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Original Article

Pathology Section

Determination of Haemoglobin Variants by High Performance Liquid Chromatography in Patients with Moderate to Severe Anaemia: A Cross-sectional Study in Tertiary Care Hospital, South Bangalore, India

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ABSTRACT

Introduction: Haemoglobinopathies are among the most common hereditary disorders in India and represent a major public health problem. Their management requires lifelong treatment such as transfusions, chelation therapy, and repeated investigations, causing significant socio-economic burden on families. Early detection through screening is essential to prevent homozygous births and enable timely genetic counselling. Cation exchange High Performance Liquid Chromatography (HPLC), along with CBC, is a reliable first-line tool for identification and categorisation of haemoglobinopathies.

Aim: To diagnose haemoglobinopathies early using cation exchange HPLC and to facilitate regional screening for prevention of homozygous disorders and genetic counselling.

Materials and Methods: This cross-sectional study was conducted in the Department of Pathology, BGS Global Institute of Medical Sciences, Bengaluru from March 2022 to September 2023 for 18 months with a sample size of 140 cases of moderate to severe anaemia. Complete blood counts were obtained from DxH520. HPLC was performed on H9 lifotronics and variants were identified on the basis of their percentages, retention times and peak characteristics. A complete blood count, including RBC indices MCV, MCH, MCHC and RDW were performed using the DxH520. Discriminant indices like mentzer index and sehgal index were calculated. Peripheral smear, reticulocyte count and sickling test were done in suspected cases. Continuous variables were expressed as mean±SD. Categorical variables were expressed as percentages.

Results: Out of 140 cases, 33 cases (23.57%) were positive for Haemoglobinopathies in which the majority of cases were β-Thalassaemia Trait (BTT) 26 cases and other cases being one case of β-thalassaemia major, one case of β-thalassaemia intermedia, one case of sickle cell anaemia, one case of sickle cell trait, one case of HbE disease, one case of HbE with β-thalassaemia and one case of sickle cell with β-thalassaemia.

Conclusion: While HPLC is an established gold standard investigation in diagnosing haemoglobinopathies, the present study focuses specifically on a regional population in Bengaluru with a high rate of consanguineous marriages (26%). This study serves as a critical regional screening baseline to identify the local prevalence of haemoglobinopathies in symptomatic patients with moderate to severe anaemia, which is essential for local genetic counselling.

Keywords: Haemoglobinopathies, HbE disease, Microcytic hypochromic anaemia, Sickle cell diseases, Thalassaemias

INTRODUCTION

Haemoglobinopathies are the most common hereditary disorders and represent a significant national health burden with a prevalence rate of 3.7% for beta thalassaemia trait in India [1,2]. Management of haemoglobinopathies is a huge socio-economic burden to the family. Treatments such as lifelong blood transfusions, allogenic stem cell transplant, chelation therapy and repeated investigations are needed. The only effective way to reduce the burden of haemoglobinopathies is to prevent birth of homozygotes. Hence, screening for haemoglobin variants and subsequent premarital and prenatal genetic counselling is a more realistic approach for reducing the burden of haemoglobinopathies [3]. India has been termed the "thalassaemia capital" of the world. The major reasons for the highest number of thalassaemia major patients in India are lack of widespread awareness, lack of genetic counselling, consanguineous marriages and traditional belief systems. Haemoglobin variants which are prevalent in India are HbS, HbC, HbE, HbD, HbH. HbE is more prevalent in north-eastern and eastern parts of India [3]. This study is undertaken to analyse all patients who present to the Outpatient Department (OPD) with moderate to severe anaemia.

HPLC system is a fully automated technique with excellent resolution, reproducibility, requires less manpower and can provide results in shorter duration. Various normal and abnormal haemoglobin fractions will be quantified based on their retention time [4,5]. Together with CBC and other ancillary tests, HPLC is effective in categorising haemoglobinopathies as traits, homozygous disorders and compound heterozygous disorders [5]. A significant proportion of moderate to severe anaemia cases may harbour underlying haemoglobinopathies, and reliance solely on peripheral smear or iron studies may result in missed diagnoses. The novelty of the present study lies in assessing haemoglobin variants by incorporating HPLC into the evaluation of unexplained microcytic anaemia enhancing the diagnostic accuracy and facilitating appropriate counselling and management.

