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Hemoglobin disorders detected in diabetic patients by HbA1c analysis

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Introduction

The development and progression of long-term complications in diabetic patients can be slowed by the use of intensive regimens to attain strict glycemic control (1). Measurement of HbA1c in diabetic patients is an established procedure for evaluating long-term control of diabetes. It is well known that, despite advances in the standardisation of methods, hemoglobinopathies cause false results in various HbA1c methods (2).

In general, when using an immunochemical method clinical silent hemoglobin-variants (Hb-variants) are not identified, while when applying a HPLC based method a majority will be identified due to the presence of an additional peak in the chromatogram besides the HbA-peak and/or an increased HbF-peak. The presence of some Hb-variants will lead to the reporting of incorrect (either false increased or false decreased) HbA1c results (3).

In our hospital, after years of immunochemical HbA1c measurement, we transferred to the use of a HPLC based method (HA-8140; Menarini Diagnostics) in November 2002 (4). This change of method inevitable led to the identification of hemoglobinopathies in some patients. Whenever HbA1c analysis revealed an additional non-glycated Hb-peak besides the HbA-peak or an increased HbF percentage, the family physician of that patient was consulted to inform whether or not further characterisation of the finding was indicated. In this way a number of Hb-variants and thalassemias were identified in 30 diabetic patients unaware of their hemoglobinopathy carrier status.

Methods

The determination of HbA1c was performed on EDTA-anticoagulated blood samples. The HA-8140 automated HPLC HbA1c method (Menarini Diagnostics, Florence, Italy) uses cation-exchange and reversed phase chromatography on a solid phase of methacrylic acid and methacrylate ester. The hemoglobin fractions are eluted by a varying pH in the mobile phase.

If HbA1c analysis revealed a deviant pattern (either an additional peak besides the HbA-peak or a HbF-peak > 3 %), subsequent hemoglobin capillary electrophoresis was performed. Whenever by capillary electrophoresis (P/ACE system 5000 with ANALIS kit, Beckman Coulter, Fullerton, CA, USA) an increased percentage HbA2, HbF (both > 3.0 %) and/or a Hb-variant was observed, relevant parts of the β-globin gene or a-globin genes were amplified and sequenced.

Genomic DNA was isolated from whole blood using Puregene (Gentra Systems, Minneapolis, MN, USA). For the β-globin gene, amplification and sequence analysis of the coding regions was performed by amplifying the 5' region and 3' region separately.

5' region (nt –103 to nt +144 in IVS-II)
Forward primer: CCCTGTGGAGCCACACCCTA
Reverse primer: ACGATCCTGAGACTTCCACAC
3' region (nt +603 in IVS-II to nt +62 in the 3' UTR)
Forward primer: TGCCTCTTTGCACCATTCTAAAG
Reverse primer: TGCACTGACCTCCCACATTCC

In one patient, identified as having a Hb-variant by capillary electrophoresis, no mutation was observed in the coding regions of the β-globin gene. Subsequently the coding parts of the two α-globin genes were amplified and sequenced using one primer-pair each, leading to a 1087 and 1076 bp PCR product for the α1- and α2-globin genes respectively.

Sequence analysis was performed using an ABI Prism 310 Genetic analyzer (Applied Biosystems, Foster City, CA, USA).

Results

Since we started using the HPLC based method, 30 diabetic patients were identified as having a Hb-disorder (see Table 1). The hemoglobinopathies observed were HbD-Los Angeles (13x), HbS (7x),

Table 1. Hemoglobinopathies identified since November 2002 in diabetic patients unaware of their hemoglobin-disorder carrier status

Hemoglobinopathy	Number of patients	Mutation (heterozygous)
HbC	3	β Codon 6 GAG→AAG
HbD-Iran	1	β Codon 22 GAA→CAA
HbD-Los Angeles	13	β Codon 121 GAA→CAA
HbG-Philadelphia	1	α2 Codon 68 AAC→AAA
HbJ-Baltimore	1	β Codon 16 GGC→GAC
Hb-Korle Bu	1	β Codon 73 GAT→AAT
HbS	7*	β Codon 6 GAG→GTG
β 0-Thalassemia	1	β IVS-I nt 1 g→a
β +-Thalassemia	1	β nt -88 to cap site c→t
β +-Thalassemia	1*	β nt -29 to cap site a→g

* one patient with an additional to –a(3.7)/aa α-thalassemia trait

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HbC (3x), β-thalassemia (3x), HbD-Iran, HbJ-Baltimore, Hb-Korle-Bu and a Hb-variant caused by a mutation in the α2-globin gene (HbG-Philadelphia).
The presence of a hemoglobinopathy may lead to an incorrect HbA1c value when it influences the erythrocyte survival, thereby decreasing the exposure time of hemoglobin to glucose. In addition, when the HPLC based method leads to the separation of the non-glycated Hb-variants but not of their glycated counterparts, or vice versa, false increased respectively decreased HbA1c percentages will be observed.
In for instance a patient known to have HbA1c values of 6.8 - 9.6 % for years, the HbA1c results suddenly dropped to 3.2 - 4.1 % after switching to the HPLC based method. Sequence analysis revealed that the patient is a HbD-Iran (β-codon 22 GAA →CAA) carrier. Detailed inspection of the HA-8140 chromatogram revealed no separation of the non-glycated Hb-types, while their glycated counterparts were separated. Only the HbA1c-peak (and not the glycated HbD-peak), but both the HbA- and HbD-peak, were included in the calculation of the HbA1c percentage. Affinity chromatography (performed by the Isala Clinics, Zwolle, The Netherlands) revealed a glycohemoglobin of 6.4 % for this patient.
On the other hand, persons having sickle cell trait will show false increased HbA1c results since the non-glycated Hb-types (HbA and HbS) are separated by the HA-8140 analyzer while their glycated counterparts are not. This results in the use of all the glycated Hb, but only the HbA-peak, in the calculation of the HbA1c percentage.

Conclusion

Characterisation of Hb-disorders observed in HbA1c analysis using a HPLC based method may lead to the identification of the carrier status for a Hb-variant or thalassemia in diabetic patients. Often, the identified Hb-disorders are clinically silent but may not be biochemically silent when it comes to HbA1c measurement. Given the fact that a HbA1c deviation of 1 % represents a change of 1.4 - 1.9 mmol/l in the average blood glucose concentration, a false increased or decreased HbA1c result may have a substantial influence on the instructions given to attain strict glycemic control. Therefore, if the clinical impression and HbA1c test results do not match, then the possible presence of a hemoglobinopathy should be examined and HbA1c values should be determined with a second method based on a different principle (5). In these cases, affinity chromatography based methods may give reliable HbA1c values, since they measure glycohemoglobin regardless of the Hb-variant present (3). In managing diabetic patients, knowledge of hemoglobinopathies influencing the locally used HbA1c method is essential because unidentified hemoglobin variants may cause mismanagement of diabetes resulting from false HbA1c results.

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