

# Study of prevalence of hemoglobinopathy in Sindhi community of Jamnagar, Gujarat: A pilot study

## Abstract

**Introduction:** Hemoglobinopathies are the most common hereditary disorders in India and pose a major health problem. A pilot study is carried out to find the prevalence of hemoglobinopathies in the Sindhi community of Jamnagar, district of Gujarat, India. **Materials and Methods:** The present cross-sectional study was undertaken among 109, apparently healthy Sindhi individual from community in Jamnagar, district of Gujarat, India from January to February 2015. All the samples were tested in automated hematology cell counter and cellulose acetate electrophoresis at alkaline pH. Hemoglobin and various red blood cell indices were derived on all samples. HbA2 level of  $\geq 3.8\%$  was taken as confirmatory of Beta-thalassemia trait (BTT). **Result:** Of 109 samples, BTT and sickle cell trait (SCT) were more common in 12–21 years of male and female, respectively. The overall prevalence of hemoglobinopathy was 21/109 (19.26%), comprised of 17 (15.59%) cases of BTT and 4 (3.66%) cases of SCT. **Conclusion:** Sindhi community has a high prevalence of hemoglobinopathy mainly BTT. Mass awareness and community screening of individual and proper counseling should be done to reduce the burden of disease.

**Key words:** Automated hematology cell counter, beta-thalassemia trait, cellulose acetate electrophoresis, sickle cell trait

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## INTRODUCTION

Hemoglobinopathy is characterized by a genetic defect that results in the abnormal structure of one of the globins of the hemoglobin (Hb) molecule. These inherited hemoglobinopathies are controlled by a single gene and are transmitted to progeny.

The carriers of Hb disorders in the world are estimated to be 269 million.<sup>[1]</sup> About 3% of the world's population (150 million people) carry beta-thalassemia genes. In India, beta-thalassemia is the most common autosomal recessively inherited monogenic disorder with approximately 30 million carrying the defective gene, with carrier frequency ranging from 3% to 17%.

As compared to other Indian states, hemoglobinopathy is more common in Gujarat. Modell and Petrou have estimated 12% incidence of major hemoglobinopathy traits in Gujarat.<sup>[2]</sup> Some ethnic groups like Sindhis, Kutchis, Lohanas, Punjabis, few Muslim groups as well as few tribal population have a higher prevalence of beta-thalassemia carriers (5-17%).<sup>[3,4]</sup>

We have observed many cases of hemoglobinopathy among the Sindhi community of Jamnagar. Thus, the Pilot study is carried out in Sindhi community, who had migrated to India from Sindh province of Pakistan at the time of partition, to measure the approximate prevalence of the beta-thalassemia trait (BTT), sickle cell trait (SCT), and incidence of anemia among trait. This study provides a base to calculate the sample size in Sindhi community for the final study of hemoglobinopathies in future.

## MATERIALS AND METHODS

A pilot study was carried out in Sindhi individual to estimate the approximate prevalence of BTT in Jamnagar, a district, located in the Western part of Gujarat, a state in India from January 2015

to February 2015. Due to the availability of limited resources, feasibility, we have enrolled 109 subjects in this pilot study. We have tried to minimize selection bias. This study was done in the city area of Jamnagar, also planned similar study in the rural area. Selection criteria for the study are apparent healthy individual from Sindhi community residing in Jamnagar city. Exclusion criteria include community other than Sindhi, person residing outside Jamnagar city, diagnosed case of thalassemia major or sickle-cell disease. An informed consent was taken from parents of children and adolescents of <18 years and individuals before inducting in the study.

The information like name, age, sex, address, phone number, previous transfusion history, sibling history, consanguinity, any family history of blood disorders, etc., was filled in pretest pro forma.

Three milliliter of blood sample was collected in ethylene diamine tetra acetic acid tubes. Complete blood count was measured on the Sysmex KX-21 fully automated hematology cell analyzer to measure Hb g/dl and red blood indices like mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH). Due to low sensitivity and specificity, screening naked eye single tube red cell osmotic fragility test was not done. Anemia is defined as Hb value in male and nonpregnant female below 13 g/dl and 12 g/dl, respectively.<sup>[5]</sup>

