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Hemoglobinopathies by High Performance Liquid Chromatography, 2 years 5 Months Study of 899 Cases in A Teaching Institute

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Abstract

Hemoglobinopathies are the most common genetic disorder with higher incidence in Middle East and Indian Subcontinent as per WHO. Hemoglobin electrophoresis carried out by High Performance Liquid Chromatography is useful method for screening and diagnosing hemoglobinopathies in developing countries like India where resources are limited. It has an advantage of quantifying HbF and HbA2 along with detecting other variants in single screening test. Retrospective study from July 2019 to December 2021 was done which included 899 cases. Complete Blood Count parameters and peripheral smears were compared with the chromatographs in all patients. The study showed prevalence of Beta Thalassemia trait in females in the age group of 21 to 40 years. Two cases showed compound heterozygous for HbS and Beta Thalassemia. Other cases of hemoglobinopathies in our study showed Sickle cell trait, Sickle Cell homozygous, Beta thalassemia major, HbD trait, HbE trait. High performance Liquid Chromatography is rapid, sensitive, specific, reproducible, less time consuming and require less man power. RBC indices, HPLC finding and family study are sufficient to detect and manage most of the

hemoglobin variants prevalent in India. However one should be aware of limitations associated with the diagnosis to avoid false negative results. Genetic studies are indicated to confirm borderline cases.

Keywords: Hemoglobinopathies, Hemoglobin Electrophoresis, High Performance Liquid Chromatography.

Introduction

Hemoglobinopathies are the most common genetic disorder with higher incidence in Middle East and Indian Subcontinent as per WHO[1,2]. These are autosomal recessive disorders of haemoglobin synthesis (thalassemia) or structure (sickle cell disorder) that are responsible for significant morbidity and mortality on a worldwide scale[3,4]. Individuals with trait (carriers) are healthy and unaware of their carrier status unless specifically screened. If couple both carry a clinically significant hemoglobinopathy trait, there is 25% chances in each pregnancy that their child will inherit a major hemoglobinopathy. The most effective approach to reduce this burden of the society is to reduce the incidence by implementation of carrier screening programme. Hemoglobin electrophoresis carried out by HPLC is useful method for screening and diagnosing hemoglobinopathies in developing countries like India where resources are limited. It has an advantage of quantifying HbF and HbA2 along with detecting other variants in single screening test. It is an automatic system with sample preparation, rapid analysis, better resolution and accurate identification of Hb variants.

Materials and methods

It was a Retrospective study carried out from July 2019 to December 2021 in the Department of Pathology, K. J. Somaiya Medical College and Research Center**.** Total cases were 899 in which ANC cases, patients with clinically suspected hemoglobinopathies, family members of patients with hemoglobinopathies were included. Hb electrophoresis was carried out on BIORAD-D 10 machine and CBC was processed on NIHON KODEN 5 part Analyser. Study included CBC parameters and peripheral smears comparison with the chromatographs in all patients.

Each Chromatograph shows peaks of HBA0, HBA2 and HBF along with C window, D window and S window with two minor peaks P2 and P3. As many HB variants are eluted in the same window, they are diagnosed considering retention time, peak areas and ethnicity of patients. The retention time for HBA2 is 3.30-3.90 minutes.

For diagnosing Beta Thalassemia Trait**,** HBA2 value should be in the range of 3.9-9 %, for Beta Thalassemia Major, HBF should be 30-90% or more; for

Sickle Cell Heterozygous, HBS should be 30-40%; for Sickle Cell Homozygous , HBS should be 70-90%; for HbD Punjab Heterozygous**,** abnormal HB (30-45%) should be eluted as unknown window with retention time of approximately 3.8 minutes, for HbE Heterozygous, abnormal HB(30%)should be eluted in the HBA2 window[5].

Results

Out of 899 cases, 20 cases were of males and 879 cases were of females. 5 out of 20 male cases and 74 out of 879 female cases were showing abnormal haemoglobin variants. This is pie diagram (chart 1) showing sex wise Distribution of haemoglobin variants.

