International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

© International Scientific Organization

Clinical heterogeneity of Moroccan patients with hemoglobin C disease

Naima Marzouki[1], Assim Mouabad[1], Wafa Quiddi[1], Adil Rabi[1], Imane harkati[1], Youness Zaid[2] and Sanae Sayagh[1]

[1]*Laboratory of medical biology, hematology Department, Arrazi hospital, CHU Med VI, Marrakech/Morocco and* [2]*Mohammed V University and Abulcasis University of Health Sciences*

Abstract

Hemoglobin C disease is an autosomal recessive disorder that results from biparental inheritance of allele encoding hemoglobin C. This mutated form reduces the normal plasticity of host erythrocytes causing a hemoglobinopathy. In homozygotes, nearly all Hemoglobin is in the Hemoglobin C form, resulting in mild hemolytic anemia. The common form of hemoglobin C disease in south Morocco is not usually benign form. His severity and heterogeneity is high. Perhaps, it is due to other local ethnic factors. Here, we studied clinical report, laboratory analysis (by capillary electrophoresis, HPLC and genealogic study) and molecular mechanism in four Moroccan families with hemoglobin C disease. Sepsis, hemostasis and cardiovascular complications can associate to hemoglobin C disease. This genetic disorder can present great health problems. Other factors interact with mutated hemoglobin C, increase oxidative stress and modulate its severity.

Keywords: Hemoglobin C disease, capillary electrophoreses, HPLC, clinical features, physiopathology

Full length article **Corresponding Author*, e-mail: namarzouki@gmail.com

1. Introduction

Hemoglobin C (Hb C) results from a SNP (rs33930165) of the HBB gene leading to an amino acidic substitution from glutamic acid to lysine (sixth codon: GAG → AAG). Individuals with the AC genotype are asymptomatic; those with the CC genotype have mild hemolytic anemia and moderate sickle cell disease [1, 2].

Hemoglobin C (Hb C) is less soluble than Hemoglobin A in red cells causing increased blood viscosity, cellular rigidity, and shortened red cell survival [3, 4]. Previous studies have shown that hydrogen peroxide (hemoglobin autoxidation) reacts with mutated hemoglobin and initiates a cascade of reactions that result in heme degradation. Mice expressing exclusively hemoglobin C had a 6.9-fold increase in heme degradation compared to other hemoglobinopathy [5]. Heme and non-heme iron accumulate in the membranes of pathological red blood cells and redox cycling of this iron (iron accumulation) is involved in membrane damage and hemolysis [6]. HbC nucleation on microscopic aggregates of hemichromes bound to the RBC membrane is an alternative possibility that cannot be completely ruled out, since RBC containing HbC exhibit hemichrome aggregates bound to the membrane [7] and enhanced membrane fragility [8]. Increased exposure of phosphatidylserine (PS) in erythrocytes postulated to contribute to the pathophysiology of Hb C cell disease because of its possible effects on blood coagulation, cell adhesion and cell clearance [9].

Efforts directed toward understanding the molecular mechanisms involved in the high propensity for liganded HbC to form crystals [10-15]. The solubility of HbC crystals is very sensitive to temperature and decreases as temperature is raised [16]. The notion that some HbC "precrystalline" structures and the RBC membrane play a secondary role in generating red blood cell (RBC) rigidity, fragmentation, and microcytosis, as observed in HbC hemoglobinopathy, has already been suggested in systematic studies of human HbC RBC by Charache et al. [8]. The results presented add to the role of the RBC membrane in this hemoglobinopathy, alterations in potassium transport observed in both human and mouse HbC RBC [17, 18], and suggest that the HbC RBC membrane or its membrane abnormalities may serve as a nucleation site for HbC crystallization. Designing drug therapies for various diseases correlated with protein aggregates, polymers, or crystals are going to be very promising [19, 20].

Here, we report four patients with hemoglobin C disease and six others with hemoglobin C trait. They originated from four south Moroccan families. We discuss their clinical severity and heterogeneity.

2. Materials and methods

2.1. Study design

Peripheral blood was obtained from 500 patients with hemoglobinopathy. Four families diagnosed with Hb C disease at the CHU Med VI Hospital (Marrakech, Morocco) were included in this study. The diagnosis was established according to capillary electrophoresis and HPLC. Patient's clinical, histological, radiological criteria and demographic characteristics were collected including questions on disease evolution, phenotype, age at onset and other clinical features.

Ethnic origin of patients and their relatives, family history concerning hemoglobinopathies, clinical data and transfusion history are noted. Recent complete blood count (i5000 sysmex) and blood smears examined to search for any abnormality. Capillary electrophoretic (capillarys 2 flex piercing, sebia) and chromatographic methods (capillary HPLC, D10) quantified different hemoglobin fractions. Genetic diagnosis by HBB gene sequencing, in four probands, confirmed hemoglobin C disease. Consent forms read and signed by all patients. This study respect ethical standards of human institution subjects.

