 DOI: 10.18231/2394-6377.2017.0011

Prevalence of Haemoglobinopathies in Punjabi population

Anju Sharma[1,*], Neha[2], Sahiba Kukreja[3], Jaskiran Kaur[4], Amandeep Kaur[5]

[1]Assistant Professor, [2,5]Associate Professor, [3]Professor & HOD, [4]Professor, Dept. of Biochemistry, Sri Guru Ram Das Institute of Medical Sciences & Research, Amritsar, Punjab

***Corresponding Author:**
Email: dranjugill30@yahoo.com

Abstract

Background: The prevalence of thalassemias and various hemoglobinopathies are a major public health problem in Punjabi population. These cases are mainly overlooked and thus only treated as a part of anaemia. Thus it is worthwhile to study its prevalence and occurrence. The main objective of our study was to find the prevalence of hemoglobinopathies in Punjab and implement strategies for preventing marriages between two carriers for effective control of these diseases.

Material and Methods: This was a case control retrospective study for screening for haemoglobinopathies of patient samples collected in biochemistry clinical laboratory, SGRDIMSAR, Vallah, Amritsar from 1 June 2015 to 30 May 2016 using automated Interlab Genios S electrophoresis system. EDTA samples were collected and 30 µL of lysate was applied on Genios S electrophoresis system in alkaline haemoglobin buffer. The results were interpreted by densitometer were obtained as a graph and interpreted.

Results: In this study, out of 187 samples we received for screening of haemoglobinopathies, total of 143 (78.5%) were found to have a normal electrophoretic pattern and 44 patients (21.4%) were found to have one or other form of hemoglobinopathy.

Conclusion: Punjabi population has high prevalence of haemoglobinopathy screening being an affordable and accessible way to detect carriers, it should be made mandatory in high schools, before marriage and antenatal clinics. Also, public education and awareness for screening in areas of high prevalence of consanguinity should be made mandatory.

Introduction

Anemia is very common in our country in childbearing women and growing children. As per National Family Survey-II conducted in 1998-1999, it was found that 73.6% child in age group of 6-35 months were anaemic.(1) Haemoglobin is a hemoprotein whose primary function is to transport oxygen from lungs to the body tissue. It was first isolated in 1849. Haemoglobin is a globular protein with a diameter of 6.4nm and a molecular mass of approximately 64000 Da. Haemoglobin consists of four globin subunits (two alpha and two non-alpha) with each looped about itself to form a pocket or cleft in which heme group nestles.(2)

Foetal haemoglobin predominates during foetal life but rapidly diminishes during first year of postnatal life. The normal adult also has between 2.3 and 3.5% of haemoglobin A2 (HbA2)($\alpha2\delta2$), and may have up to 2% HbF in circulating erythrocytes. Foetal haemoglobin F (HbF) that contains two alpha chains and two gamma chains ($\alpha2\gamma2$)(Table 1).(3)

Table 1: Normal Ranges of Adult Haemoglobins

Hemoglobin	Globin Chain Composition	Adult Concentration*
HbA	α2β2	96-98%
HbA2	α2β2	2.3-3.5%
HbF	α2β2	<2%

Functionally, fetal hemoglobin differs most from adult hemoglobin in that it is able to bind oxygen with greater affinity than the adult form, giving the developing fetus better access to oxygen from the mother's bloodstream.

