



A Baby with Abnormal Hemoglobin of a Turkish Family with a Rare Case of Homozygote Hb G-Coushatta and Heterozygote Hb D

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Abstract

This report concerns the detection of Abnormal Hemoglobins (Hb) during premarital screening in a family of whom the woman was pregnant before marriage. The presence of an abnormal Hb was confirmed in both instances by hemoglobin chain studies. Structural studies determined the two Hb variants to be heterozygous mutation of Hb D-Los Angeles (HBB:p. Glu121Gln) for mother and homozygous mutation of Hb G-Coushatta (HBB:p.Glu23Ala) for father. The fate of the baby was followed until the first year and an abnormal silent Hemoglobin (Hb) was also detected on her sample. In this report a very rare Hb variant homozygous Hb G Coushatta and also a heterozygous baby of the couple with Hb G Coushatta and HbD is presented.

Keywords: Hb G-Coushatta; Hb D-Los Angeles; Abnormal hemoglobin

Introduction

A new hemoglobin variant, Hemoglobin (Hb) G Coushatta also known as G-Saskatoon (HBB:p. Glu23Ala), has been firstly found in three generations of a family from the Alabama Coushatta Indian tribe in east Texas in 1960s [1] and was subsequently identified in several populations throughout the world in Canadian Indians, Chinese, Algerian and Japans [2-5]. As it is found as prevalent variant in Native Americans and Chinese, haplotype studies were done and they suggested a multiple origin for this variant [6,7]. In Turkey it was firstly described in a Turkish male and then in a Turkish family in 1980s [8,9].

It is abnormal hemoglobin of beta variant with a delta-like substitution. Instead of a negative charged amino acid glutamate, neutral alanine at the 22nd position of beta chains in Hb G Coushatta forms a slow migrating band in alkaline electrophoresis [10,11]. So it is sometimes hard to discriminate the band with HbD or HbS.

Hb D Punjab, also known as Hb D Los Angeles, is an abnormal type of Hb with an amino acid substitution of glutamate to glutamine at codon 121 of the beta-globin gene (HBB:p. Glu121Gln) and it is one of the most commonly observed abnormal hemoglobin worldwide [12]. Its incidence has been reported throughout Turkey with an overall frequency of 0.2% [10]. We reported before Hb D as the second most common hemoglobinopathy after beta-thalassemia trait in Kayseri province, a city in Middle Anatolia, in Turkey, accounting for 0.36% of the total, in premarital screening [13].

The both hemoglobin variants are clinically silent or with slightly microcytosis. We here report a family; a rare case with homozygous hemoglobin G-Coushatta coupled with Hb D-Los Angeles who has an eight week pregnancy.

Case Presentation

A 25 year old male and 18 year old female admitted to Department of Biochemistry in Kayseri Education and Research Hospital for premarital screening. Firstly for separation and quantitation of hemoglobin types, gel electrophoresis (Interlab G26) was used. Full blood counts and red blood cell indices in this family were determined by the Sysmex XN-9000 semi-automated hematology analyzer (Sysmex Co., Kobe, Japan). Two abnormal bands between HbA₀ and HbA₂, on HbS/D zone

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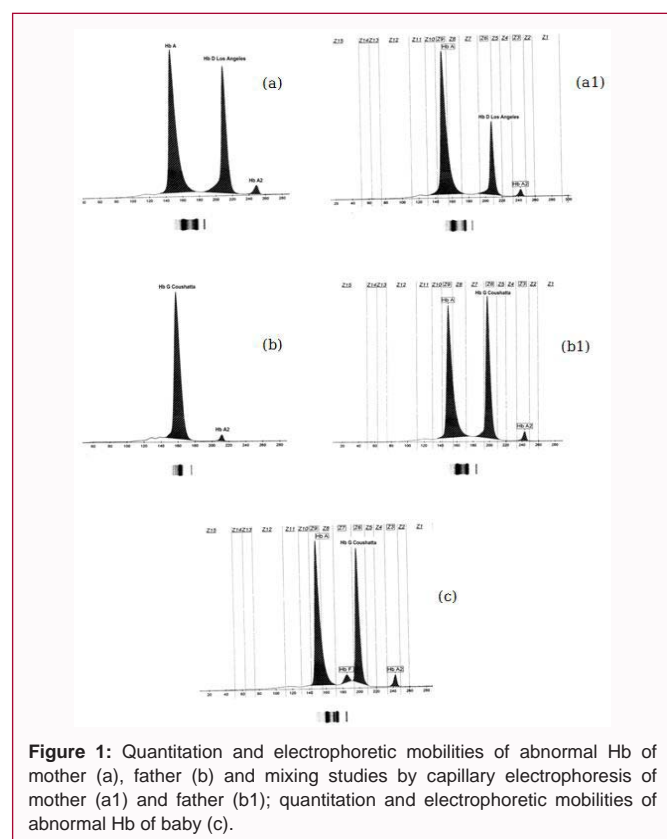


