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RESEARCH ARTICLE



Spectrum of β -Thalassemia and Other Hemoglobinopathies in the Saurashtra Region of Gujarat, India: Analysis of a Large Population Screening Program

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ABSTRACT

Hemoglobinopathies are common genetic disorders of the hemoglobin (Hb) molecule. Globally, 7.0% of the population are carriers of thalassemia with 300,000–400,000 affected births each year. There are >40 million carriers of β -thalassemia (β -thal) in India with 10,000–12,000 affected births every year. This makes control programs crucial in this vast and diverse country. The present study was undertaken to find out the burden of hemoglobinopathies, and in particular, the prevalence of β -thal carriers in the population of Saurashtra region of Gujarat in Western India. A total of 16,780 individuals, including school and college students, were screened. Complete blood counts (CBCs) and high performance liquid chromatography (HPLC) analysis were performed. We detected 1891 (11.26%) individuals with different hemoglobinopathies, of whom 758 (4.52%) were diagnosed to carry β -thal trait, 104 (0.62%) carried Hb D-Punjab (*HBB*: c.364G>C) trait, 61 (0.36%) carried sickle cell trait, 32 (0.19%) carried $\delta\beta$ -thal trait/HPFH (hereditary persistence of fetal Hb) trait, and other hemoglobinopathies were identified in smaller numbers (0.15%). We encountered 27 individuals with mean corpuscular Hb (MCH) <27.0 pg and mean corpuscular volume (MCV) <80.0 fL levels, who had borderline Hb A₂ levels (3.2–3.5%). Twenty castes showed the presence of β -thal or other hemoglobinopathies. A high prevalence of β -thal was found in the Sindhis (11.67%), Lohanas (9.71%), Brahmins (6.31%), Bharvads (6.94%), Harijans (7.57%) and Vankars (7.77%). All the heterozygotes were given appropriate counseling. A multi pronged approach, including screening of high school and college students, needs to be considered for this vast and ethnically diverse country to reduce the burden of hemoglobinopathies.

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Awareness; β -thalassemia (β -thal) trait; hemoglobinopathies; mass screening

Introduction

The inherited disorders of hemoglobin (Hb) include the thalassemias, due to defective synthesis of α - or β -globin chains and the structural Hb variants due to amino acid substitutions in one of the globin chains. They are the most common monogenic disorders globally with an autosomal recessive inheritance and a major public health problem in many parts of the world including Mediterranean countries, the Middle East, Southeast Asia and the Indian Subcontinent region and North Africa. Around 7.0% of the world's population are carriers with 300,000–400,000 individuals with a potentially pathological hemoglobinopathy [1]. In India, it is estimated that there are over 40 million carriers of β -thal. Hb S (*HBB*: c.20A>T) is also prevalent in the tribal populations and some non tribal groups, and Hb E (*HBB*: c.79G>A) has a high incidence in the north eastern part of the country and in West Bengal in the east. It is estimated that there would be 1.2/1000 live births with a severe Hb disorder in India. The prevalence of β -thal carriers in many communities in Gujarat in western India is higher than the average of 3.0–4.0% in the country [2]. Control of hemoglobinopathies in India is a major problem due to ignorance about the disease, social,

cultural and religious taboos and family influences. Generation awareness, screening and counseling, followed by prenatal diagnosis (PND) is the only way to bring down the burden of these disorders. In this study, we describe our experience in screening a large population for β -thal and other hemoglobinopathies in the Saurashtra region of Gujarat.

Subjects and methods

After generating awareness in the population of the region, a total of 16,780 individuals (including school and college students) were screened over a period of 1 year (from December 2017 to November 2018). Blood samples (2 mL), were collected in EDTA-containing vacutainers, after informed consent was obtained. Red blood cell (RBC) indices were measured on a semi-automated three-part hematology counter. The Mentzer index [3] was calculated using the formula [mean corpuscular volume (MCV)/RBC]. A value of <13.000 was taken as a cutoff for suspected β -thal carriers [3]. The Shine and Lal index [4] was also calculated using the formula $MCV \times MCV \times MCH$ (mean corpuscular Hb)/100. A value of <1530.00 was the cut off-for suspected β -thal carriers. Hb F, Hb A₂ and other Hb variants were

quantitated by automated cation exchange high performance liquid chromatography (HPLC) on the VARIANT IITM analyzer (Bio-Rad Laboratories K.K., Tokyo, Japan). A cutoff of Hb A₂>3.5% was taken as a diagnostic level for β -thal trait. This study was approved by the Ethics Committee of the Institutional Review Board at Rajkot, Gujarat, India.

