

# Prevalence of Haemoglobinopathy among Young College Students in Anand-Gujarat: A Premarital Screening Program for Carrier Detection of Hemoglobin Disorders

Minal Thakkar<sup>1</sup>, Hitesh Shah<sup>2</sup>, Utkarsh Shah<sup>3</sup>,  
Prakashbhai Parmar<sup>4</sup>, Sulabhsinh Solanki<sup>5</sup>

<sup>1</sup>Assistant Professor, Shree P M Patel College of Paramedical Science & Technology, Anand People's Medicare Society (APMS), Sardar Patel University, Opposite New Bus Station, Anand, <sup>2</sup>Professor, Department of Biochemistry, Shree Krishna Hospital & Pramukhswami Medical College, Bhaikaka University, Karamsad, <sup>3</sup>Associate Professor, Department of Community Medicine, Shree Krishna Hospital & Pramukhswami Medical College, Bhaikaka University, Karamsad, <sup>4</sup>General Secretary, Indian Red Cross Society, Gujarat State Branch, Ahmadabad, <sup>5</sup>I/C Principal and Associate Professor, CAM Institute of Allied Health Sciences and Technology & L.P.Patel Institute of Medical Laboratory Technology, Bhaikaka University, Karamsad.

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## Abstract

**Background:** Hemoglobin disorders are the leading health concern in the World including India. Effective screening programs, awareness campaign and proper prenatal diagnosis are the only ways to eradicate the disease. Very sparse data are available on the spectrum of haemoglobinopathies in the central part of Gujarat state. Hence, this study was undertaken to find out the prevalence of haemoglobinopathies among the students of Anand People's Medicare Society, Anand District, Gujarat, India.

**Methods:** In this prospective study, total 2195 students were screened for haemoglobinopathies after taking clinical and familial history. A complete hemogram report was obtained by an automated hematology counter and hemoglobin variants were quantitated by performing HPLC on Bio-Rad Variant II. The prevalence of hemoglobinopathies was 7.06%, which includes  $\beta$ -thalassemia trait (2.73 %), sickle cell trait (3.82%), homozygous sickle cell disease (0.09%), Hb D trait (0.22%), Hb E (0.09%) trait and other haemoglobinopathies (0.08%).

**Conclusion:** Population groups with high gene frequency of haemoglobinopathies requires a routine premarital screening program, awareness and education for identification, prevention of high-risk marriages and birth of thalassemic homozygotes.

**Keywords:** Haemoglobinopathies, Sickle cell anemia, Thalassemia, Premarital Screening

## Introduction

Haemoglobinopathies are severe, autosomal recessive haemoglobin disorders, if right time

identification and treatment is not provided, can be associated with high mortality and morbidity. [1] Haemoglobinopathies can be classified into three groups: hereditary persistence of fetal haemoglobin,

reduce in the number of amino acids in globin chain synthesis-Thalassemia, abnormal synthesis of haemoglobin with deletion, substitution of one or more amino acid in globin chain.<sup>[2]</sup>

The clinical spectrum of the disorders varies from asymptomatic conditions to serious disorders like thalassemia major that requires regular blood transfusions and extensive medical care. Most of children have a severe clinical presentation but are managed sub-optimally due to lack of financial resources in majority of the families. Thus, preventing the birth of affected children is the best option for India. A prerequisite for this is the knowledge of the prevalence of  $\beta$ -thalassemia and other haemoglobinopathies in different regions of the country and in particular in different ethnic groups. The best course of action for India is to understand the incidence of  $\beta$ -thalassemia and other haemoglobinopathies in various parts of the nation, particularly in various ethnic groups to prevent the birth of impacted children.<sup>[3]</sup>

The World Health Organization (WHO) estimated that 7% of people are carriers of a haemoglobin abnormality.<sup>[4]</sup>Prevalence of  $\beta$ -thalassemia carrier by community examining found as high as 17% in certain population in India because of consanguinity, caste and area endogamy.<sup>[4,5]</sup>According to Modell and Petrou et al., 12% of actual haemoglobinopathies features attributes in Gujarat whereas 4% of haemoglobinopathies are prevalent in Maharashtra and 37.18% are prevalent in Odisha.<sup>[6,7]</sup> The prevalence of HbE is 7.5% in Uttar Pradesh, while in the upper Assam region of north-eastern India has prevalence as high as 47.94% and is very less in Gujarat (0.23%).<sup>[8-10]</sup>

Screening programs should aim to identify asymptomatic carriers of haemoglobin disorders in young population to assess the risk of having children with severe form of disease. In countries with high prevalence of haemoglobinopathies, premarital screening programs are essential for the identification and prevention of high-risk marriages.<sup>[11]</sup>

The main objective of our study was to find the prevalence of haemoglobinopathies among the students of Anand People's Medicare Society (APMS) located in Anand District, Gujarat, India

and estimating the number of students who would benefit from such program.

