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ORIGINAL RESEARCH

Association of Hemoglobin A1c, 1,5-Anhydro-D-Glucitol and Glycated Albumin with Oxidative Stress in Type 2 Diabetes Mellitus Patients: A Cross-Sectional Study

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ABSTRACT

Introduction: Oxidative stress plays a central role in the development and progression of vascular complications in patients with type 2 diabetes mellitus (T2DM). We have previously shown that markers of glucose variability evaluated by continuous glucose monitoring (CGM) are positively associated with oxidative stress in patients with T2DM. However, the evaluation of the glycemic variability by CGM remains a time- and money-consuming procedure. Therefore, this study investigated the independent correlates of oxidative stress among various other clinical markers routinely measured in primary care.

Methods: This was a retrospective cross-sectional study with 234 T2DM patients to examine which clinical variables, including 1,5-anhydro-D-glucitol (1,5-AG) and glycated albumin (GA), were independently associated with oxidative stress. Oxidative stress was measured using the diacron-reactive oxygen metabolites (d-ROMs) test. The relationships between d–ROMs and clinical factors, such as blood glucose, glycated hemoglobin (HbA1c), 1,5-AG, GA, lipid parameters, and blood pressure, were examined.

Results: Multiple stepwise regression analysis revealed that 1,5-AG (inversely), GA, triglycerides, use of metformin and being female were independently associated with d-ROMs. When patients with T2DM were stratified into two groups with HbA1c $< 8.0\%$ and HbA1c $\geq 8.0\%$, 1,5-AG (inversely), HbA1c, use of metformin and being female were independently associated with d-ROMs in diabetes patients with HbA1c $< 8.0\%$, whereas GA, fasting plasma

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glucose and being female were independently associated with d-ROMs in patients with HbA1c ≥ 8.0%.
Conclusion: Our present study suggests that 1,5-AG and GA are the strongest correlates of oxidative stress in patients with well and poorly controlled T2DM, respectively.

Keywords: 1,5-Anhydro-D-glucitol; Diacron-reactive oxygen metabolites; Glycated albumin; Oxidative stress; Type 2 diabetes mellitus

Key Summary Points

Why carry out this study?

Some studies previously showed that markers of glucose variability evaluated by continuous glucose monitoring (CGM) are positively associated with oxidative stress in patients with type 2 diabetes mellitus (T2DM). However, evaluation of the glycemic variability by CGM remains a time- and money-consuming procedure.

It is probable that 1.5-AG and GA could have additional clinical value for evaluating the glucose variability.

We investigated the independent correlates of oxidative stress among various clinical markers routinely measured in primary care, including HbA1c, 1.5-AG and GA in patients with T2DM.

What was learned from the study?

1,5-AG for well-controlled T2DM patients and GA for poorly controlled T2DM patients are useful in estimating oxidative stress.

INTRODUCTION

Oxidative stress has been shown to play a central role in the development and progression of vascular complications in patients with type 2 diabetes mellitus (T2DM) [1, 2]. Since intermittent hyperglycemia has greater triggering effects on oxidative stress generation, subsequently evoking endothelial dysfunction, compared with chronic sustained hyperglycemia [3, 4], glucose variability is considered a risk factor and therapeutic target for vascular complications in T2DM [5, 6]. Indeed, our previous cross-sectional study revealed that markers of glucose variability evaluated by continuous glucose monitoring (CGM), such as mean amplitude of glycemic excursions, are positively associated with oxidative stress in patients with T2DM [7]. Moreover, we have found that improvement in glucose variability is correlated with reduction in oxidative stress levels in patients with T2DM [8]. These observations suggest that assessment of the glucose variability by CGM could help identify high-risk diabetic patients who would benefit from intensive therapy. However, evaluation of the glycemic variability by CGM remains a time- and money-consuming procedure.

