Research Article

Study of hemoglobinopathies in various subcaste of tribal medical students

Mayur J Kokani[1], Heena Parikh[2], Mayuree H Modi[3]

[1]Department of Pathology, GMERS Medical College, Valsad, Gujarat, India.
[2]Department of Pathology, Government Medical College, Baroda, Gujarat, India.
[3]Department of Pathology, GMERS Medical College, Sola, Ahmedabad, Gujarat, India.
Correspondence to: Mayur J Kokani, E-mail: rockki1234@gmail.com

Received October 26, 2015. Accepted October 26, 2015

Abstract

Background: The prevalence of hemoglobinopathies is very high in tribal castes of India. Moreover, it is also an important cause of morbidity and mortality in many tribal castes in India. The tribal students of medical college, as they are the candidates from those castes in whom hemoglobinopathies are common, can play an influential role in their own family and own society and play a key role to increase social awareness.

Objective: To find out the prevalence of various hemoglobinopathies in healthy tribal medical students.

Materials and Methods: This is a cross-sectional study. Three milliliters of blood was collected in from each participant and processed for various investigations such as complete blood count, solubility test for detection of HbS (dithionite tube turbidity test), electrophoresis, HPLC, and methemoglobin reduction test.

Result: A total of 17 participants were found with different types of hemoglobinopathies among 77 participants. Sickle cell trait was the most common (15) among the participants. One participant revealed sickle cell disease, and one showed beta thalassemia trait. Nearly 41% of all hemoglobinopathies was seen in female participants from Dhodiya Patel caste.

Conclusion: Sickle cell trait is the most common among hemoglobinopathies in tribal medical students and commonly seen in the participants of Dhodiya Patel caste. Moreover, students from other subcastes were affected by sickle cell trait.

KEY WORDS: Tribal castes, hemoglobinopathies, sickle cell trait

Introduction

Hemoglobinopathies are a group of disorders of hemoglobin (Hb), which are characterized by structurally abnormal Hb variants, and one or more of the normal Hb are synthesized at a reduced rate.[1] Inherited disorders of Hb synthesis are an important cause of morbidity and mortality worldwide. Moreover, the general incidence of thalassemia trait and sickle cell hemoglobinopathy in India varies between 3%–17%[2] and 0%–40%.[3]

Consanguinity and intracaste marriage increase the endogamy among the tribal caste and some communities, which show a very high incidence of hemoglobinopathies, making the disease as a major public health problem in our country.[4] Moreover, it increases the burden on the patients, their families, and even their communities. They are generally not curable but can be prevented by population screening, genetic counseling, and prenatal diagnosis.

The medical students are future doctors, and they are going to serve society. They can play an influential role in their own family and own society and play a key role to increase social awareness. The tribal students of medical college, as they are the candidates from those castes in whom hemoglobinopathies are common, can play an influential role in their own family and own society and play a key role to increase social awareness.

Access this article online	
Website: http://www.ijmsph.com	Quick Response Code:
DOI: 10.5455/ijmsph.2016.26102015133	

Tribal area is very large in some parts of Gujarat, and, so, the percentages of hemoglobinopathies are very much common here. Sickle cell disease is the most common hemoglobinopathy in Gujarat.[5] The aim of this study was to find out the prevalence of frequency of sickle cell disease, G6PD deficiency, and α and β thalassemia in healthy tribal medical students and to increase their own awareness about the disease they are experiencingand increase sensitization toward social awareness.

Materials and Methods

It was a prospective study carried out at Government Medical College, Baroda, from November 2012 to October 2013. Scientific and Ethical committee permission was taken before starting the study. Participants were explained about the purpose and objectives of the study before enrolling in the study. The participants who gave the written consent were included in the study. A total of 77 tribal medical students were included in the study. From each participant, 3 mL of blood was collected in EDTA vaccute and processed for investigations such as complete blood count, solubility test for detection of HbS, electrophoresis, and HPLC.[6]

- Complete blood count was done by blood cell counter,
- Solubility test for detection of HbS (dithionide tube turbidity test): Sickle cell Hb is insoluble in the deoxygenated state in the high molarity phosphate buffer .The crystals that form refract light and cause the solution to be turbid.

