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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.

Abbreviations: ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; DBP, diastolic blood pressure; DM, diabetes mellitus; DN, diabetic nephropathy; DPN, diabetic peripheral neuropathy; DR, diabetic retinopathy; FPG, fasting plasma glucose; LECT2, leukocyte cell-derived chemotaxin 2; PDR, proliferative diabetic retinopathy; PPDR, pre-proliferative diabetic retinopathy; SBP, systolic blood pressure; SDR, simple diabetic retinopathy; VEGF, vascular endothelial growth factor.

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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).