Available online at
SciVerse ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Diabetes & Metabolism 39 (2013) 363–369

Original article

Interference of the most frequent haemoglobin variants on quantification of HbA_{1c}: Comparison between the LC–MS (IFCC reference method) and three routinely used methods

S. Jaisson [a,*,b], N. Leroy [a], C. Desroches [a], M. Tonye-Libyh [a], E. Guillard [a], P. Gillery [a,b]

[a] *Laboratory of Paediatric Biology and Research, American Memorial Hospital, University Hospital of Reims, 47, rue Cognacq-Jay, 51092 Reims cedex, France*
[b] *Laboratory of Medical Biochemistry and Molecular Biology, FRE CNRS/URCA n° 3481, Faculty of Medicine, University of Reims Champagne-Ardenne, Reims, France*

Received 7 December 2012; received in revised form 18 January 2013; accepted 21 January 2013

Abstract

Aim. – Assaying HbA_{1c} in patients with haemoglobin variants has long been a technical challenge, despite methodological advances that have progressively limited the problem. The purpose of this study was to evaluate the impact of the most frequent haemoglobin variants on three routine separation methods compared with the IFCC reference method.

Patients. – Blood samples from heterozygous patients (AS, AC, AD, AE) were analyzed using the IFCC reference method (LC–MS), and the results compared with those obtained by capillary electrophoresis (CAPILLARYS 2 Flex Piercing, Sebia) and two HPLC methods using cation-exchange (Variant II, Bio-Rad) and affinity chromatography (Ultra^2, Primus).

Results. – HbA_{1c} values obtained by the IFCC reference method were comparable to those obtained by the three tested methods whatever the haemoglobin variant. Mean relative biases did not exceed the threshold of 7% (above which differences are generally considered clinically significant), although some individual values were above this limit with Variant II in samples with HbS and for all three methods in samples with HbE.

Conclusion. – This comparative study of the LC–MS reference method and three field methods has demonstrated that these assays are not clinically influenced by the presence of the most common haemoglobin variants. The present results also confirm that the interpretation of HbA_{1c} values in patients with Hb variants remains complex and depends on the assays used and should, in some cases, take into account parameters other than analytical ones (such as differences in glycation rates and half-lives of haemoglobin variants).

Keywords: HbA_{1c}; Reference method; Haemoglobin variants; Diabetes mellitus

Résumé

Interférence des variants de l'hémoglobine les plus fréquents sur le dosage de l'HbA_{1c} : comparaison entre la LC-MS (méthode de référence IFCC) et trois méthodes de routine.

But. – La présence d'un variant de l'hémoglobine est connue depuis longtemps pour être à l'origine d'interférences analytiques lors du dosage de l'HbA_{1c}, même si les avancées technologiques ont permis de limiter progressivement ce problème. Le but de cette étude était d'évaluer l'impact des variants les plus fréquemment rencontrés sur trois méthodes séparatives utilisées en pratique courante et de les comparer à la méthode de référence IFCC.

Patients. – Des échantillons de sang de patients hétérozygotes (AS, AC, AD, AE) ont été analysés à l'aide de la méthode de référence IFCC (LC-MS) et les résultats ont été comparés à ceux obtenus soit par électrophorèse capillaire (Capillarys 2 Flex Piercing – Sebia), soit à l'aide de deux méthodes CLHP au principe différent : la chromatographie échangeuse de cations (Variant II – Bio-Rad) et la chromatographie d'affinité (Ultra^2 – Primus).

* Corresponding author. Tel.: +33 3 26 78 75 62; fax: +33 3 26 78 38 82.
E-mail address: sjaisson@chu-reims.fr (S. Jaisson).

1262-3636/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.diabet.2013.01.004

Résultats. – Les résultats d'HbA_{1c} obtenus avec la méthode de référence IFCC étaient comparables à ceux obtenus par les trois méthodes testées, quel que soit le variant présent. Les biais relatifs moyens n'ont pas dépassé 7 % (seuil au-dessus duquel les différences sont généralement considérées comme cliniquement significatives), même si certains échantillons ont donné des valeurs supérieures à ce seuil, comme par exemple en présence d'HbS (pour l'automate Variant II) et d'HbE (pour les trois automates).

