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ORIGINAL RESEARCH

Study of Spectrum of Hemoglobinopathies in Pediatric Age Group Using Hematological Indices and Confirmation by High Performance Liquid Chromatography in A Tertiary Care Centre in Central India-A Prospective Study

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ABSTRACT

Background: Anaemia is defined as a reduction of the total circulating red cell mass below normal limits. Various causes of anaemia in children includes iron deficiency, glucose-6-phosphate dehydrogenase deficiency, malnutrition, infection & genetic. Plethora of haemoglobin variants is prevalent in multi-ethnic Indian population. Hemoglobinopathies are one of the major public health problems in India. Most common genetic disorder in children is thalassemia & other hemoglobinopathies. **Aims & Objectives**: To study the spectrum of hemoglobinopathies in pediatric age group and determine the prevalence of Thalassemia & other hemoglobinopathies and to also correlate & compare hematological indices and HPLC findings

Methods: Present study was conducted at Special hematology lab and Central pathology lab, G.R. Medical College, Jayaarogya Group of Hospitals, Gwalior, Madhya Pradesh from a time period of January 2021 to July2022. 150 subjects were subjected to HPLC and other relevant investigations.

Results: A total of 150 subjects were studied out of which 55 had abnormal hemoglobin fractions. Among all beta thalassemia constituted highest number of cases. Other variants found were Sickle cell trait, alpha thalassemia, HbE, HbD Punjab, and HbQ. Patient from different communities presented of which, tribal, sindhis and other backward classes found to be predominantly involved.

Conclusion: In India microcytic hypochromic anemia is a very common type of anaemia. HPLC is Rapid, automated, accurate, and reliable method for diagnosing hemoglobinopathies.

Keywords: Hemoglobinopathies, Hematological indices, High Performance Liquid Chromatography(HPLC)

INTRODUCTION

Anaemia is defined as a reduction of the total circulating red cell mass below normal limits. Various causes of anaemia in children include iron deficiency, glucose-6-phosphatedehydrogenase deficiency, malnutrition, infection & genetic. Plethora of haemoglobin

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variants is prevalent in multi-ethnic Indian population. Most common genetic disorder in children is thalassemia & other hemoglobinopathies.[1] Sickle cell anaemia and Thalassemia are the major health problems in our country. The diagnosis of thalassemias and other hemoglobinopathies can be accomplished by detailed clinical history of patient, hematological parameters, hemoglobin electrophoresis, high performance liquid chromatography and nucleic acid based methods such as polymerase chain reaction and genomic DNA sequencing along with parental screening clinching the diagnosis in certain problematic cases.[2] The basic technology of HPLC is based on the time required for gradient elution of the different hemoglobin fractions. This is called the retention time (RT). The present study aims at determining the role of HPLC and hematological parameters in the diagnosis of thalassemias and other hemoglobinopathies.[3]

Aims and Objectives

Aim

To study the spectrum of hemoglobinopathies in pediatric age Group in a tertiary care centre.

Objectives

- To determine the pattern of spectrum of hemoglobinopathies in
- pediatric age group in a tertiary care centre.
- To determine the prevalence of Thalassemia & other
- hemoglobinopathies.
- To correlate & compare hematological indices with HPLC.

MATERIALS AND METHODS

Present study was carried out in department of special haematology, Gajra Raja Medical College, Gwalior, Madhya Pradesh over a period of 18 months starting from January 2021 to June 2022.
A total of 150 blood samples of subjects aged less than 18 years who were suspected for thalassemia and other hemoglobinopathies were analysed by ADAMS ARKRAY HBAIC 8180-T. Known cases of nutritional deficiency anaemia and other hemolytic anaemias were excluded. About 3 ml of blood sample was collected in EDTA vial and was analysed in automated cell counter (MEDITECH DM 5200) for complete blood counts. Whenever required sickling test and reticulocyte counts were performed .Parental screening was done wherever possible.

RESULT

Among 150 tested samples 87 were male and 63 were female. Of the 150 samples tested 95 subjects were found to have a normal HPLC pattern. Out of remaining 55 with some form of hemoglobinopathy ,beta thalassemia trait was found to be the most common hemoglobinopathy comprising of 25 cases with a HbA2 value of 3.9% and more .Apart from beta thalassemia , other variants seen were HbH ,HbD Punjab , HbQ ,HbS , HbE. Parental screening was also helpful in clinching the accurate diagnosis.

