Clinical report

**Hb Burgos (α₁ CD64(E13)(Asp→Asn)): A new hemoglobin variant detected during follow-up of diabetic patients**

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**A B S T R A C T**

*Background and objective:* The glycated haemoglobin (HbA₁c) test by high performance liquid chromatography is a useful tool for the follow-up of diabetes mellitus patients. Some structural haemoglobin (Hb) variants are known to cause interference in the analytical measurement of HbA₁c.

*Patients and methods:* In this study, it has been characterized a new Hb variant in 4 patients during their regular control of HbA₁c.

*Results:* Selective α₁ gene sequencing showed a mutation GAC>AAC at codon 64 within exon 2. This produces a change of aspartic acid (Asp) by asparagine (Asn) that does not produce any functional alteration so the resultant molecule behaves as a silent haemoglobinopathy.

*Conclusion:* The structural Hb variants can be detected during the analysis of HbA₁c and may alter its values. Though rare, this occurrence signals the need to being aware when measuring HbA₁c.

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**Hb Burgos (α₁ CD64(E13)(Asp→Asn)): una nueva variante de hemoglobina detectada durante la monitorización de pacientes con diabetes**

**RESUMEN**

*Fundamento y objetivo:* El control de la diabetes mellitus se realiza mediante la determinación de hemoglobina glucosilada (HbA₁c) por cromatografía líquida de alta resolución. Algunas variantes estructurales de la hemoglobina (Hb) son conocidas por causar interferencia analítica en la medición de la HbA₁c.

*Pacientes y métodos:* En este estudio se ha caracterizado una nueva variante de Hb en 4 pacientes, que se detectó al realizarse un control de HbA₁c.

*Resultados:* La secuenciación selectiva del gen α₁ mostró una mutación responsable del cambio de ácido aspártico (Asp) por asparagina (Asn) en el codón 64. El cambio de Asp por Asn no produce ninguna alteración funcional de la Hb y se comporta como una hemoglobina con diabetes. Esta variedad de Hb se puede detectar durante la medición de la HbA₁c y pueden alterar sus valores. Estos casos, aunque poco frecuentes, requieren examinar a fondo los cromatogramas para detectar posibles interferencias.

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Introduction

Since the last century, the gold standard technique for haemoglobin (Hb) detection and quantification is ion-exchange high performance liquid chromatography (HPLC). This technique is also used to control diabetes mellitus by measuring the glycosylated haemoglobin (HbA1c). This last function makes it possible to identify asymptomatic variants that could not be revealed otherwise.

In some cases these variants may cause analytical interference in HbA1c measurement, risking appropriate diagnosis and treatment for diabetes.

In this study we have characterised a new variant of Hb that was diagnosed in 4 patients at the time they underwent an HbA1c control test.

Patients and methods

The patients, all of them Caucasian males native to Navarra, Madrid, and Burgos, were referred to us because they presented an anomalous peak during the HbA1c control, which was done by means of ion-exchange HPLC (Tosoh G8; Tosoh Bioscience, Tokyo, Japan).

The HbA2 and HbF quantification, as well as the Hb analysis were done by means of ion-exchange HPLC following the short-term program for the Variant™ II of Bio-Rad (BioRad Variant™ II β-thalassemia Short Program; Bio-Rad, Hercules, CA, U.S.). Furthermore, the samples were analysed by zonal capillary electrophoresis using the Sebia system (Sebia Capillaries Flex) and the reactive agents from the kit provided by the business (Capillaries Haemoglobin [E] kit; Sebia, Norcross, GA, U.S.). The globin chains were separated by reversed phase HPLC, as previously published.

Haematological data was obtained by means of an Automatic haematology analyser (Coulter® LH750 Analyzer; Beckman Coulter, Brea, CA, U.S.).

The molecular study required the automatic extraction of genomic DNA (BioRobot® EZ1™; Qiagen GmbH, Hilden, Germany) and its subsequent quantification with NanoDrop® 1000 (Thermo Scientific, Wilmington, DE, U.S.).

The most frequent mutations causing alternations in α genes were analysed with α-globin StripAssay® (ViennaLab Diagnostic GmbH, Vienna, Austria). The molecular characterization required selective amplification of genes α and their subsequent automatic sequencing in an ABI PRISM sequencer® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, U.S.) and with the commercial kit ABI PRISM® BigDye® Terminator v1.1 Cycle Sequencing Ready Reaction Kit (PE Applied BioSystems, Foster City, CA, U.S.).

Results

A new clinically silent Hb structural variant was detected, by means of ion-exchange HPLC, during HbA1c measurement, at a retention time (RT) of 1.02 min, altering the normal pattern (Fig. 1).

The Hb analysis by means of ion-exchange HPLC revealed the existence of a peak in the HbS window, involving between 13 and
34.3%; there was, as well, a 4.68 min RT adjacent peak. This reaction was confirmed by means of zonal capillary electrophoresis, with anomalous peaks in areas Z6 and Z1, respectively. In the globin chains study by reversed phase HPLC, only the β and α globin chains were separated (Fig. 1).