The present study aimed to estimate the proportions of thalassaemic syndromes using HPLC among suspected haemoglobinopathies

to facilitate screening for prevention of homozygous disorders and genetic counselling.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Pathology, BGS Global Institute of Medical Sciences, Bengaluru, Karnataka, India from March 2022 to September 2023. Ethics Committee of BGS Global Institute Of Medical Sciences, Bengaluru, approved the study with IEC number- BGSGIMS/IEC/App/2022/006. A total of 140 cases included all the patients of various age groups presenting with moderate to severe anaemia to Outpatient Department (OPD). The sample size was calculated based on the prevalence of haemoglobinopathies reported in previous regional studies.

The sample size was calculated using a study conducted by Colah R et al., in 2017 [3]. The average prevalence of β-thalassaemia carriers is 3-4% in India. At a 95% confidence level and an absolute allowable error of 3%. The sample size,

$$n = \frac{Z^2_1\text{-}\alpha/2 * p * (1-p)}{d^2}$$

n=124.2

Therefore, atleast 125 suspected haemoglobinopathies samples should be included in the study.

Patients with moderate to severe anaemia as per WHO criteria 2024were used as inclusion criteria [6]. Patients with recent history of transfusion (three months prior to sample collection), Infants less than six months of age and patients with recent history of blood loss of more than 750 mL were excluded from the study. Venous blood was collected in an Ethylene Diamine Tetra-Acetic Acid (EDTA) tube. A complete blood count, including RBC indices MCV, MCH, MCHC and RDW were performed using the DxH520. Discriminant indices like Mentzer index and Sehgal index were calculated. Peripheral smear examination and reticulocyte count were done for all cases. Special screening tests were performed as indicated. The sickling test was carried out using sodium metabisulfite to detect the presence of haemoglobin S based on red cell sickling under reduced oxygen tension. Haemoglobin H inclusion test was performed using supravital stain with brilliant cresyl blue to demonstrate HbH inclusion bodies in red blood cells, suggestive of alpha-thalassaemia.

All samples were screened for haemoglobin disorders using HPLC, H9 Lifotronics. The various normal and abnormal haemoglobin fractions were quantified based on their retention times. These retention times serve as reference points for identifying and quantifying specific haemoglobins during chromatographic analysis. Daily internal quality control was performed using manufacturer-provided control samples. Calibration of the H9 Lifotronics HPLC system was done as per standard operating protocol. Retention times were verified against standard reference windows before interpretation. Any chromatogram with abnormal baseline disturbance or poor peak resolution was repeated.

The following parameters were analysed: Haemoglobin concentration (male: 15±2 g/dL, female: 13.5±1.5 g/dL), RBC count (Male: 5±0.5 million/µL, female: 4.0±0.5 million/µL), MCV: 92±9 fL, MCH: 29.5±2.5 pg, MCHC 33±1.5 g/dL [7], Peripheral smear findings, Reticulocyte count, Discriminant indices and HPLC haemoglobin fractions.

STATISTICAL ANALYSIS

The collected data were entered into a Microsoft Excel worksheet. Qualitative variables were expressed as frequencies and percentages. Quantitative variables were expressed as Mean±Standard Deviation (SD) or median with Interquartile Range (IQR), as appropriate.

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 20.0. The Chi-square test and Student's t-test were used to assess associations between categorical and continuous variables. A p-value of <0.05 was considered statistically significant.

RESULTS

A cross-sectional study with 140 patients was undertaken for determination of haemoglobin variants by HPLC in patients with moderate to severe anaemia. The study group had 28 (20%) males and 112 (80%) females. The majority of patients in this study were females, with male to female ratio being 1:4. The most common age group included in the study was between 21-30 years of age with 70 (50%) cases followed by 31-40 years with 32 (22.9%) cases [Table/Fig-1].

Age in years	Number of cases	Percentage
>6 mnth to <1 y	06	4.3%
1-10	07	5.0%
11-20	16	11.4%
21-30	70	50%
31-40	32	22.9%
41-50	06	4.3%
51-60	02	1.4%
>60	01	0.7%
Total	140	100%

[Table/Fig-1]: Age distribution of total cases in the study.

