Serum and vitreous fibulin-1 concentrations in patients with diabetic retinopathy

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ABSTRACT

Fibulin-1, an extracellular matrix glycoprotein, is closely correlated with angiogenesis. The purpose of this investigation is to determine serum and vitreous fibulin-1 concentrations in diabetic retinopathy (DR). This cross-sectional investigation was carried out in a population of 154 diabetic patients (54 without DR, 42 with non-proliferative diabetic retinopathy (NPDR) and 58 with proliferative diabetic retinopathy (PDR)) and 49 control subjects. The diabetic group showed higher serum and vitreous fibulin-1 concentrations than the controls. Serum and vitreous fibulin-1 concentrations in PDR patients were significantly elevated compared with those in the other 3 groups. NPDR patients showed elevated levels of serum and vitreous fibulin-1 concentrations compared with patients without DR. Logistic regression analysis revealed that serum and vitreous fibulin-1 were risk factors for developing DR. Pearson correlation analysis showed that serum fibulin-1 was correlated with systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting plasma glucose and vitreous fibulin-1. Furthermore, Pearson correlation analysis showed that vitreous fibulin-1 was correlated with SBP, DBP, high-density lipoprotein cholesterol and serum fibulin-1. Serum and vitreous fibulin-1 concentrations are elevated under DR condition.

Significance of this study

What is already known about this subject?
- Angiogenesis is involved in the mechanism of diabetic retinopathy (DR).
- Fibulin-1 plays an important role in angiogenesis.
- Patients with diabetes displayed significantly higher fibulin-1 levels than those without diabetes.

What are the new findings?
- The diabetic group showed higher serum and vitreous fibulin-1 concentrations than the controls.
- Serum and vitreous fibulin-1 concentrations in PDR patients were significantly elevated compared with those in the other three groups. Non-proliferative diabetic retinopathy patients showed elevated levels of serum and vitreous fibulin-1 concentrations compared with patients without DR.
- Logistic regression analysis revealed that serum and vitreous fibulin-1 were risk factors for developing DR.

How might these results change the focus of research or clinical practice?
- Serum and vitreous fibulin-1 concentrations are elevated under DR condition.

INTRODUCTION

Diabetic retinopathy (DR), a common diabetic microvascular complication, contributes to ~4.8% of blindness over the world.[1] The pathogenesis of DR is complex and not quietly understood. A variety of evidence has focused on the key role of angiogenesis in the pathogenesis of DR, especially proliferative diabetic retinopathy (PDR).[2] The imbalance between angiogenic stimulator and inhibitor results in the angiogenic process and at last PDR development.[3]

Fibulin-1, one member of extracellular matrix (ECM) family, interacts with other ECM family members such as fibronectin, elastin and basement membranes.[4] Fibulin-1-deficient mice showed the destruction of endothelial lining in small vessels, massive hemorrhages in several tissues and ultimately early death.[5] Recent studies have shown the important role of fibulin-1 in diabetes development. Diabetic patients had relatively higher serum fibulin-1 concentrations compared with non-diabetic patients.[6] Fibulin-2 gene was found to be differentially expressed in the kidneys between streptozotocin-induced diabetic mice kidneys undergoing glomerulosclerosis and controls.[7] In addition, serum fibulin-1 was correlated with plasma glucose levels which was evaluated by hemoglobin A(1c).[6] Increased plasma fibulin-1 concentrations were found in diabetic participants with erectile dysfunction than in those without erectile dysfunction.[8] Human coronary artery atherosclerotic lesions presented strong deposition of fibulin-1, which demonstrate the pathophysiological effects of fibulin-1 in atherosclerosis.[9] Recently, fibulin-1 was found to inhibit angiogenesis and suppress tumor growth.[10] Considering the correlation of angiogenesis with DR, it is hypothesized that fibulin-1 may be involved in DR development.

We performed this cross-sectional investigation to assess serum and vitreous fibulin-1 concentrations in DR patients.

