



Original Article

Haemoglobin Variant Study by HPLC Method at a Tertiary Care Centre

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ABSTRACT

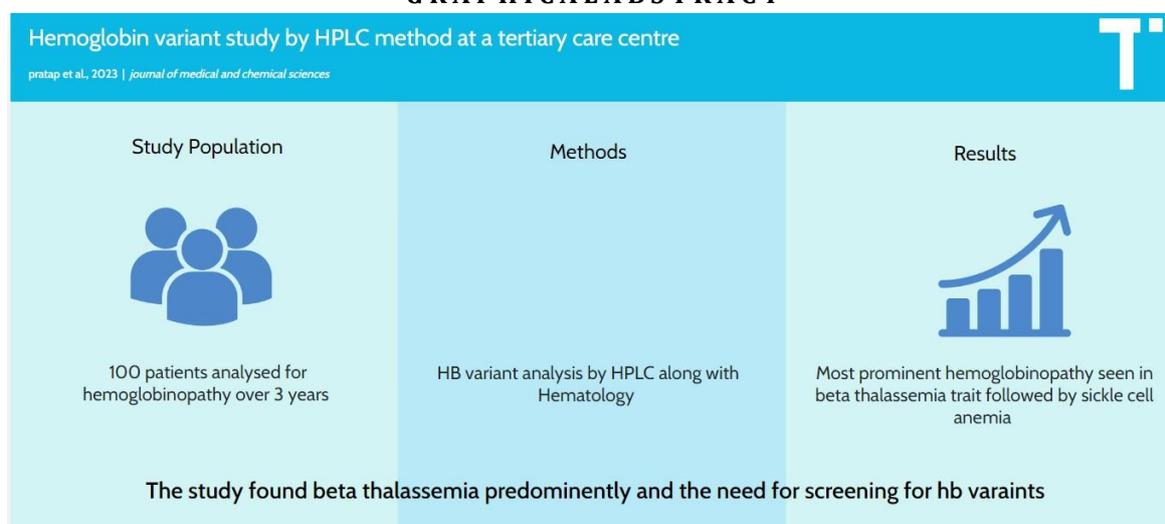
Haemoglobinopathies are genetic disorders arising due to defect in the globin chain of haemoglobin. It is relatively common amongst genetic disorders. Beta thalassemia and Sickle cell are two commonest types of haemoglobinopathy encountered and clinically range from mild or asymptomatic condition to life threatening condition requiring blood transfusions for survival. In India, haemoglobinopathies seen are both thalassemias and sickle cell syndromes. The aim of the study is to determine the prevalence of haemoglobinopathies at our tertiary care centre from the haemoglobin sample processed. Accordingly, the study estimated the haemoglobin variant by HPLC method on 100 patients over 3 years and the diagnosis of haemoglobinopathies were confirmed after taking into consideration the results of variant study, haematological parameters and clinical profile of the patients. The results revealed out of 100 patients, 27 patients showed abnormal haemoglobin variant study and the most common haemoglobinopathy observed was 17 cases of beta thalassemia trait, 4 cases of sickle cell trait, 4 cases of sickle beta thalassemia, and 1 case of haemoglobin E trait and beta thalassemia major. The abnormal haemoglobin most commonly prevalent was beta thalassemia trait which was similar to other studies done in India and many subpopulation studies. All haemoglobinopathies were advised family study for evaluation and future genetic implications. Haemoglobin E trait was diagnosed based on the retention time observed as the equipment failed to recognize the abnormal variant. One patient was on regular blood transfusion and was having beta thalassemia major haemoglobinopathy.

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GRAPHICAL ABSTRACT



Introduction

Haemoglobinopathies are amongst the most common genetic disorders. These genetic disorders cause defect in the beta globin chain of haemoglobin. Haemoglobinopathies cause a wide spectrum of clinical presentation ranging from mild anemia to life threatening anemia requiring blood transfusion for survival and being an inherited disease needs early diagnosis and management and genetic counselling, to diagnose these disorders most commonly test and technique used is haemoglobin variant study by high performance liquid chromatography or HPLC method. The 2 most common haemoglobin variant seen are Beta Thalassemia and Sickle cell anemia.

