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Detection of Hemaglobinopathies in Anemic Children by HPLC method – A Hospital Based Study

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Abstract:
Introduction*: The inherited disorders of haemoglobin synthesis are one of the important public health problems in India. Haemoglobinopathies are the most common disorders of erythrocytes. India is the home of several haemoglobin variants.*
Material and Method: *A total of 100 patients of blood transfusion patients admitted in RIMS at Paediatric Department entered the study. Materials for the present work included mainly anemic children, transfusion requiring children and their family members.*
Results: *Most commonly affected age group was that of ≤ 5 years, followed by 5- 10 years age group. Sickle cell disorders(31%) consisting of sickle cell trait (8%) , sickle cell disease (12%) and sickle β thalassemia(11%) is the most common haemoglobinopathy present in study population followed by β- thalassemia syndromes (17%) .*
Conclusion: *It can be concluded from the study that HPLC is a versatile, reproducible chromatographic technique for the estimation of haemoglobinopathies/ thalassemia.*
Keywords: *HPLC, Haemoglobinopathies.*

I. Introduction

Anemia is a global problem of immense health significance affecting persons of all ages and economic groups. The term anemia refers to a reduction of the total circulating red cell mass below normal limits. Anemia reduces the oxygen carrying capacity of the blood, leading to tissue hypoxia. Anemia is not a diagnosis by itself but is an objective sign of the presence of disease. Anemia is usually diagnosed based on a reduction in the hematocrit (the ratio of packed red cells to total blood volume) and the haemoglobin concentration of the blood to levels that are below the normal range. It is often a focus of attention in patient care because accurate quantification is easy to achieve and rational analysis of the problem is possible. A classification of anemia is important because of numerous types and useful classification would provide an orderly approach to diagnosis and therefore treatment.

The inherited disorders of haemoglobin synthesis are one of the important public health problems in India. Haemoglobinopathies are the most common disorders of erythrocytes. India is the home of several haemoglobin variants. HPLC was found to be a simple, rapid and reliable method for the detection of haemoglobin variants. An accurate diagnosis can be provided in majority of cases by use of retention time, proportion of total haemoglobin, and peak characteristics of HPLC.Haemoglobinopathies and thalassemias are the most common single gene disorders in the world. Around 7 percent of population worldwide are carriers with more than 3, 00,000 severely affected babies born every year. Worldwide, the Hb disorders are responsible for 3.4% mortality in children below 5 years of age. Inherited disorders of hemoglobin are extremely common in Indian population ranging from near structurally normal hemoglobins to severe transfusion dependant hemoglobinopathies. The disorders of Hb frequently encountered in India include β- thalassemia, HbE – β-thalassemia, HbE, HbD and sickle cell anemia. The prevalence of thalassemias and hemoglobinopathies varies with geographic locations. It has been estimated that in India, 0.37/1000 fetuses have a Hb disorder. The prevalence of β- thalassemia mutations is as high as 17% in some Indian populations[1].

Their detection is important epidemiologically and to prevent other more serious hemoglobinopathies in future generations. The inherited disorders of haemoglobin, particularly the β-thalassemias and their interaction with haemoglobin E (HbE) and haemoglobin S (HbS) are a considerable health problem in India and contribute significantly to morbidity and mortality. Earlier studies have shown that the overall prevalence of β-thalassemia is 3–4 % with an estimate of around 8,000 to 10,000 new births with major disease each year[1]. Most of these children have a severe clinical presentation but are managed sub-optimally due to lack of financial resources in majority of the families. Thus preventing the birth of affected children is the best option for India. A prerequisite for this is the knowledge of the prevalence of β-thalassemia and other haemoglobinopathies.

II. Materials And Methodology

Materials for the present work included mainly anemic children, transfusion requiring children and their family members. Cases with nutritional deficiency anemia, where a co-existent hemoglobinopathy was suspected, were also screened. The study was done in Department of Biochemistry and Department of Paediatrics, RIMS, Ranchi over 12 months period. Total number of cases included in the study was 100.

Inclusion criteria

- Patients presenting with pallor and generalized weakness
- Blood transfusion requiring patients
- Patient with nutritional deficiency anaemia, where a co-existent hemoglobinopathy is suspected

Exclusion criteria

- In patients requiring blood transfusion sampling was deferred for at least 4 week after or just before next transfusion.
- Non cooperative patients.

About 1-2 ml of blood sample was collected in EDTA vial and was analyzed in automated cell counter (Sysmex XT 1800i; Kobe, Japan) for complete blood counts and RBC indices. Follwing tests were done in each case:-

a. Total red blood cell count.
b. Total platelets count.
c. Total and differential white blood cell count.
d. Reticulocyte count
e. Hemoglobin estimation.
f. Haematocrit.
g. MCV
h. MCH
i. MCHC
j. RDW-CV
k. HPLC (High Performance Liquid Chromatography).