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MATERIALS AND METHODS

Patients

This study was performed in a consecutive population of 154 diabetic patients (54 patients without DR, 42 with non-PDR (NPDR) and 58 with PDR) who underwent vitreoretinal surgery in Department of Ophthalmology, Shandong Provincial Hospital Affiliated to Shandong University. Patients were excluded if they had vitrectomy history; other ocular disorders such as uveitis, glaucoma and corneal neovascularization; other systemic diseases including heart failure, renal failure, hematological, cardiological and metabolic disorders other than diabetes and obvious vitreal hemorrhage within the previous 3 months. Control participants included 49 subjects who matched with the cases by age and gender. Those subjects were enrolled from the subjects who underwent vitrectomy for retinal detachment in our hospital and had no systemic disease such as heart failure, renal failure, hematological, cardiological and metabolic disorders. They were confirmed having no diabetes by measuring fasting plasma glucose (<7.0 mmol/L) and 2-hour postprandial plasma glucose (<11.1 mmol/L). The study was planned according to the ethics guidelines of the Helsinki Declaration and was approved by the research ethics committee of our hospital. All subjects gave written informed consent regarding participation in this study.

LABORATORY METHODS

Vitreous samples were abstracted by manual suction to a syringe through the aspiratin line of vitrectomy, before opening the infusion line. Serum and vitreous fibulin-1 concentrations were then measured by a commercially available ELISA kit (Cusabio Biotech Company).

Statistical analysis

The results were expressed as means±SEs or median (IQR). Kolmogorov-Smirnov test was performed to analyze the data normality. The differences in variables between the four groups were performed by χ^2 tests, one-way analysis of variance or Kruskal-Wallis test. Logistic regression analysis was used to determine the risk factors for developing DR. The correlation of fibulin-1 with other parameters was analyzed by Pearson correlation analysis and multiple linear regression analysis. Statistical analysis was carried out using the SPSS V.16.0 software program (SPSS, Chicago, Illinois, USA). All analyses reported significance at the $p<0.05$ level.

RESULTS

Clinical parameters of the case and control group

Diabetic patients showed higher systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting plasma glucose (FPG) and triglycerides (TG) compared with the controls (table 1).

Serum and vitreous fibulin-1 concentrations

As shown in table 1, the diabetic group showed higher serum and vitreous fibulin-1 concentrations than the controls. Serum and vitreous fibulin-1 concentrations in PDR patients were significantly elevated compared with those in the other three groups. NPDR patients showed elevated levels of serum and vitreous fibulin-1 concentrations compared with patients without DR.

The association of fibulin-1 concentrations with DR

Serum and vitreous fibulin-1 concentrations were higher in T2DM patients with DR than in those without DR (8.96 (7.22–10.69) vs 6.74 (5.59–8.60) ng/mL, $p<0.001$ and 4.43 (3.49–5.87) vs 3.28 (2.70–4.03) ng/mL, $p<0.001$, respectively). As shown in table 2, simple logistic regression analysis and multivariate logistic regression revealed that serum and vitreous fibulin-1 were risk factors of developing DR.

Table 1 Various characteristics of diabetic patients and controls

		Diabetic patients			
	Controls (Group A)	Without DR (Group B)	NPDR (Group C)	PDR (Group D)	p Value
N	49	54	42	58	
Age (years)	57.10±7.78	54.87±10.16	55.08±10.13	55.79±9.96	0.650
Gender (M/F)	29/20	28/26	21/21	34/24	0.731
BMI (kg/m^2)	25.66±1.81	24.93±3.47	25.66±3.01	26.01±3.76	0.333
SBP (mm Hg)	124±12	133±23[a]	138±16[a]	135±17[a]	0.002
DBP (mm Hg)	77.82±7.76	83.80±14.73[a]	85.12±11.23[a]	86.03±11.58[a]	0.002
FPG (mmol/L)	5.15±0.39	8.02±3.49[a]	7.55±2.15[a]	8.01±2.71[a]	<0.001
TC (mmol/L)	4.95±0.93	5.11±1.08	5.04±0.962	5.07±1.10	0.879
TG (mmol/L)	1.09±0.59	1.68±0.86[a]	1.80±0.82[a]	1.66±0.87[a]	<0.001
LDL-C (mmol/L)	3.15±0.77	3.26±0.86	3.36±0.79	3.33±0.93	0.632
HDL-C (mmol/L)	1.43±0.29	1.37±0.32	1.27±0.22[a]	1.15±0.27[abc]	<0.001
Serum fibulin-1 (μg/mL)	5.17 (4.24–5.92)[bc]	6.74 (5.59–8.60)[a]	7.95 (6.29–10.43)[ab]	9.52 (8.15–10.87)[abc]	<0.001
Vitreous fibulin-1 (μg/mL)	2.72 (2.33–3.10)[bc]	3.28 (2.70–4.03)[a]	3.77 (3.02–4.48)[ab]	5.10 (4.16–6.62)[abc]	<0.001

