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Case Report [Olgu Sunumu]

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A case of Hemoglobin Beckman; An example of hemoglobin variants interfering with HbA1c measurement

[Hemoglobin Beckman olgusu; HbA1c ölçümünü interfere eden hemoglobin varyantlarına bir örnek]

Nihal Yücel[1],
Özlem Çakır Madenci[1],
Lale Dağdelen[1],
Derya Aksoy[2],
Asuman Orçun Kaptanağası[1]

[1]Dr. Lütfi Kırdar Eğitim ve Araştırma Hastanesi, Klinik Biyokimya Bölümü, İstanbul
[2]İzmir Bozyaka Eğitim ve Araştırma Hastanesi, Klinik Biyokimya Bölümü, İzmir

Yazışma Adresi
[Correspondence Address]

Nihal Yücel

Dr. Lütfi Kırdar Eğitim ve Araştırma Hastanesi, Klinik Biyokimya Bölümü, İstanbul
Tel.
Fax.
E-mail. yucel.nihal@yahoo.com

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ABSTRACT

Aim: It has been known for a long time that the presence of clinically silent hemoglobin (Hb) variants in blood samples could influence the measurement of glycohemoglobin (HbA1c) since these abnormal hemoglobins might interfere with some methodologies. In the present study we describe the first case of Hb Beckman in Turkey.

Material and Methods: The variant was detected during the HbA1c measurement by cation exchange high performance chromatography (CE-HPLC) in a patient with diabetes mellitus. Since a HbA1c result could not be obtained by this method, the test was repeated by boronate affinity HPLC.

Results: Further investigation performed in order to identify the variant revealed heterozygote Hb Beckman 135 GCT-GAT (Ala --> Asp) which explained the inappropriate HbA1c result by CE-HPLC.

Conclusion: This new case of Hb Beckman is thought to be a modest contribution to the subject of abnormal hemoglobins that interfere the HbA1c measurement.

Key Words: Hemoglobin Beckman, HbA1c, Diabetes Mellitus

Conflict of Interest: Authors declare that there is no conflict of interest.

ÖZET

Amaç: Klinik belirti vermeyen hemoglobin (Hb) varyantlarının glikohemoglobin (HbA1c) ölçümlerini etkileyebildiği uzun zamandan beri bilinmektedir; zira bu varyantlar bazı ölçüm yöntemleri ile interferans göstermektedir. Bu makalede Türkiye'deki ilk Hemoglobin (Hb) Beckman vakası sunulmaktadır.

Gereç ve Yöntemler: Varyant, diyabetli bir hastada katyon değişimli yüksek performans sıvı kromatografisi (CE-HPLC) ile HbA1c ölçümü sırasında tespit edilmiştir. Bu yöntemle HbA1c sonucu elde edilemediğinden ölçüm boronat afinite HPLC yöntemi ile tekrarlanmıştır.

Bulgular: Varyantı tanımlamak için yapılan ileri tetkiklerde heterozigot Hb Beckman 135 GCT-GAT (Ala --> Asp) tespit edilmiş ve böylece CE-HPLC ile HbA1c sonucu elde edilememesinin nedeni açıklığa kavuşmuştur.

Sonuçlar: Bu yeni Hb Beckman vakasının HbA1c ölçümleri ile interferans yapan anormal hemoglobinlere mütevazı bir katkı olduğu düşünülmektedir.

Anahtar Kelimeler: Hemoglobin Beckman, HbA1c, Diabetes Mellitus.

Çıkar Çatışması – Yazarlar çıkar çatışması olmadığını bildirirler.

Introduction

As a Mediterranean country, the frequencies of thalassemias and hemoglobinopathies are fairly high in Turkey. The majority of hemoglobin variants are clinically silent. They are usually recognised by chance during investigation of another health problem, mostly during the HbA_{1c} measurement.

Hb Beckman was firstly described first by Rahbar et al. in 1991 [1]. In 2010 Kim et al. reported the second case of Hb Beckman, interfering with HbA_{1c} measurement [2].

