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**Original Research Article**

# Prevalence of hemoglobinopathies detected by high performance liquid chromatography in tertiary care centre, Kota, Rajasthan

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## ABSTRACT

**Background:** Hemoglobinopathies are the most common heterogeneous group of monogenetic disorder in the world. Their prevalence varies with geographical regions. India is developing country and many studies have shown a significant burden of hemoglobinopathies in India. Our aim is to estimate prevalence of various hemoglobinopathies in our region.
**Methods:** The present study was conducted at the department of pathology, Government Medical College, Kota on patients referred for antenatal or voluntary premarital checkup, patients with clinical history and complete blood count (CBC) suggestive of hemolytic anemia, and family members of known cases of hemoglobinopathies. Transfusion-dependent patients were also included. However, patients with history of blood transfusion within the last 1 month were excluded. EDTA samples were used for CBC using 6-part differential cell counter and high-performance liquid chromatography (HPLC) using BIORAD D10 analyzer. The results were tabulated and analyzed.
**Results:** Of the 226 cases studied, 139 (61.5%) were females and 87 (38.5%) were males. Most common age group was 21 to 30 years. The number of thalassemia traits were 26 (11.5%), sickle cell traits were 7 (3.1%), sickle cell homozygous was 1 (0.4%), compound heterozygous for sickle cell and thalassemia were 2 (0.9%), while the remaining 190 patients (84.07%) were found to have normal HPLC, with presence or absence of nutritional deficiency.
**Conclusions:** In our study, we found a high prevalence of hemoglobinopathies among patients. The most common disorder detected was beta thalassemia trait. Most of the hemoglobinopathies found in our study could be accurately quantified by HPLC which is a rapid, sensitive, and reproducible method for the detection of different hemoglobinopathies.

**Keywords:** Hemoglobinopathies, Thalassemia, Sickle cell anemia, High-performance liquid chromatography

## INTRODUCTION

Hemoglobin is a major constituent of red blood cells (RBCs). It is responsible for transport and delivery of oxygen to tissues. Any abnormalities in its structure and function can result in haemoglobinopathies like thalassemia, sickle cell anaemia. These diseases are generally hereditary. In thalassemia, the quantity of hemoglobin production is affected while in sickle cell anaemia quality of hemoglobin is affected. Hemoglobinopathies have high prevalence in India and are a major public health problem. According to World Health Organization (WHO), approximately 5% of the world's population carries trait genes for haemoglobin disorders, mainly, sickle-cell disease and thalassaemia.[1] The frequency of thalassemia carriers in India is estimated to be 4% on a recent meta-analysis.[2] Thalassemia being an autosomal recessive disease can be inherited in a child, if both the parents are carrier for the defective gene, called thalassemia heterozygous or trait. High performance liquid

chromatography (HPLC) is a technique used to separate, identify and quantify each component in a mixture. It relies on pump to pressurize a liquid solvent containing the sample mixture to pass through a column filled with a solid adsorbent material. Each component in the sample interacts slightly different with the adsorbent material, leading to the separation of the components as they flow out of the column. HPLC depends on the interaction of charged group on the ion exchange material with charged group on the hemoglobin molecule. The objective of the study is to find out the prevalence of various haemoglobinopathies in the Hadoti region of Rajasthan so that we can find strategies for early identification, diagnosis and prevention of severe haemoglobinopathies. For this purpose, we are using HPLC as a diagnostic tool.

## METHODS

This is a retrospective study. The study was conducted at department of pathology, Government Medical College, Kota from August 2023 to December 2023. A total of 226 samples were received for HPLC to detect hemoglobinopathies. Patients who came for antenatal or voluntary premarital checkup, patients with clinical history and complete blood count (CBC) suggestive of hemolytic anemia, and family members of known cases of hemoglobinopathies were included in the study. Transfusion-dependent patients were also included. However, patients with history of blood transfusion within last 1 month were excluded. Detailed clinical history and family history were obtained from each patient and a signed consent form was obtained.

