ISSN: 2635 - 2826

Journal of Clinical & Biomedical Research

SCIENTIFIC Research and Community

Case Report

Open Access

Biochemical Investigation Limits in Recognizing Some Abnormal Hemoglobin Types: Hemoglobins O Arab And S

Domenico Dell'Edera[1*], Francesca Mangia[1], Rosalba Ardea Dell'Edera[2], Maria Teresa Dell'Edera[2], Francesco Larocca[1], Annunziata Anna Epifania[1] and Arianna Allegretti[1]

[1]Unit of Laboratory of Medical Genetics, "Madonna delle Grazie" Hospital, Matera, Italy

[2]Universitatea de Vest Vasile Goldis din Arad Facultatea de Medicină, Arad, Romania

ABSTRACT

Hemoglobinopathies are a major health problem worldwide. These disorders are characterized by a variable clinical and hematological situation related to a phenotypic heterogeneity. Moreover, in order to have an exact correlation between the biochemical picture and the genetic defect associated with it, it is useful and often indispensable the molecular study of alpha and beta globin genes. The present case report concerns a pregnant woman of Moroccan ethnicity who came to our observation to undergo combined first-trimester testing useful for screening of major chromosomal aneuploidies. Study of hemoglobins A, A2, and F by High performance liquid chromatography (HPLC) revealed the presence of a proportion of abnormal hemoglobin associated with hemoglobin S. Molecular investigation of globin genes excluded that the biochemical variant in question was related to hemoglobin S. In fact, molecular investigation of beta globin genes, found that the observed variant was due to the presence of a genetic defect leading to hemoglobin O Arab synthesis. Evidence suggests that molecular analysis of globin genes provides the most effective and correct way to correlate the detected biochemical picture with its associated genetic defect. The only biochemical study in the presented case determines an incorrect clinical evaluation with consequent inaccurate prognosis. The mutation detected in this work can be identified using a simple and inexpensive kit. This would generate, in economic terms, significant savings associated with a correct diagnosis.

***Corresponding author**

Domenico Dell'Edera,Unit of Medical Genetics Hospital "Madonna delle Grazie" Matera, Italy. Tel: 0835253439. E-Mail: domenicodelledera68@gmail.com

Received: September 16, 2021; **Accepted:** September 23, 2021; **Published:** January 03, 2022

Keywords: Hemoglobinopathies, Hemoglobins A (HbA), Hemoglobins A2 (HbA2), Hemoglobins F (HbF), Hemoglobins S (HbS), Hemoglobin O Arab (HbO Arab).

Background

Hemoglobinopathies are a heterogeneous group of diseases with autosomal recessive transmission. Mutations in beta globin genes can lead to changes in amino acid sequence with heterogeneous clinical effects.

This paper aimed to highlight how the study based on biochemical data alone, in some cases, is not reliable to correctly highlight the related genetic defect. This could lead to an incorrect clinical assessment resulting in an inaccurate prognosis [1,2].

The molecular study of alpha and beta globin genes is essential to have a correct relationship between the detected biochemical picture and the associated genetic defect [3].

Case presentation

The present case report concerns a pregnant woman of a thirty-one-years-old patient of Moroccan ethnicity who came to our observation to undergo combined first-trimester combined test was performed at 12 weeks of gestation. The final risk was 1:1000 for trisomy 21. Study of hemoglobins HbA, HbA2, and HbF by HPLC revealed the presence of a proportion of abnormal hemoglobin associated with HbS. The presence of HbS, in homozygous condition, is associated with sickle cell anemia.

The history taken of the husband did not reveal that he had a biochemical picture associated with malfunctioning beta globin genes. To be certain of what we observed, we repeated hematologic screening and molecular study of globin genes to the couple.

