Original Article
Serum cystatin C and Chemerin levels in diabetic retinopathy

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Abstract: Objective: To investigate the levels of serum cystatin C (CysC) and chemokine (Chemerin) in patients with diabetic retinopathy (DR) and their roles in the onset and progression of DR. Methods: A total of 150 diabetic patients were enrolled as subjects and randomly divided into three groups: the proliferative DR (PDR) group (n=50), the non-proliferative DR (NPDR) group (n=50) and the control group (diabetes mellitus (DM) alone, n=50). In addition, 50 healthy adults were selected as normal controls. The four groups of patients were compared in demographic characteristics and biomarkers including gender, age, body mass index (BMI), glycosylated hemoglobin (HbA1c), homeostasis model assessment of insulin resistance (HOMA-IR), fasting plasma glucose (FPG), triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high sensitive C reactive protein (hsCRP), as well as serum cystatin C and Chemerin levels. Besides, Pearson correlation analysis and Logistics regression analysis were performed. Results: The control group was associated with a significant increase in serum CysC and Chemerin levels as compared with the normal control group; the patients with PDR or NPDR showed significantly higher levels than those with diabetes mellitus (DM) alone; and the ones with PDR had significantly higher levels than those with NPDR. The serum CysC and Chemerin levels were significantly different across groups and increased with the severity of the disease (P<0.05). Pearson correlation analysis showed that the serum CysC levels were positively associated with systolic blood pressure (SBP), HbA1c, HOMA-IR, FPG, TG, TC and LDL-C (P<0.05), and the serum Chemerin levels were positively associated with BMI, HOMA-IR, urinary albumin and hsCRP (P<0.05). What's more, the serum CysC levels were also positively associated with the serum Chemerin levels (P<0.05). Multivariate logistic regression analysis indicated that serums CysC and Chemerin were independent risk factors for DR. Conclusion: Elevated serum CysC and Chemerin levels are risk factors for DR, and they play a role in the pathogenesis of DR. Thus they are likely to become potential markers for assessment of DR.

Keywords: Diabetic retinopathy, Cystatin C, Chemerin, diabetes mellitus

Introduction

Diabetic retinopathy (DR), one of the most common complications of diabetes mellitus (DM), is of clinical significance to improve prevention and treatment of the disease and reduce the incidence of blindness in patients with DM. It is well-known that DR and diabetic nephropathy belong to hyperglycemia-induced microvascular diseases. Early diagnosis and treatment of DR is the key to prevention and the treatment of this disease. There are no effective biological parameters for assessment of DR. Cystatin C (CysC) is a low molecular-weight, non-glycosylated basic protein belonging to the cystatin super-family of cysteine proteinase inhibitors. Its main role is to suppress the activity of endogenous cysteine protease. CysC, as a metabolic intermediate of methionine, is closely related to the onset of diabetic nephropathy. It is extensively used to assess glomerular filtration, which has been confirmed in the studies [1]. However, the studies on the association between CysC and diabetic microangiopathy are limited to diabetic nephropathy, and few studies are involved in the effect of CysC on the progression of DR. In addition, both national and international studies show that retinal inflammation is characteristic of DR. Chemerin as a fat cell factor is involved in the immuno-

lesion response by binding to many inflammatory cytokines [2, 3]. In recent years, studies have demonstrated that elevated serum Chemerin levels lead to insulin resistance and are involved in the pathogenesis of diabetic microangiopathy [4]. However, whether it is related to DR remains controversial, and there are few reports about it. The present study was designed to analyze the serum CysC and Chemerin levels in patients with DR, and to investigate the relationship between CysC, Chemerin and DR.

