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Clinical Presentation of Hemoglobin C in Albania: Case Series

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Abstract

The presence of HbC is a rare event in Europe and Mediterranean region where thalassemia and HbS are more frequently encountered. The rarely diagnosed cases are linked with the migration from the West-Central Africa. Albania is one of the Mediterranean countries where inherited hemoglobin disorders are considerably widespread. Studies have shown the presence of thalassemia, sickle cell disease, and sporadic cases of Hb O-Arab, Hb Lepore especially in the areas where malaria has been endemic. In 2006, we identified the first case with HbSC disease and until 2020, we have found 15 cases with HbC variant. In this study, we have collected and analyzed the laboratory and clinical data of HbC cases. Our data support reports that HbC combinations with HbS and beta-thalassemia are clinically important. Our data confirm the presence of the HbC variant in ex-malaric areas where thalassemia and HbS are quite widespread.

Introduction

Albania is one of the Mediterranean countries where inherited hemoglobin disorders (thalassemia and other hemoglobinopathies) are considerably widespread and constitute a major concern for public health even today. Screening studies conducted over the years 1975–1990 have shown a high frequency of beta-thalassemia carriers (7.1%) in the Western lowland areas [1], [2], [3]. The high frequency of the beta thalassemic alleles in our country has also been confirmed by studies conducted after the 90s [4]. This frequency was found 4.9% in Albanian immigrants in Greece [5]. The pilot study conducted by ASoLaM and USAID in the district of Lushnja resulted in a frequency of 4–11%, with an average of 7.58% [6].

Besides beta-thalassemia, all screening studies conducted on the Albanian population have found a high presence of another hemoglobin disorder and hemoglobin S (HbS, β6Glu-Val) in various areas of the country. The frequency of HbS was particularly high (up to 12%) in the central areas of the Western lowland, from the Lushnje-Kavaje frontier to Durres. Studies conducted up to 2006 have also identified carriers of HbO-Arabe, Hb Lepore, double heterozygotes HbS/beta-thalassemia, and some carriers of alpha-thalassemia.

Hemoglobin C (HbC) is another structural variant of normal hemoglobin caused by an aminoacid substitution at sixth position of the beta-globin chain (HbC, β6Glu-Lys). It is one of the most prevalent abnormal hemoglobin mutations globally alongside HbS and hemoglobin E (HbE, β26Glu-Lys). HbC is clinically important when combined with HbS or beta-thalassemia [7], [8]. The coexistence of βs and βc genes clinically appears as sickle cell anemia similar to HbSS (homozygous form of drepanocytosis). Chronic moderate hemolytic anemia with normal Hb and an elevated reticulocyte count is the principal finding in these subjects. All HbSS complications are encountered in this form but most of them appear later in time and with a lower frequency. Pulmonary complications such as pulmonary hypertension and acute chest syndrome are common clinical features of HbSC. These complications are associated with high mortality rates. HbC might coexist with different forms of β-thalassemia (β^0 or β□). These combinations are accompanied with moderate-to-severe anemia, splenomegaly, and hypersplenism phenomena in some cases [9], [10].

In this paper, we report the existence of hemoglobin variant HbC in the Albanian population and its appearance in various clinical forms.

Materials and Methods

This is a retrospective study. Data were collected from the results of the anemia screening and diagnosis unit of the Laboratory Department, University Hospital Center "Mother Teresa" between 2006 and 2020. Clinical data relating to geographical origin, place of birth, age, disease onset, comorbidity, and past and ongoing treatments were collected. Laboratory tests were performed as part of a routine diagnostic evaluation. CBC (complete blood count) and biochemical parameters were determined by automated routine procedures. Identification of the type of anemia was an indication for electrophoretic examination of hemoglobin fractions. Hemoglobin electrophoresis was performed in alkaline and acid agarose gel using Hydrasys SEBIA system.

Results

From 2006 to 2020, we have identified 15 cases with presence of HbC, 12 women and three men. Only one patient was in pediatric age. The median age was 33 years (range 10–52). Fourteen patients were Albanian from the central and south areas of Western lowland (one from Cerrik, one from Saranda, and 12 from Lushnja and Fier) and one patient was from Nigeria.

