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Original Research Article

Spectrum of β-Thalassemia and Sickle Cell Anemia Diagnosed by Cation Exchange High Performance Liquid Chromatography in Different Communities of Mumbai District of Western India- A study from tertiary care hospital

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Abstract: Cation exchange high performance liquid chromatography (CE-HPLC) is an important tool in rapid diagnosis of a varied spectrum of Hemoglobinopathies. The aim of the study was to detect hemoglobin variants and hematological parameters in a tertiary care hospital in Mumbai district of Western India. A total of 130 blood samples were examined on the Bio-Rad Variant CE-HPLC system by β-thalassaemia short program. The concentrations of the haemoglobin variants (%), retention times and the peak characteristics for all hemoglobin fractions were recorded. Blood indices were measured on an automated haematology counter. Out of 130 samples tested in different communities, 69 (53%) were found to have normal HPLC pattern and used as controls. Total 61 (47%) subjects were found to be hemoglobinopathies. Among those having hemoglobinopathies, 2 (3%) had β- thalassaemia major, 20 (33%) β- thalassaemia trait, 1(2%) HbE/β- thal, 2 (3%) Hb-SS, 14 (23%) Hb-S trait and HbS/β- thal respectively. The percentage of sickle-thalassemia double heterozygous subjects was 23% and their number was 22. Other variants were also found and their number was 8. Haemogram of all the study subjects with hemoglobinopathies were altered as compared to those of controls and anisopoikilocytosis, hypochromia, and abnormal red cell distribution width (RDW) were found in majority of cases. Mean corpuscular volume (MCV) was generally normal to very low. In conclusion, CE-HPLC was found to be a simple, rapid and reliable method for the quantification of HbF, HbA_2, HbS and other hemoglobin variants for screening and confirmation of common hemoglobinopathies like sickle cell anemia, β- thalassemia. Abnormal hemoglobins as HbS HbA_2 and HbF very common in our study. Scheduled caste had the highest prevalence of hemoglobinopathies.
Keywords: Thalassemia, Sickle cell anemia, Cation exchange high performance liquid chromatography

INTRODUCTION

Hemoglobinopathies, the inherited disorders of hemoglobin (Hb) synthesis as result of various genetic defects in the formation of α or β globin chains [1]. Sickle cell hemoglobin (HbS)-related disorders are common in India [2,3]. Quantitative separation of hemoglobin into major and minor haemoglobin on cation exchange-high performance liquid chromatography (CE-HPLC). CE-HPLC is increasingly being used as a first line of investigation for hemoglobinopathies and thalassemias [4].

In India, due to sociocultural practices, early marriages among individuals of the same caste or in close blood relation and this makes it important to know the prevalence of sickle cell anemia and β-thalassemia in different groups. The aim of the present study was to evaluate the utility of CE-HPLC in the diagnosis of hemoglobinopathies like sickle cell anemia, β-thalassemia in a tertiary care hospital in Mumbai district of Western India. We also tried to assess the hematological parameters in the study population.

MATERIAL AND METHODS

This study was carried out at Department of Biochemistry, Grant Medical College and Sir J.J. Group of Government Hospitals, Mumbai. One hundred and thirty (130) anemic study subjects in the age group of

18 to 35 years were studied for hemoglobinopathy screening over a period of May 2011 to August 2011.

Clearance of Institutional Ethical Committee

This study was approved by the Institutional Ethical Committee of Grant Government Medical College and Sir J. J. Group of Government Hospitals, Mumbai (vide letter No. IEC/Pharma/1133/2011, dated – 04/10/2011). Written consent was obtained from all patients enrolled in this study. These cases were taken from the wards and outpatient department of Sir J. J. Group of Hospitals and samples were received at Postgraduate Laboratory, Department of Biochemistry, Grant Government Medical College and Sir J. J. Group of Government Hospitals, Mumbai

Selection of study population

A total 130 anemic individuals were tested including all major castes and sub castes, attending hemoglobinopathies screening held at the our department. A data collection form was used to get information on the caste/ethnic group, linguistic group and religion, consanguinity in the family, any family history of blood disorders as well as to record all the laboratory findings.

