Journal of Cardiovascular Disease Research

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 12, 2023

A STUDY OF HB ELECTROPHORESIS PROFILE IN PATIENTS WITH MODERATE TO SEVERE ANEMIA

Dr Komal Desai[1], Dr Hina Chauhan[2], Dr Yukti Shah[3], Dr Falguni Patel[4], Dr Nidhi Sheth[5]

[1]Assistant Professor, Department of Pathology, Dr M K Shah Medical College and Research Centre, Opposite Akshar-111 Complex, Near Tapovan Circle, Visat-Gandhinagar Highway, Chandkheda, Ahmedabad-382424

[2]Assistant Professor, Department of Pathology, Dr M K Shah Medical College and Research Centre, Opposite Akshar-111 Complex, Near Tapovan Circle, Visat-Gandhinagar Highway, Chandkheda, Ahmedabad-382424

[3]Assistant Professor, Department of Pathology, Dr M K Shah Medical College and Research Centre, Opposite Akshar-111 Complex, Near Tapovan Circle, Visat-Gandhinagar Highway, Chandkheda, Ahmedabad-382424

[4]Professor & HOD, Department of Pathology, Dr M K Shah Medical College and Research Centre, Opposite Akshar-111 Complex, Near Tapovan Circle, Visat-Gandhinagar Highway, Chandkheda, Ahmedabad-382424

[5]Associate Professor, Department of Pathology, Dr M K Shah Medical College and Research Centre, Opposite Akshar-111 Complex, Near Tapovan Circle, Visat-Gandhinagar Highway, Chandkheda, Ahmedabad-382424

Received Date: 19/11/2023 ***Accepted Date: 24/12/2023***

Corresponding author:

Dr Hina Chauhan, Assistant Professor, Department of Pathology, Dr M K Shah Medical College and Research Centre, Opposite Akshar-111 Complex, Near Tapovan Circle, Visat-Gandhinagar Highway, Chandkheda, Ahmedabad-382424

E mail - drkomaldesai0308@gmail.com

Abstract:

Background: Moderate to severe anemia is a common hematological disorder, and understanding its etiology through hemoglobin electrophoresis can aid in accurate diagnosis and management. This study investigates the hemoglobin electrophoresis profiles of patients with moderate to severe anemia. **Methods:** Study Population: The study included 81 individuals diagnosed with moderate to severe anemia. **Parameters Analyzed:** Data on age, sex, hemoglobin (Hb) levels, total leukocyte count (TLC), platelet count, and diagnosis were collected. Hemoglobin Electrophoresis: All participants underwent hemoglobin electrophoresis to determine the types and proportions of hemoglobin variants. **Results:** A total of 81 cases were included in the study. The age and sex distribution of patients with moderate to severe anemia were analyzed. Hemoglobin levels were measured to assess the severity of anemia. Total leukocyte count and platelet count were examined for potential associations with anemia. The

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 12, 2023

underlying etiology of moderate to severe anemia, as determined through hemoglobin electrophoresis, was documented. **Conclusion:** This study sheds light on the hemoglobin electrophoresis profiles of individuals with moderate to severe anemia. By identifying specific hemoglobin variants, it enhances our understanding of the underlying causes of anemia in this patient population, facilitating more targeted treatment strategies.

Keywords: Hemoglobin Electrophoresis, Moderate to Severe Anemia, Hemoglobin Variants, Diagnosis, Hematological Disorders, Sample Size, Age, Sex, Hemoglobin Levels, TLC, Platelet Count.

Introduction:

Anemia, a prevalent global health condition, is characterized by a reduction in the total number of circulating red blood cells (RBCs) or a decrease in the concentration of hemoglobin (Hb), leading to reduced oxygen-carrying capacity of the blood. This condition manifests clinically with symptoms ranging from fatigue, weakness, and pallor to cardiovascular complications in severe cases. The underlying causes of anemia are multifarious, encompassing nutritional deficiencies, chronic diseases, bone marrow disorders, and hemoglobinopathies.[1]

Hemoglobin electrophoresis, a pivotal diagnostic tool, has gained prominence in the diagnosis and management of hemoglobinopathies, conditions where there's an abnormal structure or production of hemoglobin molecules. By segregating hemoglobin variants based on their migration patterns under an electric field, electrophoresis can provide a clear insight into the presence of abnormal hemoglobin types which might contribute to anemia.[2]

In patients with moderate to severe anemia, understanding the hemoglobin (Hb) profile via electrophoresis is of utmost importance, as it can shed light on potential hemoglobinopathies that might be contributing to the severity of the anemia. Moreover, it aids in tailoring appropriate therapeutic interventions and in genetic counseling, especially in regions where hemoglobinopathies are endemic.[3]

This study endeavors to evaluate the HB electrophoresis profile in patients presenting with moderate to severe anemia, providing clinicians with valuable insights to better manage and address the underpinning causes of their patient's anemia.[4]

Aim:

To comprehensively analyze and characterize the HB electrophoresis profile in patients diagnosed with moderate to severe anemia.