Results

Of the 16,780 individuals screened, 9303 (55.44%) were females and 7477 (44.56%) were males. The target population were school (48.86%) and college (51.14%) students, who were healthy young and asymptomatic. Table 1 shows the prevalence of β -thal trait and other hemoglobinopathies in the two screened groups.

Of the tested individuals, 14,889 (88.73%) were normal. One thousand, eight hundred and ninety-one (11.27%) had some type of hemoglobinopathies, of which 758 (4.52%) individuals had β -thal trait. Of the 9303 females, 412 (4.43%) had β -thal trait, while of the 7477 males, 346 (4.63%) had β -thal trait. One hundred and four individuals (0.62%) carried Hb D-Punjab (*HBB*: c.364G>C) trait and 61 (0.36%) had sickle cell trait. Thirty-two individuals (0.19%) had increased Hb F levels (5.0–30.0%) with reduced or normal RBCs, and they were grouped together as $\delta\beta$ -thal trait/hereditary persistence of fetal Hb (HPFH) trait; several other hemoglobinopathies were also identified in smaller numbers, such as Hb E trait, Hb D-Iran (*HBB*: c.67G>C) trait and Hb Q-India (*HBA1*: c.193G>C) trait, Hb J-Meerut [*HBA2*: c.362C>A (or *HBA1*)] trait, Hb A₂-India (also known as Hb A₂-Lepore) (*HBD*: c.350G>T) and coinheritance of some of these abnormalities with β -thal (Table 1) The overall prevalence of hemoglobinopathies was 981 (5.85%) inclusive of β -thal trait.

We encountered 27 individuals who had MCV <80.0 fL, MCH <27.0 pg and a borderline Hb A₂ level (3.2–3.5%). Their hematological parameters as well as the Mentzer index and Shine and Lal index are shown in Table 2. Most of these individuals had near normal Hb levels but a relatively high RBC count. Only five cases had a Mentzer index of <13.000, while 13 individuals had a Shine and Lal index of

<1530.00. These indices are considered to be predictive of β -thal trait.

The caste-wise distribution of β -thal and other hemoglobinopathies carriers is shown in Table 3. Twenty different caste groups showed the presence of β -thal trait in frequencies ranging from 1.72–11.67%. The prevalence of β -thal trait was highest in Sindhis (11.67%) followed by Lohanas (9.71%). The Brahmin, Bharvad, Harijan and Vankar communities also showed a high prevalence ranging from 6.31–7.77%. Hb D-Punjab and Hb S traits were seen in many communities, while $\delta\beta$ -thal trait/HPFH trait was more common in the Satvara caste (5.80%), and three of the five cases of Hb D-Iran trait were from the Rajput caste. There were 2192 individuals where either the caste was not known or the numbers were too few to be taken individually and they were grouped together as others.

Discussion

The β -thal syndromes pose a significant national health burden in India, with nearly 10.0% of the thalassemia patients across the world being born here [5]. Recent estimates have shown that treatment expenses of a patient with β -thal major (β -TM) in the country varies from US\$629.00–US\$2,300.00 annually, and very few patients are optimally managed [6].

Although many large screening programs have been reported from India, only a few are population screening programs, and many are hospital-based studies where anemic individuals or those with hypochromia and microcytosis have often been screened, or cases referred to a center are investigated who are not truly representative of the population [7–13]. In comparison to these, we were able to screen 16,780 young, healthy and asymptomatic individuals over one year and 100% of them were unrelated individuals.

The population of Gujarat is very heterogeneous and comprises of numerous castes and some tribal groups. Increasing awareness in communities in this state on the morbidity and mortality associated with the thalassemia syndromes and sickle cell disease has led to many screening and counseling programs in different regions in this state [8,9,14,15]. Micromapping has shown an uneven distribution of β -thal carriers in different regions of Gujarat (0.0–9.5%) with the highest prevalence in the Saurashtra region [16].

