

First observation of Hb Tunis [β 124(H2)Pro>Ser] in Turkey

Türkiye’de gözlenen ilk Hb Tunis [β 124(H2)Pro>Ser] olgusu

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Abstract

Hb Tunis [β 124(H2)Pro>Ser] was reported from Tunisia in 1988. This hemoglobin variant was detected by isoelectric focusing moving just ahead of Hb A. It cannot be identified by standard hemoglobin electrophoresis due to its similar mobility to Hb A. It has normal stability and oxygen affinity and does not produce any clinical symptoms. Here, we report a heterozygous Hb Tunis [β 124(H2)Pro>Ser] case discovered for the first time in Turkey in a premarital screening program. This hemoglobin variant can be identified with high performance liquid chromatography analysis confirmed with DNA sequencing. We emphasize in our study the importance of an interdisciplinary collaborative study at the provincial basis for the success of the hemoglobinopathy control program. (*Turk J Hematol 2010; 27: 120-2*)

Key words: Hemoglobinopathy, abnormal hemoglobin, Hb Tunis

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Özet

Hb Tunis [β 124(H2)Pro>Ser] ilk kez 1988 yılında Tunus’da bildirilmiştir. Bu hemoglobin varyantı alkali ve asit ortamda yapılan standart elektroforez yöntemleri ile ayıramamaktadır. İsoelektrik odaklama elektroforezinde (IEF-isoelectric focusing) ise Hb A’dan biraz daha hızlı bir davranış ortaya koymaktadır. Hb Tunis normal stabilite ve normal oksijen afinitesine sahip olup herhangi bir klinik belirti vermemektedir. Premarital tarama çalışmasında saptanan heterozigot Hb Tunis olgumuz, Türkiye ilk kez bildirilmektedir. Bu hemoglobin varyantı HPLC yöntemi ile ayrılabilmiş ve DNA dizi analizi ile doğrulanmıştır. Bu çalışmamızda ayrıca, hemoglobinopati kontrol programlarının başarılı biçimde uygulanabilmesi için yöresel düzeyde disiplinler arası işbirliğinin önemini vurgulamaktayız. (*Turk J Hematol 2010; 27: 120-2*)

Anahtar kelimeler: Hemoglobinopati, anormal hemoglobin, Hb Tunis

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Introduction

Many hemoglobin variants other than Hb S have been reported from Turkey [1,2]. In the Denizli province of Turkey, the most frequent hemoglobin variants are Hb D-Los Angeles and Hb G-Coushatta [3]. In addition to the reviewed hemoglobin variants in Turkey, Hb D-Ouled Rabah, Hb Yaizu and Hb Beograd have also been reported from the Denizli province [4-6]. Hb Tunis [β 124(H2)Pro>Ser] was reported from Tunisia by Mrad et al. in 1988 [7]. This hemoglobin variant was detected by isoelectric focusing (IEF) moving just ahead of Hb A. It has normal stability and oxygen affinity and does not cause any clinical symptoms. It is also not detectable by conventional hemoglobin electrophoresis. The Hemoglobinopathy Control Program has been applied in our province since 2005 in collaboration with the Turkish Ministry of Health Denizli Hemoglobinopathy Center and Pamukkale University Medical Faculty, leading to prenatal diagnosis in the cases of pregnancies at risk. We report herein the first observation of Hb Tunis [β 124(H2)Pro>Ser] hemoglobin variant in Turkey from the Denizli province determined during the premarital screening program.