Case and Methods

A 56 year old Caucasian man with controlled diabetes mellitus presented to the biochemistry laboratory of Dr. Lütfi Kırdar Kartal Training and Research Hospital for determination of HbA_{1c}. The HbA_{1c} value could not be determined by the Cation Exchange High Performance Chromatography (CE-HPLC) (Variant II Turbo, Biorad) method used in the laboratory because the retention time of the variant was very close to that of HbA_{1c}. The sample was remeasured by boronate affinity method (Premier Hb9210, Trinity-Biotech). An HbA_{1c} value of 5.8% (reference range: %4-6), was obtained. This was consistent with the glycemic status because simultaneous glucose level was 167 mg/dl and previous value measured one month ago was 125 mg/dl. The calibrators of this method were assigned with NGSP HbA_{1c} reference materials. Precisions of the method calculated according to Clinical and Laboratory Standarts Institute (CLSİ) EP15-A_2, were 1.79% for normal (5.5%) and and 1.79% for pathological (10.5%) control sera.

The samples were collected from the family members of the patient (mother, daughter and niece) for total blood count and the presence of the hemoglobin variant analysis. We obtained an informed consent to participation in the study from the patient and his family. The study was approved by the Ethic Committee of Dr. Lütfi Kırdar Kartal Training and Research Hospital. The hematological parameters were measured using a routine blood count analyser (Sysmex XT 2000i, Roche Diagnostic). Except for some mild decreases, most of the hematological markers were found to be between the reference range ranges.

DNA isolation was carried out using a commercially available DNA extraction kit (RTA Lab, Ltd., Sti, Türkiye). Beta globin gene regions were sequenced with an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA., USA). ABIPRISM 310 genetic analyzer (Applied Biosystems, Foster City, California, USA) was used to analyze sequence reaction.

Results

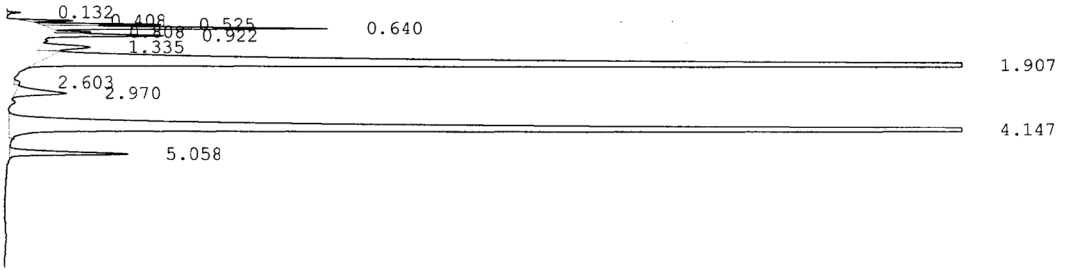
The samples of the patient and family members, evaluated for the presence of a variant by two CE-HPLC methods gave the results that are shown in Table 1. The chromatogram of the patient, showed a peak with an area of % 49.1 eluting at $LHbA_{1c}$ /CHb-1 fraction with D-10 (Bio-Rad) (figure not shown) and a peak eluting after HbA_{1c} with an area of % 46.7 with Ultra^2-Variant (Trinity-Biotech) (Fig. 1). The chromatograms of three members of the family showed the same peaks. Beta globin gene sequencing analysis revealed a heterozygote codon 135 GCT-GAT (Ala --> Asp) mutation which was identical to the Hemoglobin Beckman.

Discussion

Our patient is the first individual with Hb Beckman detected in Turkey. To our knowledge, after Rahbar's and Kim's, this is the third case reported worldwide.

Table 1. HbA_{1c}% values with ion-exchange (Variant II Turbo) and boronate affinity (Premier Hb9210) HPLC; and percent concentration of Hb fractions on ion-exchange HPLC (D10 and Ultra^2-Variant) chromatograms.