Haemoglobinopathies are relatively common genetic disorders and according to the World Health Organization (WHO), around 5% of human population have this genetic defect [1].

In India, about 15000 babies are born every year with thalassemia major [2], the prevalence of beta thalassemia carrier is around 3-4% [3-5]. Haemoglobin S is seen in tribal population of central, west and southern India and may reach upto 48% of the population [6]. Haemoglobin E is common in north eastern states where as Haemoglobin D is common in Punjab. Haemoglobin variants in India are relatively

common as compared with worldwide data, and similarly it is the most common genetic disorder seen in India [3].

Haemoglobin variants are diagnosed with the help of clinical features along with investigative modalities like blood counts, indices and Haemoglobin separation studies like electrophoresis and HPLC methods.

The Haemoglobin variants in our centre was studied by HPLC method of Haemoglobin separation which is a common alternative to electrophoresis [7, 8].

The aim of the study is to determine the prevalence of haemoglobinopathies from the haemoglobin variant test conducted over 3 years at our tertiary care centre. This will in turn reveal the magnitude of the problem and thus allow early diagnosis and genetic counselling.

The limitation of the study is the sample size of the test conducted and a larger sample size study will provide better prevalence rate of hemoglobinopathies.

Materials and Methods

The study was conducted over 3 years from beginning of 2020 to end of 2022 and involved 100 patients. Patients having history of blood transfusion in previous 3 months were excluded from the study.

Clinical history, family history, and complete blood count with red blood cell indices and peripheral blood smears were analysed for each patient. 3 ml blood samples for estimating blood counts and red blood cell indices were collected in EDTA vacutainer and analysed by fully automated 5-part differential coulter for complete blood count and analysed by Bio-Rad D10 dual mode fully automated cation exchange HPLC analyser, peripheral blood smear was stained with Leishman stain. The samples were run after running and approval of quality control materials.

The haemoglobin variant analysis by HPLC generated a chromatogram with different area peaks demonstrating individual fractions of haemoglobin separation which were identified using retention time. If the peak was not identified, it was labelled as unknown peak by the software.

Diagnosis was made using the chromatogram interpretation in co-relation with the clinical, family history, and haematological profile of the patients.

Haemoglobin electrophoresis is a similar, but old technique that can be used to study the various haemoglobin variants. However, since more than a decade HPLC method has become the

investigation of choice for studying the variants as it provides good separation and has a large databank to identify unknown variants.

Statistical analysis was done using SPSS software and Microsoft Excel. Data were expressed as mean and SD.

Results and Discussion

The Haemoglobin variant study was done on 100 patients of which 73 were having normal haemoglobin variant and 27 were having haemoglobinopathy. The age group ranged from 7 months to 84 years and mean age being 25 years. In the normal haemoglobin variant group, the male: female ratio was 20:53, where as in the abnormal haemoglobin variant group the male: female ratio was 17:10. A chromatogram is considered normal if the total area was between 1-5 million and had majorly HaemoglobinA₀ and HaemoglobinA₂ was less than 3.3% and Haemoglobin F < 0.8%. All abnormal Haemoglobin variants along with their haematological findings are shown in [Table 1](#) and along with chromatographic concentrations of haemoglobin fractions based on globin chain is summarized in [Table 2](#).

Table 1: Haemoglobin variants along with haematological findings

Diagnosis	Total no of cases	Percentage of cases (%)	Age Mean \pm SD	Haemoglobin Mean \pm SD	PBS findings red blood cell predominantly	MCV Mean \pm SD	MCH Mean \pm SD	RDW Mean \pm SD
Normal haemoglobin Variant	73	73	26 \pm 1.2	8.9 \pm 2.5	Normocytic, normochromic	78 \pm 2.9	31 \pm 2	13 \pm 1.8
Beta thalassemia Major	1	1	2	6.2	Microcytic hypochromic	64.4	27	21
Beta thalassemia Trait	17	17	26 \pm 2.4	7.5 \pm 1.5	Microcytic hypochromic	70 \pm 1.5	29 \pm 1.4	17 \pm 2.3
Sickle beta thalassemia	4	4	18 \pm 2.1	6.7 \pm 1.8	Microcytic hypochromic	66 \pm 2.5	29 \pm 1.8	19 \pm 1.2
Sickle cell trait	4	4	20 \pm 1.2	9.3 \pm 2.1	Normocytic hypochromic	72 \pm 2.1	30 \pm 1.2	15 \pm 1.3
Haemoglobin E trait	1	1	1	11	Normocytic hypochromic	76	30.2	15

