Variation in hemoglobin A1c linked to hemoglobin S : comparison of analysis results from ion exchange chromatography and an immunoturbidimetric method

Abstract

Hemoglobin S can interfere with the measurement of glycated hemoglobin, an essential tool for monitoring and diagnosing diabetes. The objective of this study is to evaluate the analytical performance of 2 glycated haemoglobin assay methods.

A prospective cross-sectional study was conducted where 186 patients (61 homozygous sickle cell disease, 61 AA type subjects and 64 AS type subjects) were recruited. Glycated hemoglobin was measured by immunoturbidimetry method and ion exchange chromatography.

The coefficient of variation (CV) of the repeatability is 2.34% and 1.13% (normal rate) ; 2.32% and 1.65% (high rate) respectively for the immunoturbidimetric method and ion exchange chromatography. In reproducibility, the CV obtained are 3.23% and 2.64% (normal rate) and 3.27% and 2.22% (high rate), respectively for the immunoturbidimetric method and the ion exchange chromatography. Linearity is satisfactory for both methods. Mean glycated hemoglobin values show no significant difference (the *P-value* is equal to 0.09, 0.17 and 0.70 respectively in subjects AA, AS and SS) in the 2 methods for patients with the same hemoglobin electrophoretic profile.

The analytical performances of the 2 methods are good but their use is not recommended in the biological diagnosis of diabetes and pre-diabetes due to interference from hemoglobin S, especially in the case of homozygous sickle cell disease or in the case of composite heterozygosity.

Keywords. Hemoglobin S, glycated hemoglobin, immunoturbidimetric method, ion exchange chromatography

1. Introduction

The determination of hemoglobin A1c (HbA1c), the main form of glycated hemoglobin characterized by the non-enzymatic fixation of glucose at the N-terminal end of the β chains of globin, today constitutes an essential element in the management of caring for the diabetic patient. Its value retrospectively reflects the average glycemia of the past 4 to 8 weeks, due to its cumulative and irreversible formation [**1**]. It is therefore an essential tool for controlling glycemic control in diabetic patients since it is correlated with the frequency and severity of microvascular complications in diabetic patients [**2**]. But the interpretation of the results of the assay sometimes remains difficult with possible analytical interference linked in particular to the presence of hemoglobin variants such as hemoglobin S [**3**]. Based on this constant, we set ourselves the objective of evaluating the analytical performance of 2 HbA1c assay methods frequently used in medical biology laboratories in Senegal.

2. Materials and methods

This is a prospective cross-sectional study that took place over a period of 6 months where 186 patients were recruited.

Included in the study were 61 known homozygous sickle cell patients regularly monitored at the National Blood Transfusion Center (Dakar, Senegal), 61 AA type subjects and 64 AS type subjects recruited after determination of their electrophoretic hemoglobin profiles at the

Biochemistry and Molecular Biology laboratory of Cheikh Anta Diop University (Dakar, Senegal).

Diabetic subjects, patients who received a blood transfusion less than 4 months ago, sickle cell patients in a period of vaso-occlusive crises, patients with kidney failure, patients on treatment that could interfere with the HbA1c assay were excluded from the study.

A venous blood sample collected on a tube containing EDTA (Ethylene Diamine Tetra-Acetate) was taken from the patients thus selected and the HbA1c assay was carried out the same day by immunoturbidimetric method (Biosystems, Barcelona, Spain) and by ion exchange chromatography (Human, Wiesbaden, Germany). Hemolyzed blood samples were discarded prior to HbA1c assay. Normal control blood and pathological control blood were also used.

The measurement of repeatability was carried out by assaying HbA1c 10 times in the same series and by the same technician on 2 different samples (normal control blood and pathological control blood). Reproducibility was assessed on the 2 samples used for the repeatability study, haemolyzed and stored at the same temperature (-20°C). The samples were assayed in 10 different series by different technicians [**4**].

The linearity study was carried out on 7 hemolysates, prepared by manually diluting by mixing in variable proportions (12.5%, 25%, 50%, 75%, 87.5%) two samples, one having a concentration in HbA1c normal (4.4%) and the other high (17.5%) [**5**].

Data were entered into Excel 2013 and R version 4.2.3 software was used for data analysis. The mean and the standard deviation were calculated and the Student's T-test was used to compare the observed results. The difference between two means was considered significant when the p-value was less than 0.05 and not significant in the other cases. Concordance was determined using the Bland-Altman diagram.

3. Résultats

The study population consists of 186 patients with a female predominance sex ratio M/F = 0.98 and an average age of 22.4 ± 12.4 years.

The within-run CV is 2.34% and 1.13%; 2.32% and 1.65% respectively for the immunoturbidimetric method and ion exchange chromatography and for low and high HbA1c levels. In reproducibility, the CV obtained are 3.23% and 2.64% on the one hand, and 3.27% and 2.22% on the other hand, respectively for the immunoturbidimetric method and ion exchange chromatography and for low and high HbA1c levels (Tables 1 and 2).