3. Results and discussions

3.1. Clinical history

3.1.1. Family I

He is a 45 years-old white man from south Morocco (Marrakesh). He had hemoptysis and Tuberculosis in his clinical history. He was hospitalized in the vital emergency department (03/06/2017) after severe sepsis, acute hypopnea pneumonia (dyspnea, stage III) and abdominal pain. He was conscious at reception, not hypertensive. Arterial blood pressure was 120/60 mm Hg with 90 beats/minute and bilateral snoring. He had painful hepatomegaly (12 cm), splenomegaly (2 cm) and bilateral lenticular inguinal adenopathy. He had severe anemia and pancytopenia (Figure 1). Hemoglobin was 5.4 g/dl, erythrocytes 1.91 million/µl, 5.16 % of reticulocytes, MCV 79.9 fl, MCH 28.3 pg, and platelets 80 000/µl (table 3). Sickling test was negative. Many target cells and micro spherocytosis observed on stained smears. Bone marrow was reactional without abnormal cells infiltration. We noted inflammatory syndrome (CRP: 515.59 mg/l), acute renal functional failure (Urea: 1.63 g/l and creatinine: 22mg/l), higher Troponin (19.22 pg/ml), hyperglycemia (1.24 g/l), Chlorine 127 mmol/L and potassium 4.1mmol/L. He had low TP (41%) but high TCA (55.5%) and D-Dimer (2.01 µg/ml). Fibrinogen and fibrin degradation products were not examined because he required a rapid transfusion. Chest X-ray showed multiple infectious pulmonary foci without any sign of pulmonary embolism. Renal function was improved after starting intravenous fluids and medications (ant biotherapy, corticoids, diuretic and unfractionated heparin). He presented Hb C (94,8 %), Hb A2 (3,8 %) and Hb A (1,4 %). We suspected hemoglobin C disease. Blood was transferred to genetic laboratory in order to confirm the diagnosis.

3.1.2. Family II

54 years old brown women, presented a C/β-thalassemia {Hb C (66.9 %), Hb A (27.8 %), Hb A2 (3.4 %) and Hb F (1 %)} along with target cells and micro spherocytosis on Blood smear (figure 2). She was hypochromic microcytic without anemia (Hb 12.2 g/dl, MCV 58.4 fl, MCH 21 pg, MCHC 35.9g/dl and platelets 218 000/µl). Heterozygous Hemoglobin C (A/C) was detected in her two children. The older one (27 years old) presented sporadic episodes of musculoskeletal (joint) pain with febrile and repetitive infections. The youngest (11 years old) had no symptoms (table 2). An 18-years–old girl examined initially in the pediatric department of University center in 1999 (at her first year of birth). She had severe anemic syndrome, asthenia, and jaundice. No improvement was noted under iron supplementation. She had hepatomegaly and splenomegaly. Severe anemia necessitated transfusion by two weeks. Her father had vesicular lithiasis. His blood analysis showed hypochromic microcytic anemia. Electrophoresis and HPLC detected beta-thalassemia trait.

First analysis of the girl noted High Hb F (23%) and absence of Hb C allele. We suspected homozygous Beta-thalassemia state. Her state was more severe, it could not be a C/β-thalassemia as for her mother. Genetic analysis confirmed homozygosity for beta-thalassemia.

3.1.3. Family III

Our patient was a 54 years homozygous Hb C woman. She had chronic gastritis (helicobacter pylori +++), hematemesis, episodic febrile, abdominal pain and hemolysis. Her hematologic parameters were anemia, microcytosis, hypochromia, elevated CMHC, reticulocytosis and splenomegaly. Blood smear revealed microcytosis, target cells, spherocytosis, and no crystallized hemoglobin. HPLC, capillary electrophoresis and gene diagnosis confirmed homozygous hemoglobin C disease (CC) (Figure 3). Her daughter had heterozygous hemoglobin C. Electrophoresis showed [34.8% of Hb C, Hb A (62%), Hb A2 (2.8%) and Hb F (0.4%)] and normal bilirubin (table 1-2).

3.1.4. Family IV

She was a 48-year-old woman who presented anemia, microcytosis, reticulocytosis and splenomegaly. Blood smear showed 12% of target cells and 5% of schizocytes (table 1).

Table 1: A Summary of clinical and laboratory data for homozygous Hemoglobin-C patient

Patients / Clinical features	(Family I) Patient I2	(Family II) Patient I2	(Family III) Patient II2	(Family IV) Patient II1
Age (years-old)	45 yrs	53 yrs	54 yrs	48 yrs
Parent origin	Ait baha	zagora	kelaa	Zagora
Hemoglobin g/dl	5.4	12.2	11.3	10.4
MCV fl	79.6	58.4	71.4	76
MCH pg	28.3	21	26.3	27
MCHC g/dl	35.5	35.9	36.8	35.7
Consanguinity	No	yes	yes	Yes
Reticulocytes/µl **%**	166.200 5.16%	96.000/µl 1.65%	123.800 2.88%	207.100 5.31%
Platelets/µl	80000	218000	119000	218000
SPLENOMEGALY	**yes**	No	YES	YES
Hemolysis	+	No	++	+
Ferritin	Normal	Normal	++	Normal
Blood smear	Absence of platelet aggregates Absence of giant platelets Anisocytosis Spherocytes Target cells	Target cells 80% Spherocytes 10%	Target cells + spherocytes	Target cells 12% + schizocytes
Hb C%	96.8	66.9	99.1	97.8
Hb A%	0	27.8	0	0.3
Hb A2%	2.4	4.3	0	1.9
Hb F%	0.8	1	0.9	0
Symptoms	TP 41%, TCA 55.5%. D-dimer 2.01 Diabetes CRP++++ acute renal failure + Troponin ++	Hypochromic microcytic migraine	Acute Gastritis(PH++), hematemesis + fewer episodes + abdominal pain+ spleen sequestration + Migraine + vomiting + nausea + vertigo	Abdominal pain Asthenia Vomiting

