

Impact of Iron-Deficiency Anemia on HbA1c Measurement in Non-Diabetic Algerian Adults

Impact de l'anémie ferriprive sur la mesure de l'HbA1c chez Des adultes algériens non diabétiques

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ABSTRACT

Introduction-Aim: Glycated haemoglobin (HbA1c) is a widely used clinical marker for assessing glycaemic control in patients with diabetes. However, certain conditions may affect the reliability of this marker, anaemia, particularly iron deficiency anaemia, being a common source of HbA1c variability. This study aimed to determine the total haemoglobin threshold below which HbA1c measurements become uninterpretable.

Methods: The study included 99 patients with iron deficiency anaemia, in whom three main parameters were measured: HbA1c, total haemoglobin and fructosamine. The population was stratified into three groups according to anaemia severity, and a correlation analysis was performed between HbA1c and fructosamine, a glycaemic marker independent of anaemia.

Results: Mean HbA1c was $5.90\% \pm 0.88$ in moderate anaemia, $5.87\% \pm 0.72$ in mild anaemia and $6.43\% \pm 1.12$ in severe anaemia. These differences were not statistically significant (Kruskal–Wallis test, $p = 0.34$). A statistically significant positive correlation between HbA1c and fructosamine was observed in the moderate ($p = 0.39$; $p = 0.007$) and mild ($p = 0.38$; $p = 0.017$) anaemia groups, but not in the severe anaemia group ($p = 0.503$).

Conclusion: Our preliminary findings show that below a total haemoglobin threshold of 9 g/dL in men and 8 g/dL in women, HbA1c is no longer a reliable marker of glycaemic control, and that the use of alternative markers, such as fructosamine, represents a valuable alternative until iron deficiency is corrected

Keywords: Glycated haemoglobin; Iron deficiency anaemia; Fructosamine; Glycaemic control; Algeria

RÉSUMÉ

Introduction-Objectif : L'hémoglobine glyquée (HbA1c) est un marqueur clinique largement utilisé pour évaluer le contrôle glycémique chez les patients diabétiques. Cependant, certaines conditions peuvent affecter la fiabilité de ce marqueur, l'anémie, en particulier l'anémie ferriprive, étant un facteur fréquent de variabilité de l'HbA1c. Cette étude visait à déterminer la limite supérieure de la concentration d'hémoglobine totale au-dessous de laquelle les mesures d'HbA1c deviennent ininterprétables.

Méthodes : L'étude a inclus 99 patients atteints d'anémie ferriprive, et trois paramètres principaux ont été mesurés : l'HbA1c, l'hémoglobine totale et la fructosamine. La population a été stratifiée en trois groupes selon la sévérité de l'anémie, et une étude de corrélation a été réalisée entre l'HbA1c et la fructosamine, un marqueur glycémique indépendant de l'anémie.

Résultats : Le taux moyen d'HbA1c était de $5,90\% \pm 0,88$ en cas d'anémie légère, de $5,87\% \pm 0,72$ en cas d'anémie moyenne et de $6,43\% \pm 1,12$ en cas d'anémie sévère. Ces différences n'étaient pas statistiquement significatives (test de Kruskal-Wallis, $p = 0,34$). Une corrélation positive statistiquement significative entre l'HbA1c et la fructosamine a été observée dans les groupes d'anémie légère ($p = 0,39$; $p = 0,007$) et moyenne ($p = 0,38$; $p = 0,017$), mais pas dans le groupe d'anémie sévère ($p = 0,503$).

Conclusion : Les résultats de nos travaux préliminaires montrent qu'à un seuil d'hémoglobine totale inférieur à 9 g/dl chez l'homme et à 8 g/dl chez la femme, l'HbA1c n'est plus un marqueur fiable du contrôle glycémique et que l'utilisation d'autres marqueurs, tels que la fructosamine, représente une solution alternative intéressante en attendant la correction de la carence en fer.

