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CASE REPORT

A Rare Hemoglobin Variant: Hemoglobin G-Siriraj, in a Moroccan Patient

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SUMMARY

Background: **Hemoglobin G-Siriraj is a rare hemoglobin variant caused by a β-globin gene mutation (HBB: c.22G>A). The focus of this paper is aimed mainly at the chromatographic and electrophoretic properties of hemoglobin G-Siriraj for a presumptive identification. Notably, this is the first documented case of hemoglobin G-Siriraj in Morocco.**
Methods: **This hemoglobin variant was discovered in a 44-year-old Moroccan patient while measuring HbA1c with high-performance liquid chromatography. The discrepancies between capillary electrophoresis and the acid agarose electrophoresis led to suspicion of heterozygous hemoglobin G-Siriraj. Only the globin gene analysis can identify definitively this rare hemoglobin variant.**
Conclusions: **Rare hemoglobin variants represent a diagnostic challenge. Contextualizing cytological and biochemical explorations based on clinical data is essential for effectively guiding diagnosis.**
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KEYWORDS

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INTRODUCTION

Hemoglobinopathies are constitutional disorders that affect the globin protein of the hemoglobin complex. They include the hemoglobin variants with a structural hemoglobin abnormality and thalassemia syndromes, resulting from a decreased synthesis of one of the globin chains.
Hemoglobinopathies represent the most common monogenic diseases worldwide. The World Health Organization estimates that approximately 5% of the population are carriers [1]. Due to the lack of national epidemiological surveys, the prevalence of hemoglobin variants in Morocco remains unknown.
The spectrum of its clinical presentation may vary from completely asymptomatic to severe life-threatening forms. Since the routine employment of high-perfor-

mance liquid chromatography for glycated hemoglobin measurement, the detection of asymptomatic hemoglobin variants has significantly increased. To diagnose a variant, the standard of care involves using three different phenotypic methods, one of them at least is an electrophoretic method [2].

In this paper, we report a case of a hemoglobin variant accidentally discovered while measuring HbA1c. Highlighting the significance of utilizing hemoglobin chromatography for detecting hemoglobin variants, especially the rarer ones, this report describes the electrophoretic profile and provides important information on the prevalence of one of the rarest hemoglobinopathies worldwide.

CASE REPORT

Mrs. T.N., a Moroccan 44-year-old female, has been treated for type II diabetes for two years, with no other notable associated pathologies. She was referred to the biochemistry laboratory of Mohammed V Military Training Hospital for the determination of glycated hemoglobin HbA1c as part of the follow-up of her diabetes. We received a venous blood sample in an EDTA tube. The analysis was performed using the ARKRAY ADAMS Ac1HA-8180V® high-performance liquid chromatography (HPLC) system.

The results revealed an HbA1c level of 44 mmol/mol equivalent to 6.2%, with the presence of a 31.5% hemoglobin variant identified by the analyzer as the hemoglobin C variant (Figure 1a).

In front of this hemoglobin variant, we proceeded to check up other related biological parameters to look for a biological impact. The blood cell count showed normal hemoglobin at 12.9 g/dL with mild microcytosis (79.9 fL) and hypochromia (26.9 pg).

The biochemical assessment revealed ferritin levels at 494 ng/mL and blood glucose at 1.88 g/L with no other abnormalities.

A further study of this variant using another technique was therefore conducted using capillary electrophoresis at alkaline pH on the Capillarys 2 Flex Piercing System® from the Sebia® laboratory. This technique demonstrated a hemoglobin variant migrating into zone D, present at 32.2%, with a decrease in hemoglobin A to 63.6% and a slight increase in hemoglobin A2 to 3.4% (Figure 1b).

Given the discrepancy between the two techniques, a third identification technique was employed using acid agarose gel electrophoresis. This revealed a variant migrating into zone C (Figure 1c). Interpreting these three different results became challenging. We contacted the Sebia® laboratory for an expert's opinion. They confirmed that it was indeed neither hemoglobin C nor hemoglobin D, but a rare variant of hemoglobin that elutes in the hemoglobin C window in HPLC and migrates to zone D in capillary electrophoresis. This variant is identified as hemoglobin G-Siriraj.

