

**HETEROZYGOTE HEMOGLOBIN G-COUSHATTA AS THE CAUSE OF A FALSELY DECREASED HEMOGLOBIN A1C IN AN ION-EXCHANGE HPLC METHOD**

## HEMOGLOBIN G-COUSHATTA KOD HETEROZIGOTA KAÖ UZROK LAŽNO SNIŽENOG HEMOGLOBINA A1C PRI METODI JONOIZMENJIVAČKE HROMATOGRAFIJE

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Glycated hemoglobin (HbA1c) is used for the assessment of glycemic control in patients with diabetes. The presence of genetic variants of hemoglobin can profoundly affect the accuracy of HbA1c measurement. Here, we report two cases of Hemoglobin G-Coushatta (HBB:c.68A>C) variant that interferes in the measurement of HbA1c by a cation-exchange HPLC (CE-HPLC) method. HbA1c was measured by a CE-HPLC method in a Tosoh HLC-723 G7 instrument. The HbA1c levels were 2.9% and 4%. These results alerted us to a possible presence of hemoglobinopathy. In the hemoglobin variant analysis, HbA2 levels were detected as 78.3% and 40.7% by HPLC using the short program for the Biorad Variant II. HbA1c levels were measured by an immunoturbidimetric assay in a Siemens Dimension instrument. HbA1c levels were reported as 5.5% and 5.3%. DNA mutation analysis was performed to detect the abnormal hemoglobin variant. Presence of Hemoglobin G-Coushatta variant was detected in the patients. The Hb G-Coushatta variants have an impact on the determination of glycated hemoglobin levels using CE-HPLC resulting in a false low value. Therefore, it is necessary to use another measurement method.

**Keywords:** Hb G-Coushatta (HBB:c.68A>C), HbA1c, cation-exchange HPLC

**Kratak sadržaj**

Glikolizirani hemoglobin (HbA1c) koristi se za procenu glikemijske kontrole kod obolelih od dijabetesa. Prisustvo genetskih varijanti hemoglobina može snažno uticati na tačnost merenja HbA1c. Ovde je prikazan izveštaj o dva slučaja gde je varijanta hemoglobina G-Coushatta (HBB:c.68A>C) uticala na merenje HbA1c metodom HPLC sa katjonskom izmenom (CE-HPLC). HbA1c meren je metodom CE-HPLC na instrumentu Tosoh HLC-723 G7. Nivoi HbA1c bili su 2,9% i 4%. Ovi rezultati ukazali su nam na potencijalno prisustvo hemoglobinopatije. Prilikom analiziranja varijante hemoglobina, otkriveni su nivoi HbA2 od 78,3% i 40,7% metodom HPLC uz korišćenje kratkog programa za Biorad Variant II. Nivoi HbA1c mereni su pomoću imunoturbidimetrijskog eseja na instrumentu Siemens Dimension. Dobijeni su nivoi HbA1c od 5,5% i 5,3%. Analiza mutacije DNK izvedena je kako bi se otkrile abnormalne varijante hemoglobina. Kod pacijenata je otkriveno prisustvo varijante hemoglobina G-Coushatta. Varijante Hb G-Coushatta utiču na određivanje nivoa glikoliziranog hemoglobina pomoću metode CE-HPLC, što ima za posledicu lažno niske vrednosti. Dakle, neophodno je primeniti druge metode merenja.

**Ključne reči:** Hb G-Coushatta (HBB:c.68A>C), HbA1c, HPLC sa katjonskom izmenom

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## Introduction

Glycated hemoglobin (HbA1c) is a well-established indicator of mean glycemia. HbA1c is the result of irreversible glycation of the N-terminal amino acid (valine) of the HbA0  $\beta$ -chain (1). Glycation of the amino group of the N-terminal residue produces a loss of a positive charge, which is the basis for methods that quantitate HbA1c by ion-exchange chromatography (2, 3). However, the presence of Hb variants may falsely produce low values for HbA1c or spuriously increased HbA1c values. Hemoglobinopathies can affect HbA1c values in 3 ways: by influencing the binding of glucose to Hb, affecting chromatography peak measurements, and increasing the risk of hemolysis and hence decreasing the life span of red blood cells (4). Hb G-Coushatta (HBB:c.68A>C) is a  $\beta$ -globin variant in which an alanyl residue occurred in place of the glutamyl group at position 22. It was first described in an American Coushatta Indian family (5). Hb G-Coushatta elute in the Hb A<sub>2</sub> window and could be mistaken for the common variants like Hb E and Hb D-Iran which also elute in the same window on HPLC (6). It was emphasized in the literature that Hb G-Coushatta causes an interference in HbA1c measurement by the ion-exchange HPLC method (7). We found that whereas this interference is very prominent in homozygote Hb G-Coushatta cases, it can be easily missed in heterozygote Hb G-Coushatta cases when unattended.