## Material and Methods

The present study was cross-sectional voluntary screening program. This study included total 2,195 students of Anand People's Medicare Society (APMS), Anand for screening of haemoglobin disorders from August, 2019 to April, 2021 at Indian red cross society, Gujarat State branch, Ahmedabad. Indian Red Cross Society has charged 150/-per student as screening token charge from all students participated in study. Awareness about haemoglobinopathies was provided to all students by pre counselling.

### • Inclusion Criteria:

1. All undergraduate and postgraduate students of Anand people's Medicare Society, Anand. 2] Age group involved ranged from 18 to 30 years.

### • Exclusion Criteria:

1. Already screened and referred to instance of haemoglobinopathies as screening test costs will be paid by understudies.

The study populations included an undifferentiated mixture of urban and rural undergraduate and postgraduate students of the age group of 18 years to 30 years. Students who already have undergone for hemoglobinopathy screening program were excluded from the study (should we include it above in exclusion criteria?). Educational talks, audiovisual presentations were provided and posters displayed for awareness generation before taking an informed consent for testing. A well-designed proforma was used to get information on the caste/ethnic group, linguistic group and religion, consanguinity in the family, any family history of blood disorders as well as to record all the laboratory findings.

**Sample Collection:** A 2-ml intravenous blood sample was collected in EDTA according to practical manual of Dacie.<sup>[12]</sup> RBC indices was measured on a haematology counter (Sysmex K4500), HbA<sub>2</sub>, HbF and other haemoglobin variants were quantitated by HPLC using the Variant Haemoglobin Testing System (Biorad Variant II) as described in the instruction manual. HbA<sub>2</sub> level of >4.0 % was used as a cut off for diagnosis of  $\beta$ -thalassemia carriers.

### Statistical analysis:

The 95 % confidence interval was calculated. The chi-square test was used to compare the distribution of various alleles causing haemoglobinopathies in Anand.

### Results

Total 2,195 students were screened for hemoglobinopathies, among them 1066 (48.5%) were male and 1129 (51.4%) were female students. No student has positive history of blood transfusion or any major illness in the past.

Of the 2,195 individuals, 155 (7.06%) students were found to be carriers of various haemoglobinopathies and 2040 (92.9%) students had normal haemoglobin pattern on HPLC. The prevalence of different haemoglobinopathies among the students shows that sickle cell trait (with co-existence / co-inheritance of one or more Alpha thalassemia gene) was the commonest haemoglobinopathy followed by typical thalassemia, sickle cell trait, Hb D Punjab trait, atypical thalassemia, Haemoglobin E trait, Double heterozygous for sickle cell anemia, Delta Beta Thalassemia trait and Hereditary persistence of fetal haemoglobin. Thus, the overall prevalence of haemoglobinopathies was 7.06% in the present study.

Table 1 shows the haematological parameters in different group of haemoglobinopathies that can differentiate the anemia in to mild, moderate and severe form. The value of haemoglobin in different haemoglobinopathies varied among the different

categories of subjects. This difference was statistically significant ( $P = 0.002$ ). Table 1 shows interpretation of haemoglobinopathies with respect to haemoglobin (g/dl) level. Out of total 155 carrier cases, maximum diagnosed cases except double heterozygous for sickle cell anemia and Hb E trait had hemoglobin level above 12 g/dl, followed by double heterozygous for sickle cell anemia and Hb E trait had hemoglobin level in between 8-12 g/dl. (Hb significantly falls in these cases).

MCV and MCH were decreased in Hb E, Double Heterozygous anemia, Hb sickle c/o and in typical thalassemia whereas it was normal in sickle cell trait, atypical beta thalassemia and Hb D Punjab. In Hb S, Hb D and in atypical thalassemia showed normal MCH values while in others it was decreased. RBC count value in all haemoglobinopathies was within the normal range. (Table 2)

HbS ranged between 36 to 38 % (on chromatogram) in the Sickle cell trait, which is less than HbA value. In the cases of Sickle cell trait (with co-existence / co-inheritance of one or more alpha thalassemia gene), Hb S was >24% with HbA2 level 3.5-7% and Hb F <1%. In typical Beta thalassemia and atypical Beta thalassemia, HbA2 level was >3.5% with Hb F <1%. In cases of double heterozygous for sickle cell anemia, HbS was >84.6%, HbA2 was ranging from 3.9 % and HbF 8.10%. Students with HbD Punjab heterozygous stated had unknown peak at D window and HbD was ranged between 32.50%. Students with HbE showed raised peak in A2 region i.e.28.30%. (Table: 3).

