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Determination of Beta Thalassemia trait and abnormal Hemoglobin variant frequency

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ABSTRACT

Introduction: Hemoglobinopathies are among the most common hereditary disorders globally, classified into thalassemias and abnormal hemoglobins. Abnormal hemoglobins arise from genetic alterations like point mutations, insertions, or deletions, with Hb S, Hb D, Hb C, and Hb E being the most prevalent in Turkey. Beta-thalassemia minor is characterized by reduced or absent synthesis of one beta-globin chain. High-performance liquid chromatography (HPLC) is widely used for screening, while genetic methods remain the gold standard for definitive diagnosis. The estimated frequency of beta-thalassemia traits in Turkey is 2–3%, and abnormal hemoglobins 0.95%. This study aimed to determine the frequency of beta-thalassemia traits and abnormal hemoglobins in a central laboratory.

Material and methods: Retrospective data from hemoglobin variant analyses conducted between November 1, 2020, and July 31, 2024, in the hospital's central laboratory were collected via the Laboratory Information Management System (LIMS). Samples from patients aged 6 months to 99 years were included. Repeated samples and those from infants under 6 months (with high HbF) were excluded. Frequencies of Hb S, D, C, and E were calculated as a percentage of total tested samples. HbA2 > 3.5% was used to identify beta-thalassemia traits. Analyses were performed using a cation-exchange HPLC-based Adams HA-8180T system.

Results: A total of 223,609 samples were analyzed. Abnormal hemoglobins were found in 0.35% (800 cases): Hb S (0.15%), Hb D (0.13%), Hb C (0.05%), and Hb E (0.01%). Beta-thalassemia traits (HbA2 ≥ 3.6%) were found in 6,017 cases (2.69%).

Conclusions: Hb S was the most frequent abnormal variant. The frequency of abnormal hemoglobins was lower than literature reports, while beta-thalassemia traits matched reported rates. HPLC is valuable for screening, but genetic testing remains essential for definitive diagnosis. These findings contribute to better prediction of hemoglobinopathy prevalence.

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Determinación del rasgo de beta talasemia y la frecuencia de variantes anormales de hemoglobina

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RESUMEN

Introducción: Las hemoglobinopatías se encuentran entre los trastornos hereditarios más comunes a nivel mundial y se clasifican en talasemias y hemoglobinas anormales. Las hemoglobinas anormales se originan por alteraciones genéticas como mutaciones puntuales, inserciones o deleciones, siendo las Hb S, Hb D, Hb C y Hb E las más prevalentes en Turquía. La beta-talasemia menor se caracteriza por la síntesis reducida o ausente de una cadena de beta-globina. La cromatografía líquida de alta resolución (HPLC) se utiliza ampliamente para el cribado, mientras que los métodos genéticos siguen siendo el método de referencia para el diagnóstico definitivo. La frecuencia estimada de rasgos de beta-talasemia en Turquía es del 2-3%, y la de hemoglobinas anormales, del 0,95%. Este estudio tuvo como objetivo determinar la frecuencia de rasgos de beta-talasemia y hemoglobinas anormales en un laboratorio central.
Material y métodos: Se recopilaron datos retrospectivos de los análisis de variantes de hemoglobina realizados entre el 1 de noviembre de 2020 y el 31 de julio de 2024 en el laboratorio central del hospital mediante el Sistema de Gestión de Información de Laboratorio (LIMS). Se incluyeron muestras de pacientes de entre 6 meses y 99 años. Se excluyeron las muestras repetidas y las de lactantes menores de 6 meses (con HbF elevada). Se calcularon las frecuencias de Hb S, D, C y E como porcentaje del total de muestras analizadas. Se utilizó una HbA2 > 3,5% para identificar rasgos de beta-talasemia. Los análisis se realizaron con un sistema Adams HA-8180T basado en HPLC de intercambio catiónico.
Resultados: Se analizaron un total de 223.609 muestras. Se encontraron hemoglobinas anormales en el 0,35 % (800 casos): Hb S (0,15 %), Hb D (0,13 %), Hb C (0,05 %) y Hb E (0,01 %). Se encontraron rasgos de beta-talasemia (HbA2 ≥ 3,6 %) en 6017 casos (2,69 %).
Conclusiones: La Hb S fue la variante anormal más frecuente. La frecuencia de hemoglobinas anormales fue inferior a la descrita en la literatura, mientras que los rasgos de beta-talasemia coincidieron con las tasas reportadas. La HPLC es valiosa para el cribado, pero las pruebas genéticas siguen siendo esenciales para el diagnóstico definitivo. Estos hallazgos contribuyen a una mejor predicción de la prevalencia de hemoglobinopatías.

