

## **Assessment of glycemic control in patients with haemoglobinopathy: concordance between HbA1c and fructosamine.**

### **Évaluation de l'équilibre glycémique chez les patients porteurs d'hémoglobinopathie : concordance entre l'HbA1c et la fructosamine.**

Deghima Sabrine, Maya Nechar, Zohra Sedoud, Kawther Nachi, Ania Outayeb, Djenouhat Kamel.

Établissement public hospitalier de Rouiba & Université Alger1

Mail : [sabrindegghima@gmail.com](mailto:sabrindegghima@gmail.com)

#### **Abstract :**

Haemoglobinopathies, such as sickle cell anemia and thalassemia, are hereditary genetic conditions very common around the Mediterranean region, characterized by qualitative or quantitative abnormalities in hemoglobin. These conditions can influence glycated hemoglobin (HbA1c) results, a crucial marker for monitoring glycemic control in diabetic patients.

In order to assess the impact of haemoglobinopathies on HbA1c results, we examined and compared two HPLC D-10 separation kits, namely the HbA1c kit, which is designed specifically to measure HbA1c, and the Dual kit, which can separate and quantify both HbA1c and other hemoglobin variants. The study was conducted on a group of 75 diabetic and non-diabetic patients with qualitative or quantitative haemoglobinopathies. Blood samples were subjected to both HPLC D-10 separation methods and plasma fructosamine assay.

The results of the study showed good agreement between the two kits for haemoglobinopathies (haemoglobinosis C, haemoglobinosis S, heterozygous beta-thalassemia, heterozygous alpha-thalassemia (silent or minor)). However, we were unable to examine the effect of haemoglobinosis E and D.

Consequently, if a hemoglobin variant (S, C) is found by chance using the HbA1c kit, the result is valid. For quantitative abnormalities such as homozygous beta-thalassaemia, homozygous sickle cell disease (HbSS), homozygous haemoglobinosis C (HbCC) and HbS/HbC composite heterozygosity, other methods of assessing glycemic control should be considered (fructosamine assay). Our results underline the importance of taking haemoglobinopathies into account when interpreting HbA1c test results, since accurate measurement is essential for monitoring diabetes. This will help to avoid long-term complications and ensure appropriate therapeutic decisions for these patients.

**Key words: Diabetes, HbA1c, Haemoglobinopathies, HPLC.**

#### **Résumé :**

Les hémoglobinopathies, telles que la drépanocytose et la thalassémie, sont des affections génétiques héréditaires très fréquentes autour de la région méditerranéenne, caractérisées par des anomalies qualitatives ou quantitatives de l'hémoglobine. Ces conditions peuvent influencer les résultats de l'hémoglobine glyquée (HbA1c), un marqueur crucial pour le suivi du contrôle glycémique chez les patients diabétiques.

Dans le but d'évaluer l'impact des hémoglobinopathies sur les résultats d'HbA1c, nous avons examiné et comparé deux kits de séparation HPLC D-10, à savoir le kit HbA1c étant conçu spécifiquement pour mesurer l'HbA1c et le kit Dual qui permet de séparer et de quantifier à la fois l'HbA1c et d'autres variants d'hémoglobine. L'étude a été menée sur un groupe de 75 patients diabétiques et non diabétiques présentant des hémoglobinopathies qualitatives ou quantitatives. Les échantillons sanguins ont été soumis aux deux méthodes de séparation HPLC D-10 et au dosage de la fructosamine plasmatique.

Les résultats de l'étude ont montré une bonne concordance des résultats entre les deux kits pour les hémoglobinopathies (hémoglobinoase C, hémoglobinoase S, bêta-thalassémie hétérozygote, alpha-thalassémie (silencieuse ou mineure). En revanche, nous n'avons pas pu examiner l'effet de l'hémoglobinoase E et D.

Par conséquent, en cas de découverte fortuite d'un variant d'hémoglobine (S, C) par le kit HbA1c, le résultat est valide. Pour les anomalies quantitatives telles que la bêta-thalassémie homozygote, la drépanocytose homozygote (HbSS), l'hémoglobinoase C homozygote (HbCC) et l'hétérozygotie composite HbS/HbC d'autres méthodes d'appréciation de l'équilibre glycémique devraient être envisagées (dosage des fructosamines). Nos résultats soulignent l'importance de prendre en compte les hémoglobinopathies lors de l'interprétation des résultats du test HbA1c dont la précision du dosage est essentielle pour le suivi du diabète ce qui permettra d'éviter les complications à long terme et de garantir des décisions thérapeutiques appropriées pour ces patients.