**Table: 1 Haemoglobin value in different group of haemoglobinopathies**

Haemoglobin value (gm/dl)	Sickle cell trait (with co-existence / co-inheritance of one or more Alpha thalassemia gene),N	Sickle cell trait, N	Double heterozygous for sickle cell anemia, N	Typical Beta Thalassemia minor, N	Atypical Beta Thalassemia minor, N	Hb D Punjab trait, N	Hb E trait, N	Hereditary persistence of fetal haemoglobin, N	Delta Beta Thalassemia trait, N	Grand Total, N(%)
> 12 gm/dl	48	10	0	28	4	5	0	1	1	97(62.5%)
Mild (12-10) gm/dl	21	0	0	21	0	0	1	0	0	43(27.7%)
Moderate (10-8) gm/dl	3	0	2	7	0	0	1	0	0	13(8.4%)
< 8 gm/dl	2	0	0	0	0	0	0	0	0	2(1.4%)
Grand Total	74	10	02	56	04	05	02	01	01	155(100%)

$\chi^2 = 48.48$ , d.f. = 24,  $P = 0.002$

**Table: 2 Haematological parameters in different group of haemoglobinopathies**

Haemoglobinopathies	Hb (g/dl) mean±S D	RBC count (million/ cmm) mean ±SD	MCV (fl) mean ± SD	MCH (pg) mean ± SD
Sickle cell trait (with co-existence / co-inheritance of one or more Alpha thalassemia gene) (74)	13.2±2.1	5.0±0.9	71.8±8.7	23.1±3.5
Sickle cell trait (10)	13.7±0.9	4.1±0.9	88.5±6.4	29±3.2
Double heterozygous for sickle cell anemia (02)	9±0.9	4.2±0.3	67.5±2.5	21.4±0.2
Typical Beta Thalassemia minor (56)	11.9±1.6	5.7±0.9	65.5±5.9	21.2±2.7
Atypical Beta Thalassemia minor (04)	14.8±0.8	4.9±0.3	88±4.3	29.9±1.1
Hb D Punjab trait (05)	14.3±0.8	4.9±0.2	86.6±3.2	29.2±1.2
Hb E trait (02)	10.7±0.8	4.6±0.1	70±5	23.1±2.5

**Table 3: shows HPLC results in different haemoglobinopathies.**

Haemoglobinopathies (N)	HbA2			Hb F			HbS			Hb D
	<3.5%	3.5-7%	>7%	<1%	1-5%	>8%	21-30%	31-40%	>80%	31-40%
Sickle cell trait (with co-existence / co-inheritance of one or more Alpha thalassemia gene) (74)	30	44	00	56	18	00	60	14	00	-
Sickle cell trait (10)	09	01	00	07	03	00	00	10	00	-
Double heterozygous for sickle cell anemia (02)	00	02	00	00	00	02	00	00	02	-
Typical Beta Thalassemia minor (56)	00	56	00	39	17	00	-	-	-	-
Atypical Beta Thalassemia minor (04)	00	04	00	04	00	00	-	-	-	-
Hb D Punjab trait (05)	05	00	00	04	01	00	-	-	-	05
Hb E trait (02)	00	00	02	01	01	00	-	-	-	-
Hereditary persistence of fetal haemoglobin (01)	01	00	00	00	00	01	-	-	-	-
Delta Beta Thalassemia trait (01)	01	00	00	00	00	01	-	-	-	-

## Discussion

India is an ethnically diverse country with marked regional variation. Anand People's Medicare Society campus has different people of varied ethnic groups of different cities and district. Due to migration, there is constant mixing of people from different regions. Hence, this study was attempted to find out the difference in the prevalence of various haemoglobinopathies among the students studying in APMS campus. Appropriate laboratory tests are required for the diagnosis and confirmation of these disorders.

