· Original article ·

Research of serum total and high-molecular-weight adiponectin levels in patients with type 2 diabetic retinopathy

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2 型糖尿病性视网膜病变患者血清总脂联素和高分子量脂联素水平研究

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摘要

目的:探讨血清高分子量脂联素、总脂联素及两者比值与2型糖尿病性视网膜病变的关系,研究非增殖性糖尿病性视网膜病变发生的危险因素。

方法:共374例研究对象纳入本研究,包括88例增殖性糖尿病性视网膜病变患者(PDR)、124例非增殖性糖尿病性视网膜病变患者(NPDR)、78例无视网膜病变的糖尿病患者(DR)和84例健康对照志愿者(对照组)。收集人口学资料、体检及实验室指标,ELISA方法测定血清总脂联素和高分子量脂联素水平。统计学分析方法包括协方差分析和累积logit模型。

结果:对照组的总脂联素水平高于其他三组(修正均数:5.717、3.310、3.288、3.822 ug/ml, $F=18.792$, $P<0.01$),PDR组的总脂联素水平高于NPDR组($P<0.05$)。对照组的高分子量脂联素水平高于其他三组(修正均数:2.490、1.425、1.409、1.633 ug/ml, $F=14.025$, $P<0.01$),其他三组的高分子量脂联素水平的差异无统计学意义($P>0.05$)。高分子量脂联素与总脂联素的比值在四组间的差异无统计学意义($F=0.650$, $P>0.05$)。服药史、高水平的高密度脂蛋白胆固醇、总脂联素和高分子量脂联素是NPDR的保护性因素,年老、糖尿病病程长、肿瘤坏死因子-α升高是NPDR的独立危险因素。

结论:较低浓度的血清总脂联素和高分子量脂联素水平可能参与非增殖性糖尿病性视网膜病变的发生,总脂联素水平可能与糖尿病性视网膜病变的严重程度有关。

关键词:脂联素;高分子量脂联素;糖尿病性视网膜病变

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Abstract

• AIM: To explore the levels of serum total adiponectin, high-molecular-weight (HMW) adiponectin and its ratio in type 2 diabetic retinopathy and to investigate risk factors of non-proliferative diabetic retinopathy.

• METHODS: Three hundred and seventy-four subjects were recruited from an epidemiological survey, including 88 patients with proliferative diabetic retinopathy, 124 patients with non-proliferative diabetic retinopathy, 78 patients only with type 2 diabetes mellitus and 84 healthy volunteers. Demographics, physical and laboratory parameters were collected. Serum total and HMW adiponectin levels were detected by ELISA. The main statistical analysis included covariance analysis and cumulative logit model.

• RESULTS: Total adiponectin level was higher in healthy group than in groups of only diabetes, non-proliferative and proliferative diabetic retinopathy (harmonic mean, 5.717, 3.310, 3.288, 3.822 μg/ml, $F=18.792$, $P<0.01$). Total adiponectin in proliferative diabetic retinopathy increased compared with non-proliferative diabetic retinopathy (harmonic mean, 3.822, 3.288 ug/ml, $P<$

0.05). HMW adiponectin level of healthy group is more than other groups (harmonic mean, 2.490, 1.425, 1.409, 1.633 μg/ml, $F=14.025$, $P<0.01$). HMW adiponectin among only type 2 diabetes mellitus, non-proliferative and proliferative diabetic retinopathy groups had no differences ($P>0.05$). The ratio of HMW adiponectin and total adiponectin had no differences among four groups ($F=0.650$, $P>0.05$). Drug history, high levels of high density lipoprotein cholesterol, total and HMW adiponectin had protective effects to the non-proliferative diabetic retinopathy ($P<0.05$). Older age, long duration of diabetes, high level of tumor necrosis factor-α (TNF-α) were independent risk factors.

• CONCLUSION: The lower concentrations of serum total and HMW adiponectin may result in the occurrence of non-proliferative diabetic retinopathy. And the total adiponectin level was related to the severity of diabetic retinopathy.

• KEYWORDS: adiponectin; high-molecular-weight adiponectin; diabetic retinopathy
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INTRODUCTION

As the leading cause of blindness among working-aged adults, diabetic retinopathy (DR) has attacked approximately 93 million people worldwide[1]. The overall prevalence of DR was 29.7% in Chinese patients with type 2 diabetes mellitus (T2DM), of which 27.3% with non-proliferative diabetic retinopathy (NPDR) and 2.4% with proliferative diabetic retinopathy (PDR)[2]. A number of factors, such as poor glycaemic and blood pressure control, lipid concentrations, body mass index (BMI), familial clustering, macrovascular complications and longer diabetes duration, were all known to be involved in DR's progression[1,3-4].