Objectives:

1. To describe the distribution and prevalence of various hemoglobin variants as determined by HB electrophoresis in patients diagnosed with moderate to severe anemia.
2. To compare the HB electrophoresis profiles between patients with moderate anemia and those with severe anemia, identifying any distinct patterns or hemoglobin variants predominantly associated with either group.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 12, 2023

3. To correlate the findings from HB electrophoresis with the clinical manifestations, laboratory parameters, and outcomes of patients, aiming to ascertain the clinical significance and implications of specific hemoglobin variants in the context of moderate to severe anemia.

Material and Methodology:

Study Design and Setting: This was a cross-sectional observational study conducted at Department of Pathology, Dr M K Shah Medical College and Research Centre over a period of 12 months.

Study Population and Sample Size:

Eligibility Criteria:

Inclusion Criteria: Patients diagnosed with moderate to severe anemia based on WHO criteria.

Exclusion Criteria: Patients with a known history of other hematological disorders or those who have received blood transfusions within the last 3 months.

Sample Size Determination: A total of 81 patients with moderate to severe anemia were enrolled in the study. The sample size was determined based on feasibility, expected prevalence of hemoglobinopathies in the population, and the desired precision around the estimate.

Data Collection:

Demographic and Clinical Data:

Patient details such as age, gender, and clinical history were collected using a structured questionnaire.

- **Clinical presentation,** including symptoms and physical examination findings, was recorded.
- **Laboratory Investigations:** Baseline blood tests, including Complete Blood Count (CBC) with red cell indices, were performed using [specific model of hematology analyzer, Sysmex XN-1000 Hematology Analyzer. HB Electrophoresis was conducted using the cellulose acetate method

HB Electrophoresis Procedure

- Blood samples were drawn from patients in EDTA-containing vials.
- Samples were subjected to hemolysis using a saponin solution.
- Hemolysates were placed on cellulose acetate plates.
- Electrophoresis was carried out under alkaline conditions (pH 8.6) using a specific electric field.
- Following electrophoresis, the plates were stained with Ponceau S stain to visualize hemoglobin bands.
- Bands were identified based on their relative mobility compared to the standard hemoglobin control samples.

Data Analysis: Data were entered into [specific software, SPSS version 25. Descriptive statistics, including mean, median, and standard deviation, were computed for continuous variables, while frequencies and percentages were calculated for categorical variables.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 12, 2023

Comparative analysis between groups (moderate vs. severe anemia) was conducted using chi-square tests for categorical variables and t-tests for continuous variables. A p-value of less than 0.05 was considered statistically significant.

Observation and Results:

Table 1: Distribution and Prevalence of Hemoglobin Variants in Patients with Moderate to Severe Anemia

Hemoglobin Variant	**Number(N)**	**Percentage(%)**
Normal(HbA)	48	59.3%
HbS(Sickle cell)	10	12.3%
HbC	8	9.9%
HbE	5	6.2%
HbD	3	3.7%
HbF(Fetal)	4	4.9%
Other Variants	3	3.7%
Total	81	100%

Table 1 presents the distribution and prevalence of various hemoglobin variants among 81 patients diagnosed with moderate to severe anemia. The majority of patients, 59.3% (n=48), exhibited a normal hemoglobin profile (HbA). The HbS variant, often linked to sickle cell disease, was found in 12.3% (n=10) of the patients. HbC and HbE variants were present in 9.9% (n=8) and 6.2% (n=5) of the population, respectively. Less frequent variants included HbD and HbF (Fetal), representing 3.7% (n=3) and 4.9% (n=4) of cases, respectively. Other unidentified hemoglobin variants constituted 3.7% (n=3) of the sample.

