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Original Research Article

Study of Spectrum of Thalassemia and Hemoglobinopathies by High Performance Liquid Chromatography (HPLC) Method at Tertiary Care Hospital

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Abstract:

Background: Hemoglobinopathies are a group of inherited disorders characterized by abnormal production or structure of the hemoglobin molecule. India, located on the thalassemic belt, has a high prevalence of beta-thalassemia and sickle cell disorders. High-Performance Liquid Chromatography (HPLC) has emerged as a reliable and efficient diagnostic tool for screening and diagnosis of these conditions.

Methods: A cross-sectional observational study was conducted among all OPD patients whose HPLC requisition forms were received at the Central Clinical Laboratory of Pathology, Civil Hospital, Gandhinagar from August 2022 to February 2024. Complete blood count was performed using Sysmex XN-350 automated analyzer, and HPLC was performed using BIO-RAD VARIANT II system. A total of 610 samples were analyzed.

Results: Out of 610 samples analyzed, 90 (14.75%) tested positive for hemoglobinopathies. Female predominance was observed (55.60% vs 44.40% males). The age group of 21-30 years showed the highest prevalence (44.40%). Beta Thalassemia Minor was the most common hemoglobinopathy (42.20%, prevalence 06.23%), followed by Sickle Cell Trait (25.60%, prevalence 03.77%) and Sickle Cell Anemia (17.80%, prevalence 02.62%). Severe anemia (Hb <07 g%) was observed in 45.60% of positive cases.

Conclusion: HPLC is a highly reproducible, excellent, and powerful diagnostic tool offering simplicity with automation, superior resolution, and rapid results for diagnosis of hemoglobinopathies. The high prevalence emphasizes the need for premarital and antenatal screening.

Keywords: Hemoglobinopathies, Thalassemia, Sickle Cell Disease, HPLC, Gujarat, Screening.

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Introduction

Hemoglobinopathies are a group of genetic disorders of hemoglobin in which there is abnormal production or structure of the hemoglobin molecule. These disorders represent the most common monogenic diseases worldwide, affecting millions of individuals across all continents. Abnormalities of hemoglobin synthesis are among the most common inherited disorders and can be broadly classified into two categories: quantitative defects (thalassemia syndromes) characterized by reduced or absent globin chain synthesis, and qualitative defects (hemoglobin variants) characterized by structural abnormalities in the globin chains. [1,2]

The molecular basis of hemoglobinopathies lies in mutations affecting the alpha-globin or beta-globin genes. Beta-thalassemia results from point mutations, small deletions, or insertions in the beta-globin gene (HBB) on chromosome 11, leading to reduced or absent beta-globin chain production. In contrast, sickle cell disease arises from a single nucleotide substitution at the sixth codon of the beta-globin gene, resulting in the replacement of glutamic acid with valine and the formation of abnormal hemoglobin S (HbS). [3,4]

Among the inherited disorders of blood, hemoglobinopathies and thalassemia constitute a major bulk of non-communicable genetic diseases in India, imposing a significant burden on healthcare systems and affected families. India is located on the thalassemic belt and there is a high prevalence of beta-thalassemia minor, which is reported to be very variable across different regions and ethnic groups. [5] The highest global prevalence of thalassemia is observed in the Mediterranean region, Central

Africa, the Middle East, and Southeast Asia, with beta-thalassemia being the most common single gene disorder in our country. [6,7]

India constitutes approximately 10.00% of the global thalassemia burden, with an estimated 10,000-15,000 children being born with thalassemia major every year. The frequency range of beta-thalassemia carriers is 03.50% to 15.00% in the Indian population, with significant regional variations. Population screening studies have identified the prevalence of beta-thalassemia carrier status as high as 17.00% in certain endogamous communities in India, including Sindhis, Punjabis, Bengalis, Gujaratis, Parsis, and Lohanas. [8,9] In Gujarat, particularly high prevalence is found among Lohanas community. [10]

Globally, the percentage of carriers of thalassemia is greater than that of carriers of sickle cell anemia, though both conditions cause significant morbidity and mortality. [11] Hemoglobin S (HbS) is prevalent in Africa, Mediterranean countries, and India. In India, sickle cell disease is predominantly seen amongst tribal populations in Central India, mainly in Madhya Pradesh, Orissa, Chhattisgarh, and Andhra Pradesh. It has been estimated that over 5,000 babies with sickle cell disease are born each year in India. [12,13]

Hemoglobin E (HbE), another clinically significant variant, is widely distributed in northeastern states including Assam, Mizoram, Manipur, Arunachal Pradesh, and Tripura. Hemoglobin D Punjab is predominantly seen in Punjab, Uttar Pradesh, Gujarat, and Jammu and Kashmir. [14,15]

The clinical spectrum of hemoglobinopathies ranges from asymptomatic carrier states to severe transfusion-dependent conditions. Beta-thalassemia minor carriers are usually asymptomatic with mild microcytic hypochromic anemia, while beta-thalassemia major patients present with severe anemia requiring regular blood transfusions and iron chelation therapy. [16,17]

High-Performance Liquid Chromatography (HPLC) has become a cornerstone in the diagnosis of thalassemia and hemoglobinopathies in tertiary care hospitals worldwide. [18,19] HPLC offers superior sensitivity and specificity in detecting and quantifying hemoglobin variants compared to traditional methods such as hemoglobin electrophoresis. [20]

HPLC can detect hemoglobin variants present in very low concentrations, as low as 00.50-01.00% of total hemoglobin. The method provides precise quantification of HbA2, HbF, and abnormal variants like HbS, HbD, HbE, and HbC. [21,22] The rapid turnaround time of 05-10 minutes per sample makes HPLC suitable for high-throughput screening programs. [23]

The accurate diagnosis of hemoglobinopathies is crucial for: (01) Genetic counseling, (02) Prenatal diagnosis, (03) Appropriate clinical management, (04) Premarital screening programs, and (05) Population-based epidemiological studies. [24,25] The present study was undertaken to diagnose thalassemia and hemoglobinopathies by HPLC method and study their distribution in the population of Gujarat.

