OBSERVATIONAL STUDY

Laboratory Profile of Adult Hemoglobinopathies Picked Up During Routine Health Check in a Tertiary Care Hospital from South India

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ABSTRACT

Background and aim: Hemoglobin (Hb) variants can clinically range from being completely asymptomatic to frequent requirement of transfusions. Some individuals may become aware of a variant only when a complete blood count (CBC), hemoglobin analysis or genetic testing is done for a different reason. These individuals are typically heterozygous for an autosomal recessive variant. A study was conducted to find out the different types of hemoglobinopathies in adults presenting to a tertiary care hospital for routine health check and its geographic distribution. **Objective:** 1) To find out the severity of anemia associated with different hemoglobinopathies. 2) To find the relevance of glycated hemoglobin (HbA1c) in the presence of various hemoglobinopathies. **Methods:** A retrospective cross-sectional observational study was done in 111 consecutive patients who were found to have hemoglobinopathies during routine health checks in a tertiary care hospital in South India from 2013 to 2021. **Results:** One hundred eleven patients were found to have abnormality in Hb electrophoresis and there was a male predominance (69 patients, 62.2%). Majority of patients with beta-thalassemia (28 patients, 63.6%) were from West Bengal. Both HbE trait (17 patients, 65.4%) and homozygous HbE (23 patients, 62.2%) were from Assam. There was statistically significant distribution (p value 0.0001). HbA1c detected Hb variant in those with HbE disease. **Conclusion:** Hemoglobinopathies constitute a huge hereditary burden and a serious healthcare concern in India. Hence, it is the need of the hour to pick up such asymptomatic cases and provide appropriate premarital and prenatal counseling. Also, it is essential to devise strategies other than routine HbA1c testing to guide blood sugar control.

Keywords: Hemoglobinopathy, HbE, HbS, beta-thalassemia, high performance liquid chromatography, HbA1c

The normal hemoglobins (Hbs) are produced during embryonic, fetal and postnatal life. Adult Hb is produced starting in the first year of life. The predominant form found is HbA (95-98%), consisting of two alpha chains and two beta chains. Red blood cells (RBCs) also contain a small portion of HbA2 (2-3%), which contains two alpha chains and two delta chains, and HbF (<2%). There are over 1,000 different variants (genetic changes) that have been described in the genes that encode the different globin chains. Hemoglobin variants can be classified based on their clinical phenotype (asymptomatic to severe anemia), by the type of hematologic changes they produce (hemolysis, reduced expression, altered oxygen affinity), by which globin chain is affected (alpha, beta or gamma), and by the type of mutation (base change, insertion and deletion). Some individuals may become aware of a variant only when a complete blood count (CBC), hemoglobin analysis or genetic testing is done for a different reason. These individuals are typically heterozygous for an autosomal recessive variant.

The present study highlights the detection of the hemoglobinopathies in adult population picked up during routine health up by high performance liquid chromatography (HPLC). This study was undertaken as there are limited studies available on the adult population hemoglobinopathies.

AIM

To find out the different types of hemoglobinopathies in adults presenting to a tertiary care hospital and its geographic distribution.

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OBJECTIVE

- To find out the severity of anemia associated with different hemoglobinopathies.
- To find the relevance of glycated hemoglobin (HbA1c) in the presence of various hemoglobinopathies.

STUDY DESIGN

A retrospective cross-sectional observational study was done in 111 consecutive patients who were found to have hemoglobinopathies during routine health checks in a tertiary care hospital in South India from 2013 to 2021. The privacy and confidentiality of patients were maintained as per norms.

DATA COLLECTION

We retrospectively collected the clinical and laboratory data of patients diagnosed with hemoglobinopathy by HPLC on their routine Master Health checks. This included epidemiological data, comorbidities of patients, laboratory parameters, like hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), ferritin and HbA1c. After collection of all required data and careful medical chart review, the clinical data was compiled and tabulated.

Inclusion Criteria

Age more than 18 years.

Exclusion Criteria

Patients with a recent history of transfusion (3 months prior to sample collection) were excluded from the study.

STATISTICAL ANALYSIS PLAN

All continuous variables are expressed as mean ± standard deviation, if they are normally distributed. Not normally distributed continuous variables are expressed as median (interquartile range). Comparisons of categorical variables are done by Chi-square test. Comparisons of normally distributed continuous variables between more than two groups are done by analysis of variance (ANOVA). Data entry has been done in Microsoft Excel 2007 spreadsheet. Data analysis has been carried out by IBM SPSS statistics for windows version 25.0, Armonk, NY: IBM CORP. All p values <0.05 are considered as statistically significant.

