CASE REPORT

DOI: 10.5336/caserep.2021-86119

The First Case of Hemoglobin Görwihl [α2β25(A2) Pro→Ala] Identified in Türkiye

Özlem ÇAKIR MADENCİ[a], Fatma ERDOĞMUŞ[a], Asuman ORÇUN[a], Berk ÖZYILMAZ[b]

[a]Kartal Dr. Lütfi Kırdar City Hospital, Biochemistry Laboratory, İstanbul, Türkiye
[b]University of Health Sciences Tepecik Training and Research Hospital, Genetic Diseases Diagnosis Center, İzmir, Türkiye

ABSTRACT We present the first individual with hemoglobin (Hb) Görwihl in Türkiye and to our knowledge, this is the 3rd case reported worldwide. During premarital thalassemia screening an abnormal unidentified Hb peak [Retention Time (RT): 4.950 min., 42.4%] was determined by cation-exchange high-performance liquid chromatography (CE-HPLC) method at Premier Resolution (Trinity-Biotech) system. Variant Hb was eluted with HbA (RT: 2.47 min., 82%) at Variant II Turbo, Bio-Rad system. He was 22 years old and didn't show any clinical symptoms or haematological abnormality. HbA1c measured with Boronate affinity method at (Premier Hb9210, Trinity-Biotech) system was 5.2% and consistent with his blood glucose value (5.5 mmol/L) while it was 4.6% with CE-HPLC method at Variant II Turbo system. β-globulin gene sequencing revealed a heterozygote codon c16C>C variant which was identical to Hb Görwihl. Although clinically insignificant, Hb Görwihl may be important in hemoglobinopathy screening and HbA1c measurements.

Keywords: Hemoglobins; abnormal hemoglobins; glycated hemoglobin A1c

Hemoglobin (Hb) Görwihl or α2β25 (A2) Pro→Ala, also known as Hb Hinchingbrooke is a rare Hb variant that was previously defined in 2 German families.[1-3] It is the heterozygote mutation at codon 5 of the β-globin gene causing cytosine to guanine transversion and replacement of proline amino acid by alanine (CCT-GCT).[2]

Hb Görwihl shows a similar electrophoretic action with HbA and variant Hb accounts for 43.7% of the total. It is a silent mutation and patients don't show any symptoms or clinical abnormalities.[2] During cation-exchange high-performance liquid chromatography (CE-HPLC) method with Bio-Rad (Bio-Rad Laboratories, USA) Variant it is eluted with HbA. In the Globin Gene Server, Hb Görwihl is 1st identified in 2001 by Giardine et al. and no case was reported after Bissé et al.'s case which was reported in 2002, Germany.[2,4]

CASE REPORT

A 22-years old Caucasian Turkish male patient was admitted to the family health center for hemoglobinopathy screening before marriage. He was healthy and his physical investigations were unremarkable. He hadn't any complaint or chronic disease and didn't use any medication. His family migrated from Bulgaria in 1950 and his parents were diabetic. In terms of the Hemoglobinopathy Prevention Program premarital thalassemia screening was performed at Dr. Lütfi Kırdar Kartal City Hospital. Besides HbA0 [Retention Time (RT): 4.628 min., 46.4%] and HbA2 (RT: 5.563 min., 3.2%); an abnormal unidentified Hb peak (RT: 4.950 min., 42.4%) was determined at Premier Resolution (Trinity-Biotech) by CE-HPLC (Figure 1A).

Correspondence: Özlem ÇAKIR MADENCİ
Kartal Dr. Lütfi Kırdar City Hospital, Biochemistry Laboratory, İstanbul, Türkiye
E-mail: ocmadenci@hotmail.com

Peer review under responsibility of Turkiye Klinikleri Journal of Case Reports.

Received: 07 Sep 2021 ***Received in revised form:*** 15 Oct 2021 ***Accepted:*** 21 Oct 2021 ***Available online:*** 26 Oct 2021

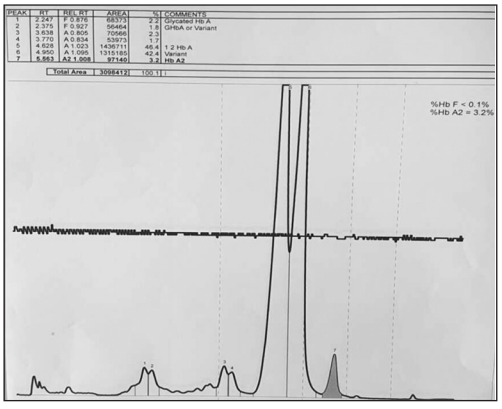

FIGURE 1A: The chromatogram of the patient obtained by Premier Resolution; Trinity-Biotech System.

