Case Report

Preliminary Identification of Hemoglobin Q-Iran in an Iranian Family from Central Province of Iran by Globin Chain Analysis on HPLC

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Abstract

Many abnormal α-chain hemoglobins (Hbs) are caused by single nucleotide mutations in α1- or α2-goblin genes. One of these Hbs is Hb Q-Iran which is resulted from a point mutation at codon 75 of the α1-globin gene (Asp→His). The identification of Hb Q-Iran was observed in two members of a family from the Central Province of Iran. In this study, Globin chain analysis on high performance liquid chromatography (HPLC) and DNA sequencing were applied. An unusual Hb variant, like HbS on alkaline pH electrophoresis was identified from samples of a father and his son from Arak city in the Central Province of Iran. The variant was further characterized by globin chain analysis and DNA sequencing methods. Globin chain analysis revealed an unknown globin chain peak after α-globin chain peak with a different retention time from β^s-globin chain, as the control in both samples. Genetic analysis led to the identification of an unknown Hb variant, Hb Q-Iran. Globin chain analysis showed the presence of an unknown globin chain, and likewise DNA sequencing revealed HbQ-Iran. In other words, Globin chain analysis procedure could preliminarily detect an unknown globin chain.

Keywords:α-globin variant, DNA sequencing, globin chain analysis,HbQ-Iran,Iran

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Introduction

More than 700 structural hemoglobin (Hb) variants have been depicted to date. Most of them are without clinical manifestations. These variants may be the representation of the globin chains with amino acid substitutions, insertions, deletions, fusion products, or cross-over products. The mutations may come about on α-chain, β-chain, or both of them.[1] Hb Q is an α-chain variant which was first described by Vella, et al. in a Chinese family.[2] This variant is caused by a point mutation in α1-globin gene (GAC→CAC; Asp→His).Three variants of HbQ are Q-India, Q-Thailand, and Q-Iran with the substitution at codon 64, 74, and 75, respectively.[3] Hb Q-Iran was identified by Rahimi and his colleagues in a family member from western Iran.[4]This variant may not cause any changes in hematologic parameters, as the involved residues are on the surface of the tetramer molecule. On the other hand, charge changes at these amino acids do not affect the characteristic of the Hb.[5–6] This Hb has the same electrophoretic migration like HbS, D, or G on alkaline pH electrophoresis. It also moves between Hb A and Hb S on citrate agar electrophoresis; thus, Hb Q-Iran band may easily be misinterpreted with Hb S band if solubility test or sickling test are not performed.[7]Hence, it is necessary to do further studies like DNA sequencing to identify this variant.[5]

In this study Hb Q-Iran was detected by Globin chain analysis on HPLC, and DNA sequencing method from a father and his son who were referred to our laboratory for detection of unknown Hb.

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Case Report

The cases included a 41-year-old man and his three-year-old son. They were presented to Globin Chain Biosynthesis Laboratory at Pasteur Institute of Iran to detect an unknown Hb variant. According to the hematologic findings (Table 1) and alkaline electrophoresis results obtained from another medical laboratory, the samples were detected as HbS, D, G, or Hb Q. Solubility test and sickling test results were also negative. Analysis of globin chains was performed by a high-performance liquid chromatography (HPLC) system on Mono-S 5/5 R column(Pharmacia, Uppsala, Sweden).[9] Globin chain analysis on HPLC revealed an unknown globin chain peak after α-globin chain peak with a different retention time from β^s-globin chain, as control in both cases (Figure1). To identify this variant, the samples were sent to Kariminejad-Najmabadi Genetics Center for molecular analysis. The samples of the patients were examined by automated direct nucleotide sequencing (ABI 377, Applied Biosystems, Foster City, California, USA) on the amplified α2- and α1-globin genes to characterize other nondeletional α□thalassemia determinants. Direct conventional sequencing revealed single G to C missense mutation (c.226G>C; *GAC>CAC*at codon 75) in the a-globin gene.[4] Genetic analysis in these cases led to the identification of a rare Hb variant, the Asp→His substitution in EF4, Hb Q-Iran.

Discussion

In Iran the prevalence of the hemoglobinopathies is unknown, since molecular techniques are not routinely used in many medi-

Table 1. Hematologic parameters.

Parameters	Father	Son
Age	41	3
RBC(10^{12}/L)	5.6	4.9
Hb (g/dL)	15.1	12.4
HCT (%)	44.2	37.3
MCV(fL)	79.1	76.4
MCH (pg)	27	25.4
MCHC (%)	34.2	33.2
Hb A (%)	65	70
Hb A2 (%)	2.8	2.6
Hb F (%)	0.8	0.8
Hb Q-Iran (%)	31.4	26.6
Sickling test	Negative	Negative
Solubility test	Negative	Negative

Hb = hemoglobin; MCHC= mean corpuscular hemoglobin concentration.

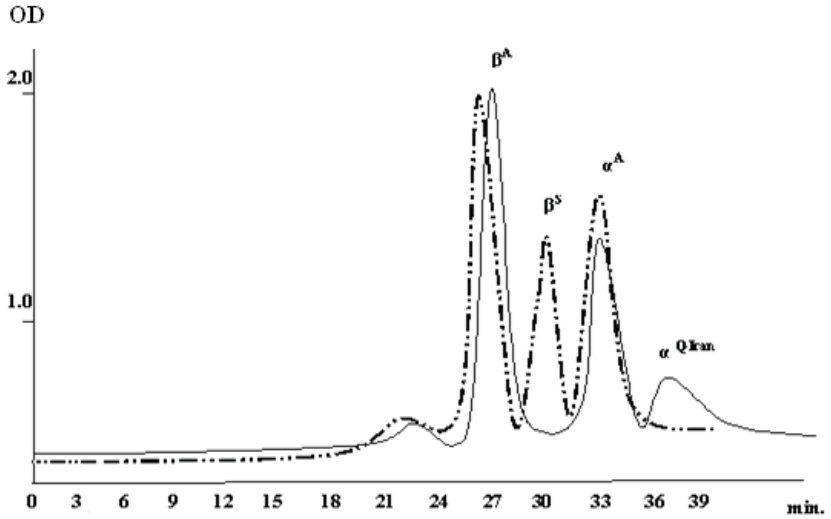

Figure 1. The chromatogram of globin chains on HPLC.

cal laboratories. Hence, a majority of unknown Hbs may not be revealed.

Conventional methods for diagnosis of abnormal Hbs are alkaline and acid electrophoresis. The disadvantage of these procedures is incomplete separation of the variants that have similar migration.[4]In this study HbQ-Iran migrated in the same zone of Hb S, D, or G on alkaline pH electrophoresis.[1]A globin chain analysis was done by HPLC method. It has been realized that the α-$^{Q\text{-Iran}}$ globin chain peak had different retention time when it was compared to retention time of β-^{S} globin chain peak. DNA analysis showed the presence of Hb Q-Iran. Hb Q-Iran is an α-chain variant which is mostly found in the heterozygous state. It shows a normal hematologic picture, because of its no changing tertiary structure.[5] The level of this Hb variant in the heterozygous state was reported 17%–19%.[10] In our report, the amount of Hb Q-Iran were 31.4% and 26.6%, in the father and his son, respectively (Table1).

Conclusion: Globin chain analysis procedure and DNA sequencing method could be used efficiently for identification of different Hb variants. In other words, Globin chain synthesis procedure could preliminarily detect an unknown globin chain.

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