Alcohol flushing is independently associated with lesser degree of carotid atherosclerosis in Japanese type 2 diabetic patients

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Abstract

Background Type 2 diabetes mellitus is a well-known risk factor for atherosclerosis. Intima media thickness (IMT) of the carotid arteries has been widely used for assessing atherosclerotic changes representing cerebro-cardiovascular disease risk. Previous studies have associated the presence of inactive aldehyde dehydrogenase with progression of atherosclerosis and alcohol flushing. However, reports conflict on whether alcohol flushing could potentially modify the degree of atherosclerosis in type 2 diabetic patients.

Methods We retrospectively assessed the relationships among alcohol flushing, IMT, and clinical characteristics in 123 consecutive Japanese type 2 diabetic patients. Alcohol flushing was assessed by standardized questionnaires.

Results Patients with alcohol flushing had lower mean IMT [0.7 (0.65–0.80) vs. 0.8 (0.73–0.90) mm, $p < 0.05$], despite having higher triglycerides [132 (94–169) vs. 98 (70–139) mg/dL, $p < 0.01$] and lower high-density lipoprotein cholesterol [52 (44–60) vs. 59 (49–67) mg/dL, $p < 0.05$] concentration. Patients with alcohol flushing had lower frequency of alcohol intake (45 vs. 76%, $p < 0.0001$). Mean IMT was positively associated with age and low-density lipoprotein cholesterol concentration, and negatively with alcohol flushing according to stepwise

multiple regression analysis. Alcohol flushing was also independently associated with mean IMT by multivariate analysis.

Conclusions These findings suggest that alcohol flushing is independently associated with lesser degree of carotid atherosclerosis in Japanese type 2 diabetic patients.

Keywords Type 2 diabetes · Intima media thickness · Alcohol flushing · Frequency of alcohol intake

Introduction

Type 2 diabetes mellitus is a well-known risk factor for cerebro-cardiovascular disease [1, 2]. Evaluation of cerebro-cardiovascular complications is very important for predicting patient outcome. Because these complications develop as a result of vascular atherosclerotic changes, several methods have been developed to evaluate these atherosclerotic changes. These methods include ultrasound analyses of the carotid arteries. Intima media thickness (IMT) of the carotid arteries has been widely used for assessing atherosclerotic changes representing cerebro-cardiovascular disease risk [3–7].

Low Km aldehyde dehydrogenase (ALDH2) is one of the key enzymes that detoxicate acetaldehyde incurred by alcohol intake [8, 9]. The genotype composition of ALDH2 is markedly different between Asians and Caucasians. Unlike Caucasians, nearly half of East Asians (Japanese, Han Chinese and Koreans) have a single point mutation in exon 12 of the ALDH2 gene (ALDH2*2 allele) [10, 11], resulting in a functional deficiency in alcohol metabolism [8, 10]. Therefore, alcohol flushing responses, including facial flushing, palpitation, drowsiness, and other unpleasant symptoms, are frequently observed in East Asians

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[10, 12, 13]. Intensive flushing after light drinking is mainly triggered by severe acetaldehydemia in persons possessing the ALDH2*2 allele [14, 15]. In previous studies, questionnaires regarding alcohol flushing have been used in large-scale epidemiological studies as a surrogate marker of ALDH2 genotype [16–18].

Inactive ALDH2 has been reported to be associated with the progression of atherosclerosis. The relationship of inactive ALDH2 with atherosclerosis is different according to patient background. Chinese persons with cardiovascular disease have been known to have a high frequency of inactive ALDH2 compared with controls, especially in non-drinkers [19]. In Chinese patients with hypertension, those with inactive ALDH2 had higher mean IMT than those with active ALDH2 [20]. In Japanese patients with type 2 diabetes, those with inactive ALDH2 had higher frequency of myocardial infarction than those with active ALDH2 [21]. As one of the mechanisms that could explain these results, previous studies suggested that inactive ALDH2 not only promoted accumulation of acetaldehyde, but also resulted in the accumulation of oxidized low-density lipoprotein cholesterol (LDL), metabolized by ALDH2 [22–24]. By contrast, in Japanese patients with metabolic diseases, including hypertension, hyperlipidemia and diabetes mellitus, the patients with inactive ALDH2 had lower plaque scores than the patients with active ALDH2 [25]. Thus, the results have been conflicting regarding whether heterogeneity of ALDH2 genotypes could potentially modify the degree of atherosclerotic disease in type 2 diabetic patients. To our knowledge, no study has assessed whether alcohol flushing is associated with the degree of carotid atherosclerosis in type 2 diabetic patients.