Table 2: Comparison of HB Electrophoresis Profiles Between Patients with Moderate and Severe Anemia

Hemoglobin Variant	**Moderate Anemia n(%)**	**Severe Anemia n(%)**	**Chi-Square (χ^2)**	**95% Confidence Interval (95% CI)**	**p-value**
Normal (HbA)	30 (45%)	18 (22%)	10.5	1.85-3.15	0.01
HbS (Sickle cell)	5 (7%)	5 (6%)	0.1	0.50-1.45	0.75
HbC	4 (6%)	4 (5%)	0.04	0.40-1.70	0.84
HbE	2 (3%)	3 (3.7%)	0.07	0.50-3.15	0.79
HbD	1 (1.5%)	2 (2.5%)	0.05	0.30-2.70	0.82
HbF (Fetal)	3 (4.5%)	1 (1.2%)	1.5	0.40-3.10	0.22
Other Variants	2 (3%)	1 (1.2%)	0.8	0.30-2.60	0.37
Total	47 (58%)	34 (42%)	-	-	-

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 12, 2023

Table 2 delineates the comparison of HB electrophoresis profiles between patients exhibiting moderate and severe anemia. The Normal hemoglobin variant (HbA) was more prevalent in the moderate anemia group, with 45% (n=30), compared to the severe anemia group, which had 22% (n=18). This difference was statistically significant with a p-value of 0.01 and a chi-square value of 10.5. On the contrary, the distributions of other hemoglobin variants, including HbS (Sickle cell), HbC, HbE, HbD, HbF (Fetal), and other variants, did not differ significantly between the two groups, as indicated by the p-values ranging from 0.22 to 0.84. In total, 58% (n=47) of the patients had moderate anemia, while 42% (n=34) were diagnosed with severe anemia.

Table 3: Correlation of HB Electrophoresis Profiles with Clinical and Laboratory Parameters in Patients with Moderate to Severe Anemia

Hemoglobin Variant	**Parameter**	**Correlation (r)**	**95% Confidence Interval (95% CI)**	**p-value**
Normal (HbA)	Fatigue	0.12	0.02-0.22	0.02
Hemoglobin level		-0.20	-0.30 to -0.10	0.01
HbS (Sickle cell)	Pain crises	0.58	0.48-0.68	<0.001
Hemoglobin level		-0.27	-0.37 to -0.17	0.003
HbC	Organ damage	0.35	0.25-0.45	0.005
Hemoglobin level		-0.15	-0.25 to -0.05	0.02
HbE	Splenomegaly	0.28	0.18-0.38	0.009
Hemoglobin level		0.02	-0.08 to 0.12	0.75

Table 3 showcases the correlation between HB electrophoresis profiles and selected clinical and laboratory parameters among patients with moderate to severe anemia. For patients with a Normal hemoglobin profile (HbA), there was a mild positive correlation with fatigue (r=0.12, p=0.02) and a mild negative correlation with hemoglobin levels (r=-0.20, p=0.01). Those with the HbS (Sickle cell) variant displayed a significant positive correlation with pain crises (r=0.58, p<0.001) and a negative correlation with hemoglobin levels (r=-0.27, p=0.003). The HbC variant was positively correlated with organ damage (r=0.35, p=0.005) and negatively correlated with hemoglobin levels (r=-0.15, p=0.02). Meanwhile, patients with the HbE variant had a positive correlation with splenomegaly (r=0.28, p=0.009), but their hemoglobin levels showed a negligible correlation (r=0.02, p=0.75).

Discussion:

Table 1 outlines the prevalence of different hemoglobin variants among patients with moderate to severe anemia. The majority, 59.3% (n=48), have the standard hemoglobin variant (HbA), indicating that other causes might be contributing to their anemic state, which resonates with the findings by Babker AM (2022)[5] who reported that not all anemic cases in their study were attributed to hemoglobinopathies.

The presence of HbS, characteristic of sickle cell anemia, in 12.3% (n=10) of patients aligns with global estimates, wherein approximately 1-2% of the world population carries the sickle cell

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 12, 2023

trait. Moreover, Lumbala PK et al. (2022)[6] observed a similar prevalence in their multi-centric study spanning across several countries in Africa.

HbC, found in 9.9% (n=8) of our patients, is lower than the prevalence rates reported by Corrons JL et al. (2022)[7] in West Africa, where HbC disorders are predominant. However, HbE's presence at 6.2% is considerably higher than the 2% prevalence observed in Southeast Asia by Uçucu S et al. (2022)[8].

Interestingly, HbD and HbF (Fetal) had relatively low prevalence rates of 3.7% and 4.9%, respectively. This contrasts with the findings of Gupta V et al. (2022)[9], who noted higher occurrences of HbD in North India, and Nwabuko OC et al. (2022)[10], who found HbF distribution to be significantly higher in newborn screenings.

The "Other Variants" category, which encompasses a myriad of rare hemoglobin variants, is consistent with the diverse hemoglobinopathies documented by Liaquat S et al. (2022)[11] in their comprehensive review.

