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Spectrum of Hemoglobinopathies by Electrophoresis at Tertiary Care Centre

Dr. Abhishek Vitthal Gawande[1], Dr. Vijay Madhukar Mulay[2], Dr. Rajan S. Bindu[3]

[1]Assistant Professor, Department of Pathology Grant Government Medical College & Sir JJ Group of Hospitals, Mumbai)
Corresponding Author Email: *drabhishekgawande10[at]gmail.com*

[2]Consultant Professor & HOD, Department of Pathology B.S.P. Medical College Chh. Sambhajinagar)
Email: *mdrvijay[at]gmail.com*

[3]Ex Professor, Department of Pathology Symbiosis Medical College for Women, Pune
Email: *rajan.bindu[at]smcw.siu.edu.in*

Abstract: ***Background: Haemoglobinopathies are among the most common inherited genetic disorders worldwide, particularly in India where diverse ethnic populations contribute to variable prevalence. Accurate and early diagnosis is crucial for clinical management and prevention. Objective: To determine the spectrum of haemoglobinopathies using hemoglobin electrophoresis at a tertiary care centre, and to evaluate hematological parameters across different groups. Methods: This prospective observational study was conducted on patients suspected of having haemoglobinopathies. Detailed clinical evaluation, complete blood count (CBC), peripheral smear, and alkaline hemoglobin electrophoresis were performed. Hematological data was analysed to identify specific patterns. Results: Out of 200 cases, the majority were diagnosed with sickle cell trait (42%), followed by sickle cell disease (28%), beta thalassemia trait (10%), beta thalassemia major (10%), sickle-beta thalassemia (4%), and hemoglobin E trait (4%). Clinical manifestations varied by genotype. Hematological parameters showed microcytic hypochromic anemia predominantly in thalassemia syndromes. Conclusion: Hemoglobin electrophoresis remains a reliable, economical, and accessible tool for diagnosing haemoglobinopathies. Early diagnosis is key for appropriate genetic counselling and clinical intervention.***

Keywords: Haemoglobinopathies, Electrophoresis, Thalassemia, Sickle Cell Disease, Hemoglobin E, India

1. Introduction

Inherited hemoglobin disorders constitute a significant burden of genetic diseases in India, contributing to substantial morbidity, moderate to severe hemolytic anemia, and even premature death in affected individuals. These disorders are broadly classified into structural hemoglobin variants and thalasaemias.[1]

On a global scale, approximately 1.1% of couples are at risk of bearing children affected by hemoglobin disorders, and around 2.7 per 1,000 conceptions are affected. Annually, over 9 million carriers become pregnant, and the chance that their partner is also a carrier ranges from 0.1% to 40%, with a global average of 14%.[2]

In India, the cumulative gene frequency of haemoglobinopathies is 4.2%. With a population exceeding one billion, this results in over 42 million carriers and more than 12,000 infants born each year with clinically significant haemoglobinopathies.[3] The prevalence of the sickle cell gene exhibits regional variation: 0–18% in Northeast India, 0–33.5% in Western India, 22.5–44% in Central India, and 1–40% in Southern India.[4]

Laboratory diagnosis of hemoglobin disorders is crucial for several purposes: (a) confirming a clinical diagnosis (especially in cases like sickle cell disease or β-thalassemia major), (b) investigating hematological abnormalities, (c) identifying disorders in the presymptomatic phase (e.g., neonates), (d) predicting serious globin chain synthesis disorders during fetal life and enabling the option of prenatal diagnosis or termination, and (e) facilitating genetic counselling for at-risk couples.[5]

Among diagnostic tools, hemoglobin electrophoresis—especially alkaline electrophoresis—is widely used. It is a rapid, reproducible method capable of distinguishing common variants like HbA, HbF, HbS, and HbC[5]. HPLC offers advantages such as rapid processing, internal sample preparation, superior resolution, accurate quantification, and minimal sample requirement.[5,6]

Given the clinical importance and high prevalence of haemoglobinopathies in India, the present study was undertaken to evaluate the spectrum of hemoglobin disorders using alkaline hemoglobin electrophoresis. Objectives included:

a) To study the spectrum of haemoglobinopathies.
b) To evaluate the patients suspected of having haemoglobinopathies with the help of hemoglobin electrophoresis.
c) To study various electrophoretic patterns of different haemoglobinopathies.

