

Research Article

Screening of Medical Students for Thalassemia and Other Hemoglobinopathies by High-Performance Liquid Chromatography for Primary Prevention in Ahmedabad City: A Cross-Sectional Study

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ABSTRACT

Background: In India, carrier detection for thalassemia and structural hemoglobin variants is a practicable primary-prevention strategy, where endogamy, regional clustering, and asymptomatic carrier states sustain the birth of affected children. Medical students are an important screening cohort because they are young adults, future reproductive decision-makers, and potential health educators. Screening them can convert laboratory diagnosis into durable community awareness and promote informed reproductive choices.

Methods: Cross-sectional design, this research was carried out in the Department of Pathology, GMERS Medical College and Civil Hospital, Sola, Ahmedabad, from July 2019 to June 2021. The participants were undergraduate and postgraduate medical students aged between 18-35 years who volunteered for screening. EDTA blood samples were analysed for complete blood count, peripheral smear morphology and hemoglobin fractions using Bio-Rad VARIANT high-performance liquid chromatography, beta-thalassemia short programme.

Results: Among 256 students, the mean age was 21.4 $\pm$ 3.4 years and 135 (52.7%) were women. HPLC showed a normal hemoglobin pattern in 246 students (96.1%). Ten students (3.9%; 95% CI, 2.1-7.0) had a carrier pattern: beta-thalassemia trait in six (2.3%; 95% CI, 1.1-5.0), sickle cell trait in two (0.8%) and Hb D trait in two (0.8%). Compared with students with a normal pattern, abnormal HPLC profiles showed higher RBC count, lower MCV, lower MCH and higher RDW. Beta-thalassemia trait had mean HbA2 of 5.0 $\pm$ 0.5%, mean MCV of 60.7 $\pm$ 14.8 fL and mean RBC count of 5.5 $\pm$ 0.5 million/cumm.

Conclusion: HPLC-based screening identified a clinically silent but preventable hemoglobinopathy carrier burden in medical students. Integrating CBC, smear review, HPLC reporting and genetic counselling in medical colleges can strengthen premarital and preconception prevention pathways in Ahmedabad.

Keywords: Beta-Thalassemia Trait; Hb D; HPLC; Medical Students; Primary Prevention; Sickle Cell Trait.

INTRODUCTION

Hemoglobinopathies such as thalassemias and structural hemoglobin variants are among the most prevalent monogenic disorders globally. Their public-health impact is magnified by the carrier state, which is usually clinically silent but capable of producing severe disease when two carriers have children. Global estimates suggest that a large proportion of the world's population carries an abnormal hemoglobin gene, and hundreds of thousands of affected births occur annually, particularly in locations where malaria-selection, consanguinity, migration and restricted access to genetic

services converge [1,2]. Prevention therefore depends less on treating established disease and more on identifying carriers prior to marriage, conception, or early pregnancy [3]. For its population size, ethnic diversity, community endogamy and marked regional variation in carrier frequencies, India has one of the largest burdens of hemoglobin disorders. A recent systematic review reported wide heterogeneity across population groups and a pooled beta-thalassemia carrier prevalence of 3.74% in general-population studies [4]. A multicentre Indian study using uniform methodology in six cities documented

beta-thalassemia trait in 2.78% overall, with important state-wise and ethnic variation, and showed that medical-college-based screening centres could deliver awareness, testing and counselling [5]. Northern and western Indian data similarly demonstrate that beta-thalassemia trait and structural variants are not confined to traditionally recognized high-risk groups [6].

Gujarat is especially relevant to the prevention of hemoglobinopathy. Epidemiological mapping conducted in western India revealed higher carrier frequencies in several Gujarati communities, and mutations that cluster by region and caste [7]. Studies in South Gujarat and Saurashtra have documented beta-thalassemia trait, sickle cell trait and Hb D-Punjab among students and community cohorts, highlighting that urban, educated populations cannot be assumed to be risk-free [9,10]. Ahmedabad, as a large educational and health-care hub, is an ideal setting for a young-adult screening model that can integrate laboratory detection with immediate counselling and peer-led health education.