The family investigation included the patient's mother, sister, and daughter, the father was deceased. We conducted a complete blood count, a biochemical assessment of hemolysis and iron deficiency, and a phenotypic study of hemoglobin using chromatographic and electrophoretic techniques (Table 1). Only the mother exhibited a hemoglobin profile like that of the index case. Additionally, she displayed no clinical or biological signs associated with this variant.

DISCUSSION

Hemoglobin variants are an inherited genetic abnormality of globin's chain structure. They are usually the consequence of single amino acid substitutions caused by point mutations in a globin gene. Currently, there are 1,426 variants affecting various globin genes listed in the HbVar database, with 953 variants affecting the β-Globin gene [3].

Hemoglobin G-Siriraj arises from a mutation in the beta-globin gene (HBB: c.22 G > A) [4], wherein the glutamic acid at position 7 of the hemoglobin beta chain is replaced by lysine (beta 7 (A4) Glu > Lys) [5]. Diverse genotypes of this abnormal hemoglobin have been described, including heterozygous [4,6,7], homozygous [8], compound heterozygous [5,9], and double heterozygous genotype [10,11].

Hemoglobin G-Siriraj is a rare hemoglobin variant [8]. It was first reported in Thailand in 1965 by Tuchinda et al. [5]. Since then, cases have appeared in Southeast Asia [4-6,8,10] as well as in Martinique [9] and Hungary [7] following migration flows. To our knowledge, our reported case is the first of its kind in the Mediterranean basin.

The literature review shows that the carriers of hemoglobin G-Siriraj generally are asymptomatic [4,6,7,12]. Consistent with prior literature, our case exhibits a clinically silent presentation.

However, it is still necessary to be alert to the possibility of an anemic syndrome or even an intermediate to severe thalassemic syndrome, in a homozygous fetus [8] or when G-Siriraj hemoglobin is co-inherited with beta-thalassemia [5] or alpha-thalassemia [10]. Compound sickle cell syndrome SG-Siriraj is a milder form of sickle cell disease that has also been described in literature [9].

G-Siriraj hemoglobin is a variant that is relatively stable [8]. The complete blood count of heterozygous cases reported in the literature reveals no hematological abnormalities. However, *in vitro*, erythrocytes are dehydrated by increased potassium efflux through the activated K-Cl co-transporter [13], responsible for microcytosis and hypochromia.

Microcytosis [8], microcytic hypochromic anemia [5,8, 11], and certain cytological abnormalities - target cells [5] and Heinz bodies [9] - have been reported in homozygous, compound heterozygous or double heterozygous carriers. Our patient presents microcytosis and hy-

Table 1. Biochemical assessment of the family investigation.

Parameters	Index case	Mother	Sister	Daughter
Hemoglobin (g/dL)	12.9	12.6	10.7	13.3
VGM (fL)	79.9	83.9	83.7	89.1
MCHT (pg)	26.9	27.9	28 .8	29.9
HPLC	Hb « C » variant at 31.5%	Hb « C » variant at 32.2%		
Capillary electrophoresis	Heterozygous variant in the D zone at 32.2%	Heterozygous variant in the D zone at 36.4%	No Hb variant	No Hb variant
Ferritin (ng/mL)	494	134	10	80
Haptoglobin (g/L)	0.32	0.69	0.08	1.4
LDH (UI/L)	241	243	208	203

