Influence of Proteinuria on Glycated Albumin Values in Diabetic Patients with Chronic Kidney Disease

Tomonari Okada, Toshiyuki Nakao, Hiroshi Matsumoto, Yume Nagaoka, Ryo Tomaru, Hideaki Iwasawa and Toshikazu Wada

Abstract

Background Glycated albumin (GA), which is an alternative glycemic marker, is influenced by factors associated with albumin turnover, and it is not clear whether proteinuria influences GA values in diabetic patients with chronic kidney disease (CKD).

Methods We enrolled 94 diabetic patients with CKD stages 3 to 5. GA, glycated hemoglobin, and urinary protein excretion (UP) levels were consecutively obtained in each patient. The correlations between GA and UP and those between changes in GA and UP were examined.

Results There was a significant correlation between GA and UP in all cases (r=-0.46, p<0.0001), however no significant correlation was found in cases with UP of 0-3.49 g/day (r=0.01). GA values in cases with UP >3.5 g/day were significantly lower than those in cases with UP <3.5 g/day [UP ≥3.5 g/day and serum albumin (Alb) ≤3 g/dL; 12.0 ± 1.3%, UP ≥3.5 g/day and Alb >3 g/dL; 17.8 ± 4.3%, 0 ≤ UP <3.5 g/day; 21.2 ± 4.2%], while no significant difference in HbA1c or glucose levels was found. In cases with a minimum of UP ≥0.5 g/day, no significant correlation was found between the difference in GA and the difference in UP at the point of maximum UP and minimum UP (r=0.04).

Conclusion Nephrotic-range proteinuria decreases GA values independent of the glycemic state, while non-nephrotic range proteinuria has no significant influence on GA values in diabetic CKD patients.

Key words: chronic kidney disease, diabetes mellitus, glycated hemoglobin, glycated albumin, proteinuria, glycemic control


Introduction

Some previous studies have shown a significant influence of glycemic control on decline in renal function or prognosis after starting chronic dialysis in patients with advanced diabetic nephropathy (1-3). Glycemic control is still important, and it is necessary to assess the validity of glycemic markers in these patients. In advanced chronic kidney disease (CKD) with diabetes mellitus, it is not clear which marker is the most adequate for the assessment of glycemic control is. Glycated hemoglobin (HbA1c) is the standard marker to assess glycemic control in diabetic patients. However, HbA1c values are affected by factors associated with erythrocyte turnover independent of glycemic control. Reduced erythrocyte survival or an increase in young erythrocytes during erythropoietin-stimulating agents (ESA) treatment decreases HbA1c values in patients with renal diseases (4, 5). Glycated albumin (GA) is an alternative glycemic marker which has been shown to be more accurate for the assessment of glycemic control than HbA1c in diabetic dialysis patients (6-8). However, GA values are influenced by several physiological factors or diseases associated with albumin turnover (9-13). Therefore, it is possible that proteinuric renal diseases may influence GA values independent of glycemic state. The majority of patients with advanced diabetic nephropathy and diabetic patients with non-diabetic renal disease have overt proteinuria. Thus, it is necessary to evaluate the validity of GA values for the assessment of glycemic control in these patients. Therefore, we attempted to
clarify the influence of proteinuria on GA values in diabetic patients with CKD.

