ORIGINAL ARTICLE

Associations between urinary 6-sulfatoxymelatonin excretion and diabetic vascular complications or arteriosclerosis in patients with type 2 diabetes

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ABSTRACT

Aims/Introduction: There are limited reports on the association between melatonin levels and vascular complications in patients with type 2 diabetes. The aim of this study was to determine the association between urinary 6-sulfatoxymelatonin, which is a urinary metabolite of melatonin, and diabetic vascular complications or arteriosclerosis in patients with type 2 diabetes.

Materials and Methods: This retrospective study included patients (167 patients with type 2 diabetes and 27 patients without diabetes adjusted for age and sex) admitted to the hospital who underwent measurement of urinary 6-sulfatoxymelatonin. The urinary 6-sulfatoxymelatonin/creatinine ratio (6-SMT) was calculated.

Results: The natural logarithmically scaled 6-SMT level (Ln 6-SMT) was significantly lower in type 2 diabetes patients (1.9 ± 1.1) compared with patients without diabetes (2.8 ± 1.0, $P < 0.001$). Multivariate linear regression analysis identified duration of diabetes, smoking status, urinary albumin-to-creatinine ratio, retinopathy and coronary heart disease as factors that could influence Ln 6-SMT levels in type 2 diabetes patients ($R^2 = 0.232$, $P < 0.001$). Ln 6-SMT was associated with decreased odds of diabetic retinopathy, even after adjustment for various confounding factors (odds ratio 0.559, 95% confidence interval 0.369–0.846, $P = 0.006$). Similarly, Ln 6-SMT was associated with decreased odds of coronary heart disease (odds ratio 0.442, $P = 0.030$).

Conclusions: Our results showed the presence of low levels of Ln 6-SMT in type 2 diabetes patients relative to patients without diabetes. Furthermore, Ln 6-SMT is an independent risk factor of diabetic retinopathy and coronary heart diseases. These findings suggest that 6-SMT could be a useful biomarker for the prediction of micro- and macrovasculopathies in patients with type 2 diabetes.

INTRODUCTION

Melatonin is a hormone secreted by the pineal body, and is involved in the regulation of the human circadian clock and regulated by exposure to light[1]. Melatonin levels follow a circadian rhythm, with a peak level at 3–5 h after the onset of sleep at night and almost no production of the hormone during daytime[2]. The hormone is reported to have protective effects against arteriosclerosis based on the reduction of low-density lipoprotein cholesterol (LDL-C) levels, lowering blood pressure and having an anti-oxidant effect through the removal of reactive oxygen species[3,4]. In addition, it has also been reported that low melatonin levels are associated with the development of atherosclerotic lesions, such as coronary heart disease and cerebrovascular disorders[5,6].

Single-nucleotide polymorphisms of the melatonin receptor (MTNR1b) are reported to be associated with increases in fasting plasma glucose (FPG) and hemoglobin A1c (HbA1c) levels, and the probability of developing type 2 diabetes[7,8]. In addition, as low melatonin levels are considered to be associated with the development of type 2 diabetes[9], they have also been shown to

adversely affect glucose metabolism. Furthermore, it is also suggested that nocturnal melatonin secretion is lower in patients with type 2 diabetes than in healthy people[10,11]. Hypomelatoninemia has been reported in type 2 diabetes patients with autonomic neuropathy[11] and proliferative retinopathy[12]. Low levels of 6-sulfatoxymelatonin, which is a urinary metabolite of melatonin, were also observed in type 2 diabetes patients with proliferative retinopathy[13]. However, there is little or no information on the association between melatonin/6-sulfatoxymelatonin and diabetic micro- and macrovasculopathies. The present study was designed to determine the association between urinary 6-sulfatoxymelatonin and diabetic vascular complications/arteriosclerosis in patients with type 2 diabetes.

