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Determination of Mean HbA2 Levels on High-Performance Liquid Chromatography in Known β Thalassaemia Trait Individuals

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Abstract

Background: Pakistan bears the largest burden of thalassemia affecting 5-7% of its population. Patients with aberrations in the beta globin chain are beta thalassemics which are further classified as trait, intermedia and major. HPLC is considered as a technique of choice for screening thalassemias due to its reliability, speed and sensitivity.
Objective: The present study was conducted to determine mean HbA2 levels of known β thalassaemia trait individuals on high performance liquid chromatography.
Methodology: A descriptive cross-sectional study was conducted from November 2018 to April 2019. Thirty patients who were diagnosed as β thalassaemia trait on cellulose acetate hemoglobin electrophoresis were included in the study. The blood samples were run-on High-Performance Liquid Chromatography (HPLC) and HbA2 levels were recorded on the proforma. Mean and standard deviation were calculated for HbA2, MCV and MCH. Frequency and percentage were calculated for gender. Effect modifiers like age and gender were controlled by stratification and post stratification independent sample t test was applied.
Results: Mean HbA2 levels of βthalassaemia trait individuals on HPLC was 5.63%. There was no effect of age (p=0.07) and gender (p=0.14) on mean HbA2 levels. Mean Hb was 9.6g/dl. Mean corpuscular volume (MCV) and Mean corpuscular haemoglobin (MCH) were reduced with mean value of 56.7 fl and 18.5 pg respectively.
Conclusion: HPLC is a fast, precise, and reliable method for the early detection and management of hemoglobinopathies and their variants especially beta thalasemia trait. Raising awareness about thalassemia and its relatively simple prevention is crucial for the success of a thalassemia control program.
Keywords: β thalassaemia trait, high performance liquid chromatography (HPLC), hemoglobin electrophoresis

Introduction

Beta thalassaemia is one of the most common inherited haemoglobin disorders which is characterised by anaemia and lifelong transfusion dependency.[1] There is a variation in the prevalence of haemoglobinopathies in different regions and population groups in the world[2]. Beta thalassaemia exists in 5% of our population as heterozygous state.[3] Beta-thalassemia is caused by the reduced or absent synthesis of the beta globin chains of the haemoglobin molecule. Three haematological and clinical conditions of increasing severity are recognized, i.e., the beta-thalaessemia trait, thalassaemia intermedia, and thalassaemia major. The beta-thalassemia carrier state, which results from heterozygosity for beta-thalassaemia, is clinically asymptomatic but has well recognised haematological features in blood CP.[4]

Individuals with thalassemia major have severe anaemia along with hepatosplenomegaly and they are usually diagnosed within the first two years of life. If untreated, affected children have severe anaemia, failure to thrive and a shortened life expectancy.[5] Haemopoietic stem cell transplant is the only curative treatment for thalassaemia major which is not available to most of the patients in Pakistan.[6]

Thalassemia intermedia is a clinical condition that falls between thalassemia trait and thalassemia major in terms of severity. It is characterized by mild to moderate anemia, which typically does not require regular blood transfusion support.[7]

electrophoresis. However, cation-exchange HPLC has now been developed for both screening and confirmation of hemoglobinopathies, offering relatively high sensitivity and specificity. HPLC is considered the method of choice for quantifying various hemoglobins due to its ease of use, strong analytical performance, and the availability of fully automated instruments. The reliable measurement of HbA2 by HPLC

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for detecting beta-thalassemia trait, without producing false positive or false negative results, is particularly advantageous. HPLC is an ideal method for rapid screening in population surveys to identify beta-thalassemia and hemoglobin variant carriers, and for preventing births of children with thalassemia major through genetic counseling.[10]

Methodology

A descriptive cross-sectional study was conducted in the Department of Hematology at Fauji Foundation Hospital, Rawalpindi, from November 2018 to April 2019. Ethical approval was obtained from the hospital's ethical committee, and informed consent was secured from all participants. The study population consisted of 30 diagnosed β-thalassemia trait individuals of both genders, aged 5 to 50 years. All participants had HbA2 levels greater than 3.5% as determined by cellulose acetate electrophoresis. Individuals with iron deficiency anemia were excluded based on RBC indices and low serum ferritin levels.