Mots-clés : Hémoglobine glyquée ; Anémie ferriprive ; Fructosamine ; Contrôle glycémique ; Algérie

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INTRODUCTION

Diabetes is a chronic condition characterized by the body's inability to produce adequate insulin (type 1 diabetes) or by resistance to insulin effects (type 2 diabetes). The function of this hormone is crucial for maintaining homeostasis in carbohydrate metabolism. According to data from the World Health Organization (WHO), the prevalence of diabetes increased from 108 million individuals in 1980 to 422 million in 2014 (1). The prevalence of diabetes has reached unprecedented levels in recent years. According to the 2021 report by the International Diabetes Federation (IDF), one in ten individuals is affected by diabetes, equating to 537 million adults worldwide (2). In Algeria, 14.4% of adults (4 million individuals) were diagnosed with diabetes in 2018 (3). Diabetes is associated with numerous complications, primarily diabetic microangiopathies and macroangiopathies, including nephropathy, neuropathy, and retinopathy, rendering it the sixth leading cause of mortality among adults globally in 2019 (4). Consequently, early detection of this disease is crucial and relies on a set of criteria that have evolved over time. In 1979, diabetes was characterised by a fasting blood glucose level of 1.40 g/l (7.7 mmol/l) (5). By 1997, the diagnostic threshold was revised to 1.26 g/l (7 mmol/l) when fasting and 2 g/l (11 mmol/l) postprandially or following an oral glucose tolerance test (OGTT) (6). In 2009, the American Diabetes Association (ADA) introduced a new definition of diabetes, which was endorsed by the World Health Organization (WHO) in 2011. This definition includes an additional criterion: a glycated hemoglobin (HbA1c) level of 6.5% (7,8).

HbA1c is defined as hemoglobin modified by the non-enzymatic binding of glucose to the N-terminal valine of one or both beta chains of hemoglobin A. Its value depends on the degree of glycation, that is, the body's exposure to glucose and the lifespan of red blood cells, making this parameter the best reflection of average blood glucose levels over a period of 8 to 12 weeks prior to measurement (9). Various methods are used to measure HbA1c, including immunological, chromatographic, and enzymatic techniques. The reference technique, as described by the IFCC in 1995, is high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) or capillary electrophoresis (10). The excellent correlation with average blood glucose levels over three months and the widespread standardisation of measurement methods have made HbA1c the marker of choice for monitoring diabetes. However, this marker loses its relevance in certain situations, which can affect its value, thereby altering the reliability of HbA1c as a marker of glycaemic balance. Several factors influence the non-enzymatic glycation of proteins, including reaction time, temperature, and glucose concentration. The first two parameters are assumed to be constant for hemoglobin. Thus, any variation in the residence time of red blood cells in the blood will affect average blood glucose levels and HbA1c: a longer residence time will increase the HbA1c value, while a shorter residence time will decrease it (11). Conditions that cause changes in the lifespan of red

blood cells and/or the rate of hemoglobin synthesis make interpreting HbA1c levels difficult and sometimes even impossible. This is the case in iron deficiency anaemia, quantitative and qualitative hemoglobinopathies (sickle cell disease, thalassaemia, hemoglobinopathies S, C, E, and D), erythrocyte abnormalities (spherocytosis), chronic haemorrhages, reticulocytosis, and taking medications such as antiretrovirals and vitamin B12 and B9 deficiency. In addition, certain factors can interfere with the HbA1c measurement method, such as aspirin use (acetylated hemoglobin), vitamin E use, the presence of carbamylated hemoglobin (kidney failure), the labile form of HbA1c (LA1c), high hemoglobin F levels (thalassaemia syndrome), liver disease (hypersplenism, haemorrhages), hypertriglyceridaemia, and hyperbilirubinaemia (12,13). Among these factors, anaemia is the most common cause that can influence HbA1c levels, especially since anaemia is often found in diabetics (14). A study in the Democratic Republic of the Congo revealed that anaemia is prevalent among individuals with type 2 diabetes, with approximately 57% of the participants affected (15).

