

Establishment of cost effective screening method for hemoglobinopathies in undergraduate medical students

Ashishkumar Agravatt¹, Amit Tyagi², Hiren Sanghani^{3*}, Rameshchandra Patel⁴, Nayna R Lakum⁵

¹Assistant Professor, ^{2,5}Professor & Head, ^{3,4}Associate Professor, ¹⁻³Dept. of Biochemistry, ^{4,5}Dept. of Pathology, ¹B.J. Medical College, Ahmedabad, Gujarat, ^{2,5}GMERS Medical College, Junagadh, Gujarat, ³Banās Medical College & Research Institute, Palanpur, Gujarat, ⁴Medical College Baroda, Vadodara, Gujarat, India

***Corresponding Author: Hiren Sanghani**

Email: hirensanghani@gmail.com

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Abstract

Introduction: Hemoglobinopathies are the most common group of single gene disorders worldwide. These disorders are major public health problem in many parts of the world including India with cumulative gene frequency of 4.2%. To reduce the burden of homozygotes of these disorders, it is essential that the carrier stage detection with genetic counseling in young individuals is done by a cost effective screening program.

Aims and Objectives: To establish screening tests like red cell indices, solubility test for Hb S and hemoglobin electrophoresis and screen students of GMERS medical college, Junagadh for hemoglobinopathies and confirming suspected cases of β -thalassemia minor and HbD at Red Cross Society laboratory, Ahmedabad.

Materials and Methods: In this descriptive cross sectional study, blood samples were collected in EDTA vacutainers from 150 students of GMERS Medical College, Junagadh, (Gujarat). Red blood cell indices, solubility test for Hb S and qualitative Hemoglobin Electrophoresis on agarose gel were performed to know the type of hemoglobin variant. Low Hb, MCV & MCH and borderline or high RBC count were suggestive of thalassemia minor. Confirmation of Hb D and suspected β -thalassemia minor cases was done by HPLC at Red Cross Society laboratory, Ahmedabad.

Results and Conclusion: Out of 150 medical students screened, 72 were males and 78 were females with mean age of 19 years. Hemoglobinopathies were detected in 18 students with prevalence of 12%. Seven cases of sickle trait, 1 case of sickle disease, 7 cases of β -thalassemia minor and 3 cases of heterozygous Hb D were detected. Red blood cell indices, Solubility test for Hb S and agarose gel Hb electrophoresis are convenient and cost effective techniques, where costly equipments are not affordable. Both medical and paramedical persons can be easily trained in these screening techniques. Detecting carriers by screening medical students for hemoglobinopathies and providing genetic counseling, we can hope to produce sensitized doctors, who will contribute in reducing prevalence of hemoglobinopathies in future.

Keywords: Hemoglobinopathies, β -thalassemia, Red cell indices, Hb electrophoresis.

Introduction

Hemoglobinopathies are common single gene disorders of hemoglobin in which there is production of an abnormal structure of the hemoglobin molecule or either partial or full absence of any one globin chains; known as thalassemias. These hereditary disorders are major public health problems in many parts of the world including South East Asian countries like India.¹ The cumulative gene frequency of hemoglobinopathies in India is 4.2%, with a population of over 1.2 billion and over 12,000 infants are born each year with a clinically significant hemoglobinopathies.² Hemoglobinopathies such as sickle cell anemia, thalassemias and variant hemoglobins together are responsible for the largest number of genetic diseases. These genetic diseases are controlled by a single gene that is transmitted from parents to offspring from one generation to another.³ Hemoglobin D-Punjab is present in a large number of people in Pakistan and North-West India and has a high frequency in Punjab with an incidence of 2-3%.⁴ Population screening has identified the prevalence of β -thalassemia carrier state as high as 17% in certain communities in India.⁵ The prevalence of hemoglobin S (Hb S) is 4.3% in India.⁶ World Health Organization figures estimate that 7% world population is

carrier for hemoglobin disorders, leading to high degree of morbidity and mortality.⁷ To reduce the birth of homozygotes and double heterozygote of these disorders, it is essential that the carrier stage detection with genetic counseling in young individuals is done by a cost effective screening program.^{8,9}