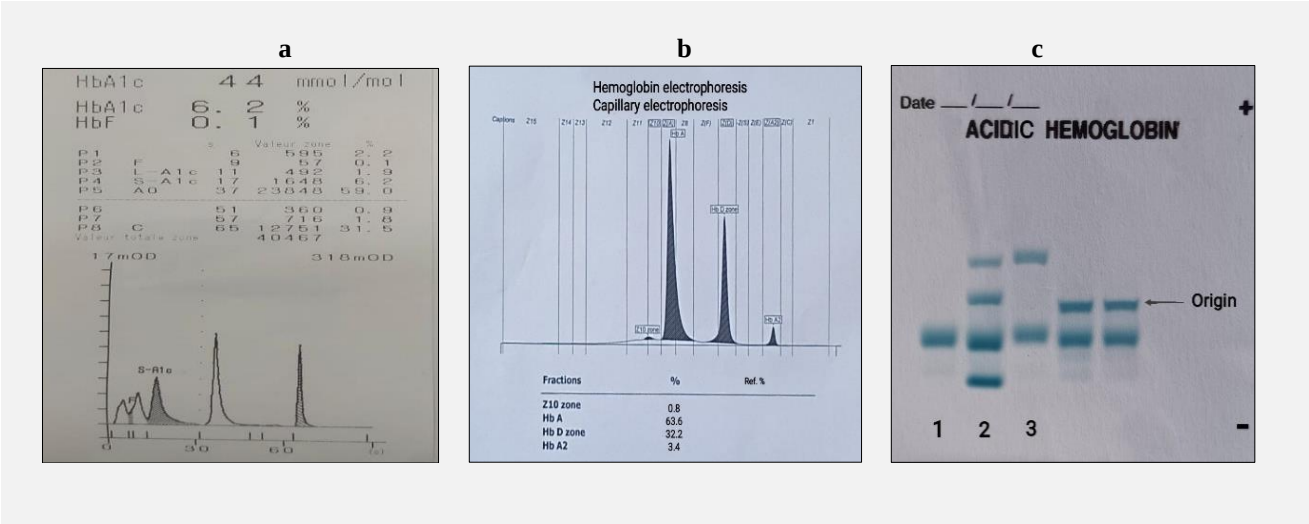

Figure 1. a) High-performance liquid chromatography results. b) Capillary electrophoresis of hemoglobin of our case. c) Acidic hemoglobin electrophoresis. Lane 1: Normal control; Lane 2: Pathological control AFSC; Lane 3: Patient profile.

pochromia with no biological signs of hemolysis.

The substitution of lysine for glutamic acid at position 7 reduces the net charge of the hemoglobin molecule by acquiring a positive charge [7]. This change can interfere with methods such as HPLC. Since the routine use of HPLC for glycated hemoglobin measurement, the incidental detection of hemoglobin variants has become common in medical laboratories. With this technique, the most common genetic variants of hemoglobin are usually recognized and quantified presumptively. They are identified based on their retention time in manufacturer-defined windows. However, rare variants may coelute with the most common ones. This was observed in our patient, where the variant had a retention time close to that of hemoglobin C.

Hence, it is essential to compare the results obtained by HPLC with those obtained using a second complementary technique based on a different principle. For our investigation, we chose capillary electrophoresis, which revealed a hemoglobin variant migrating into the hemoglobin D zone quantified at 32.2%. The studies by Bao et al. [11] and Chen et al. [8], describing cases of hemoglobin G-Siriraj, reported the same electrophoretic behavior using this technique. Hemoglobin G-Siriraj migrates into zone 6, which corresponds to the migration zone of hemoglobin D.

The third identification technique, acid agarose gel electrophoresis, revealed a hemoglobin variant with electrophoretic mobility like that of hemoglobin C. The reports by Rhoda et al. [9] and Tuchinda et al. [5] describe identical electrophoretic mobility for hemoglobin G-Siriraj.

Hemoglobin G-Siriraj exhibits a distinctive electrophoretic profile, migrating into the hemoglobin D window

in capillary electrophoresis and the hemoglobin C window in acid gel electrophoresis.
The percentage of the variant in our results closely aligns with the reported range of hemoglobin G-Siriraj in heterozygotes in the literature (32 to 40% of total hemoglobin) [7,9].
A family investigation involving first-degree relatives allows us to infer the patient's genotype. The mother exhibits the same heterozygous G-Siriraj phenotypic profile, confirming our diagnosis.
Simple hematological and biochemical tests, combined with knowledge of the clinical and family context, facilitate an effective diagnosis. However, characterizing a rare variant sometimes necessitates molecular biology for a genotypic diagnosis.

CONCLUSION

This study presents a case of hemoglobin G-Siriraj incidentally discovered through HPLC. The clinical and biological profile aligns with the typically described heterozygous forms in the literature. However, genetic analysis remains crucial for confirming the diagnosis. The description of this case contributes valuable information regarding the prevalence of this rare hemoglobinopathy worldwide.
The medical biology laboratory plays a vital role in detecting hemoglobinopathies in healthcare seekers. Additionally, these findings may motivate public health managers to conduct more extensive epidemiological investigations.

Declaration of Interest:
None.

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