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Original Article

Study of Prevalence of Hemoglobinopathies by HPLC in Central Gujarat

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Abstract
Objectives: *To evaluate Prevalence and geographical distribution of various hemoglobinopathies in central Gujarat by using HPLC.*
Method: *Hemoglobinopathies were evaluated by prospective study on BIORAD variant of HPLC (High Performance Liquid Chromatography) system in tertiary care hospital, central Gujarat. Out of 5624 cases received in our laboratotry 994 show sickling solubility test positive. A total 1203 samples were analyzed by HPLC for sickle cell and other suspected heamoglobinopathies at SSG Hospital over a period of 1 year from January 2016 to December 2016. The retention times (RT), proportion of hemoglobin (%) and the peak characteristics of all hemoglobin fractions were recorded. Many cases were advised for familial study.*
Results: *Among 5624 samples studied, abnormal hemoglobinopathies was seen in 1022(18.17%) cases, In which sickle cell trait (SCT) 681(12.10%), sickle cell disease (SCD) 272(4.83%), sickle thalassemia syndrome 38(0.63%), Beta thalassemia minor 20(0.35%), Beta thalassemia major 5 (0.09%), HBD Punjab 2(0.035%), Sickle HBD Punjab 2(0.035%), Sickle HBE 1 (0.017%), B-Thalassemia Intermediate 1 (0.017%).*
Conclusion: *In our study sickle cell trait and disease were the most prevalent hemoglobinopathies. Ethinicity wise distribution shows Rath was/Vasavas have high incidence of haemoglobinopathies .but many other casts have low incidence of haemoglobinopathies. So for effective control programme we should include all the casts. HPLC is a simple, accurate tool in the early detection and management of various hemoglobinopathies.*
Keywords: *HPLC, Hemoglobinopathies, Thalassemia, sickle cell trait and sickle cell disease.*

Introduction

Hemoglobinopathies are most common hereditary disorder which cause major public health problem in many parts of world including India. World health organization (WHO) figures estimate that 5% of world population is carrier for hemoglobin disorder.[1] The prevalence of B-thalassemia trait and sickle cell anemia in India varies between 3-17% and 1-44% respectively.[2]

The average frequency of HBS in India is 4.3% in India. The range varies from 0-44%. It is 0-18.5% in northeast zone, 0-33.5% in west zone, 22.5-44.4% in central zone and 1-40% in southern zone.[2]

There is genetical, ethnic and regional diversity in all the hemoglobin variants. So present study was undertaken to know the prevalence of hemoglobinopathies in Gujarat, located in the western part of India. Samples were analysed by HPLC which is a accurate tool in separation and quantification of various normal and abnormal hemoglobin fractions.

By this we can prevent the heterozygous hemoglobin variants like Thalassemia trait, Sickle cell trait interaction in patients which leads to serious homozygous states like thalassemia major, and sickle cell disease in offspring

Methods

Sickling solubility test was performed as a screening procedure on every sample prior to HPLC analysis.

Out of 5624 cases received in our laboratotry 994 show sickling solubility test positive. HPLC was performed on 1203 cases which were sickling solubility test positive and clinically suspicious for other hemoglobinopathies.

A total of 1203 cases were studied from Janauary 2016 to December 2016 at pathology department, Shree Sayaji General Hospital (SSGH), vadodara for hemoglobin variant analysis. 2 ml of intravenous blood sample was collected from all cases in EDTA vaccutainer. Details of clinical presentation, history of blood transfusion, family history was noted in all cases. Hemoglobin and red blood cell indices were measured on HORIBA automated five part differential cell counter. Peripheral smear examination and reticulocyte count study was done in all the patients.

All these samples were analyzed for hemoglobin disorders by BIORAD 'VARAIANT' HPLC machine. The instrument is based on High performance liquid chromatography principle. An HbA2/F Calibrator were analysed at the beginning of each run. Total area acceptable was between 1 to 3 millions.

