CASE REPORT

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The Case Report of Hemoglobin Hamadan: HBB:c.169G>C (B 56 (D7) Gly-Arg) in İstanbul

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ABSTRACT 27-year-old case with hemoglobin (Hb) Hamadan was presented. During premarital thalassemia screening an unidentified Hb peak; retention time (RT): (5.55 min, 46.2%) was determined with cation-exchange high-performance liquid chromatography (CE-HPLC) at premier resolution (Trinity-Biotech) system. Variant Hb was eluted with Hb A2 (RT): 3.76 min, 45%) at Variant II Turbo (Bio-Rad), while it was 45.7% and detected at S zone at capillary zone electrophoresis system (CZE) Sebia (Lisses, France). She didn't show any clinical symptoms or hematological abnormality. Hemoglobin A1c (HbA1c) results by Boronate affinity, CE-HPLC and CZE methods were 5.7%, 4.7%, and 5.3% respectively. Beta globin gene sequencing revealed a heterozygote codon (c.169G>C (P.Gly57Arg) identical with Hb Hamadan. Hb Hamadan does not cause clinical complaints, but may be important in prenatal examination and cause interference in HbA1c measurements.

Keywords: Hemoglobins; abnormal hemoglobins; glycated hemoglobin A1c

Hemoglobin (Hb) Hamadan; α2β256(D7) Gly→Arg), is one of the infrequent Hb variants and it was identified in an Iranian family in 1975 by Rahbar et al. It occurs as a result of the GGC→CGC change in the 56th codon of the beta-globin gene and arginine amino acid replaces glycine.[1]

The first cases from Türkiye were 4 Hb Hamadan heterozygotes belonging to one family of Turkish descent and reported by Dinçol et al. in 1984.[2] Thereafter Akar and Yüzbaşıoğlu Arıyürek identified new cases in 2003 and 2009 respectively.[3,4]

The mutation observed in Hb Hamadan does not cause any complaints or hematological abnormality in patients.[3]

CASE REPORT

Our case is a 27- years old woman who was admitted to Kartal Dr. Lütfi Kırdar City Hospital for the premarital thalassemia screening program. She was healthy and hadn't any chronic disease or drug use. There was no pathology in her physical examination. Thalassemia screening was performed at Premier Resolution (Trinity-Biotech, USA) system by cation-exchange high-performance liquid chromatography (CE-HPLC) and besides HbA0 [retention time (RT): 4.63 min, 45.1%] and Hb F (RT: 2.56 min, 0.5%), an unknown Hb peak (RT: 5.55 min, 46.2%) was observed while Hb A2 could not be detected (Figure 1).

We reached the patient, informed her about her thalassemia screening result, and requested to perform the further evaluation. We got informed consent and repeated thalassemia screening in different systems. Glucose, complete blood count (CBC), hemoglobin A1c (HbA1c), and genetic analysis were performed. Glucose was analyzed on the Cobas 8000 C 702 analyzer (Roche Diagnostics, Indianapolis, IN)

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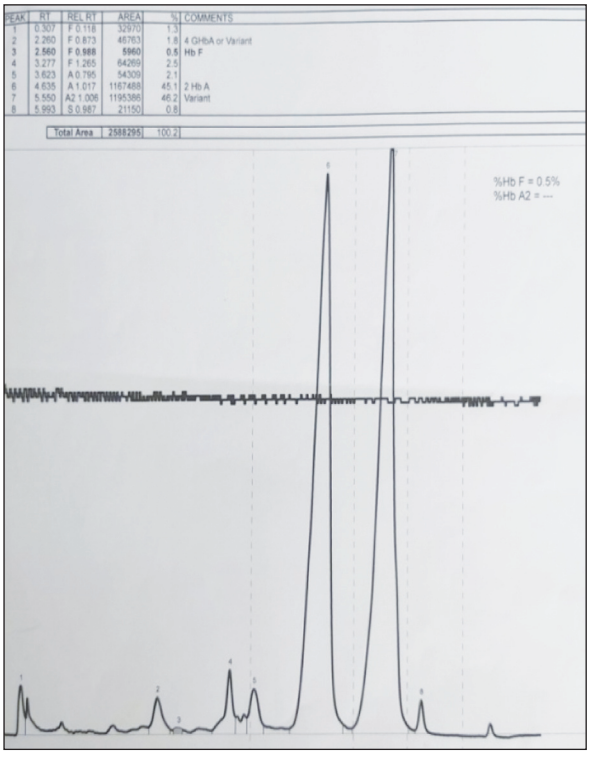