Measurement of glycated hemoglobin (HbA1c) is the gold standard method for assessing glycemic control, but HbA1c values do not reflect the glucose variability [9–11]. Actually, compared with HbAlc, 1,5-anhydro-D-glucitol (1,5-AG) and glycated albumin (GA) have been associated with postprandial hyperglycemia and could reflect the glucose variability in patients with T2DM [9–11]. Since 1,5-AG and GA have also been shown to predict future cardiovascular events in patients with T2DM [12–14], it is probable that 1,5-AG and GA could have additional clinical value for evaluating the glucose variability. Therefore, in this study, we investigated the independent correlates of oxidative stress among various clinical markers routinely measured in primary care, including HbA1c, 1,5-AG and GA, in patients with T2DM.

METHODS

Subjects and Ethics

This retrospective cross-sectional study included 234 outpatients aged > 20 years who visited

the Showa University Hospital from October 2013 to December 2018 for the treatment of T2DM. T2DM was defined according to the Japan Diabetes Society [15]. We included patients in whom oxidative stress, HbA1c, 1.5-AG and GA levels were measured and patients with diet therapy or stable oral hypoglycemic and/or insulin treatment for $\geq$ 3 months before the measurement of oxidative stress levels. Informed consents were obtained from all the patients. We excluded any patients who were using steroids or anti-inflammatory drugs; patients with diabetic ketosis and coma within 3 months before the study; patients with severe infection or trauma, malignancy, an estimated glomerular filtration rate (eGFR) < 30 ml/min/1.73 m^2 according to the Cockcroft-Gault formula [16] and severe liver dysfunction; and patients treated with the inhibitors of sodium-glucose co-transporter 2 and acarbose as well as pre- and post-surgery patients and pregnant women. This study complies with the principles laid out in the Declaration of Helsinki of 1964 and its later amendments. The study protocol was approved by the ethics committee of the Showa University School of Medicine (no. 2839). This study used an opt-out method, as shown on our hospital website and the poster at the Showa University Hospital, and subjects could opt out of the study at any time.

Clinical and Biochemical Analysis

The following clinical and laboratory parameters were measured on the morning after a minimum 8 h of fasting, as described previously [17]: body mass index (BMI), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), eGFR, blood pressure, fasting plasma glucose (FPG), HbA1c, GA and 1,5-AG. Plasma oxidant capacity against N,N-diethyl paraphenylenediamine was also measured using the d-ROMs test. Clinical data (age, sex, smoking status, duration of diabetes, diabetes therapy and antihypertensive/lipid-lowering drugs) were retrieved from medical records.

Laboratory Measurements

Oxidative stress was measured using the d-ROMs test (F.R.E.E. System, imported by LTD Tokyo from Diacron International s.r.l. Grosseto, Italy) as previously described [18, 19]. In accordance with the Wismerll kinetic procedure, the change in absorbance per minute was expressed as arbitrary units after correction (U.CARR, where 1 U.CARR = the oxidant capacity of a 0.08 mg/dl H_2O_2 solution; normal range = 250–300 U.CARR). Intra- and inter-assay coefficients of variation were 2.1% and 3.1%, respectively. Serum total cholesterol, LDL-C, HDL-C, TG and creatinine levels were measured using an automated analyzer (BM6070; Japan Electron Optics Laboratory, Tokyo, Japan). Plasma glucose was measured using the glucose oxidase method, and HbA1c was measured using high-performance liquid chromatography [20]. The 1,5-anhydro-D-glucitol level was measured by a colorimetric method (Nippon Kayaku, Tokyo, Japan). Serum GA level was measured by an enzymatic method using a Lucica GA-L kit (Asahi Kasei Pharma Corp., Tokyo, Japan).

Statistical Analysis

Data are presented as mean $\pm$ standard deviation (SD). Simple linear correlations were calculated by determining the Spearman's correlation coefficient. Multiple stepwise regression analysis was then performed with d-ROMs as a dependent variable. Independent variables included sex (female), age, duration of diabetes, BMI, smoking status (current), use of insulin, glucose-like peptide-1 receptor agonists, dipeptidyl peptidase-4 inhibitors, sulfonylureas, α-glucosidase inhibitors, metformin, thiazolidine, statins, angiotensin II receptor blockers, eGFR, FPG, HbA1c, 1,5-AG, GA, GA/HbA1c ratio, HDL-C, LDL-C, TG, systolic blood pressure and diastolic blood pressure. Analyses were performed using SPSS version 22 for Windows (IBM Corp., Armonk, NY, USA), with $p < 0.05$ indicating statistical significance.