Inclusion and exclusion criteria

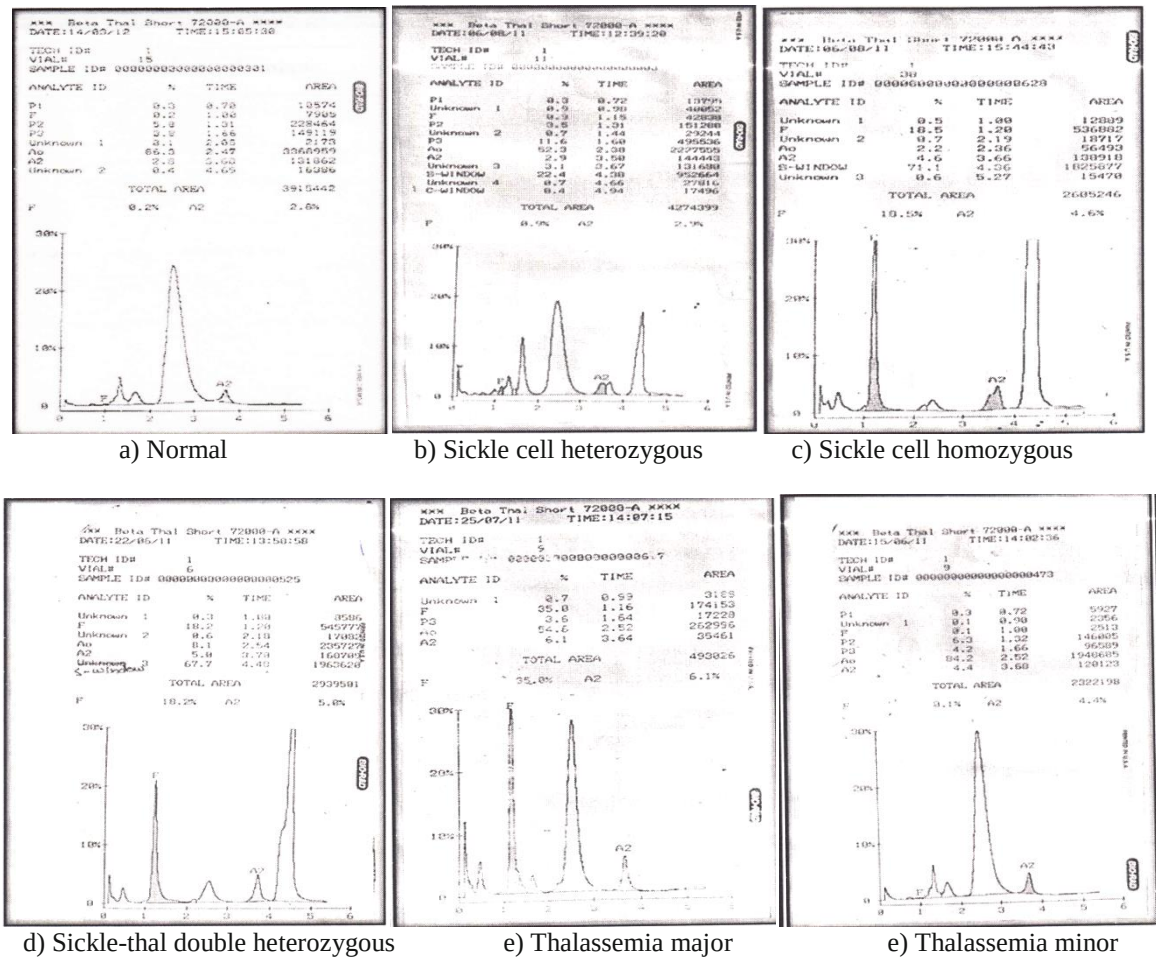
Study group included the subjects either sex, willing to give written informed consent and age group 18 to 35 years were included in this study. Study case suffering from cancer, severe infections like human immune virus, hepatitis B and C, syphilis etc, were excluded from the study.

Blood sample collection

About 5mL of intravenous blood sample was collected from study subjects in sterile lavender colored cap tube containing ethylene diamine tetra acetic acid (EDTA) as an anticoagulant. Blood sample was stored for 7 days at 2-8^{o}C.