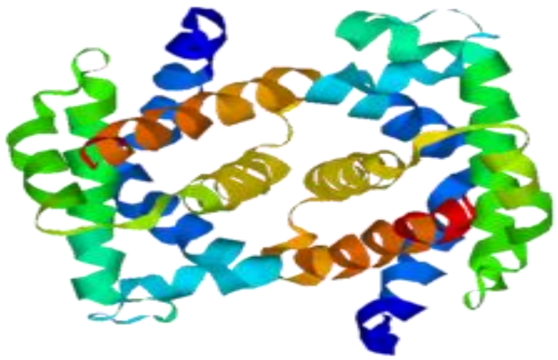

Fig. 1: Fetal haemoglobin protein structure

Fetal hemoglobin protein structure

Thalassemias and hemoglobinopathies are clinical disorders related to Hb pathophysiology although they may have similar clinical manisfestations such as anemia of varying severity.(4) The name thalassemia is derived from the Greek word for sea, thalassa, because all early cases of β-thalassemia were described in children of Mediterranean origin. Hemoglobinopathies, the most common single gene disorder in the world, are structural Hb varients arising from mutations in the globin genes, which result in substitution or disruptions in the normal amino acid residue sequence in one or more of the globin chains of Hb.(5) Congenital disorders of haemoglobin characterized by deficient synthesis of one or more hemoglobin polypeptide chains, leading to an imbalance in numbers of alpha - beta chains α-thalassemia arise from deficiencies in production of α-globin chains and are caused by deletion or point mutation in one or more of the four α-globin genes. β-thalassemias result from a reduction in the synthesis of the β-globin chains.[6,7]

Table 2[8]

Name	Description	Alleles
Thalassemia minor	Only one of β globin alleles bears a mutation. Individuals will suffer from microcytic anemia. l detection usually involves lower than normal MCV value (<80 fL). Plus an increase in fraction of Hemoglobin A2 (>3.5%) and a decrease in fraction of Hemoglobin A (<97.5%)	β^+/β or β^0/β
Thalassemia intermedia	A condition intermediate between the major and minor forms. Affected individuals can often manage a normal life by may need occasional transfusions, e.g., at times of illness or pregnancy depending on the severity of their anemia.	β^+/β^+ or β^0/β^+
Thalassemia major	If both alleles have thalassemia mutations. This is a severe microcytic, hypochromic anemia. Untreated, it causes anemia, splenomegaly, and severe bone deformities. It progresses to death before age 20. Treatment consists of periodic blood transfusion; splenectomy if splenomegaly is present, and treatment of transfusion-caused iron overload. Cure is possible by bone marrow transplantation. Cooley's anemia is named after Thomas Benton Cooley.	β^0/β^0

Sickle cell haemoglobin (HbS) results from an autosomal recessively inherited mutation in which 17th nucleotide of beta-globin gene is changed from thymine to adenine and amino acid glutamic acid is replaced by valine at position 6 in the beta-globin chain.[10,11] Sickle cell disease refers to the group of disorders that affects haemoglobin molecules(HbS). Sickle cell anaemia is the name of the specific form of sickle cell disease in which there is homozygosity for mutation that causes HbSS. Sickle cells have reduced deformability and are easily destroyed causing occlusion of microcirculationand chronic haemolytic anaemia with a median haemoglobin concentration level of 9g/dl.[12]

Table 3: Shows factors leading to Sickle Cell Crisis

Factors	
Hypoxia	Dehydration
Vascular Stasis	Fever
Cold	Acidosis

Methodology

This is an observational study concluded in clinical biochemistry laboratory of the institution. HbA1, HbA2, HbF and other variants of haemoglobin were separated from samples collected in the Biochemistry Lab. From 1/6/2015 to 30/5/2016 using Automated Interlab Genio S Gel Electrophoresis.[13] Samples were collected in EDTA vacutainers which is anticoagulant of choice for hemoglobinopathy analysis. Samples obtained were processed within three days of receipt of samples as HbF results are obtained on fresh materials and not on samples more than a week. However analysis remain fairly stable on samples stored at 4^{0}C even after 3 weeks. Lysates are prepared by the following procedures. Take EDTA whole blood and centrifuge it for 5 minutes. In a separate tube take 50 µl of RBC's (infranatant) and 250 µl of lysing solution. Centrifuge at 500 r.p.m (for 10-15 minutes). Take 30 µl of supernatant from this tube and apply as a sample on Genio S gel electrophoresis system by Liliac Diagnostics. Other reagents used for the procedure:

1. Alkaline Hb (buffer system)
2. Staining solution
3. Destaining solution – by Liliac diagnostics and the chromatogram after a single run was obtained after about one hour, Lysates can be frozen at -20^0 C (1 month) and -80^0 C for about 3 months.

Measurement Units: The relative percentage (%) on total haemoglobin is the measurement unit of choice. The factors such as high HbA1c, batch to batch variations and critical evaluation of the chromatograms may affect the results. So, the Gel electrophoresis apparatus was handled by trained personnel and controls were run with each batch.

Reference intervals: Each laboratory should calculate their own reference intervals by measuring HbF in at least 100 adult individuals who are not Iron depleted nor carriers of α/β thalassemias. The reference values calculated for clinical Biochemistry laboratory of the institution $\leq$ 2.0%.

