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Study of Haemoglobinopathies in Paediatric Age Group by High Performance Liquid Chromatography in a Tertiary Care Hospital in Gujarat

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Abstract: ***Introduction: Anemia is a major health problem in pediatric populations of India.Many factors are responsible for childhood anemia, like undernutrition, iron and vitamin deficiency, tuberculosis, HIV, chronic infections, malaria etc. Inherited hemoglobin disorders are the most common genetic disorders and their frequency and distribution varies among different racial and ethnic groups. In developing countries like India, it causes financial burden too. Aims and Objectives: This cross-sectional study was undertaken with an aim to detect haemoglobinopathies in paediatric age group in S.S.G. Hospital Baroda, Gujarat. Method: A total of 250 cases were taken of paediatric patients suspected of haemoglobinopathies. Clinical data including complete history, CBC, RBC Indices, peripheral smear findings and HPLC findings were taken. Haemoglobin typing was done by HPLC with BioRad variant II machine using EDTA samples of above mentioned paediatric patients. Results: The prevalence of Hemoglobinopathies was found to be 64.4% in the present study with sickle cell trait being the most common abnormal haemoglobin detected followed by sickle cell disease, beta thalassemia trait, beta thalassemia major and heterozygous for HbD Punjab and sickle cell disease. Conclusion: Chronic anemia cases should be screened for hemoglobinopathies. As the definitive treatment of hemoglobinopathies is still difficult to avail, genetic councelling should be considered for hrmoglobinopathy patients and their families to prevent new cases. HPLC is reliable and cost effective method for detection of various hemoglobinopathies and thalassemia.***

Keywords: Haemoglobinopathies, High performance liquid chromatography (HPLC), Thalassemia.

1. Introduction

Inherited hemoglobin disorders are the most common genetic disorders with 7% of world population being carrier and their frequency and distribution varies among different racial and ethnic groups.[1]

In India, other hemoglobinopathies diagnosed are HbD Punjab, HbE, HbQ disease, Hb C, hereditary persistence of fetal hemoglobin (HPFH), Hb Lapore, Hb M, Hemoglobin Saskatoon, Hemoglobin J Meerut, Hemoglobin Fontainbleau etc.[2] Among these, sickle cell anemia and thalassemia constitute major health problem.[3]

High performance liquid chromatography (HPLC) is a very rapid, reliable and cost effective method for early detection of various haemoglobinopathies and thalassemia. It is based on the principle of cation exchange to separate and quantify various fractions of haemoglobin. It offers a reliable tool for early and accurate detection of pathological haemoglobin, so help to prevent complications of various hemoglobinopathies. The results can be reproduced, offers simplicity with automation, has superior resolution.[4]

Prevention of complication is important step in reduction of morbidity and mortality, so for this, early diagnosis of sickling status from pediatric age is required to implement various preventive strategies.[5]

This study was done with the aims to assese the pattern of different haemoglobin variants and correlate HPLC with hemoglobin, RBC Indices and peripheral smear findings.

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Inclusion criteria

All suspected cases of haemoglobinopathies based on haemoglobin profile, RBC indices, peripheral smear findings and clinical history in paediatric age group (6 months to 12 years)

Exclusion criteria

1) Age less than 6 months
2) Age greater than 12 years.
3) History of blood transfusion in less than 3 months.

2. Method

The study is a hospital based retrospective cross sectional descriptive study from June, 2023 to October, 2023 and prospective cross sectional descriptive study from November, 2023 to June 2024, in the Department of Pathology, Baroda Medical College and Hospital, which is a tertiary care hospital located in Baroda, a district of Gujarat. Majority of patients in this hospital came from tribal area. The study was undertaken after the ethical committee approval. In present study a total of 250 suspected cases of haemoglobinopathies in which HPLC were performed was included. A detailed clinical history, haemoglobin profile, RBC Indices and peripheral smear

examination was done in every case. Haemoglobin typing was done by Bio-Rad Variant II machine using EDTA sample. Haemoglobin fractions are separated by cation exchange HPLC using a pre-programmed buffer gradient which use increasing ionic strength to the cartridge. Calibration of the instrument was performed before each run.

3. Results

A total of 250 suspected cases of haemoglobinopathies were analysed in the present study of which 105 were males and 145 females and females having abnormal Hb variants were 96 (59.6%) and males having abnormal Hb variants were 65 (40.4%) . In our study the age group of the patients ranged from 6 months to 12 years, among which maximum number of cases were found in 6 months to 3 years of age group (Table 1).

Table 1: Age wise Distribution of cases

Age Group	No. of Cases	No. of Abnormal Variants detected Hb
6 months to 3 years	101 (40.4%)	53 (21.2%)
4 years to 6 years	42 (16.8%)	28 (11.2%)
7 years to 9 years	51 (20.4%)	35 (14%)
10 years to 12 years	56 (22.4%)	45 (18%)
Total	250	161 (64.4%)

Out of the 250 cases taken for the study, a total of 161 cases (64.4%) showed abnormal haemoglobin chromatograph on HPLC.

Table 2: Distribution of different haemoglobinopathies

Interpretation	Frequency	Percent
BETA Thalassemia Major	1	0.4
BETA Thalassemia Trait	5	2.0
Heterozygous for HbD Punjab and SCD	1	0.4
Sickle Cell Trait	123	49.2
Sickle Cell Disease	31	12.4
Negative for Hemoglobinopathy	89	35.6
Total	250	100.0

The distribution of different types of haemoglobinopathies is shown in the Table 2. Sickle cell trait (49.2%) was the most common abnormal haemoglobin detected in the present study followed by sickle cell disease (12.4%) , beta thalassemia trait (2%), β thalassemia major (0.4%) and Heterozygous for HbD Punjab and sickle cell disease (0.4%) were detected by HPLC in the present study.