Eight cases resulted with HbC trait (HbAC). We have also found five cases with HbSC disease, one case with HbC homozygote (HbCC), and one case with HbC/beta+ thalassemia.

The clinical picture of our HbC trait patients was non-specific anemia. In this group, general hematological findings did not reveal any important or evident change. The correct diagnosis was confirmed by hemoglobin electrophoresis, which was requested as part of the anemia evaluation approach. In standard alkaline electrophoresis, we noticed the presence of a hemoglobin fraction (approximately 40%) in the position where HbA2 migrates. Electrophoresis at acidic pH confirmed the fact that our patients had HbC trait (Figure 1). The cases with HbC/beta-thalassemia and HbSC disease were more complicated.

The case reports which follow were chosen to illustrate some of the clinical features of the disease. We selected to report three cases with the most relevant and representative clinical manifestations.

Case 1: Anemia with splenomegaly and abdominal pain crises

A 38-year-old woman, originally from South of Albania, was referred from the Hematology Department for laboratory examination to confirm her diagnosis

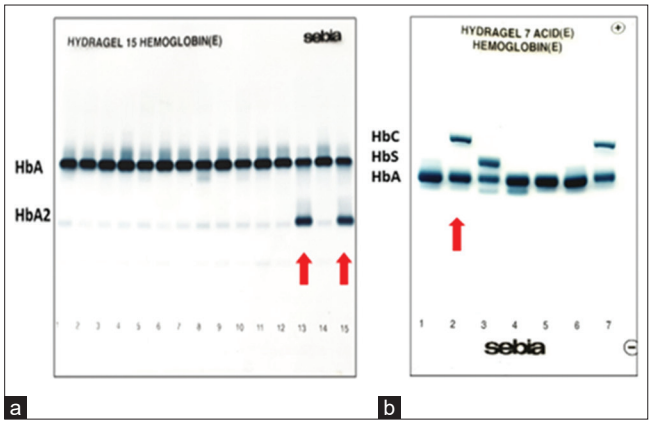

Figure 1: Hemoglobin electrophoresis in a patient with HbC trait (a) Alkaline electrophoresis: HbC trait Nr. 13 and 15, (b) Acid electrophoresis: HbC trait Nr.2

of anemia and determine its type. The patient was followed-up and treated for 5 years in the Hematology Department for anemia symptoms with splenomegaly (confirmed by ultrasonography), abdominal pain crises and recurrent weakness and fatigue.

Laboratory findings suggested microcytic anemia (Hb 10.6 g/l, MCV 68 fl) with normal MCHC (33.1pg). Biochemical findings (Table 1) showed slightly elevated bilirubin with normal liver enzymes during abdominal crisis moments. There are no data showing compromised iron metabolism and impaired erythropoiesis (erythropoietin, ferritin, and iron were normal). In blood smear, we have found mild hypochromia, mikrocytosis, aniso-poichylocytosis, ovalocytes, and target cells. Hemoglobin electrophoresis (Figure 2) at alkaline pH shows three fractions from which we could identify HbA 15%, HbF 15%, and a Hb band of 70% concentration that migrates in the area where normally does HbA2. HbA2 itself does not exceed 10% even in pathological situations. Electrophoresis performed in specific conditions, at acidic pH confirmed that the major fraction of this pattern (70%) is HbC. The molecular study (outside our institution) using PCR-based techniques confirmed the presence of beta+thalassemia allele. These two examinations confirmed the diagnosis of "Double Heterozygous HbC/beta+thalassemia".

Table 1: Laboratory findings of a patient with HbC/beta-thalassemia

Tests	Patient results	Reference range
RBC (10^{12}/L)	5.25	4.2–5.8
Hb (g/dL)	10.1	12–16.5
HCT (%)	33.3	37–50
MCV (fl)	63	80–97
MCH (pg)	19.2	26.5–33.5
MCHC (g/dl)	30.3	31.5–35.5
Ferritin (ng/mL)	33	5–204
Iron (µg/dl)	74.8	60–180
Bilirubin (mg/dl)	Total 1.6	Total: 0.3–1.2
(in abdominal pain crises)	Direct 1.3	Direct: 0–0.2