Out of 140 cases, majority of cases were Microcytic Hypochromic Anaemia (MHA) of moderate degree with 96 cases (68.5%) followed by MHA of severe degree with 34 cases (24.2%) and MHA with haemolytic blood picture in 10 cases (7.1%). Out of these, 107 (76.42%) were Iron deficiency anaemia and 33 (23.57%) of cases showed different types of Haemoglobinopathies and haemoglobin variants were detected on the basis of retention time, percentage of haemoglobin, and characteristics of the peak observed in the chromatogram. Among 140 cases, Haemoglobinopathies were detected in 33 patients and out of 33 cases, the majority were females being 20 cases (60.61%) and 13 patients (39.39%) were male patients and the ratio of male to female was 1:1.54.

Out of 33 cases, the most common age group affected in both the sexes was between 21-30 years of age [Table/Fig-2]. The most common abnormality observed in both the sexes was the β-thalassaemia trait in the age group of 21-30 years of age [Table/Fig-2].

Age in years	Male	Female	Total Beta thalassaemia trait cases
>6 mnth to <1 y	0	01	01 (3.85%)
1-10	02	02	04 (15.38%)
11-20	01	04	05 (19.23%)
21-30	03	06	09 (34.61%)
31-40	01	03	04 (15.38%)
41-50	01	0	01 (3.85%)
51-60	01	0	01 (3.85%)
>60	01	0	01 (3.85%)
Total	10	16	26 (100%)

[Table/Fig-2]: Age and gender distribution of beta thalassaemia trait cases in the study.

Association of Discriminant functions with β-thalassaemia trait on HPLC: Both Sehgal and Mentzer indices showed a statistically significant association with BTT on HPLC (p <0.01). The Mentzer index showed lower sensitivity (52%) but higher specificity (87.83%), indicating better performance in correctly identifying non-thalassaemia cases [Table/Fig-3]. The Sehgal index demonstrated higher sensitivity (69.2%) but lower specificity (64.9%), making it more suitable as a screening tool [Table/Fig-4]. Neither index alone

was sufficient for definitive diagnosis, and HPLC remains the gold standard for confirmation of BTT.

Discriminant index-Mentzer index	β-thalassaemia trait		
	Positive	Negative	Total
Positive	13	14	27
Negative	13	100	113
Total	26	114	140

[Table/Fig-3]: Association of Mentzer Index with β-thalassaemia trait on HPLC. Mentzer index showed Chi-square value of 20.9, p-value- <0.001, Sensitivity- 52%, Specificity- 87.83%, Positive predictive value- 48.14 %, Negative predictive value- 88.5%

Discriminant index- Sehgal index	β-thalassaemia trait		
	Positive	Negative	Total
Positive	18	40	58
Negative	8	74	82
Total	26	114	140

[Table/Fig-4]: Association of Sehgal Index with β-thalassaemia trait on HPLC. Sehgal index showed Chi-square value of 11.59, p-value- <0.001, Sensitivity- 69.2%, Specificity- 64.9%, Positive predictive value- 31%, Negative predictive value- 90.2%

In our study, out of 33 cases, the most common abnormal variant seen was β-thalassaemia trait with 26 cases (78.7%). HbS was detected in three cases (9.09%), of which one was sickle cell trait, one being sickle cell anaemia and other sickle cell anaemia with β-thalassaemia [Table/Fig-5,6].

HPLC chromatographic patterns. These cases were chosen as representative examples of distinct haemoglobin variant disorders encountered during the study.

Case profile of β-thalassaemia major: We had one case of β-thalassaemia major with an eight-month-old male presenting to paediatric OPD with complaints of fever, cough and generalised weakness. On general examination, the patient was thin built with presence of pallor and mild icterus. On systemic examination, mild splenomegaly was noted. CBC and peripheral smear examination was done. Reticulocyte count was 8% [Table/Fig-7]. HPLC was done which showed HbF of 80.8%, HbA of 17.1% and HbA2 of 2.1% and was diagnosed as β-thalassaemia major. In [Table/Fig-8], HPLC chromatogram shows a major peak in the F window (HbF 80.8%). The retention time for the F window is between 0.98 to 1.2 minutes. Screening of the parents and the twin sister was done. CBC showed decrease in Hb levels in all the cases and Mentzer index in both the parents was less than 13, which was indicative of β-thalassaemia trait. Peripheral smear showed MHA of moderate degree. On HPLC, they were diagnosed as β-thalassaemia trait.