Adiponectin is a novel, adipose-specific protein belonging to the collectin family, and present abundantly in the circulation[5]. Its levels were reported to be low in subjects with obesity, insulin resistance, T2DM, hypertension and cardiovascular diseases[6-8]. There are three forms in serum, including a low molecular weight (LMW) trimer, a middle molecular weight (MMW) hexamer, and a multimeric high molecular weight (HMW) form, collectively referred to as total adiponectin[5]. Of its isoforms, HMW complex is thought to be the most biologically active form and have more pathophysiological relevance in humans[9]. However, some clinical studies about the relationship between total adiponectin concentration and DR were inconsistent. Yilmaz *et al*[10] showed plasma adiponectin concentrations in patients with NPDR or PDR were significantly lower than those in patients without retinopathy. Hotta *et al*[11] reported that the presence of microangiopathy did not affect the plasma adiponectin levels in diabetic patients. In contrast, Kato *et al*[12] and Komaba *et al*[13] reported total or HMW adiponectin is increased in T2DM with retinopathy. Only one study has taken HMW/total adiponectin ratio into account in DR[12]. We found some of these studies neither control variables impacting adiponectin nor observe the dynamic trend of adiponectin from healthy status, T2DM to different stages of DR. Therefore, in our study, we aimed to explore how the serum total adiponectin, HMW adiponectin and their ratio change with the progression of T2DM and DR, and further explored the risks of NPDR.

SUBJECTS AND METHODS

Study Design and Subjects To meet the needs of research, we calculated the sample size according to the special formula. With an alpha 0.05 (two-sided test) and 90% power, at least 59 subjects were needed in each group. The definitions of T2DM and DR were used as follows. T2DM was diagnosed based on the Global Guideline from WHO and International Diabetes Federation, which was agreed implementation by Chinese Diabetes Society[14]. Diabetic retinopathy was divided into two stages based on severity, including NPDR and PDR. NPDR is the early stage of DR. The damaged retinal blood vessels leak fluid and blood into the surrounding retina. PDR is the advanced stage and the major cause of blindness in diabetics, followed with neovascularization, vitreous hemorrhage, scar tissue hyperplasia, secondary retinal detachment and blindness[15]. In Affiliated Eye Hospital of Shandong University of Traditional Chinese Medicine, the detailed history and physical examination was carried out for every patient and volunteer. Gender, age, diabetes duration, disease history, family history and drug history, height, weight and arterial blood pressure were evaluated. BMI was ratio of weight and height's square. It was worth mentioning that every subject underwent ophthalmoscopic examination and fasting plasma glucose (FPG) levels were measured. They were then stratified into subgroups.

Finally, 88 patients with proliferative diabetic retinopathy, 124 patients with non-proliferative diabetic retinopathy, 78 patients only with T2DM and 84 healthy controls were recruited. All subjects were informed of the study purpose and their consent was obtained. The study was approved separately by the ethics committee of School of Public Health of Shandong University and Affiliated Eye Hospital of Shandong University of Traditional Chinese Medicine.

Sample Collection and Laboratory Assays Fasting venous blood samples were collected and immediately centrifuged to serum at 1000g for 15min at normal atmospheric temperature, and kept frozen at −80℃ until assay analysis. All laboratory examinations were done by technicians of Medical Laboratory in Shandong University. Serum concentrations of FPG were determined by glucose oxidase method. Triglyceride (TG), total cholesterol (T-C), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C)

were measured by enzyme chromatometry. Creatinine was assayed by trinitrophenal chromatometry. Fasting insulin (FINS) and tumor necrosis factor-α (TNF-α) were determined by radioimmunity.

Serum total and HMW adiponectin were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Innogent Bioscience Inc., Shenzhen, China). Determination of the latter needed pre-treatment of serum with special digestive juice. Absorbances were measured using multiskan MK3 microplate reader (Thermo Labsystems Inc., USA).

Statistical Analysis Results were reported as numbers or mean ± standard deviation. χ^2 test was used for discrete variables. Levene's test was used to evaluate the distribution of continuous variables. One-way analysis of variance (ANOVA) was for homogeneity of variance and Kruskal-Wallis rank sum test (H test) for heterogeneity of variance. By covariance analysis, after controlling factors influencing the adiponectin, we observed the harmonic mean of total and HMW adiponectin within groups. Cumulative logit model was used for risks analysis. All analyses were performed using SAS (version 9.4, SAS Institute, Cary, NC, USA), and the $P<0.05$ was statistically significant.