High-performance liquid chromatography (HPLC) is ideally designed for this because it separates and quantifies HbA0, HbA2, HbF and common variant windows in a reproducible, automated manner. Cation-exchange HPLC has been shown to detect beta-thalassemia trait through raised HbA2 and to identify variants such as HbS, HbD, HbE and HbJ when interpreted with red-cell indices, peripheral smear findings and clinical context [8, 13, 14]. The present study screened undergraduate and postgraduate medical students of GMERS Medical College, Sola, Ahmedabad, to determine the pattern of hemoglobin fractions, identify carriers of thalassemia and other hemoglobinopathies, describe associated hematological parameters, and support primary prevention through awareness among future medical professionals. The study was deliberately framed as institutional screening rather than patient-based diagnosis because carrier detection is most useful before symptoms, marriage or pregnancy. By linking CBC, peripheral smear and HPLC data, it also examined whether routine hematological indices could guide suspicion while recognizing their limitations in structurally variant hemoglobins. This approach reflects real-world pathology practice, where the screening question is not simply whether anemia exists,

but whether a silent carrier state has been uncovered in time for counselling.

MATERIALS AND METHODS

Study Design

A cross-sectional observational study was conducted to screen medical students for thalassemia and other hemoglobinopathies using high-performance liquid chromatography (HPLC). The study was designed to determine the pattern of hemoglobin fractions, identify carrier states, and correlate abnormal hemoglobin patterns with hematological parameters.

Study Setting and Duration

The study was carried out in the Department of Pathology, GMERS Medical College and Civil Hospital, Sola, Ahmedabad, Gujarat. Data collection and laboratory analysis were performed over a two-year period from July 2019 to June 2021.

Study Participants

The study population consisted of undergraduate and postgraduate medical students of GMERS Medical College, Sola, Ahmedabad. Students aged 18–35 years were included if they voluntarily agreed to undergo screening for thalassemia and other hemoglobinopathies. Undergraduate students from first, second, and third MBBS years, as well as postgraduate students, were eligible for participation.

Inclusion Criteria

Medical students aged 18–35 years who provided voluntary consent for blood screening were included in the study. Both undergraduate and postgraduate students were considered eligible, irrespective of sex, academic year, or clinical symptom status.

Exclusion Criteria

Students who declined to provide consent for screening were excluded from the study. Students whose samples were inadequate, improperly labelled, clotted, or unsuitable for complete hematological and HPLC analysis were also excluded from final evaluation.

Ethical Considerations

The study was conducted after obtaining permission from the Institutional Ethics Committee of GMERS Medical College, Sola, Ahmedabad. Written informed consent was

obtained from all participants before sample collection. Confidentiality of participant identity and laboratory findings was maintained throughout the study. Students detected with abnormal hemoglobin patterns were advised regarding the significance of carrier status and the need for further family screening and genetic counselling.

Data Collection

Demographic and clinical details were recorded using a predesigned data collection format. Information included age, sex, academic designation, relevant clinical history, past history, and family history of anemia, blood transfusion, thalassemia, or other known hemoglobinopathies.

Sample Collection

Venous blood samples were collected from each participant under aseptic precautions. Blood was drawn into ethylene diamine tetra-acetic acid-containing vacutainers. Each sample was labelled with the participant's name, designation, and date of collection. Samples were transported to the hematology laboratory and processed according to standard laboratory protocols.

Complete Blood Count Analysis

Complete blood count analysis was performed within 8 hours of sample collection using the Horiba Pentra XLR automated hematology analyzer. The hematological parameters assessed included hemoglobin concentration, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, total leukocyte count, differential leukocyte count, and platelet count.