RESULTS

Gender Distribution

One hundred eleven patients were found to have abnormality in Hb electrophoresis. There was a male predominance (69 patients, 62.2%; Fig. 1).

Table 1 depicts gender distribution of various hemoglobinopathies present in our study.

Though there was a male predominance, there was no statistically significant gender distribution (p value 0.897).

Age Distribution

Mean age of the patients was 42 years (Range – 18-69 years). Table 2 depicts the frequency of hemoglobinopathy in different age groups of study participants.

Geographic Distribution

Majority of patients were from Assam (47 patients, 42.3%), followed by West Bengal (40 patients, 36%),

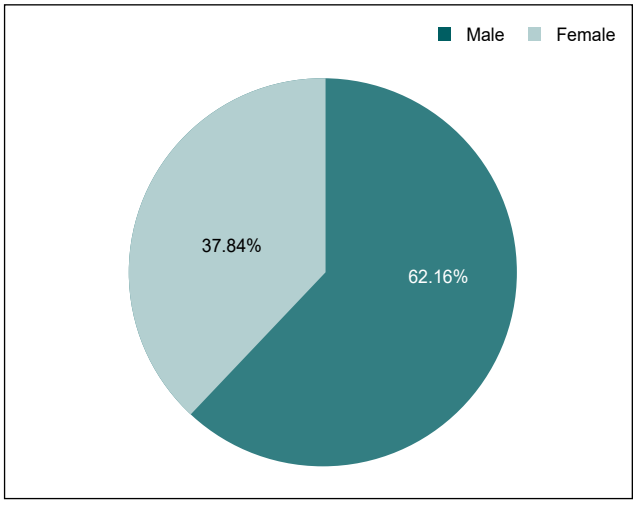

Figure 1. Gender distribution of study participants with abnormality in Hb electrophoresis.

Table 1. Gender Distribution of Various Hemoglobinopathies

Gender	Beta-thalassemia trait	HbE trait	Homozygous HbE	HbE+Beta-thalassemia	HbS disorder	HbD trait
Male	26 (37.7%)	17 (24.6%)	23 (33.3%)	1 (1.4%)	1 (1.4%)	1 (1.4%)
Female	18 (42.9%)	9 (21.4%)	14 (33.3%)	1 (2.4%)	0	0

Table 2. Frequency of Hemoglobinopathy in Different Age Groups

Age group (years)	Frequency of hemoglobinopathy	Percentage of hemoglobinopathy
<30	23	20.7
31-40	21	18.9
41-50	43	38.7
51-60	18	16.2
>60	6	5.4

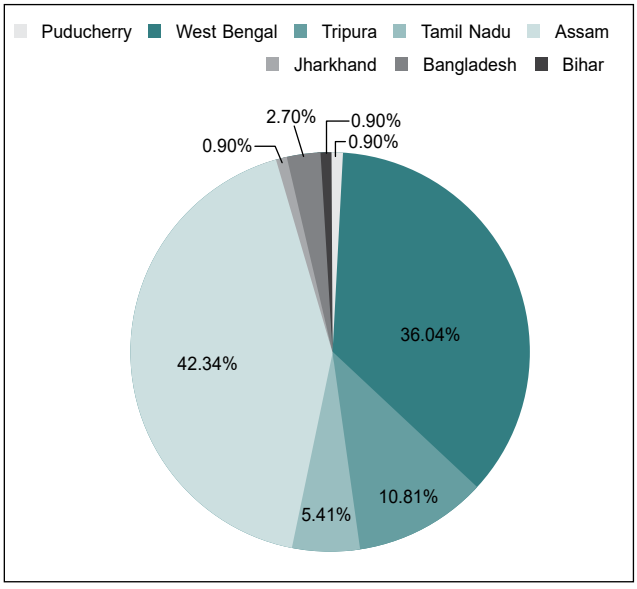

Figure 2. Geographic distribution of study participants.

Tripura (12 patients, 10.8%), Tamil Nadu (6 patients, 5.4%) and 1 patient (0.9%) each from Puducherry, Jharkhand and Bihar (Fig. 2). There were 3 patients (2.7%) from Bangladesh.

Majority of patients with beta-thalassemia (28 patients, 63.6%) were from West Bengal. Both HbE trait (17 patients, 65.4%) and homozygous HbE (23 patients, 62.2%) were from Assam. There was statistically significant distribution (p value 0.0001).

