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EXPLORING HEMOGLOBIN DISORDERS IN REPRODUCTIVE-AGE FEMALES: A PROSPECTIVE OBSERVATIONAL STUDY IN RURAL HARYANA, INDIA

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ABSTRACT

Background: Hemoglobinopathies, a diverse group of genetic disorders affecting hemoglobin synthesis or structure, pose substantial public health challenges worldwide. Understanding their prevalence and spectrum, especially among reproductive-age females, is crucial for planning effective prevention and treatment strategies. **Aim**: This prospective observational study aimed to investigate the frequency of hemoglobinopathies among reproductive-age females in a rural tertiary care centre in Haryana using high-performance liquid chromatography (HPLC).
Materials and Methods: Over the course of one year, 149 females aged 15 to 45 years were included in the study. Blood samples were collected and analysed using HPLC to identify haemoglobin abnormalities.
Results: Haemoglobin HPLC analysis revealed haemoglobinopathies in 14.1% of cases, with β-thalassemia trait being the most prevalent (11.4%). Other hemoglobinopathies identified included HbD Punjab trait and HbE trait. Haematological indices provided insights into different parameters across various hemoglobinopathies.
Conclusion: Hemoglobinopathies represent a significant healthcare challenge in India, particularly among women of reproductive age. This study emphasizes the importance of screening for carriers using haemoglobin HPLC, as it facilitates the provision of tailored counselling and management strategies to alleviate the impact of these disorders.
Keywords: Haemoglobinopathies, reproductive-age group, blood disorder, Anaemia, haemoglobin.

INTRODUCTION

Haemoglobinopathies are a heterogeneous group of inherited disorders wherein there is a defect in the synthesis or structure of the globin chains in the haemoglobin molecule. Worldwide, approximately 7% of pregnant females have been found to be carriers of inherited haemoglobin disorders with over 1% of couples being at risk of an offspring with a significant haemoglobin disorder.[1,2] The two most important haemoglobinopathies- thalassemia and sickle cell anaemia are more prevalent in malaria-endemic regions as these genetic disorders provide a selective advantage against the plasmodium species infection.[1] Haemoglobinopathies specially thalassemia and sickle cell disease pose a substantial burden on public health in India. The β-thalassemia is prevalent throughout the country with average frequency of carriers of β-thalassemia in India approximated to be 4% and almost 0.5/1000 live births expected to be affected by the disease annually.[2] The prevalence of sickle cell disease is highly variable in India with the frequency reaching 48% in certain tribal populations.[3] Almost 1.5/1000 live births are expected to be affected homozygous Hb S genotype.[2] Clinical manifestations of haemoglobinopathies show a wide variability ranging from mild anaemia at the one end of spectrum to severe transfusion dependent disease which requires regular lifelong blood transfusions

and iron chelation. The severe disease preferably requires stem-cell transplantation as the curative treatment. Requiring a wide spectrum of diagnostic and treatment modalities for their adequate management, these inherited disorders present a major health problem throughout the world.[4] In a developing country like India, haemoglobinopathies present a major healthcare and economic challenge and effective strategies are required for prevention and treatment of the disease.[2] Understanding the burden of the disease especially in the reproductive age/antenatal females is paramount for planning these various preventive and screening programmes. Current study investigates the frequency of haemoglobinopathies in reproductive age females in a tertiary care centre in Haryana using high-performance liquid chromatography (HPLC).

MATERIAL AND METHODS

A prospective study was conducted for one year (2022) in the department of Pathology in a rural tertiary care centre in Haryana. Ethical clearance from the institute ethical committee was obtained for conducting the study. The written informed consent was obtained from all included females. A total of 149 females in the reproductive age (15-45 years) were included. Three millilitres of whole blood sample of the all the subjects were collected in vacuum tubes with ethylenediaminetetraacetic acid (EDTA) as anticoagulant. No strict exclusion criteria were employed; however, for patients in need of blood transfusions, sample collection was postponed for a minimum of 4 weeks after or just before the subsequent transfusion. All the samples were subjected to complete blood count (CBC) and peripheral blood smear analysis (PBS) within 24 hours of collection. Complete blood counts were run on the 6-part haematology analyser Sysmex-XN 550 or 3-part haematology analyser Sysmex-XP 100. Haemoglobin variant analysis of the blood samples was performed using HPLC on Bio-Rad D-10 instrument (Bio-Rad Laboratories, California, USA). If haemoglobin HPLC was not performed on the blood sample on the same day, the samples were stored for a maximum of one week at 2–8°C before running on the HPLC instrument.

Analysis of HPLC of the samples was done exclusively when the calibrators and controls met the specified criteria. The chromatograms and reports produced by the Hb HPLC system were scrutinized and interpreted based on HbA2 and HbF concentration, retention time, and area percentage of other peaks for hemoglobin variants. The diagnosis of thalassemia trait was confirmed in instances where HbA2 was elevated (>4.0%). Cases with HbA2 levels ranging from 3.5% to 3.9% were categorized as borderline HbA2.

