CAN FRUCTOSAMINE BE A SURROGATE FOR HbA\textsubscript{1c} IN EVALUATING THE ACHIEVEMENT OF THERAPEUTIC GOALS IN DIABETES?

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SUMMARY - Objective: To determine if fructosamine can be used as a surrogate for HbA\textsubscript{1c} to monitor whether therapeutic goals in diabetes mellitus are achieved when HbA\textsubscript{1c} cannot be used for this purpose (hemoglobinopathies, anemia...).

Material and methods: Blood samples of 76 diabetic patients and 30 healthy subjects characterized by the absence of any risk of interference in the interpretations of HbA\textsubscript{1c} and fructosamine were studied in order to, first, deduce from the correlation a prediction of HbA\textsubscript{1c} from the fructosamine values, second, to evaluate the predictive value of such predicted HbA\textsubscript{1c} in the determination of poor metabolic control as defined by UKPDS and DCCT studies.

Results: The correlation between predicted HbA\textsubscript{1c} and actual fructosamine was fair ($r = 0.88$) in diabetic patients but not in control subjects ($r = 0.01$). It was therefore only possible to estimate HbA\textsubscript{1c} from fructosamine in diabetic patients. Nevertheless, the range of positive and negative predictive values of estimated HbA\textsubscript{1c} to detect a poor metabolic control defined by two thresholds of HbA\textsubscript{1c} (7%, 7.5%) was 91-93% and 86-87%, respectively. Then, even in this highly selected population, the risk of misclassification was around 10% when fructosamine was used to estimate HbA\textsubscript{1c}. These results were unchanged when fructosamine was corrected by plasma protein level.

Conclusions: This study shows the limitations to use fructosamine in place of HbA\textsubscript{1c} to evaluate the efficacy of antidiabetic treatments, even in a selected population.

Key-words: glycated haemoglobin, fructosamine, blood glucose control.

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FRUCTOSAMINE VS HbA1c IN DIABETES

HbA1c is considered as the best marker of metabolic control in diabetes mellitus [1, 2]. The measurement of the proportion of hemoglobin which has been glycated on the N-terminal of the β chain, is an evaluation of the quality of the glucose control during the last 2-3 months [3, 4]. This marker has been used in two large intervention trials, i.e., the DCCT [5] and the UKPDS [6] which have determined optimal therapeutic and optimal therapeutic goals in Type 1 and Type 2 diabetes, respectively, in order to prevent the occurrence of vascular complications. These threshold values are now accepted worldwide and are used as guideline for therapeutic strategy. Indeed, non-achievement of these objectives leads to an intensification of the treatment and more constraints for the patients. However, there are some situations in which the dosage of HbA1c looses any significance in terms of glucose control evaluation because either the presence of abnormal hemoglobin interfering with the HbA1c measurement or a shortening of erythrocyte lifespan. These situations are mainly represented by hemoglobinopathies, anemias, chronic hemolysis, liver cirrhosis or end stage renal failure [7-10]. Even if the number of concerned diabetic patients is rather low (for instance, the proportion of diabetic subjects with confirmed hemoglobinopathy in our department only represents 2.3%), it is obvious that it is sometimes difficult to evaluate the quality of metabolic control in such patients. The dosage of plasma fructosamine offers an alternative mean for this purpose. This marker corresponds to the measurement of the intensity of the glycation of circulating proteins mainly represented by albumin [11]. It is representative of a shorter period of time, around 2 to 3 weeks [12-14]. However, plasma fructosamine was not a marker used in the two gold standard studies, DCCT and UKPDS and thus the references similar to that determined for HbA1c are lacking. The objectives of the present study was the evaluation of the reliability of estimated HbA1c value deduced from plasma fructosamine measurement in determining the quality of the metabolic control. If this approach is relevant, it could be chosen when HbA1c cannot be used. For this purpose, the relationships between HbA1c and plasma fructosamine were studied in a population selected on the absence of any bias which could interfere with either the dosage or the interpretation of these parameters. All the subjects were devoid of any known situation interfering with either HbA1c production, i.e., hemoglobinopathy, anemia, renal insufficiency, pregnancy [7-10] or plasma fructosamine, i.e., proteinuria, dysproteinemia, denutrition, dysthyroidism, hepatopathy, pregnancy [15-18].

The present study estimates the possibility to substitute HbA1c by fructosamine when HbA1c is not available.

