Reduced serum level of leukocyte cell-derived chemotaxin 2 is associated with the presence of diabetic retinopathy

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ABSTRACT

Background: Vascular endothelial growth factor (VEGF) signaling is an important pathway in the development of diabetic retinopathy (DR). A recent report showed that leukocyte cell-derived chemotaxin 2 (LECT2) suppresses the VEGF signaling in endothelial cells. However, the clinical relevance of LECT2 in DR is unknown. This study aimed to investigate serum LECT2 levels and the presence of DR.
Methods: The study included 230 people with type 2 diabetes mellitus (DM), 95 with DR and 135 without DR. Serum LECT2 levels were measured using an enzyme-linked immunosorbent assay. Data were evaluated using Spearman's rank correlation, univariate and multivariate logistic regression.
Results: Serum LECT2 levels were significantly lower in participants with DM having DR than in those not having DR (35.6 ± 14.9 ng/ml vs. 44.5 ± 17.6 ng/ml, $P < 0.001$). Spearman's rank correlation analysis revealed a significant association between serum LECT2 levels and the presence of DR ($P < 0.001$). Multiple regression analysis revealed that serum LECT2 levels were independently related to DR ($P < 0.001$).
Conclusions: These findings indicated that serum LECT2 level is negatively associated with the presence of DR and suggest that low circulating LECT2 level is a risk factor for DR.

1. Introduction

Diabetic retinopathy (DR) is a serious microvascular complication of diabetes mellitus (DM) and the leading cause of visual impairment in people with DM [1]. The retinal extracellular fluid and/or circulating concentrations of proinflammatory cytokines and proangiogenic growth factors, such as tumor necrosis factor α and vascular endothelial

growth factor (VEGF), are upregulated in people with DR [2,3]. These inflammatory factors, which may lead to chronic micro-inflammation and the influx of leukocytes, are proposed to contribute to the onset and progression of DR [4,5]. VEGF is an important inflammatory and angiogenic factor that mediates endothelial cell proliferation, vascular permeability and cell motility. Anti-VEGF therapy is currently effective for DR, indicating a critical role for VEGF in the pathogenesis of this disease [6].

Some systemic factors had an effect on the onset and progression of DR, including blood pressure, dyslipidemia, and renal dysfunction [7]. In addition, circulating concentrations of hepatocyte-secreted mannose-binding lectin and adipocyte-secreted apelin-13 were associated with DR [8,9]. On the other hand, high circulating glycodelin prevents the progression of DR in pregnant women with diabetes [10]. Glycodelin has immunosuppressive properties and is secreted by the mammary glands and endometrium. These reports indicate that not only intraocular but also systemic factors play an important role in the development of DR.

Leukocyte cell-derived chemotaxin 2 (LECT2) is a zinc-binding plasma protein and is mainly expressed in perivenous hepatocytes [11–14]. It was originally identified from the culture fluid of the human T cell line SKW-3 during screening for a novel neutrophil chemotactic protein [15]. In humans, there is an association between fatty liver disease and systemic insulin resistance. The liver may contribute to the insulin resistance of skeletal muscle by releasing secretory proteins, now called hepatokines, including LECT2, an energy-sensing hepatokine. Serum LECT2 concentrations are positively correlated with the severity of insulin resistance and obesity [16,17]. The involvement of LECT2 in glucose metabolism was recently demonstrated, and it was suggested that LECT2 is a therapeutic target or prognostic marker for obesity-associated insulin resistance [17]. The exact molecular mechanism of LECT2 function in obesity and insulin resistance remains to be established by further studies. However, we and others have reported that LECT2 exerts an immunomodulatory effect in hepatitis, inflammatory arthritis, bacterial sepsis, and hepatic carcinogenesis [18–21]. Furthermore, two studies reported that LECT2 expression concentrations were significantly lower in human hepatocellular carcinoma (HCC) with vascular invasion than in HCC without vascular invasion [22,23]. Most recently, Chen et al. reported that LECT2 inhibits $VEGF_{165}$-induced angiogenesis through direct binding to VEGF receptor-2 (VEGFR2) [24]. These studies suggest that LECT2 is an anti-angiogenic factor.