Peripheral Smear Examination

Peripheral blood smears were prepared and stained using standard staining procedures. Smears were examined microscopically to assess red blood cell morphology, white blood cell morphology, and platelet adequacy. Particular attention was given to anisocytosis, poikilocytosis, microcytosis, hypochromia, target cells, and other morphological features suggestive of hemoglobinopathy. Peripheral smear findings were correlated with automated hematology analyzer results as part of internal quality assessment.

HPLC Analysis

EDTA blood samples were collected and stored at 2°C–8°C until HPLC analysis was conducted after complete blood count analysis. Hemoglobin fraction assay was performed with the Bio-Rad VARIANT HPLC system with the β -thalassemia short program. Hemolysate was generated by adding 5 μ L of whole blood to 1000 μ L of deionized water for each sample. The sample rack was then loaded into the HPLC analyzer. HPLC separated and quantified the various hemoglobins based on retention time and percentage area, including HbA, HbA₂, HbF, and variant hemoglobins. Diagnosis with beta-thalassemia trait, sickle cell trait, Hb D trait, and other abnormal hemoglobin patterns were derived from HPLC results, hematological indices, and peripheral smear correlation.

Awareness and Counselling Component

After screening, students were informed about the clinical and preventive importance of thalassemia and hemoglobinopathy detection. Participants with abnormal HPLC findings were counselled regarding carrier status, inheritance pattern, reproductive implications, partner screening, and the role of premarital and antenatal screening in primary prevention. Students were also encouraged to participate in awareness activities and community-level identification of carriers.

Statistical Analysis

Data were entered and analyzed using descriptive statistical methods. Categorical variables were expressed as frequencies and percentages. Continuous variables, including hematological parameters and hemoglobin fractions, were summarized as mean and standard deviation. The distribution of hemoglobin patterns and abnormal HPLC findings was tabulated. Hematological indices were compared descriptively across normal and abnormal hemoglobinopathy groups.

RESULTS

This included 256 medical students. The cohort was mainly late adolescence and early adulthood, with a mean age 21.4 $\pm$ 3.4 years and an age range of 18–29 years. The distribution of students aged 18–20 years was 121 (47.3%), 21–25 years were 92 (35.9%), and 26–29 years were 43 (16.8%). Women comprised 135 subjects (52.7%) and men 121 (47.3%). The overall mean hemoglobin concentration, after exclusion of two implausible transcription values, was 13.1 $\pm$

1.8 g/dL. Mean MCV was 81.2 +/- 8.9 fL, mean MCH was 28.1 +/- 3.1 pg and mean RDW was 13.1 +/- 2.0%.

HPLC showed a normal hemoglobin pattern in 246 students (96.1%) and an abnormal carrier pattern in 10 students (3.9%; 95% CI, 2.1-7.0). Beta-thalassemia trait was the commonest abnormality, detected in six students (2.3%; 95% CI, 1.1-5.0). Sick cell trait and Hb D trait were each detected in two students (0.8% each). Abnormal patterns were evenly distributed by sex, with five affected men and five affected women; the sex association was not statistically significant (Fisher exact p=1.000).

The beta-thalassemia trait group showed the expected hematological signature of microcytosis disproportionate to anemia. Mean RBC count was 5.5 +/- 0.5 million/cumm, mean MCV was 60.7 +/- 14.8 fL, mean MCH was 22.8 +/- 4.6 pg, mean RDW was 16.4 +/- 2.6%, mean HbA2 was 5.0 +/- 0.5%. Five out of six beta-thalassemia trait students had MCV

< 80 fL and MCH < 27 pg. Compared with students with a normal HPLC pattern, the abnormal group had significantly higher RBC count (p=0.003), lower MCV (p=0.002), lower MCH (p<0.001), higher RDW (p=0.003), higher HbF (p=0.011) and higher HbA2 among evaluable cases (p<0.001).