FIGURE 1: Hemoglobin variant analysis at Premier Resolution (Trinity-Biotech) system by CE-HPLC.
CE-HPLC: Cation-exchange high-performance liquid chromatography.

TABLE 1: Laboratory results of the patient.

	Result	Reference range
Glucose (mmol/L)	5.27	4.1-5.88
HbA1c % (Boronate affinity)	5.7	4-6
HbA1c % (CE-HPLC)	4.7	4-6
HbA1c % (CZE)	5.3	4-6
Rbc (10^6/μL)	4.85	3.91-5.31
Hgb (g/dL)	13.5	11.1-14.7
Hct %	40.5	36.9-49.1
MCV (μm^3)	83.5	82.9-98
MCH (pg)	27.8	27-32.3
MCHC (g/dL)	33.3	31.8-34.7
RDW-CV (%)	13.6	11.2-14
RDW-SD (fL)	41.1	38.9-50

HbA1c: Hemoglobin A1c; CE-HPLC: Cation-exchange high-performance liquid chromatography; CZE: Capillary zone electrophoresis; Rbc: Red blood cells; Hgb: Hemoglobin; Hct: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW-CV: Red cell distribution width-coefficient of variation; RDW-SD: Red cell distribution width-standard deviation.

(Table 1). Her fasting blood glucose was 5.27 mmol/L. CBC was evaluated at Sysmex XN 9000 (Roche Diagnostics Indianapolis, IN) analyzer and it was not noteworthy. HbA1c analysis was also performed at (Premier Hb9210, Trinity Biotech, USA) by the Boronate affinity method, Variant II Turbo by CE-HPLC method, and with the CZE at Sebia (Lisses, France) systems and HbA1c were measured at 5.7%, 4.7%, and 5.3% respectively.

Thalassemia screening was also repeated by the CE-HPLC method at Variant II Turbo and by CZE at Sebia systems. Hb A0 (RT: 2.45 min, 45.1%), Hb F (RT;1.09, 0.6%), and Hb A2 (RT: 3.76 min, 45.0%) were detected and Variant Hb was eluted in Hb A2 with Variant II Turbo system. Variant Hb was detected at the level of 45.7% in the S Zone by Sebia system (Figure 2a, Figure 2b).

For the detection of suspected *HBB* gene mutation, the patients' *HBB* gene DNA sequence was acquired by using Sanger sequencing method. For this aim, peripheral blood sample was taken and genomic DNA was isolated. Polymerase chain reaction (PCR), purification and BigDye (Cycle Sequencing) steps were completed. The acquired PCR products were run by an 8 capillary sequencing system (3500 Genetic Analyzer, Thermo Scientific, USA). DNA sequencing data of the patient was compared with the reference transcript. Bioinformatic analyis of the beta globin gene (HBB) revealed a heterozygous c.169G>C (P.Gly57Arg) variant, which was previously associated with Hb Hamadan (Figure 3).

DISCUSSION

Our patient is the 4th Hb Hamadan case identified in Türkiye. Türkiye is located at an important meeting point of the 3 continents where from history to the present, many ethnic groups are interconnected. Due to its geographical location, Türkiye has been heavily affected by migration and the evolution of Hb disorders. Therefore more than 40 variants have been reported so far.[5,6]

Most of them do not show any clinical signs. It is discovered incidentally while patients are being evaluated for another health problem or screening before marriage.[5,7] Interferences can be observed in HbA1c measurements and mutant variants, carbamylation, and acetylation of Hb significantly affect HbA1c results.[8,9] Hb variants alter the survival time