2. Materials and Methods

The study was carried out in Department of Pathology of a tertiary care hospital in Maharashtra, after the approval of the college ethical committee, over a duration of two years, from November 2016 to October 2018. Patients of all age groups who presented with clinical suspicion of haemoglobinopathies were included in the study. A total of 200 patients were studied. The suspicion was based on

clinical features such as anemia, jaundice, splenomegaly, or a positive family history of hemoglobin disorders. Hemoglobin electrophoresis (alkaline pH) and densitometry were used to identify and quantify variants. Family members were also studied to confirm diagnoses through pedigree and carrier detection. The collected data were systematically analyzed to study demographic trends, clinical manifestations, hematological parameters, and electrophoretic patterns of hemoglobin.

3. Results

Out of 200 cases, 150 cases showed normal pattern on electrophoresis. 50 cases were diagnosed as hemoglobin disorders.

Table 1: Classification of Hemoglobin disorders

Group	Diagnosis	No. of Cases
A	Sickle Cell Trait (SCT)	21 (42%)
B	Sickle Cell Disease (SCD)	14 (28%)
C	Beta Thalassemia Major (BTM)	5 (10%)
D	Beta Thalassemia Trait (BTT)	5 (10%)
E	Thalassemia Intermedia	1 (2%)
F	Sickle Cell - Beta Thalassemia (SBT)	2 (4%)
G	Hemoglobin E-Trait	2 (4%)
Total cases of Hemoglobin disorders		50

Among 50 haemoglobinopathy cases, the most common disorder was Sickle Cell Trait (42%), followed by Sickle Cell Disease (28%). Beta Thalassemia Major and Beta Thalassemia Trait each accounted for 10% of cases. Less frequent variants included Thalassemia Intermedia (2%), Sickle Cell–Beta Thalassemia (4%), and Hemoglobin E-Trait (4%).

Age ranged from neonates to 60 years. The majority of haemoglobinopathy cases were seen in the 0–10 years age group. Group A peaked in the 21–30 years age group. Cases were rare beyond 40 years.

Out of 200 patients studied, males comprised 56% and females 44%. Out of 50 patients diagnosed with Haemoglobinopathies, majority 26(58%) were females while 48% were males. Sickle cell disorders were more common in males, while thalassemia variants, especially minor and Hb E trait, affected females more. Out of 50 cases, 15 cases were having history of consanguineous marriage in family. Maximum cases with consanguineous marriages were found in Sickle Cell Disease i.e. Group B.

In this study, the majority of cases belonged to the Buddha (38%) and Muslim (24%) communities, followed by Banjara (14%). Among the 50 haemoglobinopathy cases, pallor was the most common symptom (78%), followed by splenomegaly (26%) and joint pain (18%). Jaundice was observed in 12%, hepatomegaly in 14%, and fever in 8%. Abdominal pain was reported in 6% of cases, mainly in Groups A, B, and C.

Hematological Findings:
Hematological parameters across haemoglobinopathy groups revealed the lowest mean hemoglobin levels in Groups C (4.42 g/dL) and G (3.85 g/dL), indicating severe anemia. RBC counts were generally low, with Group A having the highest (3.68 million/mm³). MCV and MCH values were markedly reduced in Group C, reflecting microcytic hypochromic anemia. MCHC was consistently low across all groups.

