Case Reports in Women's Health 28 (2020) e00265

Contents lists available at ScienceDirect

Case Reports in Women's Health

journal homepage: www.elsevier.com/locate/crwh

Fetal heterozygosity for both Hb G-Hsi-Tsou and beta thalassemia: A case report

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ARTICLE INFO

Article history:
Received 7 October 2020
Received in revised form 12 October 2020
Accepted 14 October 2020

Keywords:
Case report
Hb Hsi-Tsou
Beta thalassemia
Compound heterozygosity
Pregnancy

ABSTRACT

Introduction: This case report describes a fetus with compound heterozygosity for Hb G-Hsi-Tsou and beta thalassemia, diagnosed in a healthy pregnancy. To the best of our knowledge, this is the first documented case of compound heterozygosity and the woman is the second known case of heterozygosity for Hb G-Hsi-Tsou.
Case presentation: A 34-year-old woman during her first pregnancy underwent hemoglobin electrophoresis which revealed heterozygosity for Hb G-Hsi-Tsou. Hemoglobin G-Hsi-Tsou constitutes a hemoglobin variant with a structural abnormality of the beta chain, first described in 1972, but since then no other cases have been reported. After finding out that her husband was heterozygous for beta thalassemia, chorionic villus sampling revealed the embryo's heterozygosity for both Hb G-Hsi-Tsou and beta thalassemia. Due to lack of scientific data, the couple decided to end the pregnancy.
Conclusion: It was not possible to determine whether the fetus would present serious deficiencies in hematopoiesis, as Hb G-Hsi-Tsou is a variant which is not yet fully understood. What made this case even more complex was the simultaneous presence of the beta thalassemia allele.
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1. Introduction

Hemoglobin is the molecule responsible for transporting oxygen from the lungs to the tissues and eases the return of carbon dioxide. In the arterial circulation, hemoglobin has a high affinity for oxygen and a low affinity for carbon dioxide, whereas in the venous circulation, affinity status is the opposite [1]. In structure, hemoglobin is a tetramer, that is, it compromises four subunits, each with a polypeptide chain [1]. The normal chains of an adult are the alpha and beta chains, which have different amino acid sequences and these constitute hemoglobin A ($\alpha 2\beta 2$). There are two genes responsible for encoding the alpha chain (HBA1 and HBA2), both located on chromosome 16 [2]. The beta chain is encoded by the beta gene (HBB) and is found at chromosome 11.

'Hemoglobinopathies' is the term used to describe structural abnormalities of the globin proteins themselves (qualitative anomalies). Thalassemias constitute a type of hemoglobinopathy which mostly include reduction of the number of normal globin proteins (quantitative anomalies). A great number of pathological hemoglobin (Hb) variants have been described, occurring from alterations, usually base substitutions, of several codons responsible for either the alpha or the beta chain. Hemoglobinopathies follow the Mendelian law of inheritance, as every person obtains one allele for alpha and beta chain genes, from each parent. Hemoglobin electrophoresis is an examination done in the first trimester of pregnancy in most countries, in order to indicate whether there are any abnormal types of hemoglobin caused by genetic disorders. If a pregnant woman is found to be positive for any hemoglobin variant, the father is also tested in order to determine the possibility of having a fetus with inherited mutated globin genes.

Compound heterozygosity is the inheritance of two or more heterogeneous recessive alleles at a particular locus. These are unrelated alleles, and so these people are technically are heterozygotes, but if both are defective they can cause different aspects of a disease. In these forms, the disease may be milder, as the mutations involved are often less detrimental. Widespread confirmation of compound heterozygosity was not feasible until the 1980s, when PCR for DNA amplification made it cost-effective to sequence genes and identify polymorphic alleles.

Hemoglobin G-Hsi-Tsou constitutes a variant with a structural abnormality of the beta chain. More specifically, after sequencing, an

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A- > G mutation was detected at codon 79, resulting in the change of GAC- > GGC. As a result, there is a substitution of aspartic acid for glycine at position 79, creating an anomalous βT9 peptide. This mutation causes a mildly high oxygen affinity, normal Bohr effect and no erythrocytosis [3].