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS) Statistics 21 version for Microsoft Windows (Chicago, USA). Descriptive statistics, including range, mean ± standard deviation (SD), frequencies (number of cases), and relative frequencies (percentage), were employed to characterize the dataset appropriately.

RESULTS

This study, conducted from January to December 2022, involved 149 females aged between 15 to 45 years, with a mean age of 27.62 ± 6.14 years.

All samples were subjected to analysis using the Bio Rad D 10 HPLC analyser to identify haemoglobin abnormalities based on retention time and area percentage. The acceptable total area under the curve ranged from 1 to 5 million.

Out of the 149 females, 132 were found to be anaemic (Hb <12 g%). Among the anaemic cases, 12.9% had severe anaemia (Hb < 7g%), while 62.1% and 5% had moderate (Hb= 7.0 to 9.9g%) and severe anaemia (Hb= 10-11.9g%), respectively. Most cases exhibited microcytic RBCs (MCV<80fl) at 57.1%, while a minority had macrocytic RBCs (MCV>100fl) at 3.3%. Cases with normal MCV (80-100fl) comprised 39.6% of the total cases

Among the samples analysed using HPLC, 121 showed normal patterns, while 28 (18.8%) exhibited abnormalities related to HbA2, HbF, or abnormal peaks. Of these 28 subjects, 21 (14.1%) were classified as having beta thalassemia or other specific hemoglobinopathies, while the remaining 7 patients displayed borderline raised HbA2 levels (3.5%–3.9%). The predominant abnormality detected was β-thalassemia trait (βTT) (n = 17; 11.4%), followed by HbD Punjab trait (n = 3; 2.01%) and Hb E trait (n = 1; 0.67%) (as illustrated in Table 1).

The haematological indices of the cases included, as summarized in Table 2, provide insights into various parameters across different hemoglobinopathies. [Table 2]

Table 3 presents the distribution of HbF%, HbA2%, HbA% and variant haemoglobin% among patients with abnormal hemoglobin patterns. [Table 3]

Table 1: Distribution of patients according to haemoglobin HPLC Findings

Hb HPLC findings	No. of Patient (n=149)	Percentage (%)
Beta Thalassemia trait	17	11.4%
HbA2 (Borderline)	7	4.69%
HbD (Punjab)trait	3	2.01%
HbE trait	1	0.67%
Normal HPLC	121	81.2%
Total	149	100%

HbA2- haemoglobin A2, HbD- haemoglobinD, HbE-haemoglobin E, HPLC- high-performance liquid chromatography

Table 2: Blood Indices according to HPLC Findings

Hb HPLC findings	Hb g/dl (Mean ± SD)	RBC (million/µl) (Mean ±SD)	PCV % (Mean ±SD)	MCV (fL) (Mean ±SD)	MCH (pg) (Mean ±SD)	MCHC (gm/dl) (Mean ±SD)	RDW % (Mean ±SD)
Beta Thalassemia trait	8.72 ± 1.32	4.23 ± 0.63	28.71 ± 3.93	67.71 ± 6.12	20.73 ± 2.18	30.05 ± 2.16	19.66 ± 5.73
Borderline HbA2	9.32 ± 2.26	3.47 ± 1.16	30.32 ± 7.07	91.22 ± 15.55	28.65 ± 6.25	30.87 ± 3.09	19.81 ± 5.59
HbD trait	11.76 ±3.86	4.86 ±0.46	36.56 ±6.38	75.00 ±10.65	23.53 ±6.55	31.03 ±5.27	16.2 ±5.37
HbE trait	8.1	4.33	29.0	67.0	18.7	27.9	22.3
Normal HPLC	9.26 ± 2.16	3.86 ± 0.66	31.43 ± 7.85	79.02 ± 12.69	24.14 ± 5.46	29.5 ± 2.96	18.75 ± 5.33

RBC- Red blood cell, PCV- packed cell volume, MCV- Mean corpuscular volume, MCH- mean corpuscular hemoglobin, MCHC - mean corpuscular hemoglobin concentration, RDW- red cell distribution width

Table 3: Distribution of HbF, HbA2, HbA levels

Hb HPLC findings	HbF (%) (Mean ± SD)	HbA0 (%) (Mean ± SD)	HbA2 (%) (Mean ± SD)	Variant Haemoglobin (%) (Mean ± SD)
Beta Thalassemia Trait	1.65 ± 0.85	81.08 ± 1.29	5.43 ± 0.72	-
Borderline HbA_2	0.8 ± 0.0	84.97 ± 0.96	3.68 ± 0.13	-
HbD Punjab Trait	0.96±0.28	53.7 ± 3.47	2.23 ± 0.60	HbD 34.76± 2.7
HbE Trait	0.8	66.1	27.1	-
Normal HPLC	0.84 ± 0.24	84.40 ± 1.57	2.79 ± 0.47	-

DISCUSSION

Hemoglobinopathies encompass a diverse group of genetic disorders affecting hemoglobin synthesis or structure. These disorders can be broadly classified into two main groups: thalassemia syndromes and structural hemoglobin variants. Thalassemia is further classified into α-thalassemia and β-thalassemia, while the main abnormal hemoglobins include HbS, HbE, HbD and HbC.[4] Thalassemia syndromes and sickle cell anaemia represent some of the most severe genetic disorders and are significant public health concerns in India.