Hematological analysis

Anti-coagulated blood sample used for hematological investigations. Blood indices were measured on a haematology counter [5]. Hemoglobin variants were quantitated by CE-HPLC using the Hb-Variant testing system (Bio-Rad Laboratories, Hercules CA, USA) using variant β-thalassemia short program [6, 7]. Major hemoglobinopathies are shown in Figure 1.



a) Normal b) Sickle cell heterozygous c) Sickle cell homozygous

d) Sickle-thal double heterozygous e) Thalassemia major e) Thalassemia minor

Fig-1: Chromatogram of normal and abnormal hemoglobin variants

Statistical analysis

Statistical calculations were performed with GraphPad prism for windows (version 5.0, GraphPad Software Company). To compare the differences of the relative changes of parameters, the normal distribution *Z*-test and χ^2–test were used. In this analysis, variables showing 'p' value less than 0.05 and 0.001 were considered to be statistically significant and highly significant respectively.

RESULTS

In this retrospective study, a total of 130 cases were studied for 3 months long period. Out of these patients, 70 had normal HPLC pattern and rest 60 had some abnormality. *Retention times, band, and window of hemoglobin variants* are shown in the Table 1.

Table 2 shows significantly higher prevalence of abnormal hemoglobin in the group of III^{RD} month as compared with remaining two month group by χ^2 test ($p<0.001$).

Comparisons of hematological parameters between the subjects with abnormal Hb-variants and normal Hb-variants are shown in Table 3. Statistical significant differences were found in between the two groups.

In the Table No-3 depicts statistically significant prevalence in the group of β-Thal trait as compared with other group of hemoglobinopathy and control population by χ^2 test ($p<0.001$). The levels of Hb-variants in subjects with hemoglobinopathy were altered significantly ($p<0.001$) as compared with normal controls are shown in Table No-4.

Table 1: Bio-Rad assigned analyte identification window

Analyte Name	Retention Time (Minutes)	Band (Minutes)	Window (Minutes)
F	1.15	0.15	1.00 – 1.30
P2†	1.45	0.15	1.30 – 1.60
P3†	1.75	0.15	1.60 – 1.90
A_o	2.60	0.40	2.20 – 3.20
A_2	3.83	0.15	3.68 – 3.98
D-Window	4.05	0.07	3.98 – 4.12
S-Window	4.27	0.15	4.12 – 4.42
C-Window	5.03	0.15	4.88 – 5.18
† P2 and P3 are minor peaks associated with hemoglobin A			

Table 2: Total number of anemic subjects studied for hemoglobinopathies screening.

Period	Total subjects (n= 130)	Total Male (n=35)	Total Female (n=95)	Normal subjects (n=70)	Hemoglobinopathies Diagnosed (n=60)
I^{ST} Month	39	08	31	24	15
II^{ND} Month	30	11	19	18	12
III^{RD} Month	61	16	45	33*	28
*P <0.001					

Table 3: Hematological parameters in relation to normal and abnormal Hb-variants.

Diagnosis	n (%)	Hb (g/dL)	MCV (fl)	MCH (pg)	RBC Count (Mill/C.mm)	RDW (%)
Normal	69 (53)	11.2±1.19	89.9±5.27	29.3±3.21	4.71±0.63	42.7±1.79
Hb-SS	02 (03)	6.45±2.11*	81.9±2.31*	25.5±3.21*	2.42±0.43*	62.3±3.94*
Hb-S trait	14 (23)	11.2±3.02	79.6±13.4*	23.7±6.27*	4.37±3.78	47.8±4.81*
HbS/β-thal	14 (23)	9.21±2.64*	75.6±3.94*	24.6±3.67*	3.45±0.69*	47.5±3.96*
β-Thal major	02 (03)	5.92±2.31*	71.2±4.53*	20.3±3.54*	2.82±0.84*	50.9±9.78*
β-Thal trait	20 (33)*	9.9±3.20*	62.3±6.21*	18.2±3.52*	4.39±0.82	33.5±7.57*
HbE/β-thal	01(02)	9.5±0.00*	75.5±0.00*	24.9±0.00*	4.22±0.00	45.6±0.00*
Window P3	07 (12)	14.6±2.12*	84.6±3.98*	29.3±2.86	4.77±0.67	43.3 ± 1.08^{NS}
*P<0.001, NS= Not significant						

Table 4 : Comparison of Hb-variants between normal and subjects with hemoglobinopathies.

