Identification of patients with defects in the globin genes

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Summary

Introduction: hemoglobinopathies constitute a major health problem worldwide. These disorders are characterized by a clinical and hematological phenotypic heterogeneity.

The increase of HbA₂ is an invaluable hematological marker of the beta-thalassemia heterozygosis and of double heterozygosis for the alleles of delta and alpha globin genes or for the alleles of delta and beta globin genes which can cause the increase of HbA₂ up to normal or borderline values.

Case Report: we report the case of a 30-year-old woman (first pregnant) who was admitted to our Unit at 12 weeks for a screening for thalassemia. The outcomes of the biochemical and haematological exams (MCV, MCH, HbA2, HbF) highlighted

that the patient was a carrier of a beta-thalassemic trait. Molecular analysis of the beta globin genes highlighted a $\beta^{0}39C$ >T heterozygous mutation. Biochemical and hematological parameters of the husband (MCV, MCH, HbA₂, HbF) were normal except for the level of HbA₂ (3,6%). The molecular analysis of the beta globin genes highlighted a IVS2 nt844 C>G heterozygous mutation. Furthermore, the heterozygous mutation δ^+ cod.27G>T was detected in his δ globin gene. For this reason, he was diagnosed a δ + β Thal.

Conclusions: the aim of this paper is to highlight that biochemical diagnosis could not exhaustive and a molecular diagnostic widening is required to detect the genetic deficiency causing the thalassemic trait.

Key words: HbA₂ borderline, β -thalassemia, carrier screening, prenatal diagnosis.

Introduction

Hemoglobinopathies constitute a major health problem worldwide with a high carrier frequency particularly in certain regions where malaria has been endemic. These disorders are characterized by a clinical and hematological phenotypic heterogeneity. The laboratory analysis include the determination of RBC count, hemoglobin patterns, serum ferritin, quantifications of HbA2, HbF and detection of Hb variants by HPLC (High Performance Liquid Chromatography). The increase of HbA₂ is an invaluable hematological marker of the beta-thalassemia heterozygosis and the double heterozygosis for alleles of delta and alpha globin genes which can cause the increase of HbA2 up to normal or borderline values. Family studies and comprehensive hematological analyses provide useful insights for an accurate diagnosis of thalassemia with molecular identification of the globin gene.

Case presentation

The patient was a 30-year-old woman during her first pregnancy of a naturally conceived child. She was submitted to our Unit and the first trimester combined test was performed at 12 weeks of gestation. The final risk was 1:1000 for trisomy 21. Before carrying out the tests we carefully compiled the medical history of both the patient and her husband. The outcomes of the biochemical and haematological exams (Tab. 1) highlighted that the woman under observation was a carrier of the beta-thalassemic trait (1,2).

	Results	Unit	Normal values
Erythrocytes	5.33	10 ⁶ /µl	4.0 - 5.60
Hb	11.4↓	g/dl	12.8 - 16.5
MCV	67.9↓	fl	80 - 98
MCH	21.5↓	pg	27 - 32
HbA ₂	4.4↑	%	<3.2
HbF	1.5↑	%	<1.0
Serum ferritin	32	ng/ml	22 - 322

Table 1. Blood levels of Mrs. Lo.Gi.

Table 2. Blood levels of Mr.La.Gi.

	Results	Unit	Normal values
Erythrocytes	5.69↑	10 ⁶ /µl	4.0 - 5.60
Hb	16.7↑	g/dl	12.8 - 16.5
MCV	85.2	fl	80 - 98
MCH	29.3	pg	27 - 32
HbA ₂	3.6↑	%	<3.2
HbF	0.5	%	<1.0
Serum ferritin	67.2	ng/ml	22 - 322

The husband's biochemical and hematological parameters (Tab. 2) were normal except for the percentage of HbA₂ (3,6%) (3-5).

In order to remove all doubt the couple repeated the following exams:

- · Blood films for erythrocytes morphology.
- Complete blood count (CBC) with automated cell counter Sysmex XE 2100 (Dasit Cornaredo, Milan, Italy): red blood cells count (RBC), hemoglobin level (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 G8 system (Tosoh Bioscience S.r.l. – Turin, Italy) (6).
- Monitoring serum ferritin assay level by Elecsys 2010 (Roche Diagnostics GmbH).
- Molecular analysis of the beta globin genes.

The following mutations were also studied on the proband's partner: the mutation δ^+ cod.27(G>T) and 21 mutations in the α globin genes.

Blood samples collected in EDTA- K_3 were tested for DNA analysis. The carried molecular analysis envisaged the following steps:

- DNA isolation starting from 25 ul of blood, using the extraction kit of Promega Italy S.r.I. (DNA IQ[™] System, cod.C6701).
- 2. Polymerase chain reaction (PCR) and reverse-hybridization. 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 are obtained with the use of

commercial kits produced by Nuclear Laser Medicine (cod. AC028: genetic test aimed at the check of 21 mutations in the α globin genes; cod. AC091: genetic test aimed at the check of 25 mutations in the β globin genes).