The electrophoresis was done on cellulose acetate strip at alkaline pH 8.4 using Tris-buffer by Interlab Genios electrophoresis machine in all the cases. HbA<sub>2</sub>, HbF, and HbS were estimated by graphs obtained from densitometry of Hb pattern. Mean cell hemoglobin (MCH) is <27 pg, HbA<sub>2</sub> level of ≥3.8% indicates heterozygosity for beta-thalassemia (BTT). HbA<sub>2</sub> value between 3.5% and 3.7% was considered as borderline HbA<sub>2</sub>, subjected to repeat testing from same and fresh sample.<sup>[6]</sup>

High-performance liquid chromatography (HPLC) on Bio-Rad Hb variant system was done on samples having borderline HbA<sub>2</sub> value (3.5-3.7%). For all cases showing band in S region in cellulose acetate electrophoresis were subjected for sickle solubility test. Sickle cell Hb is insoluble in the deoxygenated state in a high molarity phosphate buffer. The crystals that form refract light and cause the solution to be turbid. Positive solubility test merely indicates the presence of sickling Hb. Further HPLC was carried out to

separate HbS from HbD and HbG and to confirm the presence of HbS. The data were collected and analyzed using Microsoft Excel software program. Abnormal results were communicated to the individual and counseling about lethal nature of the disease, nature of inheritance was done.

## RESULT

We have divided all samples in premarital (12-21 years) and marital or childbearing (22-32 years) age groups, as both age groups need a different type of sensitization and counseling. There were no subjects <12 years or >32 years of age. Of 109 samples, 84 cases were between 12 and 21 years and 25 cases were between 22 and 32 years. 56 and 53 cases were from male and female, respectively. BTT is more common in 12-21 years of male. SCT is more common in 12-21 years of female.

Of 109 samples, 13 samples have HbA<sub>2</sub> ≥3.8%, and four samples have borderline HbA<sub>2</sub> 3.5-3.7%. Repeat testing by electrophoresis and further testing by HPLC, these four samples have HbA<sub>2</sub> ≥3.8%, confirm the diagnosis of BTT. The overall prevalence of hemoglobinopathies was 21/109 (19.26%), comprised of 17 (51.59%) cases of BTT and 4 (3.66%) cases of SCT [Table 1].

Eight and 6 cases of BTT with anemia were diagnosed in male and female, respectively. Two cases of SCT with anemia were diagnosed in female [Table 2].

All 4 cases showing band in S region in cellulose acetate electrophoresis were positive for sickle solubility test. Confirmation was done by HPLC shows band of HbS.

In BTT, mean MCH was 20.15 pg/cell and 21.15 pg/cell in the male and female, respectively. Mean MCV/red blood cell (Mentzer index) was 10.83 and 12.98 in the male and female, respectively, which also support the diagnosis of BTT [Table 3].

## DISCUSSION

Hemoglobinopathy is a hematologic disorder due to alteration in the genetically determined molecular structure of Hb. The India has an extreme diverse population of 1.21 billion. It comprises numerous castes, sub castes and tribal groups in the different geographic

**Table 1: Age and gender distribution of hemoglobinopathies**

Parameters	Total number	BTT		SCT	Negative for hemoglobinopathy
		HbA <sub>2</sub> ≥3.8 (%)	HbA <sub>2</sub> 3.5-3.7 (%)		
<b>Age</b>					
12-21	84	9	3	3	69
22-32	25	4	1	1	19
Total (%)	109	13 (11.95)	4 (3.66)	4 (3.66)	88 (80.73)
<b>Gender</b>					
Male	56	8	3	1	44
Female	53	5	1	3	44
Total (%)	109	13 (11.92)	4 (3.66)	4 (3.66)	88 (80.73)

BTT = Beta-thalassemia trait, SCT = Sickle cell trait

region, each revealing different genetic traits and reservoir of hemoglobinopathies. Due to large scale migration of population and marriage across ethnic groups, the prevalence of hemoglobinopathy is quite variable.

It is estimated that about 100,000 children with transfusion-dependent thalassemia are born worldwide annually. About 8000-10,000 children with thalassemia major disease are born in India every year.<sup>[7]</sup> It is seen with highest frequency in North-West and far East of India. Several studies in the literature have reported that Gujarat has a higher frequency of beta-thalassemia and sickle cell disease.<sup>[8-11]</sup>

In our pilot study, the overall prevalence of hemoglobinopathies in Sindhi community is 19.26%. Prevalence of BTT is 15.59%, which is an accordance with other study done by Sukumaran (12.2%), Rakholia and Chaturvedi (17.2%), Mulchandani *et al.* (16.81%), Patel *et al.* (10.2%), Pande *et al.* (20.7%)<sup>[11-15]</sup> [Table 4].