Table 1. *Study of the precision of the immunoturbidimetric method*

	Repeatability		**Reproducibility**	
Whole blood sample	*Low HbA1c*	*Elevated HbA1c*	*Low HbA1c*	*Elevated HbA1c*
N	10	10	10	10
Average value	5,12	12,32	5,88	12,48
Standard deviation	0,12	0,14	0,19	0,33
Coefficient of variation %	2,34	1,13	3,23	2,64

N : *number of assays performed*

Table 2. *Study of the precision of ion exchange chromatography*

	Repeatability	**Reproducibility**
Whole blood sample	*Low*	*Low*

	HbA1c	*Elevated HbA1c*	*HbA1c*	*Elevated HbA1c*
N	10	10	10	10
Average value	4,74	12,09	5,5	12,61
Standard deviation	0,11	0,2	0,18	0,28
Coefficient of variation %	2,32	1,65	3,27	2,22

N : *number of assays performed*

The results of the linearity study are presented in Table 3. Linearity is satisfactory up to 15.8% for the immunoturbidimetric method and up to 15.7% for ion exchange chromatography.

Table 3. *Study of the linearity of the 2 methods*

Number of tube	1	2	3	4	5	6	7
Hemolysate 1 HbA1c=17,5%	240	210	180	120	60	30	0
Hemolysate 2 HbA1c=4,4%	0	30	60	120	180	210	240
Measured values (immunoturbidimetric)	-	15,8	14	11,1	7,3	5,9	-
Measured values (chromatography)	-	15,7	13,9	10,6	7,0	5,3	-
Expected values (%)	17,5	15,8	14,2	10,9	7,6	6,1	4,4

Mean HbA1c values as shown in Table 4 show no significant difference in the 2 methods for patients with the same hemoglobin electrophoretic profile.

Table 4. *Variation of mean HbA1c values in the 2 methods.*

	HbA_{1c} (chromatography)	**HbA_{1c}** (immunoturbidimetric)	***P-value****
AA patients	5,4±1,2	5,7±0,7	0,09
AS patients	5,0±1,1	5,2±0,8	0,17
SS patients	4,5±0,9	4,4±1,0	0,70

The results presented in Table 5 show for the 2 methods a statistically significant difference in mean HbA1c values in homozygous sickle cell subjects compared respectively to those of AA subjects and AS subjects. On the other hand, there is no significant difference in mean HbA1c values between AA subjects and AS subjects.

Table 5. *Variation of mean HbA1c values according to the electrophoretic profile.*

Chromatographic method		
	HbA_{1c}	***P-value****
AA patients	5,4±1,2	0,054[a]
AS patients	5,0±1,1	**0,0002[b]**
SS patients	4,5±0,9	**0,0009[c]**
Immunoturbidimetric method		
	HbA_{1c}	***P-value****
AA patients	5,7±0,7	0,46[α]

AS patients	5,6±0,8	**0,00001$^{\beta}$**
SS patients	4,4±1,0	**0,00001$^{\gamma}$**

** : P-value from Student's t-test; α : comparison between AS subjects and AA subjects; β : comparison between SS subjects and AA subjects; γ : comparison between AS subjects and SS subjects.*

The chromatographic method presents lower HbA1c values than the immunoturbidimetric method (figure 1) with a mean difference of -0.253 corresponding to the bias between the two techniques. The limits of agreement between the two techniques at 95% range from -0.438 to -0.067.