High performance liquid chromatography (HPLC) is considered the gold standard for diagnosis of hemoglobinopathies. But this test is not available at many peripheral areas because of high instrument cost as well as cost per test. So in this study we have developed cost effective program including diagnosis of sickle cell anaemia by solubility test followed by Hb electrophoresis to confirm type of sickle cell and red cell indices study to give us clue about suspected beta thalassemia minor. Low Hb, MCV & MCH and borderline or high RBC count are suggestive of thalassemia minor which can be confirmed by HPLC. Aim of this project is to establish screening tests like red cell indices, solubility test for Hb S and hemoglobin electrophoresis and screen students of GMERS medical college, Junagadh for hemoglobinopathies and detecting β -thalassemia minor cases and Hb D in suspected cases at a reference laboratory.

Materials and Methods

In this descriptive cross sectional study, blood samples were collected in EDTA vacutainers from 150 students of GMERS Medical College, Junagadh, (Gujarat) after taking written consent. Red blood cell indices, solubility test for Hb S and qualitative Hemoglobin Electrophoresis on agarose gel were performed to know the type of hemoglobin.

Solubility test is a very fast, cheap, sensitive and specific test for the demonstration of sickle cell hemoglobin. It is based on the decreased solubility of reduced Hb S in concentrated phosphate buffers. Reduced Hb S produces turbidity due to precipitation. First add 2 to 3 drops blood in pre-labeled glass tubes, filled with normal saline and mix. Then centrifuge at about 2000 rpm for 3-4 minutes. Discard the supernatant with a Pasteur pipette without dislodging the red cell button. Take about 1 ml solubility reagent in 12 x 75 mm glass tube. Add a very small amount (10 mg) Sodium dithionite powder and mix by gentle shaking. Add about 10 μ l of washed red cells in the reagent tube and mix gently by shaking. Initially it is opaque with red-violet color of reduced Hb. Wait for 3 to 4 minutes. Reduced Hb S will precipitate and produce turbidity (Fig. 1). In case of absence of Hb S,

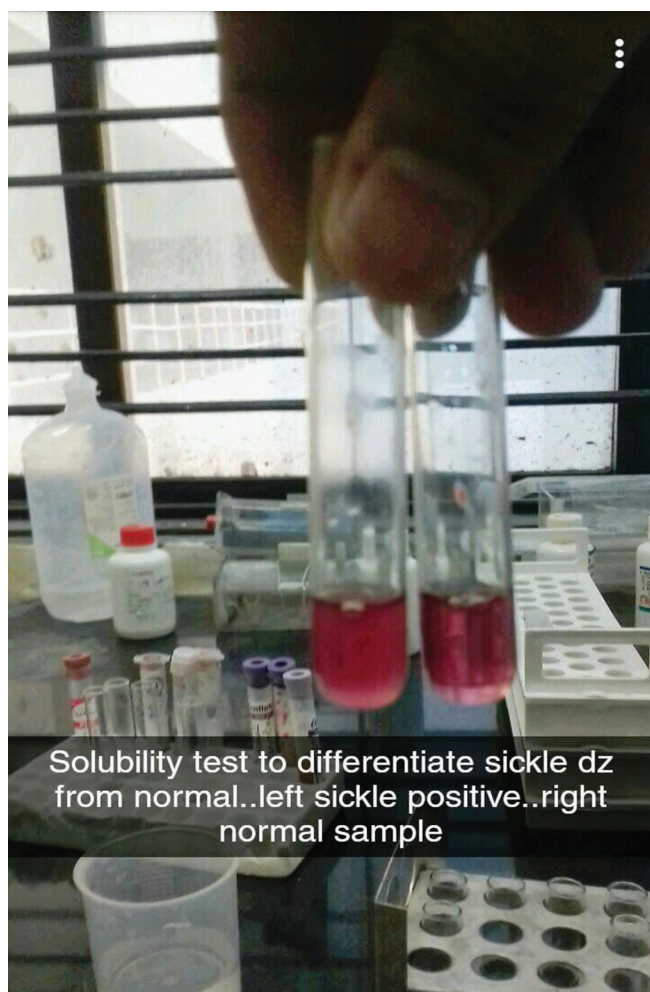


Fig. 1: Photograph showing solubility test



Fig. 2: Photographs showing material used in low cost Hb electrophoresis for making mold