Methods

The examinations repeated to the couple are as follows:

- Blood films for erythrocytes morphology.
- Detection of red cell indices with Sysmex XE 2100 automated cell counter (Dasit Cornaredo, Milan, Italy): red blood cells count (RBC), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin levels (MCH), mean corpuscular hemoglobin concentration (MCHC).
- High performance liquid chromatography (HPLC) to quantify hemoglobin subtypes in the blood samples through Tosoh HPLC G 8 system (Tosoh Bioscience S.r.l. – Turin, Italy).
- Assays of serum iron and serum ferritin through monitoring of their levels by Elecsys 2010 (Roche Diagnostics GmbH).
- molecular analysis of the alpha and beta globin genes:

Genomic DNA Preparation

The molecular analysis of the globin genes was performed following the DNA isolation, starting from 25 µl of blood, using the extraction kit of Promega Italy S.r.l. (DNA IQTM System, cod.C6701).

Study of the Globin Genes

The polymerase chain reaction (PCR) and reverse-hybridization was performed. The procedure includes two steps:

PCR amplification using biotinylated primers and hybridization of amplification products to a test strip containing allele-specific oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrates. The amplification and the reverse hybridization on a strip were obtained with the use of commercial kits produced by Nuclear Laser Medicine, Milan Italy. The genetic tests were aimed at checking 39 mutations in the β-globin genes (code n. AC104 and AC91 – figure 1A and 1B): -101[C>T]; -92[C>T];-86 [C>A]; -87[C>G]; -30[T>A]; -29 [A>G]; cap+1[A>C]; cap+33 [C>G]; HbG San José: 23 [A>G]; HbD Ouled Rabah: 60 [C>A]; HbE: 79 [G>A]; HbG Copenaghen: 142 [G>A]; Hb Camperdown: 315 [G>C]; HbD-Punjab: 364 [G>C]; HbO Arab: 364[G>A]; Hb Neapolis: 380 [T>G]; Hb Lepore-BW: [NG_000007.3: g63632_7104del]; Hb Siciliana (δβ)-O [NG_000007.3: g64336_77738del13403]; codon 5[-CT]; HbC: codon 6[G>A]; HbS: codon 6[A>T]; codon 6 [-A]; codon 8[-AA]; codon 8/9[+G]; codon 30 [G>C]; IVS 1.1 [G>A]; IVS 1.2 [T>A]; IVS 1.5[G>C]; IVS 1.6[T>C]; IVS 1.110[G>A]; IVS 1.116[T>G]; IVS 1-25; codon 39[C>T]; codon 44[-C]; codon 76 -C; IVS 2.1[G>A]; IVS 2.654 C>T; IVS 2.745 [C>G]; IVS 2.844[C>G].

The genetic tests were aimed at checking 21 mutations in the α-globin genes (code n. AC099): 3.7 single gene deletion, 4.2 single gene deletion, MED double gene deletion, SEA double gene deletion, CAL double gene deletion, FIL double gene deletion, 20.5kb double gene deletion, anti-3.7 gene triplication, α1 cd 14 G>A, α1 cd 59 (Hb Adana), α1 CD 142 Hb Hasharon, α2 init cd, α2 cd 19 [-G], α2 IVS1 [-5nt], α2 cd 59 (Hb Adana), α2 cd 125 (Hb Quong Sze), α2 cd 142 (Hb Constant Spring), α2 cd 142 (Hb Icaria), α2 cd 142 (Hb Pakse), α2 cd 142 (Hb Koya Dora), α2 poly-A1[AATAAA-AATAAG], α2 poly-A2[AATAAA-AATGAA].

Results

The outcome of biochemical and molecular tests excluded that the husband had a globin gene alteration.

Abnormal hemoglobin was detected in the wife and was identified as "variant HbS (β6 Glu→Val)" by HPLC (Figure 1). This last datum suggested that the pregnant woman was a healthy carrier of sickle-cell anemia. Molecular analysis of the globin genes revealed that the variant highlighted by HPLC was not due to the presence of hemoglobin S, but to the presence, in heterozygosity, of hemoglobin HbO Arab (β121 Glu→Lys) (figure 2A and figure 2B). In the condition of heterozygosity, HbO Arab is completely silent.

