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ORIGINAL ARTICLE

Capillary electrophoresis-based screening for haemoglobinopathies in Sindh and Balochistan, Pakistan: A cross-sectional study.

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ABSTRACT… Objective: To assess the frequency and spectrum of haemoglobinopathies in Sindh and Balochistan using the Sebia Capillarys 3 Octa capillary electrophoresis system, and evaluate its diagnostic utility in routine clinical practice. **Study Design:** Descriptive Cross-sectional study. **Setting:** Department of Hematology, Chughtai Laboratory, Karachi, Pakistan. **Period:** June 2023 to June 2024. **Methods:** A total of 1,340 patients referred for hemoglobinopathy screening were included using consecutive non-probability sampling. Capillary electrophoresis (CE) was performed using the Sebia Capillarys 3 Octa system to detect hemoglobin variants and quantify HbA2. A cut-off value of HbA2 >3.5% was used to identify β-thalassemia trait. Complete blood counts and peripheral smears were also evaluated. Patients with recent transfusions or incomplete data were excluded. **Results:** Out of 1,340 patients, 344 (25.7%) were diagnosed with haemoglobinopathies. β-thalassemia trait was most frequent (59.9%), followed by elevated HbF cases (13.9%). Females comprised 61.7% of the study population, with the 18–35 years age group most affected. Karachi reported the highest number of cases. CE showed high analytical performance in identifying common and rare hemoglobin variants. **Conclusion:** CE using the Sebia Capillarys 3 Octa system is a reliable and efficient tool for hemoglobinopathy screening. The high prevalence of β-thalassemia trait highlights the need for regional screening and prevention strategies.

Key words: Capillary Electrophoresis, Electrophoresis, Haemoglobinopathies, Thalassemia.

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INTRODUCTION

Inherited hemoglobin disorders, including hemoglobinopathies and thalassemias, are among the most common autosomal recessive conditions worldwide. These disorders can be broadly categorized into structural (qualitative) defects such as hemoglobin S, C, D, and E and quantitative (regulatory) abnormalities, which result in reduced or imbalanced globin chain production, as seen in α- and β-thalassemias.[1] Globally, approximately 7% of the population carries abnormal hemoglobin genes, with over 300,000 infants born each year with severe forms of these disorders.[2] Effective screening and prevention programs have been shown to significantly reduce the disease burden, yet such initiatives remain limited in many low- and middle-income countries.[3,4]

In Pakistan, the burden of hemoglobinopathies is particularly high, largely due to the prevalence of consanguineous marriages, which increase the likelihood of inheriting these autosomal recessive conditions.[5,6] Previous regional studies report β-thalassemia trait carrier rates ranging from 5% to over 9%, with even higher prevalence in some areas.[7,8]

Accurate laboratory diagnosis is essential for effective management and prevention. Conventional method such as gel electrophoresis, isoelectric focusing (IEF), and high-performance liquid chromatography (HPLC) have been widely used for hemoglobinopathy screening, though each comes with limitations in terms of resolution and efficiency.[4,9,10] Capillary electrophoresis (CE) has emerged as a highly effective alternative, offering superior resolution, automation, and reproducibility, particularly for quantifying HbA2 and HbF and detecting compound heterozygous states.[1,11-14]

Sebia's Capillarys 3 Octa system is an advanced CE based platform designed for the high-throughput and precise analysis of hemoglobin variants.

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This system is widely recognized for its analytical accuracy, automation, and ability to deliver consistent, reproducible separation of hemoglobin fractions. These characteristics make it particularly well-suited for the detection and characterization of both common and rare hemoglobinopathies in clinical diagnostic settings. Despite the growing global adoption of CE, there is a paucity of regional data evaluating its utility within diverse populations in Pakistan. Therefore, its application in large-scale screening and diagnosis of hemoglobin disorders in this setting warrants further exploration.[7,13,14]

This study aims to assess the frequency and spectrum of hemoglobinopathies in the coastal provinces of Sindh and Balochistan, using CE as the diagnostic modality. The goal is to evaluate the utility of CE in routine diagnostic settings and to contribute updated regional epidemiological data to support informed public health strategies.

METHODS

This descriptive cross-sectional study included 1,340 patients referred for hemoglobin disorder screening between June 2023 and June 2024 at the Department of Hematology, Chughtai Laboratory, Karachi, Pakistan. The laboratory serves as a reference center, receiving samples from multiple collection units.