The aetiology of anaemia in patients with diabetes is multifactorial. Although impaired renal function is the most common cause, numerous studies have reported a high prevalence of anaemia in patients with diabetes without renal impairment, suggesting the existence of other causal factors. In patients with type 2 diabetes, other causes of anaemia include iatrogenic factors, such as oral antidiabetic drugs, angiotensin-converting enzyme inhibitors, and angiotensin II receptor antagonists. In patients with type 1 diabetes, anaemia is often associated with autoimmune diseases, including autoimmune gastritis, pernicious anaemia, Hashimoto's thyroiditis, and celiac disease. The underlying mechanism of anaemia in cases of kidney damage is usually related to iron or erythropoietin deficiency (16). The high prevalence of iron deficiency anaemia and diabetes has led to numerous studies on the potential impact of iron deficiency anaemia on glycated hemoglobin measurements, without reaching a final consensus on the subject. This issue is particularly important because iron deficiency anaemia can influence HbA1c results by overestimating its value (11). This study aimed to evaluate the impact of iron deficiency anaemia on HbA1c measurements in a population of non-diabetic adults and to determine a total hemoglobin threshold above which HbA1c results can be considered sufficiently reliable.

METHODS

This cross-sectional study was conducted from December 2023 to June 2024 at the central medical biology laboratory of Rouïba Public Hospital in Algiers. It initially included 100 non-diabetic adult participants referred to the laboratory for routine medical examinations as part of their general health follow-up, in the absence of any specific diagnosed condition. During data review, one participant presenting a major discordance between HbA1c (12.9%) and fasting glycaemia was identified and excluded from the final statistical analyses due to the potential risk of analytical interference or an

undetected haemoglobin variant. Therefore, the final study population consisted of 99 participants.

The routine tests generally included a complete blood count, fasting blood glucose, and renal function tests. Informed consent was obtained from all participants prior to their inclusion in the study. Non-diabetic subjects were selected using a duly completed information sheet and fasting blood glucose testing, with inclusion criteria being blood glucose levels below 1.26 g/L. Participants were considered non-diabetic based on the absence of a previous diagnosis of diabetes and a fasting plasma glucose level below 1.26 g/L. HbA1c values were analysed as study outcomes and were not used as an exclusion criterion.

The study included all subjects with iron deficiency anaemia, defined by the following criteria: microcytic hypochromic anaemia without pseudo-polycythaemia, with a total hemoglobin level of <12 g/dL in women and <13 g/dL in men. The iron deficiency origin of the anaemia was confirmed by measuring iron levels, with ferritin levels of <15 ng/mL for women and <30 ng/mL for men, and CRP levels of <6 mg/L. All patients underwent serum albumin testing, and only those with albumin levels between 35 and 50 g/L were included in the study. In our study, we excluded participants who had received transfusions, pregnant women, and individuals with other forms of anaemia, including haemolytic, inflammatory, deficiency (vitamins B12, B9), and qualitative and quantitative hemoglobinopathies. For each participant, two samples were collected: one heparinised tube for fructosamine measurement and one EDTA tube for complete blood count (CBC) and HbA1c analysis. HbA1c levels were determined using ion exchange HPLC on the D-10 Biorad system (reference values < 6%), while fructosamine was quantified through a colorimetric technique employing nitrotertrazolum blue (NBT) on the Cobas Integra 400 plus (reference values 205-285 µmol/L). Total hemoglobin (THb) was assessed via spectrophotometry on the ADVIA 560 SIEMENS. Samples meeting the inclusion criteria were categorized into three subgroups based on THb levels according to WHO standards (17): severe anaemia (THb < 9 g/dL in men and < 8 g/dL in women; n = 14), mild anaemia (THb 9–10.9 g/dL in men and 8–9.9 g/dL in women; n = 38), and moderate anaemia (THb 11–12.9 g/dL in men and 10–11.9 g/dL in women; n = 47).