The selective sequencing of gene α1 showed the mutation GAC>AAC in codon 64 from exon 2 (Fig. 2A), substituting aspartic acid amino acid (Asp) for asparagine (Asn). Said variant was called Hb Burgos (α1 64(E13)Asp→Asn; HBA1:c.193G>A), because the first patient was a native of that province. This mutation was identified at the stage of heterozygosis in 3 patients, whereas in the fourth patient, it was diagnosed at the homozygote stage (Fig. 2B).

Both the haematological parameters and the genotype of study subjects are summarised in Table 1. The HbA2 values oscillated between 1.1 and 2.2%.

**Discussion**

In the Hb Burgos variant, the affected residue is the 64th in the globin chain α1, located on the external surface of the molecule (helix E13), which makes it easier to separate by means of electrophoretic and chromatographic techniques, since the replacement of Asp amino acid for Asn amino acid turns the molecule more cathodic than the HbA. This change does not imply any functional alternation to Hb, thus, from a clinical standpoint, it is asymptomatic.4,5 As a result, haematological parameters are not affected, which is evidenced by its casual and late detection: most patients are in their 70s. The quantification of HbA1c has not been affected either and, thus, there is no interference with glucose control.

The Hb Burgos percentage in heterozygosis, as it is a variant of the α chain, should be 25%, approximately. However, it is slightly lower (13–17%), both by means of ion-exchange HPLC and capillary electrophoresis, which could be due to the lower expression rate of gene α1 compared to gene α2.5 As a matter of fact, gene α2 contains HbG-Waimanalo (α2 64(E13)Asp→Asn; HBA2:c.193G>A), which has the same mutation and, still, the anomalous Hb percentage, in heterozygosis, oscillates between 17 and 22%.6

In the case of homozgyosis, there could be room for confusion, since it is separated from the HbS window by means of

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**Table 1**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Gender</th>
<th>Hb (g/dl)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>HbA2 (%)</th>
<th>HbF (%)</th>
<th>HbX (%)</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>83</td>
<td>M</td>
<td>12.9</td>
<td>96.3</td>
<td>32.9</td>
<td>2</td>
<td>0.1</td>
<td>17</td>
<td>αα*/αα</td>
</tr>
<tr>
<td>86</td>
<td>M</td>
<td>14</td>
<td>84.7</td>
<td>30.6</td>
<td>1.9</td>
<td>0.2</td>
<td>13</td>
<td>αα*/αα</td>
</tr>
<tr>
<td>68</td>
<td>M</td>
<td>14.7</td>
<td>95.1</td>
<td>31.2</td>
<td>2.2</td>
<td>0.2</td>
<td>16.2</td>
<td>αα*/αα</td>
</tr>
<tr>
<td>61</td>
<td>M</td>
<td>16.2</td>
<td>91.5</td>
<td>30.9</td>
<td>1.1</td>
<td>0.2</td>
<td>34.3</td>
<td>αα*/αα</td>
</tr>
</tbody>
</table>

B: Burgos; Hb: haemoglobin; MCH: mean corpuscular haemoglobin; M: male; MCV: mean corpuscular volume.

* Percentage by high performance liquid chromatography Variant™.
ion-exchange HPLC. Nevertheless, by means of zonal capillary electrophoresis, HbS appears in Z5, while Hb Burgos separates at Z6. Additionally, the percentage (34.3%) could lead to confusion; however, reversed phase HPLC contributes to proving that it is not a β chain variant, but rather a α variant, since only β and α chains separate globins, although judging by their homozygosis condition, higher levels should be expected (40–50%). This would confirm the fact that gene α1 has a lower rate of expression compared to gene α2. The presence of a α chain variant leads to the formation of its respective HbX2 (α2Xβ2), contributing to a reduction in HbA2. In some cases, this new variant could be separated, like in HbG-Philadelphia. In the case of Hb Burgos, both by means of ion-exchange HPLC (peak adjacent to Hb Burgos) and by zonal capillary electrophoresis (Z1), there is a reduction in the levels of Hb Burgos2 and of HbA2, ranging between 1.1 and 2.2%. In this way, the lower the chain α synthesis, the lower the HbA2 percentage shall be, which accounts for the 1.1% evidenced by the Hb Burgos homozygote case.

It has been observed that the ion-exchange HPLC is the best technique to quantify HbA1c, and considering that it is the gold standard technique for Hb separation, identification of new variants in addition to the existing ones has increased. A study conducted in 2009, at the Hospital Clínico San Carlos hospital, on the diabetic population, showed a 2% Hb variants incidence. This has made it necessary to review the chromatograms in order to detect possible interferences at the time of quantifying glucose levels, which could lead to preventing detection of HbA1c, as in Hb Porto Alegre, to undervalue it, as in Sevilla, HbD, Hb Las Palmas, HbN-Baltimore, and Hb Jerez or even to overestimate the HbA percentages, which results in failed treatment, and the all the inconveniences it entails. Notwithstanding, since there are over 1000 different variants of Hb, many of which are rare, it is necessary to pay special attention when it comes to measuring HbA1c.

Conflict of interests

The authors state they have no conflicts of interest.

References