We present the first case of compound heterozygosity for Hb G-Hsi-Tsou and beta thalassemia in a fetus. The parent elected for termination of pregnancy, following genetic counseling.

2. Case Presentation

A 34-year-old primigravida attended the obstetrics outpatient clinic after having a positive pregnancy test, in order to have a first-trimester examination. Her BMI was 22.2 and she was a smoker before pregnancy. Medical history included asthmatic bronchitis (treated with budesonide with formoterol), peptic ulceration and irritable bowel syndrome. Her mother's obstetric history was uncomplicated; her sister's included fetal growth restriction in her first pregnancy, which was attributed to thrombophilia and was successfully treated during the second gestation, with prophylactic low molecular weight heparin.

Transabdominal ultrasound showed a single intrauterine gestational sac, with a viable pregnancy. Physical examination showed no abnormal findings.

In the context of routine early pregnancy screening, several tests were performed. All tests were normal, with the exception of hemoglobin electrophoresis. Hematological examination did not show any signs of anemia, as the erythrocyte count was 4.94 M/μl, hemoglobin 13.6 g/dL and hematocrit 41.9 per cent. The MCV was 84.7 fl, the MCH was 27.5 pgr, and the MCHC was 32.4 per cent (Fig. 1).

Hemoglobin electrophoresis revealed an unknown hemoglobin variant (Position 2; Fig. 2). The analyzing method used was agarose gel electrophoresis at alkaline pH. The relative portion of the unknown variant, as shown in Table 1, was 38.8%, whereas HbA was 57.4% and HbA2 2.5%. Sickle cell anemia screening was negative.

Molecular genetic testing was performed using a peripheral blood smear, in order to identify the unknown hemoglobin variant. The methods used were GAP-PCR, high-resolution melt (HRM) analysis and nucleotide sequence analysis. Sequencing of beta globin genes revealed heterozygosity for hemoglobin G-Hsi-Tsou. In detail, a substitution at codon 79 of the beta chain was detected (A > G) in one allele, resulting in the replacement of aspartic acid by glycine. The patient's first-degree relatives were then screened for this variant. It was found that only the patient's father was a carrier of Hb G-Hsi-Tsou and he was also asymptomatic.

Due to these findings, the patient's husband was referred for electrophoresis, which showed heterozygosity for beta-thalassemia. As the

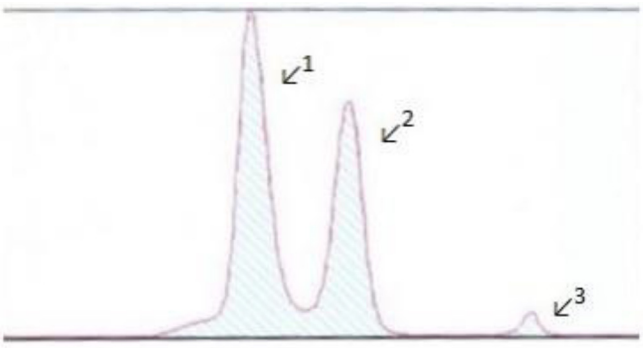

Fig. 2. Hemoglobin fractions after electrophoresis in agarose gel reveals an unknown variant (Fraction 2). Fraction 1 represents HbA and equals 57.4%. Fraction 2 cannot be identified and is 38,8%. Fraction 3 is HbA2 and has a normal fraction of 2,5%.

Table 1
Percentage of each hemoglobin variant found in our patient's electrophoresis.

Fractions	%	Ref. Range
HbA.....:	57.4	96.7–98
HbF.....:	1.30	0.00–2.00
HbA2.....:	2.50	1.50–3.20
Hb?	38.8	

TEST	RESULT/UNITS	REF. RANGE
WBC	9.3 K/μl	4.5-10.5
NE%	58.8 %	40.0 - 75.0
LY%	33.4 %	20.0 - 45.0
MO%	5.2 %	2.0 - 10.0
EO%	1.7 %	1.0 - 6.0
BA%	0.9 %	0.0 - 2.0
NE#	5.5 K/μl	1.50 - 6.50
LY#	3.1 K/μl	1.20 - 3.80
MO#	0.5 K/μl	0.20 - 1.00
EO#	0.2 K/μl	0.00 - 0.70
BA#	0.1 K/μl	0.00 - 0.20
RBC	4.94 M/μl	3.80-5.40
HGB	13.6 g/dL	11.5 - 15.5
HCT	41.9 %	36.0-46.0
MCV	84.7 fl	79.0 - 98.0
MCH	27.5 pgr	26.0 - 32.0
MCHC	32.4 %	32.0 - 36.0
RDW-CV	14.3 %	11.5 - 14.5
RDW-SD		
PLT	239 K/μl	140 - 440
PDW	16.5 fl	12 - 18
MPV	9.8 fl	7.0 - 10.5
P-LCR		