Data analysis was conducted using three software programs: Microsoft Excel V2019, R software, and IBM SPSS V29. All statistical analyses were performed after exclusion of the participant presenting a major discordance between HbA1c and fasting glycaemia. The distribution of variables was assessed using the Shapiro–Wilk normality test. Since the variables did not follow a normal distribution, comparisons between groups were performed using the Kruskal–Wallis test. Spearman's rank correlation test was used to assess the association between HbA1c and fructosamine levels within each subgroup (severe anaemia, mild anaemia, and moderate anaemia). Throughout the statistical analysis, the significance threshold was set at 5%.

RESULTS

Characteristics of study population

Table 1. Statistical summary of the study population

Descriptive Statistics			
Sex-ratio	M/F=0.45 M=31, F=68		
	Mean ± Standard deviation (SD)	Minimum	Maximum
Age (years)	33.67± 12.40	19	79
THb (g/dL)	9.96± 1.54	5.10	12.80
HbA1c(%)	5.94±0.85	4.30	8.90
Fructosamine (µmol/L)	223.91±36.24	124	297
Ferritin(ng/mL)	8.89±6.15	1.15	23.67
Glycaemia(g/L)	0.94±0.12	0.63	1.19
MCV (fL)	71.13±6.84	52.20	79.10
Shapiro-Wilk normality test			
THb	P-value=0.117		
HbA1c	P-value=0.0000000007808		
Fructosamine	P-value=0.0001313		
Total hemoglobin (THb)- mean corpuscular volume (MCV)			

Comparison of Mean HbA1c by Anaemia Severity

In the overall study population, mean HbA1c levels showed a tendency to be higher in patients with severe anaemia than in those with mild or moderate anaemia. Mean HbA1c values were 5.90% ± 0.88 in the moderate anaemia group, 5.87% ± 0.72 in the mild anaemia group and 6.43% ± 1.12 in the severe anaemia group. However, these differences were not statistically significant (Kruskal–Wallis test, p = 0.34; Figure 1).

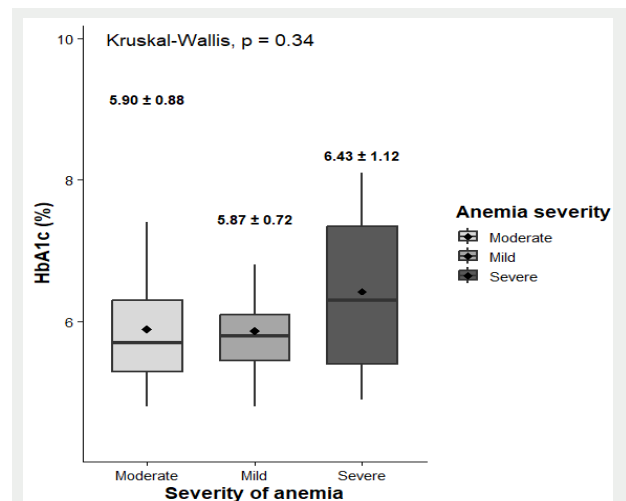


Figure 1. Comparison of HbA1c levels according to anaemia severity

Statistical analyses indicated that there were no significant differences in mean HbA1c levels when considering the severity of anaemia in both women (Kruskal–Wallis test, p = 0.509) and men (p = 0.592). Additionally, the Wilcoxon rank-sum test comparing sexes did not demonstrate a significant difference in HbA1c levels (p = 0.697) Figure 2. These findings suggest that neither sex nor the severity of anaemia significantly affects HbA1c levels in the studied population.