The software delivers a printed report showing the chromatogram, with all the hemoglobin fractions eluted. The integrated peaks are assigned to the manufacturer –defined "windows" derived specific retention time (RT).[3]

The retention time is the time that elapses from the sample injection to the apex of elution peak of normal hemoglobin fraction and common variants.[4] (table 1)

If a peak elutes at a RT that is not predefined, it is labeled as unknown. Each analytical cycle, from sampling to printing of results takes about 6.5 minutes.

Table 1: Manufacturer assigned windows for Bio-Rad variant HPLC system

PEAK NAME	WINDOW(MIN)	RETENTION TIME(MIN)
F window	1.00-1.30	1.15
P2 window	1.30-1.60	1.45
P3 window	1.60-1.90	1.75
A0 window	1.90-3.30	2.60
A2 window	3.30-3.90	3.60
D window	3.90-4.30	4.10
S window	4.30-4.70	4.50
C window	4.90-5.30	5.10

Results

In our study of the total 5624 samples studied, we put HPLC on1203 samples, 1022 (18.17%) showed abnormal hemoglobin fractions. sickle cell trait constituted the largest subgroup of abnormal hemoglobinopathies in 681(12.10%) cases followed by sickle cell disease in 272(4.83%) cases. Distributions of various hemoglobinopathies in our study are shown in graph 1.

Of the total 5624 cases 528(9.38%) cases were male and 494 (8.78%) cases were female as shown in table 2. The patients with </=14 years of age were considered as pediatric patients. The age range of patients was from 1 day to 80 years.

Of the total 5624 cases 334 (5.93%) cases were of pediatric age and 688(12.23%)cases were in adult age group. An age wise distribution of patients with hemoglobinopathies is described in table 3.

Graph 1- Bar diagram showing distribution of hemoglobinopathies; majority of cases are of sickle cell trait followed by sickle cell disease.