Reagents

1. Buffer (pH: 7): Potassium dihydrogen phosphate (KH_2PO_4)—14.35 g, dipotassium hydrogen phosphate (K_2HPO_4)—25.0 g
2. Saponin purified (S.D)—250 mg
3. Sodium dithionate (I.R.) powder ($Na_2S_2O_6$)

Preparation of reagent

One hundred milliliters of measured quantity of water (preferably deionized water) was taken. Small quantity (about 5 mL) of water was added to bulb containing saponin and mixed by shaking till it dissolved. Rest of the water was added to a beaker containing ore weighed mixture of phosphates and stirred with a glass rod to dissolve. Both the solutions were mixed and poured in a screw capped plastic bottle.

Method

Few drops of blood were taken in 12- × 75-mm tubes containing 4 mL of normal saline (9gm/dL NaCl). After mixing, the tubes were centrifuged at 2,000 rpm for 5 min. Supernatant from each tube was discarded with Pasteur pipette. This was done to avoid the false positive result owing to plasma proteins.

One milliliter of reagent was taken in a 12- ×75-mm glass tube, and a very small quantity (about 10 mg) of sodium dithionate powder was added. Tube was shaken gently to dissolve the powder. A small drop of washed red cells (about 10 μL) was added and mixed by shaking. Initially, this gave a turbid solution with pink violet color. It was again observed after 3 min. A test was read as positive if the turbidity impaired the visibility of dark bold lines on white paper held against a bright source of light at 1-inch distance. Negative test was indicated by a clear pinkish violet solution through which dark lines were easily seen. For confirmation, positive and negative controls (known positive and negative samples) were kept in parallel with test samples.

Electrophoresis

In an alkaline pH (8.2–8.6), Hb is a negatively charged molecule and will migrate toward the anode (+). The various Hbs move at different rates depending on their net negative charge, which in turn is controlled by the composition (amino acids) of the Hb molecule (globin chain).

Reagents

Tris EDTA borate buffer (pH 8.4): Tris (hydroxymethyl) amino methane (L.R) 10.2 g, EDTA disodium salt (L.R.) 0.6 g, boric acid (L.R.) 3.2 g, glycerol 20 mL, deionized or distilled water to make 1,000 mL

Method

Hemolysate preparation: Two milliliters blood was taken in 12- × 100-mm glass tube and mixed well with about 4 mL of normal saline and then centrifuged at 1,800–2,000 rpm for 10 min. The supernatant was discarded with Pasteur pipette, and the procedure was repeated twice. To the packed cell, equal amount of distilled water and carbon tetrachloride were added and shaken vigorously on vortex mixture for about 2 min and then centrifuged at 1,800–2,000 rpm for 10 min. The clear supernatant Hb solution was then carefully taken out. Hb concentrations of the samples were roughly adjusted between 8 and 10g/dL.

Plastic-coated cellulose acetate strip was held vertically and dipped slowly in a beaker containing TEB buffer with 2% glycerol. Buffer rose by capillary action in the strip, without any air bubble being trapped. The anodal and cathodal compartments of electrophoresis tank were filled with equal amounts of TEB buffer in such a way that electrodes dipped in the buffer. Appropriately cut filter paper strips were put on the bridges of both compartments.

After 5 min, the cellulose acetate strip was removed from buffer and bottled gently between two filter papers. Immediately after the blotting, strip was put with cellulose acetate surface upward on the wet filter paper wicks on the bridge between anodal and cathodal compartments of the electrophoresis tank. The either end of the strip was covered by wet filter paper to prevent drying.

For charging the strip, about 20 μL hemolysate was taken on the white plastic pad. With the help of single hemolysate applicator (semimicro, 9 mm width), the strip was charged carefully and gently at cathodal end. The applicator was allowed to remain in contact with the strip for minimum 5 s so that a uniform thin line of hemolysate was formed. Approximately, 1 μL of hemolysate was charged. With this procedure,

six different samples were applied on a single strip. The hemolysates were allowed to be absorbed uniformly on the strip.