The presence of peripheral smears further buttressed laboratory interpretation. The majority of students with a normal hemoglobin pattern sported normocytic normochromic red cells, although a minority had microcytosis most likely attributed to nutritional iron deficiency or to other non-hemoglobinopathy causes. Among 10 abnormal patterns of HPLC, six were microcytic hypochromic, with anisocytosis or anisopoikilocytosis and a number had target cells or elliptocytes. Both Hb D trait students showed normocytic normochromic smears and high variant-window fractions; thus, the structural hemoglobin carriers may be hematologically silent, and they require fraction-based

Table 1. Demographic and Hematological Profile of Screened Medical Students

Variable	Overall	Male	Female
Total participants	256	121 (47.3)	135 (52.7)
Age, years, mean +/- SD	21.4 +/- 3.4	21.3 +/- 3.2	21.5 +/- 3.6
Hemoglobin, g/dL, mean +/- SD	13.1 +/- 1.8	14.4 +/- 1.3	12.0 +/- 1.4
RBC count, million/cumm, mean +/- SD	4.69 +/- 0.61	4.96 +/- 0.57	4.46 +/- 0.55
MCV, fL, mean +/- SD	81.2 +/- 8.9	84.0 +/- 7.1	78.6 +/- 9.6
MCH, pg, mean +/- SD	28.1 +/- 3.1	29.2 +/- 2.1	27.1 +/- 3.5
RDW, %, mean +/- SD	13.1 +/- 2.0	12.9 +/- 2.5	13.4 +/- 1.5

The screened population was representative of the targeted young adult medical-student cohort, with a near-balanced sex distribution and a narrow reproductive-age range. It was physiologically anticipated that mean hemoglobin was lower in women than in men, and RBC count was slightly greater in men.

MCV and MCH mean values were nearly normal (adult reference limits), although the wide lower ranges suggested clinically silent microcytosis, and allowed for interpretation through reflex HPLC instead of relying solely on hemoglobin concentration.

Table 2. HPLC Diagnostic Spectrum and Prevalence

HPLC category	n	%	95% CI (%)
Normal	246	96.1	93.0-97.9
BTT	6	2.3	1.1-5.0
Sickle trait	2	0.8	0.2-2.8
Hb D trait	2	0.8	0.2-2.8
Any carrier pattern	10	3.9	2.1-7.0

Normal patterns of hemoglobin dominated the HPLC spectrum, yet nearly one in 25 students had a clinically important carrier result. Beta-thalassemia trait was the most common

abnormality; in contrast, Hb D and sickle cell trait appeared at lower but meaningful frequencies. As all of the conditions that were detected were carrier states, the table

demonstrates the preventive value of screening: students would otherwise be asymptomatic yet could face reproductive risk if partnered with another carrier.

Table 3. Hematological and HPLC Parameters According to Diagnostic Category

Category	n	Hb g/dL	RBC million/cumm	MCV fL	MCH pg	RDW %	HbA2 %	HbF %
Normal	246	13.2 +/- 1.8	4.66 +/- 0.59	81.8 +/- 7.9	28.3 +/- 2.9	13.0 +/- 2.0	2.74 +/- 0.51	0.35 +/- 0.27
BTT	6	11.9 +/- 1.8	5.52 +/- 0.51	60.7 +/- 14.8	22.8 +/- 4.6	16.4 +/- 2.6	4.95 +/- 0.50	0.92 +/- 0.50
Sickle trait	2	9.9 +/- 2.7	5.04 +/- 1.04	57.5 +/- 0.7	19.1 +/- 0.2	15.7 +/- 1.3	3.46 +/- 0.36	0.20 +/- 0.14
Hb D trait	2	14.6 +/- 3.5	5.54 +/- 1.63	87.0 +/- 0.0	28.0 +/- 0.6	12.4 +/- 0.7	NA	0.50 +/- 0.42

Beta-thalassemia trait separated distinctly from the normal group with elevated levels of HbA2, preserved or increased RBC count and a marked decline in MCV and MCH. Cases with sickle trait also demonstrated microcytosis and anemia, suggesting possible coexisting nutritional deficiency or alpha-thalassemia,

although molecular testing was not performed. Hb D trait was hematologically bland, underscoring the fact that structural variants can elude the observation of red cell indices and direct hemoglobin-fraction analysis may be needed.

