

Importance of DNA Sequencing for Abnormal Hemoglobins Detected by HPLC Screening

HPLC ile Tanımlanan Anormal Hemoglobinler için DNA Dizilemenin Önemi

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To the Editor,

Hemoglobinopathies are the most common health problem in Turkey. A hemoglobinopathy prevention program has been implemented by the Ministry of Health in Turkey in 33 provinces since 2003 and it spread to all 81 provinces in 2018 [1]. Our hemoglobinopathy diagnostic center has been licensed for 16 years [2]. The aim of this study was to compare the molecular genetic analysis and high-performance liquid chromatography (HPLC) results for abnormal hemoglobins. Blood samples were directed from local primary health care centers, hospitals, and laboratories in the context of premarital screening processes. Complete blood count (CBC) and HPLC methods were applied for all blood samples. Abnormal hemoglobins or abnormal bands were detected in 219 (0.67%) of 32,513 blood samples between 2013 and 2019. DNA sequencing was performed for 190 of 219 samples. Of those 190 samples, 38 were abnormal bands, 76 were HbS, 49 were HbD, 6 were HbC, and 21 were HbE. While ten different mutations were detected in 24 cases (63.2%), they were not found for 14 (36.8%) of 38 abnormal bands (Table 1). In addition, molecular analysis confirmed 69 cases of HbS (90.8%) from among 76 HbS, 42 HbD Punjab (85.7%) in 49 HbD, 4 HbC (66.7%) in 6 HbC, and 4 HbE (19%) in 21 HbE samples detected by HPLC.

Al-Madhani et al. [3] screened 3740 newborns and compared the results of CBC and HPLC with the molecular genetic analysis results for 290 newborns. They confirmed 26 cases of homozygous sickle cell anemia and 5 of homozygous β -thalassemia major by DNA sequencing among 31 newborns [3]. Warghade et al. [4] screened 65,779 cases for hemoglobinopathy using cation-exchange (CE)-HPLC and abnormal hemoglobin fractions were observed in 12,131 (18.44%) cases. They confirmed eight rare hemoglobin variants by beta-globin gene analysis for those samples that could not be distinguished by CE-HPLC [4]. Chen et al. screened couples of reproductive age using HPLC and reported 1.14% hemoglobinopathy in the Chinese city of

Table 1. Molecular analysis of the cases with abnormal bands (n=38).

Mutation	N	%
Unknown	14	36.84
Hb O-Arab (β 121 Glu->Lys)	5	13.15
Hb S (β 6 Glu->Ala)	4	10.52
Hb G-Coushatta (β 22 Glu->Ala)	4	10.52
Beta IVS2.1(G->A)	3	7.89
Hb Hamadan (β 56 Gly->Arg)	2	5.26
Hb G Norfolk (A2 85 Asp->Asn)	2	5.26
-30(T>A)	1	2.63
-56(G>C)	1	2.63
Hb Fontainebleau (A21(B2) Ala>Pro)	1	2.63
Hb ErnZ (β 123(H1) Thr->Asn)	1	2.63

Guangzhou. They reported 8 different abnormal hemoglobins by molecular techniques [5].

In the present study, the concordance of sequencing analysis with the HPLC results was 90.8% for HbS, 85.7% for HbD, 66.7% for HbC, and 19% for HbE. Interestingly, 10 different abnormal hemoglobin variants have been detected using DNA sequencing in 24 of 38 (63.2%) samples with abnormal bands. Therefore, the type of abnormal hemoglobin can be determined more precisely using molecular analysis.

In conclusion, whatever screening method is used in hemoglobinopathy diagnosis centers, all reports should include the following expression: "This is a screening test; molecular analysis should be carried out for a definite result". Taking into account the different results obtained in screening and molecular analysis, physicians working in these centers should be offered access to molecular analysis for all abnormal hemoglobins and abnormal bands.

Keywords: Hemoglobinopathy, HPLC, DNA, Sequencing

Anahtar Sözcükler: Hemoglobinopati, HPLC, DNA, Dizileme

Informed Consent: As a result of the explanations, the patients voluntarily asked for the tests to be conducted.

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Two Rare Pathogenic *HBB* Variants in a Patient with β -Thalassemia Intermedia

Bir Beta Talasemi İntermedya Hastasında İki Nadir Patojenik *HBB* Varyantı

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To the Editor,

The β -thalassemias are a group of hereditary disorders with autosomal recessive inheritance characterized by the presence of defective synthesis of the β -globin chain, an integral component of the hemoglobin molecule, resulting in either partial synthesis (β^+) or complete absence (β^0) [1]. The disease reaches a high frequency in the Mediterranean Basin, Africa, the Middle East, the Indian subcontinent, and Southeast Asia [2]. According to the World Health Organization, the frequency of abnormal hemoglobin is 7% globally [3]. β -Thalassemia major is characterized by completely inhibited synthesis of beta chains [4], and so it must be treated, generally by transfusion therapy [4]. The β -thalassemia major

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phenotype has homozygotes or compound heterozygotes for β^0 or β^+ genes. Generally, mutations targeting the coding regions of the gene and conservative regions on the exon-intron boundary lead to β^0 -thalassemia, and mutations in regions that do not encode β^+ -thalassemia. In contrast to the major type, the presence of one normal gene in heterozygotes usually leads to enough normal β -globin chain synthesis so that the affected individuals are usually asymptomatic with only hypochromic and microcytic red blood cells. This condition is referred to as β -thalassemia minor [5]. β -Thalassemia intermedia clinically differs from the major and minor ones with respect to the necessity of transfusion. The degree of anemia for β -thalassemia major is more aggravated than that for β -thalassemia intermedia. The genotype of β -thalassemia intermedia is mostly