2. Materials and methods

2.1. Study participants

For this cross-sectional study, 230 Japanese people with type 2 DM (157 men and 73 women aged 40–69 y) were recruited from the Department of Diabetes, Endocrinology, and Metabolism at the National Center for Global Health and Medicine (Tokyo, Japan) between August 2010 and September 2012. Type 2 DM was diagnosed according to the Japan Diabetes Society criteria [25]. The duration of diabetes mellitus (DM) was estimated through interviews. Patient demographics, clinical characteristics, and laboratory data were obtained from the medical records. All participants were evaluated for the presence of diabetic microvascular complications. Diabetic retinopathy was diagnosed by expert ophthalmologists, and each participant was graded according to the following categories: no diabetic retinopathy, simple diabetic retinopathy (SDR), pre-proliferative diabetic retinopathy (PPDR) or proliferative diabetic retinopathy (PDR). Diabetic peripheral neuropathy (DPN) was diagnosed by the presence of two or more of the clinical symptom components (bilateral spontaneous pain, hypoesthesia, or paresthesia of the legs), the absence of ankle tendon reflexes, and decreased vibration sensations when tested with a C-128 tuning fork [26]. Diabetic nephropathy (DN) was diagnosed as a spot urinary albumin-to-creatinine ratio greater than or equal to 30 mg/g [27]. Patients were excluded from this study if they met any of the following criteria: (1) blindness; (2) neurologic disorders unrelated to diabetic neuropathy or use of prosthetic limbs; (3) dialysis or renal transplantation; (4) the obvious macrovascular complications, such as stroke, angina, and myocardial infarction; and (5) missing values in any variable. This study was conducted with the approval of the ethical committee of the National Center for Global Health and Medicine and performed according to the Declaration of Helsinki. Written informed consent was obtained from each participant prior to study enrollment.

2.2. Measurement of serum LECT2 concentrations

Serum LECT2 concentrations were measured according to a previously reported method using a commercially available human LECT2 enzyme-linked immunosorbent assay system (Medical & Biological Laboratories, Nagoya, Japan) [16].

2.3. Statistical analyses

The results are presented as mean ± SD. Data were analyzed using IBM SPSS Statistics ver. 20. The normality of the continuous variables was analyzed using the Shapiro–Wilk test, and differences between two groups were examined by the two-tailed unpaired Student's t-test or the nonparametric Mann–Whitney U test as appropriate. Multiple comparisons between groups were performed using one-way analysis of variance (ANOVA). The χ^2 was used for the analysis of categorical variables. Spearman's rank correlation coefficients were used to evaluate associations between serum LECT2 concentrations and continuous variables. In the logistic regression analysis, a logarithmic transformation was applied to the distribution of triglycerides (TGs) and γ-glutamyl transpeptidase (γ-GTP) to approximate a normal distribution. A $P < 0.05$ were considered statistically significant.

3. Results

3.1. Characteristics of the study population

The clinical characteristics of the participants are presented in Table 1. The participants with DR had longer duration of DM ($P = 0.036$), higher systolic blood pressure (SBP, $P = 0.013$) and hemoglobin A1c (HbA1c, $P = 0.036$), and lower TGs ($P = 0.017$) and γ-GTP ($P = 0.021$). There was no significant difference between the groups with and without DR in gender, age, body mass index (BMI), diastolic blood pressure (DBP), high-density lipoprotein (HDL) cholesterol, fasting plasma glucose (FPG), or alanine transaminase (ALT). The frequency of patients using insulin was significantly higher among the participants with DR than in those without ($P < 0.001$). There was no significant difference in the frequency of the use of biguanide, statin, fibrate, angiotensin converting enzyme inhibitor (ACE-I), and angiotensin receptor blocker (ARB) between the two groups. Diagnoses of DPN and DN were made in 125 (54.3%) and 102 (44.3%) participants, respectively, with 1.6- and 1.7-fold higher incidences in the participants with DR (both $P < 0.001$). Overall, this analysis indicates that the study populations of participants with and without DR were balanced in terms of gender, age, and BMI.

Table 1
Characteristics of the participants.