## Results

Hematological tests were done in a 45 years old female non-diabetic patient suffering from anemic symptoms. Complete blood count, serum ferritin, fasting glucose, HbA1c and hemoglobin chromatogram analysis were done respectively on a Coulter LH 780 Analyzer (Beckman Coulter, Holliston, MA), DXI 800 Analyzer (Beckman Coulter, Holliston, MA), AU5800 Analyzer (Beckman Coulter, Holliston, MA), by an ion-exchange HPLC method in a HLC-723G7 Analyzer (Tosoh Bioscience, San Francisco, CA), and by HPLC using the short program for the Variant II (Bio Rad, Hercules, CA).

The results of tests were: RBC: 4.42  $10^{12}/L$ , Hb: 126 g/L, Hct: 37.1%, MCV: 83.7 f/L and ferritin: 4.6 ng/mL. Treatment for iron deficiency anemia was planned according to these results. Fasting glucose was 5.0 mmol/L and HbA1c was 2.9% (reference range: 4–6%).

Since HbA1c level was very low compared to reference range, measurement was repeated by an immunoturbidimetric assay method (Dimension RxL Max, Siemens, Malvern, PA). Actual HbA1c level was 5.6% (reference range: 4.3–6%) by this method. When we compared the results of the two methods,

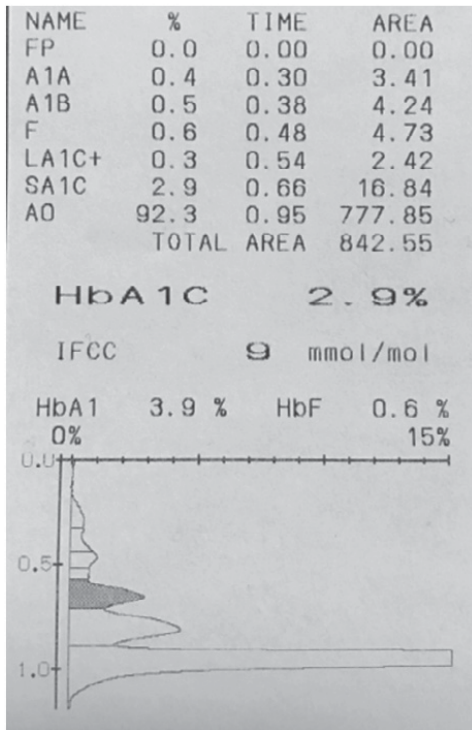
we determined that the HPLC method measured an HbA1c level 45% lower than the immunoturbidimetric assay method leading to an interference. Hemoglobin variant analysis of the patients showed an abnormal pattern (HbA0: 4.9%, HbA2: 78.3% and HbF: 0.4%). A peak of 78.3% HbA2 and low value of HbA1c (2.9%) alerted us to a possible presence of hemoglobinopathy (Figure 1). DNA sequencing showed a  $\beta$  chain variant homozygote Hb G-Coushatta (HBB: c.68A>C). Thirty-seven years old sister of the patient was also screened for  $\beta$ -thalassemia by HPLC. Analysis showed a peak of 40.7% in the HbA2 window (HbA0: 45.8%, HbA2: 40.7% and HbF: 0.1%) (Figure 1). On DNA sequencing, this variant was identified as heterozygote Hb G-Coushatta (HBB: c.68A>C). Ion-exchange HPLC method revealed 4% of HbA1c, which is in the normal range (4–6%). But, we determined the actual HbA1c level as 5.3% by an immunoturbidimetric assay. These results showed that the heterozygote Hb G-Coushatta variant causes 20% of interference by the ion-exchange HPLC method in HbA1c measurement.

Hb G-Coushatta is found in geographically separated ethnic groups. It has been reported in many countries including Algeria, Japan, Korea, Thailand, Egypt, China, Sri Lanka and Turkey (8). Hb G-Coushatta variant is the most common variant in Korea and the Silk Road region of China (9). Although the real prevalence of Hb G-Coushatta variant is unknown in Turkey (10), it is likely that the global prevalence is underestimated because Hb G-Coushatta is asymptomatic. This variant usually leads to underestimation of HbA1c (11, 12) as we noted in our patients. We found that the HbA1c level measured by an ion-exchange HPLC method was very low compared to reference range in the patient with a homozygote variant. Therefore, it is much more easy to think possible hemoglobinopathy in homozygote variants. But, we found the HbA1c level normal by an ion-exchange HPLC method in the heterozygote case.

