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SHORT COMMUNICATION

HbA_{1c} variability and the development of microalbuminuria in type 2 diabetes: Tsukuba Kawai Diabetes Registry 2

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Abstract
Aims/hypothesis The aim of this study was to examine the association between HbA_{1c} variability and the development of microalbuminuria as defined by an albumin/creatinine ratio ≥3.4 mg/mmol (≥30 mg/g) in at least two of three consecutive urine samples in Japanese patients with type 2 diabetes.
Methods HbA_{1c} level was measured in 812 serially registered normoalbuminuric adults aged 21–79 years with type 2 diabetes. After registration, a 1-year period to establish baseline values for mean HbA_{1c} and HbA_{1c} variability (measured as the intrapersonal SD of serially collected HbA_{1c}) was decided upon. The association between HbA_{1c} variability and the development of microalbuminuria was determined by Cox regression analysis after adjustment for other risk factors for microalbuminuria.
Results Microalbuminuria occurred in 193 patients during the observation period of (mean±SD) 4.3±2.7 years. Even after adjustment for mean HbA_{1c}, HbA_{1c} variability was a significant predictor of microalbuminuria independently of the mean HbA_{1c}; the HR for every 1% (95% CI) increase in mean HbA_{1c} was 1.22 (1.06, 1.40) (p=0.005), and that for HbA_{1c} variability was 1.35 (1.05, 1.72) (p=0.019). The effects of these two variables were quite similar when 1 SD was used; the HR for every 1 SD increase (95% CI) in HbA_{1c} was 1.23 (1.07, 1.43) (p=0.005), and that for HbA_{1c} variability was 1.20 (1.03, 1.39) (p=0.019).
Conclusions/interpretation HbA_{1c} variability affects the development of microalbuminuria independently of mean HbA_{1c} in type 2 diabetes. Further studies should be performed to evaluate the influence of HbA_{1c} variability on other complications and in individuals of other ethnicities with type 2 diabetes.

Keywords Haemoglobin A_{1c} · Microalbuminuria · Prospective

Abbreviation
ACR Urinary albumin/creatinine ratio

Introduction

The risk of vascular complications increases exponentially as the mean HbA_{1c} level increases [1]. Furthermore, it has been suggested that HbA_{1c} variability, which is expressed as the intrapersonal SD of serially measured HbA_{1c}, is also an independent risk factor for the development of diabetic complications in individuals with type 1 diabetes [2, 3]. However, it is unknown whether HbA_{1c} variability influences the development of microvascular complications in those with type 2 diabetes since risk factors for diabetic complications have been reported to vary depending on the type of diabetes [4]. Sone and colleagues reported that risk factors for diabetic neuropathy were different between patients with type 1 and type 2 diabetes [4]. Moreover, we are not aware of previous studies undertaking a qualitative comparison between mean HbA_{1c} and HbA_{1c} variability using 1 SD increments, which allows for a direct comparison of the effects of these two variables, except for the study of Waden et al. [3] that compared the effect of these

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two variables on categorised data using Kaplan–Meier analysis.

Therefore, we have prospectively investigated the following in Japanese patients with type 2 diabetes: (1) whether HbA_{1c} variability is associated with the development of microalbuminuria; and (2) whether mean HbA_{1c} or HbA_{1c} variability has the stronger effect. In addition, in this study, we selected a 1-year period beginning immediately after enrollment in the registry during which we calculated the mean HbA_{1c} and HbA_{1c} variability to acquire baseline information that would provide highly accurate information for Cox regression analysis.

Methods

The study population comprised 1,713 type 2 diabetic individuals aged 21–79 years who had been consecutively registered on the Tsukuba Kawai Diabetes Registry database [5] from 2000 to 2007 beginning at their first visit to the Kawai Clinic. This clinic is a typical outpatient diabetes referral centre located in a suburb of Tokyo. Participants who had fewer than two measurements of their urinary albumin/creatinine ratio (ACR; n=284), dropped out during the 1-year period after enrollment (n=29), were not normoalbuminuric (n=562), had a high serum creatinine level (>130 μmol/l; n=2), or had a history of cancer (n=24) or cardiovascular disease (n=1), were excluded. Subsequently, 812 normoalbuminuric patients (558 males, 254 females) were eligible for the current analysis.

During the 1-year period after enrollment in the registry, we accumulated data on the mean HbA_{1c} and HbA_{1c} variability for baseline information. Participants were defined as normoalbuminuric if their ACR was <3.4 mg/mmol for their first and second urine samples, and were considered microalbuminuric if the ACR was ≥3.4 mg/mmol for at least two of three consecutive urine samples. During the follow-up period, ACR was examined every 6 months by turbidimetric immunoassay (Microalbumin-HA test; Wako Pure Chemicals, Osaka, Japan) and HbA_{1c} was examined using HPLC (HLC-723; TOSOH, Tokyo, Japan). The value for HbA_{1c} was estimated as a National Glycohemoglobin Standardization Program equivalent value calculated using the formula [6]: HbA_{1c} (%)=HbA_{1c} (Japan Diabetes Society) (%)+0.4.