METHODS

Patients

Of the patients with and those without type 2 diabetes aged between 20 and 80 years who were admitted to the Hospital of the University of Occupational and Environmental Health, Kitakyushu, Japan, between September 2014 and December 2018, we retrieved the medical records of those patients who underwent testing for urinary 6-sulfatoxymelatonin levels. The patients without diabetes included in the analysis were adjusted for age and sex to those of the diabetes group. The following exclusion criteria were applied: patients with type 1 diabetes mellitus, patients with severe infection or serious trauma, patients who were found to have abnormal endocrine hormone levels (excluding those with hormone levels within the normal ranges corrected by treatment), patients with renal dysfunction (estimated glomerular filtration rate [eGFR] of <30 mL/min/1.73 m^2), patients receiving oral melatonin or MTNR1b agonists, patients with cognitive decline (Mini-Mental State Examination score ≤23) and patients who were known to have malignant tumors within the previous 5 years.

Diabetic complications were evaluated in the present study as follows: diabetic retinopathy was diagnosed based on funduscopic examination carried out by expert ophthalmologists and classified according to the Davis classification into no diabetic retinopathy; simple retinopathy; pre-proliferative retinopathy; and proliferative retinopathy. Diabetic nephropathy was considered present when the urinary albumin-to-creatinine ratio (UACR) was ≥30 mg/g creatinine and/or eGFR < 30 mL/1.73 m^2, in line with the Classification of Diabetic Nephropathy 2014 in Japan[14]. Diabetic peripheral neuropathy was diagnosed by the presence of two or more clinical features (bilateral spontaneous pain, hypoesthesia or paresthesia of the legs), absence of Achilles tendon reflexes and/or decreased vibration sensations in response to a C128 tuning fork. Diabetic autonomic neuropathy was defined as a coefficient of variation of the R-R interval of <2%, as measured by the electrocardiogram. Patients who had already been diagnosed with coronary heart disease, cerebrovascular disease or arteriosclerosis obliterans were considered to have macrovascular complications at the time of

enrollment. In addition, we evaluated the smoking status on admission (never, former, current).

The study was approved by the institutional ethics committee of the Hospital of the University of Occupational and Environmental Health, Kitakyushu, Japan (Approval No. H27-186). Informed consent was obtained from all participants.

Study protocol

The present study followed a retrospective study design. The blood samples were collected before breakfast and first morning urine samples were collected by hospital day 5.

Urinary 6-sulfatoxymelatonin excretion

The first morning urine samples were collected by hospital day 5 and stored at −80°C.

The assay for the urinary 6-sulfatoxymelatonin was carried out in the School of Medicine Laboratory in the First Department of Internal Medicine, the University of Occupational and Environmental Health, Kitakyushu, Japan. Urinary 6-sulfatoxymelatonin was measured using a competitive enzyme-linked immunosorbent assay (product code EK-M6S; Bühlmann Laboratories AG, Allschil, Switzerland). This assay has a lower limit of detection of 0.8 ng/mL for 6-sulfatoxymelatonin. The levels of 6-sulfatoxymelatonin were reported relative to urinary creatinine levels (6-SMT).

Biochemical and clinical measurements

Blood pressure, bodyweight, HbA1c, FPG, fasting plasma insulin, eGFR and UACR were measured. HbA1c levels (%) were measured by using a high-performance liquid chromatography method with a Tosoh HLC-723 G8 analyzer (Tosoh Co., Kyoto, Japan) and expressed in National Glycohemoglobin Standardization Program (NGSP) equivalent values calculated using the following equation: HbA1c (NGSP) = HbA1c (Japan Diabetes Society) (%) + 0.4%[15]. The homeostasis model assessment for insulin resistance value was calculated by the formula, FPG (m/dL) × fasting plasma insulin (μU/mL)/405. The homeostasis model assessment for β-cell function was calculated using the following formula: fasting plasma insulin (μU/mL) × 360 / (FPG [mg/dL] − 63). LDL-C was measured using the Cholestest LDL (Sekisui Medical, Tokyo, Japan) by a direct method. High-density lipoprotein cholesterol was measured using the Cholestest N HDL (Sekisui Medical) by a direct method. Triglycerides were measured using the Pureauto S TG-N (Sekisui Medical) by an enzymatic method. Total homocysteine levels were measured by using high-performance liquid chromatography (SRL Co., Tokyo, Japan). Carotid intima-media thickness (IMT) was measured by a well-trained medical technologist at the Hospital of the University of Occupational and Environmental Health. The largest IMT value was defined as the maximum IMT. The mean values of the right and left maximum IMT were used for statistical analysis. Plaques, when identified, were included in the IMT measurement. The coefficient of variation of the R-R interval was calculated by using

the Cardio Star FCP-7541 (Fukuda Denshi, Tokyo, Japan). Based on the mean R-R intervals and the R-R standard deviation, coefficient of variation of the R-R interval was calculated as R-R standard deviation / mean R-R interval $\times$ 100 (%) (reference range: >2%).