If Hb G-Coushatta was not searched for particularly, the HbA1c level would be accepted as normal leading to misdiagnosis of possible diabetes mellitus in this heterozygote case in the future. In conclusion, Hb G-Coushatta interferes with the quantitation of HbA1c. Particularly in heterozygote Hb G-Coushatta cases, HbA1c levels measured by HPLC are reduced to 20% less the actual level. So, in places where the Hb G-Coushatta variant is common, although HbA1c measured by an HPLC ion-exchange method is in the normal range, heterozygote variants must be excluded by hemoglobin variant analysis.

## Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.



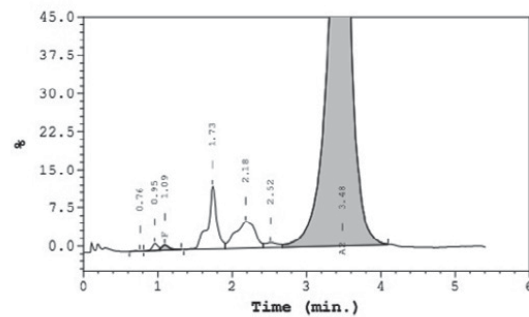
Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
P1	---	0.0	0.76	790
Unknown	---	0.4	0.95	10080
F	0.4	---	1.09	9111
P3	---	5.3	1.73	140090
Ao	---	4.9	2.18	128440
Unknown	---	0.6	2.52	15032
A2	78.3*	---	3.48	2317843

Total Area: 2,621,386

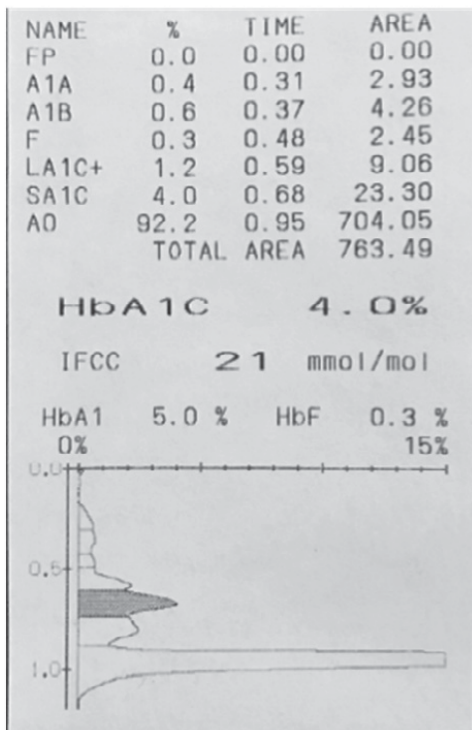
**F Concentration = 0.4 %**  
**A2 Concentration = 78.3\* %**

\*Values outside of expected ranges

Analysis comments:



Patient with homozygous Hb G-Coushatta



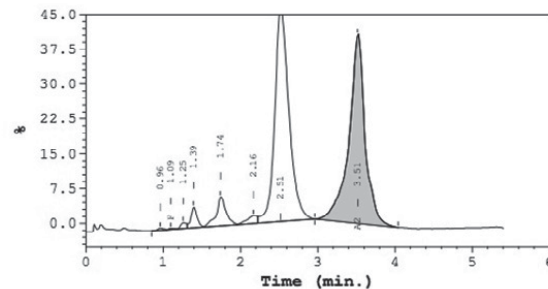
Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.2	0.96	3349
F	0.1*	---	1.09	1242
Unknown	---	0.5	1.25	9601
P2	---	1.9	1.39	32966
P3	---	4.3	1.74	75724
Unknown	---	1.3	2.16	22307
Ao	---	45.8	2.51	813163
A2	40.7*	---	3.51	815409

Total Area: 1,773,762

**F Concentration = 0.1\* %**  
**A2 Concentration = 40.7\* %**

\*Values outside of expected ranges

Analysis comments:



Patient with heterozygous Hb G-Coushatta

**Figure 1** Ion-exchange HPLC chromatogram for HbA1c and Hb variants analysis of patients with Hb G-Coushatta.

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