Patients / Clinical Features	Family II		Family III (A/C4)	Family IV (A/C3)	Sporadic (A/C)	Sporadic (A/C6)
Age years-old	11 yrs (III2) (A/C1)	27 yrs (III4) (A/C2)	60 yrs	34 yrs	26 yrs	25 yrs
Parent origin	zagora	zagora	MRK	zagora	MRK	MRK
Hemoglobin g/dl	13.2	17	13.9	13.4	13.5	12.4
MCV fl	72	76.4	80.2	79.1	83.7	72.7
MCH pg	25	28.7	28.4	28.3	30.2	25.4
MCHC g/dl	35.6	37.5	35.4	35.7		34.9
Consanguinity	yes	yes	NO	Yes	--	--

Table 2: A Summary of the hematologic data of hemoglobin-C trait patient

Reticulocytes/µl %	34000 0.66%	54600 0.92%	43100 0.88%	59300 1.25%		
Platelets/µl	438	248	269	258		
SPLENOMEGALY	No	No	No	No	No	No
Hemolysis	+	-	-	-	-	+
Ferritin ng/ml	normal	normal	normal	8ng/ml	normal	normal
Blood smear	target cells	target cells	target cells	target cells		
Hb C%	34.8	36.1	36.7	35.9	36.3	32.9
Hb A%	62.0	61.1	59.8	61.3	60	63.5
Hb A2%	2.8	2.8	3.3	2.8	3.7	3.6
Hb F%	0.4	0	0.2	0	0	0
Symptoms	sporadic episodes of joint pain in hand and foot extremities + repetitive infections	No symptom	Goiter + edema of the lower limbs + asthenia	Periodic Asthenia + Migraine + vomiting + nausea + abdominal pain	No symptom	No symptom

Table (3): Blood count evolution of patient II2, Family I.

Hematologic parameters	**Day 1**	**Day 2**		**Day 3**
Hemoglobin (g/dl)	9.6	5.4	**Transfusion with 2 GC**	7.9
MGV (fl)	80.5	79.6		81.3
MCH (pg)	29.5	28.3		28.9
Platelets (µl)	105.000	80.000		70.000
White blood cell (/µl)	4570	1730		1230
Polynuclear (/µl)	4400	1470		1060
Lymphocytes (/µl)	150	230		140
Reticulocytes (/µl)	.....	.....		166.200 /µl