## PATIENTS AND METHODS

**Patients**

Blood samples of 76 diabetic patients attending the diabetic clinic of our hospital and 30 non-diabetic controls (workers in the laboratory) were used for the study of the relationships between HbA1c and plasma fructosamine. The clinical characteristics of these populations are summarized on Table I. The diabetic population was composed of a first group of 53 patients (30 men and 23 women) treated with insulin (27 with a type 1 diabetes, 20 with type 2 diabetes and secondary oral hypoglycemic agent failure, and 6 with diabetes secondary to alcoholic chronic pancreatitis) and a second one composed of 23 patients (11 men, 22 women) with type 2 diabetes treated by diet and/or oral agents [19]. Patients with renal failure (plasma creatinine ≥ 110 µmol/l, urea ≥ 10 mmol/l), macroproteinuria (albustix® positive), dysglobulinemia or hypoprotidemia (plasma protein level below the range 61-81 g/l), hepatopathy (ASAT and ALAT ≥ 40 U/l) or dysthyroidism (TSH ≥ 4 mU/l) were excluded. No subject exhibited anemia (hemoglobin higher than 10 g/dl) or hemoglobinopathy. Pregnant subjects were excluded. In this selected population, 11 patients (9 treated with insulin, 2 receiving oral agents) were chronic consumers of aspirin (100 to 350 mg/day) for vascular protection.

**Biological analysis**

Fasting blood samples were collected by venous puncture into vacutainer tubes (Becton Dickinson Vacutainers systems, Oxford, UK). HbA1c and total hemoglobin were measured in EDTA samples. Each HbA1c measurement was accompanied by a simultaneous measurement of plasma fructosamine on the same samples.

Serum total proteins, urea, creatinine, ASAT and ALAT, and TSH also assayed using conventional methods.

HbA1c was measured by ion-exchange HPLC (High Precision Liquid Chromatography) technics on an Eurogenetics-Tosoh A1C2.2 automated analyser (Eurogenetics, Saran, France) and expressed as percentage of total hemoglobin. This method is certified by the National Glycohemoglobin Standardization Program (NGSP) and is traceable to the DCCT (reference range 4.5 - 5.7%). Intra-assay variation coefficient was 1.54% for a value of HbA1c of 5.12% and 0.75% for a value of HbA1c of 9.03%. Inter-day variability was also evaluated using internal control hemolysates at two concentrations (lyphocheck 1 and 2, Biorad, Hemel Hempstead, UK) by 30 subsequent daily measurements. The intra-batch coefficients of variation were 2.05% for an HbA1c of 5.5% and 1.10% for an HbA1c value of 9.87%.
Plasma fructosamine was determined by measuring the change in absorbance resulting from the reduction of nitroblue tetrazolium on a Hitachi 911 random access analyser (Roche-Boehringer, Mannheim, Germany). The results were expressed in micromol/liter (reference range 175-275 µmol/l). The intra-assay variation coefficients were 0.5% at a fructosamine of 244 µmol/l and 1.6% at 269 µmol/l. Inter-day variation was studied as previously described using control solution N (Roche-Boehringer, Mannheim, Germany). The variation coefficient value was 1.86% at a fructosamine of 269 µmol/l.

Statistical analysis

Relation between fructosamine and glycated hemoglobin was obtained from linear regression analysis. Predicted HbA1c values derived from fructosamine measurement were calculated using a regression straight-line equation. Differences between predicted and measured glycated hemoglobin were plotted as proposed by Bland and Altman [20] and tested by Student’s t-test and sign test.

All calculations were performed using SPSS® on a personal computer.

RESULTS

There was a statistically significant correlation between HbA1c and fructosamine levels in diabetic patients ($r = 0.88; p < 0.01$) (Fig. 1). Diverging results were obtained in controls, since no correlation was observed between the two parameters ($r = 0.01$). In diabetic subjects, the equation of the regression line was as follows: HbA1c measured = $0.0218 \times \text{fructosamine} + 1.198$. It seems possible from these data to calculate a “predicted HbA1c value” from fructosamine measurement exclusively in diabetic subjects.

But the presence of a significant correlation is not a sufficient condition to allow a switch from one parame
terto another in medical practice. We analyzed the performance of the deduced HbA1c value to predict poor metabolic control, and thus how it would lead the diabetologist to opt for alternating treatment. To define this clinical condition, we used two thresholds proposed in the aftermath of the DCCT and UKPDS trials: 7% [6] for type 2 diabetes and 7.5% [5] for type 1 diabetes. At each level of HbA1c, we evaluated sensitivity and specificity for diagnosing poor metabolic control. If we admit that the proportion of uncontrolled diabetes in our sample is not different from the prevalence in our department, it is possible to calculate positive and negative predictive values for the diagnosis of poor metabolic control. The reference in the matter was the measured HbA1c. The results of this analysis are presented in the Table II. Whatever the chosen threshold, the sensitivity of the predicted HbA1c to define poor metabolic control was rather good (more than 90%). In contrast, the specificity was lower, between 81.8 and 86.7%. It is obvious that the specificity was lowest for the stricter definition of metabolic control (HbA1c ≤ 7%). In other words, in

