International Journal of Laboratory Hematology
The Official journal of the International Society for Laboratory Hematology

Letter to the Editor

Haemoglobin kenitra identified in a Portuguese man with type 2 diabetes and pheochromocytoma

Sir, Haemoglobin (Hb) Kenitra is an asymptomatic variant of the β-globin chain [α2β2 69, Gly →Arg (GGT → CGT)], slow-moving Hb that can interfere with HbA1c and HbA2 measurements, and is therefore an occasional incidental finding.

Hb Kenitra has been described to occur both alone (in a Moroccan man) [1] and in combination with other haemoglobinopathies (e.g. with Hb Yaoundé, another β chain variant, in a Cameroonian male, [2] and with α-thalassaemia, in a Moroccan woman [3]). We report here the incidental finding of Hb Kenitra in a diabetic man during HbA1c analysis.

We report a 56-year-old Portuguese Caucasian man with type 2 diabetes, who was admitted to the Hospital Curry Cabral, Lisbon, for surgical resection of a pheochromocytoma on the left adrenal gland. Catecholamine metabolism derivative products in urine were assessed using a high-performance liquid chromatography (HPLC) system (Bio-Rad Laboratories, Hercules, CA, USA). A full blood count was performed using a Coulter LH 780 haematology analyser (Beckman Coulter, Miami, FL, USA), and biochemical parameters were measured on a Vitros® 5.1 chemistry system (Ortho Clinical Diagnostics, Rochester, NY, USA). HbA1c determination and Hb variant screening were performed using ion-exchange liquid chromatography (Variant II Dual Program System, Bio-Rad Laboratories). Absorbance was measured at 600 nm for HbA1c and 415 nm (β-Thalassemia Program) for Hb variants. The sickling test was performed using the cover slide method with reducing agent (2% sodium dithionite).

The final identification of the Hb variant was carried out by DNA sequencing.

The patient, a Portuguese man from Sesimbra, had been diagnosed with hypertension and type 2 diabetes more than 10 years earlier. In the last 2 years, he had reported paroxysmal episodes of headache, palpitations, sweating, trembling and marked anxiety. The patient reported no history of anaemia or blood transfusions.

Hormonal urinary amines were increased: norepinephrine 1 022.0 μg/day (normal range [NR]: 12.1–85.5); epinephrine 1 210.2 μg/day (NR: 1.7–22.4); normetanephrines 6 640 μg/day (NR: 162–527); metanephrines 19 657 μg/day (NR: 64–302) and vanilmandelic acid 59.2 mg/day (NR: 1.8–6.7). The patient presented no anaemia (RBC 4.74 × 10^{12}/L, Hb 14.2 g/dL, MCV 86.8 fL, MCH 30.0 pg, MCHC 34.5 g/dL). The leucocyte and platelet counts were normal, and there were no morphological abnormalities in the blood smear.

Fibrinogen was high (5.07 g/L). Iron metabolism was not analysed. Fasting blood glucose was 8.38 mmol/L.

The pre-operative analysis included a request for HbA1c determination. In the chromatogram obtained in the Variant II, A1c Dual Programme, we found an E-Window (46.9% area) where an Hb variant was eluted at a later time than HbA (retention time 1.78 and 1.63 min, respectively) – a late-eluting Hb variant (Figure 1a). The Variant II gave a HbA1c level of 7.4% (according to the National Glycohemoglobin Standardization Program – NGSP) or 58 mmol/mol (International Federation of Clinical Chemistry (IFCC) (conversion aid available at http://www.ngsp.org/convert1.asp). Using the Variant II β-Thalassemia Program, this Hb variant co-eluted with HbA2 (3.0 min) [4] with an area of 54% (Figure 1b); HbA1c was 6.5% (NGSP) and 47 mmol/mol (IFCC). The sickling test was negative.

Further analyses were performed in a reference laboratory at the Centro Hospitalar e Universitário de Coimbra, using DNA sequencing for identification of the Hb variant, and concluded that it was caused by the *HBB*: c.208G>C mutation at codon 69 (GGT→CGT; β2 Gly69Arg), previously described as Hb Kenitra [1].

No other abnormalities in the haematological, biochemical or hormonal assessments were found.

The abdominal computed tomography scan showed a well-defined lesion with tissue density measuring 6.5 × 6 × 8 cm on the left adrenal gland and moderate heterogeneous uptake of contrast medium. A histological examination of the removed tumour confirmed the diagnosis of pheochromocytoma.