**Patients and Methods**

A total of 94 type 2 diabetic patients consecutively treated at the Department of Nephrology at Tokyo Medical University Hospital between 2004 and 2009 were enrolled in this study. Enrollment criteria consisted of patients who had CKD stages 3 to 5, who had been not receiving ESA treatment, whose HbA1c, GA values, and 24-hour urinary protein excretion (UP) levels were available simultaneously, who had not previously received dialysis treatment or blood transfusion, and who had no chronic liver disease, abnormal thyroid function or malignancy. Underlying renal diseases were diabetic nephropathy (n=69), nephrosclerosis (n=14), and chronic glomerulonephritis (n=11). Underlying renal diseases were diagnosed by biopsy or clinical characteristics. Eighty-six patients were men. Baseline age was 64.0 ± 9.0 years (range; 29-79). Baseline serum creatinine was 1.93 ± 1.09 mg/dL (0.78-7.03), estimated glomerular filtration rate (eGFR) was 34.3 ± 12.8 mL/min/1.73 m² (7.5-58.1, CKD stage 3/4/5, n=56/31/7), which was calculated from the formulas for Japanese patients (14). Hemoglobin level was 12.7 ± 1.6 g/dL and serum albumin level was 4.0 ± 0.5 g/dL. Body mass index was 25.5 ± 3.5 kg/m². Thirty-nine patients received insulin, and 42 patients received oral hypoglycemic agents.

We obtained HbA1c and GA values, glucose levels, and UP consecutively 10 times. Duration of the study period was 24 ± 9 months (12-55). We examined the correlations among GA, UP and other clinical variables at baseline.

To assess any influence of intraindividual change in UP on GA values, we selected patients whose minimum UP was greater than 0.5 g/day during the study period and obtained GA, HbA1c, and UP at the point of maximum UP and minimum UP in each patient. We examined the correlations between differences in GA, HbA1c, and UP at the point of maximum UP and minimum UP.

HbA1c was measured by high performance liquid chromatography and the normal range was 4.3-5.8%. GA was measured by an enzymatic method (Lucica GA-L; Asahi Kasei Pharma Co., Tokyo, Japan) and the normal range was 11.6-16.4%. Written informed consent was obtained from all patients. The data were expressed as means ± SD. A p value of less than 0.05 was considered to indicate a statistically significant difference. Multiple comparisons of the data among groups were performed by one-way analysis of variance with Fisher’s protected least significant difference test (PLSD). Simple correlation between two variables was analyzed by Pearson’s correlation coefficient. Multiple regression analysis was performed to identify the independent variables associated with GA or HbA1c.

**Results**

**Comparison of baseline clinical variables among the groups according to UP**

Patients were classified into 5 groups according to UP (Group 1: 0-0.14 g/day, Group 2: 0.15-0.99 g/day, Group 3: 1.0-3.49 g/day, Group 4: ≥3.5 g/day and serum albumin (Alb) >3 g/dL, Group 5: ≥3.5 g/day and Alb ≤3 g/dL). Table 1 shows baseline clinical variables. Group 5 patients were significantly younger than those in Groups 1, 2, or 3, and eGFR in Group 1 was significantly greater than that in other groups. Glucose and HbA1c levels were not significantly different. GA levels in Group 4 and Group 5 were significantly lower than those in the other 3 groups individually or together (Group 1 + 2 + 3; 21.2 ± 4.2%; p<0.01, 0.001). There were no significant differences in GA values among Groups 1, 2, or 3.

**Correlations among GA, UP and other clinical variables at baseline**

Table 2 shows the correlations between GA and other clinical variables and multiple regression analysis at baseline. Figure 1 shows the correlation between GA and UP at baseline. There was a significant correlation between GA and UP in all cases (r=0.46, p<0.0001), however no significant correlation was found in cases with UP of 0-3.49 g/day (Group 1+2+3, n=69, r=0.01). Multiple regression analysis showed that glucose, albumin, and UP were significant variables associated with GA in all cases, but only glucose was a significant variable associated with GA in cases with UP of 0-3.49 g/day (Groups 1+2+3). In cases with UP greater than 3.5 g/day (Groups 4+5), multiple regression analysis showed that glucose and serum albumin were significantly associated with GA. In cases with UP of 0.15-3.49 g/day (Groups 2+3) and in cases with UP greater than 3.5 g/day (Groups 4+5), multiple regression analysis including age and eGFR as independent variables found that only glucose was a significant variable associated with GA, respectively (β=0.56, 0.65, p<0.0001, p<0.01, R²=0.36, 0.56).