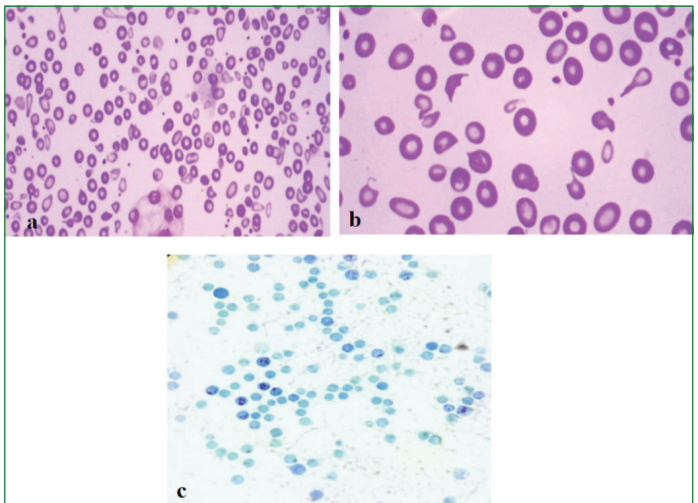
Case profile of sickle cell anaemia: A case of twenty nine year old female patient presented with cough, expectoration, fever and weakness. On routine investigations, CBC showed Hb of 7.4 gm/dL, decrease in RBC indices, and increase in RDW. Peripheral smear examination [Table/Fig-9] was done showing MHA of moderate degree along with few sickle cells, hence reticulocyte

HPLC diagnosis	HbA (%) {mean±Standard Deviation (SD)}	HbF (%) {mean±Standard Deviation (SD)}	HbA2 (%) {mean±Standard Deviation (SD)}	HbS (%)	HbE (%)
β-thalassaemia trait (26)	92.78±2.91	2.63±3.38	5.03±1.11	-	-
β-thalassaemia major (1)	17.1	80.8	2.1	-	-
β-thalassaemia Intermedia (1)	36.7	60.5	2.8	-	-
HbE disease (1)	31.9	1.8	7.7	-	58.6
HbE with β-thalassaemia (1)	47.6	25.7	4.1	-	22.6
Sickle cell trait (1)	54.4	2.4	3.5	39.7	-
Sickle cell anaemia (1)	3.5	20.9	1.7	73.9	-
Sickle cell anaemia with β-thalassaemia (1)	21	9.6	4.8	64.6	-

[Table/Fig-5]: Haemoglobin profile in each case obtained on HPLC.

HPLC diagnosis	Haemoglobin (g/dL)	RBC (million/μL)	MCV (fL)	MCH (pg)	MCHC (g/dL)
β-thalassaemia trait (26)	8.76±1.16	4.79±1.18	65.9±6.95	21.7±4	30.19±3.58
β-thalassaemia major (1)	4.6	2.35	72	19.7	27.2
β-thalassaemia Intermedia (1)	8.04	4.52	61	17.8	29.1
HbE disease (1)	8.3	4.2	72.2	18.3	28.9
HbE with thalassaemia (1)	9.1	4.4	74.6	23.2	31.1
Sickle cell trait (1)	8.42	3.02	84.6	27.9	33
Sickle cell anaemia (1)	7.4	4.8	76.8	22.5	20.4
Sickle cell anaemia with β-thalassaemia (1)	8.8	3	75.8	24.2	30.6

[Table/Fig-6]: Haematological parameters in different groups of Haemoglobinopathies.