**Mots clés : Diabète, HbA1c, Hémoglobinopathies, HPLC.**

## **Introduction**

Diabetes mellitus is a metabolic disease characterized by the presence of chronic hyperglycaemia attributable to defects in insulin secretion, insulin action or both. The prevalence of diabetes continues to rise. Current estimates suggest that 537 million adults between the ages of 20 and 79 are affected by diabetes worldwide, representing 10.5% of the global population within this age range.

Forecasts suggest that the global diabetic population is expected to reach 643 million (11.3%) by 2030, with a further increase to 783 million (12.2%) by 2045(1).

In Algeria, the prevalence of diabetes was 14.4%, which represents 4 million diabetics in 2018(2).

Glycated hemoglobin A1c (HbA1c) is the recommended marker for diagnosing and monitoring diabetes (3); it reflects glycemic control over a 12-week period prior to measurement (4). The presence of haemoglobinopathy can lead to inaccurate measurements of HbA1c and, consequently, an inaccurate estimate of previous blood glucose levels in these individuals (5), (6).

The reliability of HbA1c as a retrospective marker of glycemic control depends on two physiological factors: a normal lifespan for red blood cells (120 days) and normal hemoglobin synthesis (7).

If one of these two parameters is altered, interpretation of the HbA1c assay becomes at least delicate and at most impossible. This is the case with haemoglobinopathies (8).

Haemoglobinopathies are inherited hemoglobin (Hb) disorders that affect the structure or production of hemoglobin (9). The prevalence of these diseases varies considerably throughout the world, depending on geographical region and population. In Algeria, as in other countries in the Mediterranean region and North Africa, certain haemoglobinopathies such as thalassemia and sickle cell anemia are relatively common (10).

According to the WHO, sickle cell anemia is the most widespread genetic disease in the world. The disease is very common, particularly around the Mediterranean (11), and there are also quantitative abnormalities represented mainly by type  $\alpha$ ,  $\beta$  or  $\gamma$  thalassemia (12). More than 300,000 births a year are affected by one of these haemoglobinopathies (70% sickle cell anemia, 30% thalassemia) (13).

Despite the use of devices certified by the National Glycohemoglobin Standardization Program (NGSP) which are not affected by HbS interference, the relationship between HbA1c and the presence of haemoglobinopathy is not coherent from one study to another (5). The accuracy of HbA1c may also be affected by other factors, in particular the coexistence of several haemoglobinopathies.

Therefore, it is important to have an alternative to HbA1c testing, particularly in Algeria, which has a high prevalence of haemoglobinopathies (14).

One proposed alternative to HbA1c is the measurement of serum fructosamine.

Fructosamine is a measure of glycated plasma proteins. Like HbA1c, fructosamine assesses peripheral glucose

levels over time, but for around 2 to 3 weeks prior to measurement instead of 2 to 3 months for HbA1c. Being independent of hemoglobin levels, this makes it useful in conditions where the lifespan of red blood cells is shortened, such as haemoglobinopathies, pregnancy and hemolytic anemia (15), (16).

In this context, we aimed to assess the influence of qualitative or quantitative haemoglobinopathies on HbA1c measurement by examining the concordance between this measurement and fructosamine. We also compared two HPLC D-10 chromatographic separation kits, the standard HbA1c kit and the Dual kit, which separates and quantifies both HbA1c and other hemoglobin variants.

## **Objectives**

The aim of the study was:

- To assess the influence of haemoglobinopathies on HbA1c measurement.
- To compare two HPLC chromatographic separation kits on the Bio-Rad ® D10 automated system (standard HbA1c kit, Dual kit designed specifically for patients with haemoglobinopathies).

## **1. Material and method**

**1.1 Type of study:** This was a cross-sectional, comparative analytical study.

### **2.2. Study population**

The study involved 75 patients enrolled at EPH Rouiba over a period of seven months, from 25 October 2022 to 16 May 2023.

#### **2.2.1. Inclusion criteria**

All patients, diabetic or not, with qualitative or quantitative haemoglobinopathies from the various services of EPH Rouiba (pediatrics, internal medicine, oncology, haemobiology unit, dialysis).

