

The Contribution of High Performance Liquid Chromatography to the Incidental Discovery of Hemoglobin Variants during Glycated Hemoglobin Assay

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Abstract: HbA1c is assayed in the biochemistry laboratory using a high pressure liquid chromatography (HPLC) technique in ion exchange, and given the correlation between its value and the risk of complication, it is crucial to know it, advantages and its limits. This work highlights the contribution of HPLC in the detection of qualitative abnormalities of hemoglobin during the determination of HbA1c using the ADAMS HA-8180V ARKRAY® device. This descriptive prospective study, carried out at the Biochemistry-Toxicology department of the Mohammed V Military Hospital in Rabat, focused on all samples for which routine HbA1c testing is prescribed during the past year, external samples and from all departments. For each abnormality noted on the chromatogram, a complete study of hemoglobin was performed using other electrophoretic technique at alkaline and acid pH on the Capillarys 2 Flex Piercing® Sebia® and the Hydrasys 2 Scan Focusing® Sebia®. Almost 0.6% of the variants were detected during the HbA1c assay out of 12,944 blood samples. Only 0.04% were not identified by the machine. After confirmation, variant C was predominantly present (n = 30), followed by S (n = 28) then O-Arab (n = 8) and 1'Hb D (n = 2). The HPLC technique, for measurement of HbA1c, represents a reliable screening tool for the most common variants of Hb, with however limitations that encourage vigilance in the analysis and interpretation of the results.

Keywords: HPLC - Hemoglobin variants - HbA1c.

INTRODUCTION

Glycated hemoglobin is the product of the non-enzymatic, slow and irreversible binding of sugar to the amine functions of globin. HbA1c is the predominant form of glycated hemoglobin and its dosage is the key to manage and control glycemic balance of the diabetic patients, given the correlation between its value and the risk of complications.

On the recommendation of an international committee of experts, HbA1c has been selected by the World Health Organization (WHO) since 2011 for the diagnosis of diabetes mellitus (Zendjabil M, 2015, Gillery P. 2013).

High performance liquid chromatography (HPLC) is the reference method for the standardized assay of HbA1c, which has the advantage of being fully automated with correct specificity (Gillery P. 2013,

Meurice J *et al.*, 2011). This technique makes it possible to fortuitously highlight the variants of hemoglobin (Hb).

The present work highlights the contribution of HPLC in the detection of qualitative anomalies of hemoglobin during the HbA1c assay.

PATIENTS AND METHODS

This is a descriptive prospective study carried out at the Biochemistry-Toxicology department of the Mohammed V Military Hospital of Instruction (HMIMV) in Rabat over a period of one year between January 2020 and January 2021, which includes patients hospitalized and the outpatient consultants.

The samples were taken from venous blood at the fold of the elbow. The assay is performed on whole

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blood. The anticoagulant used is ethylenediaminetetra acetate (EDTA).

The purpose of this study is to analyze the chromatograms of each patient and the results of the HbA1c assay performed on the ADAMS HA-8180V ARKRAY® machine using the reverse phase cation exchange HPLC technique. The chromatograms which show a supernumerary peak with the mention of a hemoglobin variant, are reanalyzed by the electrophoretic technique at alkaline and acid pH on the Capillarys 2 Flex Piercing® Sebia® and the Hydrasys 2 Scan Focusing® Sebia®

All hemoglobin variants detected during the HbA1c assay were included, epidemiological duplicates have been removed. The data was processed using Microsoft Office Excel 2010 software

RESULTS

Over a one-year period, 12,944 blood samples were analyzed. The prevalence of hemoglobin variants was 0.6% (n = 72 patients). The median age of the different patients is 50 years, their age varies between 15 and 82 years, with an M / F sex ratio of 0.94 (Figure 1).

