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Level of glycation gap in a healthy subject

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Abstract

Introduction. The discordance between glycated hemoglobin (HbA_{1C}) and fructosamine (FA) estimations in the assessment of glycemia is often encountered. A number of mechanisms might explain such discordance, but whether or not they are consistent is uncertain. Nevertheless, the fact that there is a discrepancy in HbA_{1C} and mean blood glucose cannot be ignored in the monitoring of glycemic control. To address the discrepancy between HbA_{1C} and mean blood glucose, Robert Cohen proposed the measurement of glycation gap (GG). Recently, the 'Glycation Gap' (GG) has been defined as the difference between the measured HbA_{1C}. GG has improved the quality of the monitoring of glycemic control, especially for those patients whose HbA_{1C} levels do not truly reflect the mean blood glucose levels.

Objective: The aims of the statistical analyses were to estimate GG values in a healthy subject. The research was conducted among the inhabitants of the Zwierzyniec commune and nearby villages.

Material and methods: The study population consisted of 93 subjects: 63 women and 30 men, between the ages of 18-79. Measurements of HbA_{1C} and FA in the 93 people were used to calculate GG, defined as the difference between measured HbA_{1C} and HbA_{1C} predicted from FA, based on the population regression of HbA_{1C} on FA.

Conclusions: In considering the values GG in the study group, particular significance should be attributed to a progressive increase of GG with advancing age. Elderly people who are at risk of developing diabetes, or who have already developed the disease, may not exhibit the classic symptoms expected. Age-related changes can mean that some symptoms will be masked, or more difficult to spot. It is worth pointing out that HbA_{1C} together with GG must be taken into account in the correct interpretation of the glycation processes.

Key words

fructosamine, glycated hemoglobin, glycation gap

INTRODUCTION

One of the hypotheses attempting to explain the process of aging is the idea of glycation of proteins. Ageing processes can be speeded up because of the accumulation of toxic metabolic products of nonenzymatic glycation [1, 2]. Glycosylation is the reaction between the free aldehydic group of glucose and free amino groups of protein (hemoglobin, albumine, alfa 2 macroglobulin, antithrombin III, erythrocyte enzymes, fibrinogen, ferritin, HDL, LDL, transferin). A labile aldiminic adduct (Schiff base) first, then, through a molecular rearrangement, a stable ketoaminic product, slowly accumulates [3]. Advance glycosylation end products bind to specific macrophage [4] receptors inducing a release of hydrolytic enzymes, cytokines and growth factors able to promote the synthesis of the fundamental substance and acting at the intracellular level, to damage of nucleic acids [2, 3].

Glycated hemoglobin (HbA_{1c}) results from the nonenzymatic concentration-dependent covalent bonding of glucose to hemoglobin within the erythrocytes. Despite the reliability and standardization of glycated HbA_{1c} assays, clinicians still encounter discrepancies between glycated HbA_{1c} results and other assessments of glycemia among their patients [5, 6]. In addition, HbA_{1C} is expressed as a fraction of the total hemoglobin concentration, and there is disagreement as to whether fructosamine values should be corrected for protein concentrations [7]. Nevertheless, changes in the serum protein concentration or half-life may alter the fraction of protein that is glycated, and it is accepted that fructosamine should be measured when the serum albumin concentration is less than 30 g/L [7].

Discordance between HbA_{1C} and fructosamine estimations in the assessment of glycemia is often encountered. A numbers of mechanisms might explain such discordance, but whether or not they are consistent is uncertain [2]. Nevertheless, the fact that there is a discrepancy in HbA_{1C} and mean blood glucose cannot be ignored in monitoring glycemic control. To address the discrepancy between HbA_{1C} and mean blood glucose, Robert Cohen [8, 9] proposed the measurement of the 'Glycation Gap' (GG).

Currently, in the 'Glycation Gap' – the difference between the measured HbA_{1C} and that which would be predicted from another measure of glycemic control – fructosamine (FA) has been proposed as a means of identifying sources of variance in the apparent risk [9, 10].The Glycation Gap could be affected by the production and disappearance rate of glycated hemoglobin, glycated serum proteins, or both [9].

The most recent study by Rodriguez-Segade [11] concluded that GG and glycated serum proteins as measures of nonglycemic and glycemic determinates of glycation, respectively, may improve evaluation of the risk of nephropathy and of the glycemic control desirable for individual patient. The authors interpreted this finding to

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demonstrate that nonglycemic factors may determine, in part, the HbA_{1c} values [7]. In fact, the models using the GG in addition to HbA_{1C} examine the effect of fructosamine when added to the HbA_{1C} values, because the GG itself is a linear function of the HbA_{1C} and fructosamine concentrations, namely the regression residual [7]. Glycation Gap improves the quality of the monitoring of glycemic control, especially for those patients whose HbA_{1C} levels do not truly reflect the mean blood glucose level.