Multiple regression analysis regarding HbA1c as a dependent variable also showed that only glucose was significant (β=0.64, p<0.0001, R²=0.41), while eGFR, hemoglobin levels, and UP levels were not significant variables in all cases.

Figure 2 shows a significant correlation between GA and HbA1c (r=0.66, p<0.0001) in all cases. Almost all cases with UP greater than 3.5 g/day (Groups 4+5) were situated below the regression line. In cases with UP of 0-3.49 g/day (Groups 1+2+3), the correlation coefficient between GA and HbA1c was 0.69 (p<0.0001) and the regression line rose.

**Changes in GA values during the study period**

In cases with minimum UP of greater than 0.5 g/day during the study period (n=49), the maximum and minimum UP levels were 6.7 ± 3.8, 2.4 ± 1.8 g/day, respectively (p<
Table 1. Clinical Variables in Groups of Patients according to Urinary Protein Excretion at Baseline

<table>
<thead>
<tr>
<th>UP (g/day)</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>14</td>
<td>24</td>
<td>31</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>UP (g/day)</td>
<td>0.04 ± 0.06</td>
<td>0.53 ± 0.23</td>
<td>2.00 ± 0.74</td>
<td>5.11 ± 1.39</td>
<td>8.27 ± 3.32</td>
</tr>
<tr>
<td>Age (years)</td>
<td>66.2 ± 7.6</td>
<td>65.3 ± 8.3</td>
<td>65.8 ± 7.6</td>
<td>60.8 ± 8.7\textsuperscript{*}</td>
<td>54.3 ± 14.7\textsuperscript{*}</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>24.0 ± 3.2</td>
<td>25.1 ± 3.4</td>
<td>26.1 ± 3.6</td>
<td>25.7 ± 2.9</td>
<td>26.6 ± 4.9</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73m\textsuperscript{2})</td>
<td>45.7 ± 7.6\textsuperscript{c}</td>
<td>31.5 ± 15.6</td>
<td>33.0 ± 9.6</td>
<td>32.9 ± 13.9</td>
<td>30.6 ± 9.5</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.31 ± 0.26</td>
<td>4.17 ± 0.31</td>
<td>4.09 ± 0.21\textsuperscript{d}</td>
<td>3.73 ± 0.32\textsuperscript{a}</td>
<td>2.73 ± 0.27\textsuperscript{a}</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>139 ± 44</td>
<td>141 ± 34</td>
<td>156 ± 53</td>
<td>162 ± 75</td>
<td>138 ± 41</td>
</tr>
<tr>
<td>GA (%)</td>
<td>20.6 ± 5.1</td>
<td>21.6 ± 4.4</td>
<td>212 ± 3.6</td>
<td>17.8 ± 4.3\textsuperscript{a}</td>
<td>12.0 ± 1.3\textsuperscript{a}</td>
</tr>
<tr>
<td>HbA\textsubscript{1c} (%)</td>
<td>8.65 ± 0.81</td>
<td>6.91 ± 1.09</td>
<td>7.24 ± 1.01</td>
<td>7.12 ± 1.51</td>
<td>6.32 ± 0.58</td>
</tr>
<tr>
<td>GA/HbA\textsubscript{1c} ratio</td>
<td>3.00 ± 0.51</td>
<td>3.13 ± 0.43</td>
<td>2.93 ± 0.39</td>
<td>2.51 ± 0.38\textsuperscript{a}</td>
<td>1.89 ± 0.10\textsuperscript{a}</td>
</tr>
</tbody>
</table>

a: p < 0.05 vs. Group 3
b: p < 0.01 vs. Groups 1, 2, 3
c: p < 0.001 vs. Group 2, p < 0.01 vs. Groups 3, 4, p < 0.05 vs. Group 5
d: p < 0.05 vs. Group 1
e: p < 0.001 vs. Groups 1, 2, 3
f: p < 0.001 vs. Groups 1, 2, 3, 4
 g: p = 0.054 vs. Group 1, p < 0.01 vs. Groups 2, 3