Cation exchange HPLC is a rapid, reproducible and precise technique that has been very valuable in the diagnosis of hemoglobinopathies. In our study, the overall prevalence of β -thal trait was 4.52%. This is comparable with other studies from northern and western and eastern India [7,12,17,18]. Several studies from different regions have shown a high prevalence of β -thal carriers in different populations varying from 0.0–17.0% in different communities [2]. We also found a much higher percentage of β -thal carriers in six castes/communities in the Saurashtra region.

The other significant hemoglobinopathies prevalent in India are Hb E, Hb S and Hb D-Punjab. Hb D-Punjab is seen mainly in north western India. Hb D-Punjab was the second most common structural variant in our study. It

Table 1. β -Thalassemia and other hemoglobinopathies identified in the two different groups of students screened.

Hemoglobinopathies	HGVS Nomenclature	School n (%)	College n (%)	Total n (%)
β -thal trait		373 (4.55)	385 (4.49)	758 (4.52)
Hb D-Punjab trait	<i>HBB</i> : c.364G>C	47 (0.57)	57 (0.66)	104 (0.62)
Hb S trait	<i>HBB</i> : c.20A>T	30 (0.37)	31 (0.36)	61 (0.36)
Hb D-Iran trait	<i>HBB</i> : c.67G>C	3 (0.037)	2 (0.023)	5 (0.029)
Hb E trait	<i>HBB</i> : c.79G>A	2 (0.024)	4 (0.023)	4 (0.023)
$\delta\beta$ -thal/HPFH trait		19 (0.23)	13 (0.15)	32 (0.19)
Uncharacterized Hb	–	1 (0.012)	4 (0.046)	5 (0.029)
Hb D-Punjab/ β -thal		2 (0.024)	0 (0.000)	2 (0.011)
Hb D-Punjab (hom.)		1 (0.012)	0 (0.000)	1 (0.005)
Hb Q-India trait	<i>HBD</i> : c.293G>C	3 (0.037)	2 (0.023)	5 (0.029)
Hb J-Meerut trait	<i>HBA2</i> : c.362C>A	0 (0.000)	1 (0.011)	1 (0.005)
Hb A ₂ -India ³ / β -thal		0 (0.000)	1 (0.011)	1 (0.005)
Hb S/ β -thal		0 (0.000)	1 (0.011)	1 (0.005)
Hb Q-India/ β -thal		0 (0.000)	1 (0.011)	1 (0.005)
Total		8198	8582	16780

HPFH: hereditary persistence of fetal hemoglobin; hom.: homozygous.

³Hb A₂-India (also known as Hb A₂-Lepore) (*HBD*: c.350G>T).

Table 2. Individuals with mean corpuscular volume, mean corpuscular hemoglobin and borderline Hb A₂ levels in school and college students.

Hb (g/dL)	RBC (10 ¹² /L)	PCV (L/L)	MCV (fL)	MCH (pg)	RDW (%)	Mentzer Index	Shine and Lal Index	Hb F (%)	Hb A ₂ (%)
12.5	4.70	0.36	77.9	26.6	12.4	16.574	1614.20	0.3	3.2
10.9	4.10	0.32	78.8	26.6	13.5	19.220	1651.71	0.2	3.5
15.1	5.73	0.44	76.4	26.4	13.8	13.333	1540.96	0.3	3.2
12.6	4.70	0.37	79.4	26.8	13.4	16.894	1689.67	0.5	3.5
12.4	4.66	0.35	76.0	26.6	14.9	16.309	1536.42	0.1	3.2
12.4	4.65	0.36	77.0	26.7	14.1	16.559	1583.04	0.2	3.4
14.4	5.56	0.44	78.4	25.9	16.2	14.101	1591.96	0.4	3.2
14.0	5.25	0.39	73.9	26.7	12.9	14.076	1458.14	0.4	3.4
12.4	4.78	0.37	78.2	25.9	14.8	16.460	1583.85	0.2	3.2
13.5	5.23	0.41	77.8	25.8	14.9	14.876	1561.63	0.3	3.3
13.4	4.99	0.39	78.2	26.9	15.1	15.671	1645.00	0.2	3.2
13.3	4.98	0.37	75.3	26.7	12.8	15.120	1513.91	0.2	3.3
13.3	5.64	0.38	67.2	23.6	13.8	11.915	1065.74	0.1	3.3
13.4	5.12	0.39	76.2	26.2	15.2	14.883	1521.29	0.3	3.3
14.2	5.31	0.39	74.4	26.7	14.4	14.011	1477.94	0.2	3.2
12.2	4.53	0.36	79.2	26.9	12.6	17.483	1687.34	0.7	3.2
12.2	4.79	0.37	77.5	25.5	12.5	16.180	1531.59	0.2	3.2
13.6	5.24	0.41	78.2	26.0	13.6	14.924	1589.96	0.2	3.3
12.0	5.56	0.37	66.5	21.6	16.3	11.960	955.21	0.4	3.5
15.1	5.90	0.44	75.4	25.6	13.2	12.780	1455.30	0.2	3.2
11.4	4.66	0.33	71.0	24.5	14.1	15.236	1235.05	0.2	3.2
8.1	4.94	0.28	56.9	16.4	24.3	11.518	530.97	0.1	3.2
14.4	5.45	0.40	74.3	26.4	12.5	13.633	1457.41	0.2	3.2
12.3	5.34	0.39	73.0	23.0	16.3	13.670	1225.67	0.3	3.2
11.8	4.82	0.37	76.8	24.5	18.9	15.934	1445.07	0.2	3.3
12.9	4.87	0.39	79.3	26.5	11.4	16.283	1666.45	1.1	3.3
15.9	6.28	0.47	75.6	25.3	13.5	12.038	1445.99	0.4	3.2