the test solution will be transparent.¹⁰ Solubility test gives clue about presence of sickle trait and sickle disease which can be confirmed by Hemoglobin electrophoresis technique. Low cost technique of electrophoresis was developed and validated in our department in which X-ray films made transparent colorless by alkali treatment and made reasonably hydrophilic by chemical treatment. Acrylic moulds cut into required size in pairs. One of the two was carefully covered with vinyl membrane which made the surface hydrophobic. On this hydrophobic side 3 to 4 mm wide and 0.7 to 0.8 mm thick plastic strip was pasted on three sides as spacer. On the other acrylic piece, the hydrophilic PET was put and both were clamped with paper clips. Molten 1% Agarose in buffer allowed to flow in the capillary space from one end, with a pre-heated Pasteur pipette, taking care not to get any air bubble trapped. Then the mold was kept for a few minutes at room temperature. After Agarose solidified, Paper clips were removed and acrylic plate without spacer was removed carefully (Fig. 2).

After experimenting with various composition and strength of buffers, Tris-Glycine (Tris 1.5 gm and Glycine 7.2 gm in 1 liter distilled water) buffer of pH 8.2 was selected (Fig. 3). In This low ionic strength buffer Hemoglobin runs faster and less heat is generated.^{11,12} Rest hemoglobin electrophoresis technique steps are same as standard method including preparation of hemolysate, run in 1% agarose for 15 minutes at 250 volts, Fixing with methanol, drying, staining with amido



Fig. 3: Photographs showing Hb electrophoresis

black and destaining with 5% acetic acid. Band at A region in normal patients (Solubility test negative), bands at A and S region in sickle cell trait patients (Solubility test positive) & Hb D heterozygous (Solubility test negative) and band in S region was in sickle cell disease (Solubility test positive) was found in Hemoglobin electrophoresis. While RBC indices were done on Horiba Pentra XLR Fully automated Five part Hematology cell counter. Necessary statistical calculation done using epiinfo statistical software using p value. Hb <12 gm%, MCV <80 fl, MCH < 27 pg, RBC Count >4.5million and RDW borderline high raise the suspicion of thalassemia minor which were sent for confirmation by HPLC at Red Cross Society laboratory, Ahmedabad.

Table 1: Age and gender wise distribution (n = 150)

Gender	Number of Participants	Average Age
Male	72	19±1 years
Female	78	19±1 years
Total	150	19±1 years

Table 2: Distribution of variant Hemoglobins (n=150)

Hemoglobin Pattern	No. of Participants	Percentage
Normal (Hb AA)	132	88 %
Sickle cell trait (Hb AS)	07	4.65 %
Sickle cell Disease (Hb SS)	01	0.7 %
Hb AD	03	2 %
β-thalassemia trait	07	4.65 %

Results

Out of 150 medical students screened, 72 were males and 78 were females with mean age of 19 years (Table 1). Hemoglobinopathies were detected in 18 students with prevalence of 12% (Table 2). Seven cases of sickle trait, 1 case of sickle disease and 3 cases of heterozygous Hb D (including one case of Hb D Iran) were detected based on solubility test and Hb electrophoresis (Table 3). The mean±SD values of hemoglobin was 13.9 ± 2.1 g/dl, RBC count was

Table 3: Hb Electrophoresis and solubility interpretation (n=11)

Hb electrophoresis	Number of participants		Solubility Test	Inference
	Male	Female		
Bands in A and S region	0	07	Positive	Sickle cell Trait
Band in S region	0	01	Positive	Sickle cell disease
Band in A and S region	01	02	Negative	Hb D trait

Table 4: Laboratory parameters among screened subjects (n = 143) and confirmed cases of beta thalassemia minor patients (n=7)

Laboratory Parameters	Mean ± SD value among normal subjects (n=143)	Mean ± SD value among confirmed cases of beta thalassemia minor patients (n=7)	p value
Hemoglobin	13.9 ± 2.1 g/dl	10.9 ± 0.4 g/dl	p<0.05
RBC count	4.90 ± 0.58 millions/cumm	5.88 ± 0.4 millions/cumm	p<0.05
MCV	85.4 ± 10.2 fl	62 ± 3.4 fl	p<0.05
MCH	27.6 ± 4.3 pg	18.7 ± 1.11 pg	p<0.05
RDW	14.32 ± 2.2 %	16.5 ± 1.2 %	p<0.05