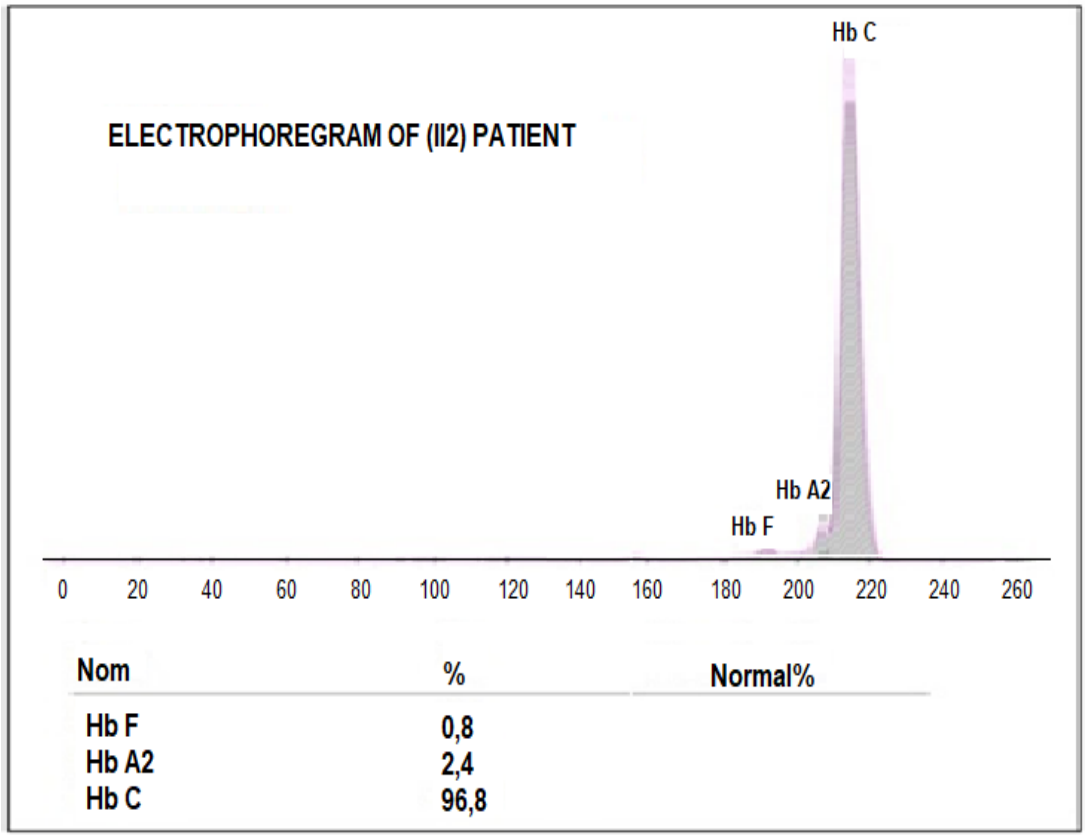


Figure 1(a): Elecrophoregram of patient II2

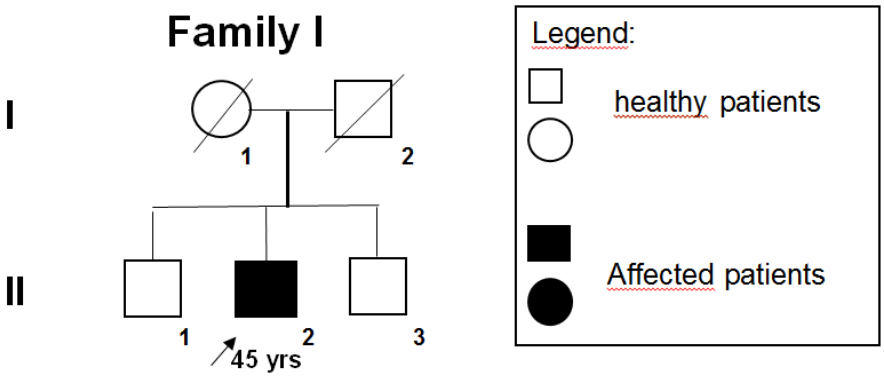


Figure 1(b): Pedigree of Family I

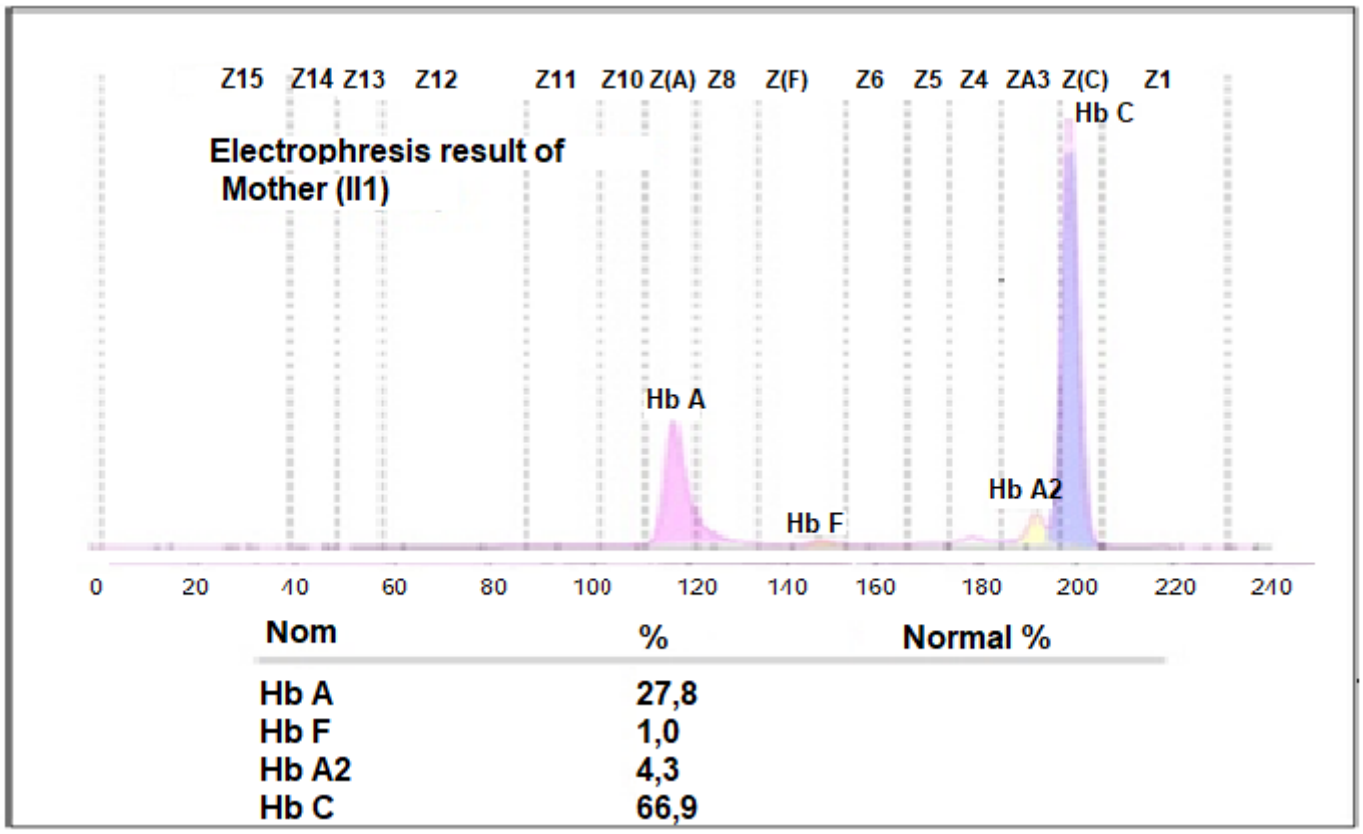


Figure 2(a): Electrophoregram of II1 Patient