The electrophoresis tank was connected with the electrophoresis power supply. The electrophoresis was run at 250 volts; 75–90 min were required for proper separation of different Hb bands. After having reasonable good separation of HbA, HbS, and HbA2, power supply was switched off, and strip was taken out of tank.

HPLC

Reagents

Lysate, buffer A, buffer B, callibrator, and distilled water. All reagents are supplied by company along with the kit and are to be stored and used as per the instruction manual.

Principle and Methods

HPLC depends on the interchange of charged groups on the ion exchange material with charged groups on the Hb molecules. A typical column packing is 5 µm spherical silica gel. The surface of the support is modified by carboxyl groups to have a weakly cationic charge, which allows the separation of the Hb molecules with different charges by ion exchange. When a hemolysate containing a mixture of Hbs is adsorbed onto the resin, the rate of elution of different Hbs is determined by the pH and ionic strength of any buffer applied to the column. Elution of the charged molecules is achieved by continually changing salt gradient; fractions are detected as they pass through an ultraviolet/visible light detector and are recorded on an integrating computer system. Analysis of the fractions under these absorption peaks gives the percentage of the fraction detected. The time of elution (retention time) of any normal or variant Hb present is compared with that of known Hb, providing quantification of both normal Hbs (A, F, and A2) and many variants.

Methemoglobin Reduction Test

Sodium nitrate converts Hb to Hi. When no methylene blue is added, methemoglobin persists; but, incubation of the samples with methylene blue allows stimulation of the pentose phosphate pathway in subjects with normal G6PD levels. The Hi is reduced during the incubation period. In G6PD-deficient subjects, the block in the pentose phosphate pathway prevents this reduction.

Reagents

Sodium nitrite, dextrose, and methylene blue.

Method

- Test: Sodium nitrite, 1.25 g; dextrose, 5 g; and methylene blue, 15 mg in 100mL distilled water
- Control: Sodium nitrite, 1.25 g; and dextrose, 5 g in 100 mL distilled water.

In the test tube, 20 μL solution and 400 μL blood sample and, in control tube, 20 μL control solution and 400 μL blood sample were taken. Both tubes were incubated at 37°C for 3 h.

Interpretation: Normal blood yields a color similar to that in the normal reference tube (clear red). Blood from deficient subjects gave a brown color similar to that in the deficient reference tube.

Result

A total of 105 medical students coming from tribal caste were counseled to participate in the study. Of them, 77 students gave consent for participation in the study.

Among 77 participants, 44 were male and 33 female students. The caste-wise distribution of male and female participants is given in Table 1. As per Table 1, the most common caste of the participants was Bhil (25), followed by Dhodiya Patel (20), Rathawa (8), Chaudhari (8), and others. The proportion of male participants was high in Bhil caste, while the proportion of female participants was high in Dhodiya Patel caste.

A total of 17 participants were found with different types of hemoglobinopathies among 77 participants. Figure 1 shows the frequency of hemoglobinopathies, which shows sickle cell trait was the most common (15) among the participants. While others were sickle cell disease (1) and beta thalassemia trait (1).

Table 2 shows the distribution of hemoglobinopathies according to caste. The caste that found the maximum number of hemoglobinopathies was Dhodiya Patel (7). All seven participants from Dhodiya Patel, who presented hemolgobinopathies, were female students and revealed sickle cell trait. Another common caste was Bhil (3), which showed two cases of sickle cell trait and one beta thalassemia trait. Other castes found with hemoglobinopathies are Rathava (2), Vasava (1), Chaudhari (1), Gamit (1), Kukna (1), and Bhagat (1). The participant from caste Bhagat showed sickle cell disease, while participants from other castes showed sickle cell trait. However, participants from Pateliya and Garasiya castes did not reveal any hemoglobinopathies.