Materials and Methods

Study Design: A cross-sectional observational study was conducted at the Central Clinical Laboratory of Pathology, Civil Hospital attached to GMERS Medical College, Gandhinagar, Gujarat, India.

Study Period: August 2022 to February 2024 (18 months).

Study Population: All OPD patients whose HPLC requisition forms were received at the laboratory. This included patients requiring recurrent blood transfusions, those with microcytic hypochromic anemia, patients with family history of hemoglobinopathies, and individuals referred for premarital or antenatal screening.

Sample Size: A total of 610 HPLC requisition forms were received and analyzed.

Laboratory Methods: EDTA anticoagulated blood samples (02 mL) were collected. Complete blood count was performed using Sysmex XN-350 automated analyzer. Parameters evaluated included Hb, RBC count, MCV, MCH, MCHC, RDW, and other blood indices. Peripheral blood smear examination was performed using Leishman stain.

HPLC Analysis: HPLC was performed using BIO-RAD VARIANT II system with the Beta-Thalassemia Short Program. HbA2/F calibrators were analyzed at the beginning of each run for quality control.

Diagnostic Criteria: Beta-thalassemia minor: HbA2 >03.50% with microcytic indices. Beta-thalassemia major: HbF >90.00% with absent HbA. Sickle cell trait: HbS 22.00-40.00%. Sickle cell anemia: HbS >50.00%.

Statistical Analysis: Descriptive statistical analysis was performed using SPSS software version 26.0.

Results

A total of 610 samples were analyzed during the 18-month study period. Out of these, 90 samples (14.75%) tested positive for various hemoglobinopathies.

Table 01 presents the comprehensive demographic and clinical profile of study participants who tested positive for hemoglobinopathies. The study population comprised 610 total samples, of which 90

(14.75%) were positive. Among positive cases, females (50 cases, 55.60%) outnumbered males (40 cases, 44.40%). The majority belonged to the 21-30 years age group (40 cases, 44.40%). Severe anemia was present in 41 cases (45.60%).

Table 1: Demographic and Clinical Profile of Study Participants

Parameter	Category	Number (n)	Percentage (%)
Study Population	Total Samples	610	-
	Positive for Hemoglobinopathies	90	14.75%
Gender (n=90)	Female	50	55.60%
	Male	40	44.40%
Age Group (n=90)	00-10 years	08	08.90%
	11-20 years	26	28.90%
	21-30 years	40	44.40%
	31-40 years	10	11.10%
	41-50 years	05	05.60%
	51-60 years	01	01.10%
Anemia Severity (n=90)	No Anemia (>13 g%)	10	11.10%
	Mild (10-13 g%)	08	08.90%
	Moderate (07-10 g%)	31	34.40%
	Severe (<07 g%)	41	45.60%