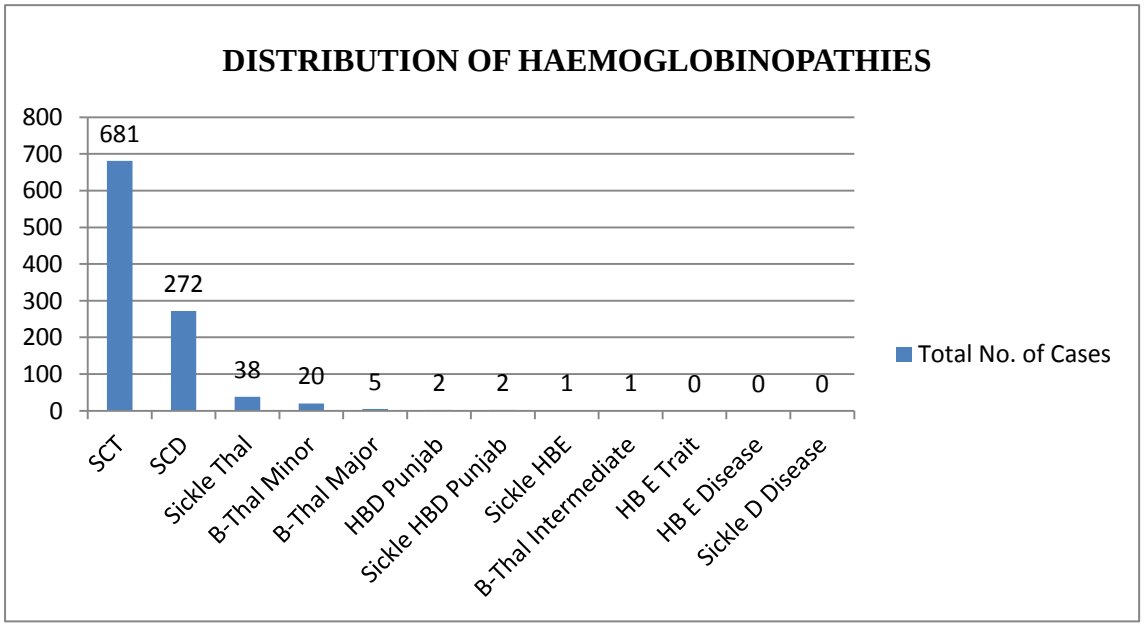

Table 2- Gender distribution of hemoglobinopathies

Haemoglobinopathies	Male	Female	Total	% out of 5624
SCT	327	354	681	12.10%
SCD	164	108	272	4.83%
Sickle Thal	20	18	38	0.68%
B-Thal Minor	11	9	20	0.35%
B-Thal Major	2	3	5	0.09%
HBD Punjab	2	0	2	0.035%
Sickle HBD Punjab	0	2	2	0.035%
Sickle HBE	1	0	1	0.017%
B-Thal Intermediate	1	0	1	0.017%
HB E Trait	0	0	0	0.00%
HB E Disease	0	0	0	0.00%
Sickle D Disease	0	0	0	0.00%
Total	528(9.38%)	494(8.78%)	1022	18.06%

Table 3- Age wise distribution of hemoglobinopathies

Haemoglobinopathies	Pediatric (<=14 yrs)	Adult (>14 yrs)	total	% out of 5624
SCT	194	487	681	12.10%
SCD	107	165	272	4.83%
Sickle Thal	17	21	38	0.68%
B-Thal Minor	7	13	20	0.35%
B-Thal Major	5	0	5	0.09%
HBD Punjab	0	2	2	0.035%
Sickle HBD Punjab	2	0	2	0.035%
Sickle HBE	1	0	1	0.017%
B-Thal Intermediate	1	0	1	0.017%
HB E Trait	0	0	0	0.00%
HB E Disease	0	0	0	0.00%
Sickle D Disease	0	0	0	0.00%
Total no. of Haemoglobinopathies	334 (5.93%)	688(12.23%)	1022	18.06%

Hb S homozygous (sickle cell disease) presents as separate S-Window with abnormal Hb S ranging from 60 to 90%. Sickling test was positive in all such cases. It constituted 272 (4.83%) cases. Hb S heterozygous (sickle cell trait) presents as an S-Window with abnormal Hb S ranging from 20 to 40% constituting 681(12.10%) cases.

There were 20 (0.35%) cases of beta thalassemia minor and 5(0.09%) cases of thalassemia major and 1 (0.017%) case of thalassemia intermedia.

Cut off level for HbA2 is taken as 4% for B-thalassemia trait and in patients with sickle cell trait/anemia along with B-thalassemia, It is taken as 5% as HbA2 level is somewhat higher in Sickle cell anaemia.

In our study Hb D-Punjab heterozygous were 2 (0.035%) cases. HPLC displayed a D Window with RT of 4.07-4.16 min.

Sickle HBD Punjab were 2 (0.035%) cases and sickle Hb E-sickle was 1 case (0.017%).

In our study Ethnic group distribution suggests that sickle cell trait and disease was commonly observed in following castes Rathwa 231 (22.60%) cases, Vasava 121 (11.80%), Bariya 83 (8.12%) cases, Parmar 82 (8.02%) cases as shown in table 4.

Table 4 Distribution of hemoglobinopathies among various caste/ethnic groups

Ethinicity	SCT	SCD	B-Thal Major	B-Thal Minor	Others	Normal	Total	%
Rathwa	148	68	0	0	2	13	231	22.60%
Vasava	86	20	0	0	2	13	121	11.80%
Bariya	42	27	0	0	3	11	83	8.12%
Parmar	33	22	0	2	11	14	82	8.02%
Muslim	36	10	0	6	5	22	79	7.73%
Tadvi	37	11	0	0	0	5	53	5.19%
Bhil	22	10	0	0	3	10	45	4.4%
Solanki	15	6	0	2	0	8	31	3.03%
Rathod	18	2	0	1	1	8	30	2.94%
Nayak	13	6	0	0	1	4	24	2.35%
Patel	14	6	0	0	2	1	23	2.25%
Chauhan	17	3	0	0	0	2	22	2.15%
Damor	7	6	0	0	2	2	17	1.66%
Bhabhor	8	4	0	0	1	0	13	1.27%
Vankar	5	5	0	0	1	2	13	1.27%
Bhilala	7	4	0	0	0	1	12	1.17%
Ninama	8	1	0	0	1	1	11	1.08%
Harijan	3	4	0	2	1	1	11	1.08%
Others	162	57	5	7	8	63	302	29.05%
Total	681	272	5	20	44	181	1203	100%

