Hb D-Los Angeles [beta121(GH4)Glu>Gln] and Hb Beograd [beta121(GH4)Glu>Val]: Implications for their laboratory diagnosis and genetic origins

Hb D-Los Angeles [beta121(GH4)Glu>Gln] ve Hb Beograd [beta121(GH4)Glu>val]: laboratuar tanıları ve gensel kökenleri

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Abstract

Objective: The aim of this study was to determine the laboratory diagnosis and genetic origins of the hemoglobin (Hb) variants, Hb D-Los Angeles and Hb Beograd observed frequently in our region.

Material and Methods: Hb variants were investigated in one Hb D-Los Angeles and two Hb Beograd families. These families were unrelated with each other. For the determination of Hb variants, alkaline/acid electrophoresis, HPLC, DE-52 micro-column chromatography procedures were applied. Mutations were determined by non-radioactive fluorescence automated DNA sequencing. Beta globin gene cluster haplotypes were identified by RFLP analysis at seven loci known as ε-Hinc II, Gγ-Hind III, Aγ-Hind III, 5'ψβ-Hinc II, 3'ψβ-Hinc II, β-Ava II ve 3'β-Hinf I.

Results: Three novel beta globin gene cluster haplotypes were identified as in relation with Hb D-Los Angeles [--+-+++], Hb Beograd [+----++ and -+-(+/-)(+/-)+(+/-)]. These haplotypes were reported for the first time in the world population

Conclusion: In this study we emphasize the importance of DNA seqeuncing and other laboratory procedures for the identification of Hb variants in premarital diagnosis. On the other hand we discuss also the genetic origins of these Hb variants. *(Turk J Hematol 2009; 26: 17-20)*

Key words: Hb Beograd, Hb D-Los Angeles, laboratory diagnosis, genetic origin, premarital diagnosis

Received: March 25, 2008 *Accepted: November 2, 2008*

Özet

Amaç: Bu çalışmamızın amacı, yöremizde sıklıkla karşılaşılan Hb D-Los Angeles ve Hb Beograd'ın laboratuar tanısı ve gensel kökenlerine ilişkin sonuçların irdelenmesidir.

Yöntem ve Gereçler: Bu çalışmada, aralarında akrabalık ilişkisi olmayan bir HbD-Los Angeles ve iki Hb Belgrad ailesi incelenmiştir. Hemoglobin (Hb) varyantlarının tanımlanmasında alkali ve asit hemoglobin elektroforezi, HPLC, DE-52 mikrokolon kromatografisi yöntemleri kullanılmıştır. Mutasyonların saptanmasında flüoresan işaretli dizi analiz yöntemi uygulanmıştır. Beta globin gen ailesi haplotiplerinin belirlenmesinde ise ε-Hinc II, Gγ-Hind III, Aγ-Hind III, 5'ψβ-Hinc II, 3'ψβ-Hinc II, β-Ava II ve 3'β-Hinf I olmak üzere toplam yedi odakta RFLP analizi yapılmıştır.

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Bulgular: Bu çalışmada, Hb D-Los Angeles ile ilgili olarak bir tane [--+-+++], Hb Beograd için ise iki tane [+----++] ve [-+-(+/-)(+/-)+(+/-)] yeni beta globin haplotipi saptanmıştır. Bu haplotipler literatürde ilk kez bildirilmektedir.
Sonuç: Bu çalışmada, özellikle premarital tanıda kullanılan yöntemler ile DNA dizi analizinin önemi vurgulanmaktadır. Diğer taraftan bu hemoglobin türlerinin gensel kökenlerine ilişkin veriler irdelenmektedir. *(Turk J Hematol 2009; 26: 17-20)*
Anahtar kelimeler: Hb Beograd, Hb D-Los Angeles, laboratuvar tanısı, gensel köken, premarital tanı