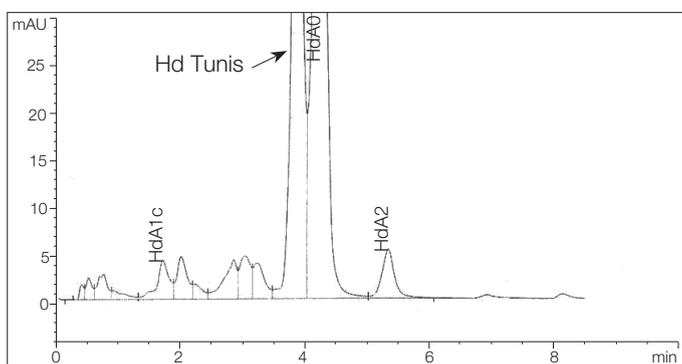


Figure 1. HPLC result of heterozygous Hb Tunis case

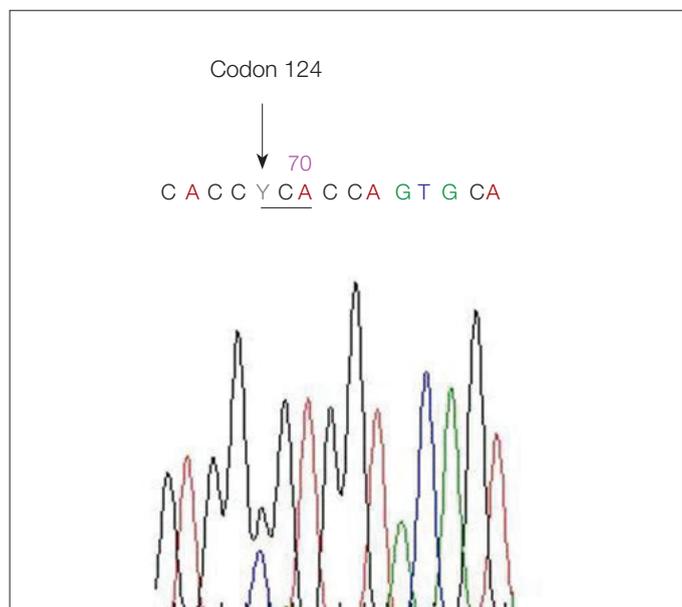


Figure 2. DNA sequencing of hemoglobin AX (beta 124, CCA>TCA, Y=C/T)

Case Report

We report in this study identification of the heterozygous Hb Tunis [β 124(H2)Pro>Ser] in a 21-year-old male living in the Denizli province, located in the Aegean region of Turkey. Written informed consent was obtained for the laboratory tests and DNA analysis from the proband, and the sample was deposited at the DNA Bank of the Department of Biophysics as an anonymous sample. Hemoglobin electrophoresis at alkaline and acid pH, DE-52 column chromatography, and non-radioactive fluorescence-based DNA sequencing as previously published were performed [8]. For the sequencing of the beta globin gene exon 3, the forward primer PAM600 (5'-CAA TGT ATC ATG CCT CTT TGC ACC-3') and reverse primer PAM603 (5'-CAC TGA CCT CCC ACA TTC CC-3') were used. High performance liquid chromatography (HPLC) was obtained with BioRad Variant II system, USA. Blood cell counts were done with Beckman Coulter AcT10 instrument, USA.

The hemoglobin variant could not be identified by alkaline or acid pH in agarose based electrophoresis and behaved like Hb A. Results of analysis of the red blood cell parameters were as follows: Hb 15.6 g/dl, RBC $5.50 \times 10^6/\text{mm}^3$, Hct 47.7%, MCV 87.0 fL, MCH 28.3 pg, MCHC 32.7 g/dl, and RDW 12.6%. In DE-52 microcolumn chromatography, Hb A₂ was eluted (3.1%) but Hb X could not be eluted with Hb S buffer (0.2 M glycine / 0.014 M NaCl / 0.01 % KCN). Changing buffer content in the NaCl concentration does contribute to the elution of Hb X. The Hb variant produces a double peak at the Hb A window slightly slower than Hb A in HPLC analysis, as shown in Figure 1. According to the HPLC results, the Hb ratios for Hb A₀, Hb X, Hb A₂ and Hb A_{1c} were 45.71%, 48.84%, 3.09% and 2.36%, respectively. DNA sequencing revealed the hemoglobin variant as a mutation at beta globin gene exon 3 codon 124 (CCA>TCA, Pro>Ser), as shown in Figure 2.