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1. INTRODUCTION

Hemoglobinopathies, genetic diseases related to hemoglobin synthesis, constitute the most common monogenic disorders worldwide. The genetic cause of this group of diseases is variants in the globin genes that encode the globin chains of the tetrameric hemoglobin protein. These DNA variants can lead to altered synthesis of α- or β-globin (α- and β-thalassemia syndromes, respectively) or to structural changes in hemoglobin, causing diseases such as sickle cell disease, hemolytic anemia, erythrocytosis, or polycythemia [1]. Approximately 7% of the world's population trait a DNA variant that causes a defect in hemoglobin synthesis, resulting in approximately 300,000 to 400,000 affected newborns; the majority of these have sickle cell syndrome and a small proportion have transfusion-dependent β-thalassemia major [2].

Abnormal hemoglobins are formed because of gene mutations such as point mutations, nucleotide additions, and nucleotide deletions. More than 90% of known abnormal hemoglobin types are caused by a single amino acid change in a globin chain, and 60% of these occur in the beta globin chain of hemoglobin [3]. Hemoglobin S, Hemoglobin D, Hemoglobin C, and Hemoglobin E are the most common pathological hemoglobins in Turkey in order of frequency [4]. Currently, hemoglobin variant analysis is primarily performed with HPLC (high-performance liquid chromatography) analysis. In clinical laboratories for hemoglobinopathies, capillary electrophoresis, classical electrophoresis, and genetic methods are also used in analysis [5].

There are two β-globin genes in the genome of each individual, one on each homologous chromosome on

chromosome 11. If there is one faulty β-globin gene, β-thalassemia minor or β-thalassemia trait occurs, and if both genes are faulty, β-thalassemia major disease occurs. Laboratory findings of β-thalassemia minor Hb electrophoresis shows an increase in HbA2 from 3.5% to 7% in more than 90% of cases, and this finding is diagnostic [6]. Although clinicians easily recognize severe forms of hemoglobinopathies, traits can be easily overlooked. Traits are usually asymptomatic and are only detected incidentally during family analysis due to an affected family member, during participation in hemoglobinopathy screening programs, or during routine hematological or biochemical analysis such as diabetes-related HbA1c analysis by HPLC or capillary electrophoresis. Molecular analysis of globin genes will support the definitive diagnosis of patients, traits, and those presenting with atypical hematological parameters [7, 8].

Studies have found the frequency of beta thalassemia traits in Turkey to be approximately 2.1% [9, 10] and the frequency of abnormal hemoglobin to be 1% [2]. In our study, we aimed to contribute to the literature by determining the frequency of beta thalassemia traits and abnormal hemoglobin variants in our central laboratory.

Table 1: Abnormal Hb variants that we screened with high-performance liquid chromatography method and genetic mutation

Hb S: β6 Glu→Val (GAG→GTG)
Hb D: β121 Glu→Gln (GAA→CAA)
Hb C: β6 Glu→Lys (GAG→AAG)
Hb E: β 26 Glu→Lys]

2. MATERIAL AND METHODS

The study was approved by the University of Healty Science, clinical research ethics committee (no: 13/13, date: 14.11.2024).

In our study, hemoglobin variant analysis data performed in our hospital's central laboratory between November 1, 2020 and July 31, 2024 were retrospectively obtained from the Laboratory information management system (LIMS). Samples of patients of both genders between the ages of 6 months and 99 years were included in the study. Samples of the same patient at different times and samples of patients aged 0-6 months whose HbF levels had not returned to normal were not included in the study. The number of abnormal hemoglobins for Hemoglobin S, D, C and E in the specified time period was determined as a percentage by dividing it into the total number of patients who underwent hemoglobin variant analysis. Those with $HbA_2 > 3.5\%$ were considered to be beta thalassemia traits [10, 11]. Hemoglobin variant analysis was performed using a cation exchange HPLC-based Adams HA-8180T (Arkray, Inc., Japan) device.