Hb Variants (%)	**Normal (n=70)**	**Hemoglobinopathies (n=60)**	'P' Value
A0	96.6±1.47	68.9±27.8	0.001
F	0.42±0.94	4.95±12.3	0.001
A2	2.84±0.38	2.92±0.28	0.001
S	0.00±0.00	34.3±20.9	0.001
P3	0.00±0.00	15.2±3.07	0.001
Normal distribution "Z" value greater than 3.50			

DISCUSSION

Hemoglobinopathies are related with the abnormal protein molecule of red blood cells. This abnormal protein molecule is due to defective synthesis of globin chains or its structure. A genetic defect that results in abnormal structure of one of the globin of the hemoglobin molecule is termed as hemoglobinopathy. These inherited genetic diseases of hemoglobin are controlled by a single gene and are transmitted from generation to the next [8].

The high prevalence rate of hemoglobinopathy could cause serious health and social problems among the anemic pregnant women and children in the Mumbai region. The prevalence of hemoglobinopathies varied from 5 to 26.7 % among the different communities/caste/ethnic groups in this study and the prevalence of β-thal trait was higher in our study cases. A large study done among the scheduled caste and tribe in central and eastern India [9]. In this study scheduled caste had the highest prevalence of hemoglobinopathies 16 (26.7 %) .We found significant difference between levels of abnormal hemoglobin variants in sickle cell anemia and β-thalassemia patients, when we compared with other normal variants**.**

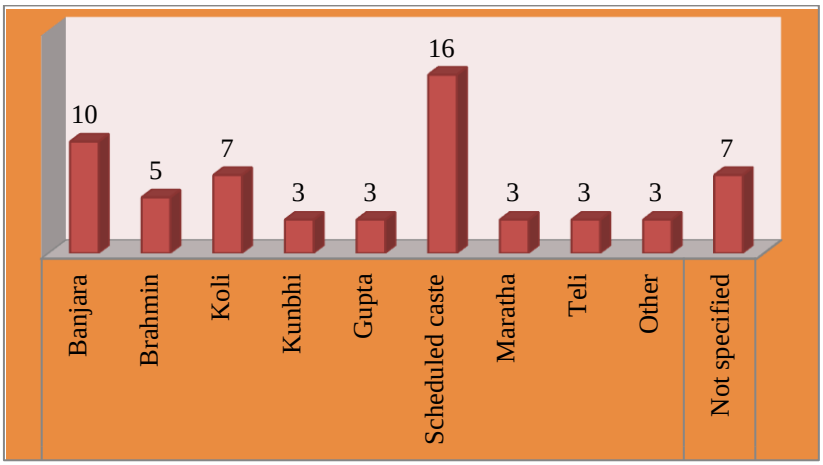


Fig-2: Distributions of subjects with hemoglobinopathies religion-wise

The incidence of β-thalassemia, sickle cell anemia and other hemoglobinopathies in different religious groups from the Mumbai city is shown in Figure No-2. Scheduled caste had the highest prevalence of hemoglobinopathies 16 (26.7 %) followed by Banjara 10(16.7%).

In India, many population groups have been screened and the sickle cell gene has been shown to be prevalent among three socio-economically disadvantaged ethnic groups, the scheduled tribes, scheduled castes and other backward classes [10-13]. Studies in different Indian regions have reported 1% to 17% prevalence of β-thal trait with mean of about 3.3% [14,15]. Comparable prevalence of 33% of β-thal trait was found in our anemic and suspected study subjects. Madan et al. showed in ICMR multi-center study reported 2.68% β-thal trait prevalence in Mumbai [16]. While in our study, we found 10 times higher than values reported by Madan *et al.*

The present study included large number of pregnant women and their family members. This is the short term community-based study done systematically in Mumbai district of Maharashtra, India. Our department and Hospital is a established centre for education, screening and counseling of people with different religion, caste, sub-caste and sociocultural practices. All the related facilities are available with free of cost in our department and we are conducting Sickle Cell Anemia Control Project.

CONCLUSION

CE-HPLC was found to be a simple, rapid and reliable method for the quantification of HbF, HbA_2, HbS and other hemoglobin variants for screening and confirmation of common hemoglobinopathies like sickle cell anemia, β-thalassemia. Abnormal hemoglobins as HbS HbA_2 and HbF very common in our study. Scheduled caste had the highest prevalence of hemoglobinopathies. Anisopoikilocytosis, hypochromia, and abnormal red cell distribution width were found in majority of cases. Mean corpuscular volume was generally normal to very low.

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