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Hemoglobin Le Lamentin in the province of Albacete, Spain: Discovery of 32 cases

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ABSTRACT

Objectives: Hemoglobin Le Lamentin (α20(*B*1)His → Gln) is a ubiquitous variant that has been previously described in a small number of isolated patients. We report the incidental observation of Hb Le Lamentin in a large population from the province of Albacete, in southeastern Spain. Our study investigates possible reasons for the elevated number of carriers of this variant and its implications for the management of diabetes in our region.

Design & Methods: The subjects are 32 diabetic patients whose hemoglobin displayed an unusual peak while they were being tested for glycated hemoglobin at the laboratory of the University General Hospital of Albacete over a 3-year period. Measurements were made by high performance liquid chromatography using a Variant™ II Turbo Kit-2.0, and subsequently the samples of the 32 patients with anomalous peaks were sent to the Hospital Clínico San Carlos (Madrid, Spain) for molecular characterization of any Hb variants.

Results: Molecular studies revealed 31 out of 32 patients heterozygous for Le Lamentin, and in one of them, Hb City of Hope was associated with Hb Le Lamentin. The remaining patient was homozygous for the Le Lamentin mutation. Additionally, most patients were native to the northeastern half of the province of Albacete and were unrelated.

Conclusions: Our study describes the largest finding to date of hemoglobin Le Lamentin in a sample of patients. The fact that our region has been perpetually depopulated, with a population that has remained stable in small localities over the centuries, may have favored the survival of the mutation. Since the presence of this variant underestimates the true value of glycated hemoglobin measured by HPLC, it is necessary to systematically review chromatograms.

1. Introduction

Hemoglobin (Hb) Le Lamentin is an α-chain (α20(*B*1)His → Gln) hemoglobin variant, first discovered in 1982 in a black family originating from the French West Indies (Martinique) [1], during an examination of cord blood for HbS and C in newborn infants. Since then, Hb Le Lamentin has been increasingly reported; it was subsequently detected in 1983 in a 73-year-old male living in Tokyo, Japan [2], and was the first case found in the Japanese population. In 1988, it was incidentally observed, by high performance liquid chromatography (HPLC), in two members of a Spanish family from the Canary Islands [3], while screening the population for β-thalassemia. Later, the variant was discovered, by electrospray mass spectrometry, in five patients from the UK [4,5], suggesting that Hb Le Lamentin might not be as uncommon as was previously thought. In 2000, one case was found in Sweden, also identified by electrospray mass spectrometry, when characterizing hemoglobin variants in the Swedish population [6]. Finally, a German long-term study (1971–2007) of the occurrence and spectrum of hemoglobinopathies in that country, revealed an immigrant patient with the variant Le Lamentin [7].

We report the incidental identification of Hb Le Lamentin in 32 diabetic patients while they were being tested for glycated hemoglobin (HbA1c) in Albacete, in southeastern Spain. HbA1c represents the fraction of hemoglobin to which glucose is bound in red blood cells, and is a measure routinely employed in the management of patients with diabetes mellitus as an indicator of long-term glycemic control. Measurements were made by HPLC, using a Variant™ II Turbo Kit-2.0, at the laboratory of the University General Hospital of Albacete. Diabetes mellitus has a prevalence of 6.7% in the province of Albacete, according to WHO statistics [8].

Hb Le Lamentin, like most of the over 1000 identified Hb variants, does not produce clinical abnormalities and has no bearing on the incidence or course of diabetes [1,2]. However, the presence of this hemoglobin variant affects the accuracy of the HbA1c result as measured by

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HPLC, tending to underestimate glycated hemoglobin values. In carriers of this variant, erroneous information may then be transmitted to clinicians regarding the evolution of patients´ diabetes, affecting treatment and management of the disease. Therefore, it is important to examine the chromatograms produced by the Variant, not only for HbA1c, but also for the hemoglobin fractions emitted. Hemoglobin variants manifest as additional peaks at different retention times on the chromatogram; further molecular studies are then required to identify the variant type.

This study describes the discovery of the largest number to date of unrelated Hb Le Lamentin carriers in a sample of patients. We discuss the possible reasons for the elevated number of carriers of this hemoglobin variant in the province of Albacete, and its implications for the management of diabetic patients in our region.

2. Materials and methods

The subjects in this retrospective study are 32 Caucasian patients with diabetes mellitus, 12 females and 20 males, with an average age of 65.9 years (range 30–91). They were being tested for HbA1c at the laboratory of the University General Hospital of Albacete, where more than 40,000 HbA1c determinations are performed annually for diagnosis and monitoring of the diabetic population of the entire province of Albacete.

We had begun revising chromatograms to ensure accuracy of the HbA1c measurements on the Variant equipment, when we made our first observation of a chromatogram with an abnormal peak in May 2012, prompting us to suspect a hemoglobin variant. A further molecular study revealed that the patient was heterozygous for the hemoglobin mutation Le Lamentin. Over the next three years, from May 2012 until February 2015, 31 more patients appeared in our laboratory with this variant. Since the percentage of patients carrying Le Lamentin was relatively high compared to the number of previous cases found in other parts of the world, we began to collect data on these 32 individuals regarding birthplace and family origins, to conduct a study describing our findings and investigate possible links between the Le Lamentin patients.

