Molecular characterization of a discrete hemoglobinopathy upon investigation for a lung hydatic cyst in an old Tunisian patient

Kyste hydatique révélateur d’une hémoglobinopathie : à propos d’un cas

Imen Moumni1*
Sadok Yalaoui2*
Najla Ghrairi2
Agnies Hamzaoui3
Amin Zoraï1
Salem Abbes1
1 Laboratory of molecular and cellular hematology, Pasteur Institute of Tunis, Tunis El-Manar University, Tunisie <salem.abbes@pasteur.rns.tn>
2 Laboratory of medical biology, Hospital A.Mami, Ariana, Tunisie
3 Service B of pneumology, Hospital A.Mami, Ariana, Tunisie
* The first two authors are equally contributors.

Abstract. We report the case of an old Tunisian patient hospitalized for a complicated hydatic cyst of the right lung. Primary laboratory investigation showed a microcytic hypochromic anemia with an abnormal hemoglobin pattern. Hemoglobin analysis and DNA sequencing of the β-globin gene revealed a compound heterozygote, HbO-Arab/cd 39 β°-thalassemia. This hemoglobinopathy was never diagnosed earlier. It spent undiagnosed until the patient presented with hydatic cyst. Coexistence of the two pathologies complicated the general state of the patient and led to a severe anemia. The patient has undergone a surgical therapy for the hydatic cyst and was advised to start a follow up for her hemoglobinopathy.

Key words: hydatic cyst, chronic anemia, Hb O-Arab, β-thalassemia, DNA sequencing

Résumé. Nous rapportons le cas d’une femme tunisienne hospitalisée pour un kyste hydatique du poumon droit. Les premières données du laboratoire révèlent une anémie microcytaire hypochrome avec un profil hémoglobinique anormal. L’analyse de l’hémoglobine et le séquençage du gène β-globine ont permis de caractériser une double héérozygotie Hb O-Arab/cd 39 β°-thalassémie. Cette hémoglobinopathie n’a jamais été diagnostiquée auparavant. La coexistence de ces deux pathologies a compliqué l’état général de la patiente et a conduit à une anémie sévère. Le kyste hydatique a été traité chirurgicalement et la patiente a été orientée afin de débuter un suivi pour son hémoglobinopathie.

Mots clés : kyste hydatique, anémie chronique, Hb O-Arab, β°-thalassémie, séquençage d’ADN

Various hemoglobinopathies responsible of severe anemia are described in the Tunisian population. The most important of them are β-thalassemia, sickle cell disease and at a lesser degree hemoglobin C [1]. Generally, these abnormalities are detected at a pediatric age requiring complete family studies and an early special care and follow up. However, many hemoglobin disorders remain undiagnosed for a long time and became observed only when they are associated with other pathologies. The most reported cases are alpha, delta and some rare beta variants such as hemoglobin D [2] and hemoglobin O-Arab (Hb O-Arab) [3]. HbO-Arab is an abnormal β variant, characterized by the substitution of a glutamic acid by a lysine at position 121 of the beta chain [4]. It is an abnormal Hb variant without significant clinical consequences either at the heterozygous or homozygous state. However, when clinical events associated with sickle cell disease, severe anemia could be observed leading to major [5]. On the other hand, when this variant is co-inherited with β-thalassemia, a discreet chronic microcytic hypochromic anemia is usually observed [6]. Consequently, patients with such a genotype might remain free of syndrome until another pathology requiring laboratory investigation occurred. We report the case of a long time ignored doubly heterozygous HbO-Arab/β°-Thalassemia discovered fortuitously upon a routine preoperative assessment and characterized by gene sequencing in a patient with severe anemia and complicated hydatic cyst of the right lung.
Current practice

Material and methods

Case presentation

The proposita is a 56 year-old Tunisian woman from a rural area of the North-Western part of the country where hemoglobinopathies are frequent [7]. She was admitted for a cough with purulent expectorations and right basithoracic pains. The first examination at the admittance shows an anemic patient with a temperature of 39 °C in spite of a preserved general state. The anamnesis did not reveal notable personal or familial history. Thoracic and pulmonary investigations revealed a complicated right lung hydatic cyst. The patient was transferred in the thoracic surgery service to undergo a kystoperikystectomy intervention for a fissured hydatic cyst of the right Fowler. Before surgical therapy, an initial hemoglobin assessment was necessary in order to avoid any complications upon anesthesia. In fact, the patient was strongly suspected to present an abnormal hemoglobin syndrome since she was anemic and originated from an endemic area for hemoglobinopathies.

Routine investigations

Blood samples were collected in EDTA as anticoagulant. Complete blood count was obtained using automatic cell counter (coulter counter ABX Micro-60 OTR. Abx Diagnostics, Montpellier, France). Cell lysates were analysed by multiple electrophoretic systems including cellulose acetate at alkaline pH (8.6), agar citrate at acid pH (6.2) and isoelectric focusing (IEF) in polyacrylamide gel using a mixture of ampholines, pH 5-8 and 7-9. Moreover, a further hemoglobin analysis and quantification were carried out by cation exchange HPLC using the D-10 system (Bio-Rad Laboratories) according to the procedure recommended by the manufacturer [8].

DNA analysis

DNA was obtained from peripheral blood leucocytes by the standard phenol/chloroform method. The three exons of the β globin gene were specifically amplified by conventional PCR as described previously [9]. Obtained amplicons were then purified and sequenced using the dye terminator method on an automatic sequencer (ABI PRISM™ 3130 DNA Genetic Analyzer; Applied BioSystems, Foster City, CA, the USA).