He was called and given information about his thalassemia screening result and invited to the laboratory for further investigation. Informed consent was obtained to participate in the study and a new sample was drawn for re-evaluation of thalassemia screening and performing clinical biochemistry, complete blood count (CBC), HbA1c, and genetic analyses. Glucose, urea, creatinine, alanine amino transferase, aspartate amino transferase, alkaline phosphatise, gamma-glutamyl transferase, lactate dehydrogenase, creatine kinase, total protein, albumin, total bilirubin, iron, iron-binding capacity, C-reactive protein were evaluated at Cobas 8000 C 702 analyzer (Roche Diagnostics, Indianapolis, IN), thyroid-stimulating hormone, FT4, ferritin, folate, vitamin B_{12} were evaluated at Cobas 8000 e 801 analyzers (Roche Diagnostics, Indianapolis, IN), and CBC was evaluated at Sysmex XN 9000 (Roche Diagnostics Indianapolis, IN) analyzer. His clinical biochemistry and CBC results were unremarkable. Clinical laboratory results were shown in Table 1. HbA1c was evaluated at (Premier Hb9210, Trinity-Biotech) system by the Boronate affinity method and it was 5.2% and consistent with his blood glucose level (5.5 mmol/L). The same sample was evaluated for both HbA1c and thalassemia screening by the CE-HPLC method at (Variant II Turbo, Bio-Rad) system. HbA1c value was 4.6% and variant Hb was eluted with HbA (RT: 2.47 min., 82%) (Figure 1B). Genetic analyses were evaluated for verification of the suspicious variant.

The genomic DNA of the peripheral blood sample of the patient was extracted according to standard protocols. HBB gene sequence analysis was carried out by the Sanger sequencing method. For this aim, polymerase chain reaction, ExoSAP purification (Affymetrix), and BigDye cycle sequencing (Thermo Scientific) reactions were performed. DNA sequencing products were cleaned up (Zymo Research) and run by an 8-capillary sequencing instrument (3500 Genetic Analyzer, Thermo Scientific). The DNA sequences were obtained via capillary electrophoresis, compared with the reference sequences. β-globulin gene se-

TABLE 1: Clinical laboratory results of the patient.

Parameters	Concentration	Reference range
Glucose (mmol/L)	5.5	4.11-5.88
Urea (mmol/L)	5.61	2.8-8.1
Creatinine (umol/L)	83.1	61.88-106
AST (IU/mL)	32.5	0-50
ALT (IU/mL)	31	0-50
ALP (IU/mL)	63	30-120
GGT (IU/mL)	13	0.60
LDH (IU/mL)	163	135-225
CK (IU/mL)	415	0-190
CRP (nmol/L)	5.33	0-47.6
Total protein (g/L)	71.6	66-87
Albumin (g/L)	43.8	35-52
Total bilirubin (umol/L)	14.36	0-20.5
Iron (umol/L)	19.8	5.9-34.5
Iron binding capacity (umol/L)	31.1	22.4-61.8
TSH (IU/L)	2.48	0.51-4.3
Free T4 (pmol/L)	18.2	12.6-20.98
Ferritin (nmol/L)	3.056	0.67-8.98
Folate (nmol/L)	19.15	10.2-73.2
Vitamin B_{12} (pg/mL)	683	197-771
Red blood cells ($x10^{12}$/L)	5.34	4.5-5.9
Hemoglobin (g/L)	157	130-169
Heamatocrit (L/L)	0.459	0.400-0.494
MCV (fL)	86	77-87
MCH (pg)	29.4	27-31
MCHC (g/L)	342	320-360
WBC ($x10^9$/L)	6.09	3.91-10.9
RDW-CV (%)	12.5	11.2-13.4
Platelet ($x10^9$/L)	204	150-450

AST: Aspartate amino transferase; ALT: Alanine amino transferase; ALP: Alkaline phosphatise; GGT: Gamma-glutamyl transferase; LDH: Lactate dehydrogenase; CK: Creatine kinase; CRP: C-reactive protein; TSH: Thyroid-stimulating hormone; MCV: Mean cell volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; WBC: White blood cell; RDW: Red blood cell distribution width.