Ethical approval was obtained from the Institutional Review Board (IRB) under letter number CIP/IRB/1189. Patients of all ages and both sexes referred for hemoglobinopathy screening were enrolled using consecutive non-probability sampling. Individuals who had received a blood transfusion within the last three months or had incomplete laboratory data were excluded.

Complete blood counts (CBCs) were performed using the Sysmex XN-1000 and Mindray BC-6200 analyzers. Peripheral blood smears were stained with Leishman's stain, and red cell morphology was assessed for hypochromia, anisocytosis, microcytosis, macrocytosis, and polychromasia using standard criteria.

Hemoglobin electrophoresis was performed using the Sebia Capillarys 3 Octa system, which employs capillary zone electrophoresis through eight parallel quartz capillaries to separate hemoglobin fractions based on electrical charge in free solution. Whole blood samples were analyzed directly using the system's cap-piercing technology. Electropherograms were evaluated for hemoglobin variants (e.g., HbS, HbC, HbE), and HbA2 quantification. A cut-off value of HbA2 > 3.5% was used for the diagnosis of β-thalassemia trait. Key performance metrics included peak resolution, coefficient of variation (CV) for HbA2 measurements, and concordance with reference diagnoses.

RESULTS

As per the inclusion criteria, a total of 1340 patients were enrolled in the study. Of the 1,340 patients, 344 (25.7%) were diagnosed with haemogobinopathies while the rest harboured a normal pattern (n=996; 74.3%). The distribution of the various hemoglobinopathies reported in the present study are displayed in Figure-1. The most frequent hemoglobinopathy observed in the current study was β-thalassemia trait (n=206/344; 59.9%) followed by cases with of high fetal hemoglobin (HbF), (n=48/344; 13.9%). These were patients with a history of blood transfusions, who showed elevated HbF levels and morphological findings suggestive of thalassemia syndrome. These patients were advised to repeat hemoglobin electrophoresis after three months. Single cases of compound heterozygous for HbE/β-Thalassemia, compound heterozygous for HbC/β-Thalassemia, and compound heterozygous for HbS/D-Thalassemia were reported in the present study (Figure-1). The present study had a female predominance (n=827; 61.7%). Based on gender, in the most common hemoglobinopathy i.e. β-thalassemia trait, more than half of the patients were females (55.8%; n=115/206) (Figure-2). Figure-2 illustrates the gender-wise distribution of various hemoglobinopathies observed in the study population. Overall, a female predominance was noted, with females accounting for 61.7% of the total participants (n=827). When evaluating the most frequently diagnosed hemoglobinopathy, β-thalassemia trait, a similar gender trend was observed. Out of the 206 individuals diagnosed with β-thalassemia trait, 55.8% (n=115) were female, indicating a slightly higher prevalence among

females compared to males.

Based on age, the most frequent age group was 18-35 years (n=585) followed by 0.02 -10.0 years (n=462). With hemoglobinpathies as a focus of study, β-thalassemia trait was the most frequent group in which majority of the patients fell in 18.0 years -35 years age group (n=102/206; 49.5%) (Table-I). Regionally, the predominance of hemoglobinopathies was observed in Karachi (n=169) followed by Quetta (n=61) (Table-II). The median (IQR) values of the electrophoresis for different hemoglobin variants are shown in Table-III.

FIGURE-1

Overall frequency of various haemoglobinopathies

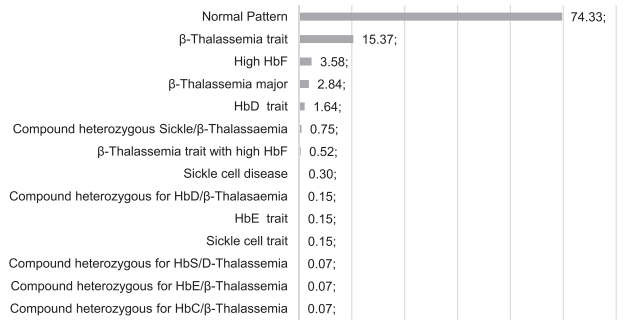
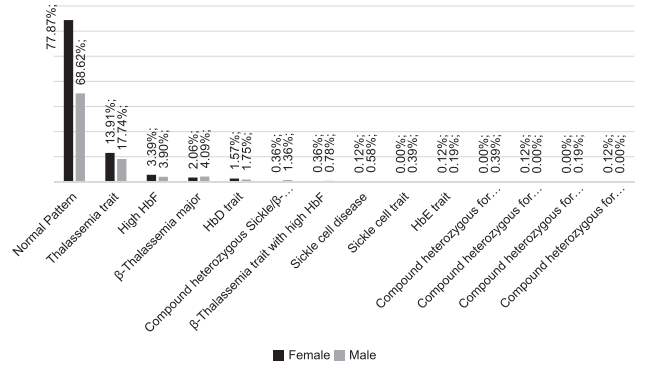