- Hemoglobin variants with retention time <1 minute- 2 cases with HbH variant were detected.
- Hemoglobin variants with retention times in the F window-4 cases of beta thalassemia major and 16 cases of thalassemia intermedia were detected in this window.
- Hemoglobin variants with retention times in A2 window- Two hemoglobin variants had elution peaks in the A2 window-HbA2 and HbE. 2 cases of HbE were reported and 25 cases of beta thalassemia trait were detected.
- Hemoglobin variants with retention times in D window -Only one variant HbD Punjab was identified in D window

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- Hemoglobin variants with retention time in the S window- Only HbS variant was seen in this window .4 cases of HbS were detected.
- Hemoglobin variant with retention time in the unknown window-1 case of HbQ India was identified

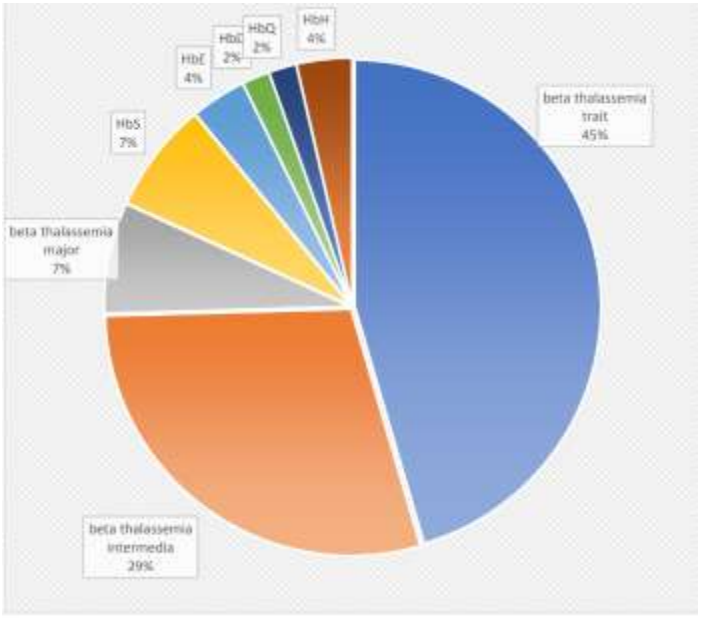

Image 1: Depicting incidence of various hemoglobinopathies

Hemoglobinopathy	Hb	MCV	MCH	MCHC	RBC	RDW-CV
Beta thalassemia minor	10.2±2.6	80.3±16.8	26.9±5.9	30.9±2.2	4.1±1.3	16.3±2.6
Beta thalassemia intermedia	6.1±2.4	70.3±9.3	21.4±3.7	29.3±2.6	2.6±1.1	20.2±5.9
Beta thalassemia major	3.9±0.3	56.9±9.0	16.3±2.5	28.6±0.1	2.4±0.5	21.8±2.4
Sickle cell trait	6.2±4.6	57.8±19.5	20.5±6.7	37.2±1.2	2.7±1.4	14.1±2.4
HbE	9.3±0.2	81.0±2.0	26.8±1.2	33.1±0.2	3.4±0.2	14.9±0.2
HbD	14.9	89.9	31.6	35.2	4.7	13.1
HbH	10.6±0.2	77.8±2	28.2±2.0	36.3±0.3	3.7±0.3	13.1±0.3
HbQ	12.9	81.6	28.3	33.6	4.8	14.6
F value	8.6	2.7	2.5	5.1	4.7	3.7
P value	0.0	0.008	0.01	0.00	0.00	0.001

Table 1: Depicting hematological indices in different hemoglobinopathies

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Hemoglob-inopathy	Tribal	Jatav	Muslim	Sindhi	Marathi	Pal	Thakur	Obc	Not available
Beta thalassemia trait	4	4	0	2	1	1	0	8	5
Beta thalassemia intermedia	3	0	2	3	0	1	1	4	2
Beta thalassemia major	0	0	0	1	0	0	0	2	1
Sickle cell trait	0	0	2	2	0	0	0	0	0
HbE	0	0	0	0	0	1	0	1	0
HbH	0	0	0	0	0	0	0	0	2
HbD	0	0	0	0	0	0	0	1	0
HbQ	0	1	0	0	0	0	0	0	0

Table 2: Shows distribution of various hemoglobinopathies in different ethnic groups