Table 2. Simple Correlation between Glycated Albumin Values and Other Clinical Variables and Multiple Regression Analysis Regarding Glycated Albumin Values as a Dependent Variable at Baseline

<table>
<thead>
<tr>
<th></th>
<th>All cases (n = 94)</th>
<th>Cases with UP of 0 - 3.49 g/day (n = 69)</th>
<th>Cases with UP greater than 3.5 g/day (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>simple correlation</td>
<td>multiple regression</td>
<td>simple correlation</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.44</td>
<td>&lt; 0.0001</td>
<td>0.47</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.23</td>
<td>0.26</td>
<td>-0.13</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.37</td>
<td>&lt; 0.001</td>
<td>0.23</td>
</tr>
<tr>
<td>UP</td>
<td>-0.46</td>
<td>&lt; 0.0001</td>
<td>-0.28</td>
</tr>
</tbody>
</table>

R\textsuperscript{2} = 0.45
R\textsuperscript{2} = 0.31
R\textsuperscript{2} = 0.52

UP: Urinary protein excretion, BMI: Body mass index

0.0001). Alb at the point of maximum UP was significantly less than at the point of minimum UP (3.6 ± 0.5, 3.8 ± 0.4 g/dL, p<0.05). There were no significant differences in GA and HbA\textsubscript{1c} between at the point of maximum UP and minimum UP (GA: 18.6 ± 5.2, 19.7 ± 5.4%, p=0.36, HbA\textsubscript{1c}: 7.1 ± 4.9 ± 1.3%, p=0.46). There were significant correlations between difference in GA and difference in HbA\textsubscript{1c} (r=0.67, p<0.0001), while no significant correlation was found between difference in GA and difference in UP at the point of maximum UP and minimum UP (r=0.04, p=0.79) (Fig. 3).

These 49 patients included 16 patients whose changes in HbA\textsubscript{1c} were less than 1% during the study period. In these 16 patients, the maximum minimum UP were 4.9 ± 3.4, 1.6 ± 1.6 g/day, respectively (p<0.001). There were no significant differences in Alb, GA, and HbA\textsubscript{1c} between at the point of maximum UP and minimum UP (Alb: 3.8 ± 0.4, 4.0 ± 0.3 g/dL, p=0.17, GA: 17.4 ± 4.0, 18.4 ± 3.0%, p= 0.40, HbA\textsubscript{1c}: 6.2 ± 0.5, 6.2 ± 0.5%, p=0.55).

Case example

We show the case of a 60-year-old man with diabetic nephropathy who received an oral hypoglycemic agent. His baseline serum creatinine was 1.60 mg/dL, eGFR was 36 mL/min/1.73 m\textsuperscript{2}, Alb was 4.0 g/dL, HbA\textsubscript{1c} was 6.2%, GA was 18.5%, and UP was 2.6 g/day. Changes in HbA\textsubscript{1c} values were less than 1% and his HbA\textsubscript{1c} values indicated a stable glycemic state during 16 months. Profiles of GA, HbA\textsubscript{1c}, and UP are shown in Fig. 4. GA values at the point of maximum UP (3.1 g/day) and point of minimum UP (0.4 g/
Figure 1. Correlation between glycated albumin and urinary protein excretion (UP) at baseline.

Figure 2. Correlation between glycated albumin (GA) and HbA1c at baseline.

Discussion

Although GA has been shown to be more accurate in the assessment of glycemic control than HbA1c in diabetic dialysis patients (6-8), it is not clear whether GA is also valid to assess diabetic predialysis CKD patients. GA values are influenced by factors associated with albumin turnover. Sev-
eral previous studies demonstrated the factors affecting GA values, including body mass index (BMI) (9, 10), chronic liver disease (11), or thyroid function (12). Our previous study showed that age, BMI, and serum cholinesterase level were significantly associated with GA values in non-diabetic hemodialysis patients (13).

