Haemoglobin Le Lamentin (α20 (B1) His → Gln) in a British family: identification by electrospray mass spectrometry

Tim M Reynolds, Fiona McMillan, Adrian Smith, Annette Hutchinson, Brian Green

Abstract
The first identification of haemoglobin Le Lamentin (α20 (B1) His → Gln) in a British patient is described. The patient was a 69 year old asymptomatic white male being screened for diabetes, whose blood was undergoing its first ever analysis for glycated haemoglobin using a Glycomat II analyser. Identification of the haemoglobin variant was by electrospray mass spectrometry.

Keywords: haemoglobin Le Lamentin; haemoglobin variants; electrospray mass spectrometry

Case report
Haemoglobin Le Lamentin (α20 (B1) His → Gln) was first described in 1983 in a black family from the French West Indies. Two other reports from Japan and the Canary Islands have also been published.

We report the identification of haemoglobin Le Lamentin in a 69 year old asymptomatic white male who was undergoing screening for diabetes. His initial pathological investigations showed no abnormalities (Hb 15.8 g/dl, red blood cell count 5.14 × 10¹²/litre, packed cell volume 0.473, mean cell volume 91.9 fl, mean cell haemoglobin 30.7 pg, mean cell haemoglobin concentration 33.4 g/dl, plasma bilirubin 16 µmol/litre). Analysis of a haemolysate for glycated haemoglobin using a Glycomat II analyser (Chiron Diagnostics, Halstead, Essex, UK) revealed an unusual peak (fig 1). Other common haemoglobins (HbA₂, HbC, HbF, HbS, metHb) were excluded because their retention times on the Glycomat analyser are characteristic and different from that of the unusual peak. Additionally, HbA₂ measured by cellulose acetate electrophoresis and densitometry was 2.3% and HbF measured by alkaline denaturation was 0.3%. In common with the other reports, the abnormal haemoglobin could not be separated by cellulose acetate electrophoresis at pH 8.9 and agar gel electrophoresis at pH 6.0. A sample was referred for electrospray mass spectrometric (MS) analysis to Micromass Ltd, Altrincham, UK.

Electrospray ionisation MS analysis using a Quattro II tandem mass spectrometer of intact α and β chains showed the abnormal haemoglobin contained an α chain variant with a mass difference of −8.9 Da from normal (fig 2), indicating a single amino acid change from His → Gln (−9.0 Da), for which there were 10 possibilities. The patient was heterozygous for the variant haemoglobin which was present as 24% of total haemoglobin. Tandem MS analysis of the variant plus normal chains showed that the altered amino acid lay in the α(1–75) fraction (fig 3) which reduced the possible variants to five. MS analysis of a tryptic digest showed that the variant was in the αT4 tryptic peptide indicating that the variant lay in the α(17–31) part of the sequence, in which there is only one probable site (α₂₀) (fig 4). Tandem MS analysis of the αT₄⁺ peak confirmed the variant to be at position α₂₀ and that the abnormal haemoglobin was Hb Le Lamentin (fig 5). Further confirmation of the His → Gln amino acid change was provided by the presence of the Gln immonium ion in the tandem mass spectrum of the variant αT₄⁺ ion with the concomitant disappearance of the His immonium ion that was present in the spectrum from the normal αT₄⁺ ion (data not shown).

Discussion
In all of the cases of haemoglobin Le Lamentin described before this report, the abnormal
haemoglobin was identified by biochemical methods. Harano et al describe their isolation and purification of the affected α chain by chromatography on a CM–cellulose column, tryptic digestion, and chromatographic analysis of altered peptide chains which showed an abnormal αT4 peptide spot with a more anodal electrophoretic mobility than normal αT4 peptide. They then carried out amino acid analysis of purified abnormal αT4 peptide which suggested a His → Gln substitution. According to the already known amino acid sequence of the α globin chain, this substitution was probably at the α20 position. Confirmation of this required successive Edman degradation of the abnormal αT4 peptide. This procedure is very slow and labour intensive.

In electrospray mass spectrometry, ions are formed from intact polypeptides by adding one or more protons (H). Mass spectrometry measures the mass to charge ratio (m/z) of these ions. Since the number of charges (n) is known, the molecular weight or mass (M) in Daltons (Da) is deduced using $M = n(m/z - H)$. In effect, tandem mass spectrometry combines the purification and sequencing of polypeptide mixtures, previously undertaken using several procedures into one operation without chromatography. The technique employs an instrument consisting essentially of

![Figure 2](http://jcp.bmj.com/)

**Figure 2**  Electro spray mass spectrum of blood from the patient (1:500 dilution). A second α chain peak can be seen, 8.9 Da lighter than the normal α chain. Assuming the mutation is a single base change, the only possibility is His → Gln.

![Figure 3](http://jcp.bmj.com/)

**Figure 3** Part of tandem mass spectrum of a chain from the patient (lower) corresponding with spectrum from normal control (upper). Variants of the $b_7$ and $b_{76}$ ions can be seen indicating that the variant lies between α and γ, inclusive. Only those peaks that are diagnostic in this case have been labelled.
Figure 4  Part of mass spectrum from tryptic digest from the patient (lower) corresponding with spectrum from normal control (upper). This shows that the mutation lies in the αT4 tryptic peptide. There is only one Hb residue in this peptide corresponding to α₂₅ implying the variant is Hb Le Lamentin (α₂₅ His → Gln).

Figure 5  Part of tandem mass spectrum from normal and variant αT4 peptides from the patient showing no change in the position of the y₁₁⁺ — y₁₁⁺ ions between normal and variant, whereas the y₁₂⁺—y₁₄⁺ ions show a decrease of 4.5 in mass to charge ratio (m/z), confirming that the mutation occurs at y₁₂⁺. The y₁₁⁺ ions in these peptides corresponds to position 20 in the α chain. The 4.5 change in m/z is equivalent to a change of 9 Da in the intact α chain, since the y₁₁—y₁₁⁺ ions are doubly charged.

Figure 6  Schematic diagram of a tandem mass spectrometer (ESI-MS-MS). There are two modes of operation: (1) MS mode: ions entering the spectrometer are analysed by MS₁ to give a mass spectrum (no gas in cell and MS₂ inoperative); (2) MS-MS mode: MS₁ selects ions of a particular mass which undergo collisions with argon in cell. Fragment ions produced in the cell are then analysed by MS₂ to give a tandem mass spectrum (MS-MS spectrum).

two mass spectrometers in series (fig 6). The first spectrometer purifies the polypeptide mixture by selecting ions with a specific m/z. These ions are then fragmented by collisions with a gas in a cell situated between the two spectrometers. The resulting fragment ions are then analysed according to their m/z in the second spectrometer to give sequence information. Fragment ions result mainly from cleavages between the amino acids in the polypeptide chain. For example in fig 5, the variant αT4⁺⁺ peptide ion was selected from a
mixture of approximately 30 tryptic peptides and sequenced to confirm its identity in one 10 minute analysis. The skill in mass spectrometry lies in identifying diagnostic peaks in complex spectra with the aid of computer programs. The complete mass spectrometry analysis of the variant haemoglobin was carried out and results were returned to the referring laboratory within four working days, a significant reduction compared with biochemical techniques.

This is the second abnormal haemoglobin we have reported that has been analysed using this technique and we recommend it as a rapid method for identification of unknown variants, although cautioning that interpretation requires considerable expertise.

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*J Clin Pathol* 1998 51: 467-470
doi: 10.1136/jcp.51.6.467