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Heterozygous Haemoglobin C/Beta Thalassemia: About a Fortuitous Discovery Case

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Abstract

Haemoglobin C is a genetic disorder caused by the synthesis of abnormal haemoglobin (Hbc) that replaces haemoglobin A. We report in our observation a case of a 50-years-old woman with heterozygous haemoglobin C/beta thalassemia, married and mother of 3 children, from Morocco. During a family assessment, the biological examinations show a discreet regenerative and haemolytic microcytic anaemia (increased reticulocytes and collapsed haptoglobin); the morphological examination of the peripheral blood shows abundant target cells and erythroblastosis. No abnormalities of haemostasis are observed, and no iron deficiency is really noticed. Without surgical or medical history, patient physical examination was purely normal. The hemoglobinopathy was confirmed by capillary electrophoresis that quantifies all different fractions of haemoglobin (HbC: 66.9%, HbF: 1% and HbA2: 4.3% for our patient) and identifies the homozygous or heterozygous character of the genetic anomaly, these results are already verified by high performance liquid chromatography (HPLC). The explored patient is heterozygous C/β-thalassemia. Early detection of these asymptomatic hemoglobinopathies makes high-risk couple's identification and genetic counselling possible and effective.

Keywords: Haemoglobin C; Beta-thalassemia; Heterozygosity

Introduction

Haemoglobin (Hb) is a tetrameric protein, constituted by two chains alpha and two chains not alpha (beta, delta, and gamma). His principal function, oxygen transport, depends on its physical and chemical characteristics (solubility, stability, oxygen affinity). However, those characteristics can be modified by genetic deteriorations of various globin genes this is what defined haemoglobinopathies. The Haemoglobinopathies include qualitative and quantitative anomalies. The qualitative anomalies or hemoglobinosis is due to production of abnormal chain of globin most frequent are HbS, HbC and HbE… and quantitative anomalies of Hb (thalassaemias) when one or more genes of alpha globin (α-thalassemia) or beta globin (β-thalassemia) are missing, without protein deterioration.

All these various anomalies can be associated. Haemoglobin C (HbC) is abnormal haemoglobin resulting from a genetic mutation, substitution of a glutamic acid residue with a lysine residue at the 6th position of the β-globin chain. This mutated form reduces erythrocytes plasticity and causes usually an asymptomatic hemoglobinopathy, except for homozygous ones when a mild haemolytic anaemia is developed. The coexistence of the two anomalies at the same individual is certainly possible but the composite hétérozygotie of HbC and beta thalassemia is unusual and responsible of benign symptoms.

We report in our observation a case of heterozygous haemoglobin C/beta thalassemia (HbC/BT) fortuitously discovered during a family assessment of a girl with homozygous beta thalassemia.

Patient and Observation

Mrs K.E, 50-years-old, married and having 3 children (consanguineous 1st degree), originating and resident in Zagoura, Morocco. Her daughter, 15 years old, followed for homozygous confirmed beta thalassemia and within the framework of the family assessment (parents and brothers) looking for a heterozygous beta thalassemia, a mom's haemoglobin C heterozygous beta-thalassemia was found. Our studied case is without notable antecedents, the clinical examination is purely normal, without cutaneous pallor, splenomegaly or hepatomegaly. The hemogramm objectifies: discrete hypochromic microcytic regenerative anaemia with haemolytic character (erythroblasts: 2% with bilirubin's slightly increased), slightly low haematocrit, normal platelets: and pseudo polycythaemia are noticed. The leukocyte count is normal (Table 1).

The cytomorphological examination of the blood smear shows microcytosis with target cells and rare erythroblastosis (Figure 1). Ferritinemia is normal, haptoglobin is slightly decreased, and LDH is slightly elevated, and there is an increase of indirect and total bilirubin. Electrophoresis of haemoglobin realised by 2 techniques (HPLC and capillary electrophoresis), allowed a quantitative and qualitative haemoglobin study with more accuracy (Figures 2-5).

According to electrophoresis results (HPLC and capillary electrophoresis), this family was diagnosed with haemoglobin C and beta thalassemia heterozygosity, the mother (our case) is composite heterozygous for HbC and beta thalassemia while dad is heterozygous beta-thalassemia, a daughter with homozygous beta-thalassemia was the result of their consanguineous marriage, the other 2 children inherited a heterozygous haemoglobin C. Ferritinemia is normal, haptoglobin is slightly decreased, and LDH is slightly elevated, and there is an increase of indirect and total bilirubin. Electrophoresis of

hemoglobin realised by 2 techniques (HPLC and capillary electrophoresis), allowed a quantitative and qualitative hemoglobin study with more accuracy. (Figures 2, 3, 4 and 5). According to electrophoresis results (HPLC and capillary electrophoresis), this family was diagnosed with hemoglobin C and beta thalassemia heterozygosity, the mother (our case) is composite heterozygous for Hb C and beta thalassemia while dad is heterozygous beta-thalassemia, a daughter with homozygous beta-thalasssemia was the result of their consanguineous marriage, the other 2 children inherited a heterozygous hemoglobin C.