Case 2. Acute chest syndrome

A 52-year-old woman originally from Lushnja was presented in the emergency department of the

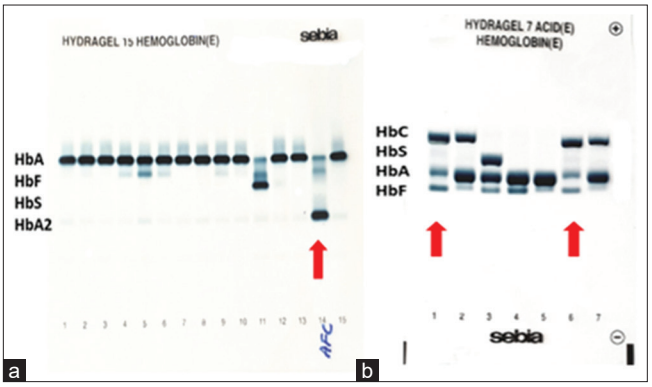

Figure 2: Hemoglobin electrophoresis in a patient with HbC/beta thalassemia (a) Alkaline electrophoresis: HbC/beta-thalassemia Nr. 14, (b) Acid electrophoresis: HbC/beta-thalassemia Nr.1 and 6

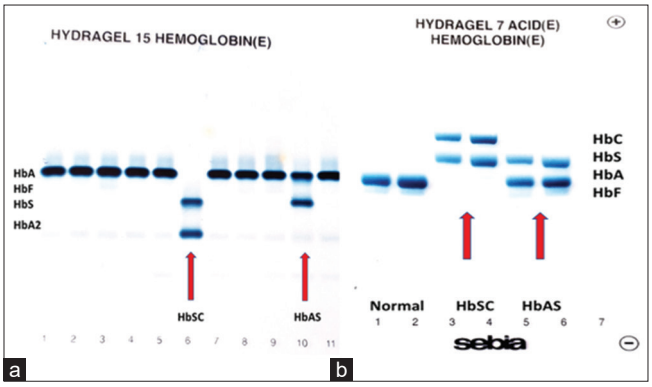

Figure 4: Hemoglobin electrophoresis in a patient with HbSC disease (a) Alkaline electrophoresis: HbSC disease Nr.6, (b) Acid electrophoresis: HbSC disease Nr.3 and 4

University Hospital of Pulmonary Diseases with fever, cough, tachypnea, and chest pain. From the anamnestic data resulted that the patient had been treated in the same hospital for "acute chest syndrome," 1 year earlier. Computed tomography of the thorax confirmed the diagnosis of acute chest syndrome, while abdominal ultrasound examination revealed the presence of hepatosplenomegaly. General laboratory findings showed the presence of hemolytic anemia (Hb 7.7 g/dl) with normal MCV, high reticulocyte count (56%), and normoblasts in the peripheral blood smear. In blood smear, we have also found an abundance of target cells, folded ("pita bread") cells, crystals, and ovalocytes (Figure 3). Hemoglobin electrophoresis was performed for further identification of the type of anemia. In the standard hemoglobin electrophoresis, the presence of two clear bands was shown, one belonging to HbS (53.4%) and the other positioned at the place where HbA2 usually migrates (46.6%). Electrophoresis at acidic pH was performed to determine the hemoglobin type. Acid electrophoresis confirmed the presence of HbC and the diagnosis of double heterozygous HbCS (Figure 4).

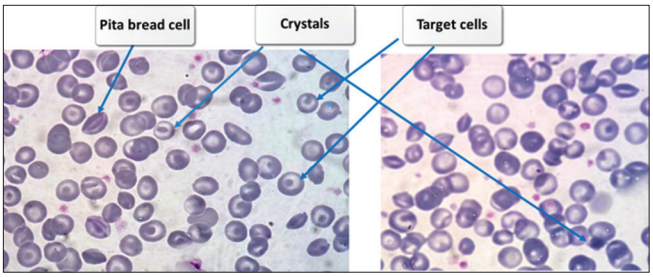

Figure 3: Blood smear in HbSC disease (May Grunwald-Giemsa Stain)