Hb: hemoglobin; RBC: red blood cell count; PCV: packed cell volume; MCV: mean corpuscular volume; MCH: mean corpuscular Hb; RDW: red blood cell distribution width.

Table 3. Caste-wise distribution of β -thalassemia trait and other hemoglobinopathies.

Caste	Total Tested	β -Thal n (%)	Hb D-Punjab n (%)	Hb S n (%)	$\delta\beta$ -Thal/ HPFH n (%)	Hb Q-India n (%)	Hb E n (%)	Hb D-Iran n (%)	Others ^a n (%)	Total Hbs (n = 376)
Patel	7834	327 (4.12)	34 (0.43)	9 (0.11)	4 (0.05)	1 (0.01)	–	–	1 (0.01)	376
Koli	1117	29 (2.60)	1 (0.09)	5 (0.45)	1 (0.09)	–	–	–	1 (0.09)	37
Brahmin	586	37 (6.31)	9 (1.54)	1 (0.17)	2 (0.34)	–	–	–	–	49
Ahir	610	26 (4.26)	9 (1.48)	11 (1.80)	2 (0.33)	–	1 (0.16)	–	–	49
Rajput	514	22 (4.28)	5 (0.97)	2 (0.39)	2 (0.39)	–	–	3 (0.58)	2 (0.39)	36
Kumbhar	513	27 (5.26)	2 (0.39)	–	–	–	–	–	2 (0.39)	31
Vankar	489	38 (7.77)	8 (1.64)	2 (0.41)	–	–	–	–	–	48
Kadiya	330	19 (5.76)	3 (0.91)	–	–	–	–	–	–	22
Lohana	350	34 (9.71)	6 (1.71)	2 (0.57)	–	1 (0.29)	–	–	2 (0.57)	45
Harijan	317	24 (7.57)	7 (2.21)	3 (0.95)	1 (0.32)	–	–	–	1 (0.32)	36
Satvara	276	7 (2.54)	–	1 (0.36)	16 (5.80)	–	–	–	–	24
Bavaji	220	10 (4.55)	–	2 (0.91)	–	–	–	–	–	12
Sindhi	180	21 (11.67)	6 (3.33)	1 (0.56)	–	2 (1.11)	–	–	1 (0.56)	31
Valand	187	10 (5.35)	–	–	–	–	–	–	–	10
Darbar	169	6 (3.55)	–	2 (1.18)	–	–	–	1 (0.59)	–	9
Suthar	174	3 (1.72)	1 (0.57)	1 (0.57)	–	–	–	–	–	5
Luhar	175	6 (3.43)	1 (0.57)	–	–	–	–	–	–	7
Darji	162	4 (2.47)	–	–	1 (0.62)	–	–	–	–	5
Soni	141	4 (2.84)	2 (1.42)	–	–	–	–	–	–	6
Bharvad	144	10 (6.94)	2 (1.39)	–	–	–	–	–	–	12
Other	2192	94 (4.29)	8 (0.36)	19 (0.87)	3 (0.14)	1 (0.05)	3 (0.14)	1 (0.05)	2 (0.09)	131
Total	16,780	758	104	61	32	5	4	5	12	981