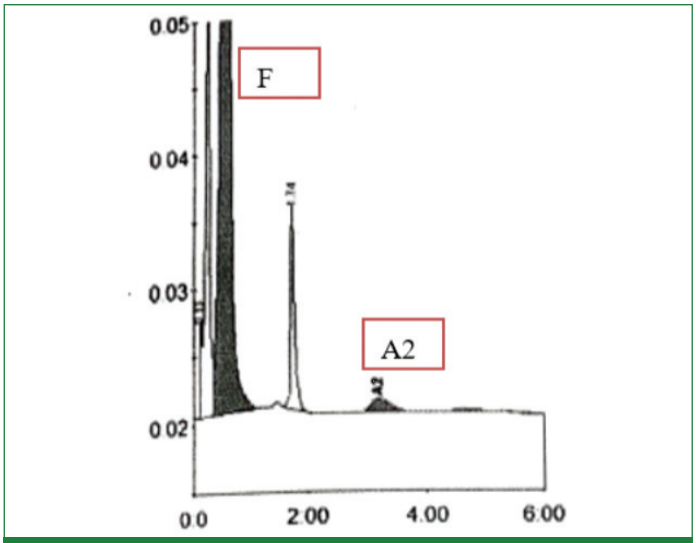


[Table/Fig-7]: a,b) Peripheral smear of β-thalassaemia major showing microcytic hypochromic RBCs, target cells, fragmented RBCs, polychromatophils and spherocytes indicating haemolytic process. {Lieshman stain, Magnification - 10x(a), 100x(b)}. Supravital stain (c)- Brilliant cresyl blue- Reticuloctyte count is increased with 8%.

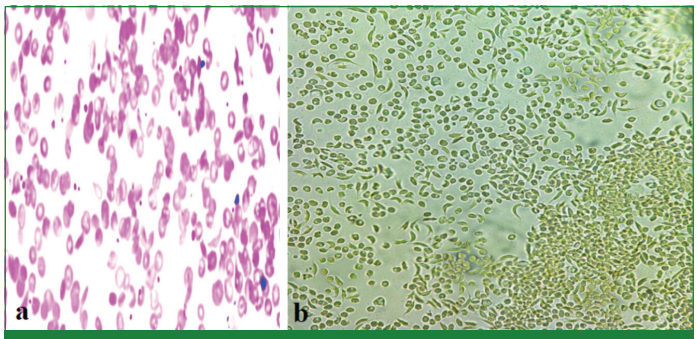
Most of the cases showed moderate anaemia with haemoglobin values between 7.4-9.1 gm/dL and β-thalassaemia major showing haemoglobin value of 4.6 gm/dL [Table/Fig-6].

Representative case descriptions of clinically significant haemoglobin variants: Selected individual cases are presented separately to highlight rare and clinically important haemoglobinopathies detected in the study, to illustrate their haemoglobin findings, peripheral smear features, characteristic

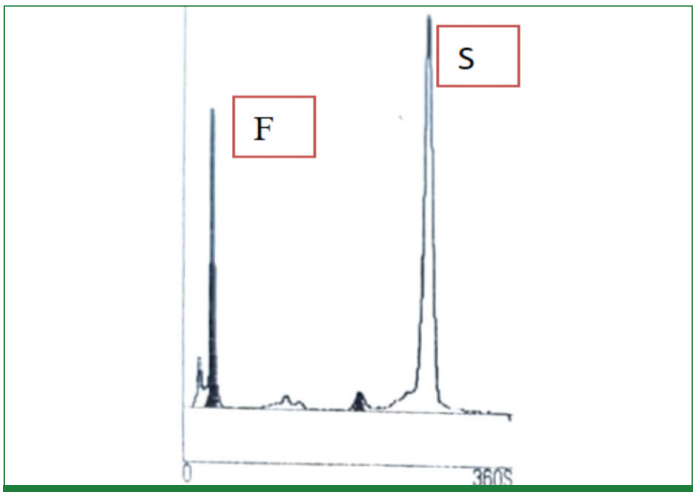
count, sickling test and HPLC were suggested to confirm the diagnosis. Reticulocyte count was 8%. Sickling test showed sickle cells with elongated and pointed ends [Table/Fig-9]. HPLC showed HbS was 73.9% with retention time for S window being 4.30-4.70 minutes, HbF 20.9%, HbA 3.5% and HbA2 1.7% confirming Sickle cell anaemia [Table/Fig-10].



[Table/Fig-8]: HPLC graph- showed HbF of 80.8%, HbA of 17.1% and HbA2 of 2.1% and was diagnosed as β-thalassaemia major.

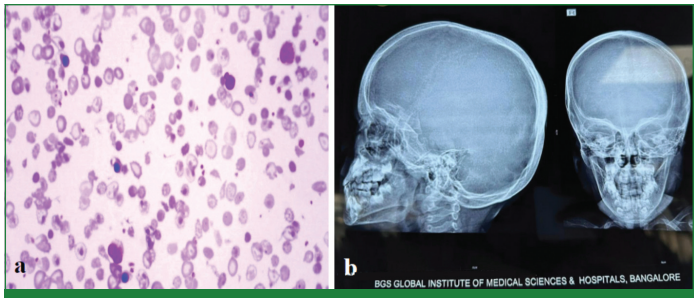


[Table/Fig-9]: a) Peripheral smear of sickle cell anaemia showing MHA along with sickle cells (Leishman stain, 400x). b) Sickling test: shows sickle cell with elongated and pointed ends.

