Original Article

COMPARISON OF CAPILLARY ELECTROPHORESIS AND CELLULOSE ACETATE ELECTROPHORESIS IN DIAGNOSIS OF BETA-THALASSEMIA TRAIT

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ABSTRACT

Objective: To determine correlation between mean HbA2 value for screening of β thalassemia trait by capillary electrophoresis and cellulose acetate electrophoresis.

Material and Methods: A cross-sectional study was undertaken at the Armed Forces Institute of Pathology Rawalpindi's Department of Hematology from September 2017 to February 2018. Cosent was taken from all patients or their immediate family members. All confirmed anemic patients with hemoglobin level below the normal limits for age and gender, MCV<75 fL and MCH <25 pg, belongs to either gender, aged 2 to 35 years, presented during the study duration were included. A written questionnaire was utilized to collect the patient information.

Results: Total 60 participants fulfilled the selection criteria according to G-Power analysis and included in the study. The mean age of (with age range 2-35 years) participants was 17.7±7.39 years. Majority of 33 (55.0%) participants were male. The mean hemoglobin of all patients was 11.06+_0.71fL and mean corpuscular volume was 67.84±5.87 fL, while mean corpuscular hemoglobin was 20.82±2.16 pg. Among all, 9 (15.0%) patients were diagnosed as β-thalassemia trait. The mean reticulocyte count was significantly higher in patients with β-thalassemia trait (2.5±0.85 vs. 1.99±0.68%). It was significantly higher in individuals with β-thalassemia trait on capillary (5.31±0.74 vs. 2.36±0.60%) and cellulose acetate (5.11±0.49 vs. 2.58±0.28%) electrophoresis.

Conclusion: Strong difference between hemoglobin A2 for screening of β thalassemia trait on capillary electrophoresis and cellulose acetate electrophoresis respective to patient's age and gender. Capillary Electrophoresis (CE) is similar to Cellulose Acetate (CA) for consistent extent of Hemoglobin (Hb) elements. Many clinical laboratories can use it to screen for hemoglobinopathies.

Key Words: Beta thalassemia, Hemoglobin A2, Capillary electrophoresis, Cellulose acetate electrophoresis.

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INTRODUCTION

One of the most frequent inherited illnesses is thalassemia disease which results from mutation or deletion of one or more of the globin gene [1]. Deficiency of α - chain production will lead to α - thalassemia whereas deficiency/decrease in β- globin production will result in β – thalassemia [2]. The disease is prevalent in tropical and sub-tropical regions including South East Asia, the Middle East, Africa, and the Indian Subcontinent [3]. In Pakistan, 5000-9000 children with -thalassemia are anticipated to be born each year, with a carrier rate of 5-7 percent and 9.8 million carriers [4].

Early detection of thalassemia carrier is the key to its prevention. Screening of carriers is important to provide prenatal genetic counseling and to assess the prevalence of the disease. Morbidity and mortality associated with thalassemia can be markedly reduced with the start of the proper treatment at an early age. The gold standard for thalassemia diagnosis is PCR, however, for identification purposes, electrophoresis is being done by various methods worldwide. The cut off value of HbA2 for thalassemia trait is more than 3.5% [5].

Capillary electrophoresis is the way of selection for identifying of thalassemia trait worldwide but it is available only in few centers in Pakistan as it requires expensive machines and skilled technical staff. For mass screening of β thalassemia trait in Pakistan, Cellulose acetate electrophoresis is being used. This method is widely available, cost effective, semi-automated and easy to perform. In this study, an effort is being made to correlate cellulose acetate electrophoresis with capillary electrophoresis for screening of thalassemia trait. Scarce data related to utilization of this method in Pakistan. This study is designed with an objective to determine correlation between mean HbA2 value for screening of β

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thalassemia trait by capillary electrophoresis and cellulose acetate electrophoresis [6].

RATIONALE

β-thalassemia is one of the most common genetic abnormalities in Pakistan. Since 1994, many efforts have been made in Pakistan to suppress the thalassemic outbreak. Despite this, there is no significant reduction in the number of children born to transfusion-dependent -thalassemia cases. It is critical to evaluate the -thalassemia trait (BTT) using a specific, low-cost, and precise mechanism of diagnosis in order to get beneficial outcomes from a nationwide thalassemia eradication effort. As a result, the current study used Capillary Zone Electrophore and Cellulose Acetate Hemoglobin Electrophoresis to determine the levels of HbA2 in samples.