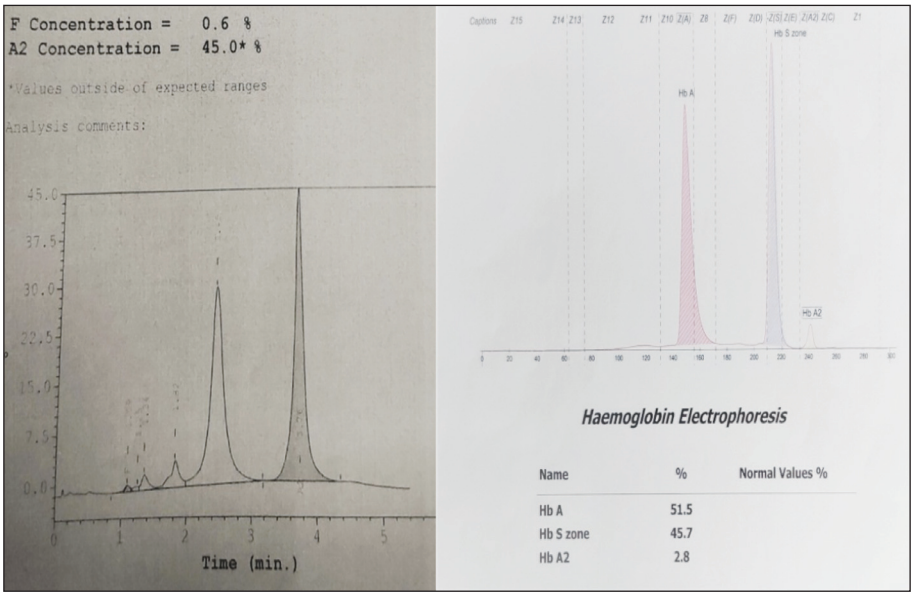

FIGURE 2: a: Variant Hb analysis by Variant II Turbo system (Bio-Rad).**b:** Variant Hb analysis by capillary zone electrophoresis system at Sebia system (Lisses, France). Hb: Hemoglobin.

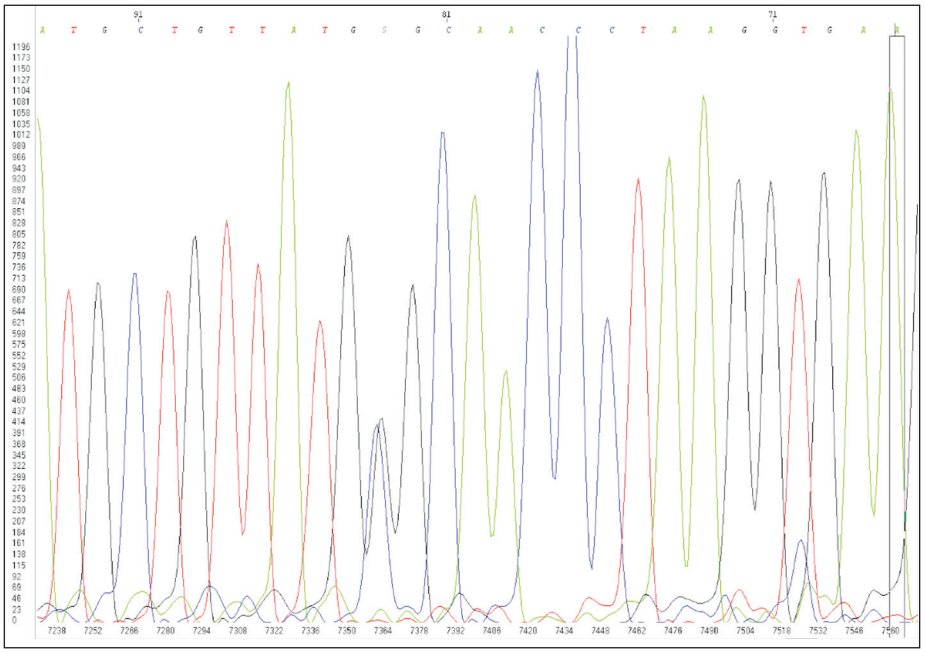

FIGURE 3: Genetic analysis at 3500 Genetic Analyzer (Thermo Scientific).

of red blood cells and cause HbA1c results which are analytically correct but clinically misleading. Unresolved HbA1c peaks may cause faulty increased or decreased HbA1c levels.[10] Immunoassay, ion-exchange HPLC, Boronate affinity HPLC, and enzymatic assays are the common methods for HbA1c analysis.

