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Case Report

Difficulty in Diagnosing a Hemoglobinopathy: Heterozygous Beta-Thalassemia Associated with Alpha Hemoglobin Variant

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Abstract: The diagnosis of a hemoglobinopathy is not always easy; it is based on a combination of clinical, hematological and biochemical findings, possibly including a genetic study of globin chains. Interpretation of biochemical profiles can be complicated when two hemoglobin abnormalities are associated. We report the case of a globin alpha variant associated with beta-thalassemia in a family of Moroccan origin that underwent a biological investigation. The discovery was fortuitous during the determination of HbA1c by high performance liquid chromatography. The diagnosis was confirmed by the use of complementary techniques, including capillary electrophoresis at alkaline pH and agarose gel electrophoresis at acid pH, associated with the hematological and biochemical data of the patient and his family.

Keywords: Hemoglobinopathy, alpha hemoglobin variant, beta thalassemia, hemoglobin electrophoresis, HbA, HbA2.

INTRODUCTION

Hemoglobinopathies group together all pathologies related to a genetic anomaly of hemoglobin. They are divided into two types: qualitative abnormalities affecting the structure of the molecule with the synthesis of an abnormal hemoglobin or hemoglobin variant (variantsβ, α and other variants), and quantitative abnormalities due to a defect in the synthesis of one of the globin chains mainly α or β, defining respectively α and β thalassemias.

In Morocco, the epidemiology of hemoglobinopathies remains unknown. The World Health Organization estimates the carrier rate at 6.5% [1].

We report the case of a α globin variant associated with β- thalassemia in a family of Moroccan origin that benefited from a biological investigation.

OBSERVATION

A 32-year-old man of Moroccan origin, married and father of one child, with no particular pathological history, was referred to the biochemistry laboratory of the Mohamed V Military Instruction Hospital for a health check-up.

The blood count showed a mild microcytic hypochromic anemia associated with polycythemia (hemoglobin level (Hb): 12.2g/dL, mean corpuscular volume (MCV): 59.1 fl, mean corpuscular hemoglobin content: 19.1 pg, red blood cell counts (RBC): 6.38.106/ul). The blood smear was not performed.

The glycated hemoglobin assay, performed in our department by high performance liquid chromatography (HPLC) on ARKRAY ADAMS A1c HA-8180V®, showed a level of 7.4%, with evidence of a supernumerary peak eluting after the HbA, with an area of 51% at the retention time 39s. The fasting blood glucose determination found a level of 0.74g/l. Because of the suspicion of the presence of a globin variant, a complementary study of the hemoglobin was necessary.

Capillary electrophoresis of Hb at alkaline pH on the Capillarys 2 Flex Piercing system (Sebia®), showed the presence of several peaks migrating in the Hb zones A (zone 9: 63.5%), F (zone 7: 1.6%), D (zone 6: 29.3%), E (zone 4: 0.7%), A2 (zone 3: 3.4%), and a last peak in zone 1 (1.5%) (Figure 1a).

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Electrophoresis of Hb at acid pH, performed on Hydrasys 2 Scan (Sebia®), showed that the abnormal variant migrates at the same level as Hb A (figure 1b).

The rest of the biochemical workup was normal, with notably the absence of iron deficiency (ferritinemia: 113ng/mL).

Electrophoresis results were in favor of the probable presence of heterozygous hemoglobinosis D (A/D) associated with a δ variant.

A family survey was conducted to study the hemoglobin of other family members including the mother, child, and spouse (the father was deceased).

- In the child, capillary electrophoresis at alkaline pH showed the presence of two supernumerary peaks, the first migrating into the Hb D zone (zone 6: 26.1%) after the Hb A zone, and the second in zone 1 (0.6%) after the HbA2 zone (Figure 1c), ferritinemia and hemogram were normal.
- In the mother, the profile was in favor of heterozygous beta thalassemia, (Figure1d).
- In the spouse, there were no abnormalities on capillary electrophoresis at alkaline pH (HbA: 97.5%, HbA2: 2.5%), with a normal blood count.