Figure 1: Quantitation and electrophoretic mobilities of abnormal Hb of mother (a), father (b) and mixing studies by capillary electrophoresis of mother (a1) and father (b1); quantitation and electrophoretic mobilities of abnormal Hb of baby (c).

were detected at the same gel. The abnormal hemoglobin bands were then confirmed with both cation exchange ultra-high performance liquid chromatography (UHPLC) (Thermo Scientifics Dionex Ultimate 3000, UHPLC) using Recipe Chemicals (Instruments GmbH, Germany) and capillary electrophoresis (Sebia Hydrasys, France). In molecular analysis the coding exon deoxyribonucleic acid from the patients were amplified by polymerase chain reaction and sequenced. For deoxyribonucleic acid sequencing and fragment sizing molecular analysis ABI 3500/Life Technologies Series Genetic Analyzer (Thermo Fisher Scientific) was used.

Results

The hematological and Hb data are summarized in Table 1. In gel electrophoresis we detected the abnormal hemoglobins of mother and father migrating as Hb S/Hb D. In capillary electrophoresis the mother had an abnormal Hb at a level of 39.9% of total Hb, in

Zone 6 suggesting Hb D; and the father had an unknown Hb variant at a level of 97.7 % of total Hb, between Zone 5 and Zone 6, an unknown hemoglobin variant (Figure 1). In UHPLC the unknown hemoglobin of mother eluted just slightly after Hb A₂ with a retention time of 6.64 minutes, while the unknown Hb of father eluted in a large peak containing Hb A₂ with a retention time of 6.01 minutes.

As both male and female had abnormal hemoglobins during premarital screening the couple was invited to the meeting for information before giving birth. In the meeting it was learned that the women was pregnant for 8 weeks. The couple was suggested for molecular analysis and genetic counseling.

Molecular analysis of father had homozygosity at codon 68 of the beta-globin gene, an amino acid substitution of glutamate to alanine (p.Glu23Ala), called as Hb G-Coushatta (beta22(B4)Glu-->Ala, HBB:c.68 A>C). And the mother revealed a heterozygosity at codon 364 of the beta-globin gene, an amino acid substitution of glutamate to glutamine (p.Glu121Gln) called as Hb D-Los Angeles (beta 121(GH4) Glu-->Gln, HBB:c.364G>C) (Figure 2a and b, respectively).

All parameters of red cell indices and hemoglobin composition of baby was in reference range after birth. So, the fate of the baby was followed until the first year and an abnormal silent Hemoglobin (Hb) was also detected on her sample. Her molecular analysis revealed heterozygous Hb G Coushatta (Figure 2c).

Discussion

Hemoglobinopathies are commonly present in populations of all Mediterranean countries, and are also common in Turkey. A review of abnormal hemoglobins reported from Turkey indicated that in addition to beta-thalassemia major, sickle cell anemia and sickle cell/β-thalassemia are major causes of public health problems [14]. HbD and Hb O-Arab are prevalent abnormal hemoglobins in Turkey with a silent clinical state after Hb S. Although Hb G Coushatta is thought to be rare in some regions it is reported to be more than expected [7]. The major problem of the reported incidence of this Hb may be disability of separation of these Hemoglobin's with Hb D and Hb Q Iran because of the same electrophoretic mobility in alkaline pH [14]. However, in this study our suspicion for the hemoglobin of father because of the homozygosity in alkaline gel electrophoresis, we could have been able to discriminate the abnormal hemoglobin with other hemoglobins by both UHPLC and capillary electrophoresis.

Presumed mutation for Hb G Coushatta is GAA->GCA at codon 68 in beta chain and is hematologically normal in heterozygote state.