Haemoglobin: Haemoglobin, PBS: Peripheral blood smear, MCV: Mean Corpuscular Volume, MCH: Mean concentration of Haemoglobin, and RDW: Red blood cell distribution width.

Table 2: Haemoglobin variants and their chromatographic concentrations of haemoglobin fraction based on globin chains

Diagnosis	Haemoglobin A0 Mean \pm SD	Haemoglobin A2 / Haemoglobin E Mean \pm SD	Haemoglobin F Mean \pm SD	Haemoglobin S Mean \pm SD
Normal haemoglobin variant	84.3 \pm 6.5	2.6 \pm 1.1	1.3 \pm 2.2	NIL
Beta thalassemia major	3.5	4.5	97.6	NIL
Beta thalassemia trait	82.9 \pm 1.5	4.8 \pm 3.5	1.4 \pm 0.9	NIL
Sickle beta thalassemia	11 \pm 1.4	4.1 \pm 2.3	21.18 \pm 3.8	60.9 \pm 9.7
Sickle cell trait	53.7 \pm 7.1	3.3 \pm 1.3	3.9 \pm 2.1	31.7 \pm 9.4
Haemoglobin E trait	60.2	26.4	3	NIL

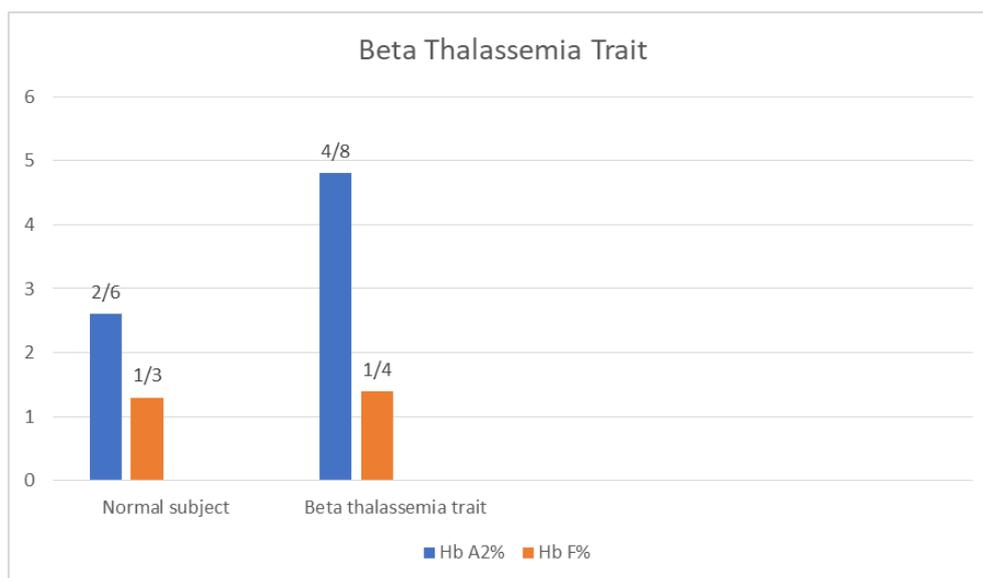
In our study, 77 cases were having normal haemoglobin fractions (77%). Most common abnormal haemoglobin variant seen was beta thalassemia trait 17 cases (17%) of mean age 26 years and having mean haemoglobin of 7.5 g/dl, the red blood cell indices, and PBS findings suggested microcytic hypochromic anemia and clinically asymptomatic. The only haemoglobin fraction elevated were HaemoglobinA₂, having a mean value of 4.8% (Figure 1).