Patients arrived fasting for their diabetes check-up at the laboratory of the University General Hospital of Albacete. Whole blood samples were collected in VACUETTE® K3-EDTA tubes (Greiner Vacuette North America Inc., USA) to measure HbA1c, using HPLC on the Variant™ II Turbo Kit-2.0 (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Serum was also collected in VACUETTE® Clot Activator tubes in order to measure glucose and fructosamine. Glucose was determined using the enzymatic hexokinase method (GLUC3), and fructosamine using the colorimetric procedure with nitroblue tetrazolium; both tests were performed employing the cobas® 8000 modular analyzer series (Roche Diagnostics, Mannheim). Hematological studies were conducted by routine procedure using an automatic cell counter (Advia®2120 System, Siemens AG, Germany).

Over the 3-year study period, the whole blood samples of the 32 patients with anomalous peaks were sent to the Hospital Clínico San Carlos (Madrid, Spain) for molecular characterization of any Hb variants. Informed consent was previously obtained from all patients, as well as approval by the Ethics Committee of the afore-mentioned hospital. HbA_2 and HbF levels were measured with the ion-exchange HPLC VARIANT™ II System (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The hemoglobin molecules were studied using capillary zone electrophoresis with the CAPILLARYS™ 2 FLEX Piercing system (Sebia, Norcross, GA), and also with the ion-exchange HPLC Variant™ II β-thalassemia Short Program (Bio-Rad Laboratories, Inc., Hercules, CA, USA). As previously reported [9], the globin chains were separated using reverse phase HPLC.

The molecular study began with the automatic extraction of the genetic material on the BioRobot EZ1workstation (QIAGEN, Hilden, Germany), and subsequent quantification with a NanoDrop 1000 (Thermo Scientific, Wilmington, DE, USA). The most frequent α-thalassemia mutations were analyzed using several PCRs with the *Alpha-Globin StripAssay* (ViennaLab Diagnostic GmbH, Vienna, Austria) commercial kit. The α2 gene was amplified with primers P1A (5′-AGCGCCGCCCG GCCGGGCGT-3′) and C3R (5′-CCATTGTTGGCACATTCCGG-3′), and the amplicon (947 bp) was automatically sequenced using primers P1A, PB (5′-CCC GCC CGG ACC CAC A-3′) and P1C (5′-AGATGGCGCCTTCCTC TCAG-3′). The sequencing of the β-globin gene was performed following the previously reported protocol [10].

3. Results

Analysis of hemolysate samples for glycated hemoglobin by HPLC revealed an anomalous peak in the chromatograms between HbA1c and HbA. In 31 patients, the peak occurred at a mean RT of 0.65 min (Fig. 1), with a mean area of 26.7%. The remaining patient, number 7 (Table 1), showed a peak area of 47.9%, at an RT of 0.61 min (Fig. 1). This additional peak, along with the observation that HbA1c levels were relatively low compared to those of serum glucose and fructosamine for these patients (Table 1), lead us to suspect the presence of a hemoglobin variant. The possibility that the peak was related to other common hemoglobins (HbF, HbC, HbS, HbD, HbE) was excluded, since their retention times on chromatograms are characteristic and distinct from that of the anomalous peak we found.

In all cases, the peaks corresponding to HbA_2 and HbA were observed using capillary electrophoresis, with HbA showing a slight protuberance, as was described in an earlier work [11]. The anomalous hemoglobin variants were separated at an average RT of 1.59 min, using ion-exchange HPLC with the VARIANT™ II β-thalassemia Short Program, as previously published [11]. The study of the globin chains by reverse phase HPLC revealed the presence of β^A chains and a double peak corresponding to α and α^x chains, while chains β^A, β^x, α and α^x were eluted from patient number 5. Sequencing of the gene α2 revealed the mutation CAC > CAA in CD20, causing a His > Gln aminoacid change which results in the Hb variant known as Le Lamentin [$alpha_2$ 20(*B*1) His > Gln; HBA2:c.63C > A]. All patients were heterozygous for Le Lamentin, except for patient number 7, in whom the mutation was homozygous [11]. In case number 5, sequencing of the β gene revealed the substitution of guanine for adenine (GGT > AGT) in codon 69 in a heterozygous state; this is a sequence corresponding to the Hb City of Hope [beta69(E13) Gly > Ser; HBB:c.208G > A] [11]. In all cases, the α-thalassemia deletion and the non-deletion were ruled out.

Hematological features of the 32 patients showed no abnormalities in association with Hb Le Lamentin (Table 1). Mean results of bloodwork were: Hb 14.4 g/dl (range 8.7–17.5 g/dl); RBC 4.70×10^{12}/l (range 3.44–5.62 $\times 10^{12}$/l); MCV 92.3 fl (range 68.7–103.7 fl); MCH 30.6 pg (range 20.6–34.3 pg); and MCHC 33.1 g/dl (range 30–34.5 g/dl). Additionally, mean levels of HbA2 and HbF were 2.0% and 0.9%, respectively. Regarding gender, hemograms of both females and males were within their respective reference ranges, with the exception of the following patients: numbers 1 and 6, whose anemia was due to chronic renal failure; number 9, who had a mechanical prosthetic mitral valve and was also heterozygous for β-thalassemia; and number 19, with an unexplained macrocytosis (ie.patient was neither a heavy drinker, nor had deficiencies of vitamin B12 or folic acid).

HbA1c results obtained with the Variant™ II Turbo Kit-2.0 are displayed in Table 1. Patients were not informed of these values since the presence of the variant hemoglobin resulted in an incorrect measurement of HbA1c, underestimating its true value. Since samples were not analyzed by any other method capable of registering accurate glycated hemoglobin levels, fructosamine determinations were used in these patients for monitoring their diabetes.

Regarding origin of patients, Fig. 2 shows a map of the province of Albacete indicating the towns, highlighted in white, where our patients were born. All patients, except for one, were native to eleven distinct localities in the province: the city of Albacete, Valdeganga, Casas de Juan