FIGURE-2

Gender distribution for various haemoglobinopathies



DISCUSSION

This cross-sectional study conducted at Chughtai Laboratory, Karachi, provides important insights into the prevalence and spectrum of hemoglobinopathies in the provinces of Sindh and Balochistan. Among the 1,340 individuals screened, hemoglobinopathies were identified in 25.7% (n=344) of cases. These findings are consistent with previous research, such as Waheed et al. (2012), who reported a 28.4% frequency of hemoglobinopathies in Islamabad, reinforcing the significant public health impact of β-thalassemia in Pakistan.[15]

Haemoglobin disorders are a major global health concern, affecting 71% of 229 countries, which together account for 89% of the world's births. Each year, over 330,000 infants are born with severe hemoglobinopathies (83% with sickle cell disorders and 17% with thalassaemias), contributing to approximately 3.4% of deaths in children under five; globally, about 7% of pregnant women carry β-thalassaemia, α^0-thalassaemia, or hemoglobin variants S, C, D Punjab, or E, and more than 1% of couples are at genetic risk. Integrating hemoglobinopathy screening and genetic counseling into routine healthcare systems is therefore essential to identify at-risk couples and reduce disease incidence through informed reproductive decisions. In Pakistan, the burden is compounded by the high rate of consanguineous marriages, which increases the inheritance of autosomal recessive traits.[6,16]

In the present study, the most prevalent hemoglobinopathy was β-thalassemia trait (59.9%), followed by cases with elevated fetal hemoglobin (HbF) levels (13.9%). Recent studies from urban centers such as Rawalpindi and Islamabad have reported similarly high prevalence rates. One study found hemoglobinopathies in 37.8% of 600 individuals, with β-thalassemia trait (47.1%) and β-thalassemia major (23.3%) as the most common forms. Another study involving 5,961 participants identified hemoglobinopathies in 11.9% of cases, with β-thalassemia trait again being the most frequent (9.5%). These findings underscore the significant burden of hemoglobinopathies in urban populations and highlight the urgent need for comprehensive screening programs and preventive strategies.[17,18] These results reflect a considerable burden in urban centers and emphasize the need for comprehensive screening and preventive measures.

A study from Peshawar detected hemoglobinopathies in 42.2% of 263 samples, with β-thalassemia minor (32.7%) and major (8.4%) as the most frequent.

TABLE-I

Age wise distribution of haemoglobinopathies

Haemoglobinopathies	Frequency	Age (years)						
		0.02-10.0 (n=462)	11.0-17.0 (n=138)	18.0-35.0 (n=585)	36.0-50.0 (n=118)	51.0-65.0 (n=25)	66.0-80.0 (n=10)	81.0-95.0 (n=2)
Normal Pattern, n (%)	996	323 (69.9)	105 (76.1)	458 (78.3)	84 (71.8)	16 (64.0)	9 (90.0)	1 (50.0)
β-Thalassemia trait, n (%)	206	44 (9.5)	21 (15.2)	102 (17.4)	28 (23.7)	9 (36.0)	1 (10.0)	1 (50.0)
High HbF, n (%)	48	40 (8.7)	2 (1.4)	6 (1.6)	-	-	-	-
β-Thalassemia major, n (%)	38	37 (8.0)	1 (0.7)	-	-	-	-	-
HbD trait, n (%)	22	5 (1.1)	4 (2.9)	11 (1.9)	2 (1.7)	-	-	-
Compound heterozygous Sickle/β-Thalassaemia, n (%)	10	4 (0.9)	2 (1.4)	3 (0.5)	1 (0.8)	-	-	-
β-Thalassemia trait with high HbF, n (%)	7	7 (1.5)	-	-	-	-	-	-
Sickle cell disease, n (%)	4	-	2 (1.4)	2 (0.3)	-	-	-	-
Sickle cell trait, n (%)	2	-	1 (0.7)	-	1 (0.8)	-	-	-
HbE trait, n (%)	2	1 (0.2)	-	-	1 (0.9)	-	-	-
Compound heterozygous for HbD/β-Thalasaemia, n (%)	2	-	-	2 (0.3)	-	-	-	-
Compound heterozygous for HbE/β-Thalassemia, n (%)	1	1 (0.2)	-	-	-	-	-	-
Compound heterozygous for HbC/β-Thalassemia, n (%)	1	-	-	-	1 (0.2)	-	-	-
Compound heterozygous for HbS/D-Thalassemia, n (%)	1	-	-	1 (0.2)	-	-	-	-