Different Types of Hemoglobinopathies

In our study group, beta-thalassemia trait (44 patients, 39.6%) was the most common hemoglobinopathy, followed by homozygous HbE (37 patients, 33.3%) and HbE trait (26 patients, 23.4%). Smaller proportion of patients also had HbE with beta-thalassemia (2 patients, 1.8%), HbS disorder (1 patient, 0.9%) and HbD trait (1 patient, 0.9%) (Fig. 3).

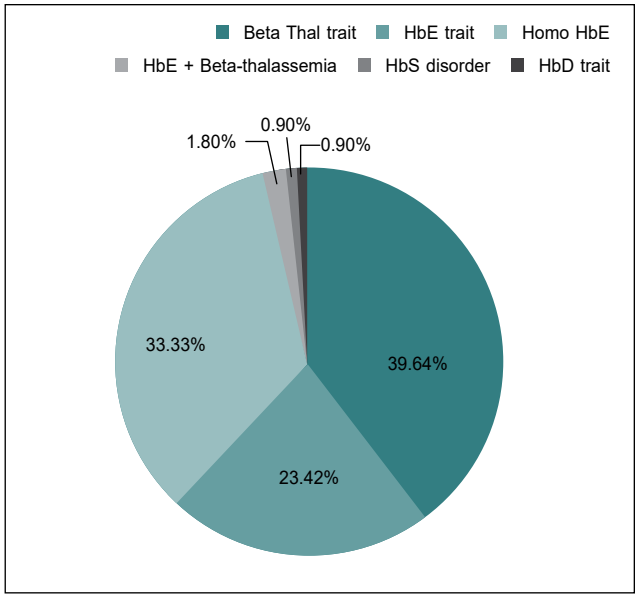

Figure 3. Presentation of different hemoglobinopathies in study participants.

Comorbidities

Various associated comorbidities in the study group were diabetes mellitus (8 patients, 7.2%), hypertension (4 patients, 3.6%), hypothyroidism (2 patients, 1.8%), asthma (2 patients, 1.8%), ankylosing spondylitis (2 patients, 1.8%) and rheumatoid arthritis, dyslipidemia, Ca stomach and benign prostatic hyperplasia in 1 patient each (0.9%).

Hemoglobin Indices

Table 3 depicts the mean value of Hb, MCV, MCH, MCHC and RBC among various hemoglobinopathies found in our study population.

HbA2

HbA2 couldn't be detected in HbE disease. Table 4 shows mean HbA2 value in different hemoglobinopathies.

HbA1c Detection in Hemoglobinopathy

HbA1c testing was done in 61 patients. Among these, mean HbA1c was 5.7% (Range: 4.1-8%). Hb variant was detected in those with HbE disease (Homozygous HbE, HbE trait and HbE with beta-thalassemia).

DISCUSSION

India is well-known for its cultural diversity. This makes it an ideal place for studying heritable disorders, like hemoglobinopathy. The understanding and

Table 3. Hemoglobin Indices among Different Hemoglobinopathies in Study Group

Hemoglobinopathy	No. of cases	Mean Hb	Mean MCV	Mean MCH	Mean MCHC	Mean RBC
Beta-thalassemia trait	44	11.15	65.20	20.61	30.93	5.59
HbE trait	26	11.45	75.04	23.85	31.85	4.79
Homozygous HbE	37	10.6	62.11	20.11	32.32	5.28
HbE + Beta-thalassemia	2	10.85	69	23	32.50	4.81
HbS disorder	1	13.2	71	21	30	6.33
HbD trait	1	14	74	24	32	5.94

Table 4. Mean HbA2 in Different Hemoglobinopathies

Hemoglobinopathies	Number of cases	Mean HbA2
Beta-thalassemia trait	41	4.85
HbS disorder	1	2.80
HbD trait	1	2.60

analysis of pooling up of hemoglobinopathies in certain geographic areas is very essential for premarital and prenatal counseling. A study by Iyer et al has reported around 40 Hb variants.[1]

In South-East Asia and the Indian subcontinent, hemoglobinopathies represent the most common monogenic blood illnesses, which represent a severe genetic and public health burden.[2,3] There are three types of Hb variants which are commonly present in India, namely sickle cell (HbS), hemoglobin E (HbE) and hemoglobin D (HbD). These structural variants of hemoglobin have geographical variations; the total allele frequency for these variants has been determined to be 5.35% in different parts of India.[4]

In India, the average allele frequency of sickle cell and HbD was found to be 4.3% and 0.86%, respectively, with HbE accounting for 10.9% gene frequency in the North-East India.[4] Sickle cell disease is prevalent in both tribal and nontribal people, particularly in Central-East India. The carrier frequency of hemoglobinopathy ranges from 3% to 17% in India.[5] Hemoglobinopathies, as a result, constitute a huge hereditary burden and a serious healthcare concern in India.