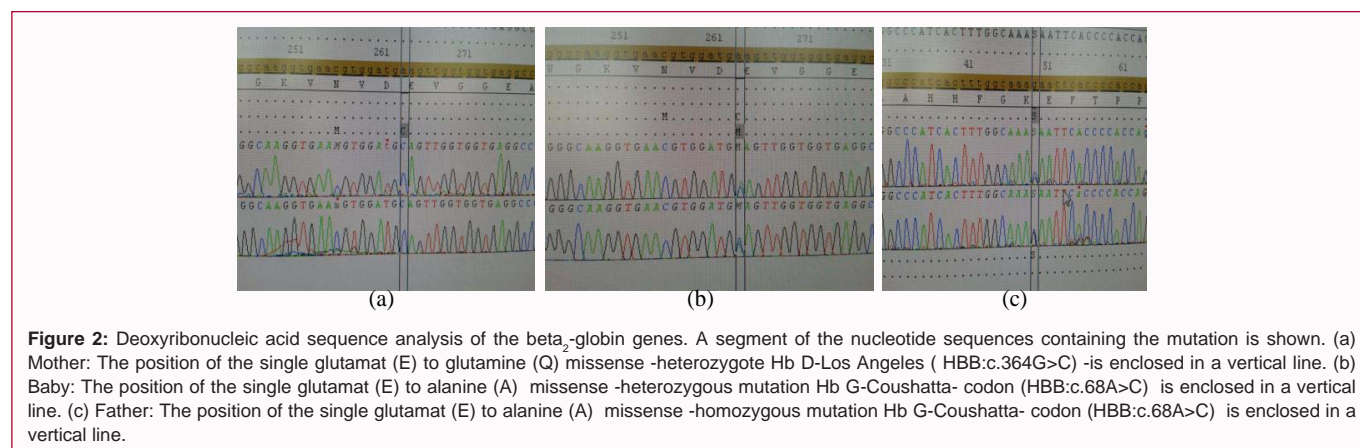


Figure 2: Deoxyribonucleic acid sequence analysis of the beta-globin genes. A segment of the nucleotide sequences containing the mutation is shown. (a) Mother: The position of the single glutamat (E) to glutamine (Q) missense -heterozygote Hb D-Los Angeles (HBB:c.364G>C) -is enclosed in a vertical line. (b) Baby: The position of the single glutamat (E) to alanine (A) missense -heterozygous mutation Hb G-Coushatta- codon (HBB:c.68A>C) is enclosed in a vertical line. (c) Father: The position of the single glutamat (E) to alanine (A) missense -homozygous mutation Hb G-Coushatta- codon (HBB:c.68A>C) is enclosed in a vertical line.

Table 1: Red cell indices and hemoglobin composition for the members of studied family.

Complete Blood Count	Father	Mother	Baby
Sex	Male	Female	Female
Age (years)	25	18	1
HbA0 (%)	0	56.2	55.4
HbA2 (%)	3.20	1.90	3.1
HbF (%)	0.00	0.00	2.7
HbD (%)	0.00	39.90	-
HbG-Coushatta (%)	97.70	0.00	38.8
White Blood Cells (x10 ³ /μl)	6.72	7.54	9.15
Red Blood Cells (x10 ⁶ /μl)	6.32	5.02	5.02
Hemoglobin (g/dl)	17.4	13.4	12.4
HCT (%)	52.4	40.1	36.7
MCV (fl)	82.9	79.9	73.1
MCH (pg)	27.6	26.7	24.7
MCHC (g/dl)	33.3	33.5	33.8
PLT (x10 ³ /μl)	282	316	394
RDW-SD (fl)	40.5	39.8	8.7
RDW-CV (%)	13.8	14.4	13.6

For that reason it is usually found during HbA1c measurement by chromatographic techniques which are dissociated with blood glucose levels [14,15]. Here we report a rare form of homozygous Hb G Coushatta at a level of 97.7 % of total Hb.

In Hb G Coushatta there is a neutral alanine instead of negative charged amino acid glutamate at the 23rd position of beta chains. Usually such a replacement would affect the distribution of charges and disturb the functional value of molecule. However 23rd position of beta chain is not connected with heme or other globin chains. So it is expected that there is no unusual effect due to such a replacement. Our patient with homozygous Hb G Coushatta was also clinically silent, consistent with these expectations. Although we cannot predict for sure, we presume that the homozygous form of Hb G Coushatta, or a combination of this variant with Hb D would not be associated with severe conditions. However, exact determination of these variants by molecular technique is important.

Both Hb G Coushatta and Hb D migrate slowly and it is hard to discriminate them with each other and Hb S by alkaline gel electrophoresis. In HPLC and capillary electrophoresis we found a slight sliding in Hb G Coushatta according to Hb D which is hard to discriminate visually. Nevertheless, some variants cannot be separated and are eventually identified by DNA sequencing if they reveal unexplained hematological anomalies. This is an important issue for an analytical decision to be taken when abnormal hemoglobins are suspected. In this case, the homozygous Hb G Coushatta and heterozygous Hb D were found by chance during a premarital screening program. Even though they both did not reveal any hematological or clinical sign, the women was pregnant and not to miss any heterozygous state of these combinations with a probability of hematologic disease molecular analysis was applied. As there was no literature finding of heterozygote state of Hb G Coushatta and Hb D, the baby was investigated for possible hemoglobinopathy.

Conclusion

Different hemoglobinopathies are frequently reported from

Turkey, but this is the first report of a very rare homozygous Hb G Coushatta mutation married with a heterozygous Hb D and got a baby with heterozygous Hb G Coushatta, those all have no clinical sign. The members of the family those will born in future may have different abnormal hemoglobins with heterozygous state. Because of the same electrophoretic mobility in alkaline pH, Hb G Coushatta can be missed and not separated with other hemoglobins like Hb D and Hb S. But it is possible to discriminate them by UHPLC and capillary electrophoresis with a careful examination.

Consent

Written informed consent was obtained from the family for publication of this report and any accompanying images.

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