The present study first showed the influence of proteinuria on GA values. Patients with nephrotic syndrome have increased albumin synthesis and increased fractional catabolic rate, resulting in rapid albumin turnover (15). Consequently, GA values might decrease independent of glycemic state in these patients. The present study showed a significant decrease in GA values in diabetic patients with nephrotic syndrome. This suggests that GA underestimates glycemic state and should not be used to assess glycemic control in such patients. On the other hand, non-nephrotic range proteinuria did not significantly influence GA values.

Many factors change UP levels in proteinuric CKD patients during the disease course. Blood pressure, antihypertensive drugs, and dietary salt or protein intake are the main factors which affect UP. Thus, UP levels might change in a very short period in these patients. GA reflects glycemic control during the previous 2 to 4 weeks (16). Our results suggest that intraindividual changes in UP levels might not necessarily influence GA levels, as shown in Fig. 3. However, it is possible that GA levels might change independent of glycemic state if serum Alb changes accompanied with changes in UP in patients with nephrotic syndrome.

In the present study, multiple regression analysis showed no significant association between GA and eGFR. Thus, renal function might not directly influence GA values. On the other hand, a recent study in the USA has shown an influ-

**Figure 3.** Correlations among difference in glycated albumin (GA), difference in HbA1c, and difference in urinary protein excretion (UP) at the point of maximum UP and minimum UP in patients with a minimum UP of greater than 0.5 g/day during the study period (n=49).

**Figure 4.** Profiles of glycated albumin, HbA1c, and urinary protein excretion and correlation between glycated albumin and urinary protein excretion during a 16-month period in a 60-year-old man with diabetic nephropathy.
ence of reduced GFR on HbA1c values, not GA values, while the association between GFR and HbA1c values has been shown to be very weak (17). In the present study, HbA1c was significantly associated with only glucose in multiple regression analysis. This result shows the validity of assessment using HbA1c as well as GA. However, it is possible that HbA1c values decrease due to ESA treatment or shortened erythrocyte survival in CKD patients as well as in dialysis patients (18, 19). Studies regarding the influence of renal function, ESA treatment, or anemia on HbA1c values are needed.

Some previous studies have shown the prognostic value of GA levels and the association between GA levels and progression of atherosclerosis in diabetic patients (20-23). In the present study, the question of which is a better marker, HbA1c, or GA, in diabetic CKD patients was not investigated. At present, it is not clear whether or not GA is more beneficial than HbA1c in the assessment of glycemic control in diabetic CKD patients.

This study has several limitations. First, this study was a cross-sectional study examining the associations among single GA, HbA1c, glucose levels, and UP levels. Therefore, it is possible that the timing of data collection during the study period might have influenced the results. Second, the number of patients with nephrotic syndrome (Group 5) was small, and HbA1c levels in this group were relatively lower than those in the other groups. Therefore, we could not exclude the possibility that GA values in patients with nephrotic syndrome might be greater than the results in the present study, if more data were available in those patients. However, the GA/HbA1c ratio in patients with nephrotic syndrome (Group 5) was significantly lower than that in the other groups. This suggests disproportionately lower values of GA. Third, we could not exclude the influence of short-term change in glycemic state on GA values during the study. GA values reflect short-term glycemic control, compared with HbA1c values, which reflect long-term values (24). Therefore, to clarify the influence of only proteinuria on GA values, non-diabetic patients should be examined. Fourth, we could not thoroughly assess the association with glucose levels. Although the majority of patients gave blood samples while fasting, we could not specify the number of patients obtained samples while fasting. It is necessary to obtain more samples of glucose within daily profiles, and to examine the correlation between GA values and glucose levels in different status of proteinuria.

In conclusion, nephrotic-range proteinuria decreased GA values independent of glycemic state, while non-nephrotic range proteinuria did not influence GA values in diabetic CKD patients. GA accurately reflected glycemic state in patients with non-nephrotic range proteinuria. Further studies regarding the validity of assessment and values of glycemic markers in diabetic CKD patients are needed.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement
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