Exact p value between different groups—SBP: A vs B: p=0.015; A vs C: p<0.001; A vs D: p=0.002. DBP: A vs B: p=0.010; A vs C: p=0.003; A vs D: p<0.001. FPG: A vs B: p<0.001; A vs C: p<0.001; A vs D: p<0.001. TG: A vs B: p<0.001; A vs C: p<0.001; A vs D: p<0.001. HDL-C: A vs C: p=0.007; A vs D: p<0.001; B vs D: p<0.001; C vs D: p=0.042. Serum fibulin-1: A vs B: p<0.001; A vs C: p<0.001; A vs D: p<0.001; B vs C: p=0.035; A vs D: p<0.001; C vs D: p=0.003. Vitreous fibulin-1: A vs B: p<0.001; A vs C: p<0.001; A vs D: p<0.001; B vs C: p=0.010; B vs D: p<0.001; C vs D: p<0.001.

[a]p<0.05 vs control; [b]p<0.05 vs diabetic patients without DR; [c]p< 0.05 vs NPDR patients. The parameter of gender was analyzed using χ^2 tests; serum and vitreous fibulin-1 were analyzed using Kruskal-Wallis test; the other parameters were analyzed using one-way analysis of variance.

BMI, body mass index; DBP, diastolic blood pressure; DR, diabetic retinopathy; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; NPDR, non-proliferative diabetic retinopathy; LDL-C, low-density lipoprotein cholesterol; PDR, proliferative diabetic retinopathy; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides.

Table 2 Logistic regression analysis for the presence of DR

	Simple regression		Multiple regression	
	OR (95% CI)	p Value	OR (95% CI)	p Value
Age (years)	1.006 (0.973 to 1.040)	0.713		
Gender (M/F)	0.881 (0.454 to 1.711)	0.708		
BMI (kg/m^2)	1.085 (0.980 to 1.201)	0.115		
SBP (mm Hg)	1.009 (0.992 to 1.027)	0.317		
DBP (mm Hg)	1.012 (0.985 to 1.040)	0.385		
FPG (mmol/L)	0.976 (0.870 to 1.095)	0.676		
TC (mmol/L)	0.957 (0.697 to 1.313)	0.784		
TG (mmol/L)	1.049 (0.708 to 1.554)	0.812		
LDL-C (mmol/L)	1.120 (0.761 to 1.649)	0.564		
HDL-C (mmol/L)	0.121 (0.035 to 0.424)	0.001	0.317 (0.071 to 1.425)	0.134
Serum fibulin-1 (μg/mL)	1.523 (1.269 to 1.828)	<0.001	1.399 (1.155 to 1.693)	0.001
Vitreous fibulin-1 (μg/mL)	2.996 (1.956 to 4.588)	<0.001	2.549 (1.603 to 4.053)	<0.001

BMI, body mass index; DBP, diastolic blood pressure; DR, diabetic retinopathy; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides.

The correlation of fibulin-1 concentrations with other parameters

As shown in table 3, Pearson correlation analysis showed that serum fibulin-1 was correlated with SBP, DBP, FPG and vitreous fibulin-1. Multiple regression analysis still revealed a correlation of serum fibulin-1 with SBP, FPG and vitreous fibulin-1. Furthermore, Pearson correlation analysis showed that vitreous fibulin-1 was correlated with SBP, DBP, high-density lipoprotein cholesterol (HDL-C) and serum fibulin-1 (table 4). Multiple regression analysis indicated that vitreous fibulin-1 was associated with HDL-C and serum fibulin-1 (table 4).