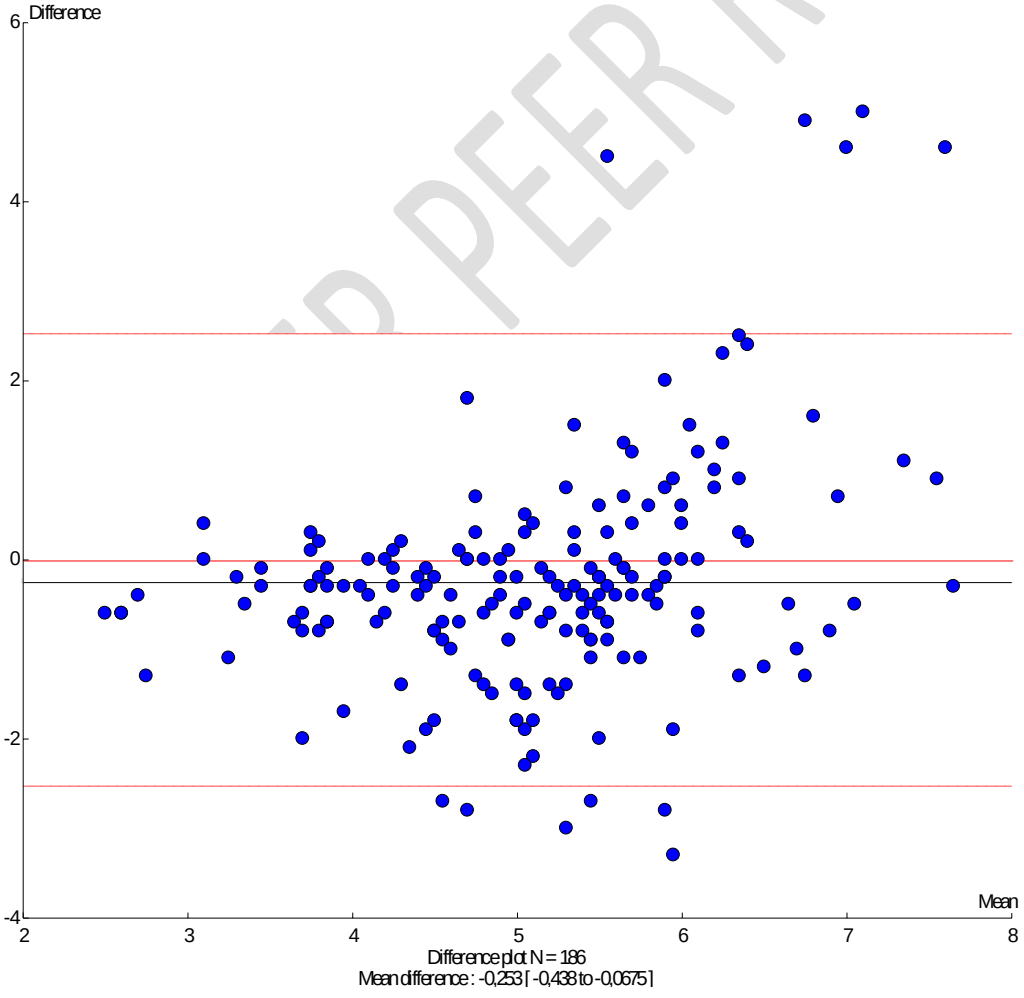

Figure 1. *Diagram of differences in mean HbA1c according to Bland and Altman*

4. Discussion

HbA1c represents a major element in the monitoring of glycemic control in diabetic patients. Its dosage, carried out using different methods, must now meet very rigorous analytical criteria in order to ensure optimum management of diabetic patients [**6**]. However, the different methods distributed on the market do not have the same characteristics, nor the same performances [**7, 8**]. It is therefore necessary to evaluate the analytical performance of 2 HbA1c assay methods (immunoturbidimetric method, ion exchange chromatography) used in several laboratories of health structures in Senegal. CV of repeatability and reproducibility respectively below 3% and 4% are considered satisfactory [**9, 10**]. These results are comparable to those given by other analyzers on the market [**11**]. The CVs obtained are proof of the high precision of these assay techniques, which have characteristics that are perfectly suited to monitoring the glycemic balance of diabetic patients [**9, 12, 13**]. The linearity of the immunoturbidimetric method is satisfactory up to 15.8% and that of ion exchange chromatography up to 15.7%. These results are quite sufficient for usual clinical needs [**11, 14, 15**]. Mean HbA1c values are higher in AA subjects, followed by AS patients, and SS patients have the lowest mean values. These results observed in SS subjects show that hemoglobin S interferes in the HbA1c assay. Interferences due to hemoglobin variants and observed in different HbA1c assay methods have been reported by several authors [**16, 17, 18, 19**] and for this reason, the risks of interference must be assessed for each assay technique. There is no significant difference between mean HbA1c levels in AA and AS subjects. In addition, the mean HbA1c values of SS sickle cell patients compared to AA or AS subjects show statistically significant differences. Farcet A and al. [**20**] state that there is an underestimation of the HbA1c value when an immunochemical method is used, which seems

to contradict our results. To assess the long-term glycemic control of heterozygous sickle cell subjects, high performance liquid chromatography is suitable for the HbA1c assay [**18**] and for SS sickle cell patients or those presenting with composite heterozygosity, instead of the HbA1c measurement, it makes more sense to use a method not based on hemoglobin, such as fructosamine assay, glycated albumin assay, or continuous glucose monitoring [**1, 17**]. Moreover, the Bland-Atman diagram shows a bias (-0.253) which is not clinically important, indicating that the 2 methods are interchangeable [**21**]. Discrepancies observed between an HbA1c test result and other parameters of glycemic control are not uncommon. Apart from an exclusively analytical problem and/or linked to a revision of the standardization which could lead to an uninterpretable result for a clinician, there are purely physiopathological reasons for misinterpretation associated, or not, with hemoglobin and with causes genetic [**22,23**]. Some authors have also noted the existence of interindividual variability of HbA1c and ethnic and racial variability [**24,25**].

5. Conclusion

The HbA1c assay is a frequently requested biochemical test. The analytical performances of the 2 methods are good but their use is not recommended in the biological diagnosis of diabetes and pre-diabetes due to interference from hemoglobin S. These differences observed between the assay techniques should lead the clinician to monitor his diabetic patients in the same laboratory and with the same method. The dosage of fructosamines or glycated albumin would be better indicated in the case of homozygous sickle cell disease or in the case of composite heterozygosity.

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