| TABLE I. Clinical characteristics of patients studied and coefficient of correlation between HbA1c and fructosamine. |
|-------------------------------------------------|-------------|------------|-------------|-------------|---------------|
| **Number** | **Age (Years)** | **Sex (M/F)** | **BMI (Kg/m²)** | **r** |
| Type 1 diabetics | 27 | 33 ± 13 | 15/12 | 24 ± 3.8 | 0.93* |
| Type 2 diabetics non insulin treated | 23 | 59 ± 11 | 11/12 | 29 ± 5.4 | 0.88* |
| Type 2 diabetics Insulin treated | 20 | 66 ± 11 | 9/11 | 30 ± 5.2 | 0.84* |
| Diabetics with Chronic pancreatitis | 6 | 48 ± 4.6 | 6/0 | 21 ± 3.3 | 0.90** |
| Controls | 30 | 41 ± 14 | 9/21 | 23 ± 2.5 | 0.01 (NS) |

* p < 0.01; ** p < 0.05; * expressed as mean ± 1 SD.
the diagnosis of poor metabolic control, the positive predictive value is lower in a range of HbA1c below 7.5%, with a risk of misclassification of around 8.7% of cases. Accordingly, the negative predictive value is lower when a stricter objective of glycaemic control is chosen, with a risk of error of almost 10.5%. This high rate of misclassification renders the switch from fructosamine to predicted HbA1c rather hazardous.

We tested some hypothesis to explain the discrepancies between truly measured HbA1c and HbA1c estimated from fructosamine. First, despite a strict selection of patients, it was possible that the inter-individual variation of plasma protein level could induce bias in the significance of plasma fructosamine level. So, we evaluated the possible gain in correlation between HbA1c and fructosamine when the later parameter had been corrected by the plasma protein level. There was no improvement of the correlation coefficient (0.88). The sensitivity, specificity and predictive values were also unchanged (result not shown). Second, we wondered whether the poor predictive value of predicted HbA1c depends on the quality of the metabolic control itself and we expressed the differences “measured HbA1c – calculated HbA1c” as a function of the measured HbA1c level (Fig. 2) according to the method described by Bland [20]. In the whole population, predicted HbA1c did not either under- or over-evaluate true HbA1c. Indeed, the sum of positive and negative differences (0.0115) was not significantly different from zero (Student’/t test: p = 0.92). But, as shown in the Figure 2, there was a trend to an association between the two parameters. More precisely, in the patients with a HbA1c below 6%, all the differences were negative (over estimation of true HbA1c) and in those with a HbA1c above 11%, all the differences were positive (under-estimation of true HbA1c). Between these limits, differences were randomly distributed on both sides of the line of zero. The 95% confidence interval has shown four patients with difference between measured and calculated HbA1c > 2 SD and one patient with difference < – 2 SD. Clinical history had allowed for explaining this difference in two cases by important and recent variations of plasma glucose values leading to more drastic changes of fructosamine than HbA1c. In three cases, we did not find any explanation. Third, it is also possible that poor agreement between HbA1c and fructosamine was due to differences in half-life kinetics of these glycated parameters. In this context, type 2 diabetics, especially if treated by oral hypoglycaemic agents, in which a more stable metabolic status is usually observed with time, could be amenable to show a good correlation between these parameters. It was not what we observed, as the correlation coefficients did not differ whatever type of diabetes or treatment; type 1 diabetes: 0.93, type 2 diabetes: 0.88, diabetes secondary to chronic pancreatitis: 0.90, patients treated with insulin: 0.88, patients treated with oral hypoglycaemic agents: 0.88. Finally, the role of chronic administration of aspirin was excluded as there was not major difference between estimation and measured HbA1c in the eleven patients chronically treated by this drug.

**DISCUSSION**

This study confirms that the estimation of HbA1c level based on plasma fructosamine measurement is not reliable enough to accurately evaluate the quality of metabolic control of diabetic subjects in accordance with the references from the DCCT [5] and UKPDS [6] studies. This disappointing conclusion is paradoxically raised despite the existence of a good correlation between the two parameters in our diabetic population. Indeed, the correlation coefficient between these two markers was 0.88, which is at the upper part of the range (0.39 to 0.86) reported by similar studies in the literature [21-24]. The specially good concordance in the present study is likely due to both the quality of

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**Table II.** Sensitivity, specificity, positive and negative predictive value of calculated HbA1c to predict a poor metabolic control as a function of different thresholds of HbA1c proposed in the literature.