Table 1 Baseline clinical characteristics of subjects

Clinical characteristics	Mean ± SD, *n* (%)
Age (years)	63.6 ± 12.5
Sex (male)	149 (63.7)
Body mass index (kg/m^2)	25.9 ± 5.1
Smoking (%)	44 (18.9)
Duration of diabetes (years)	12.7 ± 10.3
Hypertension	166 (70.9)
Dyslipidemia	182 (77.8)
Blood pressure (mmHg)	
Systolic	127.4 ± 20.9
Diastolic	73.6 ± 12.2
Low-density lipoprotein cholesterol (mg/dl)	95.9 ± 34.4
High-density lipoprotein cholesterol (mg/dl)	49.4 ± 15.0
Triglycerides (mg/dl)	129.8 ± 69.0
Estimated glomerular filtration rate (ml/min/1.73 m2)	78.1 ± 20.8
Fasting plasma glucose (mg/dl)	135.5 ± 33.8
HbA1c (%)	7.8 ± 1.4
1,5-AG (μg-ml)	8.6 ± 5.8
GA (%)	20.0 ± 4.1
GA/HbA1c ratio	2.6 ± 0.3
d-ROMs (U.CARR)	338.6 ± 66.3
Diabetes therapy	
Diet alone	26 (11.2)
Metformin	82 (35.0)
SU	48 (20.5)
Glinide	9 (3.8)
α-GI	52 (22.2)
Thiazolidine	18 (7.7)
DPP-4 inhibitor	89 (38.0)
GLP-1 receptor agonist	72 (30.8)
Insulin	88 (37.6)
Statin therapy	136 (58.1)
Angiotensin II receptor blocker	122 (52.1)
Macroangiopathy	59 (25.3)
Neuropathy	126 (54.7)
Retinopathy	76 (32.5)
Nephropathy	102 (43.6)

1 U.CARR (arbitrary unit) = the oxidant capacity of a 0.08 mg/dl H_2O_2 solution

d-ROMs diacron-reactive oxygen metabolites, *FPG* fasting plasma glucose, *HbA1c* hemoglobin A1c, *1,5-AG* 1,5-anhydro-D-glucitol, *GA* glycated albumin

RESULTS

Clinical Characteristics

The baseline clinical characteristics of the 234 patients are shown in Table 1. The 234 participants had a mean age of 63.6 ± 12.5 years, had an HbA1c level of 7.8 ± 1.4% and had had diabetes for a duration of 12.7 ± 10.3 years. The study group included more men (n = 149) than women (n = 85), and, on average, the participants were slightly overweight (BMI = 25.9 ± 5.1).

Relationship of d-Roms With Markers of Diabetic Control and Non-Glycemic Metabolic Variables

Table 2 shows the correlations between glucose metabolic variables and d-ROMs by univariate analysis. In all patients, significant correlations were observed between d-ROMs and LDL-C (r = 0.167; p = 0.010), TG (r = 0.194; p = 0.003), FPG (r = 0.235; $p < 0.001$), HbA1c (r = 0.416; $p < 0.001$), 1,5-AG (r = − 0.438; $p < 0.001$) and GA (r = 0.352; p = 0.001). In patients with HbA1c < 8%, significant correlations were

Table 2 Correlations between d-ROMs and markers of diabetic control and non-glycemic metabolic variables