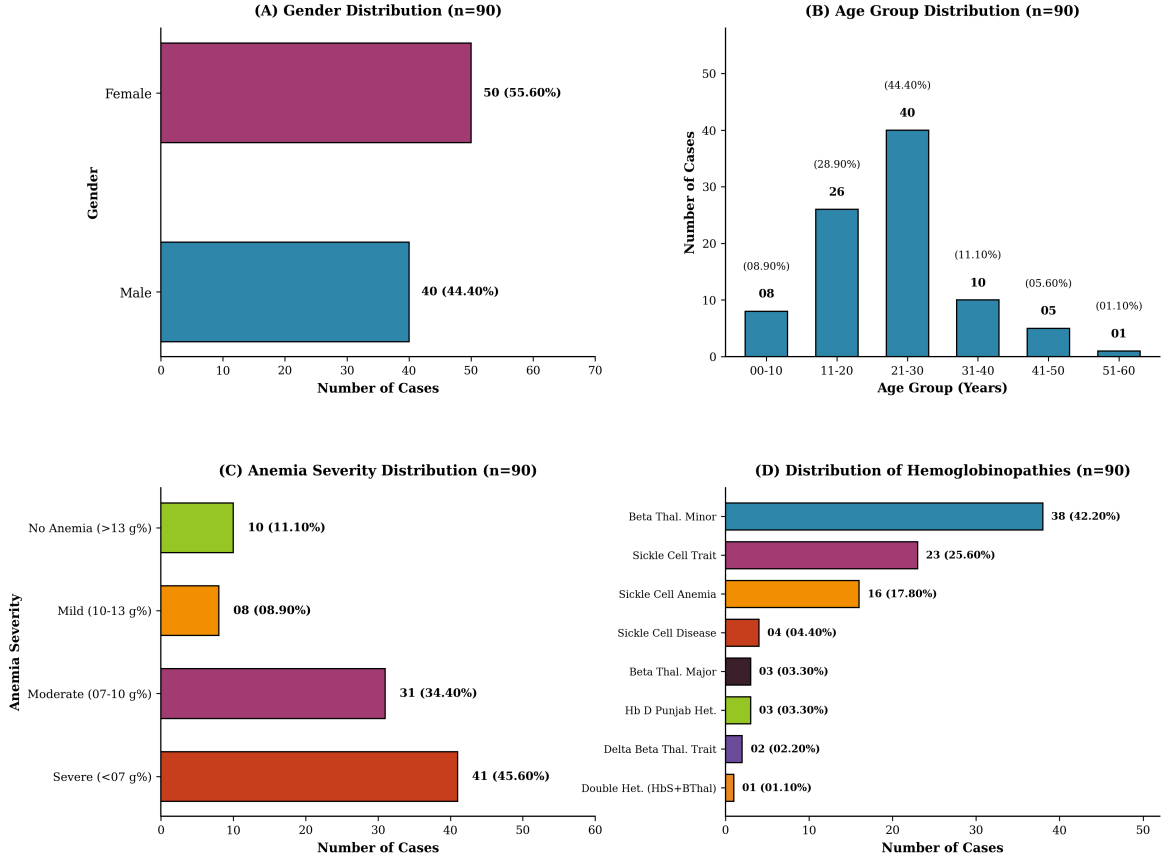

Figure 1: Demographic Profile - (A) Gender Distribution; (B) Age Group Distribution; (C) Anemia Severity; (D) Distribution of Hemoglobinopathies

Table 02 illustrates the peripheral blood smear findings. Microcytic hypochromic pattern was the most common finding (60 cases, 66.80%). Elliptocytes were most frequently observed (66 cases, 73.33%), followed by target cells (57 cases, 63.33%).

Table 2: Peripheral Blood Smear Findings (n=90)

Category	Finding	Number (n)	Percentage (%)
RBC Morphology	Microcytic Hypochromic	60	66.80%
	Normocytic Normochromic	11	12.20%
	Macrocytic Normochromic	02	02.20%
	Dimorphic (Micro + Macro)	12	13.30%
	Dimorphic (Micro + Normo)	05	05.50%
Abnormal RBC Types	Elliptocytes	66	73.33%
	Target Cells	57	63.33%
	Anisopoikilocytosis	45	50.00%
	Tear Drop Cells	39	43.33%
	Sickle Cells	35	38.89%
	Leptocytes	11	12.22%
	Schistocytes	04	04.44%

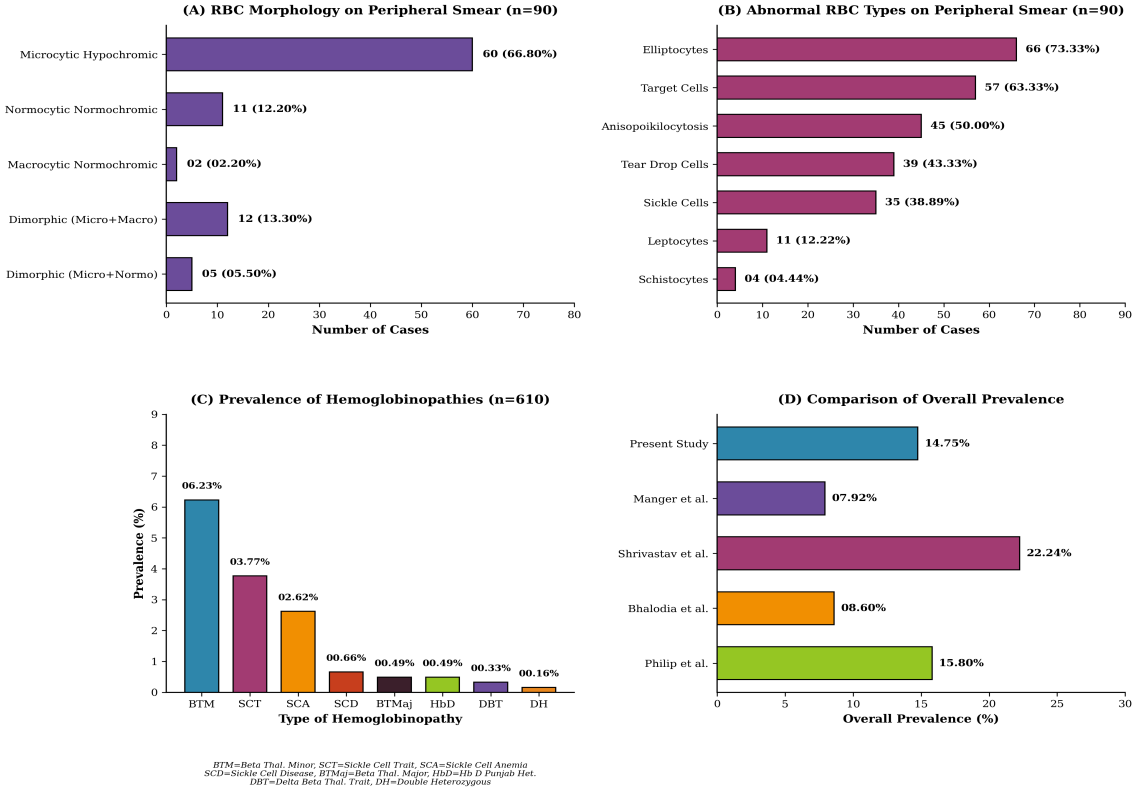

Figure 2: Peripheral Smear and Prevalence - (A) RBC Morphology; (B) Abnormal RBC Types; (C) Prevalence (n=610); (D) Overall Prevalence Comparison

