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Impact of Anemia on the Determination of Glycated Haemoglobin by High-Performance Liquid Chromatography in Diabetic Patients

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Abstract **Original Research Article**

Introduction: Glycated haemoglobin is considered an objective element in monitoring glycaemic control in diabetic patients. High-performance liquid chromatography (HPLC) is the reference method for measuring HbA1c. However, even this method can be affected by certain conditions such as anemia. ***Purpose***: To study the impact of anaemia on the measurement of glycated haemoglobin levels by HPLC in diabetic patients. ***Methodology***: We conducted a prospective, descriptive and comprehensive study from November 2022 to April 2023, on 06 months at the Mali Hospital, measuring glycated haemoglobin, blood glucose and haematological parameters (CBC). ***Results***: We included 71 diabetic patients, 42.3% were men and 57.7% women. In our study population, 33.8% were anaemic, among them 16.9% were women and 16.9% men. The age group [50-80] years constituted the majority (n = 14) of anaemic patients. The most common type of anaemia in our diabetic patients was normocytic anaemia. HbA1c >7% was found in 91.6% (n=22) of our anaemic patients. The mean haemoglobin level was not statistically significantly different from the HbA1c level (P = 0.0595). There was a statistically significant difference between MCHC and HbA1c (P = 0.04). To our knowledge, we did not observe any significant impact of anaemia on the measurement of HbA1c by the HPLC method. ***Conclusion***: Our study showed a high frequency of normocytic anaemia in diabetic patients. We also noted that the reliability of HPLC in measuring HbA1c was not affected by a minor decrease in total haemoglobin levels.

Keywords: Glycated haemoglobin, diabetes, anaemia, high performance liquid chromatography (HPLC), Mali Hospital.

1. INTRODUCTION

In the organism, proteins are frequently glycated during various enzymatic reactions when conditions are physiologically favourable. Haemoglobin is obviously not immune to this phenomenon. Glycation of haemoglobin occurs via a non-enzymatic reaction between glucose and the N-terminus of its β-chain, forming a Schiff base. During rearrangement, the Schiff base is converted into Amadori products, the best known of which is glycated haemoglobin (HbA1c) [1].

HbA1c is considered to be an objective component of glycaemic control in diabetic patients. This parameter characterises any non-enzymatic binding of glucose to haemoglobin [2].

The cardiovascular complications of diabetes are due to the high levels of HbA1c. It has been clearly established that a 1% increase in baseline HbA1c (6.5%) increases the risk of a cardiovascular event by 15-20% [3].

Diabetes is a heterogeneous group of metabolic diseases characterised by chronic hyperglycaemia resulting from a defect in insulin secretion and/or action,

responsible in the long term for the occurrence of micro and macroangiopathic complications [4].

In 2019, the International Diabetes Federation (IDF) stated that 463 million adults were living with diabetes and estimated that this figure would rise to 700 million by 2045 if the necessary efforts were made. It described the phenomenon as a veritable pandemic because of its considerable progression [5].

Uncontrolled diabetes leads to metabolic disturbances, with cardiovascular complications and the risk of premature death. Possible complications include high blood pressure (HTA), stroke, loss of vision, leg amputation and kidney failure [6]. In fact, almost half of all people living with diabetes develop kidney disease during their lifetime [7].

Diabetic kidney disease particularly affects haemoglobin balance. Erythropoietin (EPO) deficiency has also been observed in anaemic type 1 diabetic patients with severe symptomatic diabetic autonomic neuropathy [8]. However, the prevalence of anaemia in diabetic patients is difficult to estimate. A recent study of a cohort of over 4000 diabetic patients reported a prevalence of 25%, similar to that observed in other studies [9].

The measurement of glycated haemoglobin, and principally its major fraction (HbA1c), is an attractive tool in the management of diabetic patients. It provides an overall assessment of glycaemic control over the last 8 to 12 weeks. However, this tool should be used with caution.

There are various methods for measuring glycated haemoglobin, which can be divided into two groups. On one side, methods specifically measuring the HbA1c fraction, among which we have, chromatographic methods using cation exchange (minicolones or High Performance Liquid Chromatography (HPLC); electrophoresis and immunological methods using specific antibodies. On the other sidé methods based on the affinitý of glycated haemoglobin for boronate, where the totalitý of glycated haemoglobin is taken into account. This method is currently no longer used [10, 11].

These methods can be undermined by interferences such as the carbamylation of haemoglobin that occurs in patients with kidney failure, the presence of a variant of haemoglobin, labile HbA1c (formed by the unstable binding of a glucose to the N-terminal valine of one or both of the globin chains of haemoglobin), and any drop in total haemoglobin levels [12].