Results

The present cross sectional study on detection of hemaglobinopathies in anemic children was carried out in the department of biochemistry, Rajendra Institute of Medical Sciences, Ranchi. During this period total of 100 patients were analyzed by CE-HPLC (BioRad laboratories, California, USA) using Variant β-thal short program. Out of these 47 were found to have normal HPLC and 53 had one form or the other of hemoglobinopathies.

Table – I: Age distribution

Age in years	Number of cases	Percentage (%)
≤5yrs	47	47.0
5 -10 yrs	38	38.0
10-14 yrs	15	15.0
Total	100	100

Table – II: Gender distribution

Gender	Number of patients	Percentage (%)
Male	62	62.0
Female	38	38.0
Total	100	100.0

Table – III: Diagnosis based on HPLC

Hemoglobinopathies	Number of patients	Percentage (%)
Normal	47	47.0
Sickle cell disease	12	12.0
Sickle cell trait	8	8.0
Sickle β thalassemia	11	11.0
β thalassemia major	7	7.0
β thalassemia trait	10	10.0
Hb E β thalassemia	5	5.0
Total	100	100.0

Out of 100 patients studied, 47 patients were found to have normal HPLC study and 53 patients had one or the other form of hemoglobinopathies.

Table – IV: Community wise distribution of different types of hemoglobinopathies

Community	Sickle cell disorders*	Beta thalassemia syndromes**	Other Hemoglobinopathies***	Total
Non Tribals	14 (45.2%)	8 (47.1%)	4 (80%)	26 (49.1%)
Tribals	17 (54.8%)	9 (52.9%)	1 (20%)	27 (50.9%)
Total	31	17	5	53

*Includes Sickle Cell Trait, Sickle Cell Disease and Sickle Cell β□ Thalassemia cases
** Includes β Thalassemia Trait and β Thalassemia major cases
*** Includes Hb E β Thalassemia cases

Sickle cell disorders and β thalassemia syndromes are almost equally prevalent in both tribals and non tribals. Other Hemoglobinopathies were found mainly in non tribals. Out of 53 patients diagnosed with hemoglobinopathies 27 were tribals and 26 were non-tribals.

Table – V: Gender wise distribution of different hemoglobinopathies

Gender	Sickle cell disorders*	Beta thalassemia syndromes**	Other Hemoglobinopathies***	Total
Males	26	8	4	38
Females	5	9	1	15
Total	31	17	5	53

Table – VI: Age wise distribution of different hemoglobinopathies

Age	Sickle cell disorders	Beta thalassemia syndromes	Other hemoglobinopathies
≤ 5yrs	8	16	1
5-10 yrs	15	1	4
10-14 yrs	8	0	0
Total	31	17	5

III. Discussion

The laboratory diagnosis of thalassemias and other hemoglobinopathies can be achieved by a step-wise approach starting with a detailed clinical history, thorough hematologic evaluation [including hemoglobin level, complete blood count (CBC), reticulocyte count, and red blood cell (RBC) morphology], protein based analytic methods [alkaline and acid Hb-electrophoresis, isoelectric focusing (IEF) and high performance liquid chromatography (HPLC)] and nucleic acid based methods [such as polymerase chain reaction (PCR), reverse transcriptase (RT)-PCR, and sequencing of genomic DNA]1,2. Family studies also play a crucial role in clinching the diagnosis in certain problematic cases.

The aim of the present study was to evaluate the role of cation exchange HPLC (CE-HPLC) along with adjunctive tests as needed in the diagnosis of thalassemias/hemoglobinopathies and to see the profile of these in the indigenous population. It is especially important to validate the role of HPLC, as it is less labour intensive with rapid turn around time and better reproducibility compared to Hb electrophoresis. Moreover, it can replace tedious procedures like estimation of foetal hemoglobin and HbA2 quantitation by column chromatography.

There are a few studies which evaluated and emphasized the role of HPLC for diagnosis of thalassemia and various hemoglobinopathies.

Age: In this study, out of 53 patients diagnosed as having one or the other hemoglobinopathies, 25 were in the age group of less than 5 years, 19 were between 5 years and 10 years and 9 patients were between the age group of 10 to 14 year. Majority of the study cases in the paediatric age group can be well understood because most of the haemoglobinopathies present early in life and the parents seek medical attention eagerly for any complaints in child. According to Astaldi, Tolentino and Sacchetti, several grades of diseases were recognized. A severe form causing anemia early in infancy and often resulting in death in first year. A slightly less severe form of disease usually first becoming manifest in second half of first year, the child often surviving until school age. A milder form usually diagnosed in second year of life and compatible with survival until adult age.

In another study done in Western Maharashtra (B. J. Medical College, pune) in 2000, 11.3% of patients of pediatric age group showed the presence of one or the other hemoglobinopathies. Balgir et al found majority of the cases of hemoglobinopathy belong to reproductive age group,i.e. 16 to 45 years, followed by neonatal to childhood period (0-15 years) .This discrepancy can be because Balgir[2] (2005) study was a 10 yr cohort study consisting of large number of cases.(667 abnormal cases).