	Total (n = 234)	HbA1c < 8% (n = 142)	HbA1c ≥ 8% (n = 92)
Age	0.015	0.050	0.072
BMI	0.068	0.097	− 0.132
Duration of diabetes	− 0.018	0.064	− 0.082
SBP	− 0.055	− 0.091	0.162
DBP	− 0.005	0.007	0.040
LDL-C	0.167*	0.124	0.061
HDL-C	− 0.061	0.075	− 0.160
TG	0.194*	0.172*	0.144
eGFR	− 0.022	− 0.128	0.086
FPG	0.235**	0.109	0.325**
HbA1c	0.416**	0.421**	0.253*
1,5-AG	− 0.438**	− 0.515**	− 0.119
GA	0.352**	0.158	0.458**
GA/HbA1c ratio	0.029	− 0.106	0.343**

Values represent Spearman's correlation coefficients
SBP systolic blood pressure, *DBP* diastolic blood pressure, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *TG* triglyceride, *eGFR* estimated glomerular filtration rate, *FPG* fasting plasma glucose, *HbA1c* hemoglobin A1c, *1,5-AG* 1,5-anhydro-D-glucitol, *GA* glycated albumin
*$p < 0.05$, **$p < 0.01$

Table 3 Linear multivariate analysis with changes in d-ROMs as dependent variables

	Dependent variables: d-ROMs (U.CARR)			
	β coefficient	t value	p value	Full-model R^2
Total			< 0.001	0.313
1,5-AG	− 0.325	− 4.793	< 0.001	
Sex	0.244	4.404	< 0.001	
GA	0.176	2.602	0.010	
TG	0.155	2.721	0.007	
Use of metformin	− 0.126	− 2.235	0.026	
HbA1c < 8%			< 0.001	0.272
1,5-AG	− 0.483	− 6.605	< 0.001	
Sex	0.211	3.026	0.003	
HbA1c ≥ 8%			< 0.001	0.283
GA	0.363	3.643	< 0.001	
Sex	0.236	2.657	0.009	
FPG	0.205	2.051	0.043	

Multiple stepwise regression analysis, with d-ROMs as the dependent variable, adjusted for sex (female), age, duration of diabetes, body mass index, smoking status (current), use of insulin, glucose-like peptide-1 receptor agonists, dipeptidyl peptidase-4 inhibitors, sulfonylureas, glinides, α-glucosidase inhibitors, metformin, thiazolidine, statins, angiotensin II receptor blockers, estimated glomerular filtration rate, FPG, HbA1c (only used in statistical analysis for all type 2 diabetes), GA, 1,5-AG, GA/HbA1c ratio, HDL-C, LDL-C, TG, SBP and DBP
d-ROMs diacron-reactive oxygen metabolites, *TG* triglyceride, *FPG* fasting plasma glucose, *HbA1c* hemoglobin A1c, *1,5-AG* 1,5-anhydro-D-glucitol, *GA* glycated albumin, *SBP* systolic blood pressure, *DBP* diastolic blood pressure
*$p < 0.05$, **$p < 0.01$

observed between d-ROMs and TG (r = 0.172; p = 0.040), HbA1c (r = 0.421; $p < 0.001$) and 1,5-AG (r = − 0.515; $p < 0.001$). In patients with HbA1c ≥ 8%, significant correlations were observed between d-ROMs and FPG (r = 0.325; p = 0.002), HbA1c (r = 0.252; p = 0.015), GA (r = 0.458; $p < 0.001$) and the GA/HbA1c ratio (r = 0.343; p = 0.001).

A multivariate stepwise regression model was used to analyze the independent factors that affect oxidative stress (Table 3). In all patients, 1,5-AG, sex, GA, TG and use of metformin were independently correlated with d-ROMs (R^2 = 0.313). Furthermore, we stratified diabetic patients into two groups according to their HbA1c value. In patients with HbA1c < 8%, 1,5-

AG and sex were independently correlated with d-ROMs (R^2 = 0.272). In patients with HbA1c ≥ 8%, GA, sex and FPG were independently correlated with d-ROMs (R^2 = 0.283).