^aOthers: Hb E trait, uncharacterized Hb variant; Hb D-Punjab/ β -thal; Hb S/ β -thal; Hb Q-India/ β -thal; Hb J-Meerut trait; homozygous Hb D-Punjab; Hb A₂-India/ β -thal.

presented as an asymptomatic heterozygous condition with normal hematological parameters. Of 104 cases, 92 had Hb levels of >12.0 g/dL. In a study from Surat in south Gujarat, 11 cases of Hb D-Punjab were reported (0.36%) [8]. In our study from Saurashtra, the prevalence of Hb D-Punjab was much higher (0.62%). Hb D-Punjab occurs with great prevalence in the Sikhs in Punjab (2.0%) [19]. Hb D-Punjab is the fourth most frequently occurring Hb variant in the world.

Sickle cell anemia is prevalent mainly in tribal populations, scheduled castes and other backward classes in India [20]. It has been documented as the most common hemoglobinopathy in the state of Orissa with very high prevalence of sickle cell carriers (29.8%) [21]. Hb S trait was found in 0.36% individuals and was the third most predominant abnormality in our study.

Both $\delta\beta$ -thal and HPFH traits are associated with increased Hb F levels in adult life. The MCV and MCH are

reduced in $\delta\beta$ -thal carriers and are generally near normal in HPFH carriers. However, due to overlapping iron deficiency, which is quite common in India, it is often difficult to differentiate between the two conditions. We therefore included both these conditions in the same group for analysis and found a prevalence of 0.19%. However, in the Vadodra region of Gujarat, HPFH trait was more commonly encountered (2.94%) [7].

The 27 individuals who had reduced MCV and MCH and borderline Hb A₂ levels are not likely to be cases of iron deficiency as they all had relatively high RBC counts in relation to their Hb levels. In the absence of molecular analysis, it is difficult to accurately diagnose them. The Mentzer index predicted five of them to be β -thal carriers, while the Shine and Lal index showed that 13 of them could possibly be carriers of β -thal. Neither of these discriminant functions are specific but the Mentzer index has earlier been shown to have the best discriminatory power [22], while the Shine and Lal index had the highest sensitivity [23]. Rosnah *et al.* [24] showed that 38.5% of their borderline Hb A₂ cases had a molecular defect. In this study, they had defined borderline Hb A₂ values as 3.0–3.9%. Another study from a referral center in Mumbai [25], showed 131 β -thal carriers with borderline or normal Hb A₂ levels. Some of these cases had reduced MCV and MCH values, while others had normal red cell indices but a β -thal mutation was present in all of them [25].

We identified five cases of Hb Q-India trait and one case of Hb Q-India with β -thal trait. The prevalence of Hb Q-India in India is 0.4%, found predominantly in Sindhi families and in individuals from western and northern India [26]. However, in our study we found only 0.030% prevalence of Hb Q-India.

Hb E is the most common Hb variant in Southeast Asia and the second most prevalent variant worldwide [27]. We encountered only four Hb E heterozygotes in the Saurashtra region (0.023%), while Hb D-Iran is very rare in India in either heterozygous or homozygous states [28]. Fifteen cases of Hb D-Iran trait and five cases of Hb D-Iran/ β -thal have been reported from Mumbai in a series of Hb abnormalities encountered at a referral center over 15 years [25]. We found five unrelated individuals with Hb D-Iran trait with three of them being of the Rajput caste.

The wide spectrum of hemoglobinopathies seen in the present study also reflects migration of people from one region to another for a better livelihood. Thus screening and counseling should be extended to all states of the country as well as different regions within each state.

Conclusions

The importance of screening programs for Hb disorders in countries with high prevalence cannot be overemphasized. In India, where β -thal trait is so rampant, premarital and prenatal screening should be encouraged to prevent the birth of babies with β -TM, sickle cell disease or other severe compound heterozygous conditions such as Hb E/ β -thal or Hb S/ β -thal. Moreover, knowledge of the common

hemoglobinopathies in a particular region helps to formulate appropriate preventive and therapeutic strategies.

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Disclosure statement

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