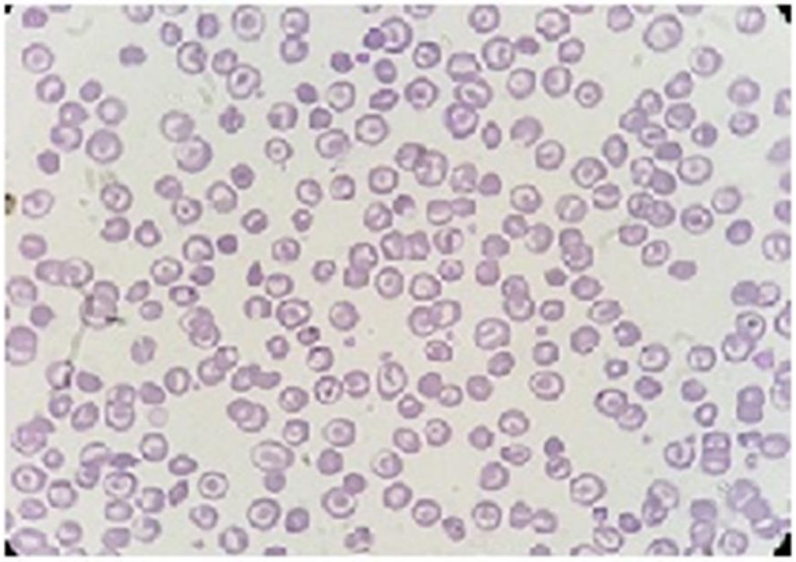


Figure 2(b): Blood smear of Mother II1

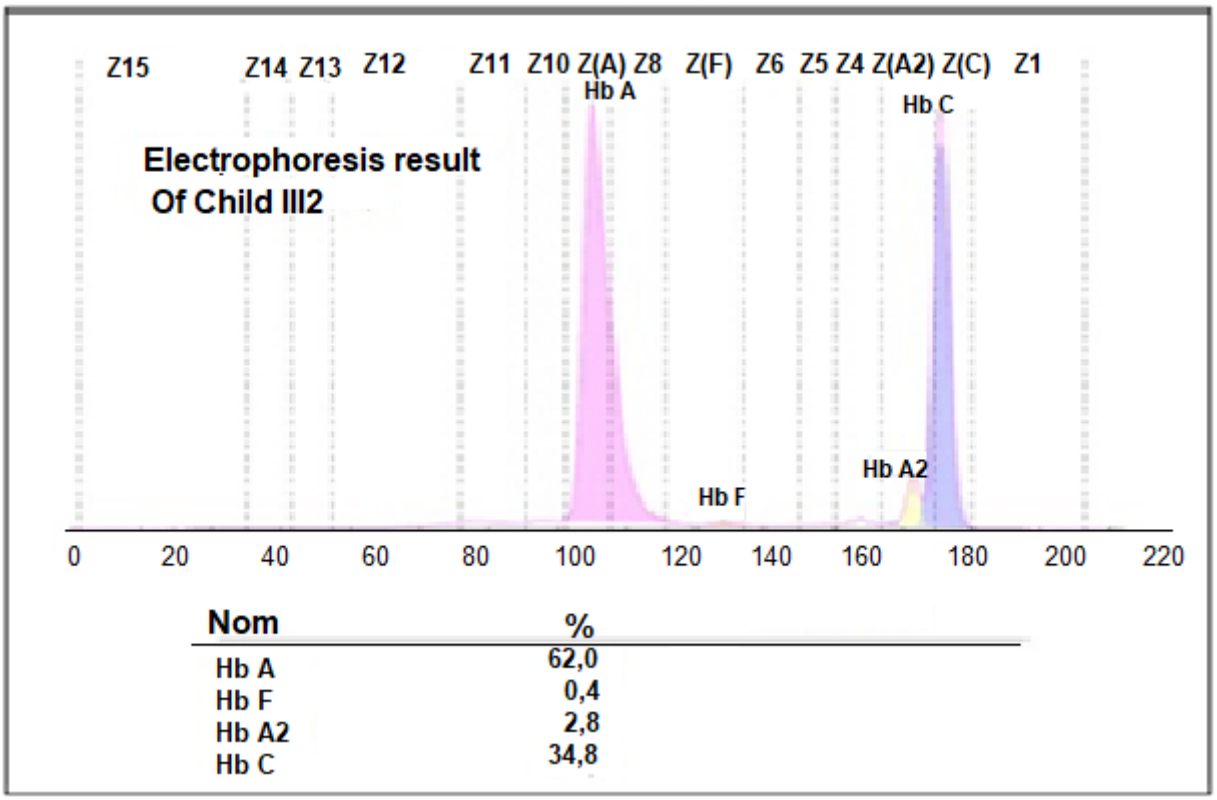


Figure 2(c): Electrophoregram of Child III2

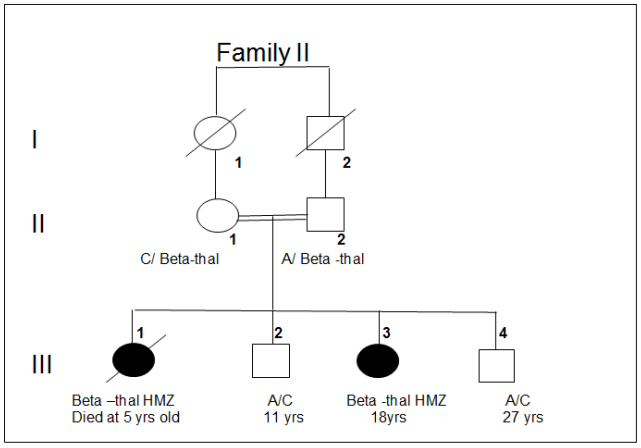


Figure 2(d): Pedigree of Family II

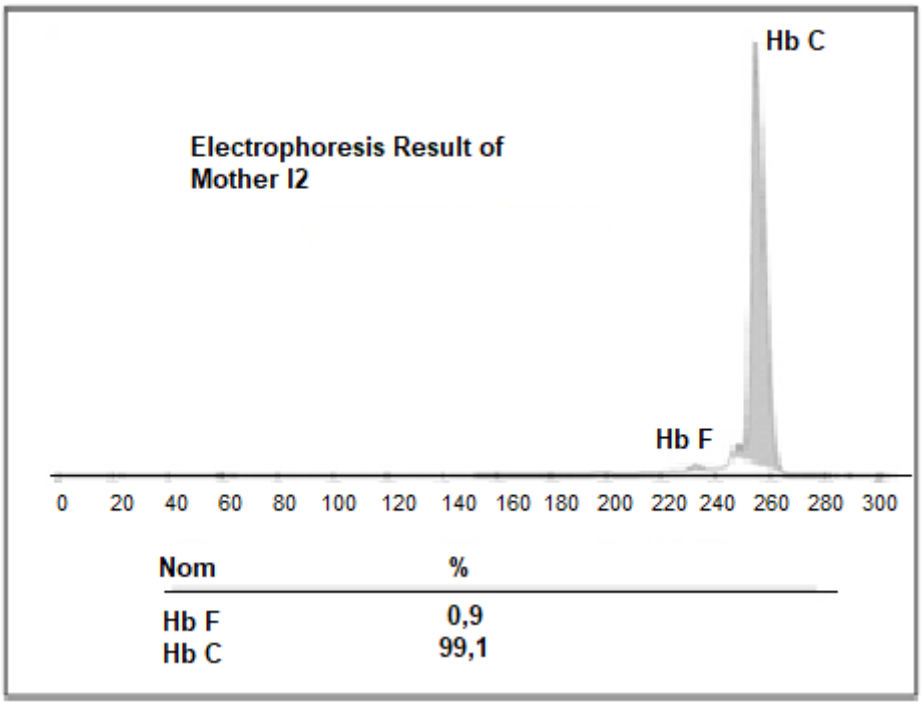