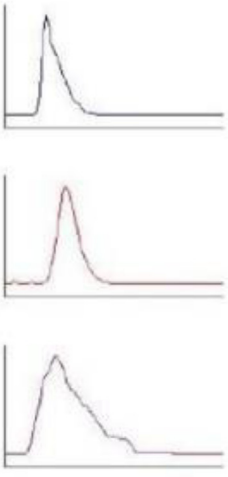

Fig. 1. Complete blood count of our patient during first trimester routine screening exams. There is no indication of anemia or any other pathology, as all components have normal values: Red blood cells are 4.94 M/μl, hemoglobin 13.6 g/dL, hematocrit 41.9%, Platelets 239 K/μl, MCV 84.7 fl, MCH 27.5 pgr and MCHC was 32.4%. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Family's genotype at first pregnancy.

Mother's genotype:	CD79 A > G (Hb G-Hsi-Tsou)/ N1
Father's genotype:	IVSI-6 T > C/ N1
Embryo's genotype:	CD79 A > G (Hb G-Hsi-Tsou)/ IVSI-6 T > C

The family's genotype after results of genetic analysis.

Table 3
Family's genotype at third pregnancy.

Mother's genotype:	CD79 A > G (Hb G-Hsi-Tsou)/ N1
Father's genotype:	IVSI-6 T > C/ N1
Embryo's genotype:	CD79 A > G (Hb G-Hsi-Tsou)/ N1

The family's genotype at the second pregnancy, according to genetic analysis.

gestational age measured by the embryo's CRL was 10 weeks (in accordance with the last menstrual period), chorionic villus sampling (CVS) was recommended in order to ascertain fetal genotype. Genetic analysis of the chorionic villi revealed the presence of heterozygosity for double hemoglobinopathy of the embryo, as alleles for both Hb G-Hsi-Tsou and beta thalassemia were present ([Table 2]).

The couple were referred for genetic counseling. Due to lack of scientific data concerning Hb G-Hsi-Tsou and the possible effects of the presence of both pathological alleles on the fetus, the couple decided to terminate the pregnancy.

One year later, the patient had a second pregnancy. Transvaginal ultrasound showed a single intrauterine gestational sac. The pregnancy resulted in spontaneous abortion at 5 weeks. The parents refused histological examination of the products of conception.

In view of the patient's sister's history of thrombophilia, she underwent thrombophilia testing. Screening for both inherited and acquired thrombophilias was negative.

Two months later, the patient had a third pregnancy. At 11 weeks and 3 days of gestation, CVS was performed. Molecular karyotyping with array-CGH showed the genomic profile of female gender and the absence of any chromosomal disorder. Results of genetic analysis were compatible with heterozygous hemoglobinopathy G-Hsi-Tsou (Table 3).

3. Discussion

Hemoglobin G-Hsi-Tsou constitutes a pathological Hb variant which is not well studied, nor well understood, as only one case of heterozygosity for Hb G-Hsi-Tsou has hitherto been reported in the medical literature. Hemoglobin G-Hsi-Tsou was first described in 1964, in two Chinese sisters, in Taiwan [3]. They were both heterozygotes; therefore, both were asymptomatic. Analyses from this case have shown that the mutation responsible for this variant is the change of GAC-> GGC, resulting in the substitution of aspartic acid for glycine, at position 79 of the beta chain [3]. This substitution creates an anomalous βT9 peptide. The only other information known for Hb G-Hsi-Tsou, reported by Blackwell et al., concerns electrophoresis of this variant. A heterozygote at pH 8.9 has both HbA and Hb Hsi-Tsou present, with the later presenting an abnormal β chain and therefore, electrophoretically, the new peptide presents a slower mobility in alkaline pH [3]. This mutation causes a mildly high oxygen affinity, normal Bohr effect and no erythrocytosis. Two other variants with amino-acid substitution at position β79 have been reported, namely Hb Tampa (Asp- > Tyr) [4] and Hb Tigraye (Asp- > His) [5]. Other properties of Hb G-Hsi-Tsou, such as the phenotype and possible morbidity of homozygosity of the pathological allele, remain unexplored.

