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Original Article

The Composition of Haemoglobin A_2 In Haemoglobin E Haemoglobinopathy by Capillary Electrophoresis

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ABSTRACT

Introduction*: Hemoglobin E (HbE) hemoglobinopathy is one of the most prevalent inherited hemoglobin disorders in Southeast Asia, including Bangladesh. Elevated hemoglobin A2 (HbA2) levels serve as an important diagnostic marker for distinguishing HbE-related conditions. This study aimed to analyze the composition of HbA2 in individuals with HbE hemoglobinopathy using capillary electrophoresis.* ***Methods & Materials****: A cross-sectional study was conducted on 50 participants clinically diagnosed with HbE hemoglobinopathy. Hemoglobin analysis was performed using the Sebia Capillarys 2 system. Data on HbA2 levels, anemia severity, and hemoglobinopathy subtypes were analyzed, and statistical significance was determined at p<0.05.* ***Results****: Elevated HbA2 levels were observed in 68% of participants, while 28% had normal levels, and 4% had below-normal levels (p=0.02). Mild anemia was the most prevalent (64%), followed by moderate (20%) and severe anemia (16%). Gender analysis revealed that moderate anemia was exclusive to females, while severe anemia was predominantly observed in males. HbE trait was the most common hemoglobinopathy type (84%), with HbE disease and HbE/β-thalassemia comprising 14% and 2%, respectively. Hemoglobin indices indicated microcytic and hypochromic anemia across the study population.* ***Conclusion****: Capillary electrophoresis is a robust diagnostic tool for quantifying HbA2 levels and characterizing HbE hemoglobinopathy. The high prevalence of elevated HbA2 highlights its significance in differentiating hemoglobinopathy subtypes, providing valuable insights for clinical management and screening programs in resource-limited settings.*

Keywords*: Hemoglobin E, Hemoglobin A2, Capillary Electrophoresis, Hemoglobinopathy, Anemia, Diagnostic Tools, Bangladesh*

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INTRODUCTION

Hemoglobinopathies are among the most prevalent inherited disorders worldwide, impacting millions of individuals and presenting significant challenges to global public health systems. Affecting approximately 7% of the world's population, hemoglobinopathies encompass a diverse range of conditions, including beta-thalassemias and hemoglobin variants such as hemoglobin E (HbE) [1]. HbE is particularly prominent in Southeast Asia, with prevalence rates of up to 30% in some populations, making it the second most common hemoglobin variant globally after hemoglobin S [2]. Within Bangladesh, a high-prevalence zone for HbE hemoglobinopathy, the carrier rate reaches 10-15% in certain regions, representing a significant public health concern [3]. Despite this, there remains a dearth of studies exploring the biochemical and clinical nuances of HbE in the context of hemoglobin A2 (HbA2), particularly through advanced diagnostic techniques like capillary electrophoresis. HbE hemoglobinopathy results from a single-point mutation in the beta-globin gene (Glu26Lys), leading to altered hemoglobin function and varying phenotypic presentations depending on the inheritance pattern [4]. In heterozygous states, individuals exhibit mild or asymptomatic phenotypes (HbE trait), while homozygous states and compound heterozygosity with beta-thalassemia (HbE/β-thalassemia) often result in more severe clinical manifestations requiring clinical intervention [5]. The diagnosis of HbE disorders relies on distinguishing them from other hemoglobinopathies, a process that is greatly aided by analyzing HbA2 levels, a secondary hemoglobin fraction normally constituting 2-3% of total hemoglobin [6]. Elevated HbA2 is a hallmark of beta-thalassemia carriers and can also be significant in the diagnosis of HbE-related conditions. However, the variability of HbA2 composition in HbE disorders, particularly within high-prevalence regions like Bangladesh, remains underexplored. Capillary electrophoresis has emerged as an advanced and reliable method for quantifying HbA2 levels, offering superior resolution and reproducibility compared to high-performance liquid chromatography (HPLC) and traditional cellulose acetate electrophoresis [7]. Its utility in diagnosing hemoglobinopathies has been demonstrated in various populations, but studies focusing on HbE hemoglobinopathy are sparse, particularly those addressing regional variations influenced by genetic and environmental factors [8]. Understanding these variations is critical for effective differential diagnosis, especially in resource-limited settings where precise and affordable diagnostic tools are essential. In Bangladesh, where the burden of HbE and beta-thalassemia is compounded by a lack of widespread screening programs, accurate diagnosis through tools like capillary electrophoresis is pivotal for improving patient outcomes and guiding public health initiatives [3]. Moreover, HbA2 plays a crucial role not