At baseline, serum lipid levels (determined by a direct enzyme method, BM-80-60; Nihon Denshi, Tokyo, Japan), blood pressure (measured by a doctor with the patient seated) and smoking status (never/ever) were also assessed.

The observation period began at the patient's first clinic visit after the 1-year period in which data were collected on mean HbA_{1c} and HbA_{1c} variability and lasted up to the date of development of microalbuminuria or to the last ACR measurement. HbA_{1c} variability was calculated as the intrapersonal SD for HbA_{1c} for each patient during the 1-year period during which the baseline was established. The difference in number of HbA_{1c} assessments between patients was adjusted according to the formula [2]: $SD/\sqrt{[n/(n-1)]}$. To compare the direct impact of mean HbA_{1c} and HbA_{1c} variability, we changed the increment from 1% to 1 SD (mean±SD being 0.0±1.0).

The study protocol was consistent with the Japanese government's Ethical Guidelines Regarding Epidemiological Studies in accordance with the Declaration of Helsinki and was reviewed by the institutional review board. All participants gave their informed consent. We used χ^2 tests to compare proportions and t tests to compare the variables between normoalbuminuria and microalbuminuria. Cox regression analysis was used to determine whether HbA_{1c} variability was an independent predictor of microalbuminuria. All statistical analyses were performed using SPSS (version 17.0 for Windows; SPSS, Chicago, IL, USA).

Results

During the 1-year period in which baseline values were established, the median (range) number of HbA_{1c} measurements per patient was 11.0 (5–12) and the mean (SD) follow-up period was 4.3 (2.7) years. At baseline, the mean age of the participants was 54.9 (10.4) years. Of these participants, 727 used glucose-lowering agents (625, oral hypoglycaemic agents; 102, insulin), and 259 patients used antihypertensive agents. The proportion of patients who used ACE inhibitors/angiotensin II receptor blockers did not differ significantly between those with normoalbuminuria and those with microalbuminuria (7.5% vs 12.2%; p=0.15). Table 1 shows the baseline characteristics of study participants who did or did not subsequently develop microalbuminuria. Participants who developed microalbuminuria (n=193) were significantly younger, had had diabetes for a longer period, and had a higher mean HbA_{1c} and a higher ACR than participants who remained normoalbuminuric. HbA_{1c} variability was not significant but was greater in participants with microalbuminuria than in those with normoalbuminuria.

The results of Cox regression analysis showed that, in addition to known predictors of nephropathy, both mean HbA_{1c} and HbA_{1c} variability were significant and independent risk factors for microalbuminuria (Table 2, model 1). By using a 1 SD increment instead of a 1% increment, we showed that the HRs of these two variables were similar (Table 2, model 2).

Discussion

We clarified that HbA_{1c} variability was independently associated with the development of microalbuminuria in our

Table 1 Baseline characteristics of those who did or did not subsequently develop microalbuminuria

Variable	Normoalbuminuria	Microalbuminuria	*p* value
n	619	193	
Age (years)	54.3±10.2	56.8±10.9	0.003
Sex (% male)	69	67.9	0.772
Duration of diabetes (years)	5.8±6.2	7.8±8.1	0.002
Mean HbA_{1c} (%)	7.0±1.0	7.4±1.2	0.001
Mean HbA_{1c} (mmol/mol)	53.4±11.1	56.8±12.8	0.001
HbA_{1c} variability (%)	0.79±0.60	0.88±0.62	0.110
Systolic blood pressure (mmHg)	125±11	128±10	0.001
Body mass index (kg/m^2)	24.7±3.6	25.4±4.0	0.031
Total cholesterol (mmol/l)	5.3±1.0	5.1±0.8	0.011
HDL-cholesterol (mmol/l)	1.4±0.4	1.4±0.4	0.248
ACR (mg/mmol)	1.1±0.5	1.7±0.6	<0.001
Ever smoker (%)	60.6	67.4	0.090
Retinopathy (%)	19.4	28.5	0.007
Neuropathy (%)	6.5	9.3	0.117

Data are expressed as numbers, means±SD, or percentages

The value for HbA_{1c} was estimated as a National Glycohemoglobin Standardization Program equivalent value calculated using the formula [6]: HbA_{1c} (%)=HbA_{1c} (Japan Diabetes Society) (%)+0.4

Japanese patients with type 2 diabetes even after adjustment for known predictors of nephropathy [5]. To our knowledge, this is the only prospective study to examine this topic in individuals with type 2 diabetes except for one Korean study that determined the progression of carotid artery intima–media thickness [7] and compared the direct impact of HbA_{1c} variability and mean HbA_{1c}. This association was in accordance with results of studies of type 1 diabetes [2, 3, 8], especially with regard to findings by Waden and colleagues in which adjustments were made for blood pressure and smoking status [3]. We found that HbA_{1c} variability had an effect on the development of microalbuminuria similar to that of mean HbA_{1c}, since the HRs of these two variables were similar, a finding that was also in accordance with results of Waden et al. [3] shown by Kaplan–Meier survival curves.