Table 03 demonstrates the distribution and prevalence of various hemoglobinopathies. Beta Thalassemia Minor was the most common (38 cases, 42.20%, prevalence 06.23%), followed by Sickle Cell Trait (23 cases, 25.60%, prevalence 03.77%) and Sickle Cell Anemia (16 cases, 17.80%, prevalence 02.62%).

Table 3: Distribution and Prevalence of Hemoglobinopathies

Hemoglobinopathy	Number (n)	Distribution (%)	Prevalence (%)
Beta Thalassemia Minor	38	42.20%	06.23%
Sickle Cell Trait	23	25.60%	03.77%
Sickle Cell Anemia	16	17.80%	02.62%
Sickle Cell Disease	04	04.40%	00.66%
Beta Thalassemia Major	03	03.30%	00.49%
Hb D Punjab Heterozygous	03	03.30%	00.49%
Delta Beta Thalassemia Trait	02	02.20%	00.33%
Double Heterozygous (HbS+Beta Thal)	01	01.10%	00.16%
Total	**90**	**100.00%**	**14.75%**

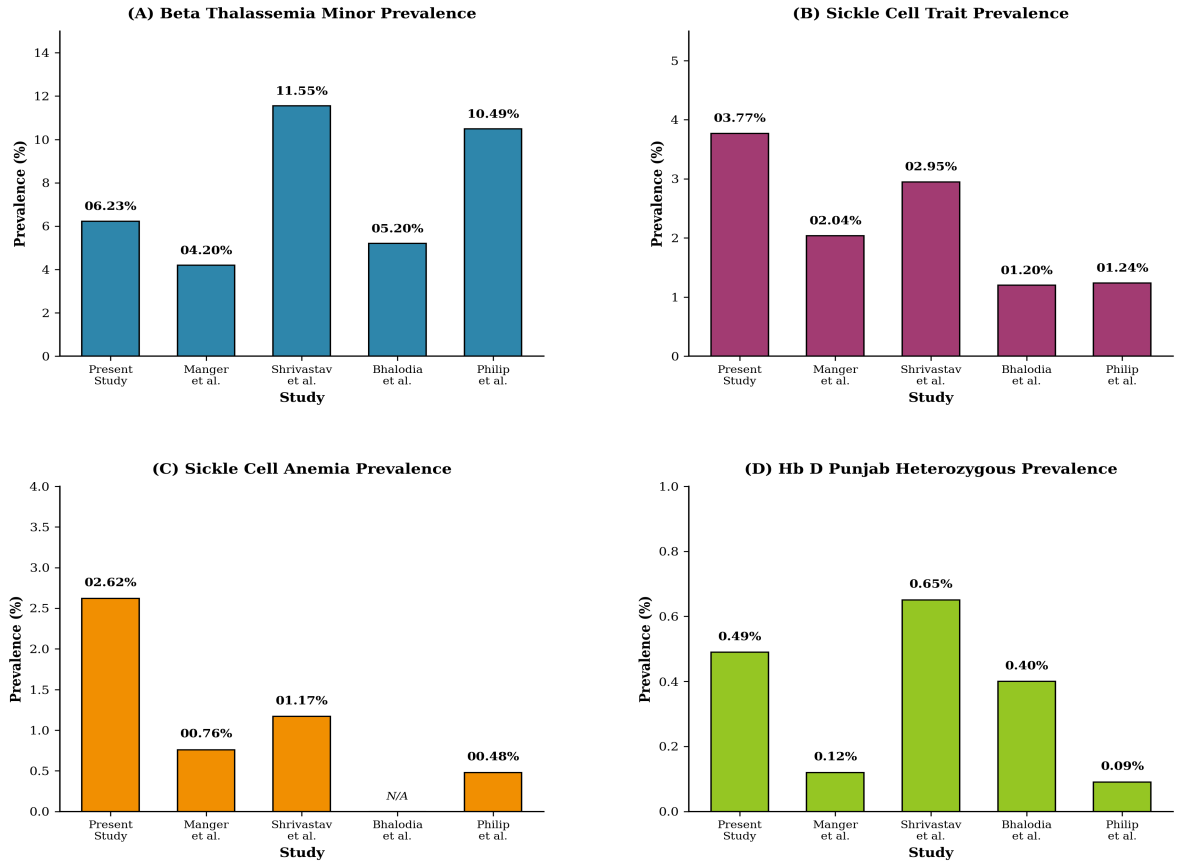

Figure 03: Comparison of Prevalence Across Studies - (A) Beta Thalassemia Minor; (B) Sickle Cell Trait; (C) Sickle Cell Anemia; (D) Hb D Punjab Heterozygous

Table 04 presents the detailed hematological parameters. In Beta Thalassemia Minor (n=38), mean hemoglobin was 09.51 g/dL with elevated HbA2 of 05.28%. In Sickle Cell Anemia (n=16), mean HbS was 79.91%. Beta Thalassemia Major (n=03) exhibited HbF levels of 91.57%.