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only in diagnosis but also in genetic counseling and management strategies. Differentiating HbE disorders from beta-thalassemia and other hemoglobinopathies is essential to prevent misdiagnoses that could lead to inappropriate interventions or missed opportunities for preventive care. The inclusion of HbA2 analysis in screening protocols can aid in identifying at-risk individuals, offering opportunities for early intervention through genetic counseling, family planning, and informed clinical management [6]. Population-based screening programs, especially in regions with high carrier frequencies like Bangladesh, are essential for reducing the incidence of severe hemoglobinopathies. However, the implementation of such programs faces challenges due to limited diagnostic infrastructure and awareness [9]. This study seeks to address these gaps by analyzing the composition of HbA2 in patients with HbE hemoglobinopathy using capillary electrophoresis within the Bangladeshi population. By investigating the diagnostic utility and variability of HbA2 in HbE conditions, this research aims to contribute to the development of more accurate and resource-efficient diagnostic protocols. The findings will not only enhance the understanding of HbA2 dynamics in HbE hemoglobinopathy but also provide valuable insights for optimizing screening programs and clinical management in resource-constrained settings.

METHODS & MATERIALS

This cross-sectional observational study was conducted in the Department of Hematology at a tertiary care medical institution in Bangladesh from January to December 2022. The study aimed to analyze the composition of hemoglobin A2 (HbA2) in patients diagnosed with hemoglobin E (HbE) hemoglobinopathy using capillary electrophoresis. Ethical approval was obtained from the Institutional Ethics Committee, and informed consent was secured from all participants. A total of 50 participants were recruited based on inclusion criteria, which comprised individuals aged 18 years and older who were clinically and hematologically confirmed to have HbE hemoglobinopathy and who provided written consent. Exclusion criteria included individuals with chronic illnesses, mental disorders, or an inability to provide informed consent. Venous blood samples were collected from all participants into EDTA tubes and processed within four hours of collection to ensure sample integrity. Hemoglobin analysis was performed using the Sebia Capillarys 2 capillary electrophoresis system, following standard operating procedures to separate and quantify hemoglobin fractions, including HbA2 and HbE. Capillary electrophoresis was chosen for its high resolution and sensitivity, making it a reliable method for evaluating hemoglobin variants. The results were verified by two independent hematologists to ensure accuracy. Data analysis was conducted using SPSS (version 25.0), with continuous variables such as HbA2 levels expressed as mean ± standard deviation (SD) and categorical data presented as frequencies and percentages. Independent t-tests and one-way ANOVA were utilized to compare HbA2 levels across subgroups, with a significance threshold set at $p<0.05$. Quality control measures included system calibration and the use of control samples to ensure reproducibility and reliability, with 10% of samples tested in duplicate. The study followed ethical principles outlined in the Declaration of Helsinki, maintaining strict confidentiality of participant data and ensuring that participants could withdraw from the study at any point without repercussions.

RESULTS

Table – I: Distribution of baseline characteristics among the participants (*n*=50)

Baseline Characteristics	Number	Percentage
Gender		
Male	18	36%
Female	32	64%
Age		
2-9	10	20%
10-20	9	18%
21-30	9	18%
31-40	12	24%
41-50	6	12%
51-60	2	4%
>60	2	4%
Mean±SD	35.4±6.5	
Marital Statues		
Married	31	62%
Unmarried	19	38%

The baseline characteristics of the 50 participants are presented in Table I. The study population comprised a majority of females, with 64% (n=32), while males accounted for 36% (n=18). The age distribution revealed that the largest age group was 31-40 years, representing 24% (n=12) of participants, followed by 20% (n=10) in the 2-9 years age group. The 10-20 years and 21-30 years groups each contributed 18% (n=9), while the 41-50 years group accounted for 12% (n=6). Smaller proportions were observed in the 51-60 years and >60 years age groups, each constituting 4% (n=2). The mean age of participants was 35.4 years (SD±6.5). Regarding marital status, 62% (n=31) of participants were married, and 38% (n=19) were unmarried.