Haemoglobin S was seen in 8 cases of which 4 were sickle trait and 4 were sickle beta thalassemia heterozygous. Sickle cell trait Haemoglobin AS were asymptomatic with mean Haemoglobin S fraction of 31.7%, mild anemia

(Haemoglobin – 9.3 g/dl), and having normal red blood cell indices and PBS findings (Figure 2).

Sickle beta thalassemia cases were clinically symptomatic, with mean Haemoglobin S fraction of 60.9%, mean Haemoglobin F was 21.18 % few cases had history of blood transfusions, mean Haemoglobin was 6.7 g/dl and red blood cell indices, and PBS findings suggested microcytic hypochromic anaemia (Figure 3).

We had 1 case of Beta thalassemia major who was a diagnosed case with history of blood transfusions having mean Haemoglobin F 97.6%, significant anemia (Haemoglobin–6.2 g/dl) with red blood cell indices and PBS showing microcytic hypochromic anemia (Figure 4).

**Figure 1:** The significant mean changes in haemoglobin variant seen between normal subjects and those having Beta thalassemia trait

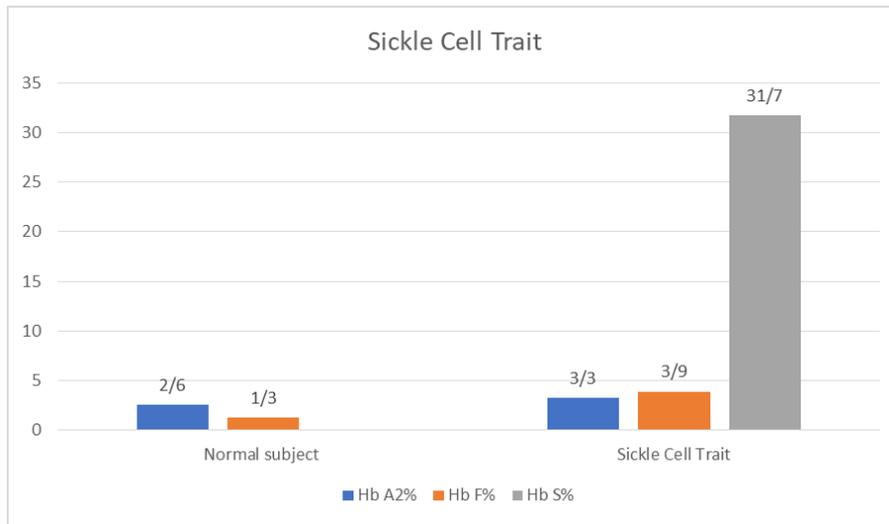


Figure 2: The significant mean changes in haemoglobin variant seen between normal subjects and those having Sickle cell trait

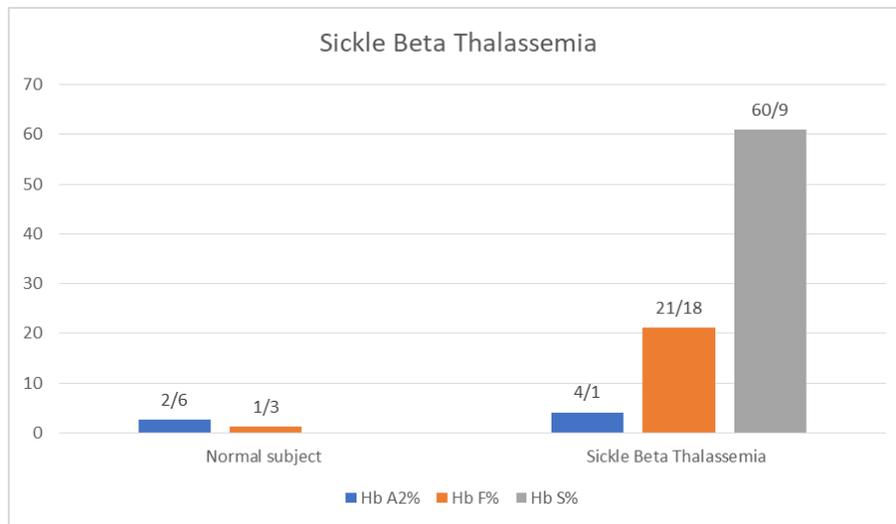


Figure 3: The significant mean changes in haemoglobin variant seen between normal subjects and those having Sickle Beta thalassemia

