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Study of Hemoglobin Disorders by HPLC at Tertiary Care Centre - 2 Year Analysis

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Abstract

Background: *The inherited disorders of blood include hemoglobinopathies as one of the major public health problems in India. This study indicates type of hemoglobinopathies in a tertiary care hospital over a period of 2 years* ***Methods:*** *A total of 269 cases between January 2017 to December 2018 were taken up for retrospective analysis and age of the patient ranged from 1 year to 63 years. Hemoglobin and Red Blood Cell indices were measured. Peripheral blood smears examination and reticulocyte count was done in all the cases along with sickling test in suspected cases of sickle cell anemia. All these samples were analyzed for hemoglobin disorders by HPLC.* ***Results:*** *Out of these 269 cases, 171(63.6%) cases displayed abnormal hemoglobin pattern on HPLC of which 72 cases were males (42.1) and 99 cases were females (57.8). Most common hemoglobinopathy observed was β Thalassemia trait 70 (26%) followed by Sickle cell trait 64 (23.8%), Sickle Homozygous 22 (8.2%), Sickle thalassemia 4 (1.5%) and raised HbF 11 (4.1%).* ***Conclusion:*** *Screening of all anaemic patients should be done for hemoglobinopathies and thus HPLC remains an accurate and reliable method for quantification of hemoglobin variants in screening of large population.*

Keywords*: Hemoglobinopathies, β Thalassemia, Sickle cell trait*

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Introduction

The hemoglobinopathies are a group of inherited disorders and is one of the major public health problems in India. WHO figures estimate that about 5% of the world population is carrier for Hb disorders [1]. The carrier frequency of hemoglobinopathy varies from 3 to 17% in different population groups of India[2]. Thus, there is a tremendous amount of burden of hemoglobinopathies in India. Beta (β)-thalassemia and sickle cell disease represent the most frequent hemoglobinopathies. The prevalence of beta Thalassemia trait and sickle cell in India varies between 3-17% and 1-44% respectively[3,4]. The thalassemias are an autosomal recessively inherited group of disorders of hemoglobin synthesis characterized by the absence or reduction in output of one or more of the globin chains of hemoglobin. The structural variants result from substitution of one or more amino acids in the globin chains of the hemoglobin molecule[5,6]. Hemoglobin A_2 constitutes less than 3% of adult hemoglobin (Hb) which has no physiological importance. On the other hand, determination of HbA2 levels is important tool in diagnosis of Beta Thalassemia trait, sickle- beta thalassemia and HbE variants[7,8]. Laboratory diagnosis of hemoglobin disorders is required for confirmation of provisional diagnosis of sickling disorders and thalassemias, explain hematological abnormalities and permit genetic counselling of prospective parents.

The use of cation exchange high performance liquid chromatography (CE-HPLC) to separate and quantify various normal and abnormal Hb fractions has been increasing [9,11]. It offers a reliable tool for early, accurate detection; thereby aiding in prevention and management of various hemoglobinopathies [10,11]. The present study highlights the detection of the hemoglobinopathies and thalassemias by HPLC on the Bio-Rad D-10 analyzer. This study is a two-year study, carried out in Prathima Institute of Medical Sciences, a tertiary care hospital in Karimnagar, and included all patients who had a clinical or familial suspicion of hemoglobinopathy and a hemoglobinopathy work up was ordered for diagnostic purposes.

Materials and Methods

2 ml EDTA Blood samples were collected in clinical hematology laboratory. Details of clinical examination, history of blood transfusion, family history and consent were taken in all cases. Hemoglobin and Red Blood Cell indices were measured on automated - five-part differential cell counter using well mixed anticoagulated blood. Peripheral blood smears examination and reticulocyte count was done in all the cases along with sickling test in suspected cases of sickle cell anemia. All these samples were analyzed for hemoglobin disorders by BIORAD D-10 HPLC machine. An HbA2/F calibrator and two levels of controls (BIO-RAD) were analyzed at the beginning of each run. The total area acceptable was between 1 and 4 million. The printed chromatogram delivered by software shows all the hemoglobin fractions eluted, the retention times, the areas of the peaks and the values (%) of different hemoglobin components. The integrated peaks are assigned to manufacturer defined "windows" derived from specific retention time (RT). This RT is the time that elapses from the sample injection to the apex of the elution peak, of normal hemoglobin fraction and common variants [Table 1]. If a peak elutes at a retention time that is not pre- defined, it is labelled as an unknown. Each analytical cycle, from sampling to printing of results takes about 6.5 minutes. HbA2 of over 3.9% was taken as cut of value for diagnosis of β-thalassemia trait [11-14].