In our patient different HbA1c results were obtained with Boronate affinity, CE-HPLC, and CZE methods and measured 5.7%, 4.7%, and 5.3% respectively. Average glucose was calculated from the formula [average glucose (mg/dL)=28.7xHbA1c-46.7] and it was 6.4 mmol/L, 4.9 mmol/L and 5.8 mmol/L with Boronate affinity, CE-HPLC, and CZE

systems.[11] CE-HPLC HbA1c was the closest result to his blood glucose level which was 5.3 mmol/L. There is not much information about the presence of Hb Hamadan and interference in HbA1c measurement. We can conclude that HbA1c measurements are affected by the presence of Hb Hamadan at different degrees from method to method.

Gunton et al reported a 36-year-old woman with gestational diabetes and her HbA1c was measured at 0.0% and 1.2% by ion-exchange HPLC.[12] DNA sequencing was identical with Hb Hamadan. Authors identified Hb Hamadan as one of the hemoglobinopathies which cause interference in HbA1c measurements by HPLC.[12]

According to World Health Organization, about 7% of the world's population is known to be affected by a hemoglobin variant. Many of these variants are clinically silent and detected with a chance.[9] Hb S is the most common and important variant in the Turkish population and other variants such as Hb D, Hb E, and Hb C are also frequently observed.[4] In association with other globin gene defects, severe anemia complicating with bleeding can be observed therefore it is important to identify these variants.[13] Since there is no radical cure for hereditary blood diseases, prenatal diagnosis studies are gaining importance for their eradication.[4]

Hb Hamadan has been first described in an Iranian family, a French Caucasian family, 4 members of a Turkish family, and in several Japanese families.[1,2,14] In Türkiye, it was first described in a heterozygous case from Western Thrace in 1984.[2] Akar and colleagues determined the homozygous cases and the association with-29 G→A β thalassemia mutation by restriction enzyme cutting method in 2003.[3] In 2009 Hb Hamadan was detected in Çukurova Region for the first time with the microarray method by Yüzbaşıoğlu et al and thereafter no new case was identified in Türkiye.[4]

In our study, all 3 hemoglobinopathy screening methods suggested the presence of the Hb variant. Premier and Sebia systems described abnormal unidentified Hb peak but the variant Hb was eluted with Hb A2 [(RT): 3.76 min, 45%] at Variant II Turbo system. HbA1c measurements also showed abnormal peaks and helped to suspect the presence of a variant by the CE-HPLC method at Variant II Turbo and by the CZE method at Sebia systems. We couldn't evaluate our patient's family for mutations which may be accepted as a limitation. The identification of those silent hemoglobinopathies is important since together with other mutations, a significant decrease may be seen in Hb levels. Hb Hamadan does not cause clinical complaints, but may be important in prenatal examination and cause interference in HbA1c measurements.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Özlem Hürmeydan, Özlem Çakır Madenci;* ***Design:*** *Fatma Erdoğmuş;* ***Control/Supervision:*** *Özlem Çakır Madenci;* ***Data Collection and/or Processing:*** *Özlem Hürmeydan, Nihal Yücel, Fatma Erdoğmuş;* ***Analysis and/or Interpretation:*** *Berk Özyılmaz, Taha Reşid Özdemir;* ***Literature Review:*** *Özlem Hürmeydan, Özlem Çakır Madenci, Fatma Erdoğmuş;* ***Writing the Article:*** *Özlem Hürmeydan, Özlem Çakır Madenci;* ***Critical Review:*** *Özlem Hürmeydan, Özlem Çakır Madenci;* ***Materials:*** *Berk Özyılmaz, Taha Reşid Özdemir.*

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