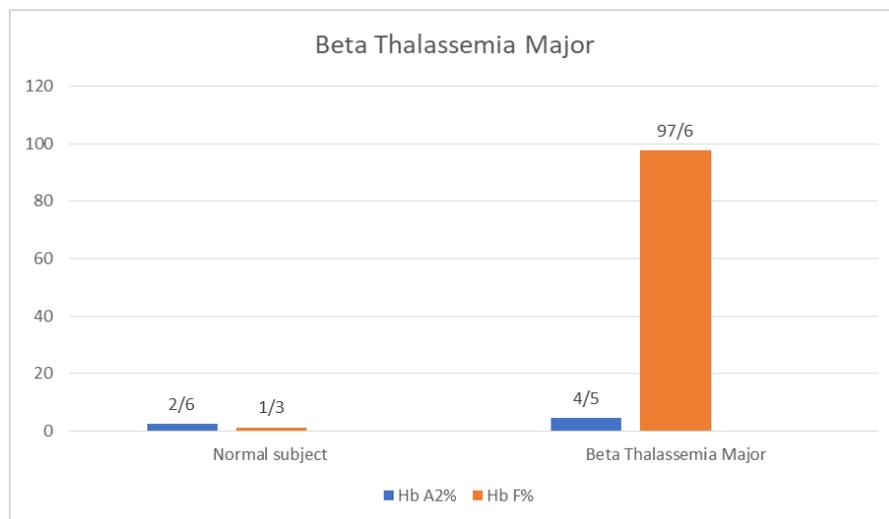


Figure 4: The significant mean changes in haemoglobin variant seen between normal subjects and those having Beta thalassemia major

1 case of Haemoglobin E trait was seen having mean Haemoglobin A₂ – 26.4%, which based on the retention time and technical limitation of the machine Haemoglobin E coelutes with Haemoglobin A₂ and having normal clinical and haematological profile a diagnosis of Haemoglobin E trait was made (Figure 5).

In this study, Haemoglobin variant was seen in 27 patients and the most common variant was beta thalassemia trait seen in 17 out of 27 patients. However, a wide range of abnormal haemoglobin variant ranging from 35 to 60% has been reported from various part of India [9, 10]. Beta thalassemia trait was in seen in 17% of total cases study and was the commonest variant seen, other studies have also found similar findings in their institution or regions [11-17]. The cases of beta thalassemia trait were characteristically asymptomatic with mild microcytic hypochromic with only abnormality noted in chromatograms were of HaemoglobinA₂ between 4-8%. Slightly raised A₂ levels as the only finding is also seen in vitamin B12/Folic acid deficiency, but it was differentiated from beta thalassemia based on red blood cell indices and vit B12/Folic acid estimation levels.

Haemoglobin S or sickle Haemoglobin (Haemoglobin S) was seen in total 8 cases (8%) out of which 4 cases presented as sickle cell trait and 4 cases as sickle beta thalassemia. The prevalence of Haemoglobin S was lower as compared to other studies like Dash *et al.* [10] (29%) and Balgair *et al.* [9] (39.3%). However, the most common type of Haemoglobin S seen was sickle cell trait Haemoglobin AS which was similar to other studies. Cases of sickle beta thalassemia were anaemic with PBS picture of microcytic hypochromic anaemia, whereas sickle cell trait was asymptomatic.

One case of beta thalassemia major was seen which was having significant anaemia and haemoglobin of 6.2 g/dl on repeated blood transfusion with typical chromatogram of Haemoglobin F as most prominent band.

One case of Haemoglobin E trait was identified which was clinically asymptomatic and haematologically normal with only prominent finding of HaemoglobinA₂ 26.4% and based on retention time diagnosis of Haemoglobin E was made.