All patients with hemoglobin variants discovered after measurement of HbA1c as part of the routine check-up in the biochemistry unit.

#### **2.2.2. Non-inclusion criteria**

- Transfused subjects.
- Subjects with a history of hemolysis.
- Subjects with anemia (total hemoglobin <8g/dl).
- Subjects with homozygous haemoglobinopathy.

## **2.3 Material**

### **2.3.1 Biological specimen**

Venous blood samples collected on EDTA tubes (for HbA1c measurement) and lithium heparinate (for fructosamine measurement).

### **2.3.2. Analyzer and technique**

The D-10 analyzer (Bio-Rad): a multiparameter high-performance liquid chromatography (HPLC) system for measuring hemoglobin A1C (HbA1c), A2, HbF and screening of hemoglobin variants. In our study, we used it as part of the HbA1c assay for monitoring diabetic patients and for screening and quantifying hemoglobin variants.

Standard HbA1c kit: Used to measure HbA1 (HbA1c), HbA0, HbF and to screen for haemoglobin variants.

Dual kit: in addition to the HbA1 and HbF fractions, this kit can also be used to determine the HbA2 fraction, providing better separation of the hemoglobin fractions (HbA1 fraction and HbA2 fraction). It is recommended for use in the presence of hemoglobin variants. It should be noted that both HbA1c assay methodologies are NGSP/DCCT (Diabetes Control and Complications Trial) and IFCC (International federation of clinical chemistry) certified.

Reference values :20 to 42 mmol/mol Hb (IFCC), 4-6 % of total Hb (NGSP).

**Fructosamine:** determination by immunoturbidimetry on Cobas integra, Roche diagnostics. Reference values:

205-285 mmol/L.

Additionally, all patients included in the study underwent concomitant measurement of albumin and total protein levels to validate the measurement of fructosamine.

Assay methodology: albumin by bromocresol green (BCG), reference values (35-52 g/L) and protein by the BIURET method, reference values (reference values) on Cobas Integra.

## **2.4. Method**

**2.4.1. Data collection:** Patients' clinical data, including age, diabetes, comorbidities, type of haemoglobinopathy and any current treatments, were collected using a completed information sheet.

### **2.4.2. experimental design:**

The evaluation was carried out within the framework of normal use of the automated system according to the recommendations for good practice indicated by the Bio-Rad company (diabetes control included in the recommended range, chromatogram validated according to the defined criteria).

Each sample was analyzed on the same day for HbA1c (using both kits) and fructosamine.

-Comparison of the two chromatographic separation methods was carried out in accordance with the instructions in the Clinical and laboratory standards institute protocol (CLSI EP09) (17).

## **2.5. Statistical analysis**

The analysis of the data was carried out using two software packages; SPSS (version 26.0) and Medcalc V23 for the comparative study in the Bland-Altman diagram.

Continuous quantitative variables are expressed as mean  $\pm$  standard deviation, minimum and maximum.

Categorical variables were expressed as percentages.

The distribution of the variables was verified using the Kolmogorov-Smirnov test.

Pearson's test was used to assess the correlations between different parameters.

Means were compared using the non-parametric Mhan-whitney test.

The accepted risk of error of the first order for these various statistical analyses is 5%, and the results will therefore be expressed within a 95% confidence interval.

## **3.1 Characteristics of the study population:**

This study involved 75 Patients whose characteristics are described in Table 1.

**Table 1:** The demographic and clinical profile of the study cohort included variables such as age, gender distribution, discovery circumstances, the specific type of hemoglobinopathy, and its defining characteristics.