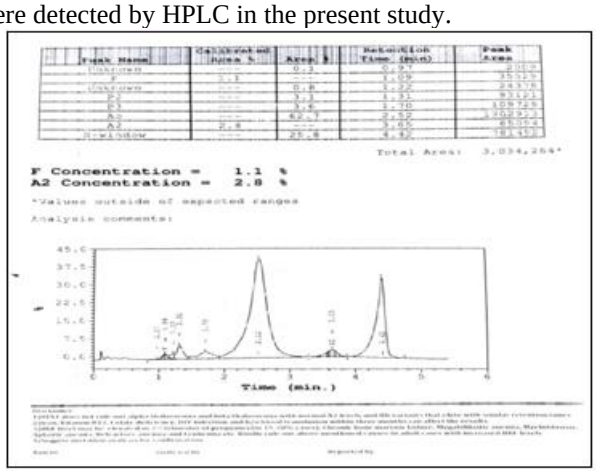

Figure 1: Sickle cell Trait

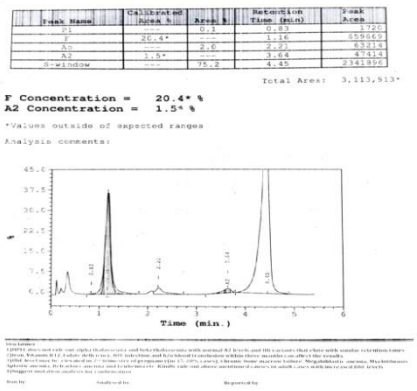

Figure 2: Sickle cell disease

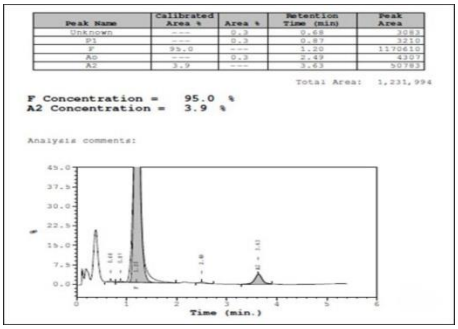

Figure 3: Beta Thalassemia Major

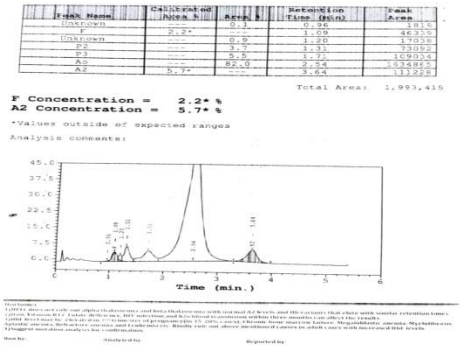

Figure 4: Beta Thalassemia Trait

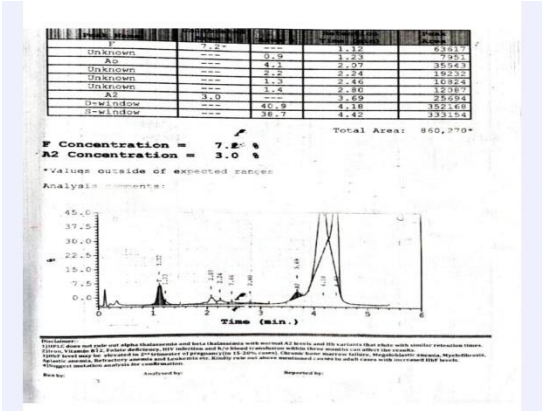

Figure 5: Heterezygous for HbD Punjab and Sickle cell disease

4. Discussion

In our study, prevalence of hemoglobinopathy is 64.4%, while study by Warghade et al.[6] For abnormal haemoglobin had shown a rate of 18.44% and sanghavi et al.

As 27%. [7] The prevalence of abnormal Hb variants was 59.11%, as seen by Baruah et al. In his laboratory-based study involving all age groups from Assam. [8] Another study by Kalita et al. Involving 100 individuals of all age group, prevalence was 55%.[9] Borah et al. From Gauhati medical college showed prevalence of hemoglobinopathies as 67.46% in a hospital-based study.

Our study shows that there is female predominance of 58%. While in Sanghavi et al. Male predominance of 59.2% was there.[7]

In our study, most common haemoglobin abnormality found was sickle cell trait (49.2%) which is supported by Mondal et al.[10] while in study by Warghade et al. , most prevalent hemoglobinopathy found was beta thalassemia trait(11.2%)[6]. At the same time huge migrant tea garden population shows a high incidence of HbS in north eastern part of India.[11,12] Various researchers from Assam have reported HbE trait as most prevalent (varying from 20% to 55%) hemoglobinopathy followed by HbE disease.[8,9,13]

This variation of prevalence of hemoglobinopathies (total and individual) in different studies, actually reflects the fact that hemoglobinopathy is a genetic disorder which is clustered among few particular communities and also the Indian practice of marriages into same community.

5. Conclusion

The purpose of our study was to assess all the pediatric age group patients suspected for hemoglobinopathies. In our study, 64.4% patients were found to have abnormal haemoglobin as a cause of anemia. Morbidity and mortality is higher in homozygous conditions like sickle cell disease and beta thalassemia major.

Continuous awareness programme and mass screening of population, especially school going children will help in early detection of heterozygous state like sickle cell trait and beta thalassemia minor, which are asymptomatic.

Hemoglobin, RBC indices and peripheral smear findings give a clue regarding necessity of HPLC for confirmation of hemoglobinopathy.

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