TABLE-II

Regional distribution of haemoglobinopathies

Haemoglobinopathies	Frequency	KHI (n=920)	HDD (n=92)	Sindh (Besides KR & HDD) (n=165)	QTA (n=122)	Balochistan Besides QTA (n=41)
Normal Pattern, n (%)	996	751 (81.6)	64 (69.6)	101 (61.2)	61 (50.0)	19 (46.3)
β-Thalassemia trait, n (%)	206	122 (13.3)	11 (12.0)	26 (15.8)	34 (27.9)	13 (31.7)
High HbF, n (%)	48	12 (1.3)	9 (9.8)	18 (10.9)	6 (4.9)	3 (7.3)
β-Thalassemia major, n (%)	38	12 (1.3)	3 (3.3)	15 (9.1)	3 (2.5)	5 (12.2)
HbD trait, n (%)	22	14 (1.5)	1 (1.1)	3 (1.8)	3 (2.5)	1 (2.4)
Compound heterozygous Sickle/β-Thalassaemia, n (%)	10	2 (0.2)	1 (1.1)	-	7 (5.7)	-
β-Thalassemia trait with high HbF, n (%)	7	4 (0.4)	2 (2.2)	1 (0.6)	-	-
Sickle cell disease, n (%)	4	-	1 (1.1)	-	3 (2.5)	-
Sickle cell trait, n (%)	2	1 (0.1)	-	-	1 (0.8)	-
HbE trait, n (%)	2	-	-	1 (0.6)	1 (0.8)	-
Compound heterozygous for HbD/β-Thalasaemia, n (%)	2	-	-	-	2 (1.6)	-
Compound heterozygous for HbE/β-Thalassemia, n (%)	1	1 (0.1)	-	-	-	-
Compound heterozygous for HbC/β-Thalassemia, n (%)	1	-	-	-	1 (0.8)	-
Compound heterozygous for HbS/D-Thalassemia, n (%)	1	1 (0.1)	-	-	-	-