|                                   |                                                                     |                                                      |
|-----------------------------------|---------------------------------------------------------------------|------------------------------------------------------|
| Age (years)                       | Mean = 40.89 ± 19,39                                                |                                                      |
| Sex-ratio (M/F)                   | 1,02                                                                |                                                      |
| Diabetes                          | Yes: N= 19 (25.3%)<br>No: N= 56 (74.7%)                             |                                                      |
| Diagnosis of haemoglobinopathies  | Fortuitous: N= 62 (82.7%)<br>known: N=13 (17.3%)                    |                                                      |
| Type of hemoglobinopathies        |                                                                     |                                                      |
| B-thalassemia n= 14 (18.7%)       | Mean HbA2 % ± standard error (Se)<br>5,77±0,103<br>Min 4<br>Max 5,6 | Mean HbF%± Se<br>1,75±0,39<br>Min 0,5<br>Max 11      |
| α-thalassaemia n= 2 (2.7%)        | Mean HbA2 % ± Se<br>2,13±0,23<br>Min 1,7<br>Max 2,5                 | Mean HbF%± Se<br>0,9±0,1<br>Min 0,8<br>Max 1         |
| Sickle cell disease n= 47 (62.7%) | Mean HbS% ± Se<br>38.55 ±0,75<br>Min 24.60<br>Max 59,20             | Mean HbF% ± Se<br>0,877±1,863<br>Min 0,1<br>Max 7    |
| Hemoglobinosis C n= 12 (16.0%)    | Mean HbC± Se<br>36.11 ± 1,82<br>Min 29.30<br>Max 42.40              | Mean HbF% ± Se<br>0.28±0,182<br>Min 0.10<br>Max 0.50 |

**Nota bene:** The diagnosis of various haemoglobinopathies is achieved through the application of two complementary methodologies, namely capillary electrophoresis and high-performance liquid chromatography (HPLC), both of which provide qualitative and quantitative data. As stipulated by the Nomenclature of Analyses of Medical Biology (NABM) (18), hemoglobin analysis necessitates the use of at least three phenotypic techniques, one of which must be electrophoretic. In our study, capillary electrophoresis was conducted utilizing the Sebia Capillarys Piercing 2. It should be noted that the Itano test (solubility test for HbS) was not performed in this study.

### 3.2. Means and standard deviations of the variables examined:

**Table 2:** Means and standard deviations of the variables studied

|                              | Mean   | SD    | Min   | Max   |
|------------------------------|--------|-------|-------|-------|
| <b>%HbA1c (Kit HbA1c)</b>    | 5.74   | 1.34  | 3.40  | 10.50 |
| <b>%HbA1c (Kit Dual)</b>     | 5.59   | 1.32  | 3.50  | 10.40 |
| <b>Fructosamine (mmol/l)</b> | 237,51 | 42,06 | 142   | 387   |
| <b>Albumin (g/L)</b>         | 43,37  | 3,42  | 33,51 | 47,81 |
| <b>Total protein (g/L)</b>   | 69,52  | 5,35  | 56,7  | 83,50 |

### 3.3. *Comparison of averages according to type of haemoglobinopathy*

We compared the mean HbA1c values of the two chromatographic separation methods according to the type of haemoglobinopathy. The results are shown in Table 3.

**Table 3:** mean HbA1c values obtained using the two methods, depending on the type of haemoglobinopathy.

|                       | Kit HbA1c | Kit Dual  | p-value |
|-----------------------|-----------|-----------|---------|
| Sickle cell disease S | 5.90±1.37 | 5.74±1.44 | 0.21    |
| Hemoglobinosis C      | 5.82±1.45 | 5.55±1.38 | 0.91    |
| β -thalassaemia       | 5.37±1.08 | 5.29±0.70 | 0.48    |
| α-thalassaemia        | 4.30±1.27 | 4.45±1.34 | 0.43    |

### 3.4. *Study of the correlation between HbA1c obtained by the two kits and fructosamine*

The Pearson correlation test showed the following results: A good correlation between fructosamine and HbA1c obtained by the DUAL kit ( $r=0.601$ ,  $p \leq 0.001$ ).

No correlation between fructosamine and HbA1c obtained by the standard kit ( $r=0.270$ ,  $p=0.053$ ).

### 3.5. *Comparison of the two HPLC separation kits (standard and dual)*

#### *a- Correlation*

The Pearson test between the two HPLC separation kits showed an excellent correlation with  $r=0.969$ ,  $r^2=0.936$  and  $p \leq 0.001$ .

#### *b- Passing Bablock regression*

Passing balock regression showed excellent agreement between the two techniques with an equation of the type  $y=0.1+1x$  where X represents the percentage of HbA1c obtained with the dual Kit and Y that obtained with the standard HbA1c Kit .

A slope of 1 indicated an excellent linear relationship between the two techniques. The slope's 95% confidence interval (95% CI<sub>a</sub>: 0.9697–1.0492) included the value of 1, indicating no proportional difference between the two methods. Similarly, the 95% confidence interval for the y-intercept (95% CI<sub>b</sub>: -0.1459–0.2909) included a value of 0, suggesting the absence of a systematic difference between the two methods (Figure 1).