RESULTS

Baseline Characteristic of Healthy Controls and T2DM Subjects with Absence or Presence of Retinopathy

Demographic, clinical and laboratory data of the patients and controls are shown in Table 1. No significant differences were observed among four groups in sex, diabetic family history, BMI, LDL-C, systolic blood pressure, diastolic blood pressure, ratio of HMW and total adiponectin ($P>0.05$).

Age in DR groups was higher than T2DM and control group ($P<0.01$); diabetic duration was also longer in DR groups ($P<0.01$); FPG in control group was lower ($P<0.01$), but other three groups had no different because they all took antidiabetic drugs ($P>0.05$); FINS was higher in DR groups ($P<0.01$); T-C, TG and HDL-C had some differences ($P<0.01$); TNF-α was higher in NPDR group than in T2DM and control groups ($P<0.01$); with the aggravation of disease, creatinine had a gradual increase ($P<0.01$); highest levels of total and HMW adiponectin appeared in control group ($P<0.01$).

Serum Total and HMW Adiponectin Levels in T2DM

Retinopathy taking the factors which affected adiponectin into account, and to observe the real levels of total adiponectin, HMW adiponectin and their ratio, we controlled interferences, including significant differences of age, hypertension history, drug history, duration of diabetes, FPG, FINS, TC, TG, HDL-C, TNF-α and creatinine ($P<0.05$). And we put them as covariates and made covariance analysis.

As shown in Figure 1A and Figure 1B, it revealed that total adiponectin level of healthy group was higher than groups of only T2DM, NPDR and PDR (harmonic mean, 5.717, 3.310, 3.288, 3.822 μg/ml, $F=18.792$, $P<0.01$). Total adiponectin in PDR increased compared with NPDR ($P<0.05$). Similarly, HMW adiponectin level of healthy group

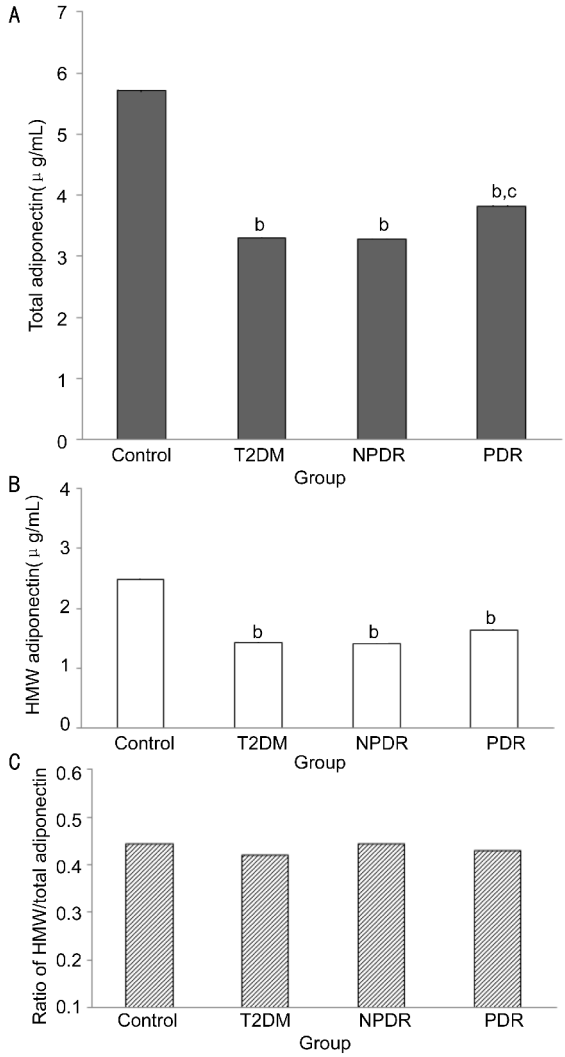

Figure 1 Serumlevels of total adiponectin A: HMW adiponectin; B: HMW adiponectin ratio; C: HMW adiponectin among groups of control, T2DM, NPDR and PDR. [b]$P<0.01$ *vs* control group, [c]$P<0.05$ *vs* NPDR group.

was more than other groups (harmonic mean, 2.490, 1.425, 1.409, 1.633 μg/ml, $F=14.025$, $P<0.01$). But HMW adiponectin among groups of T2DM, non-proliferative and proliferative diabetic retinopathy had no differences ($P>0.05$). From healthy people to diabetic retinopathy patients, the trends of total and HMW adiponectin were down at first and then up. In stage of NPDR, both were the lowest. Figure 1C showed that the ratio of HMW adiponectin and total adiponectin had no differences within groups (0.445, 0.422, 0.444, 0.432, $F=0.650$, $P>0.05$).

