Short communication

Haemoglobin J-Baltimore can be detected by HbA_{1c} electropherogram but with underestimated HbA_{1c} value

Valéry Brunel*[1], Agnès Lahary[2], Abdeslam Chagraoui[1], Christian Thuillez[1,3]

[1]Department of Medical Biochemistry, Rouen University Hospital, Rouen, France
[2]Department of Haematology, Rouen University Hospital, Rouen, France
[3]Department of Pharmacology, Rouen University Hospital, Rouen, France

*Corresponding author: valery.brunel@chu-rouen.fr

Abstract

Glycated haemoglobin (HbA_{1c}) is considered the gold standard for assessing diabetes compensation and treatment. In addition, fortuitous detection of haemoglobin variants during HbA_{1c} measurement is not rare. Recently, two publications reported different conclusions on accuracy of HbA_{1c} value using capillary electrophoresis method in presence of haemoglobin J-Baltimore (HbJ).

Here we describe the fortuitous detection of unknown HbJ using capillary electrophoresis for measurement of HbA_{1c}. A patient followed for gestational diabetes in our laboratory presented unknown haemoglobin on Capillarys 2 Flex Piercing analyser which was identified as HbJ. HbJ is not associated with haematological abnormalities. High Performance Liquid Chromatography methods are known to possibly underestimate HbA_{1c} value in the presence of this variant. This variant and its glycated form are clearly distinguished on electropherogram but HbJ was responsible for underestimating the true area of HbA_{1c}.

Capillary electrophoresis is a good method for detecting HbJ but does not seem suitable for evaluation of HbA_{1C} value in patients in presence of HbJ variant.

Key words: glycated haemoglobin; haemoglobin J-Baltimore; capillary electrophoresis

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Introduction

Glycated haemoglobin (HbA_{1c}) is considered the gold standard for assessing diabetes compensation and treatment. In addition, fortuitous detection of haemoglobin variants during HbA_{1c} measurement is not rare. Recently, an analytical evaluation of Capillarys 2 Flex Piercing (C2FP, Sebia, Lisses, France) has been published, assessing the possible interference of the most common haemoglobin variants in African immigrants to the United States (1). The authors concluded that those common African haemoglobin variants, notably heterozygous for haemoglobin S (HbS) or haemoglobin C (HbC), not only could be detected, but also do not interfere with HbA_{1c} results and do not impair the ability of HbA_{1c} to detect abnormal glucose tolerance on C2FP analyser. Although heterozygous for HbS or HbC are the most frequently encountered variants in France they are not the only ones (2). In 2013, we introduced C2FP instrument to our clinical biology laboratory at Rouen University Hospital, France to perform HbA_{1c}. Herein we report a case of haemoglobin J-Baltimore detected by electropherogram and discuss the consequences of the presence of this haemoglobin variant on accurate interpretation of HbA_{1c} result obtained by C2FP analyser.

Materials and Methods

A 28-year-old pregnant woman at 29 weeks of amenorrhoea, with no medical history, was referred by her attending physician at our Department of Endocrinology for management of newly diagnosed gestational diabetes. An oral glucose

tolerance test and HbA_{1c} measurements were done respectively on Cobas 8000 analyser (Roche, Meylan, France) and C2FP instrument (Sebia, Lisses, France) in our clinical biology laboratory at Rouen University Hospital to monitor her diabetes. Oral glucose tolerance test was performed with 75 g of glucose dissolved in 250 mL of water which was absorbed by the patient.

