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# Serum and Vitreous Levels of Visfatin in Patients with Diabetic Retinopathy

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Study Design A  
Data Collection B  
Statistical Analysis C  
Data Interpretation D  
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Literature Search F  
Funds Collection G

BCD **Yongqiang Wang\***  
EF **Ye Yuan\***  
AG **Hua Jiang**

Department of Ophthalmology, Ji'nan Military General Hospital, Ji'nan, Shandong, China

\* These 2 authors contribute equally to this article

**Corresponding Author:** Hua Jiang, w-mail: [jnmghjh@126.com](mailto:jnmghjh@126.com)  
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**Background:** Angiogenesis plays an important role in the mechanism of diabetic retinopathy (DR). Visfatin, a recently identified adipokine, is thought to possess an angiogenic effect. The aim of our study was to investigate serum and vitreous levels of visfatin in patients with proliferative diabetic retinopathy (PDR) and non-PDR (NPDR).





**Material/Methods:** A total of 280 diabetic patients (124 without DR, 56 with NPDR, and 100 with PDR) and 78 control subjects were enrolled in this study. Serum and vitreous levels of visfatin were measured by enzyme-linked immunosorbent assay (ELISA).

**Results:** Serum and vitreous visfatin levels in PDR patients were significantly elevated compared with those in the other 3 groups. NPDR patients showed elevated vitreous visfatin levels compared with patients without DR. However, no significant differences in serum visfatin levels were found between NPDR patients and patients without DR. In addition, control subjects had significantly lower levels of serum and vitreous visfatin compared with diabetic patients without DR, NPDR patients, and PDR patients.

**Conclusions:** Serum and vitreous visfatin levels are associated with the presence and severity of DR.

**MeSH Keywords:** **Adipokines • Angiogenesis Inducing Agents • Diabetic Retinopathy**

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## Background

Diabetes mellitus leads to the development of retinopathy, which is the main cause of vision loss in young adults in developed countries [1]. According to the World Health Organization (WHO), diabetic retinopathy (DR) accounts for approximately 5% of global blindness [2]. DR is characterized by gradually progressive alterations in the retinal microvasculature, leading to areas of retinal non-perfusion, increased vasopermeability, and, in response to retinal nonperfusion, pathologic intraocular proliferation of retinal vessels [3]. It is believed that the main etiologic mechanisms of DR are hypoxia-induced inflammation and angiogenesis [4].

Visfatin, a highly conserved 52-kDa protein, is a novel adipokine. Visfatin was originally cloned from human peripheral blood lymphocytes and characterized as nicotinamide phosphoribosyltransferase (Nampt), an enzyme that synthesizes nicotinamide mononucleotide from nicotinamide [5]. Visfatin is involved in the regulation of glucose homeostasis by exerting a hypoglycemic effect through the reduction of glucose release from hepatocytes and the stimulation of glucose utilization in peripheral tissues [6]. A recent study showed that visfatin exerted angiogenic effects on human umbilical vein endothelial cells through the mammalian target of rapamycin (mTOR) signaling pathway [7]. Therefore, it is hypothesized that visfatin may be involved in the pathogenesis of DR.

Although studies have focused on the relation of serum visfatin with diabetes [8], no investigation on the association of serum visfatin and DR has been performed. We aimed to determine serum and vitreous levels of visfatin in patients with DR to assess the role of visfatin in DR.

## Material and Methods

### Patients

We enrolled 280 diabetic patients [124 patients without DR, 56 with non-PDR (NPDR), and 100 with PDR] undergoing vitreoretinal surgery. Serum was obtained from all the participants and vitreous samples were drawn from the diabetic patients at the time of surgery. Exclusion criteria were chronic disease (other than diabetes), allergy, neoplasm, previous vitrectomy, photocoagulation, and intra-vitreous hemorrhages during the 3 months preceding the study. The control group consisted of 78 patients who had undergone vitrectomy for the treatment of retinal detachment (RD) with no proliferative vitreoretinopathy. Controls were free from systemic disease. This study was approved by the research ethics committee of our hospital and was conducted in agreement with the Declaration of Helsinki. Written informed consent was

obtained from the patients and healthy volunteers prior to their participation in this study.

