Inaccurate HbA_{1c} determination caused by Hb Aix-les-Bains, a rare hemoglobin variant

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Dear Editor,

the measurement of glycated hemoglobin (HbA_{1c}) in diabetic patients is used for the evaluation of glucose homeostatic control and, recently, it has also been proposed as a diagnostic tool (1-3). HbA_{1c} is a stable and irreversible product of non-enzymatic glycosylation of hemoglobin β chain by plasma glucose; moreover, its level in red blood cells depends on the mean blood glucose concentrations, exposure time to glucose and turnover of hemoglobin (Hb) itself. Because of this turnover, all the conditions shortening red blood cell life span can cause falsely reduced HbA_{1c} values, due to the reduced time of exposition of Hb molecule to the glycosilating environment. These categories include hemolytic anemias and thalassemias. Furthermore, a number of Hb variants not characterized by a consistent red cell life reduction can interfere with HbA_{1c} determination either in positive or negative way and either by pre-analytical or analytical mechanisms (4). In this paper, we report a case of unreliable evaluation of HbA_{1c} by cation exchange HPLC, caused by an unusual Hb variant, i.e., Hb Aix-les-Bains [β5(A2)Pro→Leu], first described by a French group in 2011 (5).

A 32 years old diabetic man was repeatedly observed with inconsistent HbA_{1c} values, considered too low when related to clinics (ranging 11 to 37 mmol/mol; decision level for diagnosis of diabetes, ≥48) for the presence of a poor metabolic control, as demonstrated by serum glucose concentrations (ranging 135 to 200 mg/dL) and by a fructosamine concentration of 363 μmol/L (reference interval: 205-285). As no other hematological abnormalities were found, these observations induced us to study thoroughly HbA_{1c} HPLC graphics and to perform a HPLC analysis of Hb phenotype. HPLC analyses were performed by Tosoh G8 analyzer, employing "Variant" and "β-Thalassemia" programs for HbA_{1c} and for Hb phenotype, respectively. Results are shown in Figure 1. HbA_{1c} program graphic was not able to detect any abnormality of the HbA_0 elution peak, whereas an abnormal HbA_{1c} region was recognizable, presenting a partial splitting of HbA_{1c} peak (Figure 1A). Hb "β-Thalassemia" program graphic showed an enlargement of the HbA_0 peak due to the presence of an unknown peak, which was eluted immediately [retention time (RT) 2' 52''] after the usual HbA_0 peak (RT 2' 35''), scarcely recognizable from HbA_0, but quantified as 44.9% of total Hb (Figure 1B). HPLC analyses on Variant II "β-Thalassemia" short program (Bio-Rad Laboratories) showed only an asymmetrical A_0 peak (Figure 1C) and no Hb variant or variation of Hb peak was visible by capillary electrophoresis (Sebia). The Hb isopropanol stability test was normal. DNA was prepared from whole blood with QIAsymphony (Qiagen). The β-gene was amplified by polymerase chain reaction (PCR) using primers that flank the three exons and direct sequencing of the PCR products was performed on a 3130 XL Sequencer (PE Applied Bio-Systems) using the same primers. DNA sequencing of the β-gene showed that codon 5 carried the mutation (CCT > CTT) in the heterozygous state leading to the replacement of the proline residue by a leucine. This Hb variant was previously reported by a French group in a woman of Italian ancestry, who underwent a routine workup for diabetes screening (5). The case we report here is, as far as we know, the first case described in Italy. A family study was performed, demonstrating that the patient's mother and brother were heterozygous carriers of the same defect: both relatives showed low HbA_{1c} values (15 and 18 mmol/mol, respectively) when compared with the reference interval. Physiologic fructosamine concentrations were also detected.