Geliş tarihi: 25 Mart 2008 Kabul tarihi: 02 Kasım 2008

Introduction

Premarital screening is one of the most important components in the hemoglobinopathy control programs. The correct identification of the mutations elucidates the success of the prenatal diagnostic procedures to be applied in a timely and cost-effective manner in the case of pregnancy. The premarital molecular diagnosis is also helpful in the reduction of the stress on the families under interest regarding the prenatal diagnostic approaches. Since the first chorionic villus sampling (CVS)-based reported case in 1990 [1], prenatal diagnosis has been available in different centers in Turkey like Hacettepe University (Ankara), Boğaziçi University (Istanbul), and Çukurova University (Adana) [2]. In our province, prenatal diagnosis has been applied since 2004. The premarital screening and counseling in genetics has been applied by law since 1998 in the centers established by the Turkish Ministry of Health in Hatay, Adana, Mersin and other at- risk provinces like Denizli province [2,3]. Hb Beograd [β121(GH4)Glu→Val, GAA→GTA] is a variant with normal clinical presentation in heterozygous carriers. The homozygous form of Hb Beograd is not known. It has been reported from Yugoslavia, Turkey, Australia and New Zealand [4]. On the other hand, Hb-Los Angeles (also known as D-Punjab, Hb North Carolina, D-Portugal, D-Chicago, and Oak Ridge) is an abnormal Hb at codon 121 of the beta globin gene resulting in the amino acid substitution of glutamine for glutamic acid. Hb D-Los Angeles is the most frequent abnormal hemoglobin observed in Turkey [5], especially in the Denizli province [3]. In premarital screening programs, these abnormal Hbs are easily confused with each other and also with other D-like Hbs like Hb G-Coushatta, due to their similar electrophoretic and chromatographic behavior. Since these abnormal hemoglobins reside on the same codon, they are also mixed with procedures like the Eco RI restriction enzyme protocols at the gene level. In this study, we aimed to discuss the laboratory diagnosis of Hb Beograd and Hb D-Los Angeles as well as their genetic origins according to the beta globin gene cluster haplotypes.

Materials and Methods

We analyzed three individuals from one Hb Beograd family, one unrelated heterozygous Hb Beograd and one unrelated homozygous Hb D-Los Angeles cases. These cases were from three unrelated families. All cases were detected during a premarital screening program. Blood samples were collected in EDTA vacutainers and DNA was extracted from peripheral blood using the standard phenol-chloroform procedure. Written informed consent was obtained from these individuals for DNA analysis and the samples were deposited into our Department's DNA Bank as anonymous samples. Alkaline and acid Hb electrophoresis, DE-52 micro column chromatography and non radioactive DNA sequencing were done as previously reported [2]. High performance liquid chromatography (HPLC) results were obtained with BioRad Variant II system. Beta globin gene cluster haplotype analyses were performed as previously reported elsewhere [6].

Results

Hb D-Los Angeles [β121(GH4) Glu→Gln] and Hb Beograd [β121(GH4) Glu→Val] have the same electrophoretic mobility on alkaline and acid Hb electrophoresis. Both behave like Hb S in alkaline pH and present Hb A-like mobility in acidic pH. In HPLC analysis, Hb Beograd [β121(GH4) Glu→Val] can be separated with a retention time of 4.19 min (Figure 1). Beta globin gene cluster haplotypes of the cases are shown in Table 1. In the

Table 1. Beta globin gene cluster haplotypes of the unrelated Hb D-Los Angeles cases and Hb Beograd family

	Hemoglobin	5'-ε Hinc II	Gγ Hind III	Aγ Hind III	5'-ψβ Hinc II	3'-ψβ Hinc II	5'-β Ava II	3'-β Hinf I
Case #1	Hb D-Los Angeles (homozygous)	-/-	-/-	+/+	-/-	+/+	+/+	+/+
Case #2	Hb Beograd (heterozygous)	-/-	+/+	-/-	+/-	+/-	+/+	+/-
Hb Beograd Family (*)								
Case #3	Hb Beograd (heterozygous)	+/+	-/-	-/-	-/-	-/-	+/-	+/+
Case #4	Normal	+/-	+/-	+/-	+/-	+/-	+/+	+/+
Case #5	Hb Beograd (heterozygous)	+/-	+/-	+/-	+/-	+/-	+/+	+/+
Associated Haplotype		+	-	-	-	-	+	+

(*) Case #3 propositus, case #4 mother and case #5 sister for the Hb Beograd family

Table 2. Beta globin gene cluster haplotypes in association with Hb D-Los Angeles and Hb Beograd mutations observed in world populations

Mutation	5'-ε Hinc II	Gγ Hind III	Aγ Hind III	5'-ψβ Hinc II	3'-ψβ Hinc II	5'-β Ava II	3'-β Hinf I	Ref.
Hb D-Los Angeles								
Italy	+	-	-	-	-	+	+	[09]
-Thailand	-	+	+	-	+	+	+	[10]
-Mexico	+	-	-	-	-	+	+	[11]
-Iran	+	-	-	-	-	+	+	[12]
-Turkey	+	-	-	-	-	+	+	[06]
-Turkey	-	+	-	-	+	+	+	[06]
-Turkey	-	+	+	-	+	+	+	[08]
-Turkey	-	-	+	-	+	+	+	This study
Hb Beograd								
-Turkey	+	-	-	-	-	+	+	This study
-Turkey	-	+	-	+/-	+/-	+	+/-	This study