The sickle cell hemoglobin (HbS) is a structurally abnormal variant where valine replaces glutamic acid residue at 6th position of beta-globin polypeptide chain of the molecule. HbE is caused by point mutation of beta-globin, which results in substitution of lysine for glutamic acid in position 26. HbD is a result of substitution of glutamine for glutamic acid in codon 121 of beta-globin gene.

HPLC is very helpful in the accurate diagnosis of hemoglobinopathies and thalassemias. It has exceptional resolution, reproducibility and quantification of normal and abnormal hemoglobins, thus helping with accurate diagnosis.[6]

Our study included 111 adult patients diagnosed with abnormal Hb by HPLC during routine health check. There was a male predominance (62.2%). Our study included adult patients with age ranging from 18 to 69 years. Majority of the patients belonged to the 41 to 50 years age group. Beta-thalassemia trait, HbE trait, homozygous HbE disease, HbS disorder and HbD trait were the hemoglobin abnormalities detected in our study population. Beta-thalassemia trait was the most common abnormality found in 44 patients (39.6%). Similar findings have been reported in other Indian studies as well.[7] This was followed by homozygous HbE (37 patients, 33.3%) and HbE trait (26 patients, 23.4%).

Majority of patients with beta-thalassemia (63.6%) were from West Bengal. Both HbE trait (65.4%) and homozygous HbE (62.2%) were from Assam. This geographic distribution was statistically significant with significant p value. Other studies also show clustering of such Hb variants in specific parts of India.[1]

The mean Hb was 11.15 g% in beta-thalassemia trait, 11.45 g% in HbE trait and 10.6 g% in homozygous HbE disease.

Hb variant was detected in those with structural abnormalities like homozygous HbE, HbE trait and HbE with beta-thalassemia. Antibodies that identify the N-terminal glycated amino acids in the first 4 to 10 amino acids of the beta-globin chain of hemoglobin are used in immunoassay-based HbA1c assays.[8] As a result, Hb variations with mutations in this particular area will impact HbA1c immunoassay measurements. To avoid errors in the management of diabetes patients, it is critical to understand and be aware of Hb variations that alter HbA1c values, particularly in regions with a high frequency of hemoglobinopathy. In patients with hemoglobin variations, other techniques of evaluating glycemic control, such as fructosamine, glycated serum albumin or self-monitoring of blood glucose may be better options compared to HbA1c.

LIMITATIONS

The major limitation of our study is its retrospective design and small sample size.

CONCLUSION

Hemoglobinopathies constitute a huge hereditary burden and a serious healthcare concern in India. Hence, it is the need of the hour to pick up such asymptomatic cases and provide appropriate premarital and prenatal counseling. Also, it is essential to devise strategies other than the routine HbA1c testing to guide blood sugar control.

Acknowledgments

We would like to thank Dr Prabu P, Senior Consultant Hematologist and Dr Balasubramaniam R, Statistician, for their valuable inputs.

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Melatonin Improves Sleep in Patients with MS

A pilot study, presented at the annual meeting of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS), suggested that melatonin could improve sleep time and sleep efficiency in patients with multiple sclerosis (MS) who were also having sleep disturbance.

Though the study had only 30 patients, the results suggest that melatonin could help MS patients with sleep issues, stated Wan-Yu Hsu, PhD, who presented the study. The double-blind, placebo-controlled, crossover study included participants with a Pittsburgh Sleep Quality Index (PSQI) score of 5 or more, or an Insomnia Severity Index (ISI) score more than 14 at baseline. Other assessments done at baseline included patient-reported outcomes for sleep disturbances, sleep quality, daytime sleepiness, walking ability, fatigue and mood. Half of the study subjects were given melatonin for the first 2 weeks and were then switched to placebo. The other half initially received placebo and switched to melatonin at the beginning of the third week. During weeks 2 and 4, the subjects wore an actigraph watch, and the patient-reported outcome measures were repeated at the end of weeks 2 and 4. Melatonin led to an improvement in average sleep time (6.96 vs. 6.67 hours). Sleep efficiency was also slightly improved (84.7% vs. 83.2%); however, it was not statistically significant ($p = 0.07$). Improvements in ISI (–3.5 vs. –2.4; $p = 0.07$), change in PSQI component 1 (–0.03 vs. 0.0; $p = 0.07$), and change in the NeuroQoL-Fatigue score (–4.7 vs. –2.4; $p = 0.06$) were the other trends toward statistical significance… (*Source: Medscape*)

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