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Short Communication

Hb Moncloa: A new variant of haemoglobin that interferes in the quantification of Hb A1c

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ABSTRACT

Background: In most routine laboratories in Spain, the commonly used method for evaluating HbA1c is ion-exchange high performance liquid chromatography (HPLC). The presence of a variant of Hb may interfere with the quantification of HbA1c.
Aims: Here, we report a novel haemoglobin variant, named Hb Moncloa, which was found during a routine health check at the Hospital Clínico San Carlos in Moncloa (Madrid), Spain.
Methods and results: Molecular characterization of β gene identified a novel transversion mutation [β80(EF4)Asn > Ser; HBB:c.242A > G].
Conclusions: When there is no correlation between clinical, glycemic status and glycated haemoglobin of the patient, the chromatogram of HbA1c should be carefully checked to detect the possible presence of variants that cause interference in their measurement.

1. Introduction

Structural haemoglobinopathies and thalassemias constitute the most common monogenic alterations in the world [1]. Most are clinically silent, and these changes can easily be identified during neonatal screening, in population studies and using the current methods for quantifying glycated haemoglobin (HbA1c).

HbA1c is widely accepted as the most reliable marker for monitoring long-term glucose control in patients with diabetes mellitus and thus for assessing the risk of developing the chronic complications associated with diabetes [2]. In most routine laboratories in Spain, the commonly used method for evaluating HbA1c is ion-exchange high performance liquid chromatography (HPLC). Careful inspection of the chromatogram allows detection of haemoglobin (Hb) variants through changes from the normal pattern. The presence of a Hb variant may interfere with the quantification of HbA1c. In these cases, there is a discrepancy with the rest of the analytical and clinical data, and other methods are necessary to verify the accuracy of the results [3].

Here, we report a novel haemoglobin variant, named Hb Moncloa, which was found during a routine health check at the Hospital Clinico San Carlos in Moncloa (Madrid), Spain.

2. Materials and methods

A 45 year-old Rumanian female during a routine health check was detected a novel haemoglobin, which was visualized in the chromatogram obtained in the glycated haemoglobin determination.

HbA1c quantification was performed by ion-exchange HPLC assay in an automated glycohaemoglobin analyzer HLC-723G8 (Tosoh G8; Tosoh Bioscience, Tokyo, Japan).

The hematological parameters were determined with an automated cell counter (Advia® 120 System, Siemens S.A., Germany). Haemoglobin was studied by capillary zone electrophoresis following the manufacturer's guidelines for the Sebia Capillarys Flex system using reagents provided in the Capillarys Haemoglobin (E) kit (Sebia, Norcross, GA) and HPLC analysis was performed using the manufacturer's instructions for the BioRad Variant II β-thalassemia Short Program (Bio-Rad, Hercules, CA).

Following the isolation of genomic DNA with an automatic method (Biorobot® EZ1; Quiagen GmbH, Hilden, Germany) the genomic DNA was quantified by NanoDrop 1000 (Thermo Scientific, Wilmington, DE, USA).

The β globin gene from promoter regions and the 3′ UTR was amplified by polymerase chain reaction (PCR) using the following pairs of primers: β1F 5′-TCC TAA GCC AGT TGC CAG AAG-3′ [specific for the