Venous blood samples (2.5 mL) were collected using EDTA as an anticoagulant. Complete blood counts (CBC) were performed using a Sysmex XT 2000i hematology analyzer, with hemoglobin (Hb), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) values recorded. Whole blood samples were also analyzed using high-performance liquid chromatography (HPLC) on a Bio-Rad D10 system to record HbA2 levels.

All collected data were entered into SPSS version 17 for analysis. The data included quantitative variables such as age, HbA2, MCV, and MCH. Mean and standard deviation were calculated for these quantitative variables. To control for effect modifiers such as age and gender, stratification was performed. Post-stratification, an independent sample t-test was applied, with a p-value of < 0.05 considered statistically significant.

Results

A total of 30 individuals diagnosed with β-thalassemia trait were enrolled in the study. The age of the participants ranged from 5 to 50 years, with a mean age of 25.5 years. The following laboratory parameters were analyzed:

- Hemoglobin (Hb) levels ranged from 6.7 g/dl to 11.5 g/dl, with a mean of 9.6 g/dl.
- Mean corpuscular volume (MCV) was reduced, ranging from 48.4 fl to 68.3 fl, with a mean value of 56.7 fl.
- Mean corpuscular hemoglobin (MCH) ranged from 15.7 pg to 22.6 pg, with a mean value of 18.5 pg.

These findings are summarized in Table I. HPLC was performed for HbA2 estimation in all patients, showing elevated HbA2 levels in every case. The HbA2 values ranged from 4.9% to 6.7%, with a mean of 5.63%.

Table I: Mean and standard deviation of age, hemoglobin, MCV and MCH. (N=30)

	Min	Max	Mean	SD
Age (years)	5	50	25.5	17.90
Haemoglobin (g/dl)	6.7	11.5	9.6	1.29
MCV (fl)	48.4	68.3	56.7	4.62
MCH (pg)	15.7	22.6	18.5	1.70

Effect modifiers like age and gender were stratified with HbA2 levels. Post stratification t-test was applied and p-value was calculated, however it was not significant (p value=0.07 and p-value= 0.14 respectively) as shown in Table II.

Table II: Age and Gender stratification with HbA2.

	age groups	N	Mean	SD	p-value
HbA2	< 12	6	5.30	0.52	0.07
	> 12	24	5.71	0.48	
	male	8	5.86	0.63	0.14
	female	22	5.55	0.44	

Discussion

Thalassemia is the most common inherited disorder all around the world. The carrier rate is about 5-8% in Pakistan.[11] Prevention of the disease is perhaps the most practical approach to control the increasing burden of the disease. There should be a program at the national level for screening of β thalassaemia trait individuals. However, screening at national level is not cost effective for a country like Pakistan so the best strategy is to screen the pregnant women and their partners. With this approach we can identify the carriers and the pregnancies at risk and offer preventive measures to them.[6,12] This study was conducted on 30 cases to evaluate the mean HbA2 levels of individuals with a known β-thalassemia trait using high-performance liquid chromatography (HPLC). The overall mean age of the participants was 25.5 years, with a mean hemoglobin (Hb) level of 9.6 g/dl. In aligns with our findings, Babaria SS et al. (reference 14) also reported that most cases were within the age group of 13–36 years, with a mean hemoglobin level of 9.4 ± 2.5 g/dl.

There are various screening methods for the detection of hemoglobinopathies, including cellulose acetate electrophoresis, isoelectric focusing, high-performance liquid chromatography (HPLC), and capillary zone electrophoresis. Among these, HPLC is considered one of the best methods for screening and detecting various hemoglobinopathies due to its rapid, reproducible, and precise results. HPLC is particularly recommended for detecting the β-thalassemia trait in populations, which is essential for genetic counseling to reduce the incidence and burden of thalassemia major in society.[13] Supporting evidence from the literature aligns with our findings. Babaria SS et al[14] conducted a study in India analyzing 500 blood samples using the BIO-RAD D-10™ HPLC system. They found that 69 patients had a β-

thalassemia trait, with an HbA2 level of 5.4 ± 0.9%. Similarly, Baig MA et al[15] conducted a study in 2019 in Saudi Arabia on 579 cases of hemoglobinopathies and thalassemic syndromes, in which 62 cases were found to have the thalassemia trait with an HbA2 level of 5.1 ± 1.1% using HPLC. These findings are consistent with the results of our study, further justifying the use of HPLC for the accurate detection of β-thalassemia trait.