<table>
<thead>
<tr>
<th>HbA1c</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.0 %</td>
<td>94.4</td>
<td>81.8</td>
<td>92.7</td>
<td>85.7</td>
</tr>
<tr>
<td>7.5 %</td>
<td>91.3</td>
<td>86.7</td>
<td>91.3</td>
<td>86.7</td>
</tr>
</tbody>
</table>

**Fig. 2.** Correlation between the difference between measured HbA1c and calculated HbA1c from fructosamine versus HbA1c. Vertical lines delineate two areas in which HbA1c is either over- or underestimated by determination of fructosamine. Horizontal dotted lines represent mean and the standard deviation of the differences.
the assays used and the strict selection of the subjects. However, it is worthy to note that the correlation between HbA1c and fructosamine was no longer observed when a non-diabetic population was studied. This finding suggests that the agreement between the two markers of metabolic efficacy of the anti-diabetic treatment could be very poor in patients with a metabolic control closed to the normal. These results in normal subjects have to be linked to the observation of poor intra-individual reproducibility of HbA1c values in the normal population of the Telecom Study [25]. This low reproducibility of HbA1c in non-diabetic subjects has been related to subtle variations in erythrocyte lifespan and/or glycation processes from one generation of cells to another [26].

Despite the correlation observed in the diabetic patients, the predictive value of “estimated HbA1c” to detect poor metabolic control according to the references published in the scientific literature [26, 27] is not so good. Indeed, the prediction leads to a risk of misclassification in around 10% of cases. This finding is close to that previously reported in published series [23, 26] even if the error rate is rather lower in the present study than in others. The originality of our study was the patients selection criteria aimed at excluding any bias in interpretation of both fructosamine and HbA1c, and the analysis as a function of references-values for acceptable and unacceptable metabolic control. In the previous similar series the population was only selected on the stability of diabetes and the therapeutic goals was based on either practitioner’s feelings [23] or arbitrary values of HbA1c [26, 28]. One can assume that this approach when used in routine may lead to a higher risk of misclassification and to inappropriate therapeutic consequences. The errors cannot be minimized by using a correction factor as in the range of HbA1c, corresponding to therapeutic goals “estimated HbA1c” randomly under- or over-estimated the true HbA1c. However, over-estimation was constant for HbA1c values below 6% and under-estimation for values above 11%. There are many possible explanations for the rather poor concordance between HbA1c and fructosamine values in diabetic patients. The accuracy and the reproducibility of both assays used render unlikely a bias linked to the methodology of analysis. In the present study, every patient has a plasma protein level in the normal range and the correction of plasma fructosamine by this specific factor did not improve the predictive value of estimated HbA1c. Our study can also rule out any possible influence from chronic treatment with aspirin which has been associated with acetylation of hemoglobin susceptible to interfere with HbA1c dosage [29], although it was not confirmed by Weykamp [30]. Finally, the discrepancy in the kinetics of glycation of these two substances secondary to very different life time is likely the main factor in the poor reliability in the deduction of HbA1c from fructosamine. HbA1c reflects the glycaemic environment during the previous 2 to 3 months [3, 4] and fructosamine, only the previous 2 to 3 weeks [12-14] preceding the sample. One can assume that recent hyperglycaemic imbalance should induce a relatively higher increment of fructosamine in comparison to that of HbA1c. Even if we did not observe any difference between type 1 and type 2 diabetes supposed to be more stable, we have to note that in the literature, the lower predictive value of fructosamine for metabolic control, only 54%, was found in a pediatric diabetic population more susceptible to acute hyperglycaemic episodes during infections or holidays [26]. We have previously discussed the intra-individual HbA1c variations specially noted in normal subjects. It is possible that such variation in glycation of plasma proteins may exist and amplify the discrepancy between the two parameters. In order to minimize these temporal interferences, when HbA1c is not feasible, fructosamine dosages should be frequently performed, for example one dosage per month, before changing therapeutic.

In conclusion, it appears from this study performed in a highly selected population of diabetic patients, that prediction of HbA1c from fructosamine value could be a source of misinterpretation of metabolic control in some cases. In the routine situation, the errors may even be more frequent. So HbA1c remains the reliable and validated marker of recent achievement of therapeutic goals in diabetes. When it is impossible to use this marker to evaluate metabolic control whatever the reason, fructosamine could be a surrogate despite the absence of the validation of the limits defining secure and unsecure control. The physicians using this parameter to estimate the true HbA1c value must be aware of the risk of misclassification in term of quality of metabolic control. Nevertheless, this study must be confirmed with a large cohort.

REFERENCES