DISCUSSION

To the best of our knowledge, no previous studies have investigated the association between oxidative stress and various glycemic markers, including fasting plasma glucose, HbA1c, 1,5-AG and GA, simultaneously in patients with T2DM. The present study demonstrated that oxidative stress is associated with 1,5-AG and GA in patients with T2DM. In addition, the present study demonstrated that oxidative stress is associated with 1,5-AG for good glycemic control and GA for poor glycemic control in patients with T2DM. Furthermore, this study shows that the use of metformin results in a reduction of oxidative stress. Our findings may help reduce oxidative stress in the clinical management of T2DM in the absence of CGM.

In this study, we evaluated the level of d-ROMs as a surrogate marker of oxidative stress for patients with T2DM. D-ROMs are more often detected in female patients than in males [21]. The d-ROMs are mainly composed of organic hydroperoxide; despite hydroperoxide's moderate oxidative power, its serum levels are detectable because of its relative stability compared with other free radicals. Not only is the d-ROMs test quick and inexpensive to use in clinical settings [18], but it is also predictive of morbidity and mortality [22, 23]. Recently, Yang et al. reported that d-ROMs predict future cardiovascular events in both diabetic and non-diabetic patients [24]. Therefore, d-ROMs are considered to be reliable markers of oxidative stress.

The present study demonstrates that not only HbA1c but also 1,5-AG, GA and the GA/HbA1c ratio are associated with oxidative stress in patients with T2DM. While the relationship between oxidative stress and HbA1c has been reported previously [25], our results suggest that 1,5-AG, GA and the GA/HbA1c ratio reflect glucose variability and are thereby associated with oxidative stress. However, Monnier et al. reported that the contribution of fasting plasma glucose and postprandial plasma glucose differed depending on glycemic control [26]. In addition, Monnier et al. reported the contribution of the postprandial glucose level to HbA1c values at levels < 7.5–8.0% [27]. Actually, 1,5-AG has been reported to be related to glucose variability in patients with well-controlled T2DM [9], while GA has been reported to be related to glucose variability in patients with poorly controlled T2DM [28]. Therefore, we divided the patients into two groups: those with HbA1c < 8% and those with HbA1c ≥ 8%.

The present study demonstrated the relationship between oxidative stress and 1,5-AG in patients with HbA1c < 8.0% by multivariate analysis. This result may depend on the characteristics of 1,5-AG. As excretion of glucose into the urine increases, reabsorption of 1,5-AG is inhibited competitively, and the blood level of 1,5-AG decreases. Therefore, low levels of 1,5-AG in the blood are considered a clinical marker of postprandial hyperglycemia [29]. We have demonstrated that the 1,5-AG blood level correlates with postprandial hyperglycemia in patients with HbA1c < 8.0% in T2DM [30]. The use of CGM has demonstrated a significant correlation with the mean amplitude of glycemic excursions and indices of postprandial hyperglycemia in patients with HbA1c < 8.0% [31]. In support of our findings, 1,5-AG is reported to be a useful marker for vascular endothelial dysfunction in patients with HbA1c < 8.0% [12]. It has also been reported that low 1,5-AG levels were associated with the severity of coronary artery calcification in patients with HbA1c < 7.0% [32]. On the other hand, d-ROMs did not correlate with 1,5-AG in patients with HbA1c > 8%. This was due to the contribution of basal hyperglycemia, which becomes significant when HbA1c exceeds 8.4% [26]. In addition, considering the effects of hyperglycemia on the reabsorption of 1,5-AG in the kidney, it is highly probable that a threshold exists in the reabsorption process. Therefore, 1,5-AG in patients with HbA1c > 8% may not reflect postprandial plasma glucose or glucose variability. From the above, oxidative stress, evaluated by d-ROMs, correlated strongly with

1,5-AG, suggesting the 1,5-AG level is a potentially useful predictor of oxidative stress, as well as a marker of glucose variability, in T2DM patients with HbA1c < 8%.