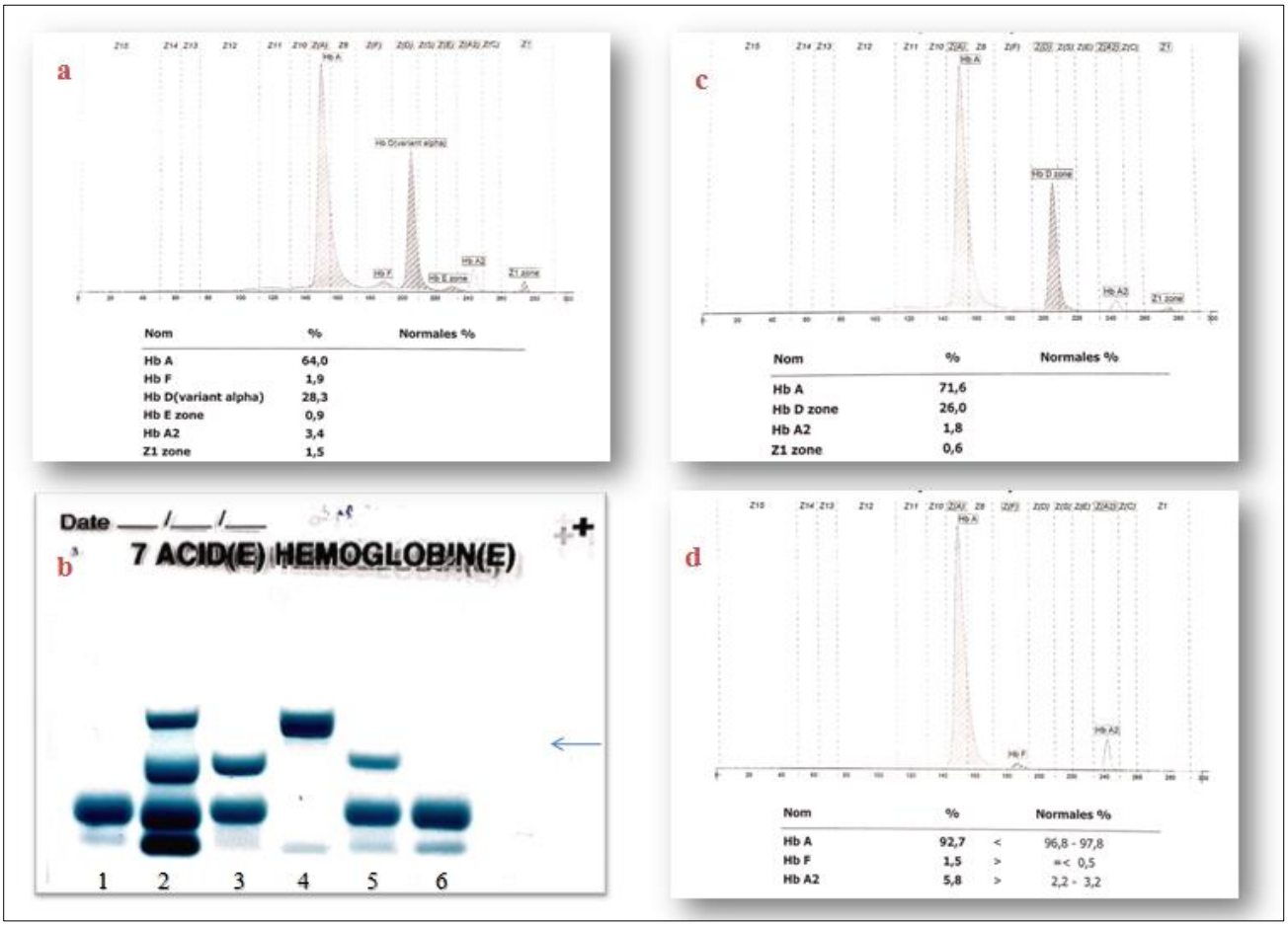

Figure 1 : a : Patient hemoglobin electrophoresis on Capillarys (Sebia ®) ; b : Patient hemoglobin electrophoresis on Hydrasys (Sebia ®) : (1 : Normal control. 2 : Pathological control (FASC). 3,4 et 5 : Other patients in the series. 6 : Patient. Deposit) ; c : Child hemoglobin electrophoresis on Capillarys (Sebia ®); d : Patient's mother hemoglobin electrophoresis Capillarys (Sebia ®).

All the biochemical results of the patient and his family combined with those of the patient's blood count were suggestive of a composite heterozygosity associating a heterozygous thalassemia with an unidentified variant of the α chain, thus making it possible to rectify the diagnosis.