Table 4. Clinico-Hematological Profile of Students with Abnormal Hplc Patterns

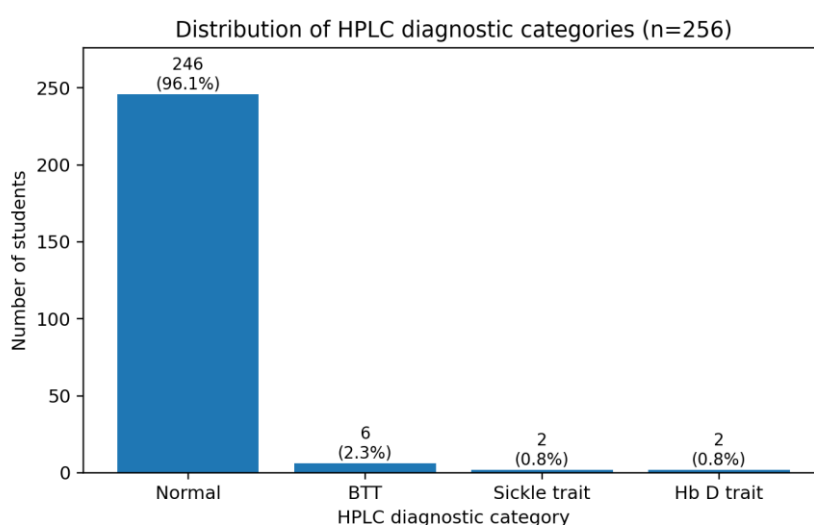
Serial	Age/Sex	Diagnosis	CBC profile	HPLC profile	Peripheral smear
6	18 y/M	Beta-thal trait	Hb 12.1; RBC 6.06; MCV 69; MCH 26; RDW 15	HbF 1.1; HbA0 86; HbA2 4.5	Mild microcytic hypochromic, anisocytosis
12	18 y/F	Beta-thal trait	Hb 10.4; RBC 5.64; MCV 52; MCH 18.4; RDW 17.5	HbF 0.7; HbA0 76.1; HbA2 4.4	Moderate microcytic hypochromic, anisopoikilocytosis, target cells, elliptocytes, tear-drop cells
14	18 y/M	Beta-thal trait	Hb 14; RBC 5.69; MCV 52; MCH 24.7; RDW 17.8	HbF 0.4; HbA0 85.3; HbA2 5.8	Moderate microcytic hypochromic, anisopoikilocytosis, target cells, elliptocytes
35	18 y/M	Beta-thal trait	Hb 14.1; RBC 4.77; MCV 85; MCH 29.5; RDW 11.7	HbF 0.9; HbA0 83.7; HbA2 4.9	Normocytic normochromic
40	18 y/F	Beta-thal trait	Hb 10.1; RBC 5.03; MCV 62; MCH 20; RDW 17.3	HbF 1.8; HbA0 82.4; HbA2 5	Mild microcytic hypochromic, anisopoikilocytosis, target cells, elliptocytes