Materials and methods

Demographic characteristics

A total of 150 patients with DM treated in our hospital from January 2014 to December 2016 were enrolled as the subjects in this retrospective study. All the patients met the diagnosis criteria listed in China Guidelines for Type 2 Diabetes (Version 2010), and met the requirements in this study. According to the Diabetic Retinopathy Staging Criteria created by the Ocular Fundus Disease Branch of Chinese Medical Association in 1984 and the results of ophthalmoscopy and fluorescein fundus angiography, the subjects were divided into three groups: 50 were in the proliferative diabeticretinopathy (PDR) group, whose ocular fundi showed neovascularization, vitreous hemorrhage or pre-retinal hemorrhage; 50 were in the non-proliferative DR (NPDR) group whose ocular fundi showed microaneurysms, more than 20 events of intra-retinal hemorrhage in any quadrant, vein beaded changes in more than two quadrants or significant intra-retinal microvascular abnormalities in more than one quadrant; 50 were in the control group whose ocular fundi showed no abnormalities. Any patient presented any one of the following conditions was excluded: autoimmune diseases, connective tissue diseases, cerebrovascular diseases, liver and kidney dysfunction, malignant tumor, acute and chronic infections, diabetes and chronic complications, pregnancy and ocular diseases for any other causes, or recently treated with large doses of hormone agents or eye surgery. In addition, 50 healthy adults were selected in the normal control group. All the healthy subjects had no hypertension, coronary heart disease, diabetes or any other disease history affected the results of the study. This study was in accordance with the medical eth-ics principles of the hospital, and qualified in the ethical reviews. All participants signed the informed written consents.

Study methods

Ophthalmoscopy

Direct ophthalmoscope was used to check first the macula regions and the posterior pole, and then the peripheral parts in order from the nasal, superior, temporal to lower retina.

Fluorescein fundus angiography

Fluorescein sodium injection (3 mL) was injected into the ulnar vein of each subject. Ten seconds after injection, 10 photographs of the posterior pole were successively shot with a Fule450-type digital retinal camera, followed by orderly shooting at the nine sites including posterior pole, temporal, nasal, superior, and lower, above and below the nose, above and below the temple at 1, 3, 5, and 10 minutes respectively. Finally at 20 minutes, the needed photographs were processed using the computer image processing software.

Determination of CysC and Chemerin

The venous blood sample (5 mL) was collected from each fasting subject in the early morning. After 30 minutes without shaking, the samples were centrifuged, from which serum was separated and cryopreserved for detection. With the use of enzyme linked immunosorbent assay (ELISA), serum CysC and Chemerin levels were detected in strict accordance with the instructions of the human CysC and Chemerin kits (Shanghai Vinhaket Biological Technology Co. Ltd). Serum CysC and Chemerin levels were compared among subjects from the four groups.

Monitoring parameters

The patients' general data on sex, age, body mass index (BMI), duration of disease, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded. The Backman AU680 automatic biochemical analyzer was used to measure glycosylated hemoglobin (HbA1c), fasting plasma glucose (FPG), triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), urinary albumin, hypersensitivity C reactive protein (hsCRP); HOMA-IR

Table 1. Demographic characteristics of patients across groups

Groups	Gender (Male/Female) (n)	Age (years)	BMI (kg/m^2)	SBP (mmHg)	DBP (mmHg)
PDR	27/23	48.9±7.2	26.4±2.46*	139.56±14.71*,#	79.31±10.21*
NPDR	26/24	47.8±7.7	25.6±2.51*	135.72±15.32*	75.72±9.82
Control	28/22	49.1±6.9	25.2±2.44*	130.11±10.64*	75.54±8.91
Normal control	25/25	50.1±6.7	21.6±1.96	121.15±9.67	73.20±4.76

Note: *P<0.05 for the comparison with the normal control group; #P<0.05 for the comparison with the diabetes control group.