3. RESULTS

The number of samples studied for hemoglobin variant analysis was 223,609. In our study, we examined the frequency of 4 variant types, namely Hemoglobin S, D, C and E (Table 1). Abnormal hemoglobin variants such as Hemoglobin S, D, C and E were detected in 0.35% of the samples included in the study (800 cases). Of these, Hb S (0.15%) was detected in 356 cases, Hb D (0.13%) in 302 cases, Hb C (0.05%) in 117 cases and Hb E (0.01%) in 25 cases. The HbA2 level of 6017 patients was 3.6 and above. We determined the frequency of beta thalassemia traits as 2.69% (Table 2).

4. DISCUSSION

Hemoglobinopathies are blood diseases caused by structural changes or synthesis disorders in the polypeptide chains of the hemoglobin molecule and are examined in two parts as thalassemia and abnormal hemoglobins [12]. In our study, we examined the abnormal hemoglobins and patients with HbA_2 >3.5 from the laboratory information system to determine the frequency of beta-thalassemia traits and abnormal hemoglobins in our central laboratory.

In our study, 0.35% (800 cases) of the 223,609 hemoglobin variant analysis samples we screened in our central laboratory were detected to have abnormal hemoglobin variants such as Hemoglobin S, D, C and E. Of these, Hb S was detected in 356 patients (0.15%), Hb D in 302 cases

Table 2: Beta thalassemia trait and abnormal hemoglobin variant frequency (%)

Parameter	Number of patients (%)	Total number of patients screened
Hb S + Hb D +Hb C +Hb E	800 (0.35)	223.609
Hb S	356 (0.15)	223.609
Hb D	302 (0.13)	223.609
Hb C	117 (0.05)	223.609
Hb E	25 (0.01)	223.609
HbA2 > %3.5	6017 (2.69)	223.609

(0.13%), Hb C in 117 cases (0.05%) and Hb E in 25 cases (0.01%). We have reviewed various studies on the subject. Among these, Dikker et al. [4] analyzed 1894 cases with HPLC in their laboratory and found abnormal hemoglobin variants in 0.95% (18 cases). They observed Hb S most frequently and then HbD variant more frequently.

In a study conducted by Güvenç et al. [13] in Adana, the frequency of abnormal hemoglobins was found to be 6.8%, with the most frequent being Hb S, Hb D and Hb E. Oğuz et al. reported the most frequent abnormal hemoglobins in our country as HbD, HbS, HbE, HbC in their study [14]. Different abnormal hemoglobins may be seen and found in different percentages among the studies. The most important reason for this may be differences in abnormal hemoglobin frequency due to the ethnic structure specific to the region, the frequency of consanguineous marriages and the presence of foreign patients coming from different countries. Our study is important in terms of determining the frequency of abnormal hemoglobin in our province. In addition, the frequency of occurrence of these abnormal hemoglobins will help predict abnormal variants that may be encountered in laboratory practice.

We also evaluated the frequency of beta thalassemia in our study. In our study, 6017 patients had HbA_2 levels of 3.6 and above out of 223,609 hemoglobin variant analysis samples that we screened in our central laboratory. We determined the frequency of beta thalassemia traits as 2.69%. Although the frequency of beta thalassemia traits is generally 2.1%, it varies between 0.6% and 10.7% depending on the region [14]. Akağaç et al. [10] reported the frequency of beta thalassemia traits as 3% in Uşak city. Keskin et al [15] reported the beta-thalassemia trait frequency as 3% in Denizli. Güvenç et al [13] reported the beta-thalassemia trait frequency as 13.46% in Adana. There may be differences in the frequency between studies for beta-thalassemia trait. The reason for this difference may be related to the ethnic structure specific to the region and the high number of consanguineous marriages.

As a result, we detected abnormal variants in our study, most frequently Hb S, followed by HbS, HbD, HbC and HbE in order of frequency. We detected abnormal hemoglobin in our study at a lower frequency than reported in studies in the literature. We detected beta thalassemia traits at a similar percentage to the rates reported in the literature. It should not be forgotten that the HPLC method is a screening test for hemoglobinopathies and that definitive diagnosis can be made with genetic methods. Our study will contribute to the predictability of the frequency of beta thalassemia traits and pathological hemoglobin.

5. CONFLICT OF INTERESTS

The authors have no conflict of interest to declare. The authors declared that this study has received no financial support.

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