Analysis of Risk Factors of Non-proliferative Diabetic Retinopathy Cumulative logit model was used for ordinal variables according to the severity of disease, including healthy control, T2DM and NPDR. We put above significant parameters into this model. Because total and HMW adiponectin had close co-linear, analysis was made twice respectively. Model was good (likelihood ratio test, $P<0.05$; goodness-of-fit test, Pearson $P>0.05$, Deviance $P>0.05$). Finally, seven variables were retained ($P<0.05$). Estimate,

Table 1 Demographic, clinical and laboratory characteristics of the patients and controls

Parameters	Control (n=84)	T2DM (n=78)	NPDR (n=124)	PDR (n=88)	P
Sex (M/F)	35/49	43/35	70/54	53/35	0.077
Age (a)	49.54±13.14	48.76±11.32	54.55±11.43	54.75±11.41	0.000[a]
Hypertension history (Y/N)	0/84	23/55	40/84	28/60	0.000[a]
Diabetic family history (Y/N)	10/75	14/64	12/112	13/75	0.356
Diabetic duration (mo)	0.00±0.00	4.76±2.93	11.50±6.14	13.33±6.14	0.000[a]
BMI (kg/m^2)	24.67±3.63	25.19±3.25	25.41±2.63	24.66±2.97	0.219
FPG (mmol/L)	5.15±0.60	6.32±1.99	6.73±3.06	6.78±3.51	0.000[a]
FINS (mIU/L)	10.17±5.38	12.08±7.32	14.07±10.57	16.03±17.55	0.000[a]
T-C (mmol/L)	5.16±1.02	5.41±1.30	4.90±1.43	4.85±0.98	0.009[a]
TG (mmol/L)	1.42±0.82	2.08±1.33	1.83±1.33	1.84±1.32	0.004[a]
HDL-C (mmol/L)	1.52±0.37	1.45±0.40	1.20±0.34	1.24±0.45	0.000[a]
LDL-C (mmol/L)	3.10±0.83	3.03±1.05	3.07±1.26	2.97±0.83	0.847
Systolic blood pressure (mm Hg)	126.19±18.00	127.44±18.81	131.46±15.83	130.69±19.45	0.126
Diastolic blood pressure (mm Hg)	83.38±10.84	85.67±11.71	85.48±9.19	84.55±11.54	0.468
TNF-α (μg/L)	7.12±1.80	7.48±1.03	8.23±2.69	7.75±2.74	0.000[a]
Creatinine (μmol/L)	54.57±8.22	57.79±4.27	63.88±10.26	71.93±24.22	0.000[a]
Total adiponectin (μg/ml)	6.17±2.21	3.34±1.33	3.06±1.71	3.68±2.25	0.000[a]
HMW adiponectin (μg/ml)	2.57±1.29	1.37±0.75	1.38±0.79	1.65±1.05	0.000[a]
Ratio (HMW/total)	0.43±0.15	0.40±0.12	0.46±0.12	0.45±0.12	0.058

NPDR: Non-proliferative diabetic retinopathy. PDR: Proliferative diabetic retinopathy. [a]P<0.01.

Table 2 Total adiponectin and other factors of non-proliferative diabetic retinopathy

Parameters	Estimate	Std. Error	χ^2	P	OR	95% Confidence Interval	
						Lower	Upper
Drug history	-2.092	0.476	19.275	0.000[a]	0.123	0.049	0.314
age	0.045	0.016	8.058	0.005[a]	1.046	1.014	1.079
duration	0.614	0.078	62.240	0.000[a]	1.848	1.587	2.153
HDL-C	-1.433	0.484	8.747	0.003[a]	0.239	0.092	0.617
TNF-α	0.195	0.088	4.960	0.026[b]	1.216	1.024	1.444
Total adiponectin	-0.379	0.115	10.911	0.001[a]	0.685	0.547	0.857

OR: Odds ratio. [a]P<0.01, [b]P<0.05.

Table 3 HMW adiponectin and other factors of non-proliferative diabetic retinopathy

Parameters	Estimate	Std. Error	χ^2	P	OR	95% Confidence Interval	
						Lower	Upper
Drug history	-2.279	0.482	22.330	0.000[a]	0.102	0.039	0.263
age	0.052	0.016	10.438	0.001[a]	1.053	1.021	1.087
duration	0.635	0.079	64.782	0.001[a]	1.887	1.617	2.203
HDL-C	-1.366	0.488	7.819	0.005[a]	0.255	0.098	0.664
TNF-α	0.195	0.089	4.796	0.029[b]	1.216	1.021	1.448
HMW adiponectin	-0.795	0.233	11.670	0.001[a]	0.451	0.286	0.712

OR: Odds ratio. [a]P<0.01, [b]P<0.05.

odds ratio (OR) and P value demonstrated comprehensively that drug history, high level of HDL-C, total and HMW adiponectin had protective effects to NPDR. Older age, long duration of diabetes, high level of TNF-α were independent risk factors. Results are shown in Table 2 and Table 3.