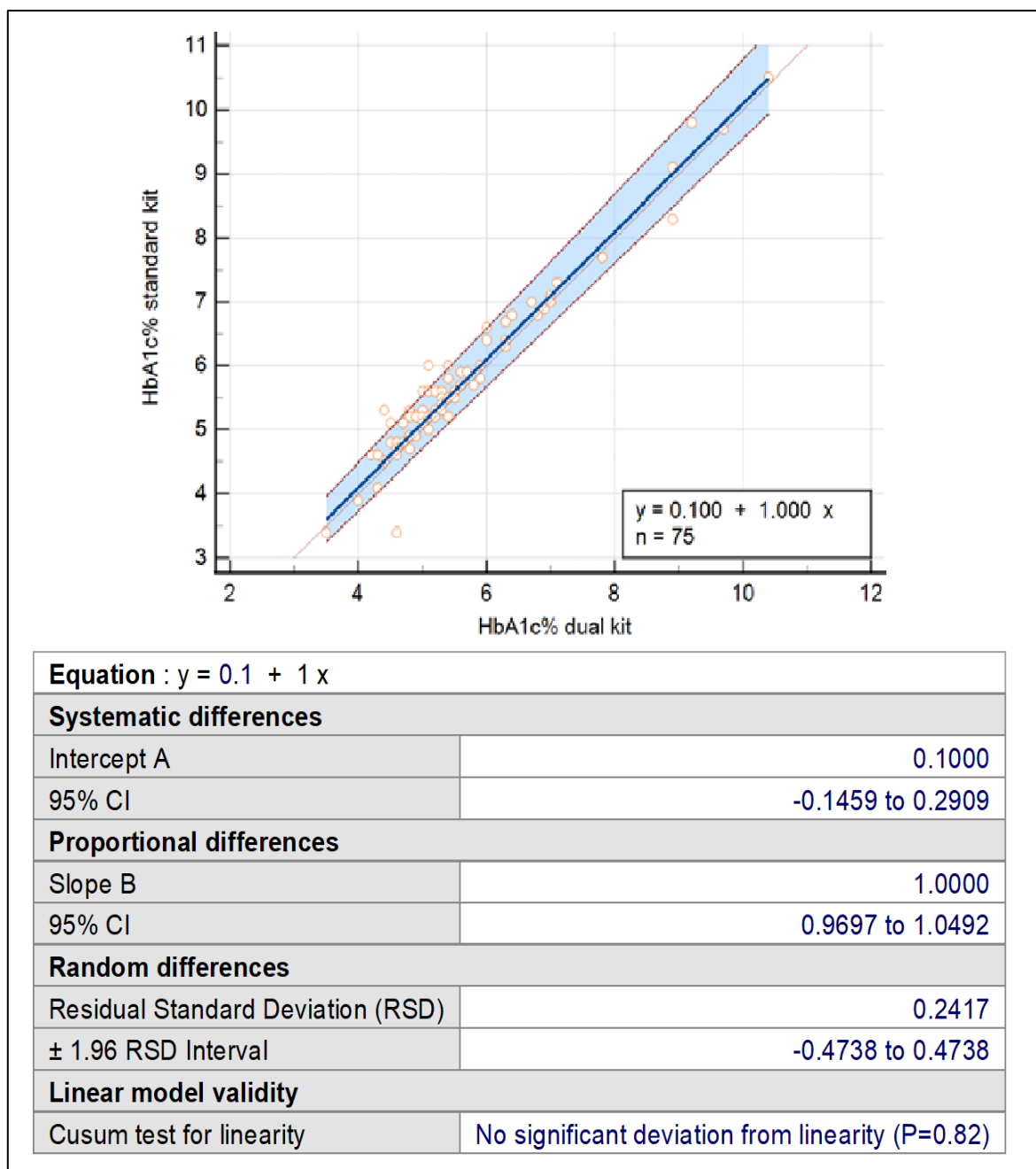