Statistical analysis

The sample size was calculated by using G*Power software (version 3.1.9.2; Franz, Universitat Kiel, Germany) to determine the number of patients required to identify whether 6-SMT varied between patients with and without diabetic microvascular complications. The sample size was based on primary outcomes, and assumed a moderate effect of 6-SMT and diabetes microvascular complication, according to Cohen[16], with an effect size of 0.5. For a power of 80%, a 0.05 level of statistical significance and 30% prevalence of diabetic microvascular complication, the sample size for patients with diabetes with or without microvascular complication was calculated to be 46 and 106, respectively. Therefore, at least 152 patients with type 2 diabetes were required.

When the 6-sulfatoxymelatonin levels were below the detection limit of the assay (0.8 pg/mL), which was the case in 24 patients with type 2 diabetes and one patient without diabetes, we substituted the value of the detection limit for normalization to creatinine. The value of 6-SMT was naturally log transformed, because the distribution of the data was skewed (Ln 6-SMT).

Data are expressed as the mean $\pm$ standard deviation. Data distribution was assessed by the Shapiro–Wilk test. Categorical variables were tested using the χ^2-test. Differences between patients without diabetes and patients with type 2 diabetes were tested by the paired t-test for normally distributed data and the Wilcoxon signed-rank test for data with skewed distribution. Correlation analyses between Ln 6-SMT level and various parameters measured at baseline were carried out using Pearson's correlation analysis for normally distributed variables and Spearman's correlation analysis for variables with skewed distribution.

Univariate linear regression analysis was carried out with Ln 6-SMT as the dependent variable and the variables listed in Table 1 as the independent variables. In the multivariate linear regression analysis, the selected independent variables were age, sex and all parameters with a P-value <0.05 in the aforementioned univariate linear regression analysis. Univariate and multivariate logistic regression analyses were also carried out to determine the association of Ln 6-SMT with diabetic vasculopathy. In the multivariate logistic regression analysis, the selected independent variables were age, sex, HbA1c and all parameters with a P-value of <0.25 in the univariate logistic regression analysis. Data are expressed as odds ratios and 95% confidence intervals. Statistical significance was set at a P-value of <0.05. All statistical analyses were carried out using SPSS version 25.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Patient demographics

Approximately 80% of the patients without diabetes were admitted to our hospital for further assessment of thyroid tumors (Table S1). The clinical characteristics of the study patients are shown in Table 1. Compared with the patients without diabetes, those with type 2 diabetes had significantly higher body mass index, were more likely to be hypertensive and current smokers, have dyslipidemia, with significantly lower high-density lipoprotein levels. For the diabetes patients, the mean duration of diabetes was 8.3 years; HbA1c levels were 9.3%, and the homeostasis model assessment for insulin resistance value was 3.5. Their blood glucose levels were poorly controlled, and insulin resistance was common. In addition, the Ln 6-SMT levels were significantly lower among the patients with diabetics (1.9 $\pm$ 1.1 and 2.8 $\pm$ 1.0, respectively; P < 0.001) than the patients without diabetes.

Relationship between Ln 6-SMT level and baseline parameters

Table 2 shows the correlation between Ln 6-SMT levels and the clinical characteristics of the diabetes patients measured at baseline. Ln 6-SMT levels correlated negatively and significantly with age and duration of diabetes, and positively and significantly with eGFR and LDL-C levels. There was no significant correlation with HbA1c or insulin secretion. In contrast, Ln 6-SMT levels correlated negatively and significantly with UACR, total homocysteine levels, and carotid IMT.

Univariate and multivariate linear regression analysis with Ln 6-SMT as the dependent variable in type 2 diabetes patients

Table 3 shows a significant positive relationship between Ln 6-SMT level and each of eGFR, LDL-C and no use of glucose-lowering agents, and a negative relationship with age, duration of diabetes, current smoking status, each of the diabetic microvascular complications, coronary heart disease, cerebrovascular disease, use of insulin, total homocysteine level, carotid IMT and carotid plaque. Multivariate linear regression analysis that included the aforementioned factors identified the duration of diabetes, smoking status, UACR, retinopathy and coronary heart disease to be significant and independent correlates with Ln 6-SMT level (R^2 = 0.232, P < 0.001).