Conclusions. – Cette étude comparative entre la méthode de référence LC-MS et trois méthodes de routine a démontré que ces méthodes ne sont pas influencées pour l'interprétation clinique par la présence des variants de l'hémoglobine les plus fréquents. Cependant, cet article rappelle que l'interprétation des résultats d'HbA_{1c} en présence d'un variant reste délicate, dépend de la méthode utilisée, et doit aussi, dans certains cas, tenir compte d'autres paramètres relatifs aux variants, comme une cinétique de glycation ou une demi-vie différentes.

Mots clés : HbA_{1c} ; Méthode de référence ; Variants de l'hémoglobine ; Diabète sucré

1. Introduction

HbA_{1c} is a widely used biomarker in the management of diabetes because it provides information on the monitoring of long-term glycaemic control and an assessment of the risk of developing complications [1–3]. Its use has also been recently proposed for the diagnosis of diabetes [4,5]. Consequently, HbA_{1c} quantification now has to meet defined quality criteria to ensure reliable results and optimal clinical use. In this regard, many technological advances have been made by manufacturers to limit analytical errors and interference, and an international standardization process has been completed by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [6,7] to ensure the traceability of methods to an internationally accepted reference system [8].

Nevertheless, HbA_{1c} use remains controversial in pathological situations that can generate analytical interference and also alter its informational value. One such case is chronic renal failure (CRF), which leads to the accelerated formation of carbamylated haemoglobin (Hb) due to the increase in uraemia, which can interfere with HbA_{1c} separation and quantification [9,10]. CRF is also responsible for an anaemic state, thus frequently necessitating erythropoietin therapy, which can distort the interpretation of HbA_{1c} because of modification of red blood cell and Hb half-lives [11]. Similar limitations are encountered in patients with an Hb variant that could also lead to haemolysis, especially in a homozygous state. In addition, Hb variants can interfere with HbA_{1c} determination. Several studies have been devoted to determining the impact of Hb variants on the analytical performance of HbA_{1c} assays [12–14], but few have emphasized the impact of the presence of Hb variants on the informational value of HbA_{1c} [15].

For this reason, the present study has evaluated the impact of the most frequent Hb variants (HbS, C, D and E) on the analytical performance of common field HbA_{1c} methods compared with the IFCC liquid chromatography–mass spectrometry (LC–MS) reference method [16]. Three systems using distinct principles for the separation and quantification of HbA_{1c} were tested: a device recently introduced into the market using capillary electrophoresis for HbA_{1c} separation and quantification (CAPILLARYS 2 Flex Piercing, Sebia, Lisses, France) [17]; a cation-exchange high-performance liquid chromatography (HPLC) method (Variant II NU kit, Bio-Rad Laboratories, Hercules, CA, USA); and a boronate affinity HPLC method (Ultra^2, Primus Corporation, Kansas City, MO, USA). The ultimate purpose of this study was to determine whether, beyond the potential analytical problems that are nowadays limited, the interpretation of HbA_{1c} results in patients with Hb variants remains a critical issue because the impact of other parameters (such as differences in glycation kinetics and quantification of a glycated variant as HbA_{1c}) on the informational value of HbA_{1c} still needs to be taken into account.

2. Patients

2.1. Samples

Whole blood samples collected in EDTA-containing tubes (Greiner Bio-One, Courtaboeuf, France) from subjects homozygous for HbA ($n = 8$), or heterozygous for S ($n = 9$) or C, D or E ($n = 10$) Hb variants, were sent to the laboratory for routine HbA_{1c} assay or were provided by Sebia (Lisses, France). The analyses covered a wide range of clinically relevant HbA_{1c} values (from 4.9–13.5%, or 30–124 mmol/mol). All samples were frozen at −80 °C before analysis. No additional samples were necessary for this study, and no sample was kept after the assays.

2.2. HbA_{1c} assays

Samples were assayed using the following analyzers: CAPILLARYS 2 Flex Piercing; Variant II NU kit; and Ultra^2. All assays were performed according to the manufacturer's instructions. Results were compared with those obtained by the LC–MS IFCC reference method. This method is based on the quantification of the N-terminal hexapeptide of Hb β-chains obtained after the digestion of Hb by a specific endoproteinase Glu-C (endo-Glu-C). The generated peptides (whether glycated or not) were then quantified by LC–MS [16]. Our laboratory belongs to the IFCC network of reference laboratories for HbA_{1c} [18] and, as such, regularly participates in the external quality-assessment scheme for reference laboratories in laboratory medicine (RELA–IFCC) [19].

2.3. Data analysis

Results obtained for each type of sample (homozygous AA and heterozygous AS, AC, AD and AE) by each method were subtracted from the values obtained by the IFCC reference