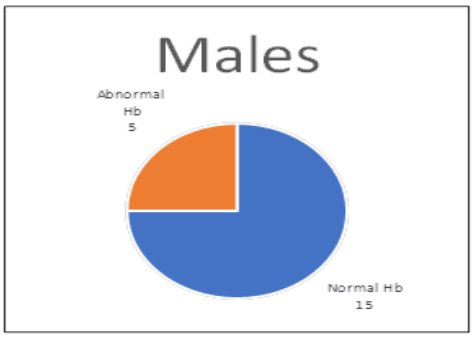

Chart 1:

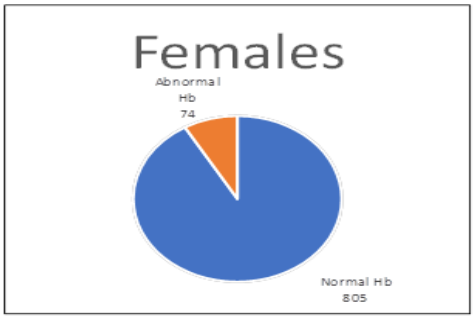

Chart 2:

Most of the cases of abnormal Hb variant were in the age group of 21-40 years as most of the cases in our study were referred from ANC OPD visits. This is the table showing age-wise Distribution of Abnormal Hb variant (Table1).

Table 1:

Age Group in years	Number of Cases	Percentage of cases %
0-20	06	7.59
21-40	68	86.07
41-60	04	5.06
61-80	01	1.26

Amongst abnormal hemoglobin variants pattern, percentage of cases of Beta Thalassemia trait were more in our study. This is the table (Table 2) of Hemoglobin pattern among study subject.

Table 2:

HB pattern	Number of cases	Percentage of cases %
Normal Hb pattern	820	91.21
Beta Thalassemia Trait	52	5.78
Thalassemia Major	01	0.11
Sickle Cell Homozygous	02	0.22
Sickle Cell Heterozygous	08	0.88
Hb D Heterozygus	03	0.33
Hb E Heterozygous	01	0.11
Compound Heterozygous for HbS & Beta Thalassemia	01	0.11
Borderline A2	11	1.22

Amongst the beta thalassemia trait (BTT), maximum cases were having hemoglobin in the range of 7-9 gm/dl and maximum cases showed MCV <82 fl, MCH < 27 pg and MCHC <32%. (Table 3) and (Table 4) show hemoglobin value of BTT and RBC indices of patients with BTT respectively.

Table 3:

Hemoglobin Value	Number of cases	Percentage of cases %
< 7 gm/dl	0	0
7-9 gm/dl	33	63.46

9-10 gm/dl	15	28.84
> 10 gm/dl	04	7.69

Table 4:

RBC Indices	Number of cases	Percentage of cases %
MCV		
<82 fl	43	82.69
82-92 fl	05	09.61
> 92 fl	04	07.69
MCH		
< 27 pg	43	82.69
27-32 pg	05	09.61
> 32 pg	04	07.69
MCHC		
< 32 %	46	88.46
32-37 %	06	11.53

These were the chromatograms of Hb variants from our studies in which figure 1 showed borderline HbA2 as HbA2 value is 3.6%. Figure 2 showed Beta thalassemia trait as HbA2 value is 4.4%. Figure 3 is sickle cell heterozygous as S-window shows 31.9%.