Diabetic retinopathy, coronary heart disease and Ln 6-SMT levels

Finally, we examined the role of 6-SMT in the progression of diabetic retinopathy and coronary heart disease, based on their significant association with Ln 6-SMT level in the multivariate linear regression analysis. In the univariate logistic regression analysis, Ln 6-SMT was negatively associated with diabetic retinopathy; it was associated with decreased odds of diabetic retinopathy, even after adjustment for various confounding factors (odds ratio 0.559, 95% confidence interval 0.369–0.846,

Table 1 | Baseline characteristics of the patients

	Without diabetes	Type 2 diabetes	*P*-value
n	27	167	
Age (years)	57.8 ± 15.7	58.6 ± 12.3	0.747
Sex (men/women)	15/ 12	99/ 68	0.715
Duration of diabetes (years)	–	8.3 ± 8.9	–
Body mass index (kg/m^2)	25.0 ± 3.9	27.2 ± 5.6	0.049
Systolic blood pressure (mmHg)	127.9 ± 14.4	132.1 ± 15.8	0.194
Diastolic blood pressure (mmHg)	78.8 ± 14.4	78.6 ± 11.9	0.934
CVR-R (%)	–	3.1 ± 1.4	–
FPG (mg/dL)	91.8 ± 7.5	155.2 ± 42.5	<0.001
HbA1c (%)	5.5 ± 0.3	9.3 ± 2.0	<0.001
Fasting plasma insulin (μg/mL)	7.4 ± 2.8	9.2 ± 6.3	0.160
HOMA-IR	1.7 ± 0.7	3.5 ± 2.4	<0.001
HOMA-β (%)	93.2 ± 35.3	42.1 ± 35.5	<0.001
Serum C-peptide (ng/mL)	2.1 ± 0.4	2.3 ± 1.1	0.380
CPR index	2.3 ± 0.3	1.6 ± 0.9	<0.001
EGFR (mL/min/1.73 m^2)	76.3 ± 17.0	79.7 ± 23.0	0.457
UACR (mg/g Cre)	8.1 ± 6.8	157.2 ± 585.7	0.215
LDL-C (mg/dL)	122.6 ± 20.5	118.7 ± 38.9	0.625
HDL-C (mg/dL)	53.7 ± 13.9	47.3 ± 12.1	0.014
Triglycerides (mg/dL)	129.5 ± 61.0	153.3 ± 84.6	0.170
6-Sulfatoxymelatonin (ng/mL)	32.7 ± 40.7	13.3 ± 21.0	<0.001
Urinary creatinine (mg/dL)	124.7 ± 79.6	102.5 ± 68.4	0.129
6-SMT (ng/mg)	25.8 ± 25.0	11.2 ± 11.4	<0.001
Ln 6-SMT (ng/mg)	2.8 ± 1.0	1.9 ± 1.1	<0.001
Carotid IMT (mm)	0.8 ± 0.3	1.0 ± 0.4	0.074
Carotid plaque (%)	50	61	0.328
Total homocysteine (nmol/L)	9.9 ± 2.5	10.8 ± 4.4	0.316
Hypertension (%)	48	69	0.003
Dyslipidemia (%)	48	75	<0.001
Antihypertensive agents (%)	37	46	0.411
Antilipidemic agents (%)	15	36	0.030
Smoking status, never/former/current (%)	70/22/7	44/25/31	0.018
Glucose-lowering agents used			
None (%)	–	38	–
DPP-4 inhibitors (%)	–	45	–
Sulfonylurea (%)	–	31	–
Glinide (%)	–	4	–
Biguanides (%)		22	–
Thiazolidine (%)		10	–
α-Glucosidase inhibitors (%)	–	11	–
SGLT-2 inhibitors (%)		1	–
Insulin (%)		16	
GLP-1 receptor agonists (%)	–	2	–
Diabetic microvasculopathies			
Retinopathy (%)	–	34	–
Nephropathy (%)	–	28	–
Peripheral neuropathy (%)	–	38	–
Autonomic neuropathy (%)	–	14	–
Diabetic macrovasculopathies			
Coronary heart disease (%)	–	7	–