Results

This study was conducted in the department of Biochemistry SGRDIMSAR, Sri Amritsar. Out of 187 cases examines 143 (78.5%) were found to have normal electrophorectic pattern and 92(21.4%) were found to have one or the other form of haemoglobinopathy.

In our study the commonest disorder is thalassemia (carrier) (17.5%), thalassemia major (2.1%), sickle cell disease (1.6%). HbD (1.09%) and HbE heterozygous (0.54%) in decreasing frequency. (Table 4)

Table 5 shows a comparative analysis of different hematological parameters for the three common hemoglobinopathies.

Table 4: Table showing percentage of various hemoglobinopathies in this study

Thalasima Carrier	Thalais major	Hbs	HbD	HbE
32	4	3	2	1
17.5%	2.1%	1.6%	1.09%	0.54%

Table 5: Comparative analysis of differenct hematological parameters for the three common hemoglobinopathies

Lab parameter	Thalassemia Major	Thalassemia Minor	Sickle Cell Disease
Hb gm%	6.1	8.57	8.8
RBC Count	2.8	5.43	5.5
MCV	72	63.4	63
HCH	21.3	20.7	21
MCHC	29.6	32	34
RDW	26.9	15.2	14.9
Abnormal Bands	HbF	HbA2	Hb3

Table 6: Shows the sex wise distribution of cases and control

Hb Electrophores	M-105	F-80	T=185
Normal	95 90.4%	48 60%	143 78.5%
Abnormal	25 23.8%	17 21.2%	42 21.4%

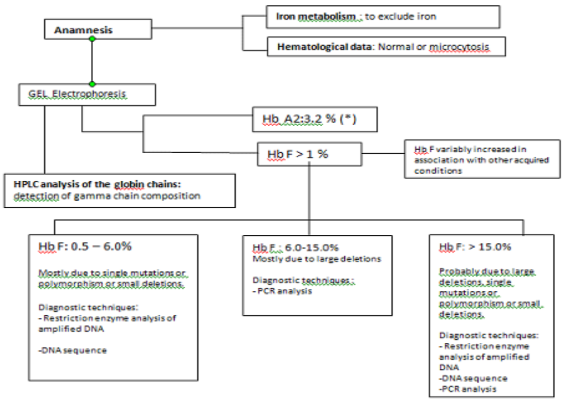

Fig. 2

Discussion

The thalassemias and hemoglobinopathies (HbS, HbE, HbD) are clinical disorders related to Hb pathophysiology. The laboratory plays a crucial role in detection and characterization of hemoglobinopathies and thalassemias. Several recommendations have been put forth for the laboratory investigation of abnormal Hbs & thalassemias. For example, International committee for standardization in hematology expert panel on abnormal hemoglobins, published recommendations for laboratory investigation of these conditions. In this initial investigation they recommended a) CBC b) electrophoresis at pH 9.2 c) tests for solubility and sickling and d) quantification of HbA2 and HbF. In thalassemias, the Hb concentration and Mean Corpuscular Volume, an index of red cell size are decreased, sometimes markedly. One study recommends that an MCV of Less than72 fL (Ref unit 80-100 fL) is maximally sensitive and specific for presumptive diagnosis of thalassemias. However, an Mean Corpuscular Hemoglobin of less than 27 pg (Ref Unit 26-35 pg) has been recommended as the decision point for further investigation for iron deficiency anaemia. Fetal hemoglobin (usually below 1% in adults) can also be a diagnostic criteria for, thalassemias an High Performance Liquid Chromatography analysis shows a major HbF peak with absence of HbA and variable A2 peak. HbF may falsely increase during pregnancy, juveline myeloid leukemia and congenital aplastic anaemia and thus these should be excluded before making the diagnosis.[14,15,16,17] Hereditary Persistance of Foetal Haemoglobin is a condition having HbF values >10%. Thus HbF plays a major role in the diagnosis of Thalassemias.

In our study the incidence of β thalassemia major and minor was 2.1 and 17.5% respectively which gives an estimate of approximate 5% of worlds population being carries for haemoglobin disorders.[18,19] The frequency of sickle cell disease is 1.6% according to our data in Punjab population. The average frequency of sickle cell anaemia in Indian population is about 4.3%. The incidence of HbE disease is more in eastern and far eastern parts of India. Only one case of HbE

(0.54%) was found in Punjabi population in two years in patients admitted in SGRDIMSAR, Amritsar. Two patients (1.09%) of HbD were detected. The percentage coincides with other reports in north Indian population.