Table 2. Comparison of biomarkers across groups

Parameters	PDR	NPDR	Controls	Normal controls
HbA1c (%)	9.96±2.55*,#	9.58±2.21*	9.22±2.11*	4.42±0.32
HOMA-IR	7.92±2.31*,#,Δ	6.85±2.26*,#	5.97±1.94*	1.69±0.26
FPG (mmol/L)	9.62±3.44*,#	9.31±2.83*	7.64±4.16*	4.79±0.79
TG (mmol/L)	2.40±1.94*	2.25±2.45*	2.04±1.31*	1.19±0.28
TC (mmol/L)	5.70±1.22*,#	5.58±0.91*	5.34±0.94*	4.42±0.66
LDL-C (mmol/L)	3.35±0.86*	3.05±0.69*	3.11±0.77*	2.65±0.49
HDL-C (mmol/L)	1.30±0.41	1.20±0.40	1.16±0.37	1.23±0.32
Urinary albumin (mg/24 h)	359±91.21*,#,Δ	50±11.92*,#	23±6.32*	3±2.24
hsCRP (mg/L)	1.83±0.58*,#,Δ	1.25±0.23*,#	0.78±0.16*	0.14±0.06

Note: *P<0.05 for the comparison with the normal control group; #P<0.05 for the comparison with the control group; ΔP<0.05 for the comparison with the NPDP group.

=FPG * fasting insulin/22.5. The factor including general information, clinical and biomarkers including gender, age, BMI, duration of disease, SBP, DBP, HbA1c, HOMA-IR, FPG, TG, TC, LDL-C, HDL-C, urinary albumin and hsCRP were compared among the subjects in the 4 groups.

Statistical analysis

The data analysis in the study was performed using SPSS software, version 12.0. The measurement data with normal distribution were presented as mean ± S.D, and the inter-group differences were compared using univariate analysis of variance (ANOVA). The qualitative data were expressed as percentages, and the inter-group differences were compared using the chi-square test. The associations of serum CysC and Chemerin levels with all other parameters were assessed with the use of Pearson correlation analysis. Multivariate logistic regression analysis was applied to determine independent risk factors for DR.

Results

Demographic characteristics of patients across groups

There were no significant differences in gender and age among the patients across groups.

BMI and systolic blood pressure were significantly different when the PDR group, the NPDR group and the diabetes control group were compared with the normal control group, respectively. The patients with PDR had significantly higher SBP values than those with DMalone (P<0.05), and also had significantly higher DBP values than those of normal controls (P<0.05; **Table 1**).

Comparison of biomarkers across groups

There were significantly reductions in HOMA-IR, urinary albumin and hsCRP levels among the patients in the PDR group, the NPDR group, the control group and the normal control group (P<0.05). Compared with the normal control group, the levels of HbA1c, FPG, TG, TC, LDL-C improved significantly among the patients in the PDR group, the NPDR group and the control group (P<0.05). The HbA1c, TC and FPG levels were significantly higher in the PDR group than in the control group (P<0.05; **Table 2**).

Serum cystatin C levels in all groups

The serum CysC levels of the patients with DMalone (0.68±0.04 mg/L) were significantly higher than those of the normal controls (0.32±0.02 mg/L) but markedly lower than the
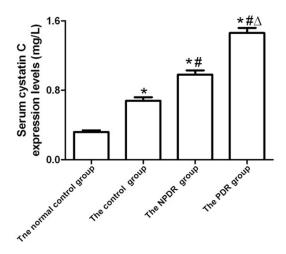
Figure 1. Comparison of expression levels of serum CysC among the patients in all the groups. *P<0.05 for the comparison with the normal control group; #P<0.05 for the comparison with the control group; ΔP<0.05 for the comparison with the NPDR group.
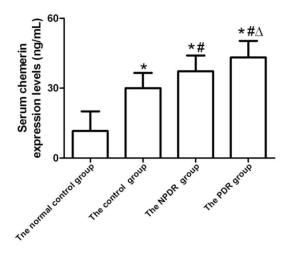

Figure 2. Comparison of expression levels of serum Chemerin among the patients in all the groups. *P<0.05 for the comparison with the normal control group; #P<0.05 for the comparison with the control group; ΔP<0.05 for the comparison with the NPDR group.

patients with NPDR (0.98±0.05 mg/L) and those with PDR (1.46±0.06 mg/L). Moreover, the serum CysC levels were significantly higher in the patients with PDR than in those with NPDR (P<0.05), on an escalating increase with the severity of disease (**Figure 1**).

