
-28 (A→G)	1.47	2.54	-	8.96	-	-	-	-	-	-	-	-	-	-	-	-	0.64
-29 (A→G)	-	-	-	0.47	-	-	-	-	-	-	-	-	-	-	-	-	0.26
Cap site +1 (A→C)	-	-	2.99	-	1.8	2.2	-	1.54	1.3	-	11.54	-	-	-	2.6	-	0.83
Init Cod T→C	-	-	-	0.94	-	-	-	-	-	-	-	-	-	-	-	-	0.054
Codon 5 (-CT)	1.47	-	2.99	-	-	-	-	-	-	-	-	-	-	-	0.3	-	1.32
Codon 8/9 (+G)	-	.85	-	-	3.6	5	-	3.08	0.7	-	11.54	-	1.32	15.21	9.1	-	4.25
Codon 15 (G→A)	16.18	13.56	-	13.21	4.5	6.1	-	1.54	17.2	6.38	-	-	7.56	-	3.4	-	7.44
Codon 15 (-T)	-	-	-	-	-	-	-	1.54	-	-	-	-	0.95	-	-	-	1.13
Codon 16 (-C)	0.74	0.85	7.46	-	1.8	-	-	-	2.25	-	-	-	1.13	1.55	5.1	7.14	1.54
Codon 17 (A→T)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.38	0.02
Codon 22/23/24 (-7bp)	-	-	1.49	-	-	-	-	-	-	-	-	-	-	-	-	-	0.02
Codon 26 (G→T)	-	0.85	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.02
Codon 30 (G→A)	1.47	-	-	1.89	-	1.9	-	-	-	-	-	-	-	-	-	-	0.46
Codon 30 (G→C)	6.62	0.85	-	-	7.21	-	-	1.54	4.2	2.13	3.85	5.88	7.75	-	3.5	-	3.16
IVS I-1 (G→A)	-	2.54	-	0.47	-	-	-	1.54	0.9	-	-	-	-	-	-	-	0.56
IVS I-1 (G→T)	-	3.39	-	0.47	0.9	6.5	20.83	9.23	1.7	-	23.08	32.35	-	25.77	4.5	-	5.55
IVS I-5 (G→C)	66.18	45.76	62.7	56.6	74.77	48.6	45.83	53.9	66.5	83	34.62	17.64	72.4	5.59	50.5	71.4	57.32
IVS I-110 (G→A)	1.47	-	1.49	-	-	-	-	-	-	-	-	-	-	-	-	-	0.08
IVS I-129 (A→C)	-	-	-	-	-	-	-	-	-	-	-	-	0.19	-	-	-	0.02

IVS I-130 (G→A)	-	0.85	-	-	-	-	-	-	-	-	-	-	-	-	-	2.38	0.02
IVS I-130 (G→C)	-	-	-	0.94	-	-	-	-	-	-	-	-	-	-	-	-	0.05
Codon 36/37 (-T)	-	0.85	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.02
Codon 41 (-C)	-	-	-	-	-	-	-	-	-	-	-	-	0.38	-	-	4.76	0.10
Codon 41/42 (-TCTT)	0.74	1.69	4.48	2.36	3.6	6.9	4.17	6.15	2	4.26	11.54	2.94	3.59	2.17	10.2	4.76	4.41
Codon 44 (-C)	-	-	-	0.94	-	2.22	-	-	-	-	-	-	-	-	-	-	0.08
Codon 62/64 (-7 bp)	-	-	-	-	-	-	-	-	-	-	-	-	0.38	-	-	-	0.05
Codon 81/87 (-22 bp)	-	-	-	-	-	-	-	-	-	-	-	-	0.19	-	-	-	0.02
IVS II-1 (G→A)	-	0.85	-	0.47	-	-	-	-	-	-	-	-	-	-	-	-	0.29
IVS II-613 (C→T)	-	-	-	0.47	-	-	-	-	-	-	-	-	0.19	-	-	-	0.29
IVS II-837 (T→G)	-	7.63	5.97	0.94	-	-	-	-	-	-	-	-	-	-	-	-	0.40
Codon 106/107 (+G)	-	-	1.49	-	-	-	-	-	-	-	-	-	-	-	-	-	0.02
Codon 110 (T→C)	-	-	-	-	-	-	-	-	-	-	-	-	0.76	-	-	-	0.10
Codon 126-131 (-17bp)	-	-	-	-	-	-	-	1.54	-	-	-	-	-	-	-	-	0.02
Poly A site (T→C)	2.94	14.41	5.97	10.85	-	-	-	-	2.25	2.13	-	-	0.76	-	-	-	1.48
IVS I 25-bp deletion	-	-	1.47	-	-	-	-	-	-	-	-	-	-	-	-	-	8.58
619-bp deletion	-	-	-	-	1.8	22.22	25	16.9	13.48	-	-	41.17	0.19	49.68	5.4	4.76	8.58
Uncharacterized deletion	-	1.69	-	-	-	-	-	-	-	-	-	-	0.19	-	4.6	-	1.05

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