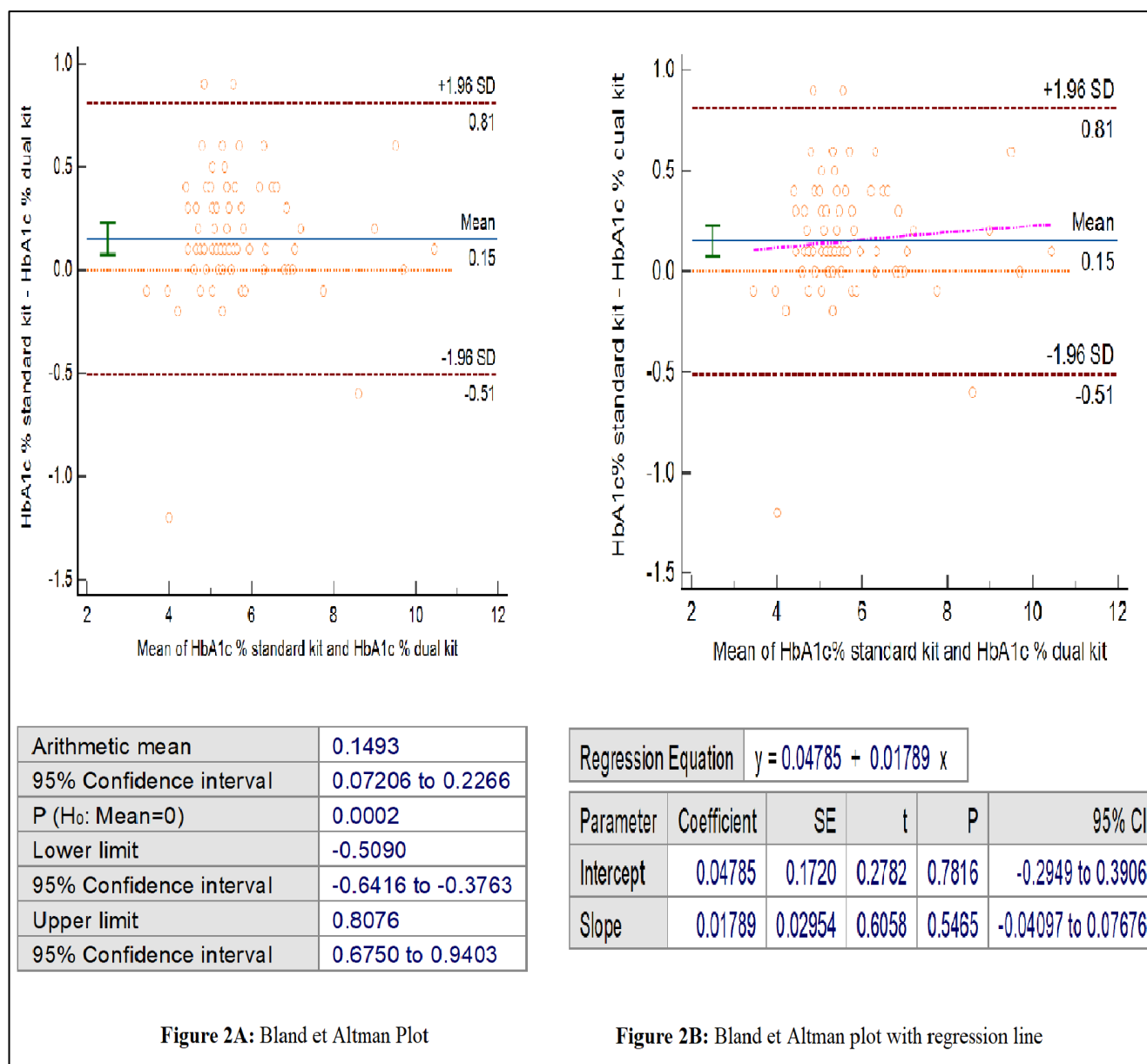