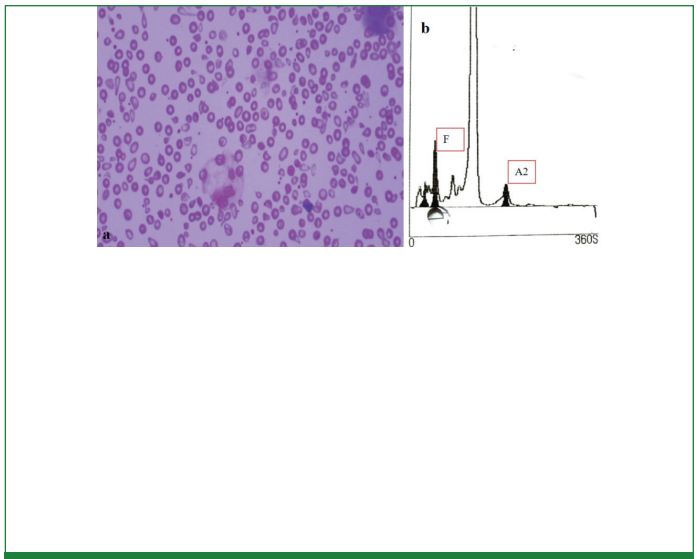


[Table/Fig-10]: HPLC graph- HbS was 73.9%, HbF 20.9%, HbA 3.5% and HbA2 1.7%. Based on the above findings, Sickle cell anaemia was diagnosed and confirmed.

Case profile of β-thalassaemia intermedia: A five-year-old male child presented with complaints of fever, cough and generalised weakness. On examination, pallor was noted and on per abdominal examination mild splenomegaly was noted. Ejection systolic murmur was noted on auscultation. CBC and peripheral smear examination was done showing the following features shown in [Table/Fig-11]. On HPLC, HbA was 36.7%, HbF of 60.5%, HbA2 of 2.8% [Table/Fig-12]. Hence, this case was diagnosed as β-thalassaemia intermedia correlating with the clinical features and investigations. Screening of both father and mother was done. CBC of mother showed Hb of 9.8 gm/dL and decrease in all the RBC indices with increase in RDW. Mentzer index was 9. Peripheral smear showed MHA of moderate degree and was positive for β-thalassaemia trait on HPLC. The family is under regular follow-up.

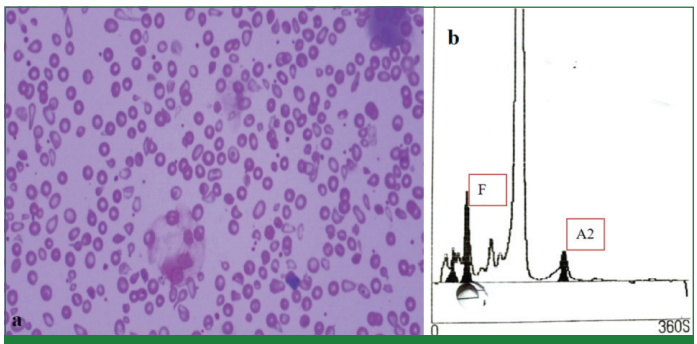


[Table/Fig-11]: a) Peripheral smear of β-thalassaemia intermedia shows Microcytic Hypochromic Anaemia (MHA) along with fragmented RBCs, target cells and Spherocytes. (Leishman stain, 400x). (b) X-ray skull: β-thalassaemia intermedia patient showing cranial vault changes compatible with haemolytic anaemia.