Settings	Patient	Reference values
Red cells	5,10^6/mm^3	4,2-5,2.10^6/mm^3
Hct	32%	37%-47%
Hb	11,5g/dl	12-16 g/dl
MCV	75 fl	80-100 fl
MCHC	30 g/dl	32-35 g/dl
PT	345000/mm^3	150-450.10^3/mm^3
White cells	8520/mm^3	4-10.10^3/mm^3
reticulocytes	121000/mm^3	
NRBC	2/100 GB	
Total bilirubin	25 mg/l	0-10 mg/l
Indirect bilirubin	21mg/l	0-8 mg/l
Direct bilirubin	4 mg/l	0-2 mg/l
ferritin	52 ng/ml	15-150 ng/ml
haptoglobin	0.20 g/l	0.25-1.75 g/l
LDH	351 U/l	120-300 U/l

Table 1: Blood test results.

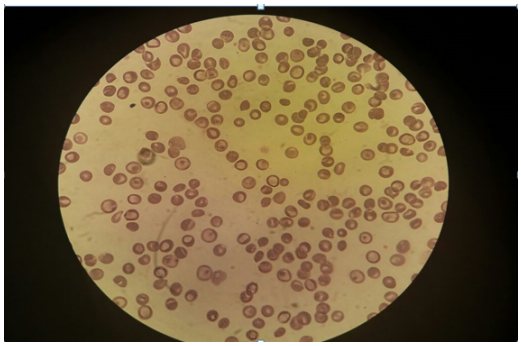

Figure 1: Microcytosis with target cells in cytomorphological examination of blood smear.

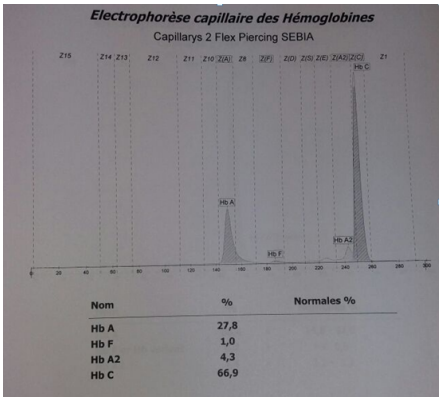

Figure 2: Capillary electrophoresis results of the mother's hemoglobin : Heterozygous haemoglobin C/beta thalassemia.

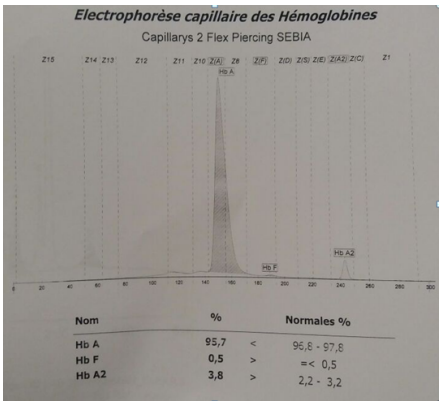

Figure 3: Capillary electrophoresis results of the father's haemoglobin: Heterozygous beta thalassemia.

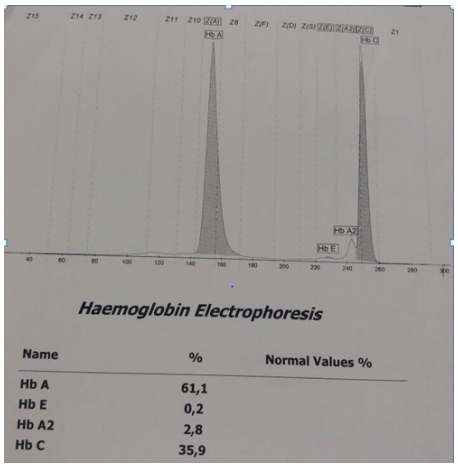

Figure 4: Capillary electrophoresis results of the first son's hemoglobin: Heterozygous hemoglobin C.