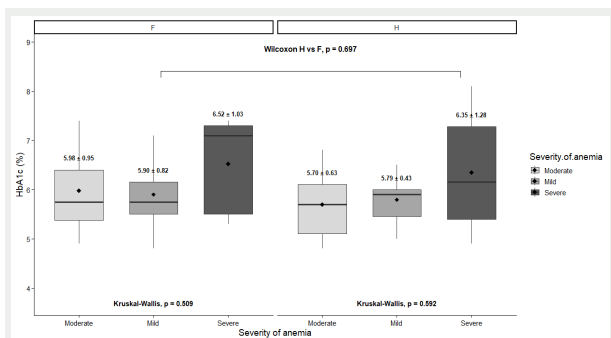


Figure 2. Comparison of HbA1c levels across anaemia severity stratified by sex

Correlation Study between HbA1c and Fructosamine

The correlation between HbA1c and fructosamine was examined in the three subgroups based on anaemia severity. A significant correlation was found in the mild and moderate anaemia groups ($p = 0.0077$ and $p = 0.017$, respectively). In contrast, the correlation was not significant in the severe anaemia group ($p = 0.503$). The results are presented in Table 2.

Table 2. Correlation between HbA1c and Fructosamine by Anaemia Severity

Variables	Groups		
	Moderate anaemia N=47	Mild anaemia N=38	Severe anaemia N=14
Mean THb (g/dl)±SD	11.21 ± 0.74	9.25 ± 0.82	7.40 ± 0.92
Min	10.0	8.10	5.10
Max	12.80	10.80	8.60
Mean HbA1c (%)±SD	5.90 ± 0.88	5.87 ± 0.72	6.43 ± 1.12
Min	4.80	4.30	4.90
Max	8.90	7.6	8.10
Mean Fructosamine±SD (μmol/l)	232.55 ± 30.04	215.54 ± 40.423	212.92 ± 38.36
Min	176.0	124.0	148.0
Max	297.0	287.0	276.0
Spearman's correlation coefficient ρ	0.39**	0.38*	0.23
P-value	0.007	0.017	0.503

NS : Not significant; * : $p < 0.05$; ** : $p < 0.01$; *** : $p < 0.001$

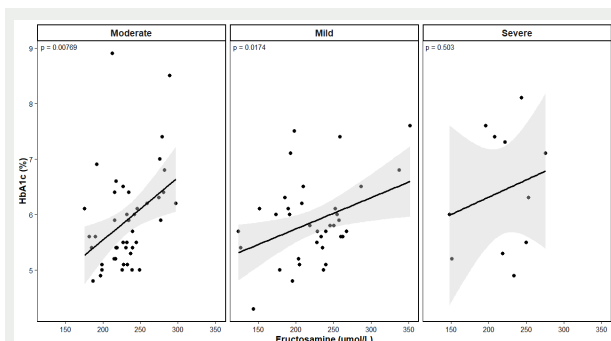


Figure 3. Scatter plot showing the correlation between HbA1c and fructosamine according to severity of anaemia

DISCUSSION

HbA1c is the preferred biomarker for monitoring diabetes mellitus, offering a practical and highly reproducible test that reflects the average blood glucose level over a three-month period. While HbA1c levels are utilized to assess glycemic control, certain conditions can affect its reliability. Our study specifically examined the impact of iron deficiency anaemia on HbA1c levels.

Our findings showed that mean HbA1c levels were comparable in mild anaemia ($5.87\% \pm 0.72$) and moderate anaemia ($5.90\% \pm 0.88$) but tended to be higher in patients with severe anaemia ($6.43\% \pm 1.12$). However, this difference did not reach statistical significance (Kruskal–Wallis test, $p = 0.34$). Although a descriptive increase in HbA1c was observed in the severe anaemia subgroup, our results do not provide sufficient evidence to support a significant relationship between anaemia severity and HbA1c levels in the overall study population. Nevertheless, previous studies have demonstrated the overestimation of HbA1c levels in the context of iron deficiency anaemia (18,19). The relationship between HbA1c and iron status is inversely proportional; a reduction in serum iron and ferritin levels correlates with an increase in HbA1c values (11). This phenomenon may be attributed to an extended lifespan of red blood cells, which alters the glycation process (18–20).