Table 1 (Continued)

	Without diabetes	Type 2 diabetes	P-value
Cerebrovascular disease (%)	–	7	–
Arteriosclerosis obliterans (%)	–	2	–

Data are the mean ± standard deviation, or n (%). Comparisons between patients without diabetes and patients with type 2 diabetes were carried out using the paired t-test for normally distributed data and Wilcoxon signed-rank test for data with skewed distribution. Categorical values were tested by the χ^2-test. P-values are for differences between the two groups. 6-SMT, 6-sulfatoxymelatonin/urine creatinine; Carotid IMT, carotid intima-media thickness; CPR index, serum C-peptide index; CVR-R, coefficient of variation of the R-R interval; DPP-4, dipeptidyl peptidase-4; EGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; GLP-1, glucagon-like peptide-1; HbA1c, glycated hemoglobin; HDL-C, high-density cholesterol; HOMA-IR, homeostasis model assessment for insulin resistance; HOMA-β, homeostasis model assessment for β-cell function; LDL-C, low-density cholesterol; Ln 6-SMT, natural logarithmically scaled 6-sulfatoxymelatonin/urine creatinine; SGLT-2, sodium–glucose cotransporter 2; UACR, urinary albumin-to-creatinine ratio.

Table 2 | Correlation coefficients between natural logarithmically scaled 6-sulfatoxymelatonin/urine creatinine levels and various parameters measured at baseline of patients with type 2 diabetes

Ln 6-SMT	r	P-value
Age	−0.172	0.026
Duration of diabetes	−0.292	<0.001
Body mass index	0.066	0.393
Systolic blood pressure	0.044	0.568
Diastolic blood pressure	0.130	0.095
FPG	0.031	0.690
HbA1c	0.065	0.404
Fasting plasma insulin	0.037	0.670
HOMA-IR	0.019	0.828
HOMA-β	0.031	0.718
Serum C-peptide	−0.027	0.731
CPR index	−0.003	0.973
eGFR	0.260	0.001
UACR	−0.220	0.004
LDL-C	0.240	0.002
HDL-C	0.005	0.954
TG	0.012	0.954
Carotid IMT	−0.175	0.025
Total homocysteine	−0.230	0.003

Data are the results of Pearson correlation analysis for normally distributed variables and Spearman rank correlation for variables with skewed distribution. 6-SMT, 6-sulfatoxymelatonin/urine creatinine; Carotid IMT, carotid intima-media thickness; CPR index, serum C-peptide index; CVR-R, coefficient of variation of the R-R interval; DPP-4, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; GLP-1, glucagon-like peptide-1; HbA1c, glycated hemoglobin; HDL-C, high-density cholesterol; HOMA-IR, homeostasis model assessment for insulin resistance; HOMA-β, homeostasis model assessment for β-cell function; LDL-C, low-density cholesterol; Ln 6-SMT, natural logarithmically scaled 6-sulfatoxymelatonin/urine creatinine; SGLT-2, sodium–glucose cotransporter 2; TG, triglycerides; UACR, urinary albumin-to-creatinine ratio.

$P = 0.006$; Table 4). Similarly, Ln 6-SMT was associated with decreased odds of coronary heart disease (odds ratio 0.442, 95% confidence interval 0.211–0.924, $P = 0.030$; Table 5).

DISCUSSION

The presence of low levels of melatonin and/or 6-SMT in type 2 diabetes patients has been described previously[10,11]. With regard to the association between melatonin and diabetic vascular complications, previous studies reported the association of melatonin with autonomic disorders and proliferative retinopathy, although the association of melatonin with macrovascular complications, such as arteriosclerosis, remains unclear. The present study is the first to show that 6-SMT is associated with the risks of diabetic microvasculopathies and macrovasculopathies. Furthermore, based on the finding that Ln 6-SMT level is an independent risk factor for retinopathy and heart disease, the present results suggest that 6-SMT can be a potentially useful biomarker for retinopathy and heart disease in patients with type 2 diabetes. As UACR was significantly associated with Ln 6-SMT levels, as shown in Table 3, we also examined the relationship between diabetic nephropathy and Ln 6-SMT levels. In the univariate logistic regression analysis, Ln 6-SMT was negatively associated with diabetic nephropathy; however, multivariate logistic regression analysis showed Ln 6-SMT was not an independent factor for diabetic nephropathy (data not shown).