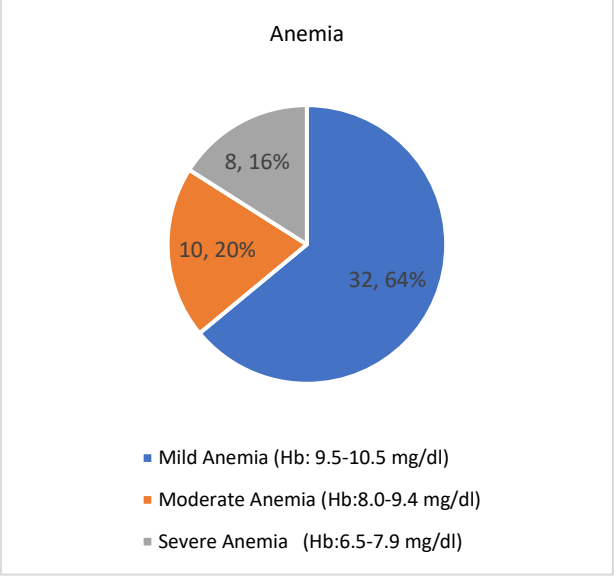

Figure – 1: Distribution of anemia among the participants (*n*=50)

The distribution of anemia among participants is illustrated in Figure 1. A majority of the participants, 64% (n=32), were classified as having mild anemia (Hb: 9.5–10.5 mg/dl). Moderate anemia (Hb: 8.0–9.4 mg/dl) was observed in 20%

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(n=10) of participants, while 16% (n=8) were categorized as having severe anemia (Hb: 6.5–7.9 mg/dl).

Table – II: Distribution of Anemia levels by gender in study population (*n*=50)

Sex	Mild Anemia (Hb: 9.5-10.5 mg/dl)		Moderate Anemia (Hb:8.0-9.4 mg/dl)		Severe Anemia (Hb:6.5-7.9 mg/dl)	
	n	%	n	%	n	%
Male	12	37.5%	0	0.0%	6	75.0%
Female	20	62.5%	10	100.0%	2	25.0%

The distribution of anemia levels by gender among the study population is presented in Table 2. Among participants with mild anemia (Hb: 9.5–10.5 mg/dl), females accounted for 62.5% (n=20), while males constituted 37.5% (n=12). All participants with moderate anemia (Hb: 8.0–9.4 mg/dl) were female (100%, n=10). Conversely, severe anemia (Hb: 6.5–7.9 mg/dl) was predominantly observed in males, who represented 75% (n=6) of this group, with females comprising only 25% (n=2).

Table – III: Haemoglobin and Red cell indices of study population (*n*=50)

Parameter	Mean (SD)	Median	Min-Max
Hb (g/dl)	09.71 (1.33)	10	6.5– 11.5
MCV (fl)	60.50 (4.74)	65.7	58.8 – 80
MCH (pg)	19.97 (2.07)	23	18 – 26

The hemoglobin and red cell indices of the study population are summarized in Table 3. The mean hemoglobin (Hb) level was 9.71 g/dl (SD ± 1.33), with a median value of 10 g/dl and a range from 6.5 to 11.5 g/dl. The mean corpuscular volume (MCV) had a mean value of 60.50 fl (SD ± 4.74), a median of 65.7 fl, and a range of 58.8 to 80 fl. The mean corpuscular hemoglobin (MCH) was 19.97 pg (SD ± 2.07), with a median of 23 pg and a range from 18 to 26 pg.

Table – IV: Distribution of the study population according to the type of haemoglobinopathy (*n*=50)

Type of the patient	Number	Percentage
Hb E trait	42	84%
Hb E disease	7	14%
Hb E/ β thalassemia	1	2%

The distribution of the study population according to the type of hemoglobinopathy is detailed in Table 4. The majority of participants, 84% (n=42), were diagnosed with Hb E trait, followed by 14% (n=7) with Hb E disease. Only 2% (n=1) of the participants were identified as having Hb E/β-thalassemia.

Table – V: Distribution of haemoglobin A2 level in study population (*n*=50)

Hemoglobin A2	Number	Percentage	p-value
Above Normal	34	68%	0.02
Normal	14	28%	
Below Normal	2	4%	

The distribution of hemoglobin A2 levels among the study population is presented in Table 5. A majority of the participants, 68% (n=34), had hemoglobin A2 levels above the normal range, while 28% (n=14) exhibited normal levels. A small proportion, 4% (n=2), had hemoglobin A2 levels below normal. The difference in hemoglobin A2 levels was statistically significant, with a p-value of 0.02, indicating a meaningful variation in hemoglobin A2 levels across the study population.