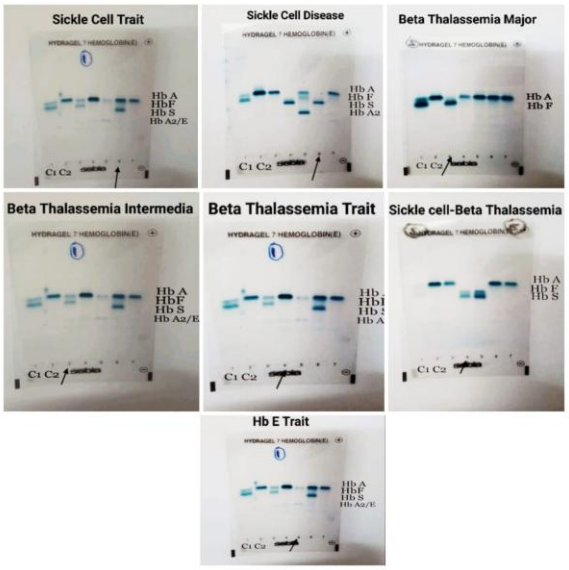

Figure 1: Electrophoretic Gel plates of various hemoglobinopathies

4. Discussion

This study highlights the diagnostic utility of alkaline electrophoresis in characterizing haemoglobinopathies. Multiple studies have highlighted the prevalence of haemoglobinopathies. They are summarized in below table.

Table 2: Studies of Hemoglobinopathies.

Other studies	Place of Study	Prevalent Hemoglobinopathy
Brig GC et al[7] (2008)	New Delhi (Hospital Based, Armed Forces)	Beta –Thalassemia trait
Dogaru M et al[24] (2007)	Romania	Beta –Thalassemia trait
Balgir RS et al[16] (2005)	Orissa	Sickle Cell trait
Baruah MK et al [22] [2014]	Upper Assam Region [North-Estern India].	HbE-trait
Mondal SK et al[23] [2014]	West Bengal	Beta –Thalassemia trait
Shrivastava A et al[8] [2013]	Western India	Beta –Thalassemia trait
Rao S et al[21] (2010)	New Delhi	Beta –Thalassemia trait
Shivashankara, A et al [9] [2011]	South India	Beta –Thalassemia trait
Patel AG et al [14] [2012]	South Gujrath	Beta –Thalassemia trait
Mandal PK et al [11] [2013]	West Bengal	Beta –Thalassemia trait

Several studies have highlighted the clinical presentations of haemoglobinopathies. Balgir RS (2010) reported 137 sickling disorder cases, noting pallor (58.6%) and vaso-occlusive crises (31%) as common in Sβ Thalassemia.[16] Panigrahi et al (2005) found pallor in 100% of HbE-β Thalassemia patients, with splenomegaly (74%), hepatomegaly (65%), and jaundice (57%).[17] Tyagi et al (2003) observed that 42.6% of 47 sickling disorder cases were asymptomatic and often detected incidentally.[18] Shah SJ et al (2012) reported pallor in all 35

haemoglobinopathy cases (100%) and icterus in 22.9%, underlining the variability in clinical manifestations across different haemoglobinopathies and patient populations.[19]

Hematological parameters in Sickle Cell Trait (Group A) show variability across studies. Chandrashekar et al (2011) and Rao et al (2008) reported higher hemoglobin levels (11.3–11.6 g/dl) and RBC counts (>4.4 million/mm³), indicating near-normal profiles.[20,21] Shrivastav et al (2013) and Mondal et al (2014) reported moderate anemia with Hb between 9.89–10.8 g/dl.[8] The present study showed comparatively lower Hb (8.19 g/dl), RBC (3.68 million/mm³), and MCHC (28 g/dl), suggesting hypochromia and mild microcytic anemia, highlighting interindividual and geographical variations in hematological expression of sickle cell trait.