67	18 y/M	Hb D trait	Hb 17; RBC 6.69; MCV 87; MCH 28.4; RDW 12.9	HbF 0.2; HbA0 50.1; HbA2 NA; HbD :43.5	Normocytic normochromic
120	18 y/M	Beta-thal trait	Hb 10.9; RBC 5.95; MCV 44; MCH 18.3; RDW 18.9	HbF 0.6; HbA0 84.2; HbA2 5.1	Moderate microcytic hypochromic, anisopoikilocytosis, target cells, elliptocytes
142	18 y/F	Sickle cell trait	Hb 11.8; RBC 5.77; MCV 57; MCH 19.3; RDW 14.7	HbF 0.3; HbA0 62.6; HbA2 3.2; HbS :25.6	Mild microcytic hypochromic, anisocytosis
146	19 y/F	Sickle cell trait	Hb 8; RBC 4.3; MCV 58; MCH 19; RDW 16.6	HbF 0.1; HbA0 68; HbA2 3.71; HbS :4.45	Moderate microcytic hypochromic, anisopoikilocytosis, elliptocytes
160	23 y/F	Hb D trait	Hb 12.1; RBC 4.38; MCV 87; MCH 27.6; RDW 11.9	HbF 0.8; HbA0 52.9; HbA2 NA; HbD :39.9	Normocytic normochromic

Case-level review demonstrated that the six beta-thalassemia-trait students clustered at HbA2 values between 4.4-5.8%, most with microcytic hypochromic smears and anisopoikilocytosis. One beta-thalassemia carrier did have normocytic indices, however,

suggesting that exclusive screening by MCV or MCH alone could miss carriers. The Hb D cases showed high HbD fractions with normal morphology, while sickle trait cases had HbS-window peaks and concomitant microcytosis.

Figure 1. Hplc Diagnostic Category Distribution among Screened Medical Students

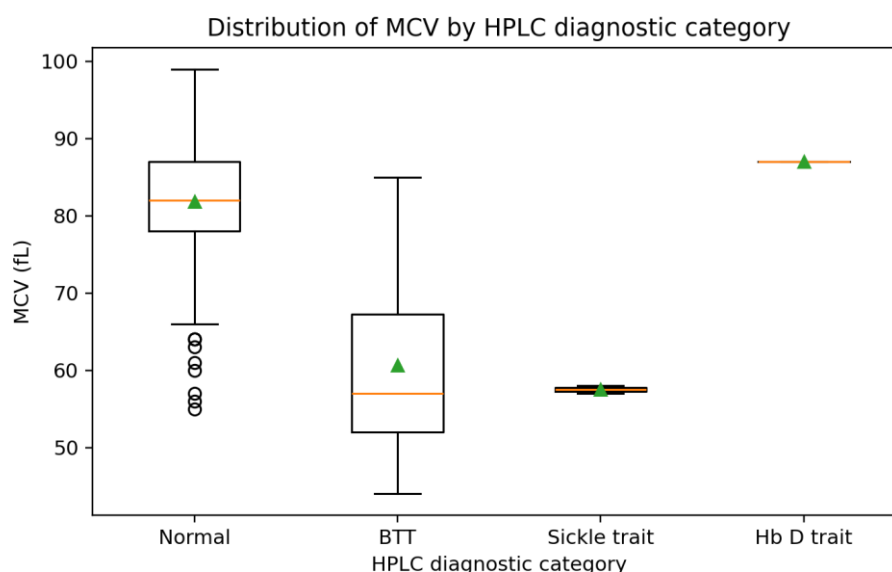


The figure highlights the low absolute but high preventive importance of carrier detection in a medical-student cohort. Predominantly normal profiles were found, but the aberrant

categories were not confined to beta-thalassemia trait. The visual separation of beta-thalassemia trait, Hb D trait and sickle cell trait supports a comprehensive HPLC

approach over a single-disease screening strategy, particularly in Gujarat where multiple hemoglobin variants coexist.

Figure 2. Mcv Distribution by Hplc Diagnostic Category.



The MCV plot plots the biological gradient of normal profiles and thalassemic or sickling carrier states. The central MCV and dispersion of beta-thalassemia trait showed the lowest values, while sickle trait cases were also microcytic. Hb D trait overlapped with normal MCV values, establishing that a normal red-cell index cannot exclude structural hemoglobin variants. This pattern validates mixing CBC-based suspicion with direct HPLC fractionation in institutional screening programs.

