The First Case of Hemoglobin Beckman Beta135(H13) ALA>ASP Identified in Turkey

Türkiye'de Tanımlanan İlk Hemoglobin Beckman Beta135(H13) ALA>ASP Olgusu

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ABSTRACT As a Mediterranean country, the frequency of thalassemias and hemoglobinopathies is fairly high in Turkey. This article describes the first case of Hemoglobin Beckman in Turkey. This variant was observed in a 56-year-old Caucasian man during the HbA1c measurement with a cation exchange high performance liquid chromatography (CE-HPLC). Gene sequencing analysis revealed an heterozygote codon 135 GCT-GAT (Ala --> Asp) mutation which was identical for the Hemoglobin Backman in the Globin Gene Server (HGVS: HBB:c.407C>A). The case was similar, in terms of DNA sequence result and clinical signs to the Hemoglobin Beckman case reported by Kim et al. in 2010, rather than the first case described for the first time by Rahbar et al. in 1991.

Key Words: Hemoglobinopathies; hemoglobins, abnormal

ÖZET Bir Akdeniz ülkesi olarak Türkiye talasemi ve hemoglobinopatilerin sıklıkla görüldüğü ülkelerden biridir. Bu makalede Türkiye'de tanımlanan ilk Hemoglobin Beckman vakası sunulmaktadır. Bu varyant, katyon-değişimli yüksek performans likid kromatografi (CE-HPLC) yöntemi ile HbA1c ölçümü sırasında 56 yaşında bir erkek hastada tespit edilmiştir. Genetik sekans analizi sonucunda heterozigot kodon 135 GCT-GAT (Ala --> Asp) mutasyonu ve bunun Globin Gene Server'da tarif edilen Hemoglobin Beckman olduğu (HGVS: HBB:c.407C>A) ortaya konmuştur. Bu vaka, DNA sekansı ve klinik bulguları bakımından 2010'da Kim ve ark. tarafından tarif edilen Hemoglobin Beckman vakası ile aynı olmakla beraber, bu varyantı 1991'de ilk tanımlayan Rahbar ve ark.'nın vakasından farklılıklar göstermektedir.

Anahtar Kelimeler: Hemoglobinopatiler; hemoglobinler, anormal

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The most common inherited diseases in humans result from mutations in the beta and alpha globin gene complex. Single nucleotide substitutions can lead to amino acid replacements that cause hemolytic anemias, such as sickle-cell disease, or hemoglobins that are unstable or have altered oxygen affinity. Substitutions or deletions, which occur in any of several regions of the genes cause the inherited anemia called thalassemia. Some other sequence changes have little or no effect on hemoglobin function, but are useful polymorphisms for genetic studies. More than 1000 hemoglobin variants have been identified to date and new variants and thalassemias continue to be discovered.[1]

Abnormal hemoglobins are the second most common hemoglobinopathies after beta thalassemia in the Turkish population. To date, more

than 40 different hemoglobin variants have been reported in the Turkish population.[2] Some of these abnormal hemoglobins were originally described in the Turkish population.[3-7]

CASE REPORT

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 the determination of HbA_{1c}. The HbA_{1c} value could not be determined by the cation exchange high performance chromatographic (CE-HPLC) (Variant II Turbo, Biorad) method used in the laboratory and an unusual peak was observed on the chromatogram.

Thereon, the samples of the propositus and the family members (mother, daughter and niece) were investigated for their hemotological status and the presence of any hemoglobin variant. An informed consent was signed by the patient and the family members.

The hematological parameters were measured by the routine blood count analyser (Sysmex XT 2000i, Roche Diagnostic). The hematological results of the propositus and the mentioned family members are shown in the table (Table 1). The hematological values of the propositus and his daughter and niece were within the reference ranges; however the mother's values indicated an anemia which was consequently diagnosed as iron deficiency anemia.

The sample of the propositus and family members were then evaluated for the presence of a variant by two CE-HPLC methods (Variant II Turbo, Biorad and Ultra^2-Variant, Trinity-Biotech); on the chromatogram of the propositus with the first system, there was a peak eluting at $LHbA_{1c}$/CHb-1 fraction with an area of 49.1% (Figure not shown). The variant generated a peak with an area of 46.7% eluting after HbA_{1c} with the second system (Figure 1). The chromatograms of tree members of the family showed the same peak with similar percentages.