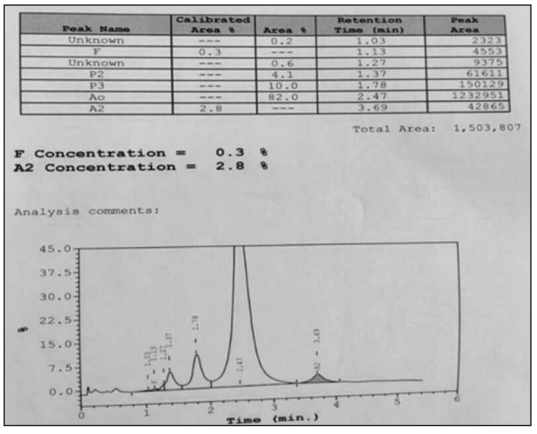

FIGURE 1B: The chromatogram of the patient obtained by Variant II Turbo, Bio-Rad System.

quencing revealed a heterozygote codon c16C>C variant which was identical to Hb Görwihl (Figure 2).

DISCUSSION

Our patient is the 3rd Hb Görwihl case reported in the world, and he is the 1st individual in Türkiye.

Migrations in the world have affected Türkiye in terms of the evolution of Hb disorders and caused the frequent observation of variant Hb.[5] Since patients usually do not have any clinical complaints, they are mostly noticed during the investigation of another health problem, HbA1c measurement, or premarital thalassemia screening.[6] HbA1c measurements are susceptible to interference and are significantly affected by mutant variants, carbamylation, and acetylation of Hb.[7,8] With Boronate affinity methods m-aminophenyl boronic acid reacts especially with cis-diol groups of glucose bound to Hb and total glycated Hb regardless of the binding sites can be measured. Thus the interference from the Hb variants is least observed with this method.[7]

In Bissé et al.'s case report, a 74-year-old male patient from Germany showed an incompatible HbA1c (1.5%) level with the CE-HPLC method. He was diabetic and when the sample was re-evaluated with Boronate affinity and immunoturbidimetric assays, HbA1c was measured at 9.7% and 4.4%, respectively. His blood glucose value was 8.6 mmol/L and consistent with the Boronate Affinity result.[2] Bissé et al. enounced that, substitution β25 (A2) Pro→Ala impairs and slows glycation of N-terminal valine during HbA1c formation by an unidentified mechanism.[2] Ito et al. replaced β5 (A2) proline with alanine in slico and created wild-type Hb mutation in an experimental media. They reported that the mutation β25 (A2) Pro→Ala would cause impaired glycation by causing an electrostatic bond between alpha-amino and beta carboxyl groups and changing the three-dimensional structure of the protein.[3]

Many of the rare variants are clinically silent but the description of them is critical because, in association with other globin gene defects, it can lead to marked low Hb levels and severe anemia needing transfusion.[1] Although Hb Görwihl is a silent mutation, it has an interfering effect on HbA1c measure-

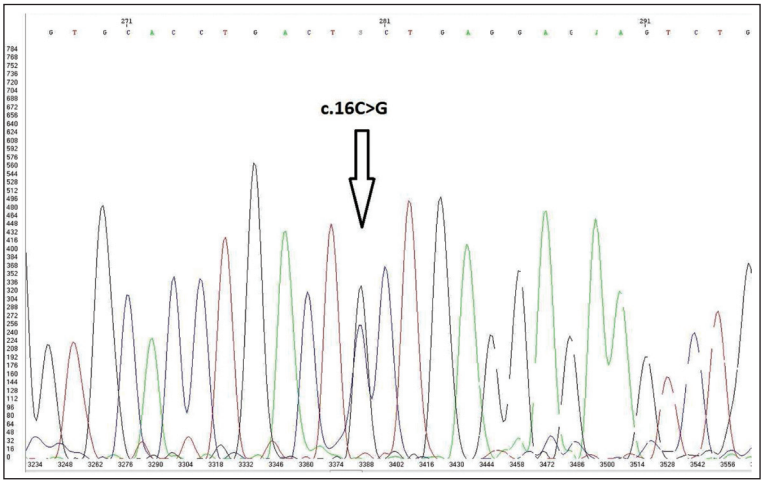

FIGURE 2: Identification of hemoglobin Görwihl in β-globin gene by Sanger sequencing analysis.

ment. Our patient's HbA1c level (4.6%) obtained with the Variant II system wasn't consistent with his blood glucose level (5.5 mmol/L) [average glucose (mg/dL)=28.7xHbA1c-46.7].[9]

In our study, 2 CE-HPLC systems show differences with the properties of the colon, buffer and the calibrators, and time of analysis. Variant Hb was eluted with HbA with Variant II Turbo Bio-Rad system, thus Hb Görwihl might have been overlooked during hemoglobinopathy screening and we can't estimate the accurate prevalence.