Patients with sickle cell disease has normocytic normochronic anaemia but majority of our patients presented with hypochromic microcytic anaemia which could be due to associated iron deficiency anaemia or α-thalassemia trait. Moreover, very high incidence of iron deficiency in patients of sickle cell disease has been reported in India.[20] As hemoglobinopathies exert a high burden on India, especially in western parts of world, screening being affordable and accessible way to detect carriers, should be made mandatory in high schools, before marriage and antenatal clinics along with ferritin, serum iron and Total Iron binding Capacity. Besides this public education and awareness for screening especially in areas of high prevalence of Consanguinity should be done.

Bibliography

1. ME Bentley, PL Griffits. The burden of Anemia among women in India European Journal of Clinical Nutrition 2003;57;52-60.
2. Higgins T, Eckfeldt JH, Barton JC, Doumas BT. Hemoglobin, Iron and Bilirubin. In Eurtis CA, Ashwood ER, Bruns DE. Tietz Textbook of clinical chemistry and Molecular Diagnostics Fth ed. Philadelphia. Elsevier Publications; 2012.P-985-1025.
3. Adachi K, Kim J, Asakuri T, Schwartz E. Characterization of two types of foetal hemoglobin: alpha 2G gamma 2 and alpha2A gamma 2. Blood 1990;75:2070.
4. Clarke GM, Higgins TN. Laboratory Investigations of Hemoglobinopathies and thalassemia review and update. Clin Chem 2000;46:1284-90.
5. Cazzola M, May A, Berganaschi G, Cerani P, Ferrillos, Bishop DF. Absent phenotypic expression of X-linked sideroblastic anemia in one of two brothers C normal ALAS2 mutation. Blood 2002;100:4236-8.
6. Hoffman: Hematolgy: Basic Principal & Practice, 6th Ed.
7. Chur DH, Ways IS. Hydrops fetalis caused by alpha thalassemia: An emerging health care problem. Blood 1998-91;91:2213-22.
8. http://ww.whonamedit.com/synd.cfm/2157.html.
9. Rees DC, William Tn, Gladwin MT. Sickle cell disease. Lancet 2010 Dec 11;376:2018-31.
10. Mousa SA, Qari MH: Diagnosis and management of sickle cell disease Methods MOI BOI. 2010;663:291-307.
11. De Montalembert M. Management of sickle cell disease. BMJ 2008 sep8;337;doi:10.1136/bmj.a1397.
12. Rees DC. Hemoglobin F and hemoglobin E β-thalassemia J Pediatr Hematol Oncol 2000;22:567-72.
13. Maier-Redelsberger, M. Et al. Diagnostic Biologique des Maladies de l'Hemoglobin. Feuillets de Biologie 170, 1989.
14. Weatherall DJ. Clegg JB, Wood WG. Et al. Foetal crythropoiesis in human leukaemia. Nature 1975:257:710-2.
15. Rautonen J, Siimes MA. Initial blood fetal hemoglobin concentration is elevated and is associated with prognosis in children with acute lymphoid or myeloid leukemia Blut 1990;61:17-20.
16. Gumruk F, Tavil B, Balta G. Unal S. Gurgey A. Significance of fetal hemoglobin values in detection of heterozygotes in Fanconi anemia reevaluation of fetal hemoglobin values by a sensitive method J Pediatr Hematol Oncol 2008;30:896-9.
17. Rassiga-Pidot AL, Cornwell 3rd GG. Melntyre OR. Paroxysmal nocturnal hemoglobinuria with elevated fetal hemoglobin Blood 1974:43:233-8.
18. Balgir RS. Genetic epidemiology of the three predominant abnormal hemoglobins in India. J Assoc Physicians India 1996;44:25-28.(s)
19. WHO-EXECUTIVE BOARD EB118/5, 118th Session Report by the Secretariat on Thalassaemia and other haemoglobinopathies: Prevalence of Hemoglobinopathies. 11 May 2006; p.1-8. (s)
20. Balgir RS. The burden of hemoglobinopathies in India and the challenges ahead. Curr Sci:2000;79:1536-1547. (s)