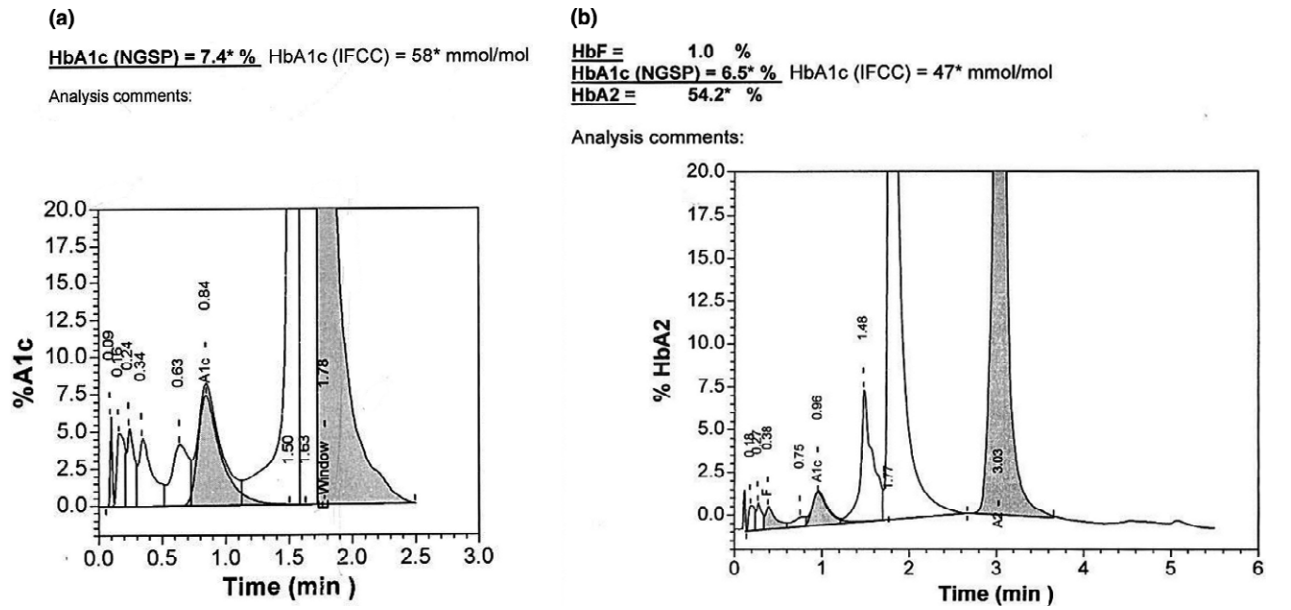

Figure 1. Chromatograms from Variant II (HPLC): (a) determination of HbA1c (V II A1c dual programme), showing an E-window, and (b) determination of HbA2 in the Variant II, β-thalassemia Program. The high HbA2 value (54.2%) leads us to suspect the presence of a haemoglobin variant.

After surgery, all previous symptoms related to adrenal medullary hyperfunction disappeared.

Currently, the patients' diabetes and high blood pressure are being well controlled with metformin, ramipril and amlodipine.

In our patient, Hb Kenitra was an incidental finding that occurred during testing to manage his diabetes; the patient had no anaemia. The HbA1c result we reported to the medical clinic – 6.5% (NR 4.3–6.1) – was obtained using the Variant II β-Thalassemia Program, following the procedure proposed by the manufacturer (Bio-Rad Laboratories) when a haemoglobin variant was detected.

This finding emphasizes the concept that it is always important to analyse the chromatogram upon validation of HPLC results. It is essential that an accurate result of HbA1c is provided when a Hb variant is present, to avoid over- or under-treatment of diabetic patients.

The patient stated that there had always been difficulties in previous HbA1c assessments when the routine tests were performed in other institutions. This was probably caused by the interference of the Hb variant in the assessment of HbA1c, affecting the accuracy of this determination [5].

In the Centro Hospitalar de Lisboa Central, which incorporates the Hospital Curry Cabral, we perform about 450 determinations of HbA1c each month (Variant II – Dual Programme), of which an average of 0.8% show a haemoglobinopathy, mostly HbS, as identified in the β-Thalassemia Program as a reflex test. Some of these haemoglobinopathies are detected for the first time in the management of diabetes and, for us, it is good laboratory practice to report these findings or to suggest future studies, such as DNA confirmation, when Hb variants are identified, if only to provide information for carriers.

Hb Kenitra has been reported in three cases in the literature to date and was associated with other haemoglobinopathies in two of these: an α^+ thalassaemia trait in a Moroccan woman [3], and another variant – Hb Yaoundé – in a black man of Cameroon origin [2]. In the third case, Hb Kenitra was reported as the sole haemoglobinopathy in a Moroccan man, using reversed-phase cation-exchange chromatography, and in this case, determination of HbA1c was not possible [1].

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 original African mutation in a Portuguese Caucasian man can be explained by the geographical proximity between the countries and by the rich historical ties between the populations of Portugal and Morocco through the centuries.

Unfortunately, in the present case, family studies were not possible.

Acknowledgements

We would like to thank Ana Mendonça, Ana Pinheiro and Ana Catarina Oliveira.

Conflict of interest

The authors have no conflict of interest to declare.

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doi: 10.1111/ijlh.12437

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