Analysis on serum Chemerin levels among the patients in each group

There was a gradual decrease in serum Chemerin among the patients with PDR

Table 3. Correlations of serum CysC levels and other parameters

	CysC	
	r value	P value
SBP	0.515	0.008
HbA1c	0.365	0.016
HOMA-IR	0.278	0.021
FPG	0.424	0.011
TG	0.586	0.006
TC	0.639	0.004
LDL-C	0.470	0.009

Table 4. Correlations of serum Chemerin levels and other parameters

	Chemerin	
	r value	P value
BMI	0.314	0.014
HOMA-IR	0.238	0.018
Urinary albumin	0.648	0.005
hsCRP	0.421	0.012

(43.21±7.12 ng/mL), with NPDR (37.25±6.8 ng/mL), with DMalone (30.04±6.53 ng/mL), and normal controls (11.65±8.41 ng/mL), respectively. The inter-group difference in serum Chemerin level was statistically significant (P<0.05; **Figure 2**).

Analyses on the correlations of serum CysC and Chemerin levels to other parameters

Pearson correlation analysis showed that the serum CysC levels were positively associated with SBP, HbA1c, HOMA-IR, FPG, TG, TC and LDL-C (r shows 0.515, 0.365, 0.278, 0.424, 0.586, 0.639, 0.470, respectively, P<0.05; **Table 3**). The serum Chemerin levels were positively associated with BMI, HOMA-IR, urinary albumin and hsCRP (r shows 0.314, 0.238, 0.648, 0.421, respectively, P<0.05). What's more, CysC levels were also positively correlated with Chemerin levels (r shows 0.863, P=0.002; **Table 4**).

Multivariate logistic regression analysis

Multivariate logistic regression analysis, taken with or without DR as dependent variables and gender, age, BMI, blood pressure, HbA1c, FPG, TG, TC, LDL-C, HDL-C, HOMA-IR, urinary albumin, hsCRP, serums CysC and Chemerin as independent variables, showed that CysC,

Table 5. Multivariate logistic regression analysis on risk factors for DR

Variables	Regression coefficients	SE	OR	95% CI	P value
CysC	2.125	0.854	8.461	1.603-40.408	0.013
Chemerin	1.524	0.689	4.949	1.203-17.938	0.024
HbA1c	1.968	0.928	4.389	1.139-43.687	0.033
LDL-C	1.517	0.678	4.591	1.168-17.956	0.026

Chemerin, LDL-C and HbA1c were risk factors for DR, and CysC and Chemerin were independent risk factors for DR (**Table 5**).

Discussion

Chronic inflammation plays a crucial role in the onset and progression of diabetic complications. One study found that retinal inflammation is decisive for the pathogenesis of DR. Inflammatory factors adiponectin and TNF-alpha made changes in the patient's aqueous humor adiponectin and serum levels and interact with each other, thereby affecting progression of DR [5]. Therefore, it is of value to assess the severity and prognosis of DR by figuring out specific biological parameters of DR.