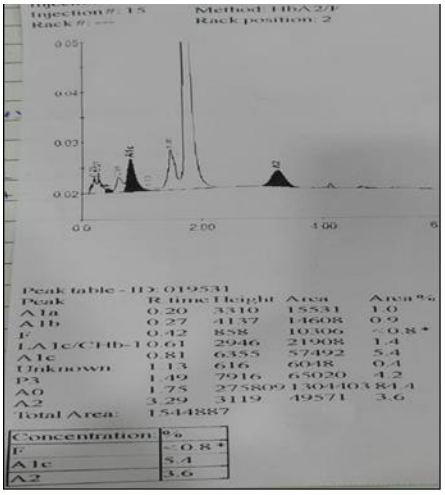

Figure 1: Borderline HbA2

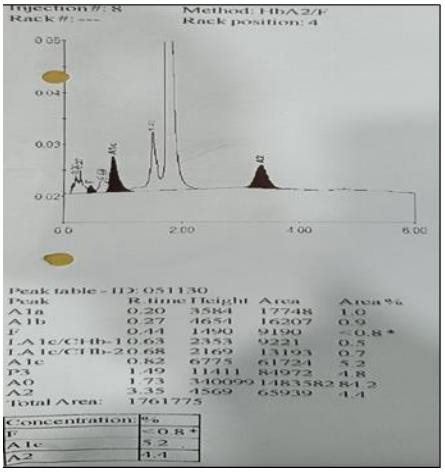

Figure 2: BTT

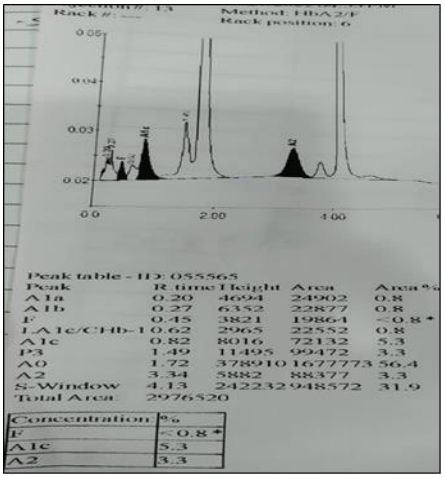

Figure 3: Sickle Cell Heterozygous

Figure 4 is sickle cell homozygous as S-window shows 77.8%. Figure 5 is HbD Punjab heterozygous as unknown window of 32.5% with retention time of 3.94

minutes. Figure 6 is HbE heterozygous as abnormal Hb eluted as 25.7% in HbA2,

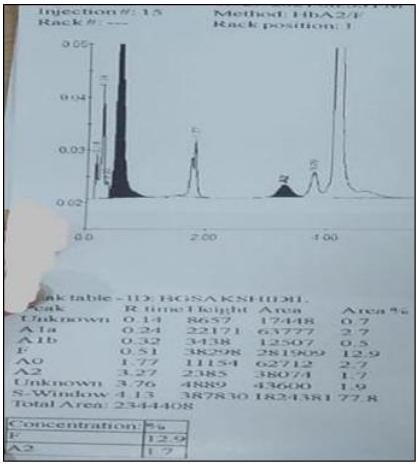

Figure 4: Sickle Cell Homozygous

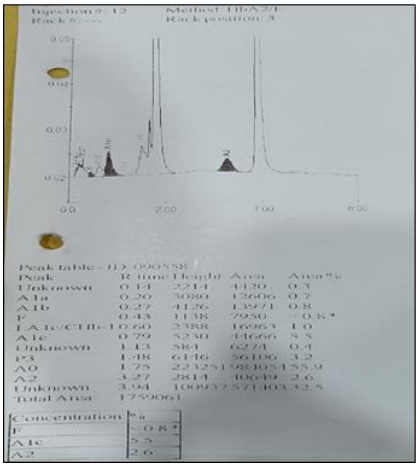

Figure 5: HbD Punjab Heterozygous

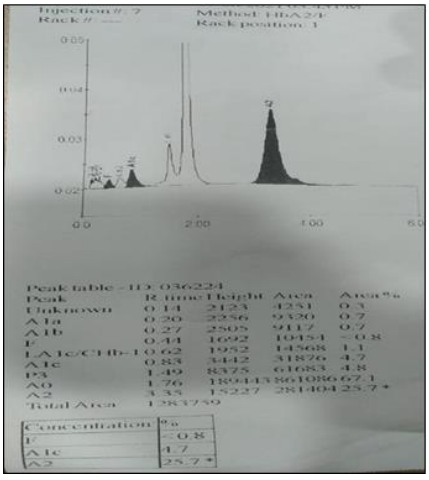

Figure 6: HbE Heterozygous

Figure 7 and figure 8 show picture of peripheral smears of sickle cells of sickle cell anemia and target cells of thalassemia respectively.