Results

Fasting glucose, 1 hour and 2 hour levels after glucose charge were measured respectively as 4.8, 10.1 and 8.5 mmol/L. We detected two peaks of unknown haemoglobin on electropherogram. The higher peak was quantified at 47.7%. The other peak, probably corresponding to a glycated form of the first, was quantified at 1.2% (Figure 1). C2FP software does not allow HbA_{1c} calculation because of insufficient separation between the peak of unknown haemoglobin and HbA_{1c} fraction. Therefore, the sample was tested by High Performance Liquid Chromatography (HPLC) Bio-Rad Variant II analyser (BioRad, Marnes-la-Coquette, France). HbA_{1c} was measured at 4.6% (27 mmol/mol) with the presence of an unknown peak in P3 window, the window on Variant's chromatogram where haemoglobin variant could be detected, quantified at 44.3%. The manufacturer's instructions define a cut-off in the window as 5% for detection of haemoglobin variant sample. The sample was analysed by the National Haemoglobinopathy Reference Laboratory (Créteil, France) which identified this haemoglobin as J-Baltimore beta 16(A13) Gly>Asp (HbJ). On electropherogram, we clearly observed insufficient separation of HbA_{1c} fraction from HbJ with an underestimation of HbA_{1c} fraction area determined by the C2FP software. We pursued our investigation to measure this interference. Thus, manual off-line recalculation of HbA_{1c} percentage was performed, excluding both unknown peaks. The formula used was $\%HbA_{1c} = [HbA_{1c}] / ([HbA_{1c} + HbA_0]) \times 100$, then we applied the calibration equation of the software, and the HbA_{1c} value obtained was 4.1% (21 mmol/mol). The laboratory did not communicate this manual off-line recalculation of HbA_{1c} to Department of Endocrinology because of the underestimation of HbA_{1c} was not quantifiable.

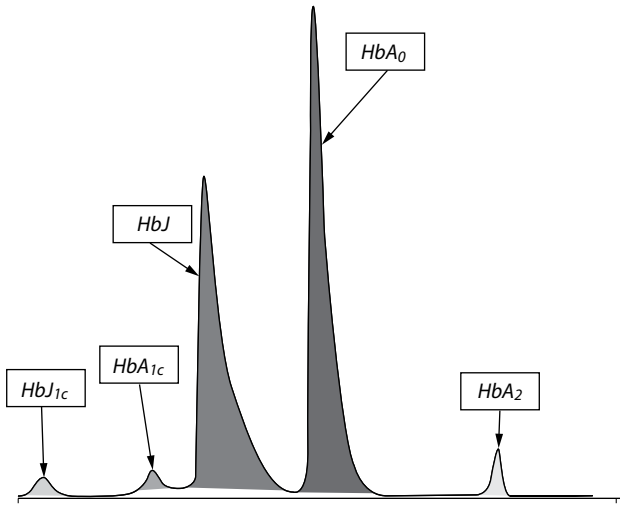

Figure 1. Electropherogram from capillary electrophoresis Capillarys 2 Flex Piercing.
Electropherogram with software alarm "*atypical profile*" presents two peaks of unknown haemoglobins. The higher unknown peak is haemoglobin J-Baltimore (HbJ) and the smaller unknown peak is glycated form of HbJ (HbJ_{1c}). We observe usual fractions of haemoglobin: haemoglobin A (HbA_0), A1c (HbA_{1c}), A2 (HbA_2). On this electropherogram, we observed insufficient separation of HbA_{1c} fraction from HbJ.

Discussion

Fortuitous identification of haemoglobin variants during HbA_{1c} measurement is not common and requires screening expertise by the clinical pathologist (3). In our laboratory, we perform 12,000 tests for HbA_{1c} measurement *per* year and we identify approximately 30 cases of haemoglobin variant *per* year. HbJ was first described in 1963 by Baglioni and Weatherall in a black American family (4). Haematological abnormalities are not usually associated with this variant. The analytical impact of this rare variant is well known on HbA_{1c} measurement by HPLC (5,6). Only a few recent publications have been published on capillary electrophoresis (CE) methods (7,8). Barakat and Roberts explained that the very light load change due to the presence of a glycine in position 16 of the haemoglobin β-chain does not allow clear separation of HbJ or HbJ_{1c} from respectively HbA or HbA_{1c} with HPLC

methods and subsequent risk of underestimating HbA_{1c} value (5,6). Rhea reported the same results between HPLC method, known with a negative bias, and CE (7). Little considered that the HbA_{1c} results of the two samples tested were accurate with CE method (8). Here, we clearly observe that we are able to identify the presence of all the fractions of interest of HbA, HbA_{1c}, HbJ and HbJ_{1c} on electropherogram obtained by C2FP analyser. We previously reported that manual off-line recalculation could be useful when CE software does not allow HbA_{1c} calculation (9).

In conclusion, in this case HbJ was responsible for underestimating the true area of HbA_{1c}, and consequently the proposed manual off-line recalculation of HbA_{1c} value. Although C2FP HbA_{1c} method can detect HbJ variant, the presence of this haemoglobin variant affects the accuracy of C2FP method for HbA_{1c} measurement.

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Potential conflict of interest

None declared.

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