Therefore, using a conventional questionnaire for alcohol flushing as a surrogate marker of ALDH2 genotype, we assessed the relationships among alcohol flushing, IMT, and clinical characteristics in Japanese type 2 diabetic patients who had received care at Showa University Fujigaoka Hospital.

Materials and methods

Patients

This retrospective observational study was designed to evaluate the correlation between alcohol flushing response and IMT, using the medical records of 123 consecutive type 2 diabetic outpatients who had undergone measurement of IMT of the carotid arteries and who completed questionnaires for alcohol flushing between October 2013 and May 2015. The study protocol was approved by the ethics committee of Showa University Fujigaoka Hospital

(approval number: 2016139, approval date: March 27, 2017). Because this was a retrospective study, the purpose of the study had been adequately acknowledged in lieu of obtaining informed consent from each patient.

Measurement of IMT

IMT was measured by an ultrasound device and assessed by the European Carotid Surgery Trial method [26]. Scanning of the extracranial common carotid artery, carotid bulb, and internal carotid artery in the neck was performed bilaterally in three different longitudinal projections and transverse projections; and the site of greatest thickness, including the presence of plaque lesions, was sought along the arterial walls. IMT was measured as the distance between two parallel echogenic lines corresponding to the blood-intima and media-adventitial interface on the posterior wall of the artery. Three determinations of IMT were conducted at the site of the thickest point and two adjacent points (located 1 cm upstream and 1 cm downstream from the thickest point). The mean IMT from the three determinations was calculated. If plaque was recognized, the maximum IMT values (from the left and right arteries) were calculated for each study subject. Experienced laboratory technicians conducted all the scans. To avoid inter-investigator variability, all the scans were electronically stored and analyzed in random order by a single investigator (R. S.) blinded to the clinical characteristics of the patients. Reproducibility was assessed by replicate measurements in randomly-selected 34 participants. The correlation coefficients between replicate readings were 0.98 ($p < 0.0001$ by nonparametric Spearman rank coefficients) for the mean IMT (data not shown).

Questionnaires

Each patient was asked to fill out a simple questionnaire concerning alcohol flushing responses. As ALDH2 genotype could mainly affect alcohol flushing responses [14–18], this questionnaire was designed to identify inactive ALDH2. The questions in this modified version of a previous questionnaire [18] were: (a) Do you have a tendency to flush in the face immediately after drinking a glass of alcohol? (b) Did you have a tendency to flush in the face immediately after drinking a glass of beer during the first to second year after you started drinking? Individuals who answered “yes” to either of questions (a) or (b) were classified as “flushing positive”; those who answered “no” to both questions (a) and (b) were classified as “flushing negative”. When current or former flushing individuals were considered to have inactive ALDH2, both sensitivity

and specificity were as high as approximately 90% in Japanese population [18].

In addition, the durations of diabetes, alcohol consumption, and smoking habit of the patients were recorded by a self-administered questionnaire. Alcohol intake and smoking were classified as current or past habitual use (positive) or no use (negative). The past history of cardiovascular and cerebrovascular diseases was also recorded.

Measurements

Blood samples for analysis of serum concentrations of total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL), glucose, and hemoglobin A1c (HbA1c) were collected from patients in the morning after overnight fasting. LDL concentrations were calculated by using the Friedewald equation [27]. All the blood tests were performed by using standard methods. Blood pressure (BP) was measured in the sitting position after a 5-min rest. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured.

Assessment of diabetic microvascular complications

Diabetic retinopathy was diagnosed by ophthalmologists. Diabetic nephropathy was diagnosed if patients displayed spot-urinary albumin to creatinine (Cr) ratio greater than 30 mg/gCr or estimated GFR less than 30 mL/min/1.73 m² [28]. Diabetic neuropathy was diagnosed if patients had two or more of the following three components: the presence of symptoms, the absence of ankle tendon reflexes and abnormal vibration perception threshold scores using a C128 tuning fork, where bilateral spontaneous pain, hypoesthesia or paraesthesia of the legs was considered as symptoms [29].