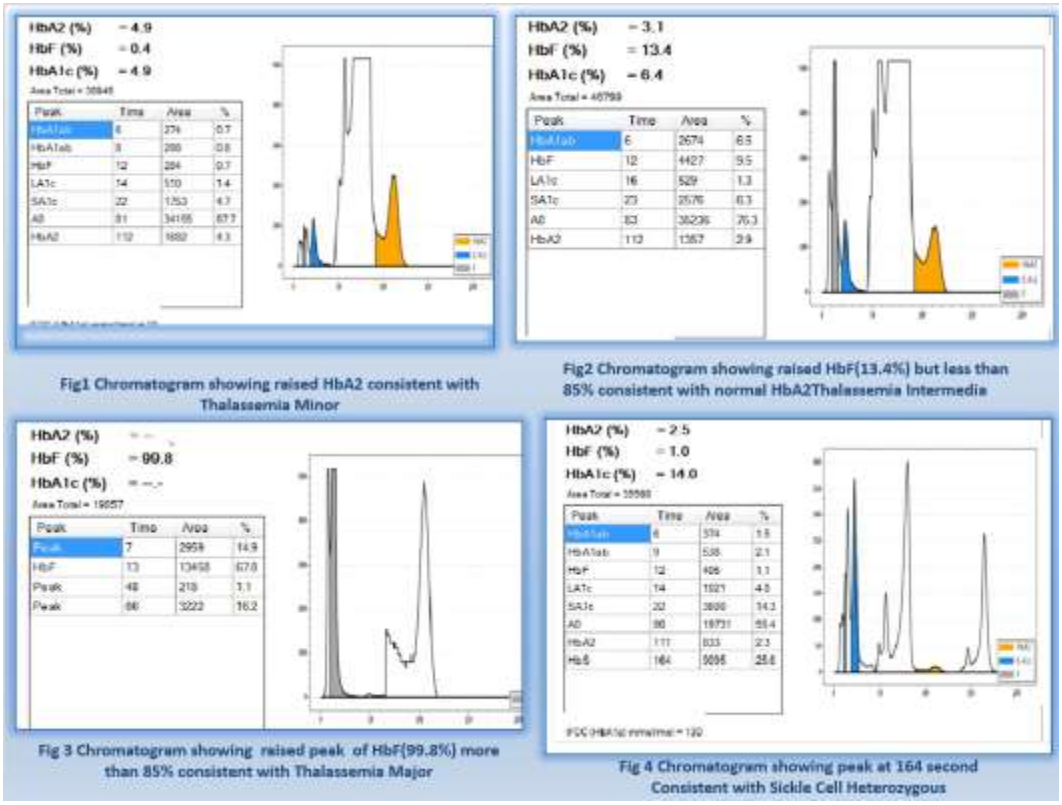

Fig1 Chromatogram showing raised HbA2 consistent with Thalassemia Minor

Fig2 Chromatogram showing raised HbF(13.4%) but less than 85% consistent with normal HbA2Thalassemia Intermedia

Fig 3 Chromatogram showing raised peak of HbF(99.8%) more than 85% consistent with Thalassemia Major

Fig 4 Chromatogram showing peak at 164 second Consistent with Sickle Cell Heterozygous