KHI: Karachi; HDD: Hyderabad; QTA: Quetta

TABLE-III

Haemoglobin electrophoresis results in various haemoglobinopathies

Haemoglobinopathy	N	HbA%	HbA2 %	HbF %	HbS %	HbD %	HbS/D %	HbE %	HbH %	HbC %
Normal Pattern, Median (IQR)	996	97.7 (97.4-98.0)	2.3 (2.0-2.5)	1.7 (1.0-2.7)	-	-	-	-	-	
β-Thalassemia trait, Median (IQR)	206	94.6 (93.9-95.0)	5.2 (4.8-5.6)	2.1 (1.5-3.3)	-	-	-	-	-	
High HbF, Median (IQR)	48	70.8 (47.5-89.5)	2.8 (2.1-3.3)	27.2 (8.6-50.1)	-	-	-	-	-	
β-Thalassemia major, Median (IQR)	38	4.7 (3-13.4)	2.4 (1.9-3.2)	97.0 (93.8-98.0)	-	-	-	-	-	
HbD trait, Median (IQR)	22	61.6 (59.3-65.2)	2.9 (2.5-3.1)	1.5 (0.43-2.3)	-	35.4 (32.1-37.4)	-	-	-	
Compound heterozygous Sickle/β-Thalassaemia, Median (IQR)	10	3.0 (3.0-3.0)	4.0 (3.8-4.6)	32.7 (26.2-38.6)	63.6 (57.4-69.3)	-	-	-	-	
β-Thalassemia trait with high HbF, Median (IQR)	7	84.7 (79.5-86.4)	4.2 (1.4-5.4)	9.8 (7.8-16.3)	-	-	-	-	-	
Sickle cell disease, Median (IQR)	4	-	1.6 (1.2-2.3)	21.3 (14.1–28.8)	77.3 (69.9–83.6)	-	-	-	-	
Sickle cell trait, Median (IQR)	2	62.0 (60.0-64.0)	2.8	-	35.2 (33.2-37.2)	-	-	-	-	
HbE trait, Median (IQR)	2	70.2 (68.1-72.3)	1.75 (0.8-2.7)	-	-	-	-	28 (25-31.0)	-	
Compound heterozygous for HbD/β-Thalasaemia, Median (IQR)	2	-	6.6 (6.4-6.8)	2.0 (1.0-3.0)	-	91.4 (90.6-92.2-)	-	-	-	
Compound heterozygous for HbE/β-Thalassemia, Median (IQR)	1	7	27.2	-	-	-	65.8	-	-	
Compound heterozygous for HbC/β-Thalassemia, Median (IQR)	1	-	6.1	1.6	-	-	-		-	92.3
Compound heterozygous for HbS/D-Thalassemia, Median (IQR)	1		3.1	3.7	-	-	93.2	-	-	

The study also reported a 37.3% association with consanguinity, underscoring the role of intra-family marriages in the transmission of these disorders. Such findings highlight the importance of family-based screening programs to reduce both the medical and financial impact on affected populations.[19]

A retrospective analysis conducted at Indus Hospital and Health Network in Karachi, using the ADAMS A1C HA-8180T analyzer, reported a hemoglobinopathy prevalence of 14.5% among 2,422 patients. The β-thalassemia trait was again the most common, accounting for 6.4% of cases.[20]

Another study from a tertiary care hospital utilizing HPLC on 3,289 samples revealed hemoglobinopathies in 21.5% of individuals.

Thalassemia minor was the most prevalent (19.6%), followed by HbD trait (1.3%), HbS trait (0.36%), and HbE trait (0.21%). No cases of HbC were reported. The mean age of patients with thalassemia minor was 24 ± 14.7 years, highlighting the asymptomatic nature of many carriers.[21]

Data from District Dera Ismail Khan pointed to notable ethnic differences in hemoglobinopathy prevalence. The Baloch, Mehsud, and Marwat tribes exhibited higher rates of β-thalassemia, while the sickle cell gene was more frequent in the Sherani, Bhatni, and Ustrana communities. These findings underscore the necessity of implementing targeted community-based screening, genetic counseling, and public education programs. In our study, most hemoglobinopathy cases were reported from Karachi, likely due to its ethnically diverse population.[22]

A comparative study at the Armed Forces Institute of Pathology, Rawalpindi, evaluated 90 newly diagnosed cases using both HPLC and CE. The study found β-thalassemia trait to be the most common (55.6%), while HbD homozygosity was the least frequent (1.1%). Notably, CE outperformed HPLC in detecting hemoglobin variants such as HbD Iran and HbE, affirming its diagnostic advantage.[23]

This study is limited by its exclusion of recently transfused patients, lack of genetic confirmation, and single-center design, which may affect the generalizability and completeness of the findings.

CONCLUSION

This study highlights the substantial burden and diverse spectrum of haemoglobinopathies in the coastal provinces of Sindh and Balochistan, with β-thalassemia trait emerging as the most frequently diagnosed variant. The demographic distribution indicates a higher prevalence among young adults and females, particularly in urban centers like Karachi. CE demonstrated high diagnostic accuracy, effectively identifying both common and rare haemoglobin variants, and offers a practical, high-throughput solution for routine clinical use. These findings emphasize the need for region wide screening initiatives, early diagnostic interventions, and public awareness programs. Future studies integrating genetic analysis and multi-center collaboration are essential to deepen our understanding and to inform national strategies for the prevention and control of haemoglobin disorders in Pakistan.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHORSHIP AND CONTRIBUTION DECLARATION	
1	**Kanwal Shafiq:** Analysis, manuscript formatting.
2	**Munazza Rashid:** Interpreted the data.
3	**Intezar Hussain:** Data analysis.
4	**Hafiza Sidra:** Data entry.