Several studies have shown that melatonin secretion, particularly nocturnal melatonin secretion, is decreased in patients with type 2 diabetes[10,11]. What is the mechanism responsible for such decrease? It is known that hyperglycemia inhibits various melatonin synthesis pathways, such as β-adrenergic receptor, cyclic adenosine monophosphate content, arylalkylamine N-acetyltransferase protein content and activity, and sodium–potassium adenosine triphosphatase activity[17]. In addition, blood melatonin levels were shown to be strongly correlated with 6-SMT levels[18]. In the present study, 6-SMT levels were significantly lower in patients with type 2 diabetes compared with those in patients without diabetes.

The present study showed a negative association between Ln 6-SMT and various diabetic vascular complications, including retinopathy, nephropathy, peripheral neuropathy, autonomic neuropathy, coronary heart disease and cerebrovascular disorders. To date, there are only limited studies on the

Table 3 | Results of univariate and multivariate linear regression analysis with natural logarithmically scaled 6-sulfatoxymelatonin/urine creatinine levels as the dependent variable in patients with type 2 diabetes

Variables	Univariate			Multivariate		
	β	95% CI	*P*-value	β	95% CI	*P*-value
Intercept					2.339, 2.874	<0.001
Age	−0.188	−0.030, −0.003	0.015			
Women (vs men)	0.170	−0.422, 0.250	0.615			
Duration of diabetes	−0.272	−0.051, −0.015	<0.001	−0.251	−0.048, −0.013	0.001
Smoking status						
Never (reference)						
Former	−0.124	−0.722, 0.100	0.138			
Current	−0.167	−0.769, −0.005	0.047	−2.663	−0.768, −0.114	0.009
eGFR	0.236	0.004, 0.018	0.002			
UACR	−0.216	−0.001, <−0.001	0.005	−0.166	−0.001, <−0.001	0.025
LDL-C	0.210	0.002, 0.010	0.007			
Carotid IMT	−0.212	−0.876, −0.145	0.006			
Carotid plaque	−0.192	−0.752, −0.087	0.014			
Total homocysteine	−0.185	−0.083, −0.008	0.017			
No use of glucose-lowering agents	0.282	0.305, 0.977	<0.001			
Use of insulin	−0.185	−0.982, −0.100	0.016			
Retinopathy	−0.290	−0.995, −0.325	<0.001	−0.211	−0.811, −0.143	0.005
Nephropathy	−0.236	−0.921, −0.207	0.002			
Peripheral neuropathy	−0.229	−0.840, −0.176	0.003			
Autonomic neuropathy	−0.209	−1.122, −0.184	0.007			
Coronary heart disease	−0.213	−1.513, −0.263	0.006	−0.212	−1.451, −0.286	0.004
Cerebrovascular disease	−0.152	−1.318, −0.002	0.049			

Multivariate linear regression analysis with natural logarithmically scaled 6-sulfatoxymelatonin/urine creatinine as the dependent variable, and age, sex and parameters that showed a significant relationship (*P* < 0.05) on univariate regression analysis as the independent variables. Carotid IMT, carotid intima-media thickness; CI, confidence interval; eGFR, estimated glomerular filtration rate; LDL-C, low-density cholesterol; UACR, urinary albumin-to-creatinine ratio.

association of melatonin secretion with diabetic vasculopathies. These studies reported the presence of low blood melatonin levels in type 2 diabetes patients with autonomic neuropathy[11] and proliferative retinopathy[12]. The same studies also showed decreased 6-SMT in type 2 diabetes patients with proliferative retinopathy[13]. The association between melatonin secretion and autonomic neuropathy could be explained by the presence of MTNR1bs on the suprachiasmatic nucleus, which regulates the autonomic nervous system. With regard to the association between melatonin and retinopathy, based on the role of oxidative stress in the progression of retinopathy, it is considered that reduced secretion of melatonin, which has an anti-oxidative stress action, might lead to the progression of retinopathy. Although we did not measure in the present study markers of oxidative stress, our results showed a significant negative association between Ln 6-SMT and total homocysteine levels. In addition to reports that homocysteine increases reactive oxygen species and enhances oxidative stress[19], melatonin is known to suppress homocysteine production[20,21]. These findings suggest that melatonin might promote the progression of retinopathy through oxidative stress, such

as through the effects of homocysteine. In addition, previous studies reported that melatonin exerts protective effects against peripheral neuropathy and nephropathy by reducing oxidative stress in rats[22,23]. The present results add support to this conclusion.