Figure 3(a): Electrophoregram of Mother I2

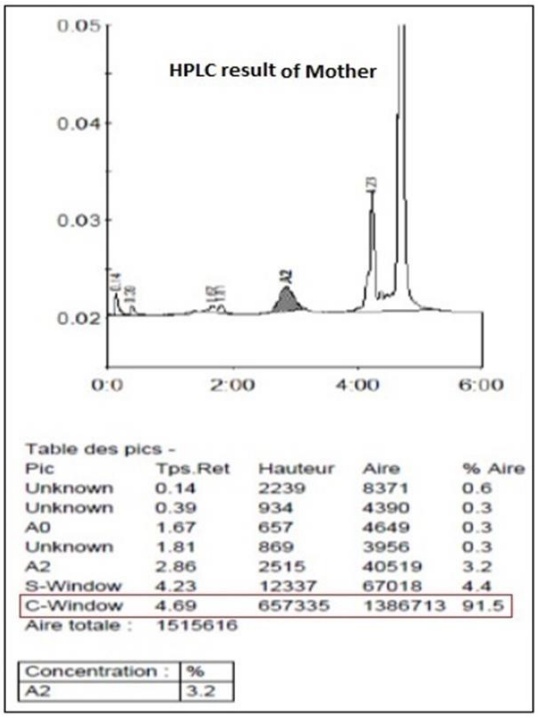


Figure 3(b): HPLC result of Mother I2

Figure 3(c): Pedigree of Family III

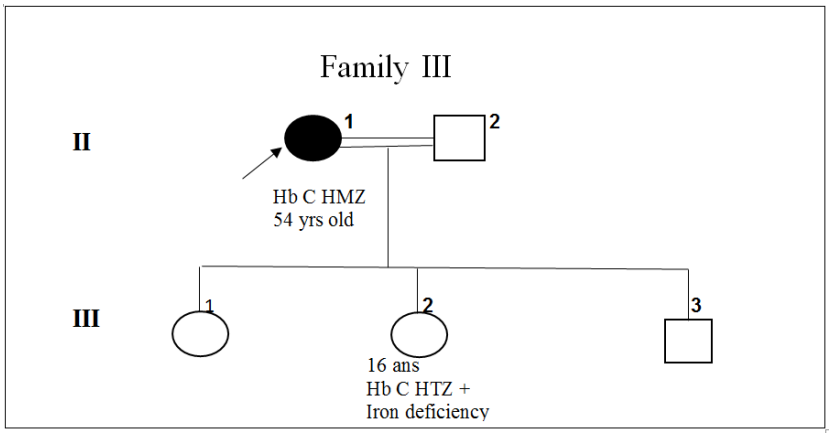
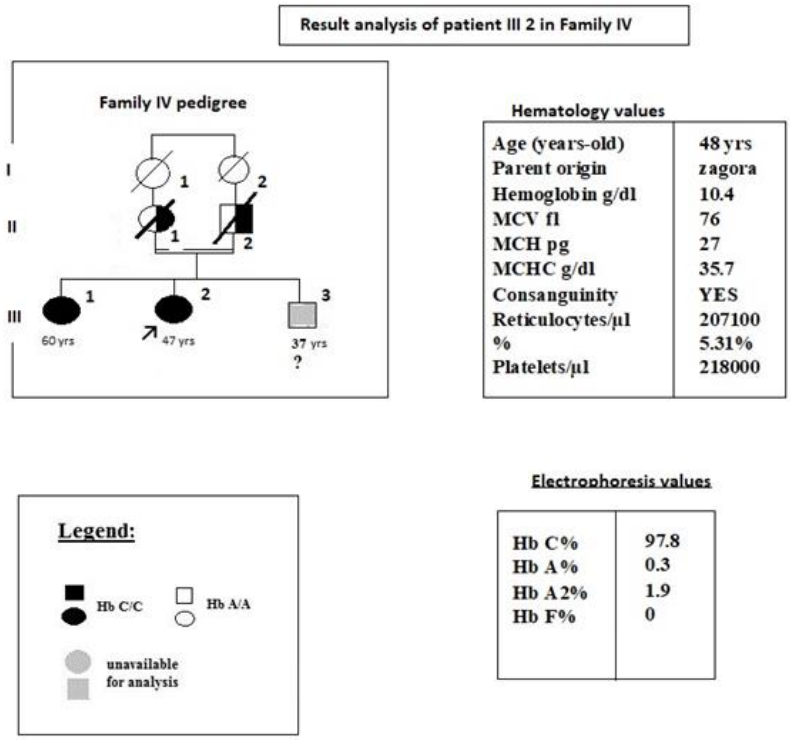