CysC is an ideal parameter for glomerular filtration rate [6]. The kidney and micro-vessels of Type 2 diabetic patients have been reported to express serum CysC [7]. Another study reported that CysC increases the permeability of retinal blood vessels and stimulates the growth of intraocular neovascularization [8]. It enhances the inflammatory response of the retina, leading to immunolesions. Serum CysC also reduces the consumption of glucose, further increases the intensity of insulin resistance and worsens prognosis [9, 10]. These results suggest that CysC is a risk factor for DR. In the present study, serum CysC was detected in patients with PDR, NPDR or diabetic mellitus alone as well as healthy controls. The results showed that the serum CysC levels of the patients with diabetes alone were significantly higher than those of the normal controls but markedly lower than the patients with NPDR and those with PDR. Moreover, the serum CysC levels were significantly higher in patients with PDR than in those with NPDR, on an escalating increase with the severity of disease. The inter-group difference in serum CysC levels was significant. Multivariate logistics regression analy-sis also showed that serum CysC was one of the independent risk factors for DR, indicating that serum CysC, to some degree, affects the onset and progression of DR. The reasons may be that serum CysC improves the pro-liferation of smooth muscle cells in micro-vessels of the retina of patients with DR, leading to microvascular occlusion which causes retinal isch-emia and hypoxia, and ultimately to the presence of lesions. As an inflammatory mediator, serum CysC makes direct damages to the microvascular functions and structure. Another study showed that serum CysC promotes inflammatory response, and high glucose-induced microvascular inflammatory injury is the pathogenesis of DR, which further implies that serum CysC is closely related to diabetic microangiopathy [11].

Chemokin as a fat cell factor is involved in the inflammatory response of the body [12-14]. It has been found that Chemerin also mediates angiogenesis, which plays an essential role in the onset and progression of DR [15]. Chemerin inhibitor has been found to reduce the levels of serum CysC, tumor necrosis factor alpha and C-reactive protein, which is conducive to elimination of inflammatory reaction [16]. This suggests that high glucose-induced Chemerin release not only makes direct damages to micro-vessels of retina in diabetic mellitus leading to DR, but also enhances inflammatory response and aggravates inflammatory damages to DR. The results of the present study showed that the serum Chemerin levels increased with the aggravation of DR. Pearson correlation analysis showed that serum Chemerin levels were positively associated with CysC levels, and Chemerin is one of the independent risk factors for DR. In addition, epidemiological studies show that serum Chemerin levels were higher in patients with type 2 diabetes than in those of healthy controls. With effective hypoglycemic treatment, there was a reduction in the serum Chemerin levels and in the intensity of insulin resistance. This further shows that the serum Chemerin levels interact with HOMA-IR [17, 18].

Besides, the Pearson correlation analyses on serum CysC and Chemerin levels, demographic characteristics and various parameters showed

that serum CysC and Chemerin levels were positively associated with HOMA-IR, which fully indicates that the positive correlations of serum CysC and Chemerinin levels to insulin resistance in patients with type 2 diabetes aggravate dysglycemia. National and international scholars hold that Chemerin promotes the release of inflammatory factors, such as tumor necrosis factor alpha, C- reactive protein etc. [19-21]. This suggests that Chemerin may be concomitantly implicated in the pathogenesis of both type 2 diabetes mellitus and insulin resistance. The findings of the present study showed that the serum CysC levels were positively associated with HbA1c, HOMA-IR, FPG, TG, TC and LDL-C (P<0.05); and the serum Chemerin levels were positively related to BMI, HOMA-IR, urinary albumin and hsCRP (P<0.05). What's more, the serum CysC levels were also positively associated with the serum Chemerin levels (P<0.05). This shows that in the onset and progression of DR, elevated CysC and Chemerin levels lead to disorders of lipid metabolism, dysglycemia, and intense insulin resistance, resulting in abnormal microcirculation, and ultimately in such lesions as retinal ischemia and hypoxia and neovascularization. This further indicates that changes in serum CysC and Chemerin levels are closely related to the onset and progression of DR, and of significance to the inflammatory response in DR.

Due to the small sample size and single-center in nature, there are still some limitations and selection of bias in the present study. Additional multi-centered, large-sampled, randomized controlled studies are required to further improve the research.

In conclusion, significant increases in serum CysC and Chemerin levels were observed in patients with DR. The serum CysC and Chemerin levels are not only implicated in the onset and progression of DR, but also to a certain extent express the severity of damages to retinopathy in diabetic patients. It indicates that elevated serum CysC and Chemerin levels are risk factors for DR and likely to become a marker for DR.

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Disclosure of conflict of interest

None.

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