Figure 2 shows percentage of HbS in participants with sickle cell anemia, which shows that most of the participants (14) presented HbS in the range of 20%–30%. Two participants presented 30%–40% and one more than 90% HbS.

Table 1: Subcaste-wise distribution of students

Subcaste	Male	Female	Total
Bhil	20	5	25
Dhodiya Patel	6	14	20
Vasava	2	1	3
Rathwa	7	1	8
Chaudhari	3	5	8
Gamit	1	1	2
Kukna	3	3	6
Garasiya	0	2	2
Bhagat	0	1	1
Pateliya	0	2	2

Table 2: Analysis of hemoglobinopathies according to caste from samples of tribal students

Subcaste	Male	Female	SCT		SCD		B-Thalassemia		G6PD deficiency		Total
			M	F	M	F	M	F	M	F	
Bhil	20	5	1	1	0	0	1	0	0	0	3
Dhodiya Patel	6	14	0	7	0	0	0	0	0	0	7
Vasava	2	1	1	0	0	0	0	0	0	0	1
Rathwa	7	1	2	0	0	0	0	0	0	0	2
Chaudhari	3	5	0	1	0	0	0	0	0	0	1
Gamit	1	1	1	0	0	0	0	0	0	0	1
Kukna	3	3	0	1	0	0	0	0	0	0	1
Garasiya	0	2	0	0	0	0	0	0	0	0	0
Bhagat	0	1	0	0	0	1	0	0	0	0	1
Pateliya	1	1	0	0	0	0	0	0	0	0	0
Total			15		1		1		0		17

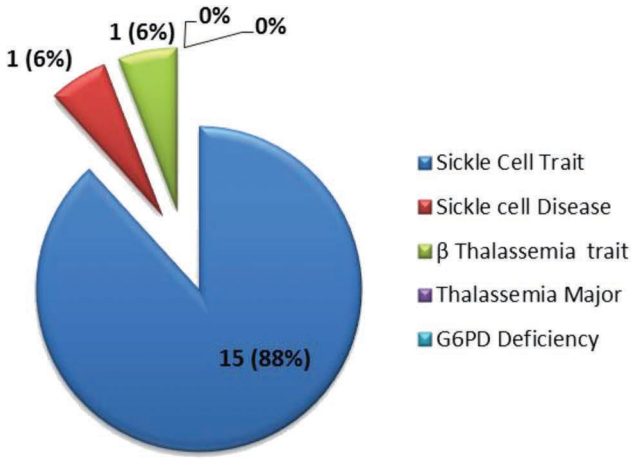

Figure 1: Frequency of different hemoglobinopathies diagnosed in this study.

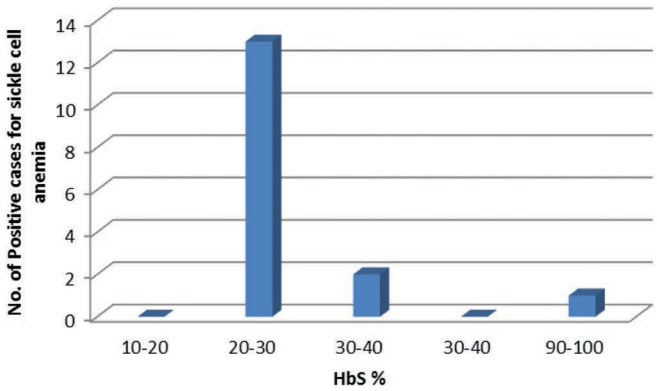

Figure 2: Percentage of HbS by HPLC in participants with positive sickle cell anemia.

Discussion

The large Indian population is multiethnic and divided into subgroups. As per the 2001 census, there are about 635 biological isolates (tribes and subtribes) that constituted 8.08% (about 84.3 million) of the total population of India. Tribal communities in India constitute the largest tribal population in the world. Most of them have been practicing endogamy for a long period of time, for which tribal communities are highly vulnerable to various hereditary diseases. As the frequencies of hemoglobinopathies are increased by the consanguinity and endogamous mating, it may be assumed that the tribal communities in India are facing the problem at large scale.[7]

A total of 77 tribal medical students of various semesters were included in this study. There were many studies found done on general population[7–9]; but, this study was done specifically in medical students. Moreover, in this study, subcaste-wise distribution of hemoglobinopathies was studied, which is not done by other studies. Male:female ratio was 1.33:1 in this study. However, it was also found that male: female ratio was the reverse in Dhodiya Patel (i.e., 1:2.3). The main reason was feeling apprehension and social stigma for not giving consent for the screening for hemoglobinopathies.