Table 4: Hematological Parameters in Major Hemoglobinopathies

Parameter	Beta Thal. Minor (n=38)	Sickle Cell Trait (n=23)	Sickle Cell Anemia (n=16)	Beta Thal. Major (n=03)
Hb (g/dL)	09.51 ± 02.49	08.15 ± 04.07	07.01 ± 02.40	07.60 ± 02.16
RBCs (x10^6/uL)	04.68 ± 01.16	03.47 ± 01.63	03.13 ± 01.05	02.69 ± 00.55
MCV (fL)	69.03 ± 12.21	77.34 ± 23.96	70.85 ± 09.05	79.33 ± 11.30
MCH (pg)	23.99 ± 10.21	23.69 ± 07.15	23.26 ± 03.98	27.17 ± 03.54
MCHC (g/dL)	30.74 ± 01.61	31.94 ± 03.09	31.79 ± 03.17	33.17 ± 02.78
RDW (%)	17.76 ± 05.14	22.07 ± 08.27	20.86 ± 04.63	24.13 ± 11.42
HbA2 (%)	05.28 ± 00.67	02.93 ± 00.41	03.23 ± 00.98	04.30 ± 01.42
HbF (%)	01.24 ± 00.94	01.83 ± 02.02	13.93 ± 05.32	91.57 ± 03.02
HbS (%)	-	29.59 ± 05.69	79.91 ± 04.90	-

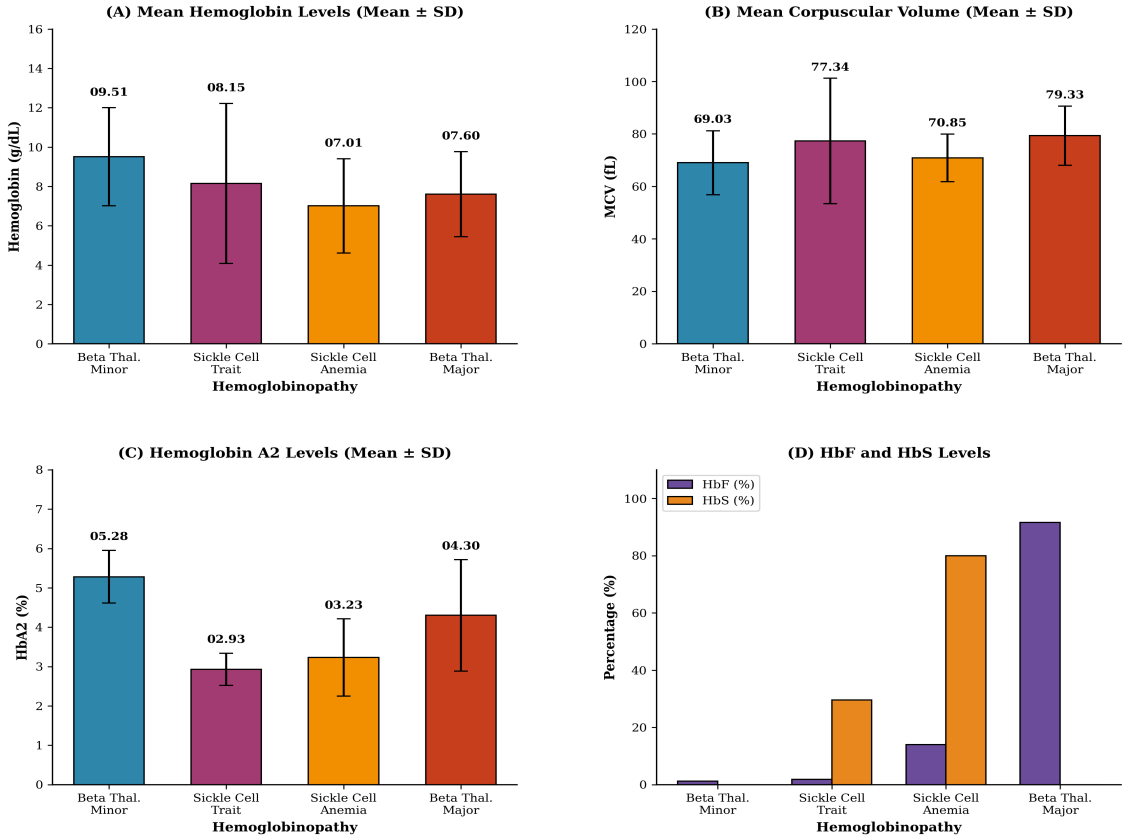

Figure 4: Hematological Parameters - (A) Mean Hemoglobin Levels; (B) Mean Corpuscular Volume; (C) Hemoglobin A2 Levels; (D) HbF and HbS Levels

Table 05 compares the prevalence of various hemoglobinopathies with other published studies. The overall prevalence (14.75%) was higher than Manger et al. (07.92%) and Bhalodia et al. (08.60%), but comparable to Shrivastav et al. (22.24%) and Philip et al. (15.80%).