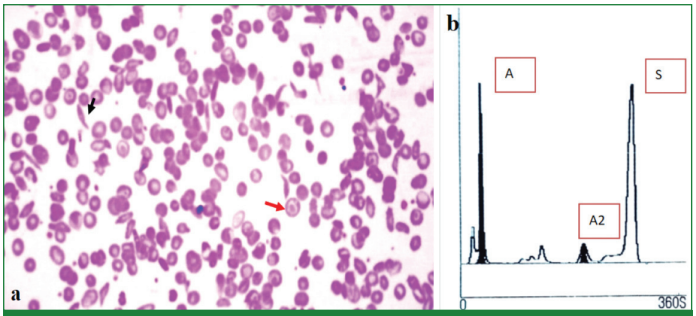
[Table/Fig-12]: HPLC graph- HbA was 36.7%, HbF of 60.5%, HbA2 of 2.8% Hence, this case was diagnosed as β-thalassaemia intermedia correlating with the clinical features and investigations.

Representative peripheral smear and HPLC features of β-thalassaemia trait: In this study, 26 cases of β-thalassaemia trait were identified. Peripheral smear findings showed microcytic hypochromic RBCs along with few target cells. HPLC revealed a mild increase in HbA2 and HbF levels [Table/Fig-13].



[Table/Fig-13]: a) Peripheral smear shows microcytic hypochromic cells and occasional target cells. HPLC graph (b): Mild increase in HbA2 and HbF levels- β-thalassaemia trait.

Case profile of HbS with β-thalassaemia: A 17-year-old male patient visited the OPD with complaints of Upper Respiratory Tract Infection (URTI), fever, and joint pains. According to the parents, the patient has a clinical history of experiencing episodes of joint pain and URTI over the past six years. He was investigated in a primary health care facility and no underlying pathology was found to explain the symptoms. Hence, a detailed work-up was done to rule out the cause of recurrent infections in the child. CBC revealed Hb of 8.8 gm/dL and peripheral smear examination showed MHA of moderate degree with sickle cells and target cells [Table/Fig-14] and iron profile done showed normal values. HPLC showed 64.6% of HbS, 9.6% of HbF, 21% of HbA and 4.8% of HbA2 suggesting sickle cell with β-thalassaemia [Table/Fig-14].



[Table/Fig-14]: a) Peripheral smear of HbS with β-thalassaemia shows MHA along with sickle cells (black arrow) and few target cells (red arrow). (Leishman stain, 400x). HPLC graph (b): showed 64.6% of HbS, 9.6% of HbF, 21% of HbA and 4.8% of HbA2 suggesting sickle cell with β-thalassaemia.

Case profile of HbE disease: A 32-year-old female patient presented with complaints of generalised weakness, fatigue, giddiness and headache. On general examination, pallor was noted. On peripheral smear examination, MHA was seen along with poikilocytes like target cells and elliptocytes. HPLC showed 58.6% of HbE, 31.9% of HbA, 7.7% of HbA2 and 1.8% of HbF, consistent with HbE disease.

DISCUSSION

Haemoglobinopathies represent a significant global public health challenge, particularly in countries like India, where they impose a substantial burden on both health services and finances [8]. Given the geographical variability of haemoglobinopathies, conducting regional studies becomes crucial to accurately determine their prevalence [8]. Early and precise diagnosis is paramount in preventing clinically significant manifestations of these lifelong disorders, thereby alleviating the economic and psychological burdens associated with them [8].

Consanguineous marriages are more common in India compared to other countries, largely influenced by cultural, religious, and social practices. Consanguineous marriages are particularly prevalent in the southern states and Union Territories of India, including Tamil Nadu, Puducherry, Andhra Pradesh, Telangana, and Karnataka, where the rate can reach as high as 28% [9,10]. According to a study done by Nalini et al., it was observed that consanguinity was notably more prevalent among families with a history of genetic disorders, such as thalassaemia and sickle cell anaemia [10]. One more study by Ramachandra et al., found that consanguinity was associated with a higher prevalence of genetic disorders, including haemoglobinopathies and chromosomal abnormalities [10]. This correlates with the study where 26% of cases were from consanguineous marriage. Prenatal and pre-marital screening and counselling, and comprehensive education on genetic risks is essential to address the burden of thalassaemia.