The sensibility of different HbA1c assay techniques to a decrease in a patient's haemoglobin level may vary. Immunological methods, particularly those used in tests available in clinical practice, may be more sensitive to changes in haemoglobin levels than some of the more specialised techniques, such as high-performance liquid chromatography (HPLC), which remains the gold standard of assay [13]. However, even this method can be affected by certain conditions such as anaemia [14].

With this aim, we propose to study the impact of anaemia on the determination of glycated haemoglobin by high-performance liquid chromatography in diabetic patients at Mali Hospital.

2. METHODOLOGY

The study was conducted in the medical biology and anatomopathology laboratory of the Mali Hospital. We conducted a prospective, descriptive and exhaustive study from November 2022 to April 2023. Our study concerned type 1 or 2 diabetic patients followed up on an outpatient basis at Mali Hospital or from various health facilities and who had undergone diabetes monitoring (fasting glycaemia and HBA1c) and blood counts during the study period.

The following variables were determined:

- Nominal qualitative variables: gender, diabetes follow-up.

Diabetes follow-up was determined on the basis of the frequency of patient consultations with a healthcare professional.

Diabetes follow-up: Yes Frequency of consultation > 1 time/quarter
No: Frequency of consultation < 1 time/quarter

- Ordinal qualitative variables: type of diabetes, type of anaemia, physical inactivity.

Quantitative variables:

Weight, age, BMI, height, fasting blood glucose, HbA1c, duration of diabetes, mean corpuscular volume, haematocrit, reticulocyte count, haemoglobin count, mean corpuscular haemoglobin concentration.

The mean corpuscular volume was used to classify the type of anaemia. We have therefore distinguished:

- Microcytic anaemia: VGM< 80 fl
- Normocytic anaemia: 80fl <MV<100 fl
- Macrocytic anaemia: VGM > 100 fl

The mechanism by which the anaemia is compensated by the production of new cells was also considered, and this was determined by the reticulocyte count:

- Regenerative anaemia: Reticulocyte count > 2%.
- Anegenerative anaemia: reticulocyte count < 2%.

Weight and height measurements were used to calculate the body mass index to determine whether the

patient was underweight, overweight, moderately obese or morbidly obese. The following formula was used to calculate BMI:
BMI=Weight/[(Height)]^2
BMI is expressed in (Kg/m2), with weight in "Kg" and height in "m".

Calculating BMI has enabled us to categorise body mass using the following common values:

- < 18, 5 Kg/m2: Undernutrition
- 18, 5 - 24, 99 Kg/m2: Normal weight
- 25 - 29, 99 Kg/m2: Overweight
- 30 - 34,99 Kg/m2: Moderate obesity
- 35 - 39,9 Kg/m2: Severe obesity
- ≥ 40 Kg/m2: Morbid obesity

The duration of the diabetes was determined according to the time of diagnosis, and was classified using the following ranges:

- < 5 years
- [5 - 10 [years
- [10-20 [years
- [20-40 [years
- ≥ 40 years

The HbA1c level was used to determine diabetic control:

- Balanced diabetes: HbA1c < 7%.
- Unbalanced diabetes: HbA1c > 7%.

Verbal, free and informed consent was obtained from patients prior to their inclusion in the study. The information provided by each patient was kept completely confidential and was not divulged. It was used exclusively for research purposes. Each patient's personal information was coded with a number that could not be used to identify the patient when the study results were published. Good medical practice, the dissemination of results and patient dignity were respected.

The data collected was analysed and processed using SPSS version 25.0 software. The graphs were produced using Excel 2016 and the data were entered using Word 2019. The significance threshold for all statistical tests was set at $P < 0.05$.

3. RESULTS

During the study period, 71 patients satisfying the inclusion criteria were registered. The majority were women (57.7%) and men (42.3%). The sex ratio was 0.73. The average age of our patients was 52±14.2 years, with extremes of 13 and 77 years; the [50-80] age group was the most represented, with 60.6%.

Type 2 diabetes accounted for 93% of cases. Almost half of the study population (49.3%) had diabetes for less than 5 years.

Fasting blood glucose levels were high in 76% of our patients.

Obesity accounted for 23.9% of cardiovascular risk factors, as did a sedentary lifestyle in 23.9% of our patients. Physical inactivity was observed in 28% of our patients.

About 70% of our patients were being followed by a healthcare professional. Only 7% had controlled diabetes.