MATERIAL AND METHODS

The cross-sectional research was directed after the approval through the principled committee of Pakistan armed forces institute Rawalpindi at Department of Hematology with IRB Number ERC/ID/14. Permission was taken from all patients or their immediate family members. All confirmed anemic patients with hemoglobin level below the normal limits for age and gender, MCV<75 fL and MCH <25 pg, belongs to either gender, aged 2 to 35 years, presented during the study duration were included (7). A pre-designed written proforma was used to collect information of all the patients.

Under supervision of senior pathologist, 5 ml venous blood in EDTA tube was collected. Blood CP was performed on Sysmex KX-21 automated hematology blood-stained steamer was examined for DLC and morphology. The inclusion criteria were strictly followed to control confounding variables and bias in the study results. The demographic and clinical data of all the patients including age, gender, occupation, residence, Hb value, MCV, MCH, reticulocyte count, serum ferritin levels was recorded in the proforma. Samples were then tested on acetate cellulose electrophoresis and capillary electrophoresis machines for estimation of HbA2.

SPSS version 19 was used to enter all of the gathered data. Numerical variables; age, hemoglobin concentration, reticulocyte count, MCV, MCH, serum ferritin levels and HbA2 value by both the techniques have been presented by descriptive statistical analysis (mean & standard deviation). Variable that is categorical in nature i.e., gender has been reported as a percentage and frequency. t has been calculated as a difference between HbA2 by capillary and cellulose acetate electrophoresis. To account for effect modifiers, data was stratified by age and gender. A post-stratification correlation was used, with a p value of 0.05 considered significant.

RESULTS

Majority of the patient's mean age was 17.70±7.39 years. In the study group, there were 33 (55.0%) male patients and 27 (45.0%) female patients, for a male to female ratio of 1.2:1. The patients' haemoglobin levels varied from 8.9 to 11.9 g/dl, with a mean of 11.060.71 g/dl while the reticulocyte count ranged from 1% to 3% with a mean of 2.0±0.69%. The mean corpuscular volume (MCV) ranged from 49.3 fL. to 74 fL with a mean of 67.84±5.87 fL, while the mean corpuscular hemoglobin (MCH) ranged from 13.9 pg to 24 pg with a mean of 20.28±2.16 pg. Serum ferritin level ranged from 30µg/L to 110µg/L. Red cell distribution width (RDW) ranged from 30 fL to 52 fL with a mean of 40.41±4.73 as revealed in Table-I.

Table-I: Characteristics of the Study Population at the Outset (n=60).

Characteristics	Participants
Age (years)	17.70±7.39
Gender	
Male	33 (55.0%)
Female	27 (45.0%)
Hemoglobin (g/dl)	11.06±0.71
Reticulocytes (%)	2.00±0.69
MCV (fL)	67.84±5.87
MCH (pg)	20.28±2.16
RDW (fL)	40.41±4.73
Serum Ferritin (µg/L)	30-110

In this study, mostly females (55%) were diagnosed with β-thalassemia trait (Table-II).

Table-II: Frequency of β-thalassemia trait (n=60).

β-Thalassemia Trait	Frequency (n)	Percent (%)
Yes	09	15.0
No	51	85.0
Total	60	100

Mean score of majorities of the patient's age range was 21.68 who diagnosed with β- thalassemia trait except for reticulocyte count which was significantly higher in patients with β- thalassemia trait (2.5±0.85 vs. 1.9 ±0.68%) as shown in Table-III.

Table-III: Parameters of RBCs are compared between Normal and B Thalassemia Trait Patients (n=60)

β-Thalassemia trait		
Age	Yes	No
	21.68±6.2	16.35±7.32
Gender		
Male	3	30
Female	6	21
Hemoglobin (g/dl)	11.37±0.43	10.95±0.75
Reticulocytes (%)	2.5±0.85	1.99 ± 0.68

MCV (fL)	59.08±4.78	70.76±2.22
MCH (pg)	18.06±2.48	21.02±1.47
RDW (fL)	34.61±2.42	42.34±3.67
Serum Ferritin (µg/dl)	20.47 ± 4.6	6.69 ±3.25

The HbA2 level ranged from 1.5% to 6.6% on capillary electrophoresis with a mean of 3.1±1.42%. It was significantly higher in individuals with β-thalassemia trait (5.31±0.74 vs. 2.36±0.60%). On cellulose acetate electrophoresis HbA2 level ranged from 2% to 5.5% with a mean of 3.21±1.15%. It was significantly higher in individuals with β-thalassemia trait (5.11±0.49 vs. 2.58±0.28%) as shown in Table-IV.