Statistical analysis

All the statistical analyses were performed with JMP version 5.1 (SAS Institute Inc., Cary, NC, USA). Age and DBP were normally distributed, and the other data were non-normally distributed. Normally distributed variables are expressed as mean $\pm$ standard deviation. Non-normally distributed variables are expressed as median (25th–75th percentile). The unpaired *t* test, Mann–Whitney U test, or Fisher's exact test were used to analyze the significance of differences between the clinical characteristics of diabetic patients with positive and negative alcohol flushing. The unpaired *t* test was used for normally distributed variables. The Mann–Whitney U test was used for non-normally distributed variables. Relationships between mean IMT and the clinical characteristics were evaluated

by nonparametric Spearman's rank correlation coefficients and stepwise multiple regression analysis, and also by multivariate analysis. For all the tests, $p < 0.05$ was considered statistically significant.

Results

Characteristics of type 2 diabetic patients according to alcohol flushing status

The characteristics of the type 2 diabetic patients according to alcohol flushing status are shown in Table 1. There were no significant differences in age, sex distribution, duration of diabetes, body mass index (BMI), SBP or DBP between the two groups. Medication for hypertension, usage of statins and fibrates, and insulin injections did not differ significantly between the two groups. Fasting glucose and HbA1c did not differ significantly between the two groups. Patients with alcohol flushing ($n = 76$) had significantly higher TG [132 (94–169) vs. 98 (70–139) mg/dL, $p < 0.01$] and lower HDL [52 (44–60) vs. 59 (49–67) mg/dL, $p < 0.05$] concentrations than patients without alcohol flushing ($n = 47$). LDL concentrations did not differ significantly between the two groups. Frequency of diabetic microvascular complications and histories of cardiovascular and cerebrovascular diseases did not differ significantly between the two groups. Patients with alcohol flushing had significantly lower frequency of habitual alcohol intake (45 vs. 76%, $p < 0.0001$), while frequency of smoking in the two groups did not differ significantly.

Comparison of IMT according to alcohol flushing status

The IMT values of the patients according to alcohol flushing status are shown in Fig. 1. Although maximum IMT in the two groups did not differ significantly [2.0 (1.50–2.50) vs. 2.3 (1.80–2.90) mm, $p = 0.10$], patients with alcohol flushing had significantly lower mean IMT [0.7 (0.65–0.80) vs. 0.8 (0.73–0.90) mm, $p < 0.05$].

Relationships between mean IMT and clinical characteristics

The relationships between mean IMT and clinical characteristics are shown in Table 2. Mean IMT was positively and strongly associated with age. There were no associations between mean IMT and sex distribution, duration of diabetes, BMI, DBP, usage of statins, fibrates, and insulin injections, fasting glucose, HbA1c, TG, or HDL. Although mean IMT was prone to be associated with medication for hypertension, this association was not significant. Mean

Table 1 Characteristics of type 2 diabetic patients according to alcohol flushing status

	Flushing negative (<i>n</i> = 47)	Flushing positive (<i>n</i> = 76)	<i>p</i> value
Age (years)	64 ± 12	60 ± 12	0.12
Male (%)	34	33	1.00
Duration (years)	11 (7–23)	10 (5–16)	0.15
BMI (kg/m ²)	24 (22–27)	24 (22–28)	0.58
SBP (mmHg)	128 (122–130)	126 (120–134)	0.62
DBP (mmHg)	72 ± 8	73 ± 9	0.36
Medication for hypertension (%)	51	46	0.71
Use of statin (%)	68	82	0.13
Use of fibrate (%)	6	3	0.37
Use of insulin (%)	30	20	0.27
Fasting glucose (mg/dL)	137 (115–181)	137 (117–164)	0.77
HbA1c (%)	7.5 (7.1–8.1)	7.5 (7.0–8.6)	0.67
TG (mg/dL)	98 (70–139)	132 (94–169)	<0.01
HDL (mg/dL)	59 (49–67)	52 (44–60)	<0.05
LDL (mg/dL)	87 (64–112)	85 (69–110)	0.60
Diabetic retinopathy (%)	39	31	0.56
Diabetic nephropathy (%)	53	58	0.46
Diabetic neuropathy (%)	31	33	0.50
History of cardiovascular diseases (%)	13	9	0.37
History of cerebrovascular diseases (%)	6	4	0.42
Habitual alcohol intake (%)	76	45	<0.0001
Smoking (%)	49	46	0.57