Discussion

HPLC has been shown to be a sensitive, specific, and reproducible alternative to electrophoresis. With automation and quantitative power, it appears to be a sensitive and accurate technique for direct identification and quantification of normal and abnormal haemoglobin fractions[5,6] .

Our study included predominantly central Gujarat population which belongs to western part of India. The gender distribution in our study 621(9.38%) cases were male and 582 (8.78%) cases were female. no gender preponderance was found in the studies which coincides with other studies by Archana et al and Sahu et al.[7,8]

In our study of total 1203 samples studied, most predominant hemoglobinopathy was sickle cell trait 681 cases (12.10%) followed by sickle cell disease 272 cases (4.83%) and sickle thalassemia syndrome 38 cases(0.44%). The highest frequency of sickle cell gene in India is reported in Orissa (9%), followed by Assam (8.3%), Madhya Pradesh (7.4%), Uttar Pradesh (7.1%), Tamil Nadu (7.1%) and Gujarat (6.4%).[9,10,11]

The high incidence of sickle cell trait requires need of premarital counseling and also antenatal screening to prevent sickle cell disease in off springs.

In our study the prevalence of beta thalassemia minor was 20 cases(0.35%), thalassemia major was 5 cases(0.09%) and b-thalassemia interme-diate was one case (0.017%). the patients of thalassemia major manifest in early age of life and require regular blood transfusion. Early detection of thalassemia major is important for early management and prevention of complications. Thalassemia trait is asympatomatic, but its detection is important to prevent thalassemia major in off springs.

In our study double heterozygous for sickle and thalassemia were 38 (0.68%) cases. The compound heterozygous disorders (Hb S D-Punjab, Hb S E, and Hb S-b thalassemia) or unusual variants (Hb Q India, Hb D Punjab, and Hb D Iran) are all clinically significant with varying degree of severity, making precise identification is important.[12,13] None of these can be conclusively identified by a single electrophoretic technique.[14] Definite identification usually requires DNA analysis

or amino acid sequencing. HPLC has been shown to be a sensitive, specific, and reproducible alternative to electrophoresis.[5]

HbD Punjab was prevalent in Punjab (northest India)frequency of 2%.[15] in Gujarat region its frequency drops one half. The HbD Punjab if heterozygous has no symptoms. if it is homozygous, rarest form of inheritance, is not commonly related to symptoms. But if HbD Punjab is associated with other heamoglobinopathies like thalesemia or sickle cell it gives symptoms. if HbD Punjab is associated with HbS then clinical manifestation is similar to HbSS. In our study there were two cases of double heterozygous sickle HbE.

In India, Hb S has been detected in more than 50 distinct subgroups predominantly tribals. In Gujarat also it is prevalent in tribes like Rathwa, Vasava, Bariya, Parmar solanki castes. But heamoglobinopathies can be found in any casts so premarital screening of all the population is very useful to prevent symptomatic haemoglobinopathies.

Screening is affordable and an accessible way to detect carriers, and can be offered in a range of settings in different societies: in high school, before marriage, or in antenatal clinics.

Conclusion

In our study sickle cell trait and disease were the most prevalent hemoglobinopathies. Ethinicity wise distribution shows Rathwas/Vasavas have high incidence of haemoglobinopathies .but many other casts have low incidence of haemoglobinopathies. So for effective control programme we should include all the casts. HPLC is a simple, accurate tool in the early detection and management of various hemoglobinopathies.

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