Table-IV: Comparison of Mean HbA2 on Capillary Electrophoresis and Cellulose Acetate Electrophoresis between Individuals with and without β-Thalassemia Trait (n=60)

β-Thalassemia Trait		
Electrophoresis	Yes (n=9)	No (n=51)
Capillary	5.31±0.74	2.36±0.60
Cellulose acetate	5.11±0.49	2.58±0.28
P-value	0.102	0.008*

There was significant difference between mean HbA2 on capillary electrophoresis and cellulose acetate electrophoresis (3.1±1.42% vs. 3.21±1.15%; p=0.63; r=0.91) as shown in Table-V

Table-V: Difference between capillary and cellulose acetate electrophoresis over HbA2 (n=60).

Technique	**Hemoglobin A2 (HbA2)**	**P-value**	**Correlation Coefficient**
Capillary Electrophoresis	3.1±1.42%	0.63	0.91
Cellulose Acetate Electrophoresis	3.21±1.15%		

DISCUSSION

Analysis of erythrocyte indices and morphology, as well as quantification of HbA2 using traditional methods such as HPLC, electrophoresis, and microcolumn techniques, can be used to diagnose -thalassemia [8]. HbA2 quantification is difficult since its level is low and only slightly increases in disease, and other Hb variations frequently interfere with its assessment [9]. CA is commonly used; however, it is ineffective. HPLC is the preferred procedure, although it is expensive and not widely available. Previous research has demonstrated that the newly developed CE effectively distinguishes HbA2 from HbE, HbC, and HbS, with CV levels adequate for screening [9]. Excellent efficiency (many samples can be performed in parallel), high precision, and full automation are all advantages of this method.

The CBC as a diagnostic tool for hemolytic Thalassemia Trait (TT) was previously found to have lower mean values of blood parameters such as RBC, Hb, and mean corpuscular haemoglobin in individuals with TT when compared to the control group [10]. In thalassemia, oxidative stress in erythrocytes accelerates cell breakdown, resulting in a lack of oxygen delivery. The average Hb level in normal controls was 139 g/L in a recent study. The mean Hb and HbA levels in individuals with TT and Thalassemia Disease (TD) were 112 g/L and 86 g/L, respectively. Based on other blood characteristics, such as HbA, HbA2, and HbS, Hb electrophoresis is effective in identifying thalassemia and sickle cell disease [11].

CA patterns were more difficult to decipher than CE patterns. While the latter requires technical knowledge, the former offers objective results as well as a variety of settings for interpretation, recording, and archiving. The existence of HbA, on the other hand, is required for CE to get suitable zones for confirming the identity of the Hb [12].

The use of cellulose acetate at an alkaline pH is a feasible approach for routine Hb electrophoresis. It distinguishes Hbs A, F, S, C, and A2 in a quick and repeatable manner. The use of acid pH citrate agar electrophoresis allows for the separation of many common types that move together on cellulose acetate: S from D and G, and C from E and O [13]. Nonetheless, because it is slow and difficult to standardise, citrate agar electrophoresis is not extensively employed [14]. The agar plates must be produced fresh, and due to the citrate agar's comparatively high electrical resistance, electrophoretic mobilities are typically inconstant, resulting in varied effects on the Hb [14].

The mean value of HbA in controls was 97 percent in earlier research, but it was much lower in individuals with thalassemia mild and major [15]. Furthermore, the control group's mean HbA2 level was 2.62. A mean score of more than 3 but less than 5 suggests borderline disease, while a score of more than 5 implies disease [16].

AUTHOR CONTRIBUTION

Aqsa Noreen: Literature search and write up.
Irfan Ahmed: Data interpretation.
Aneela Faisal: Data analysis.
Sadia Ali & Faiqa Aslam: Data collection
Altaf Hussain Chowdhary: Study design and proof reading.

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