DISCUSSION

The present study aimed to analyze the composition of hemoglobin A2 (HbA2) in individuals with hemoglobin E (HbE) hemoglobinopathy using capillary electrophoresis, with a focus on identifying patterns and implications for diagnostic accuracy. Elevated HbA2 levels were observed in 68% of participants, a finding consistent with the role of HbA2 as a diagnostic marker for differentiating β-thalassemia carriers and other hemoglobinopathies. Previous studies have underscored the importance of HbA2 quantification, with Mosca et al. emphasizing that elevated HbA2 is a hallmark of β-thalassemia carriers, while HbE-associated conditions often exhibit variable HbA2 levels depending on genetic and environmental factors [1]. The statistically significant difference in HbA2 levels (p=0.02) highlights the sensitivity of capillary electrophoresis in identifying these variations. Comparatively, Prasing and Pornprasert demonstrated that HbA2 levels above 6.0% were effective in differentiating β-thalassemia/HbE from homozygous HbE, supporting the use of elevated HbA2 as a diagnostic tool [10]. Similarly, Singha et al. highlighted that regional genetic factors, such as the presence of α-thalassemia or δ-globin chain mutations, could influence HbA2 levels, potentially explaining the high prevalence of elevated HbA2 observed in this study [11]. These findings underscore the necessity of localized studies, as variations in genetic backgrounds may lead to diagnostic challenges in regions like Bangladesh. The gender-specific distribution of anemia in this study revealed a higher prevalence of moderate anemia among females and severe anemia among males. Such gender-based disparities align with findings from Adamu et al., who reported that women are disproportionately affected by anemia due to biological and socio-environmental factors, including nutritional deficiencies and healthcare access [12]. However, elevated HbA2 levels among females in this study may indicate underlying hemoglobinopathies, necessitating further investigation. In terms of diagnostic methodologies, capillary electrophoresis proved to be a highly effective tool for quantifying HbA2 levels, corroborating the findings of Yang et al., who demonstrated the reliability of capillary electrophoresis in identifying hemoglobin variants [13]. This technique's superior resolution compared to HPLC has been previously reported by Guo et al., making it particularly suitable for resource-limited settings like Bangladesh, where accurate and cost-effective diagnostic methods are critical [7]. Additionally, the distribution of hemoglobinopathy types in this study highlights the predominance of HbE trait (84%) compared to HbE disease (14%) and HbE/β-thalassemia (2%). These

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findings are consistent with the regional prevalence patterns described by Mondal and Mandal, who observed similar distributions in neighboring regions of Southeast Asia [14]. Such data reinforce the importance of targeted screening programs for HbE-related hemoglobinopathies to prevent misdiagnoses and ensure effective management. The clinical relevance of elevated HbA2 levels in HbE hemoglobinopathy cannot be overstated. Studies such as Higgins et al. have shown that accurate quantification of HbA2 is essential for distinguishing HbE hemoglobinopathy from other conditions with overlapping hematological profiles [15]. The use of capillary electrophoresis in this study provided robust and reproducible measurements, supporting its application as a standard diagnostic approach. In conclusion, this study adds to the growing body of evidence highlighting the diagnostic significance of HbA2 levels in hemoglobin E hemoglobinopathy. The use of capillary electrophoresis demonstrated its efficacy in accurately quantifying HbA2 and differentiating between various hemoglobinopathy types. Future research should focus on the interplay of genetic and environmental factors influencing HbA2 levels to optimize diagnostic strategies in regions with high hemoglobinopathy prevalence.

Limitations of The Study

The study was conducted in a single hospital with a small sample size. So, the results may not represent the whole community.

CONCLUSION

This study highlights the critical role of hemoglobin A2 (HbA2) in the diagnosis and characterization of hemoglobin E (HbE) hemoglobinopathy, using capillary electrophoresis as a precise and reliable diagnostic tool. The findings demonstrate a high prevalence of elevated HbA2 levels in the study population, underscoring its diagnostic significance in differentiating various hemoglobinopathy subtypes. The gender and severity distributions of anemia further emphasize the clinical heterogeneity of HbE-related conditions, with implications for targeted diagnostic and management strategies. By addressing a key gap in region-specific research, this study provides a foundation for improved screening programs and clinical interventions in resource-limited settings like Bangladesh. Future studies should explore the genetic and environmental factors influencing HbA2 variability to optimize diagnostic accuracy and therapeutic outcomes.

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