History of consanguinity was noted and Family study of cases was carried out wherever possible, to confirm the diagnosis as family study is effective for centers which do not have facility for genetic analysis. Mother, father, siblings, son and daughter of patient were studied.

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

Peak name	Retention Time (minutes)	Window (minutes)
A1a	0.21	0.16-0.26
A1b	0.3	0.24-0.36
F	0.52	0.42-0.62
LA1c/CHb-1	0.775	0.62-0.93
LA1c/CHb-2	0.795	0.66-0.93
A1c	0.87	0.70-1.04
P3	1.43	1.23-1.63
A0	1.7	1.55-1.85
A2	3.15	2.80-3.50
S	4.16	4.02-4.30
C	4.75	4.65-4.85

Results

A total of 269 cases from January 2017 to December 2018 were taken up for retrospective analysis and age of the patient ranged from 1 year to 63 years (Table 2). Out of these 269 cases, 171 cases displayed abnormal hemoglobin pattern on HPLC of which 72 cases were males (42.2) and 99 cases were females (57.8) thus having female preponderance (Table 3). Mean values of hematological parameter of these cases is shown in Table 4 and mean values of hemoglobin fractions in all groups are shown in Table 5. The major abnormality observed in thalassemia cases was high HbA_2. A cut of over 3.9% was taken for diagnosis of Beta Thalassemia Trait. A total of 70 cases of beta Thalassemia trait were diagnosed (Fig. 1). The retention time for HbA_2 ranged between 2.80 to 3.50 minutes. Peripheral blood smear showed microcytosis, hypochromia and target cells.

HbS homozygous presents as separate S-window with abnormal Hb ranging from 50-90% which constituted 22 cases (8.2%) and in HbS heterozygosity abnormal Hb range was 30 - 40 % constituting 64(23.8%). There were 4 cases diagnosed as double heterozygous for HbS-BTT.

Table 2: Type of hemoglobin

Hemoglobin pattern	Patient (%), n=269
Normal Hb pattern	36.4% (98)
Beta Thalassemia trait (BTT)	26.0%(70)
Thalassemia major	0
Hb S Homozygous	8.2%(22)
Hb S Heterozygous	23.8%(64)
HPFH	0
Raised Hb F	4.1%(11)
Sickle thalassemia	1.5%(04)

Table 3: Sex-wise distribution

Group	Male	Female	Total
Beta Thalassemia trait (BTT)	25	45	70
Hb S Heterozygous	31	33	64
Hb S Homozygous	10	12	22
Sickle thalassemia	1	3	04
Raised HbF	5	6	11

Table 4: Haematological parameters in different group of hemoglobinopathies

Hemoglobinopathies (n)	Hb (g/dl) mean±SD	RBC count (million/cmm) mean±SD	MCV (fl) mean±SD	MCH(pg) mean±SD	MCHC(g/dl) mean±SD
Beta-thalasssemia trait (BTT)	7.8±1.48	4.4±1.3	64.58±8.46	22.3±5.14	27.6±7.04
HbS heterozygous	8.3±1.6	4.2±0.53	68.01±5.2	19.25±2.02	32.13±2.3
HbS Homozygous	6.64±1.16	3.8±1.58	70.14±5.62	24.42±4.25	29.57±2.84
Sickle -thalassemia	7.32±1.86	4.1±1.47	70.33±8.85	24.22±4.19	29.34±3.23
Raised HbF	4.9±1.7	4.2±1.2	71.13±5.7	28.10±4.7	35.76±5.4

Table 5: Mean Values of hemoglobin fractions of Hb disorders on HPLC

GROUP	HbF	HbS	HbA_2
Beta-thalasssemia trait (BTT)	1.38± 1.16	-	5.19 ± 0.8
HbS heterozygous	1.09 ± 0.89	34.74±2.63	2.38± 1.04
HbS Homozygous	18.3± 6.56	70± 7.7	0.88 ± 1.14
Sickle -thalassemia	0.85 ± 0.63	33 ± 3.7	4.47 ± 0.28

Discussion

HPLC has been shown to be a sensitive, specific and accurate technique for direct identification and quantification of normal and abnormal hemoglobin fractions[10, 15-17].

There are few studies from India which evaluated and emphasized the role of HPLC for diagnosis of thalassemia and various hemoglobinopathies [6, 12, 17].

In our study we found that total of 171 (63.6%) cases have abnormal hemoglobin fractions. Beta thalassemia trait formed the largest subgroup of abnormal Hb 70(26%) cases. High incidence of traits underscores the need for antenatal screening for prevention of thalassemia major in offspring.