In this study, HbS test positive was observed in (16) 19.48% of study population, which is quite comparable with the study done by Paunipagar et al.,[10] which reported 21.69% and 17.20% HbS-positive cases. Among them, sickle cell disease was found in 1.29% participants, which is similar to the studies done by Patel et al. (1.70%) and Nag (1.72%). Subcaste-wise distribution shows that 50% of females (7/14) of Dhodiya Patel caste were positive for sickle cell trait, while no male was positive for HbS in the same caste. Moreover, female participants were high in Dhodiya Patel caste.

Various investigations such as sickle solubility test, electrophoresis, and HPLC were done in this study. It was found that that HPLC is easy, reproducible, and accurate in most

cases. But, one has to be cautious when dealing with compound heterozygous states of sickle cell disease and β thalassemia and β thalassemia and HbD. These states require family study to have exact presumptive diagnosis. Moreover, HbD Iran and HbE cannot be differentiated with confidence on HPLC alone. One has to do electrophoresis to reach at conclusive diagnosis.

Presently, there is no effective and specific treatment of sickle cell disease and disease states of other hemoglobinopathies. So, there should be blanket screening of tribal students, to have better assessment of load and proper counseling to stop marriages between defective gene carriers.

Conclusion

Sickle cell trait is the most common among hemoglobinopathies in tribal medical students and commonly seen in the participants of Dhodiya Patel caste. Moreover, students from other subcastes are affected by sickle cell trait.

References

1. Greer JP, Foerster J, Lukens JN, Rodgers GM, Paraskevas F, Glader BE, et al. *Wintrobe's Clinical Hematology*, 11th edn. Philadelphia, PA: Lippincott Williams and Wilkins, 2004. p. 1240.
2. Sengupta M. Thalassemia among the tribal communities of India. Internet J Biol Anthropol 2007;1(2):1–9.
3. Serjeant GR, Sergeant BE. *Sickle Cell Disease*. 3rd edn. Oxford: Oxford University Press, 2007.
4. Desai D, Dhanani H. Sickle Cell Disease: History And Origin. Internet J Hematol 2003;1(2).
5. Balgir R. Epidemiology, population health genetics and phenotypic diversity of sickle cell disease in India. Internet J Biol Anthropol 2007;1(2).
6. Laboratory techniques for the identification of abnormalities of globin chain synthesis. In: Bain BJ (Ed.). *Hemoglobinopathy Diagnosis*, 2nd edn. Malden, MA: Blackwell Publishing, 2006.
7. Nadkarni A, Phanasgaonkar S, Colah R, Mohanty D, Ghosh K. Prevalence and molecular characterization of alpha-thalassemia syndromes among Indians. Genet Test 2008;12(12):177–80.
8. Dhanani H. *Interaction of Sickle Cell with α Thalassemia in Tribal Population around Vadodara*. Dissertation. Maharaja Sayajirao University of Vadodara, 2003.
9. Verma IC, Saxena R, Thomas E, Jain PK. Regional distribution of beta-thalassemia mutations in India. Hum Genet 1997; 100(1):109–13.
10. Paunipagar PV, Patil SKB, Singh CM, Arya RC. Sickle cell gene in tribal area of Rajnandgaon district of Chhattisgarh. Indian J Prev Soc Med 2006;37:142–7.

How to cite this article: Kokani MJ, Parikh H, Modi MH. Study of hemoglobinopathies in various subcaste of tribal medical students. Int J Med Sci Public Health 2016;5:505-509

Source of Support: Nil, **Conflict of Interest:** None declared.