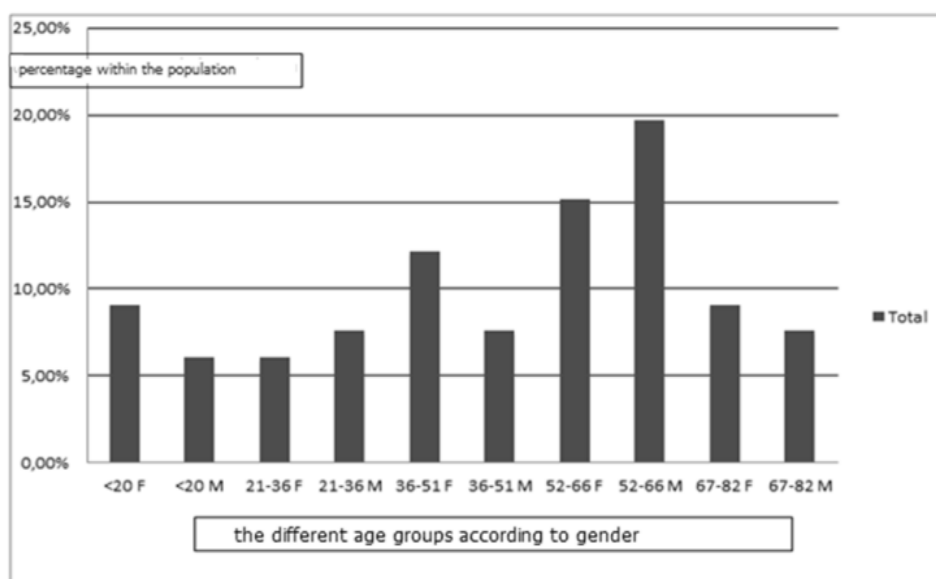


Figure 1: Population distribution by age and gender

Nearly 72% of the variants collected were detected in outpatient consultants (n = 52) versus 28% in hospitalized patients (n = 20) (Figure 2).

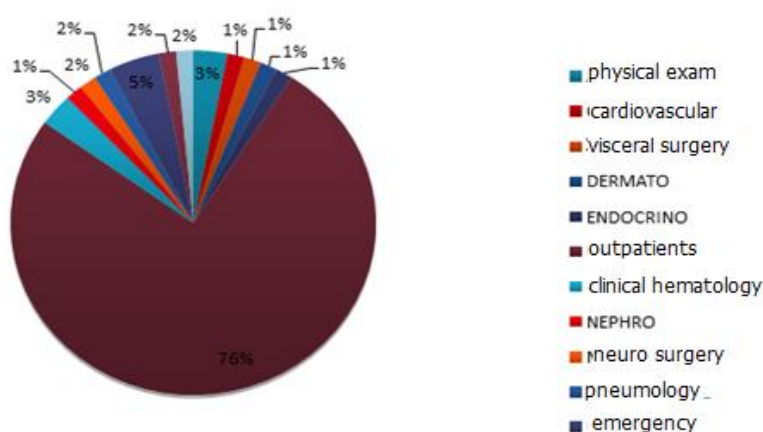


Figure 2: Global distribution of the population within the services of HMIMV in Rabat

The hemoglobin variant mainly found is Hb C (n = 38 i.e. 0.30%), Hb S represented 0.22% (n = 28),

Hb D nearly 0.02% (n = 2) and 4 patients (i.e. close to 0.03%) not identified by the machine (Figure 3).

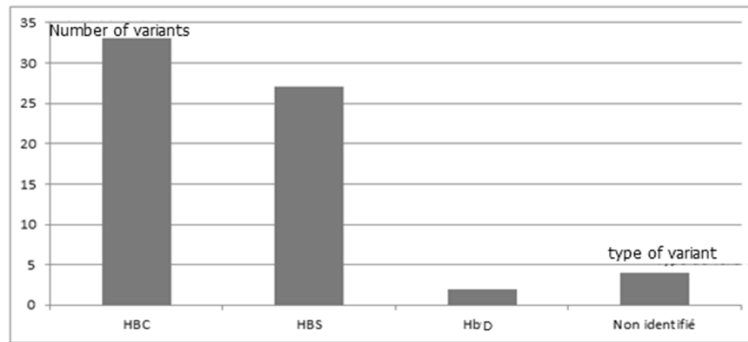


Figure 3: Global distribution of variants detected during the HbA1c assay

Electrophoresis of Hemoglobin (Hb) at alkaline and acidic pH was performed in 72 patients. The heterozygous S, C and D variants detected by HPLC were confirmed by the electrophoretic techniques used.

Except 8 cases detected Hb C by HPLC turned out to be Hb O-Arab cases, while the 4 patients not identified by HPLC, were identified by the other electrophoretic techniques as composite heterozygotes S/C, C/β+ and C/C, S/S in the homozygous state.

DISCUSSION

HbA1c is the gold standard for the assessment of blood sugar control in diabetes mellitus. Its dosage reflects changes in blood sugar over a period of approximately two months prior to collection (Gillery P, 2000, Krishnamurti U *et al.*, 2001).

The semiological value of HbA1c as a retrospective and cumulative marker of glycemic balance is conditioned by a normal synthesis of hemoglobin (97 to 99% of Hb A) and a normal lifespan of erythrocytes (120 days). If one of these parameters is modified, the balance between synthesis / degradation reactions and non-enzymatic glycation reactions is disturbed, leading to an underestimation or

overestimation of the HbA1c level (CAMARGO JL *et al.*, 2004). Indeed, Bisse and coll. have shown that in some cases in heterozygous subjects, a mutation on the beta chain slows down the glycation kinetics of the non-mutated chain (BISSE E *et al.*, 2003).

