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Discordance in the levels of hemoglobin A1C and glycated albumin: Calculation of the glycation gap based on glycated albumin level

Mee Kyoung Kim, Kyung-Jin Yun, Hyuk-Sang Kwon, Ki Hyun Baek, Ki-Ho Song *

Department of Internal Medicine, The Catholic University of Korea, Seoul, Korea

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ABSTRACT

Objective: The glycation gap (G-gap) is an empirical measure of the extent of the difference between HbA1C and fructosamine levels. Several studies have shown that the presence of a G-gap is linked to diabetic nephropathy, but possible artifacts caused by dependence of the fructosamine level on the extent of serum protein metabolism require careful consideration. We investigated the consistency of G-gaps measured by assaying glycated albumin (GA) levels to identify factors associated with G-gap variations.

Method: A total of 457 pairs of observations, like an HbA1c and GA measurement at the same clinic visit, were obtained from 170 Korean patients with type 2 diabetes.

HbA1c and GA levels were measured simultaneously in two or three separate occasions. Each G-gap was calculated as the difference between the measured HbA1c level and that predicted by the GA level. All patients underwent abdominal computed tomography, and the areas of subcutaneous and visceral fat were measured.

Results: The G-gaps were all significantly inter-correlated over time ($\gamma = 0.755$, $P < 0.001$).The direction of each G-gap was consistent. The body mass index (BMI), visceral fat area, and the estimated glomerular filtration rate (eGFR) increased linearly from the lowest to the highest G-gap quartile (all P values <0.05). Upon multivariate analysis, both visceral fat area and the eGFR were significantly associated with a G-gap.

Conclusions: A G-gap determined using GA measurements is consistent within an individual over time. The G-gap is associated with visceral fat and kidney function in patients with type 2 diabetes.

1. Introduction

The hemoglobin A1C (HbA1c) level affords a measure of glycemic control within the intra-erythrocyte space, whereas glycation of serum proteins occurs in the extracellular compartment. Cohen et al. (2006) suggested that a glycation gap (G-gap), defined as the difference between the actual HbA1C concentration and that predicted by the fructosamine concentration, could explain inter-individual variations in HbA1C levels (Cohen et al., 2006). A G-gap is reproducible over time, despite variations in the extent of glycemic control as evidenced by HbA1c and fructosamine levels (Cohen, Holmes, Chenier, & Joiner, 2003; Cohen et al., 2006; Cosson et al., 2013; Rodriguez-Segade, Rodriguez, Cabezas-Agricola, Casanueva, & Camina, 2011; Rodriguez-Segade, Rodriguez, Garcia Lopez, Casanueva, & Camina, 2012; Zafon, Ciudin, Valladares, Mesa, & Simo, 2013).

The intra-cell relative to extra-cell glucose concentration increased with HbA1c and with the G-gap, but not with the corresponding serum fructosamine concentration (Khera et al., 2008). Thus, the G-gap might be a useful predictor of complications associated with intracellular glucose metabolism. A G-gap is associated with macroproteinuria independently of HbA1c and albumin levels and other confounding factors, suggesting that susceptibility to intracellular glycation specifically triggers changes in the glomeruli of the kidney (Cosson et al., 2013). However, serum fructosamine levels depend on the extent of serum protein metabolism. Such dependence may possibly interfere with G-gap calculations, and careful consideration is thus required. Albuminuria affects plasma fructosamine concentrations (Chan, Yeung, Cheung, Swaminathan, & Cockram, 1992). Both fructosamine and glycated albumin (GA) are indicators of short-term glycemia. GA levels are not influenced by the albumin concentration; the glycation level is calculated as a ratio of modified to total albumin (Ogawa et al., 2012). In the present study, we used GA rather than fructosamine levels to calculate G-gaps. Previous studies showed that an increased G-gap in diabetic patients was associated with nephropathy (diagnosed primarily by evaluation of proteinuria) (Cohen et al., 2003; Cosson et al., 2013; Rodriguez-Segade et al., 2012). We explored whether the G-gap was associated with renal impairment measured estimated glomerular filtration rates (eGFRs). We also explored factors affecting the G-gap.

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* Corresponding author at: Department of Internal Medicine, Yeouido St. Mary's Hospital, The Catholic University of Korea, 62 Yeouido-dong Youngdeungpo-gu, Seoul 150-713, Korea. Tel.: +82 2 3779 1557; fax: +82 2 780 3132.
E-mail address: kihos@catholic.ac.kr (K.-H. Song).

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2. Materials and methods

2.1. Subjects

Our retrospective cohort consisted of Korean patients with type 2 diabetes who attended clinics at Yeoido St. Mary's Hospital between 2010 and 2012. We selected patients whose HbA1c and GA levels had been measured on the same days. These paired estimations were performed 3–6 months after admission, when HbA1c values were stable. Exclusion criteria included any known hemoglobinopathy, anemia (hemoglobin <10 g/dL), hypoalbuminemia (serum albumin <3.0 g/dL), renal failure (creatine clearance <30 mL/min/1.73 m^2), pregnancy, and liver cirrhosis. Finally, 170 patients were enrolled. Of these, 117 had undergone three repeat HbA1c-GA estimations 3–6 months apart. HbA1c and GA levels were measured simultaneously in two or three separate occasions from each patient (1st visit at admission, 2nd visit and 3rd visit during routine outpatient clinical care). A total of 457 pairs of observations, like an HbA1c and GA measurement at the same clinic visit, were obtained from 170 patients. The protocol was reviewed and approved by the institutional review board (IRB) at The Catholic University of Korea and the patient informed consent requirement was waived by the IRB, because information obtained in routine analyses was recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects.