Table 2 Cox regression models for the development of microalbuminuria

Variable	HR (95% CI)	*p* value
Age (years)	1.02 (1.00, 1.04)	0.013
Male sex (%)	0.70 (0.47, 1.06)	0.089
Duration of diabetes (years)	1.03 (1.01, 1.05)	0.005
Systolic blood pressure (mmHg)	1.01 (1.00, 1.03)	0.090
Body mass index (kg/m^2)	1.05 (1.01, 1.10)	0.016
Total cholesterol (mmol/l)	0.87 (0.73, 1.03)	0.097
HDL-cholesterol (mmol/l)	0.90 (0.59, 1.40)	0.650
Ever smoker (%)	1.68 (1.13, 2.49)	0.010
Model 1: 1% increment		
Mean HbA_{1c} (%)	1.22 (1.06, 1.40)	0.004
HbA_{1c} variability (%)	1.35 (1.05–1.72)	0.019
Model 2: 1 SD increment		
Mean HbA_{1c}	1.23 (1.07, 1.43)	0.005
HbA_{1c} variability	1.20 (1.03, 1.39)	0.019

Model 1: effect of 1% increments in mean HbA_{1c} and HbA_{1c} variability (intrapersonal SD of HbA_{1c})

Model 2: comparison of direct effect of mean HbA_{1c} and HbA_{1c} variability using 1 SD increments

The observation period was the time variable in the Cox proportional hazards model. All models were adjusted by various known predictors of nephropathy in addition to those related to HbA_{1c} (upper section of the table). Mean HbA_{1c} and HbA_{1c} variability were calculated during the 1-year period before start of the study, and other variables were obtained at the start of the study

HRs of variables except mean HbA_{1c} and HbA_{1c} variability were determined by multivariate analysis from model 1. The result did not change in model 2

Although the mechanisms of the association between HbA_{1c} variability and the development of vascular complications are unclear from this observational study, increasing oxidative stress is speculated to be one explanation since unstable glucose fluctuation induces greater oxidant production [9]. Monnier and colleagues showed a strong relationship between oxidative stress and daily glucose variability [9]. However, it is yet to be clarified whether there is a correlation between short-term glucose variability and HbA_{1c} variability. Future study is expected to clarify the pathway.

The strength of the current study is that we used a 1-year period to calculate the baseline data of mean HbA_{1c} and HbA_{1c} variability for use in Cox regression analysis. Another strength is that we measured HbA_{1c} much more frequently than in previous studies [2, 3, 7, 8], which could contribute to the reliability of the data. Such a testing frequency is routine in clinical settings in Japan. In addition, because the use of monthly measurements of HbA_{1c} might cause partial interdependence, we performed a reanalysis using 3-monthly measurements of HbA_{1c} and found that

results were no different (HbA_{1c} variability for 1% increase: HR [95% CI]=1.33 [1.02, 1.72]; p=0.036).

In contrast, the fact that the study participants were from a single clinic in Japan is one of the limitations. However, the characteristics of our study participants are similar to those reported in a previous large-scale study in Japan [10]. Another limitation is that the hyperglycaemic treatment modalities (i.e. oral agents and insulin) or use of an ACE inhibitor was not explored in the context of HbA_{1c} variability. However, the results of the present study are very much in line with those of previous studies of type 1 diabetes [2, 3, 7, 8]. In addition, changes in HbA_{1c} during the entire observation period before the development of microalbuminuria were not taken into account in this study. However, this is also a limitation of Cox regression analysis since this method is a way to predict future risk using present data, and further analysis is needed to clarify it.

In conclusion, HbA_{1c} variability might be a risk factor for the development of microalbuminuria in patients with type 2 diabetes, and the impact of the effect might be similar to that of the mean HbA_{1c}. Further studies are recommended to evaluate complications other than nephropathy and complications in other ethnic groups with type 2 diabetes.

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Contribution statement AS designed and conducted the study, analysed the results and wrote the manuscript. KK, SM and KY contributed to the acquisition of data, review of the data, and discussion of the data, and reviewed and edited the manuscript. KS, SK, YY, RH, HSh and HSo designed and conducted the study, analysed the results, and critically reviewed and edited the manuscript. All the authors gave final approval of the version to be published.

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