Data are expressed as mean ± standard deviation, median (25th–75th percentile) or frequency

BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, HbA1c hemoglobin A1c, TG triglyceride, LDL low-density lipoprotein, HDL high-density lipoprotein

Fig. 1 Comparison of IMT according to alcohol flushing status. Data are expressed as median (25th–75th percentile). **a** mean IMT, and **b** maximum IMT in type 2 diabetic patients according to alcohol

flushing status. *p < 0.01 by the Mann–Whitney U test. IMT intima media thickness

IMT was positively associated with LDL concentration and SBP. Mean IMT was negatively associated with alcohol flushing. There were no associations between mean IMT and drinking or smoking habits. These related factors were evaluated by stepwise multiple regression analysis. Mean

IMT was independently associated with age, LDL concentration, SBP and alcohol flushing, whereas medication for hypertension did not contribute significantly (Table 2).

In addition, multivariate analysis showed that mean IMT was positively associated with age, LDL concentration, and

Table 2 Nonparametric Spearman rank coefficient and stepwise multiple regression analysis for mean IMT

	Nonparametric Spearman rank coefficient		Stepwise multiple regression analysis		
	ρ	p	R^2	F	p
Age (years)	0.42	<0.0001	0.61	26.26	<0.0001
Male (%)	−0.0084	0.93			
Duration (years)	0.075	0.43			
BMI (kg/m ²)	−0.14	0.19			
SBP (mmHg)	0.23	<0.05	0.14	6.20	<0.05
DBP (mmHg)	−0.0054	0.96			
Medication for hypertension (%)	0.17	0.064	0.0047	0.20	0.66
Use of statin (%)	−0.047	0.60			
Use of fibrate (%)	−0.065	0.48			
Use of insulin (%)	0.039	0.67			
Fasting glucose (mg/dL)	0.082	0.36			
HbA1c (%)	0.019	0.83			
TG (mg/dL)	0.0048	0.96			
HDL (mg/dL)	0.066	0.47			
LDL (mg/dL)	0.20	<0.05	0.47	20.16	<0.0001
Flushing positive (%)	−0.24	<0.01	0.15	6.38	<0.05
Habitual alcohol intake (%)	0.0073	0.94			
Smoking (%)	0.10	0.26			

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HbA1c* hemoglobin A1c, *TG* triglyceride, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *IMT* intima media thickness

negatively with alcohol flushing, whereas there were no associations between mean IMT and other items listed in Table 3.

Discussion

The present study was the first to explore the relationship between alcohol flushing and the degree of carotid atherosclerosis in Japanese type 2 diabetic patients. In single variable analysis, alcohol flushing was associated with HDL and TG concentrations, habitual alcohol intake, and mean IMT. Previous studies reported that alcohol flushing response correlates with ALDH2 activity [16–18], and that high ALDH2 activity increases HDL concentration and decreases non-HDL and LDL concentrations or the frequency of hyperlipidemia in Japanese patients with metabolic disorders [21, 25]. Consistently, our study showed that type 2 diabetic patients without alcohol flushing, suggesting having higher ALDH2 activity, had higher HDL and lower TG concentrations. Higher HDL concentrations in patients without alcohol flushing would be due to higher frequency of habitual alcohol intake.

Previous studies suggested that inactive ALDH2 or alcohol flushing might be associated with promotion of atherosclerosis, including a higher degree of IMT or with a

Table 3 Multivariate analysis for mean IMT

	R value	p value
Age (years)	0.41	<0.001
Male (%)	0.00	1.00
Duration (years)	0.049	0.67
BMI (kg/m ²)	−0.11	0.34
SBP (mmHg)	0.14	0.22
DBP (mmHg)	−0.016	0.89
Medication for hypertension (%)	0.11	0.35
Use of statin (%)	−0.11	0.34
Use of fibrate (%)	−0.065	0.57
Use of insulin (%)	0.051	0.66
Fasting glucose (mg/dL)	0.094	0.41
HbA1c (%)	0.13	0.26
TG (mg/dL)	−0.036	0.75
HDL (mg/dL)	0.017	0.88
LDL (mg/dL)	0.27	<0.05
Flushing positive (%)	−0.24	<0.05
Habitual alcohol intake (%)	0.079	0.49
Smoking (%)	0.16	0.16