Hematological parameters in Sickle Cell Disease (Group B) vary across studies. Chandrashekar et al (2011) reported Hb 7.8 g/dl and MCHC 31.5 g/dl, while Baruah et al (2012) observed lower RBC (2.2 million/mm³) with higher MCV (93.5 fl).[20,22] Rao et al (2008) noted Hb 8.3 g/dl and MCV 90.5 fl, indicating macrocytosis.[21] Shrivastav et al (2013) found moderate anemia with Hb 7.46 g/dl and RBC 3.44 million/mm³. In comparison, the present study showed more severe anemia (Hb 5.68 g/dl), low MCHC (26.21 g/dl), and microcytosis (MCV 73.28 fl), suggesting more severe hemolysis and red cell abnormalities in the studied population.

In patients with Beta Thalassemia Major (Group C), severe anemia and pronounced microcytosis is found in all studies. Chandrashekar et al (2011), Baruah et al (2012) and Rao et al (2008) revealed hemoglobin of 3.8-5.4g/dl and RBC counts (1.9-2.4 million/mm³) with MCV being 66.3-74.9fl.[20,22] The value of MCHC (34.11g/dl) reported by Shrivastav et al (2013) was slightly higher.[8] In the present study (2016) values of Hb 4.42g/dl and RBC count 2.42 million/mm³ with MCV 56.6 fl and the lowest MCHC (27.4g/dl) show more severe degree of microcytic hypochromic anemia corresponding to the degree of severity of the disease.

In Beta Thalassemia Trait (Group D), studies consistently report mild anemia with microcytosis and hypochromia. Hemoglobin values ranged from 7.9 to 10.4 g/dl and RBC counts were relatively preserved or elevated (3.6–5.38 million/mm³) (Chandrashekar et al, 2011; Rao et al, 2008; Shrivastav et al, 2013). MCV values typically ranged between 62.1 and 71.1 fl, and MCH ranged from 19.4 to 23.6 pg.[20,21,8] The present study (2016) recorded Hb of 7.7 g/dl, RBC count of 3.42 million/mm³, MCV of 68.8 fl, MCH of 23.6 pg, and MCHC of 26.8 g/dl—indicating microcytic hypochromic anemia consistent with previous findings for carriers of the β-thalassemia gene.

In cases of Sickle-Beta Thalassemia (Group F), lower Hb (4.6 g/dl), RBC (2.4 million/mm³), and MCHC (24 g/dl) was observed. Hemoglobin levels across studies ranged from 5.7 to 9 g/dl, with RBC counts between 2.4 and 4.1 million/mm³ (Chandrashekar et al, 2011; Rao et al, 2008; Baruah et al, 2012; Shrivastav et al, 2013; Mondal et al, 2014). MCV values generally ranged from 70.28 to 78 fl, MCH from 21 to 24.3 pg, and MCHC from 24 to 32.1 g/dl. [20,21,8,23]

Hematological parameters in HbE-trait cases (Group G) exhibit mild to moderate anemia with microcytosis and hypochromia. Hemoglobin levels across studies ranged from 6.2 to 10.3 g/dl, with RBC counts between 3.2 and 4.3 million/mm³ (Chandrashekar et al, 2011; Rao et al, 2008; Mondal et al, 2014). MCV values ranged from 63.9 to 75.5 fl, MCH from 16.3 to 24.2 pg, and MCHC from 29.4 to 31.7 g/dl.

Limitations include lack of molecular confirmation and use of only electrophoresis without HPLC. However, for primary screening, electrophoresis offers a cost-effective method.

5. Conclusion

This study provides a comprehensive overview of the spectrum and distribution of haemoglobinopathies in a tertiary care setting. Sickle Cell Trait emerged as the most common haemoglobinopathy, followed by Sickle Cell Disease and various forms of Thalassemia. Hemoglobin electrophoresis is a powerful and economical tool for the diagnosis of haemoglobinopathies in India. Its application in community and hospital settings can lead to early diagnosis, better clinical management, and effective genetic counselling.

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