CBC reports were noted for mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), hemoglobin (Hb), hematocrit (Hct) and other required data. For CBC, 3 ml blood was collected in ethylene diamine tetrachloride acetate (EDTA) vacutainer and analyzed in a 6-part differential automated cell counter on the same day. The HPLC samples were collected in EDTA vacutainer and analyzed within one week. The test was performed on BIORAD D10 which works on principle of cation exchange HPLC. The BIORAD D10 device can work on two modes that gives a chromatogram. The chromatogram has a graph showing different peaks of different type of hemoglobin like HbA1a, HbA1b, HbF, HbA1c, HbA0, HbA2 and some unknown peaks that are identified on the basis of their retention time. The chromatogram shows height, area and percentage of hemoglobin variants. The reagent for BIORAD D10 HPLC analyzer has a blood primer, two calibrator and elusion buffer 1 and 2 with different ionic strength, wash reagent that acts like mobile phase and it takes about 6 minute 18 seconds for each analytical cycle. Hbs were identified by their retention time and quantified by computing the area under the corresponding peak in the elution profile.[3] All values obtained were tabulated and analysed using Microsoft excel to get the final result. Further confirmatory tests could not be performed due to unavailability at our center.

## RESULTS

A total of 226 cases were studied, out of which 139 (61.5%) were females and 87 (38.5%) were males. The ratio is 1.6: 1. The number of females are high because they are advised HPLC during their first antenatal visit. (Table 1).

**Table 1: Gender distribution of patients.**

| Sex | Samples (%) |
|---|---|
| **Male** | 87 (38.5) |
| **Female** | 139 (61.5) |

The patients in age group from 1 to 10 were 5 (2.2%), from 11 to 20 were 28 (12.3%), from 21 to 30 were 78 (34.5%), from 31 to 40 were 38 (16.8%), from 41 to 50 were 25 (11.1%), from 51 to 60 were 25 (11.1%), from 61 to 70 were 15 (6.6%), from 71 to 80 were 11 (4.8%), from 81 to 90 were 1 (0.44%), the youngest patient was of 1 year and the maximum age was 86 years, out of all the cases maximum number of patients were from age group of 21 to 30 which is the general reproductive age group (Table 2).

**Table 2: Age-wise distribution of patients.**

| Age group (years) | Samples (%) |
|---|---|
| **1 to 10** | 5 (2.2) |
| **11 to 20** | 28 (12.3) |
| **21 to 30** | 78 (34.5) |
| **31 to 40** | 38 (16.5) |
| **41 to 50** | 25 (11.1) |
| **51 to 60** | 25 (11.1) |
| **61 to 70** | 15 (6.6) |
| **71 to 80** | 11 (4.8) |
| **81 to 90** | 1 (0.44) |

The number of thalassemia heterozygous or trait were 26 (11.5%), sickle cell heterozygous or trait were 7 (3.1%), sickle cell homozygous was 1 (0.4%), compound heterozygous for sickle cell and thalassemia were 2 (0.9%), while the remaining 190 patients (84.07%) were found to have normal HPLC (Table 3).

**Table 3: Prevalence of various hemoglobinopathies in study population.**

| Hemoglobin variant | Number |
|---|---|
| **Normal** | 190 |
| **Beta thalassemia trait** | 26 |
| **Sickle cell trait** | 7 |
| **Sickle cell-beta thalassemia compound heterozygous** | 2 |
| **Sickle cell homozygous** | 1 |

Among the 26 thalassemia trait cases, 6 were male (23%), 20 were female (76%), from age group 1 to 10 were 1

(3.8%), from age group 11 to 20 were 4 (15.3%), from 21 to 30 were 12 (46%), from 31 to 40 were 3 (11.5%), from 41 to 50 were 2 (7.6%), from 51 to 60 were 3 (11.5%), from 61 to 70 were 1 (3.8%). Out of 26 cases, 18 possibly also had iron deficiency anemia, suggested by their high RDW value as shown (Tables 4 and 5).