**Figure 1: Passing Bablock regression**

### **c- Bland and Altman difference diagram**

Analysis of the Bland and Altman diagram is used to determine the bias and the limits of agreement. The bias corresponds to the average of the differences and is used to assess the accuracy of the measurements. It represents the average systematic deviation of one series of values from another. The bias was +0,149%, **95% CI of bias** (0.0720 to 0.226), limit of the agreement (-0.508 to 0.807).

**Figure N°2:** Bland et Altman plot showing difference between HbA1c standard kit and HbA1c dual kit: Figure

2A Bland et Altman plot Bias=0,149, 95%CI of bias= 0,072 to 0,226, limit of agreement= -0,509 to 0,807. Figure 2B: Bland et Altman plot with regression line Slope 0,0178(95%CI:-0,0409 to 0,0767), Intercept 0,0478(95%CI:-0,294 to 0,390)

## 2. Discussion

The mean age of the subjects was  $40.89 \pm 19.39$  years, with a sex ratio of 1.02, indicating an approximately equal distribution between males and females in the study.

From the 75 subjects enrolled, only 19 were diabetic (25.3%) due to random recruitment. Most of the subjects included in our study (82.7%) had haemoglobinopathy discovered after an HbA1c test in the biochemistry unit (incidental finding).

Subsequent to the incidental detection of the variants, the patients were invited to participate in a family investigation, and a second control sample was obtained. Two analytical techniques were employed to confirm the diagnosis: capillary electrophoresis (Sebia Capillarys 2) and high-performance liquid chromatography (HPLC) (D10 Bio-Rad, Variant Kit).

This can be explained by the absence of significant symptoms associated with the presence of a haemoglobinopathy and the high frequency of these pathologies in the Algerian population. Our results are

similar to those of studies carried out by C. Désidéri- Vaillan and D. Kone et al on the circumstances of discovery of haemoglobinopathies, which describe an incidental finding of these disorders in most cases (19) (20).

The distribution of patients according to the type of haemoglobinopathy showed a clear predominance of sickle cell disease (62.67%), followed by  $\beta$ -thalassemia (18.7%), haemoglobinosis C (16.0%) and  $\alpha$ -thalassemia (2.7%), in agreement with data found in the literature describing a predominance of sickle cell disease, which alone accounts for approximately 70% of haemoglobinopathies (21).

However, it is essential to emphasise that the results of our study cannot be generalised to the entire population, as the sample studied is not representative of the general population. Moreover, the study protocol does not permit conclusions to be drawn regarding prevalence.

The study of the correlation between the HbA1c obtained by the two kits and fructosamine, considered to be the reference test for assessing glycemic control in patients with haemoglobinopathies, found a good correlation between the dual kit and fructosamine ( $r=0.601$ ,  $p \leq 0.001$ ) while there was no correlation with the standard kit, suggesting that the HbA1c obtained by the dual kit is more appropriate for assessing glycemic control in this population.

In order to verify the validity of measuring HbA1c using the standard kit in this population, we compared the two kits according to the CLSI EP09 guidelines. The findings were as follows:

An excellent correlation  $r=0.969$ ,  $r^2=0.938$ ,  $p \leq 0.001$ , showing an excellent linear relationship between the two kits. Our results are similar to those of C. Marzullo et al, 2008, who found a correlation coefficient of 0.998 (22). The passing bablock regression shows excellent agreement between the two techniques with an equation of the type  $y=1x+0.10$  where  $x$  represents the percentage of HbA1c obtained with the dual kit and  $y$  that obtained with the standard kit.

The slope of 1 indicated an excellent linear relationship between the two techniques. The slope's 95% confidence interval (95% CI<sub>a</sub>: 0.9697–1.0492) included the value of 1 indicating no proportional difference between the two methods. Similarly, the 95% confidence interval for the y-intercept (95% CI<sub>b</sub>: -0.1459–0.2909) included a value of 0, suggesting the absence of a systematic difference between the two methods (*Figure 1*).

Analysis of the Bland and Altman plot (*Figure 2*) revealed fairly good agreement of HbA1c results between the two modes, although a moderate overestimation of HbA1c values was noticed with the standard kit with a low bias of 0.149% (95% CI: -0.508 to 0.807%) and

tight limits of agreement (-0.509 to 0.807) which means that 95% of the differences between the methods fall within this interval, indicating moderate variation.

Similar results were found in the study by H. Chemsu et al, 2020, with a bias of 0.244% (23).

The analysis of the Bland–Altman plot, incorporating the regression line, was conducted to ascertain whether the bias was influenced by the measured value.

The intercept (0.04785,  $p=0.7816$ ) and slope (0.01789,  $p=0.5465$ ) were both statistically non-significant, indicating the absence of proportional bias. Therefore, the difference between the two methods remains consistent across the range of measured values.

-Validity of HbA1c results in the presence of hemoglobin variants:

HbA1c was measured in 59 patients with heterozygous hemoglobin abnormalities (HbS; N=47, HbC; N=12). The presence of the hemoglobin variant was detected in all cases as a peak with a retention time later than that of HbA0. HbS and HbC are identified on the chromatogram as such (S and C window respectively).