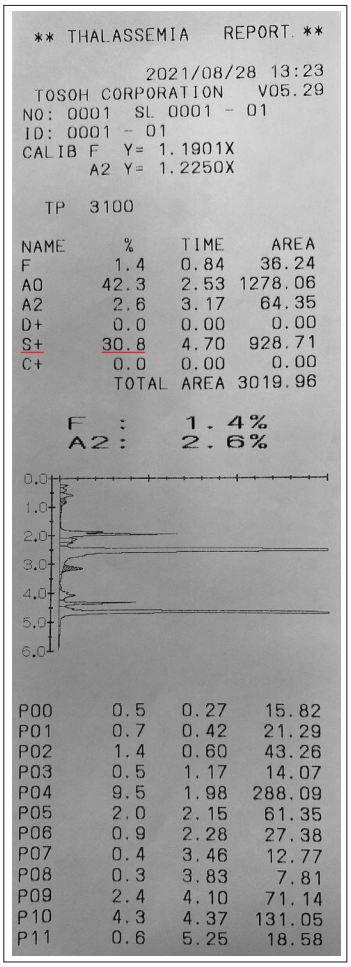
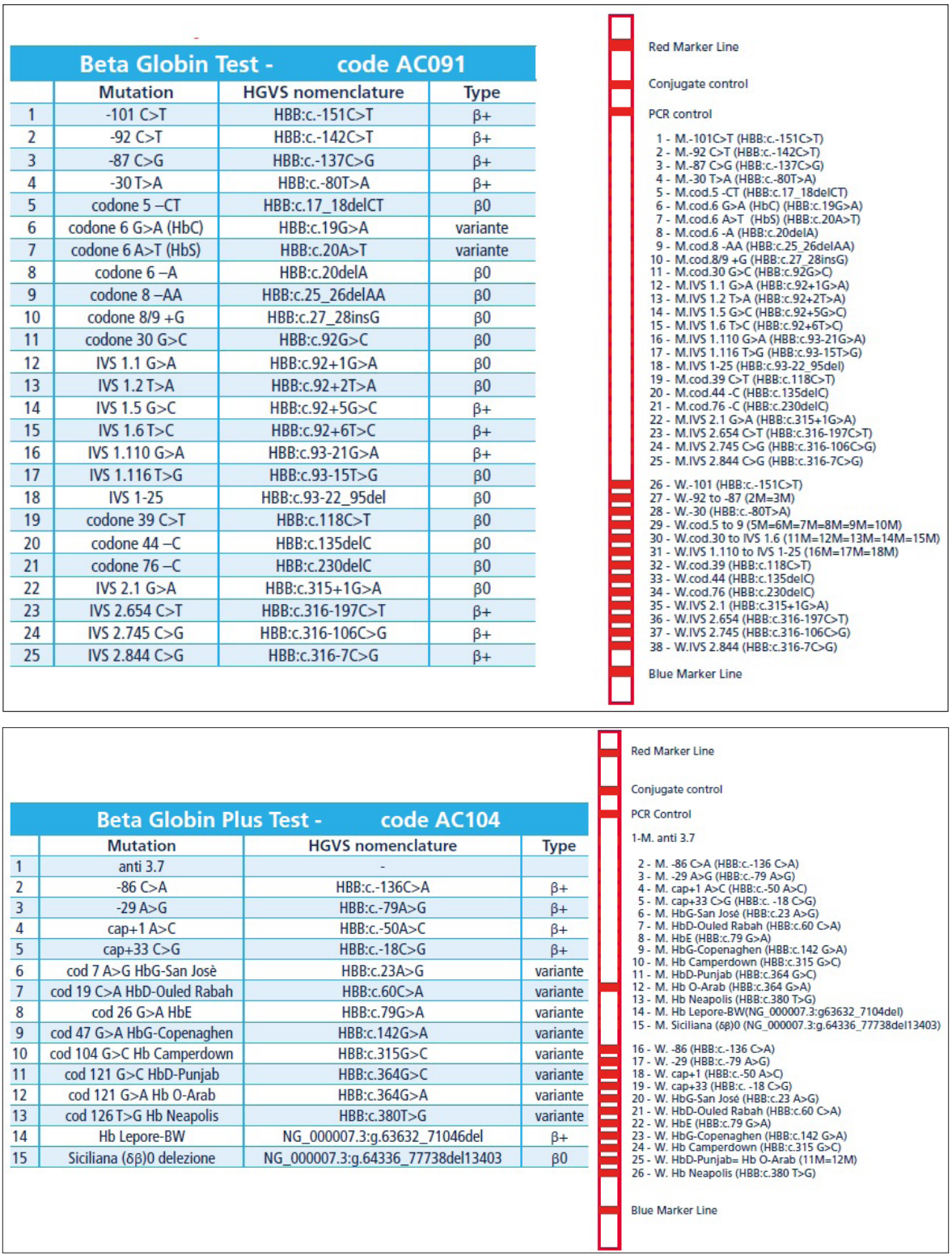