**Table 4: Age-wise distribution of thalassemia carriers at diagnosis.**

| Age group (years) | Thalassemia trait (%) |
|---|---|
| **1 to 10** | 1 (3.8) |
| **11 to 20** | 4 (15.3) |
| **21 to 30** | 12 (46) |
| **31 to 40** | 3 (11.5) |
| **41 to 50** | 2 (7.6) |
| **51 to 60** | 3 (11.5) |
| **61 to 70** | 1 (3.8) |

**Table 5: Range of red cell distribution width (RDW) among thalassemia carriers.**

| RDW | Number |
|---|---|
| **12 to 16** | 8 |
| **16 to 20** | 15 |
| **Above 20** | 3 |

## DISCUSSION

Thalassemia and other hemoglobinopathies are autosomal recessive inherited disorders, primarily affecting the globin moiety of the Hb molecule. These disorders, which were mainly confined to certain areas, religions, castes, and tribes, particularly with endogamous norms of marriages, are now widely prevalent all over the world. This is because of the migration of various races over the ages and hence, being home to an assortment of sociocultural, linguistic, and ethnically diverse people.[4]

Hemoglobinopathies are one of the major health problem in India which causes financial, social, psychological and medical issues. Early diagnosis of these diseases is required so that the next generation can be safeguarded against these diseases. For this purpose, various methods can be used like gel electrophoresis and HPLC. Nowadays, HPLC is the most common diagnostic procedure for haemoglobinopathies in India as it is more accurate and rapid.

In our study, 226 samples were screened from the state of Rajasthan. We found that 36 patients had various haemoglobinopathies which is about 15.9% of total cases. Result of this study was compared with the results from previous studies. The prevalence of haemoglobinopathies found by Philip et al and Gupta et al were 15.8% and 14.3% respectively, which are comparable with our study.[5,6] While the study conducted by Patil et al had 20.5% prevalence, and that by Mondal and Mandal reported 12.17% prevalence in West Bengal.[7,8]

Colah et al reported that nearly 1.5% of the world's population was carriers of β thalassemia.[9] Several studies reveal that in most parts of India, β thalassemia trait was the commonest Hb disorder. In our study, the proportion of thalassemia trait was 11.5%, with 26 cases. While prevalence reported by Philip et al was 10.49%, by Gupta et al was 9.53%, by Biswas and Philip was 8.03%, by Mondal and Mandal was 4.60% and by Patil et al was 1.6%.[5-8,10]

Sickle cell trait was the second most common disorder in our study with 7 cases (3.1%), while it was found in 1.25% cases by Philip et al, in 1.31% cases by Biswas et al, in 10.9% cases by Gupta et al and in 14.22% cases by Patil et al.[5-7,10]

Sickle cell homozygous state was observed in 1 case (0.4%) in our study, whereas Philip et al noted 0.12%, Gupta et al noted 2.2% prevalence and Patil et al noted 2.51% prevalence.[5-7]

In our study 2 cases (0.9%) were found with double heterozygosity for beta thalassemia and sickle cell trait. There was a similar prevalence in studies by Philip et al (0.48%), Gupta et al (1.5%) and Patil et al (0.42%).[5-7]

### *Limitations*

Limitations of HPLC are that it cannot detect alpha thalassemia, relies on retention time which can be overlapping for various variants of haemoglobin and also the reagents are expensive. Also, during interpretation of chromatograms, nutritional anemias must always be taken into account. A low level of HbA2 may be induced by iron deficiency, thus masking β thalassemia trait. Similarly, cobalamin or folate deficiency may raise HbA2 level, leading to a false diagnosis of thalassemia trait and whenever necessary, HPLC must be followed by molecular studies, such as polymerase chain reaction (PCR), amplification refractory mutation system (ARMS), and other similar tests to determine specific mutations responsible for the Hb disorder.[11,12]

## CONCLUSION

By comparing all the data and finding out about the hemoglobinopathy prevalence in the population, we conclude that determination of these haemoglobinopathies is a need of the time so that they can be prevented in future generations. A premarital counselling can also be done on the basis of the HPLC results so that the disease may not occur in the child. HPLC is the preferable mode of diagnosis as it is rapid and sensitive and can be performed in most of the clinical laboratories, having the appropriate devices and reagents. In the study, we also found out that anaemia is quite prevalent in India. So, there is also a need for screening for anaemia. As hemoglobin deficiency may also cause many health problems.

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