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Research Paper

Hemoglobinopathies Contributing To Anemia: A Tertiary Care Centre Study from Jharkhand

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***ABSTRACT**: Anemia is a common health problem in our country. Among its various causes hemoglobinopathies are most common inherited red cell disorders. It is very important to identify these disorders for its prevention and management. Our study aims to detect hemoglobin disorders in patients with anemia with the help of HPLC method. It is a retrospective study done in a tertiary care centre in Jharkhand. A total of 285 cases were studied. Complete blood counts were taken and hemoglobinopathies were detected by HPLC method in BIO RAD Variant II analyzer. Out of 285 cases 145 (50.87%) cases showed abnormal hemoglobin. Beta thalassemia trait was the most frequently detected hemoglobinopathy (17.89%) followed by sickle cell disease (9.47%).*

***KEYWORDS** – anemia, hemoglobinopathy, thalassemia,HPLC*

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I. INTRODUCTION

Anemia is a reduction of the total circulating red cell mass below normal limits [1]. There are various causes of anemia in different parts of the world. In developing countries as in India anemia due to nutritional deficiencies are common. Prevalence of anemia in India is very high as compared to world prevalence[2]. Hemohlobinopathies are the group of genetic disorders of haemoglobin in which there is a qualitative or quantitative abnormality in haemoglobin molecule[3,4]. Beta thalassemia and sickle cell disorders are the most common hemoglobinopathes[4,5]. In India the cumulative gene frequency of hemoglobinopathies is 4.2%[6]. Prevalence of hemoglobinopathies is quite heterogenous with the geographic locations and ethnic groups[7]. High performance liquid chromatography is a precise, sensitive and widely used method for detection of abnormal haemoglobin variants because it is easy to do, quick and reliable.

II. AIM

To assess the burden of anemia due to hemoglobinopathies in a tertiary care centre in Jharkhand.

III.MATERIALS AND METHODS

This is a retrospective observational study done in department of laboratory medicine, RIMS, Ranchi. A total of 285 cases from January 2016 to September 2021 were selected for the study. Data was taken from the records. Anemic patients suspected for hemoglobinopathies were tested. Blood samples were collected in 2ml EDTA vaccutainer. Complete blood count was done in five part differential cell counter(sysmex XT-2000i) using well mixed anticoagulated blood sample. Hemoglobin values of the cases ranged from 4g/dl to 11g/dl. History of blood transfusion and relevant personal history was taken in all cases. All samples were analyzed for abnormal haemoglobin by BIO-RAD Variant ii HPLC machine (beta thalassemia short program). It uses the principle of high performance liquid chromatography (HPLC). HbA2/F calibrator and high and low controls were analyzed at the beginning of each run. The total area acceptable was between one million to three millions. Sample ratio was increased in case of low total area and vice versa. The software delivers a printed report showing the chromatogram, with all the eluted haemoglobin fractions. The integrated peaks assigned by the

manufacturer- "windows" derived from specific retention time (RT). It is the time that elapses from the sample injection to the apex of the elution peak, of normal haemoglobin fractions and common variants.

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

PEAK NAME	WINDOW (MIN)	RETENTION TIME(MIN)
F window	1.00-1.30	1.15
P2 window	1.30-1.60	1.45
P3 window	1.60-1.90	1.75
A0 window	1.90-3.30	2.60
A2 window	3.30-3.90	3.60
D window	3.90-4.30	4.10
S window	4.30-4.70	4.50
C window	4.90-5.30	5.10

Table 1 shows windows of established ranges in which common variants have been observed to elute in the variant ii beta thalassemia short program. The printed chromatogram shows all the haemoglobin fractions eluted, the retention times, the areasof the peaks and the values (%) of different haemoglobin components. If a peak elutes at a retention time that is not pre-defined, it is labelled as unknown. Each analytical cycle, from sampling to printing of results takes approximately 6.5 minutes.

IV. RESULT

As shown in table 2, total of 285 cases were studied out of which 145(50.87%) cases showed abnormal haemoglobin fractions on HPLC. 51 cases (17.89%) of beta thalassemia trait were diagnosed. The major abnormality observed in thalassemia cases was raised HbA2. A cut off over 3.9% was taken for diagnosis of beta thalassemia trait [8].

Second commonest abnormality was sickle cell disease (27 cases, 9.47%) with HbS window and retention time of 4.30-4.70 min. there were 23 cases (8.07%) of beta thalassemia major with HbF upto 90%. We had 17 cases (5.96%) each of sickle cell trait and double heterozygous for sickle cell and beta thalassemia. HbE variant included four cases of HbE trait (1.40%); two cases of HbE homozygous(0.70%) and four cases of double heterozygous for HbE and beta thalassemia.

Table 2: Type of haemoglobin pattern in our study subjects

Haemoglobin pattern	**Patients (%), n=285**
Normal Hb	140 (49.12)
Beta thalassemia trait	51(17.89)
Sickle cell disease	27(9.47)
Beta thalassemia major	23(8.07)
Sickle beta thalassemia	17(5.96)
Sickle cell trait	17(5.96)
Beta thalassemia HbE	4(1.40)
HbE trait	4(1.40)
HbE disease	2(0.70)

Table 3. showing age and sex distribution of the study subjects

Upto 10 years		11 -20 years		21-30 years		31-40 years		>40 years	
Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
38	35	32	30	38	71	21	14	1	5

V. DISCUSSION

There are multiple causes of anemia. It could be primary or secondary. Thalassemias are the most common gene linked hemoglobin disease in the world[9]. Hemoglobinopathies constitute a major cause of anemia as well as morbidity to the affected populations. They are distributed widely in different geographic areas, populations and ethnicity.

In the present study a total of 285 cases (130 males and 155 females) were included. Of these 285 cases,145(50.87%) cases showed abnormal hemoglobins. The most common hemoglobin disorder in our study was beta thalassemia trait(17.89%) which is in accordance with study of Rao S et al[10]. Sickle cell disease was 9.47% in the present study which is in accordance with study done by Mukesh et al [11]. In the tribal populations of central India and eastern parts of Orissa and Jharkhand HbS is the predominant hemoglobinopathy[12].

According to study done by Rachna Nagar et al Jharkhand recorded 3.3% of HbS but the frequency of sickle gene in the tribals was higher than in non tribals (8.9% vs 4.5%)[13] in contrast to the findings of Mukesh et al that showed almost equal prevalence of sickle disorders and beta thalassemia in both tribals and non tribals [11].

Beta thalassemia major constituted 8.07% of cases in our study. We got 10 cases HbE disorders (4 cases(1.40%) each of HbE trait and double heterozygous for beta thalassemia and HbE and 2 cases (0.70%) of HbE disease in contrast to 37.38% of HbE homozygous/ Ebeta thalassemia in one study[14].

It is very important to identify hemoglobin variants for the proper treatment and also to prevent possible complications in the patients. Persons who are heterozygous should be counselled properly regarding the nature and inheritance of hemoglobinopathies.

VI. CONCLUSION

HPLC is a rapid, accurate, cost effective and reliable technique for early diagnosis and proper management for hemoglobinopathies. Anemia is a major burden in our country. Nutritional deficiencies are the culprit most of the times. But considering our ethnicity, tribes and social customs of marrying close relatives hemoglobinopathies must be considered during the investigation of anemia.

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