The present study also showed the association of 6-SMT with atherosclerotic lesions, such as heart disease, cerebrovascular disorders, carotid IMT and carotid plaque. These lesions also appeared to be related to the low levels of melatonin, which has anti-oxidative stress and anti-homocysteine actions, causing vascular endothelial dysfunction. In addition, the results of univariate linear regression analysis showed a positive association between Ln 6-SMT and LDL-C among the items listed in Table 3. Although this result seemed to be contradictory, Ln 6-SMT levels tended to be lower in patients with dyslipidemia (1.8 ± 1.1 ng/mg) than non-dyslipidemia (2.2 ± 1.0 ng/mg, *P* = 0.085). As patients with dyslipidemia included those with LDL-C levels controlled to be low by antilipidemic agents, Ln 6-SMT could be associated with LDL-C levels. Similarly, the data in Table 5 implied that SBP was a protective factor for coronary heart disease in type 2 diabetes. Careful assessment

Table 4 | Results of binary logistic regression analyses of potential risk factors for diabetic retinopathy in patients with type 2 diabetes

	Univariate logistic regression			Multiple logistic regression		
	Wald χ^2	P	OR (95% CI)	Wald χ^2	P	OR (95% CI)
Age	0.028	0.868	1.002 (0.976–1.029)			
Women (vs men)	2.979	0.084	1.075 (0.925–3.405)			
Duration of diabetes	5.328	0.021	1.043 (1.006–1.081)			
Systolic blood pressure	4.610	0.032	1.023 (1.002–1.045)			
HbA1c	0.551	0.458	1.064 (0.903–1.255)			
CPR index	5.268	0.022	0.593 (0.380–0.927)			
eGFR	2.657	0.103	0.988 (0.973–1.002)			
UACR	7.368	0.007	1.003 (1.001–1.005)			
Carotid IMT	4.442	0.035	2.190 (1.056–4.540)			
Total homocysteine	4.329	0.037	1.082 (1.005–1.166)			
Ln 6-SMT	13.158	<0.001	0.545 (0.393–0.757)	7.580	0.006	0.559 (0.369–0.846)
No use of glucose-lowering agents	10.673	0.001	0.261 (0.116–0.584)			
Use of insulin	11.303	0.001	4.403 (1.855–10.447)			
Nephropathy	21.3.07	<0.001	5.549 (2.681–11.488)	5.439	0.020	2.884 (1.184–7.025)
Peripheral neuropathy	35.823	<0.001	9.565 (4.566–20.037)	22.627	<0.001	7.680 (3.316–17.789)
Autonomic neuropathy	5.932	0.015	3.053 (1.244–7.498)			
Coronary heart disease	1.517	0.218	2.100 (0.645–6.839)			

Age, sex, HbA1c and factors with $P < 0.25$ on univariate logistic regression analysis were included in this multiple-factor logistic regression analysis. Carotid IMT, carotid intima-media thickness; CI, confidence interval; CPR index, serum C-peptide index; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; Ln 6-SMT, natural logarithmically scaled 6-sulfatoxymelatonin/urine creatinine; OR, odds ratio; UACR, urinary albumin-to-creatinine ratio.