2.2. Laboratory measurements

HbA1C levels were measured via automated HPLC (HLC-723 G7, Tosoh Corporation, Tokyo, Japan); the reference range was 4.0%–6.0%. The intra- and inter-assay coefficients of variation were 0.89% and 1.56%, respectively, at an HbA1C level of 5.6%. GA levels were measured using a Toshiba 200FR analyzer (Toshiba Medical Systems Co., Tokyo, Japan) and an enzymatic method involving an albumin-specific proteinase, ketoamine oxidase, and an albumin detection reagent (Lucica GA-L; Asahi Kasei Pharma Co., Tokyo, Japan).

2.3. G-gap calculation

G-gaps were calculated using the method of Cohen et al.; each G-gap was defined as the difference between the measured HbA1c level and that predicted from the GA level, calculated using the HbA1c-GA regression equation. The regression equation is based on all the data in this study's subjects. We examined the correlation between HbA1c and GA using a linear regression analysis; Information on all patients at all visits was included. The following equation was established (Supplementary Fig. 1): Predicted HbA1c level $= 0.146 \times$ GA level $+ 4.722$ ($r = 0.749$; $P < 0.001$). The G-gap at each visit was calculated as the measured HbA1c level minus the level yielded by the regression equation. Rodriguez-Segade et al. (2011) suggested that G-gaps should be calculated when glycemic control is relatively stable, to avoid any effect of short-term blood glucose fluctuations. Thus, we classified subjects into four groups by their 2nd visit G-gap quartiles (all patients exhibited relatively stable glycemic control at 2nd visit). To explore G-gap reproducibilities (Zafon et al., 2013), we sought correlations between the G-gaps calculated at different times in each individual. The G-gaps on 2nd visit (the x-axes) were plotted against the product of both later G-gaps (2nd visit × 3rd visit; the y-axes). The product of any two concordant G-gaps (thus either positive or negative) must always be positive; any disagreement in the direction of the G-gap between the two determinations would thus appear in the negative region of the y-axis (the product of the two G-gaps).

2.4. Abdominal fat levels

Computed tomography (CT) was performed at level L4–5 to measure the cross-sectional areas of abdominal total fat (TFA), visceral fat (VFA), and subcutaneous fat (SFA), using previously described methods (van der Kooy & Seidell, 1993). First, the TFA adipose tissue (−190 to −30 Hounsfield units) was measured. VFA was distinguished from SFA by manually tracing the abdominal muscular wall separating the two adipose tissue compartments. The VFA area was measured and the SFA area calculated by subtracting the former area from the TFA.

2.5. Statistical analysis

We explored the clinical characteristics of type 2 diabetic patients by 2nd visit G-gap quartiles. The chi-squared test (χ^2) and analysis of variance (ANOVA) were used to compare proportions and means, respectively, between groups. Pearson's correlation coefficients between G-gap values and experimental variables were calculated. Multiple linear regression models were used to identify factors affecting the G-gap. Predictors that achieved a P value <0.05 in univariate analysis were assessed for inclusion in the multivariate model. The potential predictors evaluated were age, sex, BMI, SFA, VFA, eGFR, and hemoglobin and serum albumin levels. All data were analyzed using the SPSS statistical package (SPSS Inc., Chicago, IL, USA), and a P value <0.05 was considered to indicate statistical significance.

3. Results

3.1. Clinical characteristics of the study subjects by G-gap status

The mean patient age was 58.3 years and the mean duration of diabetes 12.0 years. The mean HbA1c level on routine outpatient visits 3–6 months after admission (2nd visit) was 8.1%. The anthropometric and metabolic characteristics of patients in the four G-gap quartiles are shown in Table 1. BMI, VFA, and eGFR increased linearly from the lowest (Q1) to the highest quartile (Q4) (all P values <0.05). The numbers of patients with urine albuminuria ≥100 mg/g creatinine tended to decrease linearly from the lowest to the highest G-gap quartile. Patients with negative G-gaps (Q1) were older, less obese, and more likely to exhibit albuminuria and renal impairment (a decreased eGFR) than were those with positive G-gaps (Q4). The overall mean HbA1c level increased linearly as the G-gap quartile rose, whereas the mean GA level did not differ significantly among the G-gap groups.

3.2. Variables affecting the G-gap

Pearson correlation analysis showed that the G-gap value was positively correlated with BMI ($r = 0.285$), abdominal SFA ($r = 0.212$), VFA ($r = 0.298$), the eGFR ($r = 0.318$), and hemoglobin level ($r = 0.207$) (all P values <0.01; Table 2). The G-gap value was correlated negatively with age ($r = -0.203$, $P = 0.008$). Upon univariate analysis, all factors other than the GA level were correlated significantly with the G-gap. However, after adjustment of relevant covariates, the only significant relationships were those between the G-gap and both visceral fat mass and the eGFR (Table 3).

3.3. Reproducibility and consistency of the G-gap

The correlation between the 2nd visit and 3rd visit G-gaps was $\gamma = 0.755$ ($P < 0.001$). There was no subjects who had a G-gap product more negative than −1.0 (G-gap 2nd visit × 3rd visit; Fig. 1A). A significant correlation was evident between the 1st visit and the 2nd visit G-gap, although the HbA1c level had been ameliorated by intensive treatment over this time (1st visit vs. 2nd visit; 10.0 vs. 8.1%; Table 4). A negative value of the product < −1.0 (G-gaps 1st visit × 2nd visit) was noted in only seven patients (Fig. 1B).