The type of treatment most frequently used in our study was a combination of ADO and MHD (40.8%).

The mean glycated haemoglobin level was 10.64±3.07, with extremes of 4% and 20% (see Table I). Poor glycaemic control was associated with fasting blood glucose >6 mmol/l in 50 patients in our sample. There was a statistically significant correlation (P=0.004) between fasting plasma glucose and glycated haemoglobin (see Table II).

Table I: Statistical parameters of glycated haemoglobin level (HbA1c)

Number	71
Mean value	10.64
Mode	8.00
Standard deviation	3.07
Minimum	4.00
Maximum	20.00

Table II: Distribution of diabetic patients according to glycated haemoglobin and fasting blood glucose levels

	Glycated haemoglobin			***p***
Fasting blood glucose level (mmol/l)	**< 7 %**	**>7 %**	**Total**	
< 3,9	0	6	6	
3,9-6	1	10	11	
> 6	3	**50**	53	**0,004**
Total	**4**	**66**	**70**	

In the [50-80] age group, there were 40 patients with glycaemic imbalance.

There was no statistically significant correlation (P=0.692) between fasting plasma glucose and glycated haemoglobin.

Fasting blood glucose >6 mmol/l was found in 29 overweight/obese patients.

There was no statistically significant difference between body mass index and fasting plasma glucose P= 0.404.

The mean haemoglobin level in our sample was 12.95±2.4 with extremes of 6.40 g/dl and 20.90 g/dl. (See Table III). The prevalence of anaemia was 33.8% in our diabetic patients. The proportion of anaemic patients was 16.9% in 41 female subjects and 16.9% in 30 male subjects. Anemia was predominant (n=14) in the [50-80] age group.

Table III: Statistical parameters for haemoglobin levels

Number	**71**
Mean	12,9549
Standard deviation	2,45623
Variance	6,033
Minimum	6,40
Maximum	20,90

There was no statistically significant difference between haemoglobin level and age P=0.323. Normocytic anaemia was the most common type of anaemia in our sample, accounting for 22.53%. The mean GMV was 85.3±13.1 fl with extremes of 11.4 fl and 99.5 fl. The mean MCHF was 333.5±17.88 g/dl with extremes of 221 g/dl and 360 g/dl. An increase in the reticulocyte count was observed in 36% of our patients.

HbA1c > 7% was found in 91.6% of our anaemic patients. The mean haemoglobin level showed no statistically significant difference from the HbA1c level (P>0.05) (See Table IV).

Table IV: Distribution of diabetic patients according to haemoglobin level and glycated haemoglobin level (HbA1c)

Haemoglobin level (g/dl)				
HbA_{1c} (%)	**Number**	**Mean± Standard deviation**	**Min-Max**	***p***
<7	5	13,00±0,14	12,9-13,1	**0,0595**
>7	66	12,95±2,49	6,4-20,9	
		X^2=3,5504		

There was no statistically significant difference between GMV and HbA1c (P>0.05).

There was a statistically significant difference between the HLCM and HbA1c (P<0.05).

4. DISCUSSION

The aim of our study was to determine the impact of anaemia on glycated haemoglobin determination in diabetic patients. The study was conducted in the medical biology laboratory of the Mali Hospital for a period of 6 months, from November 2022 to April 2023. We included a total of 71 type I and type II diabetic patients, including 24 with anaemia and 47 patients with normal haemoglobin levels:

Difficulties:

- Lack of reagents: the often prolonged absence of the Mindray H50p reagent was a major obstacle to the progress of our study. This reagent is eluent B (phosphate buffer), which is essential for stabilising the pH during sample analysis.

Limitations:

- Sample size,
- Low proportion of anaemic patients: the size of our sample did not allow for sufficient representation of anaemic patients. The non-inclusion of hospitalised patients in this study also contributed to this.

Despite these limitations and difficulties, the results obtained enabled us to open a discussion with the data in the literature.

The majority of patients in our study were female (57.7%) compared with 42.3% male (sex ratio 0.73).

This result reflects the population as a whole, compared with the results of A. Keita, who obtained 55% women and 45% men, with a sex ratio of 0.81 [15]. This female predominance could be explained by the fact that women are more obese and sedentary than men in our society. This is corroborated by B. Traoré *et al.*, who obtained a female representativeness of 72.20% during their study on the "epidemiological and clinical aspects of obesity in the medicine and endocrinology department of the Mali hospital" [16], as well as by J. Matta *et al.*, 2018 who report a worldwide prevalence of overweight and obesitý of 36.9% for men and 38% for women [17].