DISCUSSION

DR is a microangiopathy of the retina from which nearly all diabetic patients eventually suffer. Regular ophthalmological examinations, timely laser therapy depending on the stage of the disease, and close interdisciplinary cooperation are essential to prevent visual loss [15]. Adiponectin is a key adipocytokine in T2DM and DR[5]. It was discovered during gene-expression profiling of human adipose tissue conducted by the human cDNA project. Located on chromosome 3q27, a locus for diabetes susceptibility, adiponectin encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a collagenous region (Gly-X-Y repeats), and a globular domain[16]. It exists as full-length

or a smaller, globular fragment; however, almost all adiponectin appears to exist as full-length adiponectin in plasma. Adiponectin belongs to a family of proteins possessing a collagen-like domain and can be divided to three styles by different molecular weight, including LMW trimer, MMW hexamer and a multimeric HMW form[17].

Our findings in this study were that serum total adiponectin was decreased in T2DM and diabetic retinopathy compared with healthy controls. The levels of total adiponectin in PDR higher than NPDR illustrated that it was positively correlated with the progression of diabetic retinopathy. Scilicet, hypoadiponectinemia was closely related to the occurrence of diabetic retinopathy, which was consistent with Kato *et al*'s[12] and Pradeepa *et al*'s[18] studies. The mechanism may be a physiological response to mitigate retinal microvascular injury and to prevent the further progression of diabetic retinopathy through its anti-inflammatory and anti-atherogenic effects. On the other hand, when proliferative diabetic retinopathy appeared, diabetic nephropathy often accompanied. Serum adiponectin increased in the end stage of diabetic nephropathy[19]. In this study, serum creatinine levels increased gradually up to 71.93 umol/L in the group of PDR. This maybe renal function was damaged to some extent and the rate of clearance to adiponectin reduced, resulting in total serum adiponectin rising in serum.

Serum HMW adiponectin levels were also found to be associated with diabetic retinopathy, independent of age, hypertension history, drug history, duration of diabetes, FPG, FINS, TC, TG, HDL-C, TNF-α and creatinine. In response to endothelial dysfunction, it may be increased compensatorily to repair microvascular lesions because one report considered that it induces endothelia nitric oxide production *in vitro*[20]. Conversely, HMW adiponectin may worsen diabetic microangiopathies, although this appears to be contrary to the concept of adiponectin as a beneficial hormone to diabetes and cardiovascular disease[6-8]. Diabetic retinopathy itself is a chronic long-term inflammatory reaction[21]. HMW adiponectin was reported to have dual action, both pro and anti-inflammatory. An initial period of NF-κB activation by HMW adiponectin might be proinflammatory, but it could be counteracted by activation of AMP-activated protein kinase (AMPK)/eNOS, which lead to a potential reduction in a second activation of NF-κB against inflammatory stimuli[22]. In short, that increase of adiponectin is cause or result of the serious stage of diabetic retinopathy needs to be studied further.

The ratio of HMW adiponectin and total adiponectin had no differences within T2DM and its retinopathy. Kato *et al*[12] also said the ratio was not correlated with the diabetic retinopathy stage. Ratio of plasma level of HMW adiponectin to that of total adiponectin had better predictive power for the prediction of insulin resistance and metabolic syndrome than plasma total adiponectin level[9]. This relationship may be existed only in diabetic macrovascular disease, not in microvascular disease.

In study of diabetic retinopathy risks, we found drug history, high level of HDL-C, total and HMW adiponectin had protective effects to the non-proliferative diabetic retinopathy. Older age, long duration of diabetes, high level of TNF-α were risk factors. These were consistent to some researches[1,23-24].

The study had several limitations. Firstly, because of the case-control study, we cannot determine the causal relationship between total or HMW adiponectin and diabetic retinopathy. Prospective in-depth investigations with larger sample sizes are required to clear this important question[25]. Secondly, whether HMW adiponectin involved in the inflammatory process of diabetic retinopathy requires further study.

In summary, the results from our study suggested that serum total adiponectin and HMW adiponectin were the chief contributors to the generation of both type 2 diabetes and non-proliferative diabetic retinopathy. Future prospective studies with greater numbers of patients are recommended to establish a direct relationship between serum adiponection concentrations and the severity of diabetic retinopathy.

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