Figure (4): Pedigree, Cells Blood Count and electrophoresis result in patient III2, family IV.



Figures (1, 2, 3 and 4): Families (I, II, III and VI) pedigree, electrophoresis and HPLC results

Diagnosis of hemoglobin C disease (CC) was confirmed by genetic analysis after capillary HPLC and electrophoresis. Her sister presented the same symptomatology. Their parent's blood was unavailable for analysis (figure 4).

3.2. Discussion

Homozygous hemoglobin C is known as an autosomal recessive benign disease. It is characterized by mild anemia and chronic tolerable hemolysis. It is not usually benign in our population. Cases of current study present significant clinical heterogeneity. Some homozygous cases in the south of Morocco show a severe form of hemoglobin C disease. They necessitate hospitalization in vital emergency department. The association of mutated Hb C and severe sepsis with organic complications was not described before. It can suppose a simple coincidence. However, the high level of free radicals in both entities [21, 22], suppose a possible molecular relation.

In moderate forms of homozygous hemoglobin C, we have observed migraine attacks, abdominal pain, nausea, vomiting, vertigo and asthenia. A periodic spleen sequestration has also occurred and required frequent hospitalizations. While other forms of homozygous hemoglobin C showed only slight anemia with tolerated splenomegaly.

Even heterozygotes presented remarkable clinical heterogeneity: asymptomatic forms and forms with periodic joint, musculoskeletal pain attacks and repetitive infections. This can interrupt academic life of young patients and sometimes their state can become severe without adequate therapeutic care. Votaw equally described a heterozygous hemoglobin C with acute renal failure, thrombocythemia and diabetes [23]. Understanding of the pathophysiology of Hb CC disease was advanced by the classical observations of Charache et al. [24] and by the work of Murphy [25] and Lessin et al. [26]. Nevertheless, several issues remain unresolved.

Although extensive research on the animal model, with hemoglobin C disease, has been a major advancement, the pathophysiology still lacks precision. Studies have shown that free radicals are more prevalent in Hb C than in other hemoglobinopathy. During sepsis, antioxidant defenses are overwhelmed [8, 9] and reactive oxygen species cause cellular damage, contributing to organ dysfunction. Therefore, oxidative stress which is strongly induced by mutated Hb C is the main cause of sepsis with its various complications. Our study suggests that homozygous hemoglobin C and sepsis association is not a mere coincidence. Therefore, a patient with sepsis must be asked for his hemoglobin and conversely patient with hemoglobin C disease must be monitored to avoid sepsis and its complications.

4. Conclusion

Intra- and inter-familial variability of this disorder suppose other intrinsic factors interacting with mutated hemoglobin C and changing its evolution from one case to another. Further studies are needed to more understand the pathophysiological mechanism and to improve diagnosis, prognosis and therapeutic management of each patient.

Acknowledgement

The authors appreciate family members who kindly contributed to this study.

Conflict of interest

The authors declare no conflict of interest.

Informed consent

Consent forms read and signed by all patient's family

Author's contributions

NM has designed and performed the study. NM, SS, WQ and AR have drafted the manuscript and did critical editing. MN, MA, IH, SS and WQ have assisted and supported in sample collection and subsequent analysis with statistics. YZ, MA, SS and MN have carefully supervised this manuscript preparation and writing.

Data availability

The authors declare that data supporting the findings of this study are available within the article (and its supplementary files).

The source of funding

There is No source of funding.

References

[1] Rees, D.C., Williams, T.N. and Gladwin, M.T. (2010). Sickle-cell disease. The Lancet. 376(9757): 2018-2031.

[2] Ware, R.E., de Montalembert, M., Tshilolo, L. and Abboud, M.R. (2017). Sickle cell disease. The Lancet. 390(10091): 311-323.

[3] Hirsch, R.E., Raventos-Suarez, C., Olson, J.A. and Nagel, R.L. (1985). Ligand state of intra-erythrocytic circulating HbC crystals in homozygote CC patients. Blood. 66(4): 775-777.

[4] Fabry, M.E., Kaul, D.K., Raventos, C., Baez, S., Rieder, R. and Nagel, R.L. (1981). Some aspects of the pathophysiology of homozygous Hb CC erythrocytes. The Journal of Clinical Investigation. 67(5): 1284-1291.

[5] Rice-Evans, C., Omorphos, S.C. and Baysal, E. (1986). Sickle cell membranes and oxidative damage. Biochemical Journal. 237(1): 265.