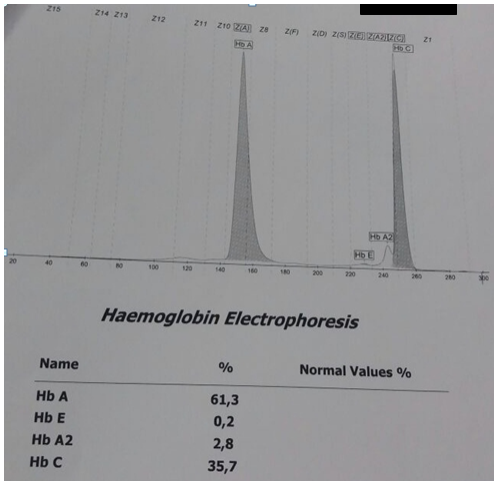

Figure 5: Capillary electrophoresis results of the second son's hemoglobin: Heterozygous hemoglobin C.

Discussion

HbC is present in areas where HbS is itself prevalent. Epidemiologically, the mutation frequency reaches 1 to 10% in North Africa (Morocco and Algeria) and 20% to 50% in West Africa (Ghana, Ivory Coast and Burkina Faso). The clinical syndrome prevalence is estimated at between one in 3000 and one in 6000 inhabitants from African origin. In the United States, the prevalence of HbC is 2.4 per 100000 in black population [2]. Heterozygous individual with HbC are asymptomatic although they may have a moderate hematological microcytosis with increased osmotic resistance. HbC-BT patients are composite heterozygous for Hbc and beta-thalassemia.The prevalence of this disorder is not really known but it is mainly found in African populations. Blood transfusion is rarely necessary.

Patients are usually asymptomatic and diagnosed by routine medical examinations, on the other hand, clinical manifestations are almost like a CC homozygosity: compensated hemolysis with splenomegaly whose complications may include hypersplenism, cholelithiasis, folate deficiency, joint pain and skeletal muscle, retinopathy, abnormal dental training [3-9] and rare complications during pregnancy [10]. The C / beta-thalassemia is more severe and may resemble an intermediate betathalassemia [11].

The discovery of moderate anemia and pseudoglobulia with microcytosis should prompt the search for hemoglobinopathy. The reticulosis and haptoglobin collapse reflect the haemolytic character of anemia. Iron supplementation is, of course, contraindicated. The erythrocyte cytomorphological abnormalities seen on the blood smear are characteristic, especially target cell abundance in more than 90% of cases, characteristic HbC crystals with straight and parallel edges, and irregularly contracted cells showing signs of thalassemia.

The fortuitous observation of a large number of target red blood cells on a blood smear should prompt a search for HbC and using a reticulocyte count which is clearly increased (40%-60%) and reflects the haemolytic character of anemia. Cholestasis secondary to haemolytic anemia is reported in 25% of cases [12].

The diagnosis is based on the detection of Hbc-betathalassemia in the heterozygous state. Electrophoresis at alkaline pH (8.6) on agarose gel or cellulose acetate is part of the basic screening for hemoglobinopathies [4-9] [13-17] and is used to diagnose homozygous or heterozygous hemoglobin abnormalities. However, this technique confuses HbC with HbE, HbA2, HbO-arab ... For the differentiation of this character, a confirmation by using other more efficient techniques to separate the different fractions of Hb, with quantification and phenotyping, is necessary. We have adopted for our patients HPLC and capillary electrophoresis.

These chromatographic methods allow a quantification of all different fractions of hemoglobin, which makes them a valuable tool to specify, quantify and confirm the identification of abnormal hemoglobins [18-19]. HPLC distinguishes HbC from HbE, HbA2, HbO-arab ... it is the only technique that allows exact dosage of HbF and HbA2 [1].

Capillary electrophoresis is technically between electrophoresis and HPLC. The proteins separation is due to an electric field such as conventional electrophoresis but using a separation liquid and its detection mode brings it closer to the chromatography. There is no protein staining, which saves time and precision. Its instrumentation allows automated analysis and data computer storage.

HPLC and capillary electrophoresis [8,14] are more resolutive for separating the different fractions of normal or abnormal Hb and allow today to overcome the electrophoretic techniques limitations on agarose gel at alkaline or acid pH.

Conclusion

Fortuitous observation of target cells associated with cytomorphological abnormalities of the red blood cells (microcytosis, deformed red blood cells, etc.) must encourage the search of hemoglobinopathy. Routine hemoglobin testing is simple and accessible to all multipurpose medical laboratories. The results interpretation is difficult without anamnestic data (age, ethnographic origin, transfusion ...) what makes a clinical-biological connexion very necessary. Using different techniques of hemoglobin testing is the way for precise results, HPLC and capillary electrophoresis now allow to overcome technical limits of routine electrophoresis.

Data Availability

The data used to support the findings of this manuscript are included within the article.

The conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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