	Non-DR	DR	P value
n	135	95	
Women [%]	41 [30.4%]	32 [33.7%]	NS
Age (y)	60.8 ± 6.4	61.1 ± 6.2	NS
Duration of DM (y)	11.2 ± 7.4	13.6 ± 9.8	0.036
BMI (kg/m^2)	25.5 ± 3.9	25.1 ± 4.6	NS
SBP (mm Hg)	126 ± 13	131 ± 18	0.013
DBP (mm Hg)	74 ± 10	73 ± 12	NS
HDL cholesterol (mg/dl)	53 ± 16	51 ± 15	NS
TGs (mg/dl)	123 (91–179)	110 (76–145)	0.017
FPG (mg/dl)	145 ± 42	147 ± 47	0.752
HbA1c (%)	7.4 ± 1.0	7.7 ± 1.3	0.036
ALT (U/l)	30 ± 18	27 ± 20	0.259
γ-GTP (U/l)	30 (21–46)	23 (17–35)	0.021
Insulin therapy [%]	13 [9.6%]	29 [30.5%]	<0.001
Biguanide [%]	92 [68.1%]	61 [64.2%]	NS
Statin [%]	58 [43.0%]	44 [46.3%]	NS
Fibrate [%]	12 [8.9%]	6 [6.3%]	NS
ACE-I and/or ARB [%]	54 [40.0%]	48 [50.5%]	NS
DPN [%]	58 [43.0%]	67 [70.5%]	<0.001
DN [%]	47 [34.8%]	55 [57.9%]	<0.001

Values are presented as means ± SD for normally distributed data and as median (interquartile range) for non-normally distributed data (i.e., for TGs and γ-GTP).

3.2. Comparison of serum LECT2 concentrations between type 2 DM patients with and without retinopathy

Serum LECT2 concentrations were significantly lower in the participants with DR compared to those without (35.6 ± 14.9 vs. 44.5 ± 17.6 ng/ml, $P < 0.001$) (Fig. 1A). Consequently, we compared serum LECT2 concentrations between the different stages of DR (Fig. 1B). Serum LECT2 concentrations of participants with SDR ($n = 65$), PPDR ($n = 10$), and PDR ($n = 20$) were 36.0 ± 15.7, 29.7 ± 12.1, and 37.3 ± 13.3 ng/ml, respectively. These differences were not statistically significant. These results suggest that serum LECT2 concentrations are not correlated with the stage of DR, but are associated with the presence of DR.

3.3. Impact of clinical parameters, medications, diabetic neuropathy, and diabetic nephropathy on serum LECT2 concentrations

Table 2 presents the results of correlation analyses to determine whether clinical parameters and other diabetic microvascular complications, i.e., DPN and DN, were associated with serum LECT2 concentrations. Serum LECT2 concentrations were significantly correlated with BMI ($\rho = 0.256$, $P < 0.001$), HDL cholesterol ($\rho = -0.164$, $P = 0.013$), log-transformed TGs ($\rho = 0.164$, $P = 0.013$), ALT ($\rho = 0.157$, $P = 0.017$), log-transformed γ-GTP ($\rho = 0.215$, $P = 0.001$), insulin therapy ($\rho = -0.153$, $P = 0.020$) and the presence of DR ($\rho = -0.252$, $P < 0.001$), but not with gender, age, duration of DM, SBP, DBP, FPG, HbA1c, oral medications (biguanide, statin, fibrate, ACE-I, and ARB), DPN, or DN. After adjustment for gender, age, duration of DM, and BMI, the correlations with serum LECT2 concentration that remained significant were those with HDL cholesterol (partial $\rho = -0.175$, $P = 0.008$), ALT (partial $\rho = 0.158$, $P = 0.018$), log-transformed γ-GTP (partial $\rho = 0.176$, $P = 0.008$), and the presence of DR (partial $\rho = -0.250$, $P < 0.001$). These results indicated that serum LECT2 concentration is only associated with the presence of DR and not with DPN and DN in diabetic microvascular complications.

3.4. Association of serum LECT2 concentrations with diabetic retinopathy

Table 3 presents the results of uni- and multivariate logistic regression analyses to elucidate independent factors associated with DR. The univariate regression model showed significant positive correlations between DR and duration of DM ($P = 0.039$), SBP ($P = 0.016$), HbA1c ($P = 0.038$), insulin therapy ($P < 0.001$), DPN ($P < 0.001$), and DN ($P < 0.001$), and significant negative correlations between DR and log-transformed TGs ($P = 0.018$), log-transformed γ-GTP ($P = 0.022$), and serum LECT2 concentration ($P < 0.001$). There was no relationship

Table 2
Spearman's rank correlation coefficients between serum LECT2 levels and clinical parameters.