Figure 1: Abnormal hemoglobin was detected in the wife and was identified as "variant HbS by HPLC



Beta Globin Test - code AC091

	Mutation	HGVS nomenclature	Type
1	-101 C>T	HBB:c.-151C>T	β+
2	-92 C>T	HBB:c.-142C>T	β+
3	-87 C>G	HBB:c.-137C>G	β+
4	-30 T>A	HBB:c.-80T>A	β+
5	codone 5 –CT	HBB:c.17_18delCT	β0
6	codone 6 G>A (HbC)	HBB:c.19G>A	variante
7	codone 6 A>T (HbS)	HBB:c.20A>T	variante
8	codone 6 –A	HBB:c.20delA	β0
9	codone 8 –AA	HBB:c.25_26delAA	β0
10	codone 8/9 +G	HBB:c.27_28insG	β0
11	codone 30 G>C	HBB:c.92G>C	β0
12	IVS 1.1 G>A	HBB:c.92+1G>A	β0
13	IVS 1.2 T>A	HBB:c.92+2T>A	β0
14	IVS 1.5 G>C	HBB:c.92+5G>C	β+
15	IVS 1.6 T>C	HBB:c.92+6T>C	β+
16	IVS 1.110 G>A	HBB:c.93-21G>A	β+
17	IVS 1.116 T>G	HBB:c.93-15T>G	β0
18	IVS 1-25	HBB:c.93-22_95del	β0
19	codone 39 C>T	HBB:c.118C>T	β0
20	codone 44 –C	HBB:c.135delC	β0
21	codone 76 –C	HBB:c.230delC	β0
22	IVS 2.1 G>A	HBB:c.315+1G>A	β0
23	IVS 2.654 C>T	HBB:c.316-197C>T	β+
24	IVS 2.745 C>G	HBB:c.316-106C>G	β+
25	IVS 2.844 C>G	HBB:c.316-7C>G	β+

Beta Globin Plus Test - code AC104

	Mutation	HGVS nomenclature	Type
1	anti 3.7	-	
2	-86 C>A	HBB:c.-136C>A	β+
3	-29 A>G	HBB:c.-79A>G	β+
4	cap+1 A>C	HBB:c.-50A>C	β+
5	cap+33 C>G	HBB:c.-18C>G	β+
6	cod 7 A>G HbG-San Josè	HBB:c.23A>G	variante
7	cod 19 C>A HbD-Ouled Rabah	HBB:c.60C>A	variante
8	cod 26 G>A HbE	HBB:c.79G>A	variante
9	cod 47 G>A HbG-Copenaghen	HBB:c.142G>A	variante
10	cod 104 G>C Hb Camperdown	HBB:c.315G>C	variante
11	cod 121 G>C HbD-Punjab	HBB:c.364G>C	variante
12	cod 121 G>A Hb O-Arab	HBB:c.364G>A	variante
13	cod 126 T>G Hb Neapolis	HBB:c.380T>G	variante
14	Hb Lepore-BW	NG_000007.3:g.63632_71046del	β+
15	Siciliana (δβ)0 delezione	NG_000007.3:g.64336_77738del13403	β0

Figure 2: List of mutations researched in the beta globin genes and mutations detected. A) Molecular analysis of the globin genes revealed that the variant highlighted by HPLC was not due to the presence of hemoglobin S, B) Presence, in heterozygosity, of hemoglobin HbO Arab.