DNA isolation from blood samples collected in EDTA tubes 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), according to the manufacturer's instructions; and finally, ABIPRISM 310 genetic analyzer (Applied Biosystems, Foster City, California, USA) was utilized to analyze sequence reaction. This beta globin gene sequence analysis revealed an heterozygote codon 135 GCT-GAT (Ala —> Asp) mutation which was identical to the Hemoglobin Beckman (HGVS: HBB:c.407C>A). (Figure 2).

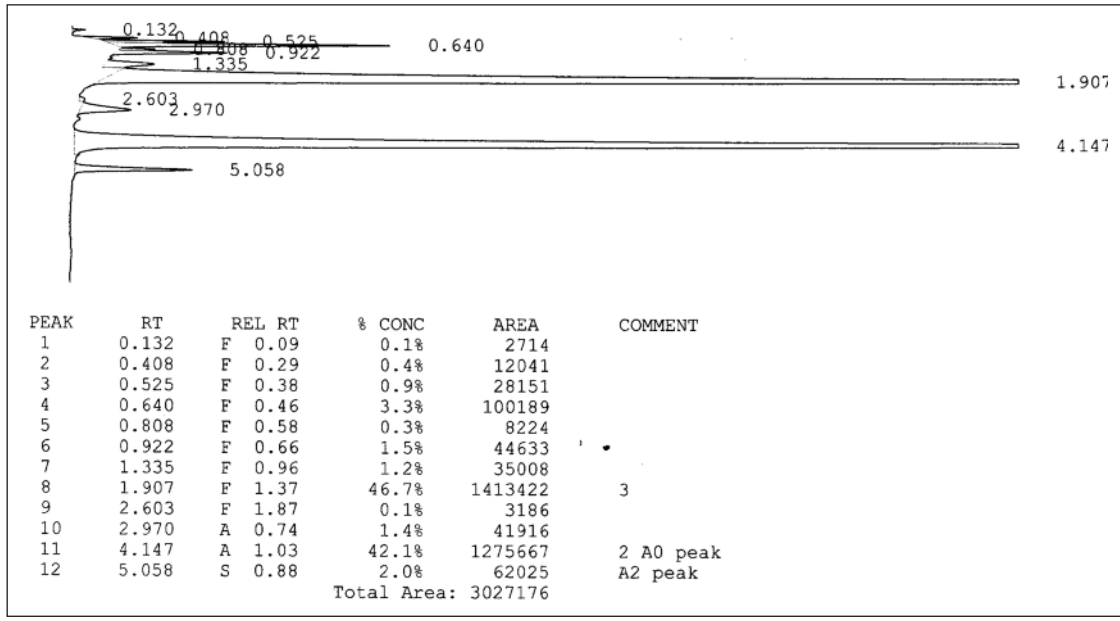
TABLE 1: Hematological parameters of the propositus and the relatives having Hemoglobin Beckman.

	Propositus (56-M)	Mother (96-F)	Daughter (28-F)	Niece (28-F)
Rbc (106/μL) Ref: M:4.6-6.0, F:3.9-5.4	4.69	3.27	3.93	3.98
Hb (g/dL) Ref: M:14-18, F: 12-16	14.6	9.4	12.2	13.1
Hct (%) Ref: M: 42-52, F: 37-47	40.3	30.8	38.5	37.1
MCV (fL) Ref: 81-99	85.9	94.2	92.9	88.8
MCH (pg) Ref: 27-31	29.0	28.7	31.0	29.9
MCHC (g/dL) Ref: 32-36	33.7	30.5	33.4	33.7

Age and sex of the individuals were given in parantheses.
Rbc: Red blood cell; Ref: Reference range; Hb: Hemoglobin; Hct: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration.

DISCUSSION

As mentioned above, disorders resulting structurally abnormal hemoglobins and decreased capacity of globin chain syntesis (thalassemia) are the most common genetic hematological problems; as a Mediterranean country, the frequency of thalassemias and hemoglobinopathies is fairly high in Turkey and more than 40 variants have been reported so far.[2] The majority of the abnormal hemoglobins do not show any clinical signs and are discovered during the investigation of another health problem. In the Globin Gene Server, Hb



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: Chromatogram of the patient obtained with Premier Hb9210.
Peak No. 11 with retention time of 4.147 is the peak of Hb. A0.
Peak No. 12 with retention time of 5.058 is the peak of Hb. A2.
Peak No. 8 with retention time of 1.907 is the peak of Hb. Beckman.