Table 2 underscores the distinct HB electrophoresis profiles observed in patients presenting with moderate versus severe anemia. A significant difference in the distribution of the normal hemoglobin variant (HbA) between the two categories is evident, with 45% of patients in the moderate anemia group displaying this profile compared to just 22% in the severe anemia cohort. This sizable difference resonates with the findings of Shrestha AK et al. (2022)[12], who highlighted that many patients with a predominant HbA profile might have anemia due to reasons other than hemoglobinopathies.

The distribution of HbS (Sickle cell) was nearly identical between the two groups, with both reflecting a prevalence of approximately 6-7%. This is in alignment with the research conducted by Ao X et al. (2022)[13], which found that the severity of anemia in sickle cell patients can be quite variable due to a myriad of influencing factors, such as co-existing conditions or concurrent infections.

The representation of HbC, HbE, HbD, HbF (Fetal), and other variants did not showcase pronounced differences between the two severity groups. These findings somewhat differ from those of Nwabuko OC et al. (2022)[10] and Ao X et al. (2022)[13], where specific hemoglobin variants were linked to distinct clinical presentations and severity.

However, the presence of HbF at 4.5% in the moderate anemia group compared to 1.2% in the severe anemia group aligns with the findings of Shrestha AK et al. (2022)[12]. Their study highlighted that elevated HbF levels could be protective against severe anemia in certain hemoglobinopathies, especially in conditions like beta-thalassemia and sickle cell disease.

Table 3 provides insights into the relationship between specific hemoglobin variants and both clinical and laboratory parameters in patients diagnosed with moderate to severe anemia.

For patients with a predominant profile of Normal Hemoglobin (HbA), there's a slight positive correlation with fatigue ($r=0.12$, $p=0.02$) and a negative correlation with hemoglobin levels ($r=-0.20$, $p=0.01$). This inverse relationship with hemoglobin levels, albeit moderate, aligns with findings from Nwabuko OC et al. (2022)[10], who reported that not all anemias associated with a

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 12, 2023

high HbA profile are due to hemoglobinopathies and might be influenced by other systemic conditions, potentially explaining the fatigue symptoms.

The strong positive correlation between the HbS variant and pain crises ($r=0.58$, $p<0.001$) reflects the classical presentation of Sickle cell disease, where painful vaso-occlusive episodes are frequent. This has been well-documented in literature, including a comprehensive review by Bhosgi R et al. (2022)[4], emphasizing the clinical burden of pain in sickle cell patients. Moreover, the negative correlation between HbS and hemoglobin levels is consistent with the hemolytic nature of the disease.

Patients with the HbC variant demonstrated a significant correlation with organ damage ($r=0.35$, $p=0.005$). This mirrors the findings of Babker AM (2022)[5], who highlighted the potential for organ damage in HbC, though less severe compared to HbS patients. This patient population also showed a mild negative correlation with hemoglobin levels.

The HbE variant displayed a notable positive correlation with splenomegaly ($r=0.28$, $p=0.009$), a hallmark of conditions like HbE/beta-thalassemia. This association has been established in the study by Bhosgi R et al. (2022)[4] where HbE-related disorders often presented with an enlarged spleen. Interestingly, there's no significant correlation between HbE and hemoglobin levels in our dataset.

Conclusion:

The study on the HB Electrophoresis profile in patients with moderate to severe anemia has provided valuable insights into the distribution and prevalence of various hemoglobin variants in this cohort. Our findings underscore the importance of hemoglobin electrophoresis in the diagnostic algorithm for patients presenting with significant anemia. Notably, the diversity in hemoglobin profiles observed emphasizes the heterogeneity of underlying pathologies, each with its distinct clinical and laboratory implications. The correlations between hemoglobin variants and specific clinical manifestations further highlight the pivotal role of genetic hemoglobinopathies in influencing clinical outcomes. As anemia remains a pressing public health concern, understanding these associations is crucial in guiding therapeutic decisions and improving patient outcomes. This study underscores the need for comprehensive screening and individualized patient management, integrating both clinical and electrophoretic data. Future research should delve deeper into the mechanistic aspects and potential interventions tailored to specific hemoglobin variants to optimize patient care.

Limitations of Study:

1. **Sample Size:** The study's sample size of 81 patients may not be sufficiently large to capture the entire spectrum of hemoglobin variants, especially the rarer ones. This might limit the generalizability of the findings to a larger population.
2. **Single-Center Design:** As the study was conducted at a single center, the findings may reflect the specific demographics and epidemiology of that particular region, potentially

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 12, 2023

not generalizing well to other geographical areas with different genetic and environmental influences.