Haemoglobinopathies are common around the world; in any case, it is progressively common in some geological territories. In India, according to medical clinic-based study, normal recurrence of sickle cell quality is around 5%.<sup>[13]</sup> The most noteworthy recurrence of sickle cell quality in India is accounted for in Orissa (9%), pursued by Assam (8.3%), Madhya Pradesh (7.4%), Uttar Pradesh (7.1%), Tamil Nadu (7.1%) furthermore, Gujarat (6.4%).<sup>[13]</sup> The appropriation of beta thalassemia isn't uniform in the Indian subcontinent. In spite of the fact that certain networks are recognized to have

high predominance, it has been identified in pretty much every Indian populace. The predominance of beta thalassemia characteristic changes from 1-17% in various populaces of India.<sup>[11]</sup> Earlier studies show prevalence of beta thalassemia in different district of Maharashtra 1-6% and in Gujarat 0-9.5%.<sup>[14]</sup>

In our study, most common haemoglobinopathies observed were Sick cell trait (with co-existence / co-inheritance of one or more Alpha thalassemia gene (3.37%), followed by typical beta thalassemia minor (2.55 %), sickle cell trait (0.45 %), Hemoglobin D trait (0.22%), atypical beta thalassemia minor (0.18%), Sick cell disease (0.09%) and haemoglobin E trait (0.09%). Frequency of haemoglobin E in Gujarat was very less.<sup>[15]</sup> This is comparable to the frequency reported by Shah et al. (2018), Patel et al. (2012) and Bhukhanwala et al. (2013).<sup>[16-18]</sup>

In the present study, increased value of HbF between 1-3% was observed in 30% cases of beta thalassemia trait and increased value of HbF>5% was not observed in any cases which is less compare to Greer et al. (50%) and Shukla et al. (42%).<sup>[19,20]</sup>

In our study HbS was ranged between 35 to 39% in cases of sickle cell trait and HbS value was less than HbA value. Hb value was above 12 gm% in all haemoglobinopathies. HbS was ranged between 21-34% with HbA2 <3.5% and HbF <1% with low blood indices in sickle cell trait (with co-existence / co-inheritance of one or more Alpha thalassemia gene) patients. The similar finding was reported previously in sickle cell trait cases.<sup>[21]</sup> Elevated level of HbA2 >3.5% is signet for identification of  $\beta$ -thalassemia carriers and marginal HbA2 level may be due to co-existence / co-inheritance of one or more Alpha thalassemia gene identified by HPLC and further confirmation can be achieved by molecular methods like AMRS-PCR, whole genome sequencing.<sup>[22]</sup>

In this study HbS was >84.6%, HbA2 was 3.9 % and HbF was 8.10% in cases of double heterozygous for sickle cell anemia. In the study of Bhukhanwala et al. reported that level of HbF is up to 20%<sup>[18]</sup>. In present study students with HbD Punjab heterozygous state had unknown peak at D window. HbD was ranged between 32.50% and HbE showed raised peak in A2 region about 28.30%. Incidence of Hb D & Hb E is very less in Gujarat.<sup>[16]</sup>

The present study shows the prevalence of  $\delta\beta$ -thalassemia and HPFH in Gujarat with low incidence of 0.04%. This finding is important as an earlier large multicenter study covering cities from different regions also reported few subjects with  $\delta\beta$ -thalassemia and HPFH <1% in Indian population. Carrier detection of  $\delta\beta$ -thalassemia and HPFH is necessary as combination with  $\beta$ -thalassemia can result in major complications.<sup>[14]</sup>

#### **Limitation:**

The limitation of present study is voluntary participation of the subjects. So, it may not be able to represent population of interest and whole community level prevalence. Further large-scale population-based screening should be carried out to find out the real status of hemoglobinopathies in different community and geographical area.

#### **Conclusion**

Our study was an effort to find out the carrier status for sickle cell anemia, thalassemia and other haemoglobinopathies among the participants. Identification of these individual is very important as they may be transmitting abnormal gene and can give rise to various combination of haemoglobinopathies and thalassemia in their progeny which may lead to high morbidity and mortality. As hemoglobinopathies are not curable, the possible ways for reducing the incidence in India are by generating knowledge, mass campaign, population screening, genetic counseling and prenatal diagnosis. Thus, it can reduce the possibility of hemoglobin disorders of offspring, mental and physical disturbance of affected patients and socio-economic burden of the family. Hence suitable control measures need to be undertaken in India.

**Ethical considerations:** The research protocol was approved by the Institutional Ethical Committee of H M Patel center for medical care & education, Karamsad under the faculty of medicine.

**Conflict of Interest:** None

**Source of Funding:** Nil

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