As a limitation of the study, we couldn't investigate our patient's family for mutations since they didn't live in İstanbul and couldn't participate in the study. It was especially important for his parents to be informed since they were diabetic and HbA1c measurement could be interfered with according to the method.

Although clinically insignificant, Hb Görwihl may be important in hemoglobinopathy screening and HbA1c measurements.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

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 Çakır Madenci, Fatma Erdoğmuş, Asuman Orçun, Berk Özyılmaz;* ***Design:*** *Özlem Çakır Madenci, Fatma Erdoğmuş, Asuman Orçun, Berk Özyılmaz;* ***Control/Supervision:*** *Özlem Çakır Madenci, Fatma Erdoğmuş, Asuman Orçun, Berk Özyılmaz;* ***Data Collection and/or Processing:*** *Özlem Çakır Madenci, Berk Özyılmaz;* ***Analysis and/or Interpretation:*** *Özlem Çakır Madenci, Fatma Erdoğmuş, Asuman Orçun, Berk Özyılmaz;* ***Literature Review:*** *Özlem Çakır Madenci, Asuman Orçun;* ***Writing the Article:*** *Özlem Çakır Madenci, Asuman Orçun;* ***Critical Review:*** *Özlem Çakır Madenci, Fatma Erdoğmuş, Asuman Orçun, Berk Özyılmaz;* ***References and Fundings:*** *Özlem Çakır Madenci, Fatma Erdoğmuş;* ***Materials:*** *Özlem Çakır Madenci, Berk Özyılmaz.*

REFERENCES

1. Vinciguerra M, Passarello C, Leto F, Cassarà F, Cannata M, Maggio A, et al. Co-inheritance of the rare β hemoglobin variants Hb Yaounde, Hb Görwihl and Hb City of Hope with other alterations in globin genes: impact in genetic counseling. Eur J Haematol. 2015;94(4):322-9. [Crossref] [PubMed]
2. Bissé E, Schauber C, Zorn N, Epting T, Eigel A, Van Dorsselaer A, et al. Hemoglobin Görwihl [alpha2beta(2)5(A2)Pro-->Ala], an electrophoretically silent variant with impaired glycation. Clin Chem. 2003;49(1):137-43. [Crossref] [PubMed]
3. Ito S, Nakahari T, Yamamoto D. Relationship between impaired glycation and the N-terminal structure of the Hb Görwihl [beta5(A2)Pro-->Ala] variant. Hemoglobin. 2010;34(2):151-6. [Crossref] [PubMed]
4. Giardine B, van Baal S, Kaimakis P, Riemer C, Miller W, Samara M, et al. HbVar database of human hemoglobin variants and thalassemia mutations: 2007 update. Hum Mutat. 2007;28(2):206. [Crossref] [PubMed]
5. Kılınç Y. Hemoglobinopathies in Turkey. Turk J Hematol. 2006;23:214-6. [Link]
6. Altay Ç. Abnormal hemoglobins in Turkey. Turk J Haematol. 2002;19(1):63-74. [PubMed]
7. Little RR, Roberts WL. A review of variant hemoglobins interfering with hemoglobin A1c measurement. J Diabetes Sci Technol. 2009; 3(3):446-51. [Crossref] [PubMed] [PMC]
8. Bry L, Chen PC, Sacks DB. Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin. Clin Chem. 2001;47(2):153-63. Erratum in: Clin Chem 2001;47(6):1141. [Crossref] [PubMed]
9. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ; A1c-Derived Average Glucose Study Group. Translating the A1C assay into estimated average glucose values. Diabetes Care. 2008; 31(8): 1473-8. Erratum in: Diabetes Care. 2009; 32(1):207. [Crossref] [PubMed] [PMC]