Results

Laboratory investigations

Hematological data [RBC: 3.8 T/L, Hb: 68 g/L, MCV: 64.7fL, MCH: 20.1 pg] highlighted a microcytic hypochromic anemia in spite of an elevated ferritinemia (365 μg/L). Hb pattern obtained mainly by HPLC and IEF (figure 1 A and B) showed a major fraction of Hb O Arab expressed at 89.7%, increased levels of Hb F and A2 expressed respectively at 6.2 and 4.1% (values obtained by HPLC). This profile suggested a homozygote Hb O-Arab or a doubly heterozygote Hb O-Arab/β-thalassemia since we are in presence of microcytic anemia with elevated Hb A2 level.

In front of this situation and in the absence of family study (death of the two parents), a molecular investigation was undertaken allowing to obtain a final diagnosis of a doubly heterozygote O-Arab/β-thal. Indeed, the β-globin gene sequencing showed two heterozygote mutations: a structural mutation at codon 121 (GAA > AAA) characteristic of the Hb O-Arab and a stop codon at position 39 CAG > TAG leading to β-thalassemia (figure 2). These two alleles were later observed in two of her descent.

Figure 1. Phenotypical characterization of Hb O-Arab. A) Cation exchange HPLC (Biorad D-10) showing three fractions corresponding to HbO, HbF and Hb A2 identified by their retention times. B) Isoelectric focusing on polyacrylamide gel; line 1: Proposita with Hb O arab, lane 2: Hb S/C control, lane 3: mixture of proposita and S/C control, 4 : heterozygote β-thalassemia.
Lung hydatid cyst and hemoglobinopathy

A1: normal Sequence: $\beta$121 GAA (Glu)

B1: normal Sequence: $\beta$39 CAG

A2: heterozygote HbO-Arab: $\beta$121 AAA (Lys)

B2: heterozygote $\beta$39 TAG (STOP)

Figure 2. Fluorescent-based automated DNA sequence analysis of the $\beta$-globin gene: A1) Normal sequence of codons 120, 121 and 122. B1) Normal sequence of codons 38, 39 and 40; A2) Hb O-Arab, A $\rightarrow$ G substitution at codon 121; B2) Abnormal sequence C $\rightarrow$ T: at codon 39.

(three months after), one of them was heterozygous $\beta^2$-thal (cd 39 CAG $\rightarrow$ TAG) the other was heterozygote HbO-Arab.

Surgical operation

Since The initial biological analysis led to the absence of sickle cell disease, the surgical coverage put no more particular attention, the patient was treated by cystectomy. Histological examination confirmed a hydatid cyst and the post-operative result was satisfactory with relief of the compression and improvement in phonation point. However, hematological disturbance persists and the patient was oriented to hematological service for further investigations and follow up.

Discussion

The first case of hemoglobin O-Arab was described in 1960 in an Arab child suffering from sickle cell disease syndrome [10]. The genetic alteration leading to this Hb was revealed by protein sequencing on 1962 [11]. In Tunisia, although several hemoglobinopathies were described with high incidence, HbO-Arab remains until now as one of rare variants detected so far. Its incidence doesn’t exceed 0.11% and the most cases were doubly heterozygote of HbO with other hemoglobin abnormalities. They were reported in or in fortuitous associations with other pathology [12]. Whereas, the present one constitute an exception by the fact that, the diagnosis of hemoglobinopathy have been done only at the age of 56.

The patient had a chronic microcytic anemia previously considered as a common refractory iron deficiency. No hemoglobinopathy was suspected until the discovery of the hydatid cyst and the need for surgical intervention. To our knowledge, it’s the first time worldwide that a doubly heterozygote Hb O-Arab/$\beta^2$-thalassemia is reported in association with such a pathology. Compared to other subjects with HbO/$\beta^2$ thalassemia, our patient looks to have the deepest anemia and a worst general state. This situation is expected since two causes of anemia are present at the
same time: a major hemoglobinopathy and a lung Hydatic cyst. This association tends to complicate the general state of the patient by a double respiratory inefficiency due to a lack of functional hemoglobin (for the hemoglobinopathy) and a lung infringement (for the hydatic cyst).

Generally, when such a situation occurs, physicians give more attention to the faced disease and take care of the visible things. Unfortunately, the hereditary side spend ambiguous with its risk and could be considered for a long time as an idiopathic disease as long as suffering doesn’t impose any biological and molecular exploration. Indeed, the old recorded hematological data of the patient does not notify any problem other than a discreet microcytic anemia compatible with a minor thalassemia.

However, and according to previous clinical reported data on Tunisian HbO-Arab beta thalassemia, some of them could present, during their life, episodes of severe hemolytic anemia requiring blood transfusions childhood [13]. On the other hand, the evolution of hydatidoses is characterized by thrombopenic complications, splenomegaly, osteo-articular pains and vesicular lithiases [14]. These same complications might happen also in severe forms of hemoglobinopathy. The coexistence of both pathologies in the same patient tends to maintain a deep anemia and increases the risks of handicapping complication.

Finally, it is of great importance to notify that biological and molecular investigation might be performed when we face anemia especially in endemic region for both hydatic cyst and hemoglobinopathies. Moreover, it would be judicious to explore hydatidoses for hemoglobinopathy in order to improve the assumption of responsibility and to give the patient a complete and final diagnosis for a better prevention as well as an adapted treatment.

Acknowledgments. This work has been supported by « la direction de la recherche scientifique, ministère de l’enseignement supérieur et de le recherché scientifique Tunisie, laboratoire « LR05SP04 ».

Conflicts of interest: none.

References