[6] Nagababu, E., Chrest, F.J. and Rifkind, J.M. (2003). Hydrogen-peroxide-induced heme degradation in red blood cells: the protective roles of catalase and glutathione peroxidase. Biochimica et Biophysica Acta (BBA)-General Subjects. 1620(1-3): 211-217.

[7] Reiss, G.H., Ranney, H.M. and Shaklai, N.U.R.I.T.H. (1982). Association of hemoglobin C with erythrocyte ghosts. The Journal of Clinical Investigation. 70(5): 946-952.

[8] Charache, S., Conley, C.L., Waugh, D.F., Ugoretz, R.J. and Spurrell, J.R. (1967). Pathogenesis of hemolytic anemia in homozygous hemoglobin C disease. The Journal of Clinical Investigation. 46(11): 1795-1811.

[9] Olson, J.F., Ware, R.E., Schultz, W.H. and Kinney, T.R. (1994). Hemoglobin C disease in infancy and childhood. The Journal of Pediatrics. 125(5): 745-747.

[10] Hirsch, R.E., Samuel, R.E., Fataliev, N.A., Pollack, M.J., Galkin, O., Vekilov, P.G. and Nagel, R.L. (2001). Differential pathways in oxy and deoxy HbC aggregation/crystallization. Proteins: Structure, Function, and Bioinformatics. 42(1): 99-107.

[11] Vekilov, P.G., Feeling-Taylor, A.R., Petsev, D.N., Galkin, O., Nagel, R.L. and Hirsch, R.E. (2002). Intermolecular interactions, nucleation and thermodynamics of crystallization of hemoglobin C. Biophysical Journal. 83(2): 1147-1156.

[12] Vekilov, P.G., Feeling-Taylor, A. and Hirsch, R.E. (2003). Nucleation and crystal growth of hemoglobins. The case of HbC. Methods in Molecular Medicine. 82: 155-176.

[13] Dewan, J.C., Feeling-Taylor, A., Puius, Y.A., Patskovska, L., Patskovsky, Y., Nagel, R.L. and Hirsch, R.E. (2002). Structure of mutant human carbon monoxy hemoglobin C (βE6K) at 2.0 Å resolution. Acta Crystallographica Section D: Biological Crystallography. 58(12): 2038-2042.

[14] Ferrone, F.A., Hofrichter, J. and Eaton, W.A. (1985). Kinetics of sickle hemoglobin polymerization: II. A double nucleation mechanism. Journal of Molecular Biology. 183(4): 611-631.

[15] Patskovska, L.N., Patskovsky, Y.V., Almo, S.C. and Hirsch, R.E. (2005). COHbC and COHbS crystallize in the R2 quaternary state at neutral pH in the presence of PEG 4000. Acta Crystallographica Section D: Biological Crystallography. 61(5): 566-573.

[16] Vekilov, P.G., Feeling-Taylor, A.R., Petsev, D.N., Galkin, O., Nagel, R.L. and Hirsch, R.E. (2002). Intermolecular interactions, nucleation and thermodynamics of crystallization of hemoglobin C. Biophysical Journal. 83(2): 1147-1156.

[17] Brugnara, C., Kopin, A.S., Bunn, H.F. and Tosteson, D.C. (1985). Regulation of cation content and cell volume in hemoglobin erythrocytes from patients with homozygous hemoglobin C disease. The Journal of Clinical Investigation. 75(5): 1608-1617.

[18] Romero, J.R., Suzuka, S.M., Nagel, R.L. and Fabry, M.E. (2004). Expression of HbC and HbS, but not HbA, results in activation of K-Cl cotransport activity in transgenic mouse red cells. Blood. 103(6): 2384-2390.

[19] Wheby, M.S., Thorup, O.A. and Leavell, B.S. (1956). Homozygous Hemoglobin C Disease in Siblings: Further Comment on Intraerythrooytio Crystals. Blood: Journal of Hematology. 11(3): 266-72.

[20] Canterino, J.E., Galkin, O., Vekilov, P.G. and Hirsch, R.E. (2008). Phase separation and crystallization of hemoglobin C in transgenic mouse and human erythrocytes. Biophysical Journal. 95(8): 4025-4033.

[21] Exline, M.C. and Crouser, E.D. (2008). Mitochondrial mechanisms of sepsis-induced organ failure. Frontiers in Bioscience: A Journal and Virtual Library. 13, 5030.

[22] Ruggieri, A.J., Levy, R.J. and Deutschman, C.S. (2010). Mitochondrial dysfunction and resuscitation in sepsis. Critical Care Clinics. 26(3): 567-575.

[23] Votaw, M.L., Spannuth Jr, C., Krish, G. and Guarderas, J. (1990). Acute renal failure in a patient with essential thrombocythemia, diabetes mellitus, and heterozygous hemoglobin C disease. Southern Medical Journal. 83(1): 57-59.

[24] Charache, S., Conley, C.L., Waugh, D.F., Ugoretz, R.J. and Spurrell, J.R. (1967). Pathogenesis of hemolytic anemia in homozygous hemoglobin C disease. The Journal of Clinical Investigation. 46(11): 1795-1811.

[25] Murphy, J.R. (1968). Hemoglobin CC disease: rheological properties of erythrocytes and abnormalities in cell water. The Journal of Clinical Investigation. 47(7): 1483-1495.

[26] Lessin, L.S., Jensen, W.N. and Ponder, E. (1969). Molecular mechanism of hemolytic anemia in homozygous hemoglobin C disease: electron microscopic study by the freeze-etching technique. The Journal of Experimental Medicine. 130(3): 443-466.