The presence of abnormal Hb can only be suggested when the HbA1c assay technique demonstrates the qualitative abnormality such as HPLC and capillary electrophoresis. With the other assay methods, the presence of a variant goes unnoticed while the analytical interference varies greatly depending on the type of Hb abnormality and the method used (Nathalie Mario *et al.*, 2007). On the other hand, the importance of signaling one's presence is recognized internationally.

In this study, the technique used is HPLC, a qualitative and quantitative separation technique, based on the modification of the charge. It is a reverse phase cation exchange chromatography with a measurement wavelength of 420 nm to 500 nm (double wavelength colorimetry) (ADAMS™ A1c HA-8180V/Manuel, 2006); at the same time allows the determination of HbA1c and the detection of the most common Hb variants S, C (Figure 4).

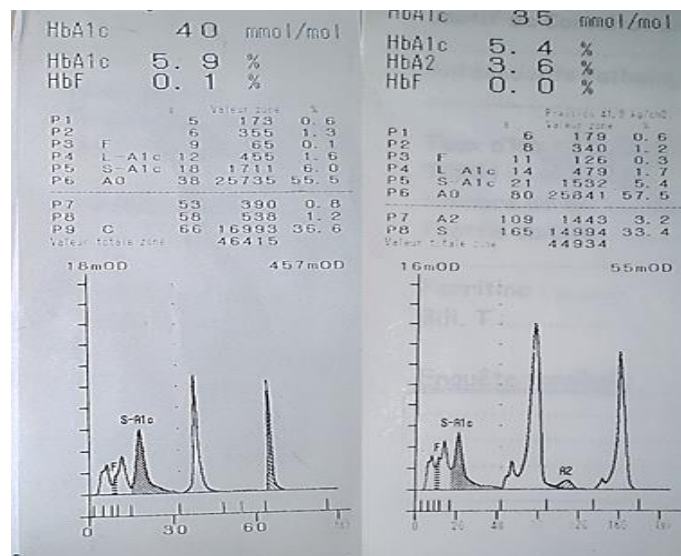


Figure 4: The most detected variants by HPLC

The other variants are generally eluted as unidentified supernumerary peak, the presence of which must always be taken into account for identification by other complementary techniques, in particular electrophoretic (Lemée V *et al.*, 1999).

The prevalence of Hb variants during the HbA1c assay is not unusual since it represents 0.6% in our experience. This value is clearly much lower than that found in the literature, in comparison with the Tunisian study by Kahena *et al.*, (2014). Who found a prevalence of 2.33% over a one-year period.

The variant mainly found is Hb C with 0.30%, Hb S represented nearly 0.22% and Hb D is 0.02%. The predominance of variant C is mainly present in West Africa and in the African-American and Magrebian populations (Gulbis B *et al.*, 2004, Couque N, 2013).

Sickle cell disease is the most common hemoglobinopathy in North Africa. Its average frequency is 1.89% in Tunisia (Fattoum S, 2009). It is 0.80 to 3.50% in Algeria and 1.20% in Morocco (Haj Khelil A, 2010). In our study, its prevalence of 0.22% seems consistent since it does not represent the overall Moroccan population. However, it is much lower than the value found by Kahena *et al.*, (2014), estimated at 2.05%.

Electrophoretic methods have shown their contribution in the phenotypic study of Hb. In fact, the variants S, C and D detected in the heterozygous state by HPLC were confirmed by the electrophoretic techniques used, except for 8 cases detected by Hb C by HPLC which turned out to be cases of Hb O-Arab, while the 4 patients not identified by HPLC were identified as composite heterozygotes S/C, C/ β + and S/S, C/C in the homozygous state.

Given the importance of the HbA1c result for therapeutic adjustment in the diabetic subject, it is important that biologists know the characteristics and limitations of the method they use in order to signal the possible presence of hemoglobinopathy (BRY L, *et al.*, 2001). Another retrospective marker can be used is the fructosamines which reflect the glycemic balance of a shorter period than that reflected by the HbA1c (2 to 3 weeks instead of 2 to 3 months) the concentration of fructosamines is not modified by the existence of hemoglobinopathy (Pileire B *et al.*, 1988, Martina WV *et al.*, 1993) but the uncertainty will relate to the decision threshold because there is no consensus on the relationship HbA1c - fructosamines (COHEN RM, 2003). Another envisaged alternative is the use of the HbA1c obtained during the first determination, while monitoring of the patient with the same technique so that he is his own "model" (GILLERY P, 2000).

CONCLUSION

The measurement of HbA1c is the key examination in the management of the diabetic patient, given the correlation between its value and the risk of complications.

Biologists must then recognize the characteristics and limitations of the method used for this assay in order to have a critical look at the result and to provide interpretation aid in the event of a qualitative hemoglobin abnormality.

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Authors' Contributions

Dr. Houari Mouna first author.

Pr Biaz Asmae second author and supervisor.

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