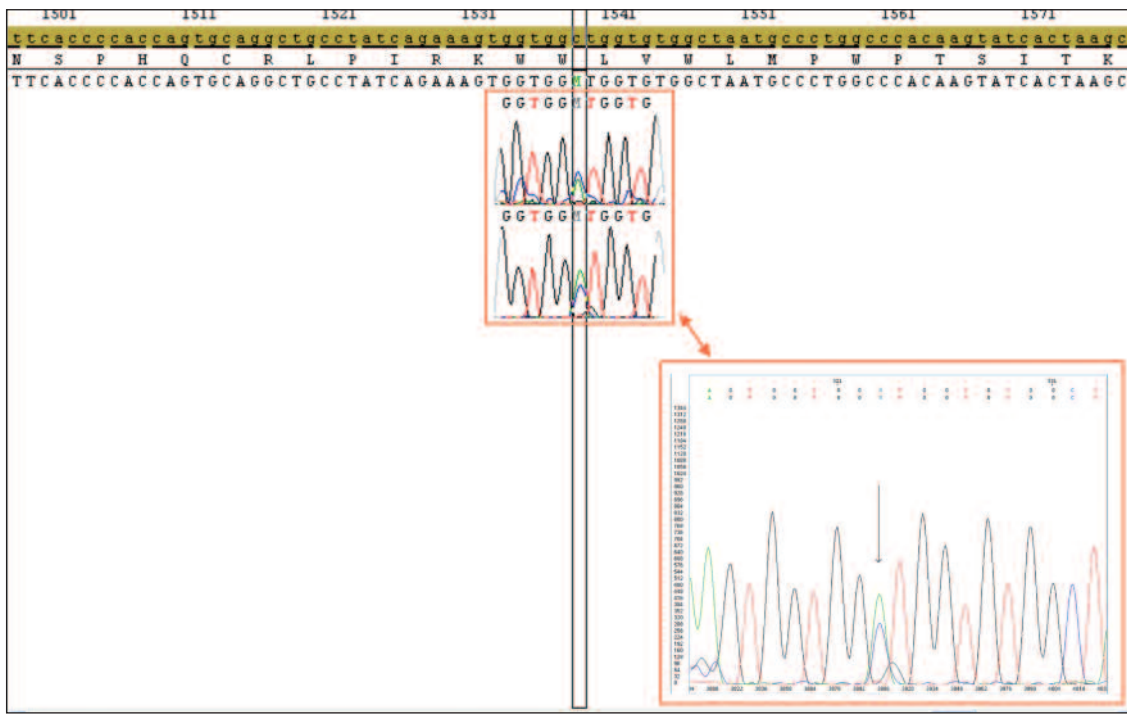


FIGURE 2: Beta globin gene sequence analysis result; the indicated area shows the mutation site of heterozygote codon 135 GCT-GAT (Ala --> Asp).

Beckman is referred to the case of Rahbar et al. in 1991, described in an 32-year-old African-American female presenting with chronic anemia, microcytosis and splenomegaly. In 2010 Kim et al. reported the second case of Hb Beckman in a 61-year old Korean man with no clinical signs.[8] Our patient and his fam-

ily are the first individuals with Hb Beckman detected in Turkey. They were all heterozygotes and otherwise healthy.

These two previous cases were inconsistent in terms of clinical status and DNA sequencing analysis. The case of Rahbar et al. presented with microcytic anemia while the other case was clinically silent. The amino acide sequences of two cases were also discrepent. The first experimental case of Rahbar et al. was described p.Ala136Glu; Hb Beckman alpha2 beta2 135(H13) ala-to-glu. In the Globin Gene Server the Hb Beckman variant was reported as beta 135(H13) Ala —> Asp with a comment noting that an Ala —> Glu change would require a GCA or GCG mutation to GAA or GAG and that the codons GCA and GCG do not occur in the beta-globin gene; GCA is in the gamma gene and GCG in the alpha gene. Landin et al. included Hb Beckman in the group of variants whose reported amino acid substitutions are not compatible with single point nucleotide substitutions.[9] Additionally, this data in the Globin Gene Server was a result of computed information drawn from Rahbar's case, rather than a direct experimental evidence. Under the circumstances, the case of Kim et al. should be the first single point nucleotide substitution, experimentally confirmed by direct sequencing of Hb variant of p.Ala136Asp.

Our case which is an heterozygote, codon 135 GCT-GAT (Ala —> Asp) mutation seems sequencially and clinically identical to the case of Kim et al. and is seen for the first time in Turkey. We expect that this new case would contribute to elucidate the controversy on the definition of Hb Beckman.

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