Over 70 laboratory techniques are currently employed for HbA_{1c} determination: the majority of them are based on

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immunochemical principles and HPLC; various Hb variants can affect different methods in a different way (4, 6). A Hb variant not able to form stable adducts with glucose or presenting glycation kinetics different from HbA_0 can generate a HbA_{1c} underestimation on the basis of biological/pre-analytical effects, affecting in this way a variety of laboratory techniques. Hb variants characterized by mutations in the N-terminal sequence of β chain can heavily influence HbA_{1c} evaluation by immunochemical methods, based on antibodies directed against this glycated portion of Hb molecule. In the case of HPLC or separative methods, the following conditions can be found: a) the Hb variant (HbX) and its glycated form (HbX_{1c}) are clearly separated from HbA_0 and HbA_{1c}, respectively, so that HbA_{1c} is correctly estimated, b) HbX and HbX_{1c} partially overlap with HbA_0 and HbA_{1c}, respectively, with errors in estimation of HbA_{1c}, which can be under- or overestimated, c) HbX is eluted with HbA_{1c} (as for Hb Camperdown), which results in an unreliable estimation of HbA_{1c} (overestimated) (7, 8). The case reported here clearly corresponds to the second scenario.

As the evaluation of HbA_{1c} has a crucial role in diabetes monitoring, it is essential that results supplied by laboratory are reliable. Clinical pathologists performing HbA_{1c} determinations by HPLC should thoroughly inspect chromatograms before reporting HbA_{1c} results and, if there is a discrepancy between HbA_{1c} results and the clinical/biochemical status of the patient, a careful search for possible Hb variants should be performed. Fructosamine or glycated albumin determinations can be proposed as an alternative tool, keeping in mind, however, that they are able to monitor glucose metabolism for a period of 2-3 weeks in spite of 4-6 weeks for HbA_{1c} (9).

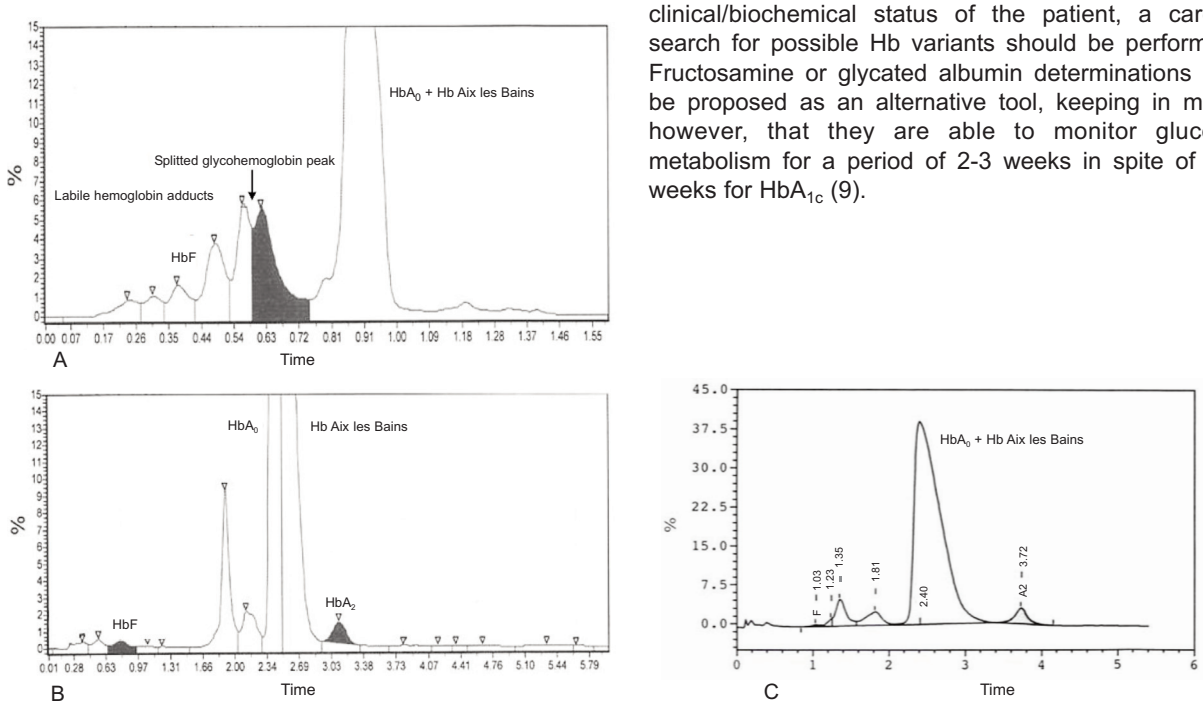

Figure 1
HPLC separations of patient's blood sample, obtained by Tosoh G8 "Variant" program (A), Tosoh G8 "β-Thalassemia" program (B), and Bio-Rad Variant II β-Thalassemia short program (C).

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