DISCUSSION

Fibulin-1 is one of the ECM proteins highly expressed in circulation. Recent studies have showed that fibulin-1 is demonstrated to be a biomarker for assessing the condition of diabetes.[6 11–13] Patients with diabetes displayed significantly higher fibulin-1 levels than those without diabetes.[6 11] Furthermore, higher fibulin-1 protein concentrations were found in artery extracts from patients with diabetes than those in controls.[6] Plasma fibulin-1 concentrations were found to be positively correlated with glycated hemoglobin.[6 12] In addition, our study also demonstrated that serum fibulin-1 was correlated with FPG. Scholze *et al*'s investigation indicated that diabetes was an independent predictor of plasma fibulin-1 after a multivariable regression model. Skov *et al*[13] reported that plasma fibulin-1 was elevated in diabetic patients during the 2-year follow-up. And metformin treatment markedly reduced plasma fibulin-1 concentrations in those diabetic patients.[13] It seems that serum fibulin-1 was correlated with plasma glucose. And higher serum fibulin-1 may be responsive to diabetes disease condition. Therefore, further

Table 3 The correlation of serum fibulin-1 concentrations with other parameters

	Pearson correlation analysis		Multiple regression analysis	
Parameters	r	p Value	β	p Value
Age (years)	0.127	0.116		
Gender (M/F)	−0.076	0.349		
BMI (kg/m^2)	−0.036	0.654		
SBP (mm Hg)	0.380	<0.001	0.203	0.018
DBP (mm Hg)	0.355	<0.001	0.046	0.585
FPG (mmol/L)	0.272	0.001	0.193	0.001
TC (mmol/L)	−0.034	0.680		
TG (mmol/L)	−0.038	0.636		
LDL-C (mmol/L)	0.036	0.653		
HDL-C (mmol/L)	−0.126	0.120		
Vitreous fibulin-1 (μg/mL)	0.654	<0.001	0.567	<0.001

BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides.

Table 4 The correlation of vitreous fibulin-1 concentrations with other parameters

	Pearson correlation analysis		Multiple regression analysis	
Parameters	r	p Value	β	p Value
Age (years)	0.005	0.955		
Gender (M/F)	−0.132	0.103		
BMI (kg/m^2)	−0.007	0.932		
SBP (mm Hg)	0.248	0.002	0.001	0.987
DBP (mm Hg)	0.260	0.001	0.052	0.540
FPG (mmol/L)	0.129	0.112		
TC (mmol/L)	−0.084	0.299		
TG (mmol/L)	−0.059	0.470		
LDL-C (mmol/L)	−0.008	0.923		
HDL-C (mmol/L)	−0.381	<0.001	−0.307	<0.001
Serum fibulin-1 (μg/mL)	0.654	<0.001	0.597	<0.001

BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides.

investigation on animals should be performed to determine the pathogenesis of fibulin-1 involved in diabetes.

Fibulin-1 is also a new factor in cardiovascular disease. It was found that plasma concentration of fibulin-1 predicted all-cause and cardiovascular mortality in diabetic patients.[6] Recent study showed a correlation between plasma fibulin-1 concentrations and cardiovascular risk markers in diabetic patients.[12] Patients with peripheral arterial disease and coronary artery disease had higher fibulin-1 concentrations than the controls.[14] This indicates that fibulin-1 was correlated with diabetic macrovascular complication. However, no investigation has been performed about the association between fibulin-1 and diabetic microvascular complication. DR is an important diabetic microvascular complication. Then our results showed that serum and vitreous fibulin-1 were elevated under DR condition.

Angiogenesis is a potential mechanism of DR. Fibulin-1 was found to serve as an angiogenesis inhibitor. It was found that fibulin-1 overexpression contributed to suppressed tumor growth, induced tumor cell apoptosis and inhibited angiogenesis in the bladder cancer and renal cell carcinoma condition.[15 16] ADAMTS-1 is a metalloprotease which is involved in the inhibition of angiogenesis. Lee *et al*[17] reported that fibulin-1 could enhance the binding of ADAMTS-1 to cleave aggrecan when colocalized with ADAMTS-1. This indicates that fibulin-1 could inhibit the angiogenesis by promoting ADAMTS-1 activity. Therefore, fibulin-1 is hypothesized to play a role in DR development through an antiangiogenesis effect. However, this role is controversy with relatively higher serum and vitreous fibulin-1 concentrations in DR. This may be explained that as angiogenesis is an important mechanism of DR development and progression, more fibulin-1 concentrations were released to inhibit angiogenesis process through its anti-inflammatory role under the condition of DR disease.

In conclusion, serum and vitreous fibulin-1 concentrations are elevated under DR condition.

Contributors BH researched literature and conceived the study. MT and JW were involved in protocol development and data analysis. YW and QL wrote the first draft of the manuscript.

Competing interests None declared.

Patient consent Obtained.

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