In our case report, the fetus in the first pregnancy was heterozygous for both Hb G-Hsi-Tsou and beta thalassemia. Heterozygosity for beta thalassemia is due to expression of one mutated beta-globin gene, resulting in underproduction of beta chains of HbA [6]. Heterozygosity for Hb G-Hsi-Tsou also affects the beta globin, but in this variant, it is the quality of beta chain that is impaired, not the quantity. Therefore, it becomes apparent that compound heterozygosity would result in one allele coding lower beta globin production, but of normal molecular structure, and the second allele coding physiological beta globin quantity, but with a modified composition. However, what remains to be clarified is whether this specific structural variation of HbA, if coexisting with lower production of normal beta chains, would in reality cause any clinical or biochemical pathology, and if so to what extent.

Hemoglobinopathies result in variable phenotypes ranging from severe anemia to clinically asymptomatic individuals. There are hemoglobin variants which in heterozygous genotypes alone cause mild or no symptoms, but when they coexist with another anomalous beta chain gene, the phenotype adversely affects quality of life. On the other hand, some variants do not cause any pathology and even homozygosity or double heterozygosity with other alleles, constituting a normal variation without any clinical symptomatology. Taking the example of sickle cell thalassemia, patients may suffer from a wide range of conditions: anemia, repeated infections, frequent episodes of pain or even pulmonary hypertension, stroke and acute chest syndrome [7]. Prognosis largely depends on the amount of normal hemoglobin produced; the condition is generally associated with a shorter life expectancy [7]. On the other hand, taking the example of Hb Iran, a variant resulting from a point mutation at position 22 GAA-> CAA (Glu–>Gln) [8], homozygosity of a qualitative hemoglobin variant may create a normal phenotype. Thus, all cases of heterozygous (25 cases) and homozygous (2 cases) of Hb Iran were asymptomatic, with differences in the hematological profile, as the latter presented mild anemia, similar to thalassemia minor [8]. What is worth mentioning from this study, in relation to our case, is a patient heterozygous for both Hb Iran and beta thalassemia, whose symptomatology included anemia, fatigue and weakness.

Thus, it is unknown whether the fetus in the first pregnancy would have serious deficiencies in hematopoiesis. In our case report, both alleles concerned the beta chain, and both in heterozygous form do not cause any signs or symptoms. Taking into account that no data concerning the phenotype of Hb G-Hsi-Tsou are available as no cases of homozygosity have been reported, fetal clinical outcome is unknown. However, the example of Hb Iran demonstrates that hemoglobin variants, even in homozygous form, can present a silent phenotype by not causing any deficiencies. Nevertheless, genetic counseling cannot be based on examples and hypotheses, as every variant can differ significantly from the other and the phenotype can be unpredictable, especially in double heterozygosity with beta thalassemia. This case demonstrates the need for further examination and analysis of hemoglobin variants, their structure, stability and oxygen affinity. Beta thalassemia carriers are frequent in several countries, especially in the Mediterranean and the Aegean regions. Most pregnant women undergo hemoglobin electrophoresis in the context of routine screening during the first trimester and it is then that several hemoglobinopathies are discovered. As the patient, her father and fetus were heterozygous for Hb G-Hsi-Tsou, it is safe to conclude that this variant presents a Mendelian type of inheritance. Taking into consideration the above, it is possible that many pregnant women will face the same dilemma where there is compound heterozygosity of the embryo for unknown hemoglobin variants.

Contributors

The authors organized the visits and follow-up of this patient and interpreted the results. All authors contributed to planning, data collection and writing the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

No financial compensation was received for this case report.

Patient Consent

Consent for publication was obtained from the patient.

Provenance and Peer Review

This case report was peer reviewed.

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