We could then assume that the mother transmitted the β –thalassemia heterozygous mutation and that the deceased father (we have no biological data concerning him) transmitted the α variant mutation to our patient, thus passing this variant on to his child (figure 2).

A genotyping study was advised to determine this variant accurately.

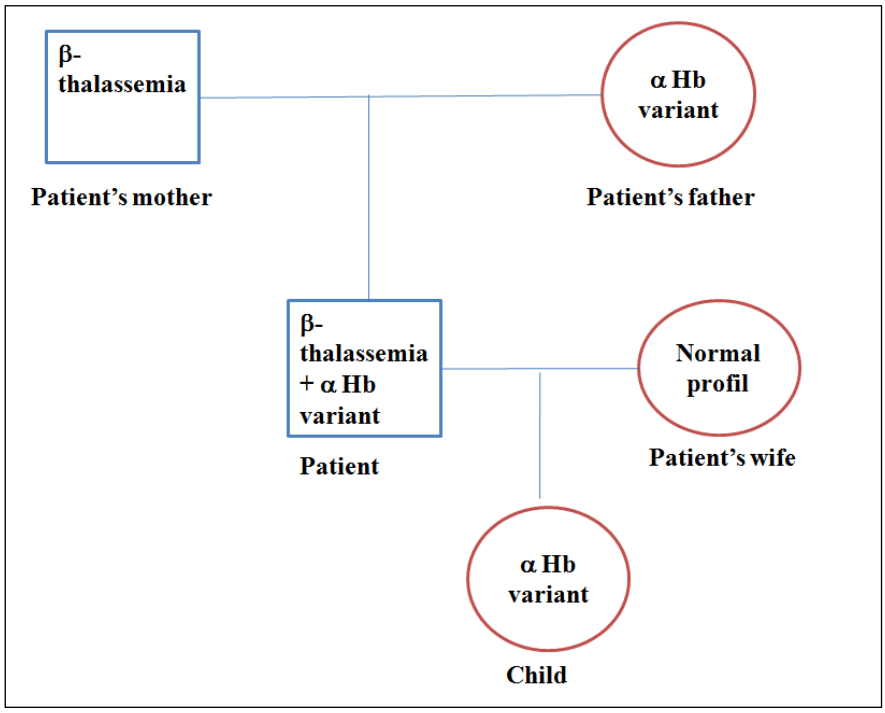

Figure 2: Patient's family tree

DISCUSSION

To date, more than 1000 hemoglobin variants are counted, the β variants are largely in the majority, those of the α chain are very rare, and 93 variants have been listed in France, 31 of which are not yet identified [2]. In Morocco, no statistics are available.

This rarity can be explained by the fact that they are under-diagnosed because they are rarely pathological and in the absence of clinical anomaly their formal identification is not necessary [3].

There are many circumstances in which a biological study of hemoglobin in the laboratory is required [3, 4]: systematic study of patients from countries at risk, particularly newborns (neonatal screening), pregnant women and during preoperative check-ups; exploration of clinical and hematological signs suggestive of hemoglobinopathy (pallor, cyanosis, jaundice, hemolytic anemia, polycytosis, microcytosis, etc.); family investigation; chance discovery of a hemoglobinopathy (e.g., the presence of a hemoglobin deficiency).); family investigation, incidental finding during Hb A1c determination.

According to the nomenclature of medical biology acts, the search for an Hb abnormality must include 3 distinct phenotypic tests, including at least one electrophoretic technique. Among the 3 techniques used, one must be quantitative [3]. In our laboratory, biochemical exploration of hemoglobinopathies is done by 3 complementary methods including HPLC, capillary electrophoresis at alkaline pH and agarose gel electrophoresis at acid pH. For our patient, the discovery was fortuitous during the determination of HbA1c by HPLC. Knowing his profile, the identification of the fractions becomes less difficult, so the supernumerary peak eluting after the Hb A0 corresponds to the alpha variant which has separated from the Hb A0 while the Hb F and HbA1c fractions are not separated from their mutated fractions, they have co-eluted, this results in an overestimation of the HbA1c. In fact, HbA1c results measured by chromatography will be inaccurate when the Hb variant or its glycated derivative cannot be separated from HbA or HbA1c [5]. Thus, the HbA1c level would be overestimated in case of its co-elution with the Hb variant, or in case of separation of HbA from the Hb variant with co-elution of their glycated derivatives (HbA1c and glycated fraction of the variant) [6]. The latter situation was observed in our patient where the Hb A1c level was 7.4% contrasting with a fasting blood glucose level of 0.74g/l.