 Amplification, followed by enzymatic digestion for the research of the mutation δ⁺+cod.27(G>T) (replacement of a single base of the codon 27, first exon of the δ gene). The restriction enzyme used is the HaeIII (BioLabs, London, New England). The resulting product is subjected to electrophoresis on agarose gel (3%) in TAE 1X buffer and subsequent ethidium bromide staining.

Discussion

Table 1 shows the haematological results of the patient. In Table 2 are displayed the haematological results of the partner. Her biochemical and hematological data showed features associated with a beta thalassemia trait (MCV \downarrow , MCH \downarrow , HbA₂ \uparrow , HbF \uparrow). Molecular analysis of the beta globin genes highlighted the β^{0} 39C>T heterozygous mutation (Fig.1a) (7).

Patient's husband showed a normal biochemical phenotype except for HbA₂ (3.6%). The molecular analysis of the beta globin genes indicated that he had the heterozygous mutation IVS2 nt844 C>G (Fig.1b). Furthermore, the heterozygous mutation δ^+ cod.27G>T was detected in his δ globin gene (Fig.2). For this reason, he was diagnosed a $\delta+\beta$ Thal. Non mutation were found in the alpha globin gene showed (8).

Therefore, only a careful HbA₂ evaluation allowed us to highlight a silent mutation in the β -globin gene (IVS2 nt844 C>G) and a mutation in the δ -globin gene (δ^+ cod.27G>T), both in heterozygosity. These mutations may be undetected without performing such molecular analysis. The identification of the so-called "silent forms" is of crucial significance especially in prenatal diagnosis. The correct identification of healthy carriers is of primary importance, leading to a subsequent in-depth investigation of the partner.

Conclusions

Our case showed that when a member of the couple is found having a thalassemic trait and his/her partner is found to be a carrier of $\delta+\beta$ Thal, the couple should be informed about the possibility of conceiving a baby with non-transfusion-dependent thalassemia. However it is not our aim to submit to prenatal diagnosis at risk couples. Our purpose is to identify at-risk couples and offer them comprehensive and accurate information on the clinical implications of the genetic defect.

Competing interests

The authors have declared that no competing interests exist.



Figure 1. Reverse dot blot analysis. 1a. Mrs. Lo.Gi presents a mutation in heterozygosity: β 039C>T. 1b. Mr La.Gi. presents a mutation in heterozygosity: IVS2 nt844 C>G.



Figure 2. Mutation research δ^+ cod.27G>T electrophoretic trace obtained after amplification and digestion with engyme HealII. In the presence of the mutation HealII enzyme cuts the fragment amplified in three pieces. In the absence of mutation the enzyme cuts the fragment in two pieces. Wells 1 and 2: Amplified DNA and digested subject known for not having the mutation in the gene δ (presence of two electrophroretic band). Wells 3: Amplified and digested DNA of Mr. La.Gi. present mutation in heterozygosity: δ^+ cod.27G>T (presence of three electrophroretic bands).

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References

- 1. Thein SL. Genetic modifiers of beta-thalassemia. Haematologica 2005; 90:649-660.
- Kan YW, Chang JC. Molecular diagnosis of hemoglobinopathies and thalassemia. Prenat Diagn 2010; 30:608-610.
- Mosca A, Paleari R, Ivaldi G, Galanello R, Giordano PC. The role of haemoglobin A(2) testing in the diagnosis of thalassaemias and related haemoglobinopathies. J Clin Pathol 2009 Jan;62(1):13-7. doi: 10.1136/jcp.2008.056945.
- Préhu C, Ducrocq R, Godart C, Riou J, Galactéros F. Determination of Hb F levels: the routine methods. Hemoglobin 1998; 22:459-467.
- Mosca A, Paleari R, Leone D, Ivaldi G. The relevance of hemoglobin F measurement in the diagnosis of thalassemias and related hemoglobinopathies. Clin Biochem 2009; 1797-1801.
- Huisman THJ. Gamma chain abnormal human fetal haemoglobin variants. Am J Hematol 1997; (55):159-163.

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- Dell'Edera D, Epifania AA, Malvasi A, Pacella E, Tinelli A, Capalbo A, Lioi MB, Di Renzo G. Incidence of βthalassemia carrier on 1495 couples in preconceptional period. J Matern Fetal Neonatal Med 2013 Mar;26(5): 445-448.
- Dell'Edera D, Malvasi A, Tinelli A, Mazzone E, Leo M, Monti V, Epifania AA. Importance of the molecular diagnosis in the screening of alpha-thalassemia. Recenti Prog Med 2011 Jul-Aug;102(7-8):302-306.