The analysis of HbA1c variation in relation to anaemia severity and sex revealed a p-value of 0.697, indicating no significant interaction between these variables. This suggests that the effect of anaemia severity on HbA1c levels is consistent across sexes. Furthermore, the correlation study between HbA1c and fructosamine, a marker of glycaemic control unaffected by anaemia, demonstrated a statistically significant positive linear relationship in individuals with moderate anaemia ($p=0.39$, $p=0.007$) and mild anaemia ($p=0.38$, $p=0.017$). However, this correlation was not present in the severe anaemia group ($p=0.503$).

These findings suggest that severe anaemia may influence the reliability of HbA1c as a marker of glycaemic status. The weaker association observed between HbA1c and fructosamine in the severe anaemia subgroup may indicate that HbA1c does not fully reflect glycaemic exposure in these patients. Nevertheless, given the limited number of subjects with severe anaemia, these results should be interpreted with caution. Our findings are in agreement with those reported by Bhargava et al (20).

Other studies have demonstrated that anaemia significantly affects both mild and severe anaemia groups (21). It is important to acknowledge that the absence of correlation in our severe anaemia subgroup may be attributed to the limited sample size ($N=14$), which constrains the statistical power of the analysis. Further research with larger sample sizes is necessary to validate these findings.

The study also identified a correlation in the moderate and mild anaemia subgroups, indicating the potential utility of HbA1c within the hemoglobin range. However, this correlation is moderate, and the results should be

interpreted with caution. Any discrepancies between HbA1c and other glycaemic control markers (fasting glucose, self-monitoring, CGM) should prompt clinicians and medical biologists to investigate potential sources of variability.

Our exploratory findings suggest that severe anaemia, defined as total haemoglobin (THb) levels below 9 g/dL in men and below 8 g/dL in women, may be linked to a decreased concordance between HbA1c and fructosamine measurements. Nonetheless, this observation necessitates validation in larger cohorts to determine any clinically relevant threshold.

Consequently, caution is warranted when interpreting HbA1c levels in these clinical ranges, and its use for the diagnosis or monitoring of diabetes should be carefully evaluated in light of alternative methods. In such instances, measuring fructosamine presents an interesting alternative for assessing glycaemic control until anaemia is corrected. However, fructosamine has its own limitations: it reflects glycaemic control over a shorter period (2-3 weeks) and can be influenced by conditions affecting protein metabolism, such as nephrotic syndrome, liver cirrhosis, or thyroid disorders (22).

These findings provide preliminary insights into the relationship between severe anaemia and glycated hemoglobin. However, their interpretation requires caution due to the specific clinical context of this study. The analysis was limited to non-diabetic patients, which may restrict the external validity of the results and precludes direct extrapolation to diabetic populations. As a result, the observed associations may not fully capture the complexity of HbA1c interpretation in patients with altered glucose metabolism. Further well-designed studies, including diabetic patients, are necessary to elucidate the impact of iron deficiency anaemia on HbA1c within a broader clinical context.

CONCLUSION

These findings indicate that although glycated haemoglobin (HbA1c) remains the standard marker for assessing long-term glycaemic control, its interpretation may be more challenging in patients with severe iron deficiency anaemia (total Hb <9 g/dL in men and <8 g/dL in women).

In such cases, HbA1c values may not accurately reflect glycaemic status and should therefore be interpreted with caution. The use of complementary or alternative glycaemic markers, such as fructosamine, may be helpful in selected patients until the anaemia is corrected.

However, given the limited sample size of the severe anaemia subgroup in the present study, these findings should be considered preliminary and require confirmation in larger populations before definitive conclusions can be drawn.

Further research is needed to better elucidate the mechanisms underlying this interference and to identify the most reliable biomarkers of glycaemic status in patients with severe iron deficiency anaemia.

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