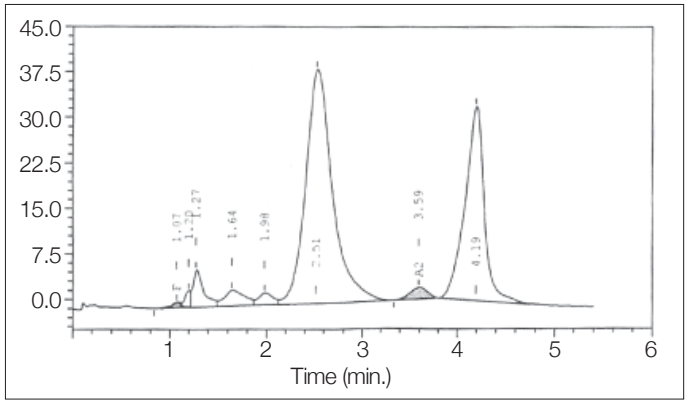

Figure 1. High performance liquid chromatography profile of Hb Beograd

case of Hb Beograd, there were two different haplotypes, as [+----++] and [-+-(+/-) (+/-) + (+/-)], in two unrelated families. For the homozygous Hb D-Los Angeles case, the related haplotype is a novel haplotype, as [--+-+++].

Discussion

Since the retention times of Hb D-Los Angeles [β121(GH4) Glu→Gln] and Hb Beograd [β121(GH4) Glu→Val] are almost similar as observed in D-window, they cannot be differentiated easily in premarital screening using HPLC. Laboratory identification of the Hb D-Los Angeles and Hb Beograd is not possible with electrophoretic and chromatographic techniques in premarital screening programs. As far as molecular identification is concerned, Eco RI restriction enzyme digestion protocol is also not sufficient. Since EcoRI digestion cannot differentiate Hb Beograd from Hb D-Los Angeles, as the mutation lies in the same codon, EcoRI/Tsp509I dual restriction enzyme digestion can be used to differentiate these variants. The restriction enzyme digestion protocol is convenient, rapid and feasible, especially in populations where these mutations are prevalent [7]. DNA sequencing is an important issue for the application of the exact determination of the abnormal hemoglobins, especially in the regions in which these types of hemoglobins are prevalent.

Hb Beograd cases show two different beta globin gene cluster haplotypes, as observed in Turkey. In the first case, Hb Beograd mutation is linked with the Mediterranean haplotype I [+----++] deduced from the family study. In the second case, although the family study could not be applied, the first three loci of the 5'-haplotype had a different structure compared to the Mediterranean haplotype I. These results show the different beta globin gene cluster haplotypes of the Hb Beograd cases in Turkey. Since there is no information about the previously published cases, the case could not be compared with the other Hb Beograd cases regarding their genetic relationships. Four different beta globin gene cluster haplotypes for the Hb D-Los Angeles mutation have been published [6,8-12]. In this study, we report the novel haplotype of [--+-+++]. The beta globin gene cluster haplotypes for the Hb D-Los Angeles and Hb Beograd in world populations are summarized in Table 2. The most common haplotype linked with the Hb D-Los Angeles in the world is Mediterranean haplotype I, as in Turkey. Three different haplotypes, [+----++], [-++-+++] and [-+--+++], were reported from different populations in Italy, Thailand, Mexico, Iran and Turkey [6,8-12]. The Asian and Mediterranean connections could be deduced only for the Mediterranean haplotype I and Thai haplotype, but the other two haplotypes are observed only in Turkey. These uncommon haplotypes could reflect different genetic origins and/or other molecular events like gene conversions or gene rearrangements. The presence of the other reported Turkish haplotypes should be studied in more detail at the molecular level in order to understand their mutation age, mutation mechanisms and genetic linkages.

In conclusion, laboratory analysis of the abnormal hemoglobins has a complex structure at the molecular level, emphasizing the importance of DNA sequencing, especially in the pre-

marital screening programs leading to prenatal diagnosis. In the case of prenatal diagnosis, molecular identification of the abnormal hemoglobins is an important issue in the genetic counseling, especially in regions like the Denizli province of Turkey. As far as genetic linkages of the beta globin mutations and the beta globin gene cluster haplotypes are concerned, this should be investigated with more genetic data to determine their connections and mutation mechanisms.

Acknowledgements

This study was supported by Pamukkale University Research Fund, Project Nos. 2005SBE001 and 2005SBE002.

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