Table 5: Comparison of Prevalence with Other Studies

Hemoglobinopathy	Present Study	Manger et al.	Shrivastav et al.	Bhalodia et al.	Philip et al.
Beta Thal. Minor	06.23%	04.20%	11.55%	05.20%	10.49%
Beta Thal. Major	00.49%	00.24%	04.02%	00.20%	00.55%
Sickle Cell Trait	03.77%	02.04%	02.95%	01.20%	01.24%
Sickle Cell Anemia	02.62%	00.76%	01.17%	-	00.48%
Hb D Punjab Het.	00.49%	00.12%	00.65%	00.40%	00.09%
Overall Prevalence	**14.75%**	**07.92%**	**22.24%**	**08.60%**	**15.80%**

Table 06 presents comparative analysis of hematological parameters. For Beta Thalassemia Minor, mean hemoglobin (09.51 g/dL) and HbA2 (05.28%) were comparable to other studies. For Sickle Cell Anemia, mean HbS (79.91%) confirmed severe disease.

Table 6: Comparison of Hematological Parameters with Other Studies

Parameter	Present Study	Shrivastav et al.	Kapadia et al.	Shah et al.
Beta Thal. Minor				
Hb (g/dL)	09.51 ± 02.49	10.40 ± 01.87	10.08 ± 01.11	09.88 ± 00.87
MCV (fL)	69.03 ± 12.21	62.10 ± 05.72	65.57 ± 04.52	64.69 ± 05.81
HbA2 (%)	05.28 ± 00.67	05.19 ± 00.82	04.50 ± 00.82	05.03 ± 00.71
Sickle Cell Trait				
Hb (g/dL)	08.15 ± 04.07	09.89 ± 02.40	09.23 ± 01.06	10.12 ± 00.97
HbS (%)	29.59 ± 05.69	35.30 ± 04.30	32.25 ± 04.78	34.11 ± 05.16
Sickle Cell Anemia				
Hb (g/dL)	07.01 ± 02.40	07.46 ± 02.26	-	-
HbS (%)	79.91 ± 04.90	73.90 ± 08.90	-	-

Discussion

The present study was conducted to diagnose thalassemia and other hemoglobinopathies among patients attending a tertiary care hospital in Gujarat using HPLC. The study provides valuable insights into the spectrum and distribution of hemoglobinopathies in this region.

Prevalence of Hemoglobinopathies: Out of 610 patients examined, 90 (14.75%) tested positive for hemoglobinopathies. This prevalence is notably higher than that reported by Manger et al. [26] (07.92%) and Bhalodia et al. [27] (08.60%), but comparable to Shrivastav et al. [28] (22.24%) and Philip et al. [29] (15.80%). This variation can be attributed to study population characteristics, inclusion criteria, regional ethnic composition, and referral patterns.

The diversity in hemoglobinopathy prevalence across different regions reflects the complex genetic landscape of India. Endogamous marriage practices have contributed to high carrier frequencies in specific populations. [30,31]

Gender Distribution: Our study found higher prevalence among females (55.60%) compared to males (44.40%). This aligns with Bhagat et al. [32] Several factors contribute to this: Increased healthcare-seeking behavior among women during antenatal care, Mandatory hemoglobin screening during pregnancy, Greater awareness about thalassemia screening among women of reproductive age.

However, hemoglobinopathies being autosomal conditions have equal genetic predisposition in both genders. Balgir et al. [33] reported balanced gender distribution (females 50.20%, males 49.80%) in their population-based study.

Age Distribution: The highest prevalence was observed in the 21-30 years age group (44.40%), followed by 11-20 years (28.90%). This pattern is consistent with Shrivastav et al. [28] and Kumar et al. [34] The predominance in the reproductive age group has significant public health implications for premarital and antenatal screening programs.

The lower proportion in pediatric age group (08.90%) may reflect: Mild clinical presentation not prompting evaluation, Inadequate pediatric screening, and Potential under-diagnosis.[35]

Anemia Severity: Our study demonstrated that 45.60% had severe anemia (Hb <07 g%) and 34.40% had moderate anemia. These findings are consistent with Weatherall and Clegg [36] and De Sanctis et al. [37] who reported approximately 70.00-80.00% of patients with beta-thalassemia major exhibit severe anemia.

The high prevalence of severe anemia underscores the clinical severity and importance of early diagnosis. Regular transfusion programs and iron chelation therapy have significantly improved survival and quality of life. [38]

Beta Thalassemia Minor: Beta Thalassemia Minor was the most common hemoglobinopathy (42.20%, prevalence 06.23%). The mean HbA2 of 05.28% is diagnostic for beta-thalassemia trait (values >03.50% are confirmatory). [39] Gujarat has particularly high prevalence due to Lohanas, Khatris, and Banias communities. [40]

Sickle Cell Disorders: Sickle Cell Trait was second most common (25.60%, prevalence 03.77%), followed by Sickle Cell Anemia (17.80%, prevalence 02.62%). The higher prevalence is attributable to referral of patients from tribal areas. [26,29]

The mean HbS of 29.59% in sickle cell trait cases is consistent with the typical range of 22.00-40.00%. In sickle cell anemia, mean HbS of 79.91% and elevated HbF of 13.93% reflect the pathophysiology. [41]

Peripheral Blood Smear Findings: Microcytic hypochromic anemia was observed in 66.80% of positive cases. Elliptocytes (73.33%), target cells (63.33%), and sickle cells (38.89%) provide valuable morphological clues.[43] Target cells are characteristic of thalassemia while sickle cells are pathognomonic of sickle cell disorders.[44]