The average age of our patients was 52±14.2 years with extremes of 13 and 77 years; the [50-80] age group was the most represented with 60.6%. This high frequency can be explained by the predominance of

T2DM, which is considered to be the diabetes of the second age.

In the United States, the age of diagnosis of T2DM is 51.5 years (50.8 years for men and 51.3 years for women) [18]. The predominance of this age group has also been observed in other studies reporting approximately the same frequency, 84.5% for patients aged 40-80 years [19]. These values are similar to those of the study conducted by Sangaré *et al.*, where the mean age was 53.26±9.69 years [20]. They were also consistent with those of Diao A. and Kahina *et al.*, whose studies reported mean ages of 54.82±11.93 years [21] and 54.59±4.87 years [22]. These different values reflect a disturbed metabolic activity under the effect of risk factors for the disease and can be explained by the fact that the prevalence and incidence of T2DM increase sharply in both sexes and from the age of 40 onwards [23].

The majority of our patients were type 2 diabetics (93%). This observation is also reported in the literature, which estimates that approximately 90% of diabetics are type 2 [26]. Our results are similar to those of Coulibaly I., who found that 94% of patients had T2DM [24].

The duration of diabetes was less than 5 years for 49.3% of our patients and between 10 and 20 years for 21.1% of them. This result is similar to that of F. Ousmane, who reported 47.69% of patients with diabetes for less than 5 years and 21.54% of patients with diabetes for 10 to 20 years [25].

These results are also comparable to those of K-F. Kamissoko, where 56.25% of patients had been diagnosed for less than 5 years, compared with 22.5% who had been living with diabetes for more than 10 years [19].

Fasting blood glucose levels were high in 76% of our patients. This high rate of hyperglycaemia is justified by the poor glycaemic control found in 93% of our patients, with an average glycated haemoglobin level of 10.64±3.07 and extremes of 4% and 20%.

We noted that glycated haemoglobin levels changed in proportion to fasting blood glucose levels. We obtained a statistically significant correlation P=0.004 between fasting blood glucose and glycated haemoglobin.

This correlation between fasting plasma glucose and HbA1c is clearly reported in the literature, by A. Bissan *et al.*, also by H. Kouame *et al.*, [26, 27].

There was no statistically significant difference between fasting plasma glucose and BMI in our sample (P=0.404). However, Inserm describes obesity as a cause of hyperglycaemia [28].

Physical activity was practised habitually by 35.5% of our patients. Smoking accounted for only 2.8% of our sample, and alcohol consumption for 5.6%.

These low values can be explained by the high proportion of women in our study, smoking being mainly found among males in our society [86]. This observation was reported by I. Coulibaly, who found only one female patient who smoked during his study of cardiovascular risk factors in diabetic patients in Bamako [24].

A sedentary lifestyle and obesity were the most common risk factors, with 23.9% each found in the study population.

This balance between obesity and physical inactivity could be explained by the undeniable link between these two factors and their strong involvement in the onset of type 2 diabetes [30].

On the other hand, a sedentary lifestyle could explain the importance of exercise in maintaining endothelial function. Regular physical activity has been shown to normalise lipid marker levels and reduce factors linked to inflammation, oxidative stress and endothelial dysfunction [31, 32].

Good diabetes control was noted in 7% of our patients. At the time of the survey, 70% of the patients in the sample were being monitored regularly by a healthcare professional.

Compared with the results of Keïta A, who obtained 15% of patients with good diabetic control for 65% of regular follow-up, glycaemic imbalance was more marked in our sample. This difference could be linked to the predominance of T1DM (57.5%) in his study population, which was mainly composed of patients aged 16-25 years (31.3%); but also to the low rate of obesity (8.8%) observed during his study [15].

The type of treatment most frequently encountered in our study was a combination of ADO and MHD (40.8%). This is clearly explained by the high proportion of T2DM in our sample [33].

The combination of insulin, OADs and MHD was used by 22.5% of our patients. This result is due to the frequent use of insulin in the therapeutic strategy for T2DM. It is estimated that at the time of diagnosis of type 2 diabetes, insulin secretion is reduced by 50% and that six to eight years later, this deficit reaches 75%. For the majority of patients, the progressive nature of diabetes means that, sooner or later, they will require insulin treatment to maintain glycaemic control within defined targets [34].

In the [50-80] age group, about 56% of patients had an HbA1c > 7%. The prevalence of T2DM increases

sharply with age. It is estimated that 10-15% of people over the age of 65 have T2DM [35].