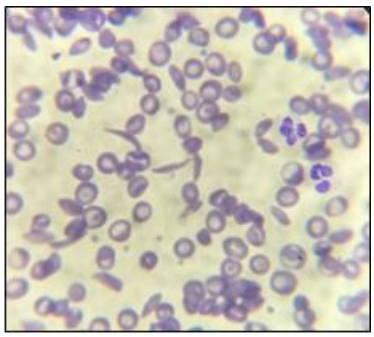

Figure 7: sickle cells

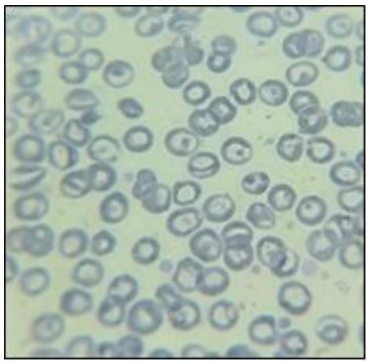

Figure 8: target cells

Discussion

Disorders of hemoglobin are most common gene linked disease in the world[1,8]. Total number of cases included in our study were 899 out of which 879 (97.7%) were females and 20 (2.22%) were males. Age-wise distribution of hemoglobinopathies showed 68 (7.56%) cases were in the age group of 21-40 years. This female predominance and more number of cases in the age group of 21-40 years are because of most of the patients were referred from antenatal OPD screening. Among 79 abnormal Hb variants, 52 cases (65.8%) were of Beta Thalassemia Trait comparable with Sharma et al studies (66.6%)[13] followed by 8 cases (10.1 %) sickle cell heterozygous, 2 cases (2.5%) of Sickle cell Homozygous comparable with Chauhan et al studies (13.3%)[12], 3 cases(3.8%) of HbD heterozygous, and remaining 1 case

(1.2%) of each Thlassemia major, Hb E heterozygous and compound heterozygous for HbS and Beta Thalassemia.

Table 5:

Hb Variants	Present study (n=79)	Chauhan et al (n =49)	Sharma et al (n=21)
BTT	65.8	81.6	66.6
SCD	12.6	13.3	4.7
HbD trait	3.8	1.66	14.2
THAL major	1.2	1.66	-
HBE trait	1.2	1.66	4.7
Coumpound heterozygous for HbS and Beta Thalassemia	1.2	-	-

Thalassemia and sickle cell anemia are the most severe forms of genetic disorders and hence are of great importance to be dealt with from public health point of view in India. Screening of Beta thalassemia trait is important because if couple with both having traits decide a family, there is one in four chances that their child could inherit beta thalassemia major, one in four of a child being normal and one in two chance of the child also being a carrier[11]. In our study, Borderline A2 cases were 11(13.9%). Antenatal patients having borderline or suspicious results, whose partner has the one or the other hemoglobinopathy or is having borderline results should be referred for the genetic counselling and testing[9]. In all borderline A2 cases, genetic studies with DNA analysis are advised after thorough workup with CBC, RBC indices, peripheral smears, iron studies and work up for megaloblastic anemia. Concomitant beta thalassemia trait and iron deficiency which is more common in children and pregnant woman are difficult to diagnose on HPLC as iron deficiency tends to reduce HbA2 according to some studies[6,7]. Hence simultaneous iron studies along with HPLC can be used for screening of borderline cases[8]. Similarly, Megaloblastic anemia also cause false elevation HbA2 values. Refractory hypochromic microcytic anemia with borderline or reduced HbA2 on HPLC may be investigated for alpha thalassemia by molecular genotyping to avoid unnecessary iron therapy. Follow up is required if HbF value is in between 5-10% with MCH <27 pg or HbF over 10%, as it could be beta thalassemia inermedia or delta beta thalassemia or HPFH trait[14]. Thus adequate measures and screening procedures especially HPLC should be performed concurrently with the aim to reduce the possibility of Hb disorders in offspring, mental and physical trauma of affected patients and socio economical burden of the family. Screening is affordable and can be offered in a range of settings in different societies like before marriage or in antenatal clinic[9].

Conclusion

Hemoglobinopathies can present in adults without symptoms related to red blood cell disorders. Many a times routine CBC may not reveal the exact morphology needed to identify the hemoglobinopathy. With automation and quantitative power, HPLC appears to be most sensitive and accurate and less time consuming technique for direct identification and quantification of normal and abnormal haemoglobin fractions[10]. RBC Indices, HPLC finding and family studies are sufficient to detect and manage most of the haemoglobin variants prevalent in India. However one should be aware of limitations associated with the diagnosis to avoid false negative results. Appropriate screening, detection by HPLC and counselling of patients at risk are the most

important measures for reduction of morbidity and mortality due to hemoglobinopathies.

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