

Spectrum of Haemoglobinopathies in Sikh population of Bangalore: A pilot study

Amrit Kaur Kaler^{1*}, Uma Bai KR², Vishwanathan V³, Chandrashekhar P⁴

¹Associate Professor, Dept. of Pathology, The Oxford Medical College & Research Centre, ²Associate Professor, Dept. of Pathology, MVJ Medical College and Research Hospital (MVJMC), Bangalore, ³Professor and HOD, Dept. of Pathology, Indira Gandhi Institute of Child Health, Bangalore, Karnataka, ⁴Professor and HOD, Dept. of Medicine, MVJ Medical College and Research Hospital (MVJMC), Bangalore, India

***Corresponding Author:**

Email: amrit_kaler@yahoo.co.in

Abstract

Objectives: To study the burden of different Hemoglobinopathies in the Sikh community of Bangalore. To study their patterns on gel electrophoresis (EPH) and compare the results with hematological parameters and peripheral smear. To confirm the results by cation exchange- High Performance Liquid Chromatography (CE-HPLC) wherever necessary.

Materials and Methods: A prospective pilot study was conducted on one hundred and fifty one cases who visited Gurudwara for a regular visit every Sunday. About 2-3 ml. intravenous blood samples were collected after obtaining informed consent from each individual. Background data of each individual were recorded like age, sex, caste, place of origin, consanguinity, etc. Hematological indices were measured using MS4 Cell Counter. Hemoglobin electrophoresis was carried out for the quantification of A2 fraction of hemoglobin by elution method. The use of high performance liquid chromatography is used to separate and quantify abnormal haemoglobin (Hb) fractions that were inseparable on Gel electrophoresis.

Results: Most common hemoglobinopathies observed were β -thalassemia trait (5.3%), thalassemia major (0.6%), Hb D trait (1.9%), HbD- β -thalassemia (0.6%), sickle cell trait (0.6%), HbQ disease (0.6%).

Conclusions: Proper screening camps and counselling needs to be done on the population at risk about awareness and detection of heterozygous carriers; to prevent the birth of homozygous Beta Thalassemia Major Child.

Keywords: Sikh population, Hemoglobinopathies, Electrophoresis, High Power Liquid Chromatography.

Introduction

Hemoglobinopathies are the most common group of inherited genetic disorders of hemoglobin in which there is an abnormal production or structure of the hemoglobin molecule. World Health Organization (WHO) estimated that 7% of the world population is a carrier for hemoglobin disorders.¹ These hereditary disorders are major public health problem in South East Asian countries like India and Pakistan. The prevalence of β -thalassemia trait and sickle cell in India varies between 3-17% and 1-44% respectively in various studies.^{2,3} About ten thousand children of beta Thalassemia are born every year due to consanguinity; hence the identification of β -Thalassemia minor is essential for screening as it may prevent the birth of beta Thalassemia major.⁴ Different mutations are restricted in this population probably due to lack of awareness, ignorance and consanguinity marriages. Proper screening camps need to be organized in the affected population as a part of Thalassemia control programmes of Government of India and necessary for genetic counselling to reduce the incidence and burden of Thalassemia major in the society. Mandatory screening test at birth of the child might help to prevent the spread of the disease in affected areas.

The main objective of this study was to assess the burden of different Hemoglobinopathies in the Sikh community of Bangalore. The preliminary step in this study is the screening of all the samples by Hemoglobin electrophoresis with hematological parameters and

randomizing all the positive samples for the quantification and confirmation by HPLC.

Materials and Methods

A prospective descriptive pilot study was carried in a camp organized in the Sikh Gurudwara of Ulsoor, Bangalore in all age groups, where all the Sikhs gather every Sunday for prayers over a period of 6 months from June 2012 to January 2013. An approval by the Ethics Department of MVJ Medical College and Research Hospital was taken to commence the study. Motivational Lectures and pamphlets were distributed in order to spread awareness among the population, highlighting the incidence of Hemoglobinopathies among the Sikh Population. Inclusion criteria were volunteers of all ages and both sexes. Exclusion criteria include pregnant women and patients with co-morbidities. An informed consent was taken; EDTA samples were collected, stored in special packs and transported to Indira Gandhi Institute of Child Health, Jayanagar, and Bangalore for evaluation. Family history and pedigree analysis was done in all cases. All the samples were subjected to CBC analysis using MS4 cell counter. Hematological parameters like RBC count $>5/\text{million}$, MCV <72 and RDW <17 were taken as cut off. Subsequently all samples were taken for Hemoglobin electrophoresis (EPH) for HbA2 estimation by alkaline cellulose acetate electrophoresis machine model Beckman Coulter.⁵ Serum ferritin was also analysed to rule out Iron deficiency anemia. Peripheral Smear examination was done using