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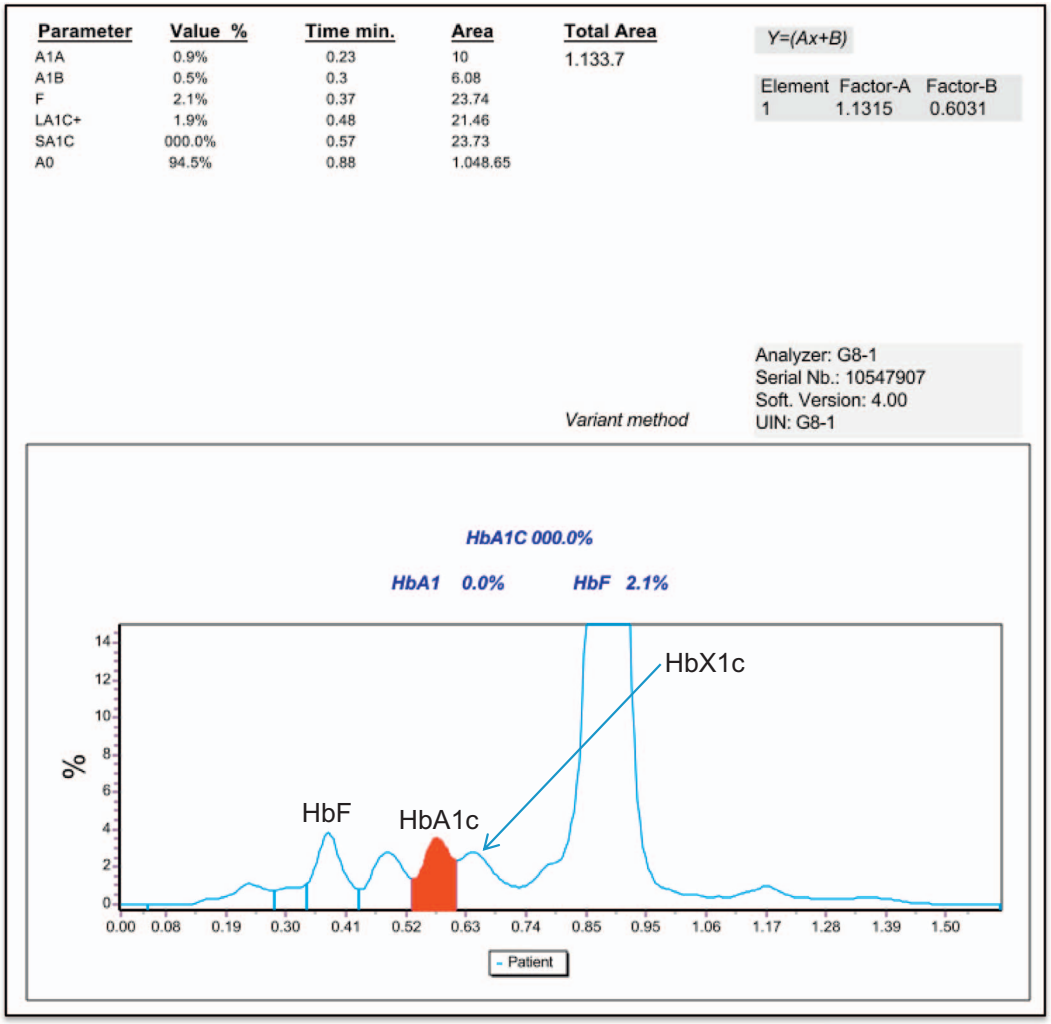

Fig. 1. Chromatogram obtained using a Tosoh G8 showing HbF (2.1%) and HbA1c (not quantified); HbX1c indicates abnormal findings originating from the Hb variant.

5′ region of the β-globin gene (from nucleotide (nt) − 160 to − 142)] and β1R 5′-TGC TTC GTC TGT TTC CCA TTC TAA AC-3′ (from nt + 610 to + 585); CD1 5′-TGC CTC TTT GCA CCA TTC TA-3′ (from nt + 1098 to + 1118) and CD2 5′-GAC CTC CCA CAT TCC CTT TT-3′ (from nt + 1659 to + 1643) (all positions are given relative to the Cap site, nt 1 from NCBI GenBank®). The PCR products (770 and 575 bp long, respectively) were purified and directly sequenced with β1F and CD1 primers using the BigDye Terminator V3.1 Cycle Sequencing kit and an ABI prism 3130 Genetic Analyzer (Applied-Biosystems, Foster City, Ca, USA). All of our hematological indices and clinical findings were collected with the prior informed consent of the patient.

3. Results

The chromatogram obtained by the ion-exchange HPLC assay using a Tosoh G8 showed the following Hbs, concentrations (%) and retention time (RT): LA1c (1.9%) at 0.49 min, HbA1c (not quantified) at 0.58 min, HbA0 (92.5%) at 0.88 min, and an abnormal peak (RT 0.65 min) just after the HbA1c peak. However, no other suspicious major peak for a haemoglobin variant was observed (Fig. 1).

To elucidate the nature of this interference, we used protein chemistry and molecular biology techniques.

The patient's blood cell counts were unremarkable (Hb 14.4 g/dL, Mean Corpuscular Volume (MCV) 89.5 fL, Mean Corpuscular Haemoglobin (MCH) 29.3 pg and Red Distribution Width (RDW) 13.6%).

The study of haemoglobins by capillary zone electrophoresis showed no anomalous peaks, but a variant peak was detected via cation exchange with the Variant II β-thalassemia Short Program from Bio-Rad. The peak accounted for approximately 38% of the Hb, eluted before HbA0, was not totally separated from HbA0, and had an elution time of 2.66 min (Fig. 2).

Since the percentage of anomalous haemoglobin was greater than 25%, we thought that the haemoglobin could be have a variant in the β chain. Molecular characterization of the β gene by automatic sequencing identified the novel transversion mutation HBB:c.242A > G, which resulted in an amino acid change from Asn → Ser at codon 80 of exon 2 in the heterozygous state [β80(EF4)Asn > Ser; HBB:c.242A > G] (Fig. 3). This new haemoglobin was named Moncloa because the hospital is situated in this area of Madrid.

4. Discussion

Hb Moncloa is the first change described in the second base of this codon 80 (AAC > AGC). Genomic evolutionary rate profiling (GERP) indicates that this nucleotide (A) is conserved in nature (3.94 rejected substitutions), and the undescribed nucleotide substitution (G) is not reported in either the Exome Aggregation Consortium (ExAc) database