In fact, non-enzymatic glycation has been strongly related to hyperglycemic conditions, and therefore to chronic complications associated with diabetes mellitus and renal failure, as well as degenerative changes occurring in the course of aging [12]. In a more recent clinical study, GG was found to be a better indicator than HbA_{1C} for assessing the risk of death and hospitalization in diabetic dialysis patients [8, 13, 14].

The aims of the presented statistical analyses were to estimate Glycation Gap values in a healthy subject.

MATERIAL AND METHODS

The research was conducted among the inhabitants of the Zwierzyniec commune and nearby villages in Lublin province, south-eastern Poland. The inhabitants took the opportunity of medical counseling during a socio-scholarly camp organized by the Medical University in Lublin. The study population consisted of 93 subjects: 63 women and 30 men, between the ages of 18-79. The participants provided information about their age, weight and height. In fasting serum samples, with the use of routine laboratory methods, the concentration of glucose, triglycerides, total cholesterol and high-density lipoprotein cholesterol (low-density lipoprotein cholesterol was calculated according to the Friedewald formula), as well as the levels of fructosamine and HbA_{1C} in whole blood. For the fructosamine assays, a Rosche kit was used - Cobas Integra 800 autoanalyzer with a nitrobluetetrazolium reaction, using a fructosepolylysine, intraassay coefficient of variation 2%. Determination were performed by single assay of samples stored at -80°C. Determination of HbA_{1C} was based on the interaction of antigen and antibody to direct determination of HbA_{1C} concentration in the whole blood. Each patient's Glycation Gap was calculated by the method of Cohen et al. [9] A regression equation of HbA_{1C} on fructosamine was derived from data from all patients in the study. A predicted HbA_{1C} was then be computed from the regression equation (HbA_{1C} = -0,0099 × FA + 7,8703); (r = -0.3) by substitution of the individual patient`s observed FA. The Glycation Gap for each patient was calculated as the difference between measured HbA_{1C} and predicted $HbA_{1C:}$

$$GG = \text{measured } HbA_{1C} - \text{predicted } HbA_{1C}$$

By this definition, GG is negative if the measured HbA_{1C} is less than HbA_{1C} predicted from FA, and positive if the measured HbA_{1C} is greater than that predicted. GG is zero when HbA_{1C} and FA are concordant [9].

All values are expressed as mean and standard deviations (Tab. 1). Distributions of the analyzed variables were tested using the Shapiro-Wilk test. For a comparison of the obtained results of investigations in the case of normally distributed variables, the Student t-test was applied. For variables that did not demonstrated compliance with the normal distribution, the non-parametric Mann-Whitney test was used. Correlations between variables were investigated using Pearson`s or Spearman's test. In all tests, a p-value of <0.05 was considered significant. All statistical analyses were conducted using Statistica 8.0 software.

Table 1. Baseline characteristic of healthy subject

Parameter	All (n = 93)	Women (n = 63)	Men (n = 30)
Glucose [mg/dl]	90.82±8.3	90.07±8.52	92.4±7.72
HbA1C [%]	5.5043±0.6	5.46±0.62	5.58±0.55
Predicted HbA1C [%]	5.5053±0.18	5.49±0.17	5.53±0.2
Fructosamine [µmol/l]	238.89±18.4	240.25±17.35	236.03±20.44
HGB [g/dl]	13.83±1.48	13.26±1.31*	15.04±1.03
Glycation Gap	- 0.001±0.57	- 0.025.65±0.6	0.049±0.5
CHOL [mg/dl]	199.15±40.01	200.5±43.11	196.3±33.05
TG [mg/dl]	102.23±57.97	96.92±57.05	113.4±59.25
LDL – cholesterol [mg/dl]	110.83±32.37	110.68±34.69	111.16±27.39
HDL – cholesterol [mg/dl]	67.94±15.97	70.49±15.71*	62.57±15.41

* p < 0.05 in compare with the men group

RESULTS

The mean value of measured FA (238.89 µmol/l, p = 0.3), HbA_{1C} (5.5043%, p = 0.52) predicted HbA_{1C} (5.5053%, p = 0.27) and GG (-0.001, p = 0.78) in the examined population were without significant differences between males and females (Fig. 1). Spearman ranking correlation analysis showed that