The homozygosity condition is rare and has only occasionally been reported to cause very mild hemolytic anemia with some splenomegaly. Significant hemolysis and/or anemia or microcytosis with hemoglobin O-Arab should prompt further investigation for co-inheritance of thalassemia or sickle cell hemoglobin. Interestingly, the position of the substituted amino acid in HbO Arab (ß121 Glu → Lys) is the same as in HbD Los Angeles (ß121 Glu → Gln). In HbO Arab the amino acid present at position 121 is lysine (Lys), whereas in HbD Los Angeles the amino acid present at position 121 is glutamine (Gln). This represents a critical point for tetramer assembly, and the resulting hemoglobin is slightly unstable, resulting in mild hemolysis in both variants.

Arab HbO was reported for the first time in an Arab family in Israel4. Regarding the origin of Arab HbO, it has been shown that it appeared about 2000 years ago on a rare haplotype, characteristic of Greek Pomaks. Its frequency increased as a consequence of high genetic drift within this population, and it subsequently spread throughout the Mediterranean basin and the Middle East 5.

Discussion

The case we describe demonstrates that biochemical screening alone is in many cases insufficient to accurately determine the genetic defect causing a hemoglobinopathy. In these cases, a precise diagnosis will allow to offer exact and detailed information on the implications related to the genetic defect in question.

Thus, correct identification of the healthy carrier and extension of the analysis to the spouse and family members is necessary. In our case, the molecular study of globin genes, led not only to a correct molecular diagnosis, but also to define a genetic condition that from the biochemical point of view had been confused for HbS [4,5].

Conclusion

This paper has shown that biochemical diagnosis alone, in some cases, is not reliable enough to detect carriers of hemoglobinopathies/thalassemias.

Molecular analysis of globin genes provides the most effective way to detect carriers of hemoglobinopathy with certainty. He mutations detected in this work can be identified with a simple and inexpensive kit. This means, in economic terms, significant savings to healthcare spending.

We can say that in the field of prevention, knowing also means recognizing a specific condition, which means faster diagnosis, prevention of complications, a great impact on the quality of life of patients and reduction of social costs of a potentially disabling disease.

Funding

This research was not supported

Conflicts of interest/Competing interests

The authors declare that they have no competing interests.

Availability of data and material

The data of the present paper are available

Ethics approval

As they are anonymous data it was not necessary to ask for approval.

Consent to participate

As they are anonymous data it was not necessary to ask for approval.

Consent for publication

As they are anonymous data it was not necessary to ask for approval.

Acknowledgments

The authors thank "Association Gian Franco Lupo – Un Sorriso Alla Vita" (ONLUS: no-profit organization of social utility).

References

1. Dell'Edera D, Epifania AA, Milazzo GN, Leo M, Santacesaria C, et al. (2013) Identification of patients with defects in the globin genes. J Prenat Med 7: 47-50.
2. Dell'Edera D, Epifania AA, Malvasi A, Pacella E, Tinelli A, et al. (2013) Incidence of β-thalassemia carrier on 1495 couples in preconceptional period. J Matern Fetal Neonatal Med 26: 445-448.
3. Dell'Edera D, Pacella E, Epifania AA, Benedetto M, Tinelli A, et al. (2011) Importance of molecular biology in the characterization of beta-thalassemia carriers. Eur Rev Med Pharmacol Sci. 15: 79-86.
4. Ramot B, Fisher S, Remez D, Schneerson R, Kahane D, et al. (1960) Haemoglobin O in An Arab Family. Br Med J 2: 1262-1264.
5. Papadopoulos V, Dermitzakis E, Konstantinidou D, Petridis D, Xanthopoulidis G, et al. (2005) HbO-Arab mutation originated in the Pomak population of Greek Thrace. Haematologica. 90: 255-257.