DISCUSSION

This study estimated a hemoglobinopathy carrier burden among medical students in Ahmedabad of 3.9%, and that beta-thalassemia trait represents the majority of the abnormal profiles. The beta-thalassemia trait prevalence of 2.3% was similar to the reported 2.78% overall prevalence in the six-city Indian multicentre study and general-population pooled estimates in the recent Indian systematic review [4,5]. It was lower than some Gujarat-based community and student studies (such as those with South Gujarat and Saurashtra cohorts that reported beta-thalassemia trait approximately 4.4-4.5% [9 and 10]), which included caste and regional populations with higher background risk. The hematological phenotype was consistent with established beta-thalassemia trait biology. Carriers showed relatively high RBC counts, low MCV and MCH, raised RDW, and raised

HbA2. Similar clinico-hematological correlations were found in Khera et al., who demonstrated that HPLC reliably identifies high-HbA2 beta-thalassemia trait even when iron deficiency may partially lower HbA2 [8]. The literature, from northern and western India, West Bengal, Uttarakhand antenatal cohorts and HPLC-based tertiary-centre series, has also found that raised HbA2 with microcytosis is the core laboratory signature of beta-thalassemia trait, although borderline or coexisting nutritional deficiencies may make interpretation problematic [6,11-15].

The detection of sickle cell trait and Hb D trait is clinically relevant despite low frequencies. Gujarat identified clustering of sickle cells at the region, particularly based on tribal belts, and population analyses in South Gujarat reported sickle cell trait alongside beta-thalassemia trait in student and community sources [9]. Hb D-Punjab is generally silent in the heterozygous state, although clinically relevant if acquired with HbS or beta-thalassemia; it has been detected at the molecular and HPLC levels in northwestern India and Gujarat [10,16]. In the current series, nearly normal CBC and smear morphology was presented for both Hb D trait students, indicating that variant carriers would have been missed in the absence of HPLC.

Our results are consistent with screening medical students as a prevention strategy with multiplier value. International experience

shows that screening works best when laboratory detection is paired with education, counselling and reproductive-risk communication [3,17]. Testing students reaches individuals before marriage and pregnancy, allowing time for informed partner testing and family counselling, unlike opportunistic antenatal screening. Medical students additionally become informed advocates who can recognize microcytosis, avoid unnecessary iron therapy in carriers, and explain autosomal-recessive risk to patients and peers. This is particularly relevant in busy outpatient and antenatal settings where mild anemia is often treated empirically; a trained clinician can distinguish nutritional anemia from inherited microcytosis and can refer families for appropriate partner testing rather than repeated non-specific therapy.

The study had limitations. It was single-centre and voluntary; prevalence estimates should not generalize to all Ahmedabad students. No molecular confirmation was performed; therefore, alpha-thalassemia, silent beta-thalassemia mutations and compound states could not be excluded. Iron studies were unavailable, limiting interpretation of microcytosis in students with normal HPLC results and in sickle trait. Some isolated transcription errors in continuous variables required field-level exclusion. Further studies should combine HPLC with ferritin, CRP and targeted molecular testing and should assess post-test knowledge retention, partner testing and counselling uptake. A longitudinal design would also ascertain whether screened students retain their carrier cards, disclose results appropriately, and translate knowledge into safer reproductive counselling for relatives and patients.

CONCLUSION

HPLC screening of medical students in Ahmedabad revealed a measurable burden of silent hemoglobinopathy carriers with beta-thalassemia trait being the most prevalent alongside Hb D and sickle cell trait. It demonstrates how a medical college-based model can detect carriers prior to making reproductive decisions while simultaneously creating a trained arm of future doctors equipped to prevent thalassemia. CBC and smear findings were informative but fall short on their own, especially for Hb D trait and occasional beta-thalassemia carriers with near-normal indices. Incorporating HPLC, counselling and education into the student-

centered health programmes might promote primary prevention and decrease future births with severe hemoglobin disorders. This model is feasible, educational and can be widely spread for other medical colleges in Gujarat.

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