Leishman's staining. The suspicious and the positive cases were subjected to High Performance Liquid Chromatography (HPLC) using Bio – Rad Variant Haemoglobin Testing system (Hercules, CA), is a totally automated CE – HPLC instrument was used for the definite quantification of HbA₂, HbF and any other abnormal Hb variant.⁶ In this system, the samples are mixed on the Variant I sampling station, diluted with the specific haemolysing wash buffer and injected into an assay-specific analytic cartridge. The separated hemoglobin fractions pass through a flow cell and absorbance is measured at 415 nm while background noise is reduced with the use of a secondary wavelength at 690nm. The raw data are integrated and a chromatogram report is generated.⁶ Statistical analysis: Master chart was prepared by collecting data from all the volunteers and tabulated by using Microsoft excel. The statistical analysis was done using software SPSS version 20.0 such as mean, standard deviation, t test & chi square. The project was funded by Medical Education and Research Trust, Karnataka.

Results

All 151 Sikh volunteers came forward for the screening and investigations included 98 males and 53 females with ages between 9 months and 45 years. Of

these, 15 (9.93%) showed were found to have one or the other form of Hemoglobinopathy as noted on electrophoresis. In our study, common disorder was Thalassemia Minor (5.2%), followed by HbD Punjab (1.9%), Sickle cell (0.6%), Thalassemia Major (0.6%), combined HbD and Thalassemia minor (0.6%), and one rare case of HbQ disease (0.6%) was also found. (Table 1) All Thalassemia minor and HbS trait cases had hemoglobin <12g/dl among women and <14g/dl among men. Majority of the cases had RBC count >5/ul, MCV <72pg and RDW <17. (Table 2) Other Hemoglobinopathies like HbD and HbQ disease displayed no clinical signs and symptoms of anemia, and RBC count was more than 5/ul, MCV >72pL and RDW <17. (Table 2) Serum ferritin was normal in all the Thalassemia and Hemoglobinopathy cases. These positive and suspicious 15 cases were confirmed and quantified by HPLC, which was very helpful in solving the problematic cases. Family studies were proven to be positive in these cases. The only limitation of the study was the cases missed on Hemoglobin electrophoresis as only the positive samples on EPH were subjected to HPLC due to lack of funds. Further study on genetic mutations could not be carried out due to the similar reason.

Table 1: Percentage of various hemoglobinopathies in this study

Hemoglobinopathy	Thal Minor	HbD	Thal Major	Sickle cell trait	HbD + Thal Minor	HbQ Disease
Cases	8	3	1	1	1	1
Percentage	5.2%	1.9%	0.6%	0.6%	0.6%	0.6%

Table 2: Comparative analysis of hemoglobin electrophoresis and hematological parameters

Lab parametres	Thal Minor	HbD	Thal Major	Sickle cell trait	HbD + Thal Minor	HbQ Disease
Hb	<12g/dl	>12g/dl	<12g/dl	<12g/dl	<12g/dl	>12g/dl
RBC Count	>5 M/uL	>5 M/uL	>5 M/uL	>5 M/uL	>5 M/uL	<5 M/uL
MCV	<72 fL	>72 fL	<72 fL	<72 fL	<72 fL	>72 fL
RDW	<17	<17	<17	<17	<17	<17
HbA	>95%	>60%	<20%	<55%	>60%	>70%
HbA ₂	>3.5%	<3.5%	<3.5%	<3.5%	>3.5%	<3.5%
HbF	<1%	<1%	>75%	<1%	<1%	<1%
Others		HbD>35%		HbS>45%	HbD>35%	HbQ>16%

Discussion

The hereditary disorders of haemoglobin may be classified into two broad groups, the Hemoglobinopathies and the Thalassemias. The Hemoglobinopathies are characterized by the production of structurally defective haemoglobin due to abnormalities in the formation of the globin moiety of the molecule such as haemoglobins S, C, D, E, etc. The Thalassemias are characterized by reduced rate of production (synthesis) of normal haemoglobin due to absence or decrease in the synthesis of one or more types of globin polypeptide chains.⁷

The distribution and burden of various Hemoglobinopathies in the Sikh population of Bangalore was determined. The most common abnormality detected in this study is Thalassemia minor carrier with an average of 5.2%. The values are in comparison to Sukumaran and Masters who also reported the prevalence of Thalassemia in Punjab to be 6.5%.⁸ A study conducted by Madan N and co-investigators showed the overall gene frequency of β Thalassemia trait reported in northern and western India was 4.05%.⁹ These patients were clinically normal and

presented with mild anaemia (mean Hb level of 10.5 gm/dl). Only one case (0.6%) of 15 abnormal cases was diagnosed on EPH as beta – homozygous Thalassemia with increased HbF values (76%), and severe pallor requiring regular blood transfusion and had marked splenomegaly.