Spectrum of different Haemoglobinopathies: Majority of cases in the present study were sickle cell disorders(58.5%) consisting of sickle cell trait (15.1%) , sickle cell disease (22.6%) and sickle β thalassemia(20.8%). As there is paucity of study about the various hemoglobinopathies present in Jharkhand , there are few studies with which analysis can be done. In a study "Efficacy of High Speed Super Solubility Test

In Detection Of Sickle Cell Diseases at Rajendra Institute of Medical Sciences, Ranchi, Jharkhand, India" done by Chandrahas Prasad (Associate Professor, Department of Laboratory Medicine), Hemant Narayan Rai (Associate Professor ,Department of Cardiology), and Shashi Bhushan Singh(Lecturer Cum Statistician, Department of Preventive and Social Medicine), 63 patients were diagnosed to have one or the other hemoglobinopathies by HPLC. Out of which, 12 were sickle homozygous, 10 were sickle cell trait, 7 were sickle thal trait, 13 beta thal trait, 9 delta beta thal trait, 5 beta thal major. The present study closely resembles this study[3].A study done by Balgir et al[4] in Orissa, which is also a hot bed of sickle cell genes , found presence of sickle cell disorder in 39.1% cases (sickle cell trait - 29.8%, sickle cell disease - 7.6% , sickle β thalassemia 1.7%). Jharkhand has a population of 32.96 million with 28% tribal peoples, 12% Scheduled Castes and 60% others[5,6]. In tribals because of consanguinity, and area endogamy, these communities show a very high incidence, making the disease a major health problem in these communities. Most of the tribal population are poor and illiterate and they live in far flung areas.So they don't seek medical attention for any disease unless it has acquired serious proposition and also they don't undergo any premarital genetic testing and counselling for any suspected haemoglobin disorders.

This can be one of the reason for proportions of different sickle cell disorders in present study.Beta thalassemia syndromes constituted 32.1 % cases with β- thalassemia trait 18.9% and β- thalassemia major 13.2%. Present study shows close similarity with the study done Balgir et al[2] (2005) in which 23.5% of the referred cases were suffering from β thalassemia syndromes (β thal trait -18.2% and β thal major 5.3%). In another study done by Jain BB[7] et al for screening of thalassemia and other hemoglobinopathies in a tertiary care hospital of West Bengal it was found that incidence of β thalassemia trait was 55.84 % and β thalassemia major was 9.46 %. High incidence in this study was because it was screening study and majority of the subjects were in high risk group. In a study done in Western Maharashtra by S. S. Ambekar, M. A. Phadke and others, amongst the total 891pediatric subjects studied, 790 (88.66%) were detected to be normal, 101 (11.3%) showed the presence of one or the other hemoglobinopathy[8]. Thalassemia major was detected in 76 (8.5%) subjects, beta thalassemia trait in 4 (0.5%), sickle cell disease in 16 (1.8%) HbE in 4 (0.5%) and HbD disease in 1 (0.1%). This high frequency of thalassemia is because, the distribution of beta thalassemia gene is high in Maharashtra(1.9%) with highest frequency in Gujarat. As reported in that study the highest frequency of sickle cell gene is in Orrisa followed by Assam, Madhya Pradesh and other eastern states.

IV. Conclusion

The inherited disorders of hemoglobin synthesis are one of the important public health problems in India. Haemoglobinopathies are the most common disorders of erythrocytes. India is the home of several hemoglobin variants. The most common haemoglobinopathy in this study was sickle cell disorders followed by β thalassemia syndromes. This study provides a comprehensive database on the spectrum of haemoglobinopathies in the region. The prevention and control of haemoglobinopathies is an uphill task for the planners,policy makers and medical and health care machinery. Along with sickle cell disorders, β-thalassemia is also prevalent in this region. In this perspective, it is emphasized that a routine premarital screening program is needed for identification and prevention of high-risk marriages. Nevertheless, mass awareness, knowledge generation, and genetic counseling are still a vital requisite. It can be concluded from the study that HPLC is a versatile, reproducible chromatographic technique for the estimation of haemoglobinopathies/ thalassemia. Since this work was carried out in a limited period of time with a small number of cases, it is envisaged that a long term comprehensive study in this line will throw more light and help in formulating ways to prevent and for early diagnosis and treatment of different hemoglobinopathies prevalent in Ranchi and near by areas.

Most commonly affected age group was that of ≤ 5 years, followed by 5- 10 years age group. Sickle cell disorders(31%) consisting of sickle cell trait (8%) , sickle cell disease (12%) and sickle β thalassemia(11%) is the most common haemoglobinopathy present in study population followed by β- thalassemia syndromes (17%). Sickle cell disorders(54.8%) as well as β thalassemia syndromes(52.9%) were mainly present in tribal population as compared to non-tribal population. As expected tribals were most commonly affected by sickle cell disorders with a high prevalence of sickle cell disease. Other hemoglobinopathies constituted only 5% of the study population which included Hb E β thalassemia.

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