4.9 ± 0.58 millions/cumm, MCV was 85.4 ± 10.2 fl, MCH was 27.6 ± 4.3 pg and RDW was 14.32 ± 2.22 among the students. In beta thalassemia minor students, the mean $\pm$ SD values of hemoglobin was 10.9 ± 0.4 g/dl, RBC count was 5.88 ± 0.4 millions/cumm, MCV was 62 ± 3.4 fl, MCH was 18.7 ± 1.11 pg and RDW was 16.5 ± 1.2 (Table 4.) showing statistically significant difference in Hb, MCV, MCH, RBC count and RDW as compared normal students.

Discussion

Hemoglobinopathies constitute the most commonly inherited genetic disorders, the distribution of which varies geographically and by community.¹³ The overall prevalence of hemoglobinopathies that included sickle trait (AS), beta -thalassemia trait and HbD (heterozygous) was 12%. Wide prevalence of thalassemias and hemoglobinopathies has been attributed to migration of people from one region to another and marriages between different communities.¹⁴ In our study 16 students had Hb <12 gm%, MCV <80 fl, MCH < 27 pg, RBC Count >4.5 million and RDW borderline and among them diagnosis of beta thalassemia minor was confirmed in seven students by HPLC at reference laboratory with sensitivity of 44%. However similar indices picture is seen in patients with Iron deficiency anaemia which can be differentiated by Mentzer Index, Srivastav index etc. Mentzer index is calculated from the mean corpuscular volume (MCV, in fL) divided by the red blood cell count (RBC, in Millions per microLiter). If ratio is less than 13, thalassemia is said to be more likely. If the result is greater than 13, then iron-deficiency anemia is said to be more likely.^{15,16} In my study 19 students had MCV/RBC ratio less than 13 and among them diagnosis of beta thalassemia minor was confirmed in seven students with sensitivity of 37%. Srivastav index is calculated from the the Mean Corpuscular Hemoglobin (MCH, in picogram) divided by the red blood cell count (RBC in millions per microLiter) is less than 4.4, thalassemia is said to be more likely. If the result is greater than 4.4, then iron-deficiency anemia is said to be more likely.¹⁷ In my study 24 students have MCH/RBC ratio less than 4.4 was found and among them diagnosis of beta thalassemia minor was confirmed in seven students with sensitivity of 30%. Thus from red cell indices only, we could predict thalassemia minor cases. Low Hb, MCV & MCH and borderline or high RBC count are suggestive of thalassemia minor which can be confirmed by HPLC. Sonali Datar et al. conducted study on premarital screening of college students & found 10% prevalence of hemoglobinopathies among students.¹⁸ In our study sickle cell trait was found in 7 students and sickle cell disease in 1 student. All sickle positive students were from tribal community. In tribal population prevalence of sickle cell is as high as 35% and they inhabit remote areas where advanced health facilities are scarce and government run hospitals can not afford costly equipments like HPLC and capillary electrophoresis,

this low cost Hb electrophoresis and solubility test is very effective tool for diagnosis and prevention of sickle cell disease. If premarital screening of undergraduate medical students, such as in our study, is carried out on large scale with proper genetic counseling, we could reduce the disease burden of hemoglobinopathies in the society and create more awareness among medical fraternity for hemoglobinopathies.

Conclusion

Red blood cell indices, Solubility test for Hb S and agarose gel Hb electrophoresis are convenient and cost effective techniques, where costly equipments like HPLC are not affordable. Both medical and paramedical persons can be easily trained in these screening techniques. A nationwide program should be started to screen and diagnose hemoglobinopathies by using Red blood cell indices, Solubility test for Hb S and agarose gel Hb electrophoresis. Those found to have asymptomatic hemoglobinopathies should be given counseling regarding marriage so as to reduce the birth of children with major hemoglobinopathies. Detecting carriers by screening medical students for hemoglobinopathies and providing genetic counseling, we can hope to produce sensitized doctors, who will contribute in reducing prevalence of hemoglobinopathies in future.

Conflict of Interest: None.

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