We found no significant difference between the HbA1c measured by the two kits, and since the study of concordance between the two kits is also satisfactory, we can conclude that in the presence of an HbS variant or an HbC, in the heterozygous state, the HbA1c

result obtained by the standard kit is valid, as reported by Mangia et al, 2008 and D. kalkuran et al, 2021 (24,25) and on the NGSP website, which confirms that there is no interference from these variants on the assay technique in question (26).

On the other hand, HbS or HbC mutations in the homozygous state and the presence of both mutations simultaneously (composite heterozygous HbSC), do not produce hemoglobin A, so it is impossible to measure HbA1c and in this case fructosamine should be used to assess glycemic control.

For HbE and HbD, the retention time is too close to that of HbA0, so there could be co-elution with the A0 fraction (7). It is therefore more appropriate to return the HbA1c value measured using the Dual kit, which provides better peak separation. The same recommendation must be followed for any hemoglobin variant other than S or C whose retention time is later than that of HbA0(23). Unfortunately, we were unable to verify this as we did not have any variant E or D in our sample.

-Validity of the HbA1c result in the presence of quantitative abnormalities:

We found no significant difference between the HbA1c level obtained by the standard kit and that obtained by the Dual Kit in patients with heterozygous  $\beta$ -thalassemia (N=14) contrary to what is described in the literature, this is probably due to the small sample size.

The presence of a  $\beta$ -thalassemia trait may affect HbA1c levels. Ineffective erythropoiesis as well as peripheral hemolysis may decrease HbA1c levels due to the reduced lifespan of erythrocytes in  $\beta$ -thalassemia carriers (27). In addition, extremely high fetal hemoglobin (HbF) levels (>10-15%), which are sometimes found in  $\beta$ -thalassemia carriers, could affect the reported HbA1c result, leading to falsely low HbA1c values (27).

In our sample, subjects with beta thalassaemia, the mean HbF was  $1.75 \pm 0.39$  with a minimum of 0.5% and a maximum of 11%. According to the official NGSP website, there is no interference from HbF up to 12.5% with the HbA1c measurement technique we used.

Moreover, in the presence of  $\beta$ -thalassemia, the increase in HbA2 cannot be detected with the HbA1c programme. This is only possible with the Dual program (long program retention time: HbA0 1.67 min, HbA2 3.11 min). Therefore, it is strongly recommended to use the dual kit for HbA1c measurement in a population with a high prevalence of  $\beta$ -thalassemia (23).

In the case of alpha-thalassemia, which shares a similar pathophysiological background to  $\beta$ -thalassemia, HbA1c levels are affected to a degree that depends on the number of missing  $\alpha$ -chain genes and the severity of the resulting hemolytic state (27), in our study we found no significant difference in HbA1c levels measured by the two methods in this population (N=2). but the sample is too small to make any conclusions; a study on a larger sample would be valuable.

## **Conclusion**

Haemoglobinopathies are a group of inherited genetic disorders affecting the structure or the production of hemoglobin. They are common in some parts of the world, particularly around the Mediterranean region such as Algeria.

The most prevalent hemoglobinopathies, namely sickle cell disease and thalassemia, are characterized by abnormalities in either the quality or quantity of hemoglobin. These disorders can influence HbA1c levels, a crucial marker for monitoring blood glucose levels in individuals with diabetes, by affecting the lifespan of red blood cells (120 days) and the synthesis of hemoglobin.

This study highlighted the need to take haemoglobinopathies into consideration when interpreting HbA1c test results, as the accuracy of the assay is crucial for the effective monitoring and management of diabetes and for ensuring appropriate therapeutic decisions for these patients.

In the light of our results, we can conclude that in the event of the incidental discovery of a hemoglobin variant (heterozygous HbS, HbC) by the standard HbA1c kit, the result is valid, whereas in other cases, such as heterozygous beta-thalassemia, alpha-thalassemia, HbE, HbD, it is necessary to use a special variant kit (Dual kit). In other rarer situations, such as homozygous beta-thalassemia, HbSS, HbCC and HbSC, it is impossible to measure HbA1c. In these cases, other methods of assessing glycemic control (measurement of fructosamine) can be used. However, it is important to note that fructosamine measurement has its own limitations. It reflects glycaemic control over a shorter period of time (2-3 weeks) compared to HbA1c, and can be influenced by conditions that affect protein metabolism, such as nephrotic syndrome or liver disease.

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