Case Report

HAEMOGLOBIN KENITRA IDENTIFIED IN A PORTUGUESE MAN WITH TYPE 2 DIABETES AND PHEOCHROMOCYTOMA

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Summary
The periodical evaluation of diabetic patients, for the assessment of glycohemoglobin by High-Performance Liquid Chromatography (HPLC), allows us to detect by chance haemoglobin's structural variants.
We describe here a clinical report from a Portuguese man previously diagnosed for type 2 diabetes and high blood pressure that developed a typical symptomatic case of pheochromocytoma that was due to a tumour located at the left adrenal gland.
When the assessment of glycated haemoglobin (HbA1c) by HPLC was performed, we found by chance, that he was a carrier of an haemoglobin variant. By DNA sequencing the variant was identified as being Hb Kenitra (beta 69(E13) Gly>Arg HBB:c.208G>C), an asymptomatic Hb variant.

Introduction

Haemoglobin Kenitra is an asymptomatic haemoglobin (Hb) variant of the β-globin chain, α2β2 69, Gly →Arg (GGT →CGT), slow moving Hb, that can interfere with HbA1c and HbA2 measurements, therefore often an occasional finding.
It has already been described alone (a Moroccan man)[1] or in association with other haemoglobinopathies (a man from Cameroon, where it was associated with another β chain variant, Hb Yaoundé [β134(H12)Val>Ala] [2] -and a woman from Morocco – here associated with α- thalassemia) [3] .

Patient and Methods

A 56-year-old Portuguese man was admitted at Hospital Curry Cabral, Lisbon for surgical resection of a pheochromocytoma located on the left adrenal gland, in May 2010. Pre and posoperative analytical control was done in the laboratory of this

hospital. Variant II System (BIO–RAD Laboratories, Hercules, CA, USA), HbA1c Dual Program/ β Thalassemia Program was used for automatic separation of haemoglobins, by ion-exchange high-performance liquid chromatography, allowing the determination of HbA1c (absorbance measured at 600 nm) and Hb variant screening (absorbance measured at 415 nm) [5].
Full blood count was performed in Coulter LH 780 (Beckman Coulter, Miami, USA); sickling test was done using coverslide method with reducing agent (2% sodium dithionite). Catecholamine metabolism derivative products in urine were assessed using an HPLC System (BIO–RAD Laboratories, Hercules, California).
The final identification of the Hb variant was carried out by DNA sequencing.

Results

The patient, a Portuguese man from Sesimbra, presents hypertension and type 2 diabetes, both diagnosed more than 10 years before. In the last two years, he reported paroxistic episodes of headache, palpitations, sweating, trembling and marked anxiety. The patient reported no history of anemia or blood transfusions.
In preoperative analysis, a determination of HbA1c was requested. In the chromatogram obtained in Variant II, A1c Dual Program, we found an E-Window, where an Hb variant elutes at a later time than HbA (retention time 1.78 and 1.63 minutes respectively) – late eluted Hb variant (figure 1). The HbA1c was 7,4 % (National Glycohemoglobin Standardization Program-NGSP), 58 mmol/mol (International Federation of Clinical Chemistry (IFCC). In Variant II, B-thal program, this Hb variant co-elutes with HbA2 (3,0 min) with a total peak area of 54% (figure 2). The HbA1c was 6.5 % (NGSP), 47 mmol/mol (IFCC).
At the routine total blood count, the patient presented no anemia (RBC 4,74 $x10^{12}$/L, Hb 14,2 g/ dL, MCV 86,8 fL, MCH 30,0 pg ; MCHC 34,5 g/dL). The leucocytes an platelet count were normal. We have not found morphological abnormalities in blood smear. The sickling test was negative.
Fibrinogen was high (507 mg/dL). Iron metabolism was not performed.
Fasting blood glucose : 151 mg/dL.
The hormonal urinary amines were increased: Norepinephrine 1022,0 µg/ day (Normal range: 12,1- 85,5); Epinephrine 1210,2 µg/day (NR: 1,7- 22,4); Normetanephrines 6640 µg/day (NR: 162-527); Metanephrines 19657 µg/day (NR: 64-302); Vanylmandelic acid (59,2 mg/day (NR: 1,8-6,7).
Further analysis were performed at a Reference Laboratory in Centro Hospitalar e Universitário de Coimbra, using DNA sequencing for identification of the Hb variant, and concluded that it was caused by a HBB:c.208G>C mutation at codon 69 (GGT→CGT; β2 Gly69Arg, already described as Hb Kenitra (ref)
No other abnormalities at the haematological, biochemical or hormonal assessments were found.
The abdomen computed tomography scan showed, at the left adrenal gland, a well-defined lesion with tissue density, measuring 6,5 x 6 x 8 cm and showing moderate heterogeneous uptake of contrast medium.
The histological examination of the removed tumor confirmed the diagnosis of pheochromocytoma.
After surgery all previous symptoms related to adrenal medullary hyperfunction disappear. Presently, the patient remains well controlled for diabetes and high blood pressure, under therapy with metformin, ramipril and amlodipine.