Cellulose acetate electrophoresis (EPH) at alkaline pH (8.9–9.1) is the most widely used method, simple, rapid, inexpensive and effective in separating the common haemoglobin variants.¹⁰ In the present study, only one case (0.6%) of heterozygous sickle cell anaemia, followed a milder clinical course with few target cells and sickles on peripheral smear; showed an increase in HbS on electrophoresis and HPLC. (Fig. 2 & 3) The average frequency of sickle cell anaemia in Indian population is about 4.3% while the frequency of sickle cell disease is 1.6% according to our data in Punjab population.^{11,12} Three (1.9%) volunteers showed the presence of Hb-D Punjab trait on electrophoresis and HPLC displayed a D window with variant percentage ranging from 33% to 38%. The blood parameters were essentially normal. Hemoglobin D-Punjab is one of the most commonly encountered abnormal hemoglobins worldwide.¹³ It 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%.^{14,15}

Among the red cell parameters, Red cell count (RBCs) was consistently increased (>5.0 Million), which proved to be quite a significant marker in all cases. The increase in count (RBCs) might be possibly to compensate for the reduced amount of oxygen carried by hemoglobin in the blood. Ayesal et al also stated that RBC count is one of the most accurate parameters available with the highest sensitivity (94.8%)¹⁶. MCV (<72) was consistently decreased in the majority of cases. Schaefer and Schaefer also proved that MCV also provides a useful tool for characterizing red blood cells in patients with anemia.¹⁷ Peripheral blood film (PBF) showed anisopoikilocytosis and microcytic hypochromic blood picture and target cells; it is of especial relevance in the laboratory diagnosis of Thalassemia. (Fig. 1) RDW was consistently found below 17 in all the 15 cases. These hematological parameters were found to be reliable, convenient and cost effective and correlated well with the results of Hemoglobin electrophoresis. In areas where modern equipments for diagnosis are not available, these a simple morphologic criterion based on microcytic red cells, and RBC count and MCV can be used to assess the possibility of Thalassemia.

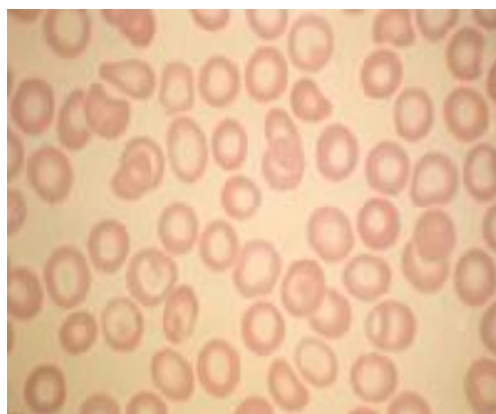


Fig. 1: Peripheral smear shows microcytosis and target cells suggesting Thalassemia minor