Variable	Simple correlation		Adjusted for age, gender, duration of DM, and BMI	
	ρ	P value	Partial ρ	P value
Gender (0, man; 1, woman)	0.129	0.051	–	–
Age	−0.025	NS	–	–
Duration of DM	−0.082	NS	–	–
BMI	0.256	<0.001	–	–
SBP	−0.062	NS	−0.057	NS
DBP	0.026	NS	0.022	NS
HDL cholesterol	−0.164	0.013	−0.175	0.008
TGs[a]	0.164	0.013	0.121	NS
FPG	0.033	NS	0.051	NS
HbA1c	0.019	NS	0.046	NS
ALT	0.157	0.017	0.158	0.018
γ-GTP[a]	0.215	0.001	0.176	0.008
Insulin therapy (0, no; 1, yes)	−0.153	0.020	−0.096	NS
Biguanide (0, no; 1, yes)	0.061	NS	0.049	NS
Statin (0, no; 1, yes)	0.095	NS	0.103	NS
Fibrate (0, no; 1, yes)	0.050	NS	0.057	NS
ACE-I and/or ARB (0, no; 1, yes)	−0.005	NS	−0.044	NS
DR (0, no; 1, yes)	−0.252	<0.001	−0.250	<0.001
DPN (0, no; 1, yes)	0.035	NS	0.054	NS
DN (0, no; 1, yes)	−0.070	NS	−0.049	NS

[a] Log scale.

between DR and gender, age, BMI, DBP, HDL cholesterol, FPG, ALT, or oral medications. The multivariate regression model revealed that DR was correlated independently with serum LECT2 concentration after adjusting for gender, age, duration of DM, and BMI (Table 3, Model 1; $P < 0.001$). Even after adjustments for SBP, log-transformed TGs, HbA1c, log-transformed γ-GTP, insulin therapy, DPN, and DN, serum LECT2 concentration was still strongly independently correlated with DR (Table 3, Model 2; $P < 0.001$).

4. Discussion

The pathogenesis of DR includes angiogenesis and inflammation. Although LECT2 is a secretory blood protein with anti-angiogenic and immunomodulatory effects, there has been no reported study that associates circulating LECT2 concentrations with DR. Here, we performed Spearman's rank correlation analysis to examine whether changes in serum LECT2 concentration is associated with retinopathy in participants with type 2 DM that indicated a negative correlation between serum LECT2 concentration and the presence of DR. Other diabetic microvascular complications, namely DPN and DN, were not
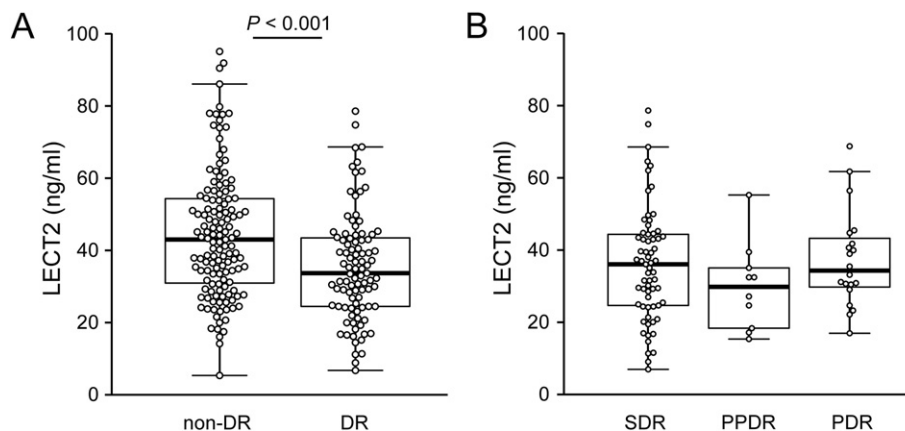

Fig. 1. Distributions of serum leukocyte cell-derived chemotaxin 2 (LECT2) levels within the groups. (A) Serum LECT2 levels were significantly decreased in the participants with diabetic retinopathy (DR, $n = 95$) compared to those without (non-DR, $n = 135$). (B) Serum LECT2 levels did not differ significantly between the three stages of diabetic retinopathy: simple diabetic retinopathy (SDR, $n = 65$), pre-proliferative diabetic retinopathy (PPDR, $n = 10$), and proliferative diabetic retinopathy (PDR, $n = 20$).