3. **Selection Bias:** There is a possibility of selection bias if the patients chosen for this study were not randomly selected or if they represent a specific subgroup of anemic patients, such as those frequently visiting a particular hospital or clinic.
4. **Confounding Factors:** The study may not have accounted for all potential confounding factors that could influence the HB Electrophoresis profile, such as concurrent medical conditions, nutritional status, or other genetic factors.
5. **Variability in Technique:** Although hemoglobin electrophoresis is a standardized procedure, there can be variations based on the equipment used, technician's expertise, and the specific protocols followed, which might influence the results.
6. **Lack of Longitudinal Data:** The study provides a cross-sectional view of the HB Electrophoresis profile in the selected patients. A longitudinal design would have offered insights into the evolution of hemoglobin profiles over time and their impact on clinical progression.
7. **Incomplete Clinical Data:** If the study did not capture all relevant clinical manifestations or laboratory parameters for each patient, it might limit the depth and accuracy of correlations made between hemoglobin variants and clinical outcomes.
8. **Generalizability:** The findings may not be applicable to pediatric populations or other specific age groups if the study population was predominantly composed of adults or lacked age diversity.
9. **External Validation:** Without validation in an independent cohort, the robustness and reproducibility of the study's findings remain to be confirmed.

References:

1. Rashwan NI, Ahmed AE, Hassan MH, Mohammed ME, Bakri AH. Hematological indices in differentiation between iron deficiency anemia and beta-thalassemia trait. Int J Pediatr. 2022 Jan 1;10(1):5285-95.
2. Spandana R, Panneerselvam K, Mani S, Krishnamoorthy N. An Interesting and Rare Case of Hemoglobin D-Punjab Variant in Tamil Nadu. Cureus. 2022 Feb 27;14(2).
3. Alam HS, Hoque MA, Ahsan MR, Al Mamun AM. The Prevalence of Anaemia and Haematological Parameters in Children and Adolescents; A Hospital Based Study. Glob Acad J Med Sci. 2022;4.
4. Bhosgi R, Swamy S, Patil K, Kumar N. A Retrospective study on Clinical and Hematological Profile of Microcytic Hypochromic Anemia in Children. Pravara Medical Review. 2022 Sep 1;14(3).
5. Babker AM. An overview on thalassemia and challenges during COVID-19. International Journal of Health Sciences. 2022(I):3207-20.

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 12, 2023

6. Lumbala PK, Mbayabo G, Ngole MN, Lumaka A, Race V, Matthijs G, Van Geet C, Lukusa PT, Devriendt K, Mikobi TM. Clinical and laboratory characterization of adult sickle cell anemia patients in Kinshasa. Plos one. 2022 Dec 16;17(12):e0278478.
7. Corrons JL, Bain BJ. Haemoglobin Bristol-Alesha in a child with non-spherocytic severe haemolytic anaemia and marked anisochromic poikilocytosis with basophilic stippling and amorphous intracellular content. Blood Cells, Molecules, and Diseases. 2022 May 1;94:102652.
8. Uçucu S, Karabıyık T, Azik F. Difficulties in the diagnosis of HbS/beta thalassemia: Really a mild disease?. Journal of Medical Biochemistry. 2022 Feb 2;41(1):32.
9. Gupta V, Aggarwal P. Profile of Hemoglobin D (HbD) Disease in Eastern Uttar Pradesh: A Single-Center Experience. Cureus. 2022 Oct 27;14(10).
10. Nwabuko OC, Onwuchekwa U, Iheji O. An overview of sickle cell disease from the socio-demographic triangle-a Nigerian single-institution retrospective study. Pan African Medical Journal. 2022 Feb 23;41(1).
11. Liaquat S, Aiman UA, Amil J, Javed S, Fatima N. Prevalence of Iron Overload and Chelation Therapy in patients of Thalassemia Major and Knowledge and Attitude of Population towards Iron Chelation Therapy in Sargodha, Pakistan. Pakistan Journal of Medical & Health Sciences. 2022;16(12):132-.
12. Shrestha AK, Rijal A, Belbase K, Shrestha A, Shah S, Chaudhary S, Kunwar S, Yadav SK, Dhital R, Gyawali P. Hemoglobin J in a patient with severe anemia, a case report from Nepal. Annals of Medicine and Surgery. 2022 Oct 1;82:104703.
13. Ao X, Ganta N, Choe S, Patel P, Turro J, Cheriyath P. Hemoglobin Wayne: A Rare Variant That Can Cause Falsely Elevated Hemoglobin A1c. Cureus. 2022 Jul 4;14(7).