Hb D Punjab Heterozygous: Our study identified 03 cases (00.49%) of Hb D Punjab. The mean HbD of 34.80% is consistent with heterozygous state.[45]

Rare Hemoglobinopathies: We identified 02 cases (02.20%) of Delta Beta Thalassemia Trait with elevated HbF (06.20%) and borderline HbA2 (02.55%).[46]

Clinical Significance: The high prevalence (14.75%) has significant public health implications. With approximately 35 million beta-thalassemia carriers and 10,000-15,000 affected children born annually in India, the burden is enormous.[47]

The findings support implementation of: Mandatory premarital screening, Routine antenatal screening, Cascade family screening, Newborn screening, and Community awareness programs. [48,49]

Role of HPLC: HPLC has emerged as the gold standard offering rapid turnaround time (05-10 minutes), high precision, and comprehensive quantification of all hemoglobin fractions.[50]

Study Limitations: Limitations include: Hospital-based study with selection bias, Lack of molecular confirmation, Absence of family studies for all cases, Possible influence of iron deficiency on HbA2 levels, and Limited follow-up data.

Conclusion

HPLC is a highly reproducible, excellent, and powerful diagnostic tool offering simplicity with automation, superior resolution, and rapid results for diagnosis of hemoglobinopathies. It is suitable for large-scale screening programs.

The prevalence of hemoglobinopathies (14.75%) is significantly high in Gujarat. Beta Thalassemia Minor was the most common (42.20%, prevalence 06.23%), followed by Sickle Cell Trait (25.60%, prevalence 03.77%) and Sickle Cell Anemia (17.80%, prevalence 02.62%).

These findings have important implications: Routine HPLC use should be encouraged, Premarital and antenatal screening should be strengthened, Carrier couples should receive genetic counselling, Community awareness programs should be conducted, and A hemoglobinopathy registry should be maintained.

By implementing comprehensive screening and prevention programs, the burden of hemoglobinopathies can be significantly reduced.

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References

1. Kultarn F. Diagnostic approach to hemoglobinopathies. Hemoglobin. 2007;31(02):243-250.
2. Parikh UR, Goswami HM, Mehta RC, et al. Incidence of hemoglobinopathies in women attending antenatal clinics in Gujarat. NHL J Med Sci. 2014;03(01):63-67.
3. Baxi A, Manila K, Kadhi P. Carrier screening for β-thalassemia in pregnant Indian women: Experience at a single center. Indian J Hematol Blood Transfus. 2013;29(02):71-74.
4. Baig MA, Swamy KB, Baksh AD, et al. Evaluation of role of HPLC in diagnosis of hemoglobinopathies: A hospital-based study. Indian J Pathol Microbiol. 2021;64:518-523.
5. Biswas AK, Philip J. Incidence of hemoglobinopathies using HPLC in 6500 anemic patients at a tertiary care center. Indian J Appl Res. 2016;06:214-218.
6. Vaz FE, Thakur CB, Banerjee MK, et al. Distribution of β-thalassemia mutation in the Indian population referred for prenatal diagnosis. Hemoglobin. 2000;24:181-194.
7. Singh T. Atlas and textbook of hematology. 4th ed. New Delhi: Avichal Publishing Company; 2018.
8. Sengupta M. Thalassemia among the tribal communities of India. Internet J Biol Anthropol. 2007;01(02):01-09.
9. Bain BJ. Hemoglobinopathy diagnosis. 2nd ed. Oxford: Blackwell Publishing; 2006:01-25.
10. Mohanty D, Colah RB, Gorakshakar AC, et al. Prevalence of β-thalassemia and other hemoglobinopathies in six cities in India: A multicentre study. J Community Genet. 2013;04(01):33-42.
11. Lewis SM, Bain BJ, Bates I, et al. Dacie and Lewis practical hematology. 11th ed. Philadelphia: Elsevier; 2011:301-329.
12. McPherson RA, Pincus MR. Henry's clinical diagnosis and management by laboratory methods. 22nd ed. Philadelphia: Elsevier; 2011:575-588.
13. Traeger-Synodinos J, Harteveld CL. Advances in technologies for screening and diagnosis of hemoglobinopathies. Blood Cells Mol Dis. 2014;52(02-03):119-127.
14. Makroo RN, Raina V, Chowdhry M, et al. Prevalence of hemoglobinopathies and thalassemia among blood donors in Northern India. Indian J Pathol Microbiol. 2017;60(03):543-548.
15. Giordano PC. Strategies for basic laboratory diagnostics of hemoglobinopathies in multiethnic societies. Int J Lab Hematol. 2013;35(05):465-479.
16. Modell B, Darlison M. Global epidemiology of hemoglobin disorders and derived service indicators. Bull WHO. 2008;86(06):480-487.
17. Piel FB, Patil AP, Howes RE, et al. Global epidemiology of sickle hemoglobin in neonates: A contemporary geostatistical model-based map and population estimates. Lancet. 2013;381(9861):142-151.
18. Stephens AD, Angastiniotis M, Baysal E, et al. ICSH recommendations for the measurement of haemoglobin A2. Int J Lab Hematol. 2012;34(01):01-13.
19. Joutovsky A, Hadzi-Nesic J, Nardi MA. HPLC retention time as a diagnostic tool for hemoglobin variants and hemoglobinopathies: A study of 60000 samples in a clinical diagnostic laboratory. Clin Chem. 2004;50(10):1736-1747.
20. Wild BJ, Bain BJ. Detection and quantitation of normal and variant hemoglobins: An analytical review. Ann Clin Biochem. 2004;41(Pt 05):355-369.
21. Colah RB, Surve R, Sawant P, et al. HPLC studies in hemoglobinopathies. Indian J Pediatr. 2007;74(07):657-662.
22. Srinivasan S, Batmanabane G. Analysis of hemoglobin variants using HPLC: A South Indian study. Clin Chim Acta. 2012;413(13-14):1172-1175.