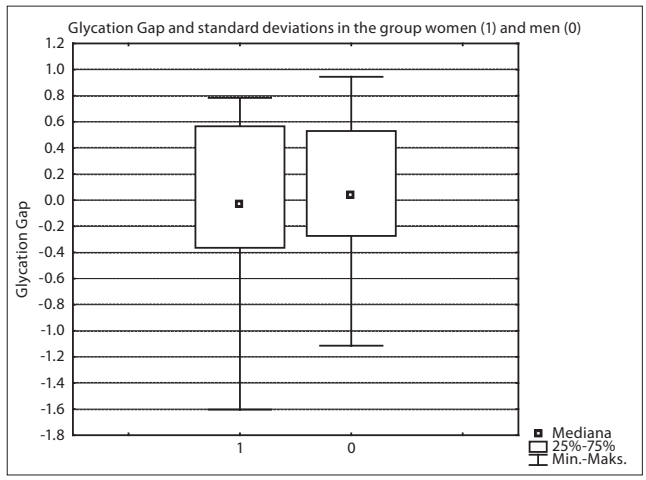

Figure 1. Mean value of glycation gap and standard deviations in the group of women (1) and men (0)

in the whole group there was a significant positive correlation between age and GG values (r_s = 0.34, p = 0.0006) (Fig. 2); HbA_{1C} and age (r_s = 0.43, p = 0.000013) (Tab. 2), FA and HbA_{1C} (rs = -0.3, p = 0.002) (Fig.3). Pearson correlation analysis revealed a significant negative correlation between fructosamine and age (r = -0.3, p = 0.003), and also a positive correlation with age and glucose (r = 0.47, p = 0.001). The existence was also shown of a significant positive correlation between GG values and total cholesterol (r_s = 0.34, p = 0.0006), triglycerides (r_s = 0.3, p = 0.0031), and low-density lipoprotein

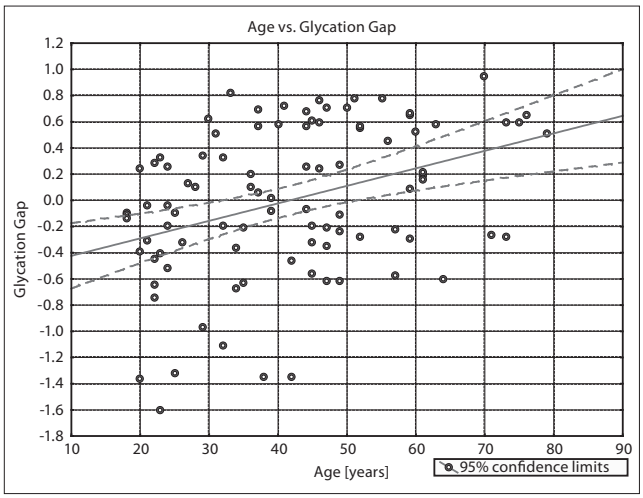

Figure 2. Correlation between glycation gap and age

Table 2. Correlation to age in health subject

Correlates	Correlation coefficient		
	All (n = 93)	Women (n = 63)	Men (n = 30)
Age vs. GG	rs= 0.34, p = 0,0006*	rs = 0.29, p = 0.018*	r = 0.52, p = 0.001*
Age and HbA1C	rs = 0.43, p = 0.000013*	rs = 0.38, p = 0.002*	rs = 0.57, p = 0.0008*
Age vs. Fructosamine	r = - 0.3, p = 0.003 *	rs = -0.22, p = 0.08	rs = - 0.34, p = 0.064
Age vs. Glucose	r = 0.47, P = 0.001*	rs = 0.57, p = 0.000001*	rs = 0.31, p = 0.09

* $p < 0.05$

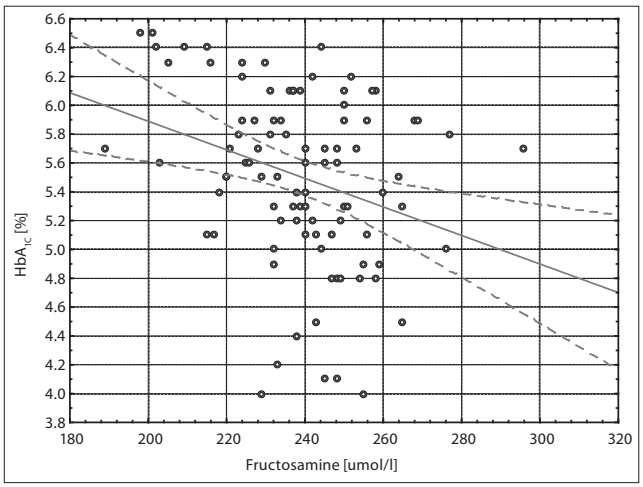

Figure 3. Correlation between HbA_{1C} and FA measured on the same sample in 93 patients

(r_s = 0.32, p = 0.001) in the whole group. In the group of women, Wilcoxcon tests showed significant differences between age and GG (p = 0.000001). The GG values also correlated with age (r_s = 0.29, p = 0.018), total cholesterol (r_s = 0.34,p = 0.0005), triglycerides (r_s = 0.29, p = 0.019), and low-density lipoprotein (r_s = 0.29, p = 0.018). In the group of men, t-test revealed that the mean values of GG significantly differed between ages, (p = 0.0001); Pearson correlation analysis also showed a positive correlation of GG values with age (r = 0.52, p = 0.001). There was no correlation between lipids profile in the group of men.