HbS homozygous presents in 8.2% as an S-window with abnormal Hb ranging from 50-90%. Sickling test was positive in all such cases, whereas HbS heterozygous presents in 23.8% as an S-window with abnormal Hb ranging from 30 -40%. Double heterozygous HbS+ β Figure 4: Sickle/ Beta thalasssemia constituted 4 cases with 1.5%.

This compares with other studies [3, 17, 18] (Table- 6) where β thalassemia trait was noted to be common disorder. In the study by Bhokare SB[8] sickle cell

trait was noted to be common as it is a hospital-based study but not community-based study.

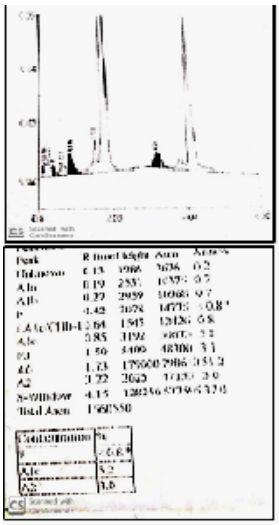

Figure1: Sickle cell trait

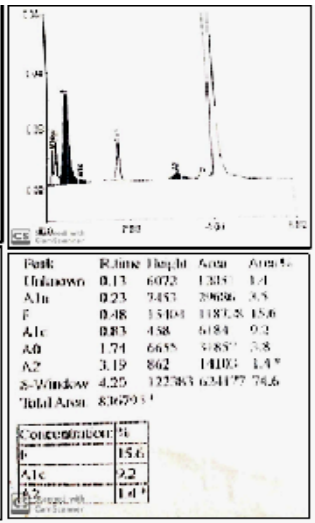

Figure 2: Sickle cell disease

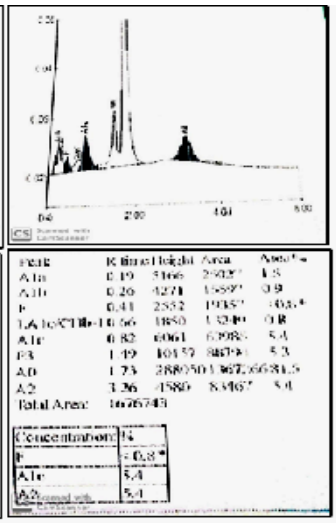

Figure 3: Beta thalassemia trait

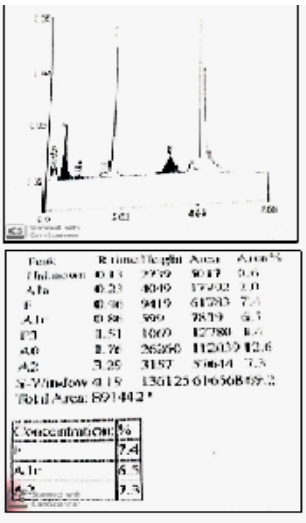

Figure 4 : Sickle/ Beta thal

Table 6: Comparison with other studies

Study	Our	Sachdev et al.	Jignasa N et al.	Rao et al.	Bhokare SB et al.
Beta-thalasssemia trait (BTT)	26	8.9	5.2	18.1	6.6
HbS heterozygous	23.8	-	1.2	1.4	18.8
HbSHomozygous	8.2	0.03	-	0.5	2.8
Sickle -thalassemia	1.5	0.07	-	0.8	5.4

In addition, some cases (n=11) showed isolated raised HbF, where no other abnormality related to hemoglobinopathy was found and family study was negative. Anemia is considered a major public health problem in India[19,20]. Moderate to severe degree of anemia was seen in majority of our patients. Low hemoglobin concentration is a result of many factors such as malnutrition, hemorrhagic conditions or hereditary conditions such as hemoglobinopathies[3]. It is difficult to set cut-off values in the red cell indices for these disorders as suggested by some authors[20,21]. These facts help us in using new techniques for early detection, prevention and treatment of this disease.

Clinicians are attuned to ask for an Hb variant analysis for a cause of anemia or coexistent hemoglobinopathy. History of recent blood transfusion must be also being soughed along with correct age so as to aid in an accurate diagnosis.

Conclusion

Hemoglobinopathies exert significant burden on various developed and developing countries of world including India and therefore adequate measures and screening procedures should be adopted to reduce this burden. All anemic patients should be subjected for screening for hemoglobinopathies and also a routine premarital screening program should be done for identification and prevention of high –risk marriages inorder to prevent psychological trauma of bearing a transfusion –dependent child for life.And thus, HPLC remains an accurate and reliable method for quantification of hemoglobin variants in screening of large population.

Conflict of Interest: *None declared*
Source of Support: *Nil*
Ethical Permission: *Obtained*

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