Whereas an underestimation of the HbA1c level would be observed in case of co-elution of the HbA and the variant with separation of their glycated derivatives [6].

The capillary electrophoresis has allowed the separation of the different fractions, so we can see that each normal fraction corresponds to an abnormal fraction. This is explained by the presence of the globin chain α in Hb A and Hb A2, hence the high number of peaks. Since our patient has an associated β - heterozygous thalassemia, which is characterized by an

increase of HbF in addition to Hb A2, this has further increased the number of peaks corresponding to HbF, also containing the globin α and to its variant.

Indeed, in the case of a α heterozygous variant, the classical profile in an adult results in the presence of 4 fractions: HbA(αβ) the HbA variant (α'β) HbA2(αδ) and the HbA2 variant(α'δ) with migration of supernumerary peaks (α'β) and (α'δ) respectively in the D and Z1 areas. The HbA2 appears decreased whose true value corresponds to the sum of the percentages of HbA2 and zone1, for our patient it is: 4,9%(3,4% + 1,5%).

Migration into zone D with the presence of HbA may be confused with heterozygous hemoglobinosis D. In this case the level of the D variant must be between 30 and 40% [4], which is not the case here where the level is 28.3% for the patient and 26% for the child, suggesting an associated α -thalassemia or martial deficiency, not found in our patients.

Nevertheless, when only one alpha gene is mutated, the fractions (α'β) and (α'δ) have a respective rate around 25% and 0.75% [7], this is consistent with the results of our patients in whom the rates are respectively 28.3% and 1.5% for the patient and 26% and 0.6% for the child.

Hemoglobin electrophoresis at acid pH was not useful in this situation for diagnostic orientation, since it did not allow the elimination of a D variant (comorbid with A, A2, E and Lepore) nor did it objectify the presence of a α variant.

The confrontation of biochemical data with hematological data, was in agreement; the blood count was normal in the child carrying the alpha variant, while we noted a slight microcytic hypochromic anemia with polyglobulia in our patient which is explained by the β -thalassemia added with an erythrocyte index of Mentzer (MCV/RBC) at 9.26 which evokes a thalassemia if it is lower than 13 [7].

CONCLUSION

The α variant is a very rare entity very little documented in the literature, the interpretation of HPLC and Capillary electrophoresis profiles can be complicated, especially in case of association with other hemoglobinopathies or in the newborn making the diagnosis difficult. This underlines the importance of interpreting all epidemiological, clinical, biochemical and hematological data with possible genotypic confirmation.

REFERENCES

1. L. R. (2018). Profil des hémoglobinopathies au service de biochimie de l'hôpital Avicenne. Thèse de doctorat en médecine. Université Cadi Ayyad, Faculté de médecine et de pharmacie de Marrakech.
2. BRUNENGO, D. Epidémiologie qualitative des variants rares de l'Hémoglobine en France métropolitaine au travers de la collaboration HCL - Eurofins Biomnis-Poster-2017
3. Mario, N., & Sala, N. Diagnostic biologique des hémoglobinopathies. Revue Francophone des Laboratoires- Avril 2016-N°481
4. Saoud, M. Z., Biaz, A., Rachid, A., El Amin, G., Dami, A., & Ouzzif, Z. (2020). Association d'hémoglobinose D-Punjab et de β-thalassémie dans une famille marocaine. *Ann Biol Clin*, *78*(1), 61-9.
5. Bry, L., Chen, P. C., & Sacks, D. B. (2001). Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin. *Clin. Chem*, *48*, 153-163.
6. Mario, N., & Lasnier, E. Les difficultés d'interprétation du dosage de l'hémoglobine a1c, Revue Francophone des Laboratoires, mai 2006, N~382
7. Condom, C., Castex, M. P., & Dubois, F. (2022). Enjeu et difficulté du diagnostic des hémoglobinopathies chez le nouveau-né: à propos d'un cas. *Ann Biol Clin*, *80*(5), 455-9.