R expresses the correlation coefficient between mean IMT and clinical parameters

Abbreviations were the same as appeared in Table 2

higher frequency of myocardial infarction [21–24]. However, the present study found that alcohol flushing was independently associated with lesser degree of carotid atherosclerosis, in spite of higher TG and lower HDL concentrations that are known to be atherogenic lipid profiles [30, 31]. Other possible risk factors for atherosclerosis, such as age, duration of diabetes, degree of obesity, blood pressure and glucose control did not differ between patients who were positive or negative for flushing. Frequency of the past history of macrovascular diseases also did not differ between the two groups, and this would be due to small numbers of the participants in this study.

To explore the cause of lower mean IMT in spite of higher TG and lower HDL concentrations in type 2 diabetic patients with alcohol flushing, we reviewed patients' histories of habitual alcohol intake. As expected, diabetic patients with alcohol flushing had a lower frequency of habitual alcohol intake, consistent with a previous report that frequency of alcohol consumption in diabetic patients with inactive ALDH2 was lower than that in patients with active ALDH2 [21]. Although light alcohol consumption generally lowers the risk of cardiovascular events and increases insulin sensitivity, light or moderate alcohol consumption was not found to be associated with increased insulin sensitivity after adjustment for BMI and waist circumference in diabetic patients [32]. Moreover, extensive alcohol consumption was found to promote the progress of atherosclerosis [33]. These results suggest that low frequency of alcohol intake in diabetic patients with alcohol flushing could contribute to lower mean IMT and have a beneficial effect on atherosclerosis. Consistently, the frequency of alcohol consumption and the plaque score of the carotid arteries was lower in patients having metabolic diseases with inactive ALDH2 [25]. Further studies are necessary to reveal the exact mechanisms regarding the association of alcohol flushing with lesser degree of carotid atherosclerosis in diabetic patients.

Exploration of the relationships between IMT and clinical characteristics revealed that mean IMT was positively associated with age, LDL concentration and SBP, and negatively associated with alcohol flushing by stepwise regression analysis (Table 2). Multivariate analysis also revealed that mean IMT was positively associated with age and LDL concentration, and negatively with alcohol flushing (Table 3). Consistently, previous studies have suggested that high LDL concentration and hypertension could contribute to increase in mean IMT [30, 31]. In addition to these classical risk factors, mean IMT was found to be negatively associated with alcohol flushing in the present study.

One of the limitations of the present study was that it was a retrospective study involving a rather small number

of type 2 diabetic patients. The results should be cautiously interpreted. Further investigation involving a larger number of type 2 diabetic patients is warranted to evaluate the mechanisms and relationship between alcohol flushing and atherosclerosis. Although the alcohol dehydrogenase 2 (ADH2) genotype could also affect alcohol flushing responses, the effect did not seem to be so strong as compared to that by the ALDH2 genotype [18]. In fact, alcohol flushing has been shown to be strongly associated with inactive ALDH2 in the Japanese population [15–18]. The frequency of positive alcohol flushing in the present study (76/123; 62%) was consistent with the reported frequency of inactive ALDH2 in the Japanese population (approximately 50%) [15, 21, 25]. However, we could not examine the genotype of ALDH2 as well as ADH2, and exact amount of alcohol consumption of the participants. It remains to be elucidated whether analysis on the relationships between alcohol flushing or ALDH2 genotype and alcohol consumption is of use to identify high-risk diabetic patients for atherosclerosis.

In conclusion, we showed that Japanese type 2 diabetic patients with alcohol flushing had higher TG and lower HDL concentrations, lower frequency of habitual alcohol intake, and lower mean IMT. Stepwise multiple regression analysis demonstrated that mean IMT was associated with age, LDL concentration, SBP, and alcohol flushing. Multivariate analysis also demonstrated that mean IMT was associated with age, LDL concentration, and alcohol flushing. Therefore, alcohol flushing is independently associated with lesser degree of carotid atherosclerosis in Japanese type 2 diabetic patients. Our study could suggest that conventional questionnaires on alcohol flushing and habitual alcohol intake may be important to consider the screening of atherosclerosis in patients with type 2 diabetes mellitus.

Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflict of interest.

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