**Table 2: Gender and hemoglobin level distribution**

Gender	Hemoglobin (g/dl)	Total number	BTT	SCT
Male	<10	1	0	0
	10-12.9	26	8	0
	≥13	29	2	1
Female	<10	4	0	1
	10-11.9	19	6	1
	≥12	30	1	1

BTT = Beta-thalassemia trait, SCT = Sickle cell trait

**Table 3: Gender distribution of mean hemoglobin, MCV, MCH and RBC count in hemoglobinopathies**

Parameters	Hemoglobin (g/dl)	MCV (fl)	MCH (pg/cell)	RBC (million/cumm)	MCV/RBC (Mentzer index)
BTT					
Male	11.97	65.17	20.15	6.014	10.83
Female	11.18	69.35	21.15	5.34	12.98
SCT					
Male	14.5	80.8	25.5	5.68	
Female	9.96	80.9	27.3	3.6	

BTT = Beta-thalassemia trait, SCT = Sickle cell trait, MCV = Mean corpuscular volume, MCH = Mean corpuscular hemoglobin, RBC = Red blood cell

**Table 4: Result of prevalence studies in sindhi community**

Study conducted by	Number tested	Prevalence of BTT (%)	State, region
Sukumaran <i>et al.</i>	82	12.2	Ulhasnagar, Maharashtra
Rakholia and Chaturvedi	550	17.2	Wardha, Maharashtra
Mulchandani <i>et al.</i>	446	16.81	Nagpur, Maharashtra
Patel <i>et al.</i>	127	10.2	South Gujarat
Pande <i>et al.</i>	511	20.7	Jabalpur, Madhya Pradesh

BTT = Beta-thalassemia trait

Prevalence of SCT is 3.66%. All four cases were confirmed by HPLC Bio-Rad system as SCT. In cellulose acetate electrophoresis, HbS migrates along with HbD and HbG. Hence, it is necessary to confirm the diagnosis by sickle solubility test and HPLC.

In the present study, out of 21 cases of hemoglobinopathies, 16 cases were anemic comprised of 8 cases of male and female, respectively. In our study, anemia was not treated before performing HbA2 estimation.

Severe iron deficiency anemia (Hb <8 g/dl) can reduce the HbA2 level by up to 0.5%. Anemia is to be treated, and the Hb analysis repeated when the patient is iron replete.<sup>[16]</sup>

Iron deficiency anemia is commonly misdiagnosed in BTT and SCT subjects. Inadvertent iron treatment may harm the patient and physician should advise estimation of HbA2 to diagnose BTT in high-risk community.

An increased HbA2 level (≥3.8%) along with MCH <27 Pg is the hallmark of heterozygosity of BTT. HbA2 level <3.7% probably due to silent mutation, co-inheritance of  $\delta$  and  $\beta$ -thalassemia, some mild  $\beta$ -gene mutations,  $\gamma\delta\beta$ -thalassemia, HbH disease, severe iron deficiency, erythroleukemia, and sideroblastic anemia should be repeated on HPLC and may be analyzed by molecular methods.<sup>[17]</sup>

Artifact in the presence of HbS, hypothyroidism, megaloblastic anemia, patient on anti-retroviral therapy and hypertrophic osteoarthropathy may cause elevated HbA2 value.<sup>[17]</sup> These preanalytic factor is important while interpreting elevated HbA2%.

With best of our Knowledge as this is a first study conducted in this community in Jamnagar city. One of the limitations of this study is small sample size, so that could affect the prevalence of hemoglobinopathy. There is a need for screening of whole population by calculating sample size from the pilot study in high-risk Sindhi community in near future.

Abnormal results are explained, and genetic counseling was done to individual affected. Those unmarried individual, explained regarding inheritance of disease, and the need to prevent marriage to traits. Those married individual explained regarding prenatal diagnosis, nature of inheritance, and selective termination of pregnancy of the affected fetus and preconceptional counseling for future.

## CONCLUSION

Sindhi community has higher prevalence of hemoglobinopathy, mainly BTT. The most effective approach to minimize the problem of hemoglobinopathies in India is to sensitization and screening of individual, genetic counseling, proper health education regarding the nature of inheritance and prenatal diagnosis in a high-risk community. More efforts are needed to increase awareness in high-risk communities like Sindhi regarding thalassemia before the dream to control hemoglobinopathy in India.

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