Unlike the association between 1,5-AG and oxidative stress, GA and oxidative stress were associated with HbA1c ≥ 8% in the multivariate analysis of this study. GA, an early Amadori-type glycation protein of the nonenzymatic glycation reaction between glucose and serum albumin, is an index that reflects the average glucose level over the previous 2–3 weeks. GA is not only an indicator of intermediate glycemic control but also reflects glucose variability as well as the mean plasma glucose level [33]. In addition, the GA/HbA1c ratio is reported to be an indicator that reflects glucose variability [34]. Actually, it reported that GA correlates with diabetic complications such as retinopathy progression [35], neuropathy [36] and cardiovascular disease [37, 38], and the GA/HbA1c ratio correlates with cognitive impairment [39]. In this study, GA was associated with oxidative stress in patients with HbA1c ≥ 8% by multivariate analysis. In support of our results, Suwa et al. demonstrated that GA is an indicator that has a closer relationship with glucose variability compared with HbA1c and 1,5-AG in patients with poorly controlled T2DM [28]. However, the GA/HbA1c ratio was not associated with oxidative stress in patients with HbA1c ≥ 8%. Glucose variability has been reported to increase with a GA/HbA1c ratio > 2.8 [34], but the patients in our study had a mean GA/HbA1c ratio of 2.6, which may have contributed to this result. On the other hand, FPG was also associated with oxidative stress in patients with HbA1c > 8%. This result is considered to be due to the fact that the contribution of FPG increases the HbA1c at levels > 7.5–8%. It has been reported that elevated FPG induces oxidative stress and interferes with normal endothelial function via ROS overproduction [40]. From the above, it is suggested that GA is a good marker for oxidative stress because FPG contributes to oxidative stress as well as glucose variability when glycemic control is poor.

Several studies have reported on the relationship between metformin and oxidative stress [41, 42]. There are a variety of mechanisms by which metformin reduces oxidative stress. Metformin reduces ROS formation, suggesting a diminishing effect of oxidative stress [41, 43]. It has been reported that, in aortic endothelial cells, the activation of AMPK by metformin limits the endothelial cell damage caused by oxidative stress under hyperglycemic conditions through the inhibition of the protein kinase C-NAD(P)H oxidase pathway [44]. Furthermore, metformin may partially protect against oxidative stress through regulation of serum insulin levels [45]. Metformin was shown to lower the risk of diabetes-related complications, cardiovascular disease, stroke, and all-cause mortality in the UKPDS study [46].

Although the relationship between LDL-C and oxidative stress has been reported previously [7, 47], there are no reports about the relationship between TG and oxidative stress. In this study, we have demonstrated that TG is associated with oxidative stress by multivariate analysis. Previous epidemiologic studies have reported that hypertriglyceridemia is an independent risk factor for cardiovascular disease [48, 49]. In addition, it has been reported that not only high LDL-C but also hypertriglyceridemia is a risk factor for cardiovascular disease in Japanese patients with T2DM [50]. However, the mechanism by which hypertriglyceridemia induces oxidative stress remains unclear. It may be related to insulin resistance, but further research is needed to elucidate this mechanism.

The present study had several limitations. First, the determination coefficient of the independent variable was low for the model employed in this study, with the adjusted R^2 value being approximately 0.3 in the multivariate analysis. The sample size was relatively small; therefore, the obtained results require further confirmation in a large number of patients. Second, this study was cross-sectional, precluding the evaluation of any cause-effect relationship between glucose metabolism, 1,5-AG, GA and oxidative stress. Whether intervention aimed at reducing glucose metabolism via 1,5-AG and GA should be administered needs further examination.

CONCLUSION

In conclusion, 1,5-AG and GA are useful markers for estimating oxidative stress in patients with well and poorly controlled T2DM, respectively.

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Compliance with Ethics Guidelines. The study protocol was approved by the ethics committee of Showa University School of Medicine (no. 2839). All patients provided informed consent according to the provisions of the Declaration of Helsinki of 1964 and its later amendments. This study used an opt-out method, as shown on our hospital website and the poster at the Showa University Hospital, and subjects could opt out of the study at any time.

Date Availability. The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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