23. Old JM. Screening and genetic diagnosis of haemoglobin disorders. Blood Rev. 2003;17(01):43-53.
24. Weatherall DJ. The inherited diseases of hemoglobin are an emerging global health burden. Blood. 2010;115(22):4331-4336.
25. Angastiniotis M, Modell B. Global epidemiology of hemoglobin disorders. Ann N Y Acad Sci. 1998;850:251-269.
26. Manger U, Panchal N, Patel V, et al. Detection of hemoglobinopathies through HPLC in Northern Gujarat. SSR Institute Int J Life Sci. 2024;10(04):Article 35.
27. Bhalodia JN, Oza HV, Modi PJ, et al. Study of hemoglobinopathies using HPLC in Western India: Experience of a single tertiary care center. Natl J Community Med. 2015;06(01):35-40.
28. Shrivastav A, Patel U, Joshi JR, et al. Study of hemoglobinopathies in Western India population using HPLC: A report of 7,000 cases. J Clin Diagn Res. 2013;07(12):2825-2828.
29. Philip J, Sarkar RS, Kushwaha N. Microcytic hypochromic anemia: Should HPLC be used routinely for screening hemoglobinopathies? Indian J Pathol Microbiol. 2013;56:109-113.
30. Colah R, Gorakshakar A, Nadkarni A. Global burden, distribution and prevention of β-thalassemias and hemoglobin E disorders. Expert Rev Hematol. 2010;03(01):103-117.
31. Balgir RS. The burden of haemoglobinopathies in India and the challenges ahead. Curr Sci. 2000;79(11):1536-1547.
32. Bhagat S, Das Gupta S, Kaushik S, et al. Gender distribution of hemoglobinopathies in India: A multicenter study. J Clin Diagn Res. 2016;10(04):EC01-EC04.
33. Balgir RS. Spectrum of hemoglobinopathies in the state of Odisha, India: A ten year cohort study. J Assoc Physicians India. 2005;53:1021-1026.
34. Kumar A, Babu K, Singh B, et al. Demographic profile of patients with thalassemia attending a tertiary care center in North India. Indian J Pediatr. 2017;84(04):302-306.
35. Verma IC, Saxena R, Kohli S. Past, present & future scenario of thalassaemic care & control in India. Indian J Med Res. 2011;134(04):507-521.
36. Weatherall DJ, Clegg JB. The thalassemia syndromes. 4th ed. Oxford: Wiley-Blackwell; 2001:287-356.
37. De Sanctis V, Kattamis C, Canatan D, et al. β-thalassemia distribution in the Old World: An ancient disease seen from a historical standpoint. Mediterr J Hematol Infect Dis. 2017;09(01):e2017018.
38. Rachmilewitz EA, Giardina PJ. How I treat thalassemia. Blood. 2011;118(13):3479-3488.
39. Kutlar A, Kutlar F, Wilson JB, et al. Quantitation of hemoglobin components by high-performance liquid chromatography: Its application to the determination of HbF and HbA2 levels. Am J Hematol. 1984;17(01):39-53.
40. Patel AG, Shah AP, Sorathiya SM, et al. Hemoglobinopathies in South Gujarat population and incidence of anemia in them. Indian J Hum Genet. 2012;18(03):294-298.
41. Steinberg MH. Genetic etiologies for phenotypic diversity in sickle cell anemia. Sci World J. 2009;09:46-67.
42. Serjeant GR, Serjeant BE. Sickle Cell Disease. 3rd ed. Oxford: Oxford University Press; 2001.
43. Bain BJ. Blood Cells: A Practical Guide. 5th ed. Oxford: Wiley-Blackwell; 2015.
44. Clark BE, Thein SL. Molecular diagnosis of haemoglobin disorders. Clin Lab Haematol. 2004;26(03):159-176.
45. Torres LS, Okumura JV, Silva DG, et al. Hemoglobin D-Punjab: Origin, distribution and laboratory diagnosis. Rev Bras Hematol Hemoter. 2015;37(02):120-126.
46. Thein SL. The molecular basis of β-thalassemia. Cold Spring Harb Perspect Med. 2013;03(05):a011700.
47. Saxena A, Phadke SR. Thalassemia control by carrier screening: The Indian scenario. Curr Sci. 2002;83(03):291-295.
48. Angastiniotis M, Kyriakidou S, Hadjiminas M. How thalassemia was controlled in Cyprus. World Health Forum. 1986;07:291-297.
49. Cao A, Kan YW. The prevention of thalassemia. Cold Spring Harb Perspect Med. 2013;03(02):a011775.
50. Harteveld CL, Higgs DR. Alpha-thalassemia. Orphanet J Rare Dis. 2010;05:13.