Table 5 | Results of binary logistic regression analyses of potential risk factors for coronary heart disease in patients with type 2 diabetes

	Univariate logistic regression			Multiple logistic regression		
	Wald χ^2	P	OR (95% CI)	Wald χ^2	P	OR (95% CI)
Age	4.050	0.044	1.064 (1.002–1.131)	4.630	0.031	1.078 (1.007–1.154)
Women (vs men)	1.269	0.260	0.462 (0.120–1.772)			
Duration of diabetes	2.040	0.153	1.044 (0.984–1.107)			
Systolic blood pressure	3.960	0.047	0.957 (0.916–0.999)	7.481	0.006	0.920 (0.867–0.977)
HbA1c	0.101	0.750	0.950 (0.695–1.300)			
LDL-C	5.575	0.018	0.978 (0.959–0.996)			
HDL-C	1.907	0.167	0.960 (0.905–1.017)			
Carotid IMT	3.929	0.047	2.876 (1.012–8.175)			
Carotid plaque	3.708	0.054	7.663 (0.964–60.887)			
Total homocysteine	2.189	0.139	1.082 (0.975–1.012)			
Ln 6-SMT	6.675	0.010	0.425 (0.222–0.814)	4.707	0.030	0.442 (0.211–0.924)
Dyslipidemia	1.601	0.206	3.826 (0.479–30.580)			
No use of glucose-lowering agents	2.895	0.089	0.165 (0.021–1.314)			
Use of insulin	5.406	0.020	4.318 (1.258–14.820)			
Retinopathy	1.517	0.218	2.100 (0.645–6.839)			
Nephropathy	2.853	0.091	2.780 (0.849–9.108)	5.665	0.017	6.753 (1.401–32.553)
Peripheral neuropathy	4.134	0.042	3.636 (1.048–12.623)			
Cerebrovascular disease	1.929	0.165	3.244 (0.616–2.384)			

Age, sex, glycated hemoglobin (HbA1c) and parameters with $P < 0.25$ on univariate logistic regression analysis were included in this multiple-factor logistic regression analysis. Carotid IMT, carotid intima-media thickness; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Ln 6-SMT, natural logarithmically scaled 6-sulfatoxymelatonin/urine creatinine; OR, odds ratio.

showed that the number of prescribed antihypertensive agents was significantly larger among type 2 diabetes patients with coronary heart disease, because these patients required stricter control of blood pressure (data not shown). Therefore, type 2 diabetes patients with coronary heart disease had a significantly lower systolic blood pressure compared with those without coronary heart disease (with coronary heart disease 123.3 ± 17.7 mmHg; without coronary heart disease 132.8 ± 15.5 mmHg; $P = 0.046$), which might have affected the results presented in Table 5.

The usefulness of melatonin, which has an anti-oxidant action against oxidative stress, has been reported in both *in vitro* and *in vivo* studies[22-24]. It is assumed that treatment with melatonin or MTNR1b agonists is useful for type 2 diabetes patients with abnormally low melatonin secretion. Indeed, a randomized controlled trial showed that melatonin therapy reduced HbA1c levels in type 2 diabetes patients[25]. In contrast, another trial showed that the administration of ramelteon, a MTNR1b agonist, did not improve HbA1c levels in type 2 diabetes insomniac patients[26]. However, the findings of the latter trial might have been influenced by the inclusion of type 2 diabetes patients with favorable HbA1c levels and the small sample size. Further studies are required to determine the usefulness of melatonin treatment in type 2 diabetes patients with glucose metabolism disorders, microvascular complications and arteriosclerosis.

The present study has several limitations, including the retrospective design and inclusion of only Japanese participants who were admitted into a teaching hospital. The number of patients without diabetes was relatively small and unequal to that of those with type 2 diabetes ($n = 167$). This could be considered an important limitation of the present study. This study was carried out in a real-world clinical setting, and it was difficult to include an equal number of inpatients without diabetes matched for age and sex. The melatonin level is reported to be lower in type 2 diabetes patients than in patients without diabetes[10,11]. In the present study, we were able to confirm the aforementioned finding, even with the small sample size of the control group.

At this stage, it is not known whether treatment of diabetes increases urinary 6-sulfatoxymelatonin excretion. To verify this, urinary 6-sulfatoxymelatonin excretion should be examined before and after treatment of diabetes in a prospective study.

In conclusion, the present results showed low Ln 6-SMT levels in type 2 diabetes patients relative to patients without diabetes. Furthermore, Ln 6-SMT levels were identified as an independent risk factor of diabetic retinopathy and coronary heart diseases. These suggest 6-SMT could be a useful biomarker for the prediction of micro- and macrovascular complications in patients with type 2 diabetes.

ACKNOWLEDGMENTS

DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Prevalence of comorbidities in patients without diabetes.