DISSCUSION

The widespread recognition that aging is a major risk factor for the development of diabetes has led some to believe that glucose intolerance is an inevitable outcome of aging. Approximately 20% of individuals over 65 years of age have diabetes mellitus, and almost half of these individuals have not been diagnosed [11].

Most studies reveal that both fasting and postprandial blood glucose show an increase in the level as age advances. Fasting blood glucose increases by 1-2 mg/dl per decade, while postprandial glucose increases by up to 15 mg/dl per decade increase in age [15]. Routine blood sugar testing should be carried out every 3 years in individuals over 45 years of age [16]. HbA_{1c} has low sensitivity and specificity in the elderly [16]. Serum fructosamine is an alternative screening test that is well standardized in the young, but the data is limited in elderly [15]. The problem is even greater with the large discrepancies between the HbA_{1C} and glucose, which raisees important questions concentrating on the widespread introduction of HbA_{1C} and the Glycation Gap for the diagnosis of prediabetes and diabetes particularly in the elderly.

In the presented study, HbA_{1C}, FA and GG were the most important risk factor for aging in study population. HbA_{1C} reflects blood glucose concentration over the predicting 8-12 weeks; however, fructosamine reflects blood glucose concentration over 1-3 weeks. It is a fact strictly correlated with the mean plasmatic glycemia. Thus, it is clear that the results of this study show that blood glucose concentration were positively correlated with serum FA and HbA_{1C}.

The presented study has demonstrated a negative correlation between HbA_{1C} and fructosamine. This is in accordance with Cohen et al. [9] who reported that the important difference between plasma glucose and HbA_{1c} is that the former reflects the physiology of glucose in the extracellular space, whereas HbA_{1c} reflects nonenzymatic glycosylation (and depends on glucose concentration) in the intraerythrocyte compartment. FA was selected for comparison because it represents a clinically accessible measure of nonenzymatic glycation of proteins in the same compartment as plasma glucose, and should integrate plasma glucose fluctuations.

On the other hand, patients with a high GG and low FA in relationship to the HbA_{1C} would therefore be the direction anticipated if albumin has a shorter survival time in the circulation, upon loss of glomerular selectivity with aging [9, 17, 18]. It should also be noted that serum fructosamine itself may be subject to variability in protein turnover, serum albumin concentration, and obesity [9]. Valeri et al. reported that serum fructosamine concentration correlates closely with HbA_{1C} because it reflects glycemic control that lasts 2-3 weeks, and HbA_{1C} reflects glycaemic control lasting 4-6 weeks. Any reduction in HbA_{1C} is likely to reduce the risk of complications, with the lowest risk being in those with HbA_{1C} in the normal range [19]. However, HbA_{1c} levels do not accurately predict their average glucose levels and diabetic complications. Recent studies have found that there are considerable interindividual HbA_{1C} variations that are affected by nonglycemic factors, such as genetic and age [10].The positive correlation between GG and age may be due, in part, to the glucose intolerance of aging, including alternations in glucose-induced insulin release and resistance to insulin- mediated glucose disposal [20]. Another paper

revealed that the HbA_{1C} levels are positively associated with age in the non-diabetic population [21].

As mentioned earlier, according to Calisti and Tognetti, glycosylation is the reaction between the free aldehydic group of glucose and free amino groups of protein (low-density lipoprotein) [3]. Obesity, especially with a central distribution of body fat and reduction in physical activity, occurs progressively with aging, and both of these factors are associated with abnormal carbohydrate metabolism [22]. Furthermore, exercise and adapting a diet can be more difficult for elderly people, and problems can arise in these areas.

CONCLUSION

In considering the values of the Glycation Gap in the study group, particular significance should be attributed to the progressive increase in Glycation Gap with advancing age. Elderly people who are at risk of developing diabetes, or who have already developed the disease, may not exhibit the classic symptoms expected. Age-related changes can mean that some symptoms will be masked, or more difficult to spot. It is worth pointing out that $HbA_{1C,}$ together with the Glycation Gap, must be taken into account for the correct interpretation of the glycation processes. This may provide a platform for future investigation of the underlying metabolic mechanisms on the effect of ageing in subjects, as well as improving evaluation of the risk of glycemic control desirable for individuals patients.

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