Discussion

Haemoglobin Kenitra has been reported in three cases in the literature until now. In two of this cases, it was associated with other hemoglobinopathies: an α^+ talassemia trait in a Moroccan woman [3]; and another variant – an Hb Yaoundé [β134(H12)Val>Ala] [2] in a man of Cameroon origin.

In the third case, haemoglobin Kenitra was reported alone [1],in a Maroccan man, while screening for hemoglobinopathies.
The case we report here, the hemoglobin variant was found in routine control of diabetes and the patient had no anemia.
In Centro Hospitalar de Lisboa Central, that owns Hospital Curry Cabral, we do about 450 determinations of HbA1c monthly, in which an average of 0,8% patients show an hemoglobinopathy, mostly HbS, some of them detected for the first time.
This finding emphasizes the concept that it is always important to analyze the chromatogram upon validation of the results obtained by HPLC.
The HbA1c result we reported to the medical clinic - 6.5 % (NGSP), 47 mmol/mol (IFCC) - was obtained in B-thal program, following the procedure proposed by the manufacturer, when a variant is detected in Dual Program.
Our patient stated that there had always been difficulties in previous HbA1c assessments when the routine tests were performed in other institution. This was probably caused by the interference of the haemoglobin variant in the assessment of HbA1c, affecting the accuracy of this determination.
Hb Kenitra has been described in patients with some ancestral relationship with north Africa. Kenitra is a city in northern Morocco.The occurrence of an African original mutation in a Portuguese man can be explained by the geographical proximity between countries and by the rich historical ties between populations from Portugal and Morocco through the centuries. Unfortunately, in the present case, family studies were not possible.

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CONFLICT OF INTEREST:
The authors have no conflict of interest to declare.

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HbA1c (NGSP) = 7.4* % HbA1c (IFCC) = 58* mmol/mol

Analysis comments:

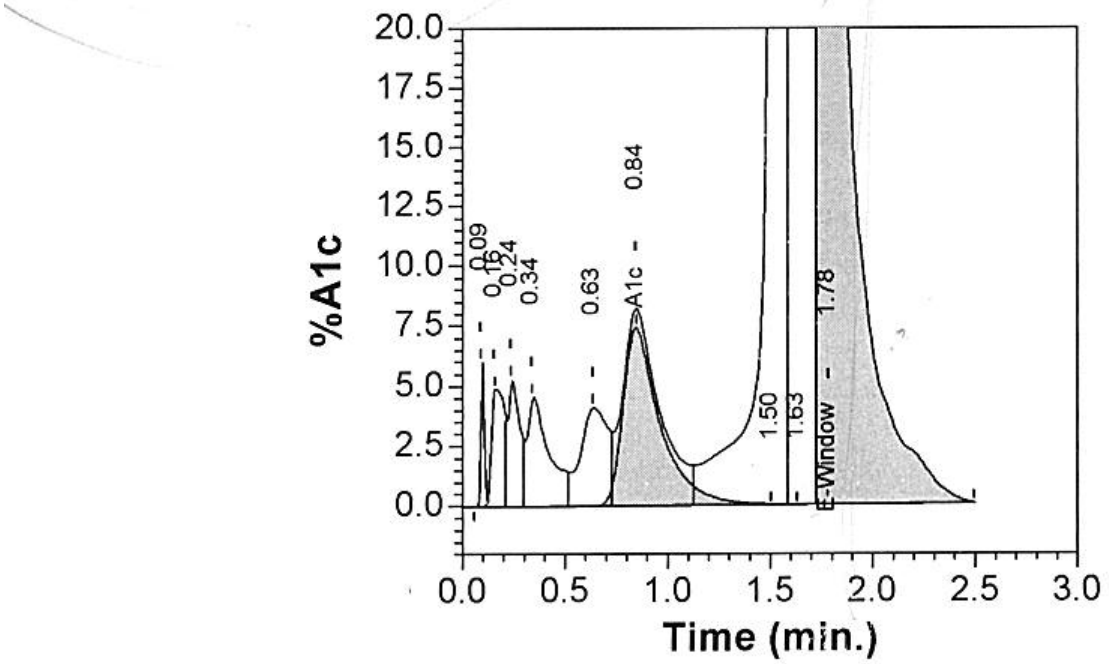

Fig 1- Determination of HbA1c by HPLC (Variant II A1c dual program).

Peak Name	NGSP %	Area %	Retention Time (min)	Peak Area
A1a	---	0.7	0.182	14604
A1b	---	0.6	0.267	13587
F	1.0	---	0.382	21969
LA1c	---	0.6	0.752	13150
A1c	6.5*	---	0.955	49060
P3	---	6.9	1.478	150370
Ao	---	41.3	1.773	900649
A2	54.2*	---	3.029	1019823

*Values outside of expected ranges Total Area: 2,183,213

HbF = **1.0 %**
HbA1c (NGSP) = 6.5* % HbA1c (IFCC) = 47* mmol/mol
HbA2 = **54.2* %**

Analysis comments:

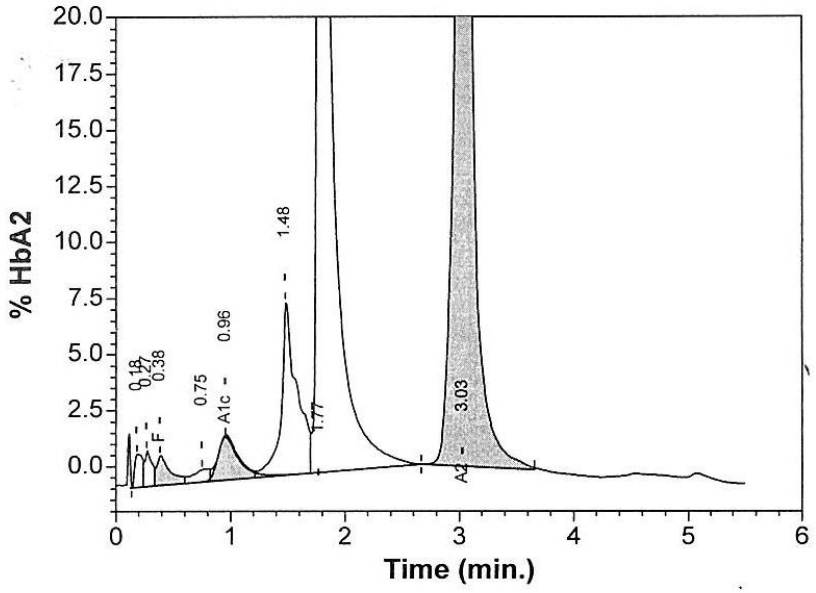

Fig 2-Determination of HbA2 in variant II , β-talassemia program. The high value of HbA2 (54, 2%) leads us to the suspition of the presence of a haemoglobin variant.