Table 3
Logistic regression analysis for diabetic retinopathy.

Variable	Univariate				Multivariate Model 1				Multivariate Model 2			
	B	Wald	P value	Odds ratio (95% CI)	B	Wald	P value	Odds ratio (95% CI)	B	Wald	P value	Odds ratio (95% CI)
LECT2 (ng/ml)	−0.034	14.144	<0.001	0.966 (0.949–0.984)	−0.035	13.316	<0.001	0.965 (0.947–0.984)	−0.040	13.310	<0.001	0.961 (0.940–0.982)
Gender (women vs. men)	0.152	0.282	0.595	1.165 (0.664–2.042)	0.335	1.168	0.280	1.399 (0.761–2.570)	0.623	2.889	0.089	1.864 (0.909–3.821)
Age (10 y)	0.060	0.079	0.779	1.062 (0.698–1.614)	−0.126	0.261	0.610	0.882 (0.544–1.429)	−0.161	0.320	0.571	0.851 (0.488–1.486)
Duration of DM (y)	0.033	4.273	0.039	1.034 (1.002–1.066)	0.029	2.910	0.088	1.029 (0.996–1.064)	0.012	0.386	0.534	1.012 (0.974–1.052)
BMI (kg/m^2)	−0.022	0.455	0.500	0.979 (0.919–1.042)	0.016	0.192	0.661	1.016 (0.947–1.089)	0.022	0.284	0.594	1.022 (0.943–1.108)
SBP (10 mm Hg)	0.223	5.825	0.016	1.249 (1.043–1.497)					0.180	2.815	0.093	1.197 (0.970–1.476)
DBP (10 mm Hg)	−0.083	0.434	0.510	0.920 (0.718–1.179)								
HDL cholesterol (mg/dl)	−0.006	0.434	0.510	0.994 (0.977–1.012)								
TGs (mg/dl)[a]	−1.502	5.610	0.018	0.223 (0.064–0.772)					−1.429	3.199	0.074	0.240 (0.050–1.147)
FPG (mg/dl)	0.001	0.101	0.751	1.001 (0.995–1.007)								
HbA1c (%)	0.246	4.292	0.038	1.279 (1.013–1.614)					0.245	3.086	0.079	1.277 (0.972–1.677)
ALT (U/l)	−0.009	1.263	0.261	0.991 (0.976–1.006)								
γ-GTP (U/l)[a]	−1.222	5.220	0.022	0.295 (0.103–0.841)					0.203	0.087	0.768	1.225 (0.317–4.738)
Insulin (no vs. yes)	1.417	14.894	<0.001	4.124 (2.008–8.467)					0.838	3.944	0.047	2.313 (1.011–5.290)
Biguanide (no vs. yes)	−0.176	0.388	0.533	0.839 (0.482–1.459)								
Statin (no vs. yes)	0.136	0.254	0.614	1.145 (0.676–1.942)								
Fibrate (no vs. yes)	−0.370	0.507	0.476	0.691 (0.250–1.911)								
ACE-I and/or ARB (no vs. yes)	0.427	2.493	0.114	1.532 (0.902–2.601)								
DPN (no vs. yes)	1.156	16.521	<0.001	3.177 (1.819–5.547)					1.247	13.448	<0.001	3.481 (1.787–6.781)
DN (no vs. yes)	0.946	11.794	<0.001	2.574 (1.501–4.416)					0.742	4.877	0.027	2.099 (1.087–4.054)

[a] Log scale.

correlated with serum LECT2 concentrations. The association between DR and decreased serum LECT2 concentrations did not change markedly after adjusting for known DR risk factors and potential confounders. These results indicate that serum LECT2 concentration is specifically associated with the presence of retinopathy in people with DM.