HPLC is widely used and is a gold standard for diagnosis of haemoglobinopathies. As HPLC can detect all the Hb variants based on their retention times, it is better than the other haemoglobin electrophoresis in detecting haemoglobinopathies as few of the variants get overlapped and cannot be diagnosed. Haemoglobin electrophoresis separates haemoglobin variants based on charge and size, but while cellulose acetate at alkaline pH often shows co-migration of similar variants, citrate agar at acidic pH allows clearer differentiation, enabling accurate identification of haemoglobins like HbF, HbC, and HbS [11]. HPLC separates haemoglobin types based on their interactions with a stationary phase in a column and a mobile phase (solvent) that passes through it. Haemoglobins are separated based on their affinity for the column material and their different chemical properties offers higher resolution and greater sensitivity than electrophoresis. It can distinguish between haemoglobin variants that are very similar and can quantify the relative amounts of different haemoglobin types with precision [1].

The HPLC offers greater sensitivity and resolution compared to haemoglobin electrophoresis, allowing it to detect and quantify haemoglobin variants that may be challenging to distinguish using electrophoresis alone. While citrate agar electrophoresis is effective for certain variants, HPLC provides even higher resolution and quantitative analysis, making it more suitable for precise haemoglobin variant identification.

A study done by Khera R et al., studied 62 cases of β-thalassaemia trait with HbA2 of 5.7±0.9%, HbF of 1.5±1.3% and Bhokare S et al., showed HbA2 of 5.27±0.55% and HbF of 1.18±1.62% [12,13]. These values were comparable with the present study. In β-thalassaemia trait, there is an increase in HbA2 levels. β-thalassaemia trait typically does not present with symptoms and is often discovered either incidentally or through prenatal screening. The study observed a predominance of females, likely because pregnant women were screened to detect haemoglobinopathies and β-thalassaemia trait may be more commonly detected through such screening programs.

Baig MA et al., studied 38 cases of β-thalassaemia major with HbF of 62±21%, Rao S et al., studied 23 cases with HbF being 52.5±35.2% [5,14]. These studies were comparable to the present study where it showed increased HbF levels with 80.8%.

In cases with extremely low haemoglobin levels (<6 g/dL), results may be undetectable or misinterpreted as errors; therefore, an outside dilution method is done, which can identify Hb variants even with very low Hb levels. In our case, the outside dilution method-mixing 30 µL of patient sample with 1.5 mL of lyse and running in dilution mode- enabled accurate detection of haemoglobin variants, revealing elevated HbA2 and HbF levels.

Khera R et al., studied five cases of β-thalassaemia intermedia with HbF of 62.37%, HbA 30.4±31.6% and HbA2 of 5.5 ± 2.6% and was comparable with the present study and other studies [12]. β-thalassaemia intermedia and β-thalassaemia major has to be differentiated on the basis of HPLC values, clinical presentation and also the age of presentation. The term "β-thalassaemia intermedia", describes patients whose clinical severity lies between the mild features of β-thalassaemia minor and the severe symptoms of β-thalassaemia major, typically presenting later in childhood or adulthood [15].

HbS/β-thalassaemias are categorised into two main types: HbS/β0 thalassaemia, where there is a complete absence of HbA, leading to a severe clinical course similar to homozygous HbS disease; and HbS/β+ thalassaemia, characterised by the presence of 20-30% HbA, resulting in a milder clinical course. In our case, it was diagnosed as HbS/β+ thalassaemia as HbA was 21% and hence the clinical manifestations were less significant [16]. Rao S et al., studied four cases of sickle cell anaemia with HbS of 74.5±6.7% and HbF of 18.3±8.2% which was comparable with present study and other studies [14].

Khera R et al., studied eight cases of HbE with β-thalassaemia with HbE of 52.8±17.9%, HbF of 18.7±22.2%, HbA of 24.4±18.1% [12]. The present study showed HbE of 22.6%, HbF of 25.7%, HbA 47.6% and HbA2 4.1% and was comparable with other studies.

Limitation(s)

This study was a single-center, hospital-based cross-sectional study. The female predominance observed likely reflects antenatal screening attendance rather than true population distribution. Detailed socio-economic and ethnic data were not analysed. Molecular confirmation of haemoglobin variants was not performed, and long-term follow-up of diagnosed patients was beyond the scope of the study. Larger multicentric studies with molecular analysis are recommended for broader epidemiological validation.

CONCLUSION(S)

Haemoglobinopathies pose a major national health challenge in India. Managing these conditions places a substantial socio-economic burden on families. Antenatal screening plays a vital role

in the identification of thalassaemia and other haemoglobinopathies. HPLC has proven to be a powerful diagnostic tool in the diagnosis and management of haemoglobinopathies.

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