This observation is supported by a study conducted in China by Yang Y. *et al.*, who described the evolution of age itself as a factor favouring the rise in HbA1c [36]. Nevertheless, we found no statistically significant difference between glycated haemoglobin levels and age P=0.692. These results are similar to those of Samaké N. who concluded that there was no significant difference between age and glycated haemoglobin with P=0.093 [37].

In our study, the MCHF and HbA1c levels changed proportionally (P=0.04). This result is comparable to that of Zong-Hui Guo *et al.*, who stated following their study that the HGLC was significantly correlated with HbA1c [38].

In our study, the prevalence of anaemia in diabetic patients was 33.8% with an mean of 10.49±2.05g/dl and a perfect balance of 16.9% between the male and female populations. This is comparable with the results of Kehailou and colleagues who obtained a prevalence of anaemia 20.44% lower than ours but with a similar mean Hb level of 10.67 ± 2.02 g/dl [39].

The prevalence of anaemia in our study sample was higher than that found in the literature, which is close to 30% in the general population (89), despite the low representativeness of anaemic patients. Our results are similar to those obtained in Ethiopia by A. Bekele *et al.*, 2019, who revealed that 34.2% of T2DM patients also suffered from anaemia [40].

These results are consistent with those reported in the literature, where several studies based on small numbers of patients with overt kidney disease have suggested that the prevalence of anaemia is higher in diabetic patients. The first large-scale systematic survey assessing the prevalence and predictors of anaemia (a cross-sectional survey of 820 patients) in diabetic patients without nephropathy reported that a total of 190 patients (23%) had unrecognised anaemia. This prevalence was two to three times higher than in the general population [8].

According to Ronald *et al.*, the prevalence of anaemia is 35.5% among diabetics in Africa [41].

Although anaemia is common in people with diabetes, it is often of moderate severity [42]. However, an NGSP study mentions severe anaemia and major blood loss as interferences that can affect the results of HbA1c testing [43].

The presence of anaemia was associated with an HbA1c level > 7% in almost 91.6% of our patients, as described in the literature by Wenjia G. *et al.*, for cases of iron deficiency anaemia in diabetic patients [44].

In a study in Morocco, Kehailou *et al.*, observed a prevalence of anaemia of 78.86% in diabetics with a high HbA1c level [39].

The type of anaemia found most frequently in our study was normocytic anaemia, with a percentage of 22.53%. Microcytic anaemia was the least represented in our study with a percentage of 11.27%. Our results are comparable to those of I. Coulibaly and F. Ousmane (81, 82) who obtained 33.9% and 54.69% for normocytic anaemia compared with 1.9% and 29.68% for microcytic anaemia.

Iron deficiency anaemia is a condition frequently associated with falsely elevated HbA1c. Studies in patients with and without diabetes have shown that treatment of iron deficiency anaemia lowers HbA1c [45].

Other conditions leading to reduced red blood cell turnover are also associated with falsely elevated HbA1c, including vitamin B-12 and folate deficiency anaemias [46, 47].

However, chronic hyperglycaemia associated with high HbA1c levels also has an impact on inflammation and can modify the haematological parameters of diabetes as described by S. Antwi-Baffur *et al.*, [48]. Haemoglobin functions such as O2 supply, tissue perfusion and HbO2 dissociation are therefore affected by the non-enzymatic glycation of proteins associated with chronic hyperglycaemia [49].

However, our results differ from those obtained by A. Zinebi *et al.*, in their general population study on the aetiological profiles of anaemia in internal medicine, with microcytic anaemia coming out on top (56% of anaemias found), followed by macrocytic anaemia (23%) and then normocytic anaemia, which was the least represented (21%) [50].

We did not find a statistically significant difference between haemoglobin and glycated haemoglobin levels (P=0.0595), compared with the study conducted by N. Samaké which found a significant correlation with P=0.01264 (94).

In line with the results reported by Patel *et al.*, anaemia was predominant in patients aged between 50 and 80 years [51]. However, there was no statistically significant difference between age and haemoglobin level (P=0.323).

5. CONCLUSIONS

The aim of this study was to explore the impact of anaemia on the determination of glycated haemoglobin by HPLC in diabetic patients at the hospital in Mali. As HPLC is the reference method for measuring HbA1c, its reliability was relatively unaffected by the

slight fall in haemoglobin levels found in our study population. A more detailed study would be needed to establish a reference value below which the reliability of HbA1c would be called into question in diabetic patients presenting with anaemia, which would make it possible to alert healthcare professionals and ensure better management of diabetic patients.

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