Discussion

In this study, Hb Tunis was identified by DNA sequence analysis, which confirmed the C-to-T mutation at the beta globin gene codon 124. Hb Tunis was reported once in the world populations by Mrad et al. [7] from Tunisia. To the best of our knowledge, our case is the second observation in the world population and is the first reported case in Turkey. This mutation causes the replacement of proline residue into serine. Proline has no ionizable groups, and all conformations like cis- and trans- are almost isoenergetic. When proline is in the protein structure, it cannot donate a hydrogen bond for stabilizing the protein structure. On the other hand, serine is a neutral polar molecule that has a tendency to remain on the surface of the proteins. The replacement proline into serine at beta globin codon 124 does not change its standard electrophoretic characteristics at alkaline and acid medium. Only IEF electrophoresis can detect Hb Tunis [β 124(H2)Pro>Ser] as a slightly faster moving band compared to Hb A [7] due to the slight difference in the isoelectric points of the proline and serine residues. The presence of serine also changes the chromatographic behavior of the hemoglobin molecule slightly. This

change cannot be observed in DE-52 microcolumn chromatography, but it can be detected by the HPLC system, which has resolution capacity. In the HPLC system, we observed a slightly slower peak at the Hb A window. This behavior is due to the weak interaction of the serine residue with the HPLC column matrix. In the HPLC system, the parameters should be standardized very carefully. If the experimental HPLC analysis parameters are not controlled and standardized, such hemoglobin variants can be bypassed easily.

Beta globin gene codon 124 resides in the external region of the hemoglobin molecule and is located in the $\alpha_1\beta_1$ contact region of the hemoglobin tetramer. During transition of the hemoglobin molecule from oxy-form to deoxy-form, the relative displacement of the β -chain to the α -chain is greater at the $\alpha_1\beta_2$ -contact than at the $\alpha_1\beta_1$ -contact [9]. Therefore, it is expected that the effect of the mutation on the structure and function the hemoglobin molecule is absent or very small and thus does not manifest in clinical symptoms. This heterozygous Hb Tunis case is an apparently healthy male. The place of the codon 124 with its characteristic features confirms this issue. Since homozygous Hb Tunis and other combinations with alpha and beta thalassemia are not yet reported, the clinical significance of this mutation remains unclear in such conditions.

Concerning the historical background, North African populations were affected by different Mediterranean populations due to the population movements. The Turkish influence was particularly notable in Tunisia and Algeria during the 14th-19th centuries [10]. Although molecular genetic data like beta globin gene cluster haplotypes is absent, Hb Tunis could have been introduced in the Denizli province gene pool due to these historical population movements.

In premarital screening programs, the nature of any hemoglobin variant detected by HPLC that is of potential clinical relevance should be confirmed by alternative techniques [11]. Premarital screening leading to prenatal diagnosis of the hemoglobinopathies has been applied in our province since 2004. Identification of the hemoglobin variants poses problems in the premarital control program in Denizli province due to the heterogeneous structure of the province. According to the results of our registered cases, Hb D-Los Angeles and Hb G-Coushatta were observed frequently [3]. Hb Beograd, Hb Yaizu and Hb D-Ouled Rabah have also been reported from our province [4-6]. We would also like to emphasize the importance of an interdisciplinary collaborative study at the provincial basis to facilitate the success of the hemoglobinopathy control program.

In conclusion, we report Hb Tunis [β 124(H2)Pro>Ser] for the first time in Turkey from the Denizli province. We emphasize that the exact identification of many hemoglobin variants can be easily bypassed with the application of limited laboratory techniques like standard hemoglobin electrophoresis and even with HPLC methods. The standardization of the protocols and

algorithmic approach used in hemoglobinopathy control programs is another important issue to be addressed.

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Conflict of interest

No author of this paper has a conflict of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included in this manuscript.

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