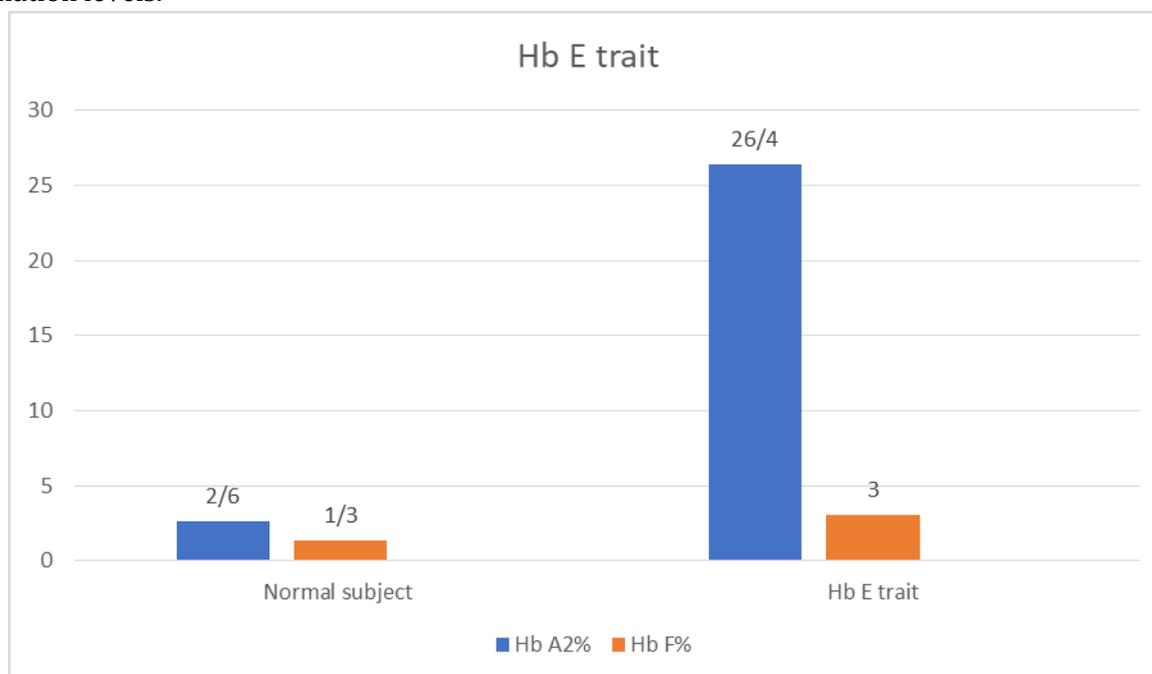


Figure 5: The significant mean changes in haemoglobin variant seen between normal subjects and those having HbE trait. Co-elution of HbE and HbA₂ is seen

Conclusion

Beta thalassemia trait is very common in most parts of India, which was also seen in this study. The condition although is asymptomatic but holds significant clinical outcome if both parents are beta thalassemia trait and the child becomes beta thalassemia major, a significant clinical, mental, and financial burden to the whole family. Hence, the identification of beta thalassemia trait, evaluation, and genetic counselling can help and guide the patient and family, similarly this is true for all haemoglobinopathies. The novelty of the study is that it addresses to an extent the prevalence of haemoglobinopathy amongst the local population accessing the tertiary care centre and help provide necessary services.

HPLC is a simple, sensitive, and accurate method to identify Haemoglobin variant in an individual, but the chromatogram data should always be interpreted with clinical findings and family history of the patient.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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Ethics approval and consent to participate

All clinical investigations must be conducted according to the [Declaration of Helsinki principles](#). The authors must comply with the guidelines of the International Committee of Medical Journal Editors (www.icmje.org) with regard to the patient's consent for research or participation in a study. Patients' names, initials, or hospital numbers must not be mentioned anywhere in the manuscript (including figures). Editors may request that authors provide documentation of the formal review and recommendation from the institutional review board or ethics committee responsible for oversight of the study.

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