Fig 1-shows various chromatograms with different hemoglobinopathies

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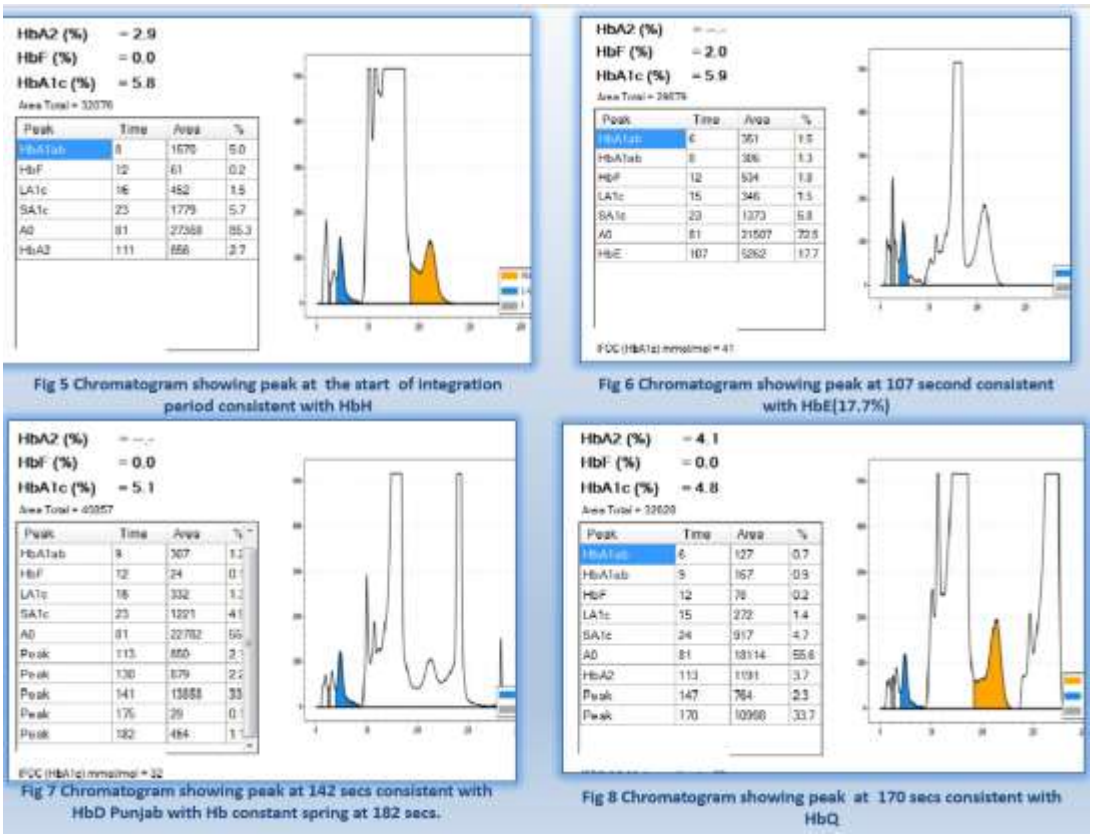

Fig 5-8 shows various chromatograms with different hemoglobinopathies

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. Different reports have addressed the precision of the retention times obtained with stored normal and abnormal sample. There are a few studies from India which evaluated and emphasized the role of HPLC for diagnosis of thalassaemia and various haemoglobinopathies.[4]

In the present study a total of 150 subjects who were suspected for hemoglobinopathies clinically. Out of total 150 cases, 55 cases (37%)were confirmed to have hemoglobinopathy by HPLC .In a study by Mondol et al 35% cases were confirmed to have hemoglobinopathies. In present study beta thalassemia minor comprised of 25 cases (47%), followed by beta thalassemia intermedia (30%) as shown in table Other hemoglobinopathies found were beta thalassemia major (7.3%),sickle cell trait (7.3%), alpha thalassemia (3.6%), HbE (3.6%) and 1 case each of HbD Punjab (1.8%)and HbQ (1.8%) each .Beta thalassemia accounted for majority of diagnosed hemoglobinopathy similar to Indian study by Warghade et al., in which most prevalent hemoglobinopathy was found to be beta-thalassemia trait (11.2%).[5] In the study carried out by Sanghavi et al sickle cell anaemia was found to be most prevalent6. In present study there were total 55 cases of hemoglobinopathies out of which 28(50.9%) were male and 27 (49.1%) were female , male to female ratio being 1.03:1 as shown in table number 5. In present study majority of subjects were from other backward classes (26.7%), followed by muslim population(14.7%), tribal population (10.7%),sindhis (8.0%), Jatav population (8%), pal (2.7%), marathis (2.7%), Thakur (1.3%),kushwah (1.3%).

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CONCLUSION

In India microcytic hypochromic anemia is a very common type of anemia. Most common cause of anaemia is iron deficiency and next to it is thalassemia. Diagnosis of microcytic hypochromic anemia can be made by complete hemogram and peripheral smear and differential diagnoses can be confirmed by various special tests. HPLC is Rapid, automated, accurate, and reliable method for diagnosing hemoglobinopathies. In present study male preponderance was observed. Cases with hemoglobinopathies predominantly had microcytic hypochromic anaemia. Beta thalassemia was the most frequent diagnoses. Other hemoglobinopathies observed were sickle cell trait, HbE, HbH, HbD Punjab and HbQ.

It is very important that the population be screened so that carriers could be detected and informed about the various complications and reproductive risks. In addition, preventive measures could also be adopted in the form of genetic counselling, prenatal diagnosis and termination of the affected babies.

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