	Proband (56-M)	Mother (96-F)	Daughter (-F)	Niece (-F)
HbA_{1c} (Variant II Turbo)	-	-	-	-
HbA_{1c} (D10)	-	-	-	-
Hb A_0 (D10)	37.9	37.0	39.6	39.2
Hb A_2 (D10)	1.8	1.7	1.9	2.2
Hb Beckman (D10)	49.1	49.7	49.3	47.3
HbA_{1c} (Premier Hb 9210)	5.8	4.8	4.5	4.8
HbA_{1c} (Ultra^2-Variant)	-	-	-	-
Hb A_0 (Ultra^2-Variant)	43.3	41.8	43.7	43.1
Hb A_2 (Ultra^2-Variant)	2.1	2.0	2.3	2.2
Hb Beckman (Ultra^2-Variant)	45.5	47.0	46.9	47.3



PEAK	RT	REL RT	% CONC	AREA	COMMENT
1	0.132	F 0.09	0.1%	2714	
2	0.408	F 0.29	0.4%	12041	
3	0.525	F 0.38	0.9%	28151	
4	0.640	F 0.46	3.3%	100189	
5	0.808	F 0.58	0.3%	8224	
6	0.922	F 0.66	1.5%	44633	
7	1.335	F 0.96	1.2%	35008	
8	1.907	F 1.37	46.7%	1413422	3
9	2.603	F 1.87	0.1%	3186	
10	2.970	A 0.74	1.4%	41916	
11	4.147	A 1.03	42.1%	1275667	2 A0 peak
12	5.058	S 0.88	2.0%	62025	A2 peak
			Total Area:	3027176	

Figure 1. The chromatogram of the patient obtained by CE-HPLC method with Ultra²-Variant (Trinity-Biotech).

The Hb Beckman case reported by Kim et al. was a 61-year old Korean man with no clinical signs. The HbA1c value of the patient could not be determined with CE-HPLC. According to this data our case seems sequencially and clinically identical to the case of Kim et al. and is seen for the first time in Turkey.

Although the silent hemoglobinopathies are not a threat for the health of the individuals, they have some interfering effects in the measurement of HbA_{1c}. An Hb variant that change erythrocyte life span or glycation of the Hb may cause an erroneous HbA_{1c} result regardless of the methodology. Other interferences are method dependent. This issue was widely investigeted by several researchers and most of the HbA_{1c} measuring methods were studied with regard to their interferences with various Hb variants [3, 4].

HbA_{1c} analysing methods are mainly based on either molecular charge (ion exchange chromatography, electrophoresis) or structure (immunoassays, boronate affinity chromatography, mass spectrophotometry). All of these methods, except for mass spectrophotometry which is an expensive and complicated one, are widely used in routine laboratories for HbA_{1c} measurement. The former methods are affected by the posttranslational modifications or mutations that alter the charge of the molecule resulting in co-elution or co-migration of the variant with Hb A or HbA_{1c}. On the other hand, any mutation at the N-terminal of β chain that affects the antibody recognition may cause an incorrect HbA_{1c} result in immunoassay methods. The boronate affinity methods measure only glycated Hb regardless of the glycation site. Although there are few exceptions like Hb Raleigh [5] or Hb Himeji [6], the boronate affinity methods are not affected by most of the variants.

Although HbA_{1c} measurement is the best indicator of the long-term glycemic control, the laboratories must be aware of the presence of Hb variants that could interfere with their methodology and should have an alternative way to obtain a true HbA_{1c} value. The measurement of fructosamine, daily multiple testing of glucose or continuous glucose monitoring for hospitalized patients are suggested for this purpose. These tests can also be useful in patients with altered erythrocyte life span or altered glycation. However, measurement of HbA_{1c} with a boronate affinity method can be a reliable diagnostic tool in most of the cases like ours.

This new case of Hb Beckman is thought to be a modest contribution to the subject of abnormal hemoglobins that interfere with HbA_{1c} measurement. We presume that in countries like Turkey where Hb variants are quite frequent, the awareness of the interference of these abnormal hemoglobins by the laboratorians and the clinicians is crucial.

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