Fig. 2: Electrophoresis showing HbS/HbD/HbQ band

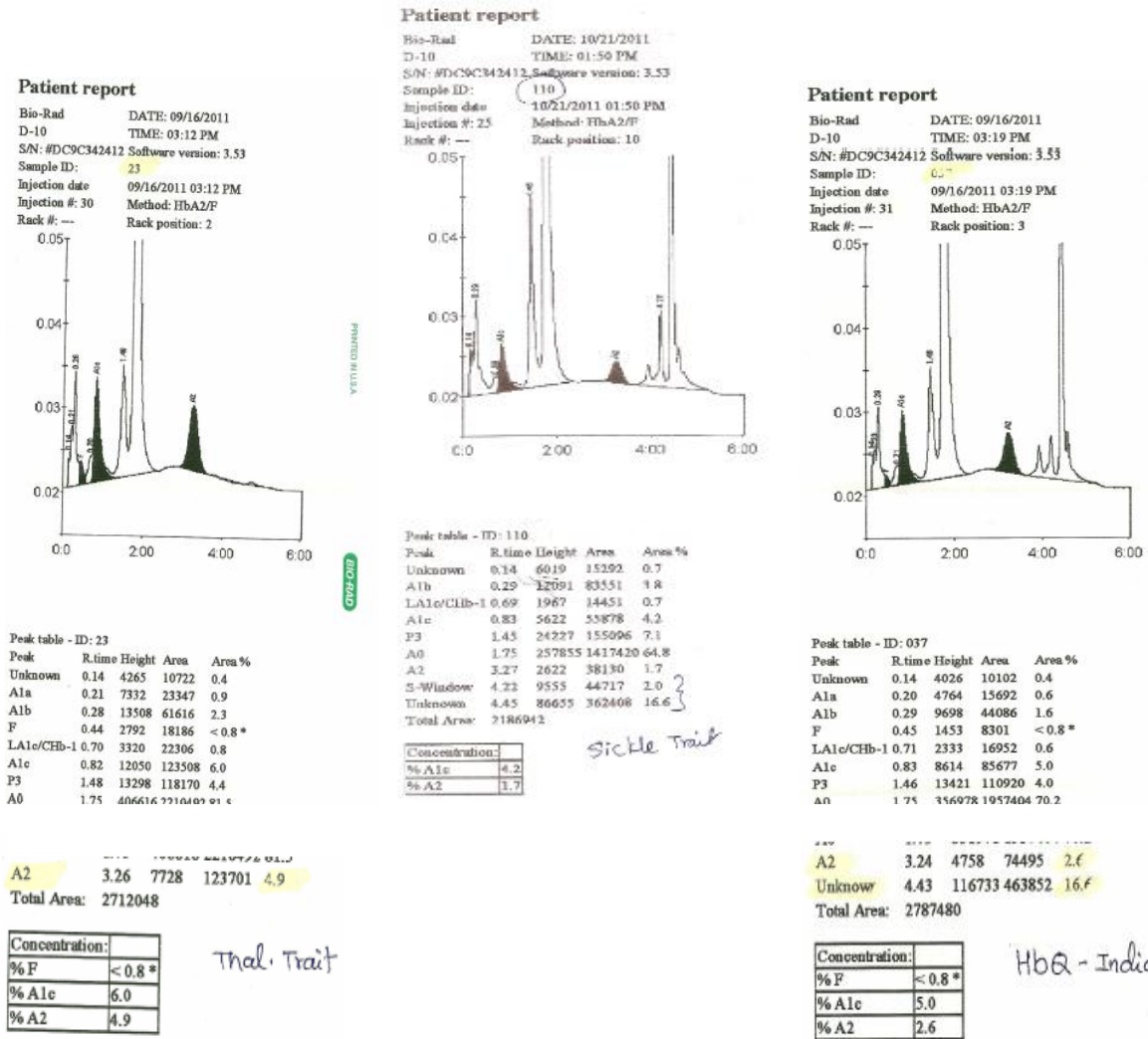


Fig. 3: HPLC showing quantification of different Hemoglobinopathies like thalassemia trait, sickle cell trait, HbQ disease

But the accurate quantification of HbA₂ by High performance liquid chromatography (HPLC) is essential for the diagnosis of β thalassaemia trait, in which the HbA₂ is elevated, typically > 3.5% and even so in borderline cases.¹⁸

This was proved in one of the volunteer, in a two year old girl child who showed all the symptoms and laboratory findings of Thalassemia minor but both the parents were positive for heterozygous Hb- D on EPH Gel electrophoresis and a weak band on HbA₂. This discrepancy was solved by HPLC which showed that the father had borderline levels of HbA₂ levels 3.6% in addition to the HbD levels of 36%. Hence, HPLC is a fast, sensitive and accurate method of separation and quantification of Hemoglobinopathies and can be used as a method of choice for large screen population testing in endemic areas.¹⁹

HPLC also allows identification of some rare variants of Hemoglobinopathies not possible by EPH. A similar case showed discrepancy on electrophoresis in a 35 year old male, diagnosed probably as HbS/HbD on

the same with negative sickling test, showed a retention time of 4.76 minutes with an intense peak for this abnormal Hb variant when the sample was run on HPLC, proving it to a case of heterozygous Hemoglobin Q disease. (Fig. 3) The first case of HbQ India was described by Sukumaran in 1972 in a Sindhi family in association with beta Thalassemia and later by Desai et al.^{20,21}

A community based approach was used with the families and parents of volunteers and a positive correlation was found between the increases in the burden of β-thalassemia with increased tendency of consanguinity. It was very interesting to note that the majority of the positive cases were ancestral migrants from Pakistan and belonged to Jat Sikh and Khatri families.

It has been estimated that the lifetime cost of healthcare, premature mortality and lost earnings versus a national screening program including antenatal diagnosis is much higher and only 10-15% of all Thalassemia children born per year get optimal therapy in the form of regular blood transfusions and

chelation.²² The curative treatment like bone marrow transplantation is very costly and not affordable by the poor patients. Thus preventing the birth of affected children is the best option for India. A prerequisite for this is the knowledge of the prevalence of β -thalassemia and other haemoglobinopathies in different regions of the country and in particular in different ethnic groups and then organizing screening of the population with genetic counselling birth rate of β -thalassaemia major can be reduced by as much as 90%.^{23,24}

Conclusion

It is important to know the high incidence of Hemoglobinopathies in the Sikh population of the Indian subcontinent. This can be achieved by screening the mass population under National Programs for Thalassemia especially in Punjab. Moreover, knowledge of common Hemoglobin patterns in Sikh population helps to formulate appropriate preventive and therapeutic strategies. Thus, premarital and antenatal screening should be mandatory to prevent the birth of offspring with β thalassemia major child.

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