Case 3: Acute abdominal pain and gallstones

A 45-year-old woman patient from Cerrik was hospitalized in Gastro-Hepatology Department with acute abdominal pain, jaundice, fever, and anemia. The illness had a 1-year duration. Ultrasound examinations revealed hepatosplenomegaly and presence of multiple gallstones. Biochemical-hematological examinations showed the presence of anemia with low MCV and normal MCHC, alterations of the hepatic function with hyperbilirubinemia (total bilirubin 7.8 mg/dl, direct bilirubin 5.1 mg/dl), and increased enzymatic activity (ALT 207 U/L, AST 211U/L, ALP 267U/L, Amylase 158U/L, and GGT 85U/L). Standard hemoglobin electrophoresis showed the presence of two clear bands, one belonging to HbS (58.4%) and the other positioned in the place where usually HbA2 migrates (41.6%). Electrophoresis at acidic pH confirmed the presence of HbC and the diagnosis of compound heterozygous HbC/HbS.

Discussion

The presence of HbC is a rare event in the Europe and Mediterranean region where thalassemia and HbS are more frequently encountered [11]. The rarely diagnosed cases are linked with the migration of people from the West-Central Africa and their movements in the trade routes that connected these areas with Europe in centuries [12]. The subjects found in our population do not refer any descent indicative for mutation migration. An additional reason for HbC presence in Albania might also be the past presence of malaria. Until the mid-20th century, malaria has been the principal medical and social cause influencing the reduction of the number of the Albanian population. This disease was endemic in Western lowlands, which was the origin of the above-mentioned patients. Many studies have confirmed that homozygous HbCC patients were strongly protected against severe malaria, and heterozygous (HbAC) were mildly protected [13], [14].

The clinical presentation, as also confirmed in our patient cohort at an adult age, is discrete and unclear [15], [16], [17], [18]. HbC carriers might never be diagnosed because they are asymptomatic. The

first signs that lead patients to diagnosis in most cases are non-specific such as splenomegaly and heterogeneous changes of erythrocyte series in a blood sample without obvious findings of anemia [19]. The red cells findings in the blood film are particularly characteristic in HbSC with crystals containing cells and tendency for membrane folding and HbC/beta-thalassemia with simultaneous presence of target cells. Diagnosis may also be established after complications which, as referred in the literature [20], can be fatal. The most serious clinical presentation belongs to HbCS forms where the sickling phenomenon might lead to pulmonary complications (Case 2), biliary stones (Case 3), retinal phenomena, osteonecrosis, etc. From a morbidity and mortality point of view, the HbC presence, particularly when combined with HbS or thalassemia, is problematic during gestation, especially in the perinatal period [21], [22]. Our patients with compound heterozygosity HbCS come from areas where thalassemia and hemoglobinopathies have a considerable frequency. Since clinical and laboratory data are evident, neonatal screening for hemoglobinopathies with CBC and hemoglobin electrophoresis is a necessity in these areas.

Our study showed that the correct diagnosis of the HbC presence cannot be confirmed with the standard methods used for the screening of thalassemia and sickle cell disease in our country. Research shows that hematological changes might be absent or to a degree that is not an indication for diagnosis [23]. The changes in the peripheral blood smear, although characteristic, do not confirm the diagnosis because they can also be encountered in other forms of hemoglobinopathy. The common electrophoresis in alkaline pH gives information only about the presence of a band that migrates in HbA2 position, which can be HbC, HbO-Arab, or HbD. Electrophoresis at acidic pH confirms the diagnosis because it does not only identify HbC, but it also gives accurate data on the relative percentage of HbC and the other Hb fractions of the patient. In cases where a combination of HbC with α or β thalassemia is suspected, the diagnosis can be confirmed only by molecular biology methods [24]. Chromatography is also a method of choice to correctly diagnose hemoglobinopathies including HbC presence, due to the short time of examination and comparable cost regarding other methods [25].

Conclusions

HbC seems to be a hemoglobin variant with a non-negligible frequency in areas reported as endemic of hemoglobinopathies in Albania. It is presented in various clinical manifestations related also to the combinations of HbC with other hemoglobinopathies such as HbS or beta-thalassemia trait. Correct diagnosis of HbC patients requires a proficient examination of CBC and hemoglobin electrophoresis.

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