A recent study reported that LECT2 inhibits VEGF$_{165}$-signaling through binding to VEGFR2 in human umbilical vein endothelial cells [24]. The decreased circulating LECT2 concentration may contribute to exacerbating VEGF/VEGFR2-signaling of the retinal endothelial cells in DR. These findings together with the results of the present study suggest that LECT2 plays an important suppressive role in retinal microvascular dysfunction by reducing chronic micro-inflammation. Moreover, circulating LECT2 concentration is not associated with the presence of DPN and DN, although the contribution of VEGF to these microvascular complications was reported [28,29]. This difference might be explained by vitreous VEGF-induced leukocyte–endothelial interaction in DR. Vitreous VEGF also can induce intercellular adhesion molecule-1 expression and leukocyte adhesion in retina [30]. The leukocyte–retinal endothelial interaction plays an important role in the early phase of the development of DR [31]. LECT2 was originally named for its possible neutrophil chemotactic activity in vitro [15]. However, in the present study serum LECT2 concentration was lower in the participants with DR than in those without. The study data imply that VEGF-induced leukocyte adhesion is increased in lower circulating LECT2 circumstance and suggested that the neutrophil chemotactic function of LECT2 differs between in

vivo and in vitro. Certainly this speculation is consistent with our previous study that the synovial membranes of arthritis-induced LECT2-deficient mice are more markedly infiltrated by neutrophils than in the wild-type mice [19]. This study also revealed that serum LECT2 concentrations did not differ significantly between the stages of DR. We consider that the lower circulating LECT2 condition leads to the onset of DR and may be a risk factor for DR. Further investigation is needed to clarify this issue. We intend to further investigate these possible molecular mechanisms in DR.

We previously reported that increased serum LECT2 concentration is positively correlated with obesity and insulin resistance but not correlated with FPG and HbA1c after adjusting for potential confounders [16]. The present study supported the abovementioned findings: serum LECT2 concentrations in participants with DM were positively correlated with BMI but not correlated with FPG and HbA1c. These findings indicated that LECT2 does not have a direct impact on the blood glucose concentration. After adjustment of gender, age, duration of DM, and BMI, serum LECT2 concentration was also correlated with HDL cholesterol, ALT, and γ-GTP, which was consistent with our previous data based on the serum of healthy participants [16].

LECT2 is mainly secreted by hepatocyte. In this study, liver function markers (ALT and γ-GTP) were correlated with serum LECT2 concentration but not with the presence of DR. The frequency of the use of oral medications, including biguanide, was not correlated with serum LECT2 concentration or the presence of DR. Moreover, serum LECT2

concentration is more associated with the presence of DR than with liver function markers. These results suggest that the liver function-independent LECT2 alterations are related to the onset of DR.

Limitations of this study included the relatively small number of participants when divided according to the different vitreoretinal conditions of DR, involvement only of a single center, and the study's non-random, non-blinded nature. All these factors may be associated with bias. Because this study is a cross-sectional case-control study, it is uncertain whether the onset of DR leads to the decreased serum LECT2 concentration or the lower serum LECT2 concentration condition leads to the onset of DR. This could be resolved by comparing the serum LECT2 concentration before and after the onset of DR in a multicenter cohort study.

We did not conduct an interview regarding life style factors in this study. However, we have previously reported that alcohol consumption was not statistically associated with serum LECT2 concentration in healthy subjects [16]. Additionally, we obtained data on the smoking and coffee-drinking habits from the lifestyle questionnaire filled by the healthy subjects and assessed the relationship between serum LECT2 concentration and these habits. We found that these habits were not associated with serum LECT2 concentration in healthy subjects (unpublished data). To date only one human study has been reported on the effect of exercise on circulating LECT2 concentration in a conference presentation [32]. This report indicates that cardiorespiratory fitness and physical activity are not independently associated with plasma LECT2 concentration in human. On the other hand, Lan et al. reported that mouse serum LECT2 concentration was significantly reduced by exercise [17]. Future experiments are needed to validate this finding.

This was the first study to examine the associations of DR with LECT2 and showed that serum LECT2 concentrations were significantly lower in people with DR compared with those with DM but without DR. This finding suggests that circulating LECT2 is specifically associated with the presence of DR and plays a functional role in the pathogenesis of DR. The possible causal relationship between low circulating LECT2 and the presence of DR may be due to VEGF-signaling inhibition and immunomodulatory properties of LECT2.

Acknowledgements

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