β-thalassemia is widespread throughout India, with carriers comprising an average of 3-4% of the population. Certain communities, including Sindhis, Punjabis, Gujaratis and Bengalis, exhibit a higher prevalence of the condition.[5] Tribal populations in Southern, Central, and Western states of India show a significant prevalence of HbS,[6] Estimates suggest that India may have approximately 100,000 patients with β thalassemia syndrome and around 150,000 cases of sickle cell disease.[5]

Current study aimed to identify the frequency and spectrum of various haemoglobinopathies in reproductive age group among the patients visiting a rural tertiary care centre in North India. There is limited research available on hemoglobinopathies among females of reproductive age in North India.

Out of the 149 female participants in this study, 21 individuals (14.1%) were identified as having a haemoglobin disorder. Similar findings were observed in studies conducted by Balgir et al,[7] and Shah et al,[8] where the prevalence of hemoglobinopathies among antenatal females was reported to be 14.8% in Madhya Pradesh, Central India, and 14.9% in Meghalaya, North-Eastern India, respectively. In contrast, Narang et al,[9] found a lower frequency of hemoglobinopathies (6.7%) in reproductive-age females from Punjab, Northern India, while Jain et al,[10] reported a prevalence of 5.5% among anaemic females. It is important to note that this study is not population-based, and the frequency of hemoglobinopathies may vary depending on the frequency of reference for Haemoglobin HPLC examination.

The predominant haemoglobin abnormality observed in this study was β-thalassemia trait, with 17 cases accounting for 80% of all detected hemoglobinopathies. Following β-thalassemia trait, HbD Punjab trait was the next most common, observed in 3 cases, while HbE trait was identified in 1 case. This distribution aligns with findings from other studies conducted in North India,[9,10] as well as in a multicentric study by Mohanty et al,[11] where β-thalassemia trait was reported as the most prevalent hemoglobinopathy in Northern India, followed by HbD Punjab. Notably, HbD Punjab has been documented to affect around 2% of the population in Punjab.[6]

In a study conducted among reproductive-age females in West Bengal (Eastern India), a higher prevalence of HbE (18.7% of all hemoglobinopathies) was observed, with β-thalassemia trait being the second most prevalent haemoglobin disorder.[12] HbE trait was the most common haemoglobin disorder reported in antenatal females in Meghalaya, North Eastern India.[8] whereas in antenatal females from Madhya Pradesh, Central India, HbS trait was identified as the most prevalent hemoglobinopathy.[7]

The prevalence of various haemoglobinopathies varies widely across different states and communities in India. Most common haemoglobin abnormality in the current study was βTT. High prevalence rates of βTT in states like Punjab,

Gujarat, Maharashtra, and Tamil Nadu.[2,3,5] While heterozygous individuals with β-thalassemia are often asymptomatic and are typically identified during routine anaemia screenings, homozygous patients experience significant morbidity. At the severe end, hemoglobinopathies can significantly affect quality of life, necessitating lifelong care often involving regular blood transfusions, and may lead to a shortened life expectancy. Allogeneic stem cell transplant remains the only curative option for β thalassemia major patients, with a success rate exceeding 90% in patients with good risk features. However, the high cost of transplantation is prohibitive for many families with a thalassemia major child. Thus, prevention of the birth of an affected child is a feasible and realistic option. The birth of a child with β-thalassemia major presents substantial emotional and financial challenges for families and society.[4]

Population-wide screening, premarital and genetic counselling, and prenatal diagnosis are vital strategies for managing the impact of haemoglobinopathies. Screening of reproductive age females for haemoglobinopathies can help identifying couples at risk of having an offspring with severe haemoglobin disorder and discuss reproductive options with them. For couples already pregnant, early identification and prenatal diagnosis options are available to prepare for potential outcomes. While fetal hemoglobinopathy diagnosis typically does not affect ongoing obstetric care, it provides valuable information for decision-making regarding the pregnancy. Early detection through routine haematological tests and advanced techniques like High-Performance Liquid Chromatography (HPLC) helps prevent affected births. Increasing awareness among healthcare professionals and the general population is vital for the success of screening programs and reducing the disease burden in India..

CONCLUSION

In conclusion, this study sheds light on the prevalence and spectrum of hemoglobinopathies among reproductive-age females in a rural tertiary care center in Haryana, India. Detection of these disorders, particularly β-thalassemia through screening programs including haemoglobin HPLC, holds promise for preventing affected births and reducing the disease burden. However, to effectively address this challenge, increased awareness among healthcare professionals and the general population is paramount. By prioritizing screening, genetic counselling, and appropriate management, we can work towards alleviating the burden of hemoglobinopathies in India and improving the quality of life for affected individuals and their families

Limitation of the study: There are few limitations of present study firstly, small sample size secondly, we did not record any associated medical complication arises from haemoglobinopathies, and thirdly any family history of this disorder was not recorded.

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