Another study conducted by Mahajanin 2022 in India included102 adult patients with anemia suspected of haemoglobinopathy. The distribution of patients according to abnormal Hb & thalassemic trait showed that 81 (79.4%) of patients were having a normal Hb while 17 (16.7%) patients showed the prevalence of thalassemia trait with mean value of Hb and HbA2 as 9.15 and 5.49%. respectively.[16]

Studies have been conducted worldwide to determine the percentage of HbA2 in individuals with β-thalassemia trait using HPLC. Fabella et al. conducted a study in the Philippines on 622 patients, of whom 181 were diagnosed with thalassemia. Among these, 65 subjects (10.45%) were identified with the β-thalassemia trait. In this group, HbA2 levels ranged from 3.6% to 8%, which is notably broader than the ranges reported in other studies.[9] Colaco et al. conducted a study assessing the diagnostic value of borderline HbA2 levels (3.2% to 3.9%) in identifying individuals with β-thalassemia trait using HPLC. The study highlighted that these cases were often identified only after the individuals had given birth.[17] However, none of the β-thalassemia trait individuals in our study fell into this borderline HbA2 category. Cases of borderline HbA_2 levels have also been documented in Middle Eastern populations and in Pakistan, which may be attributed to inter-country adoptions and inter-racial marriages, leading to the spread of gene defects.[17]

Mukhopadhyay *et al* conducted a study on the HPLC based analyzer in which 1458 healthy antenatal mothers It was observed that all the RBC parameters including mean HB, mean RBC, mean packed cell volume, mean MCV, and mean MCH were significantly lower among the Beta thalasemia trait when compared with the normal population. However its results were in accordance with our study except for the mean value of HbA2 which is higher in this study MEAN 7.8%.[18]

Although elevated HbA2 levels are diagnostic for β-thalassemia trait, hemoglobin levels, MCV (mean corpuscular volume), and MCH (mean corpuscular hemoglobin) also play significant roles in diagnosing β-thalassemia trait, providing important clues. Hemoglobin levels in individuals with β-thalassemia trait are typically normal or slightly below normal, while MCV and MCH values are disproportionately low relative to the degree of anemia. In our study, the mean values for Hb, MCV, and MCH were 10.0 g/dl, 67.8 fl, and 20.2 pg, respectively, indicating a reduction in these parameters. This aligns with findings from other studies on β-thalassemia trait, which also reported decreased Hb, MCV, and MCH, thereby validating our results.

However, this study has limitations, including a relatively small sample size, which may not fully represent the broader population. Additionally, it did not account for other hemoglobinopathies that may present with similar hematological profiles, potentially affecting the specificity of the findings. Further large-scale studies are recommended to include a broader range of hemoglobinopathies, thereby enhancing the generalizability and specificity of the results. Incorporating genetic analysis could also improve diagnostic accuracy for β-thalassemia and provide better insights into the clinical spectrum of the trait.

Moreover, expanding screening programs using HPLC for early identification of carriers and providing appropriate genetic counseling is crucial. This approach could significantly reduce the burden of thalassemia major in the community.

Conclusion

A key feature of beta-thalassemia trait is an elevated level of HbA2, which is best measured using automated HPLC. Early detection of thalassemia traits is crucial in preventing the birth of children with thalassemia major. Routine premarital screening is essential to avoid high-risk marriages, especially given the prevalence of hemoglobin abnormalities. A sustained effort in education and awareness is necessary to reduce the suffering associated with repeated blood transfusions and related mortality.

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