### Measurements

Serum was obtained from blood samples by centrifugation and stored at  $-80^{\circ}\text{C}$  until analysis. Vitreous samples were collected undiluted by manual suction to a syringe through the aspiration line of vitrectomy, before opening the infusion line. Vitreous samples were transferred on ice, centrifuged, and supernatants were stored at  $-80^{\circ}\text{C}$  until analysis. The serum and vitreous samples were analyzed for visfatin using a commercially available ELISA (R&D Systems Inc., Minneapolis, MN, USA).

### Statistical analysis

Data are presented as means  $\pm$ SD or median (interquartile range). Data normality was analyzed using the Kolmogorov-Smirnov test. The differences in characteristics between patients with PDR and NPDR, diabetic patients without DR, and control subjects were compared using chi-square tests, one-way ANOVA, or Kruskal-Wallis test. Statistical analysis was carried out using SPSS version 13.0 software program (SPSS Inc, Chicago, Illinois). All analyses reported significance at the  $P < 0.05$  level.

## Results

### Baseline clinical characteristics

The clinical characteristics of diabetic patients and control subjects are presented in Table 1. Increased levels of systolic blood pressure (SBP), diastolic blood pressure (DBP), HbA1c, fasting plasma glucose (FPG), 2-h postprandial plasma glucose ( $P_{2h}$ PG), and triglycerides (TG) were found in the diabetic group compared with healthy controls. The levels of HDL-C were significantly elevated in NPDR and PDR patients compared with controls. Furthermore, diabetic patients without DR had relatively higher low-density lipoprotein cholesterol (LDL-C) levels than the other 3 groups.

### Serum and vitreous visfatin levels between the three groups

Serum and vitreous visfatin levels in controls, diabetic patients without DR, NPDR patients, and PDR patients are shown in Table 2. Serum and vitreous visfatin levels in PDR patients were significantly elevated compared with those in the other 3 groups. Control subjects had significantly lower levels of serum and vitreous visfatin compared with diabetic patients without DR, NPDR patients, and PDR patients. In addition, NPDR patients showed elevated vitreous visfatin levels

**Table 1.** Clinical and biochemical characteristics of diabetic patients and controls.

	Controls	Diabetic patients			P
		Without DR	NPDR	PDR	
N	78	124	56	100	
Age (years)	56.72±7.68	55.98±10.27	54.75±10.27	56.03±11.10	0.737
Gender (M/F)	40/38	54/70	27/29	49/51	0.497
BMI (Kg/m <sup>2</sup> )	25.45±1.83	25.20±3.78	25.37±3.23	25.98±3.87	0.386
SBP (mmHg)	123.04±12.03	142.86±27.65*	144.91±27.16*	145.50±29.01*	<0.001
DBP (mmHg)	77.71±7.56	85.97±14.02*	85.27±15.62*	85.06±17.01*	<0.001
HbA1c (%)	5.04±0.72	9.05±2.26*	8.88±2.51*	9.20±2.34*	<0.001
FPG (mmol/L)	5.10±0.37	7.56±2.37*	7.36±2.10*	7.87±2.78*	<0.001
P <sub>2h</sub> PG (mmol/L)	5.92±0.66	16.96±4.08*	16.20±3.52*	16.99±3.90*	<0.001
TC (mmol/L)	5.07±0.88	5.20±1.31	5.14±1.03*	5.18±1.22	0.886
TG (mmol/L)	1.12±0.31	1.96±0.48*	1.93±0.45*	1.98±0.42*	<0.001
LDL-C (mmol/L)	3.24±0.73	3.34±1.05	3.42±0.83*	3.41±1.06	0.640
HDL-C (mmol/L)	1.45±0.27	1.37±0.31	1.12±0.18*	1.22±0.35***	<0.001

\* P<0.05 vs. control; \*\* P<0.05 vs. diabetic patients without DR.

**Table 2.** Serum and vitreous visfatin levels in controls, diabetic patients without DR, NPDR patients, and PDR patients.

Visfatin (ng/mL)	Controls (n=78)	Without DR (n=124)	NPDR (n=56)	PDR (n=100)	P value
Serum	23.17 (18.63–27.78)****	28.76 (22.68–33.83)*	30.85 (23.98–34.19)*	34.59 (29.68–38.56)****	<0.001
Vitreous	12.72 (10.20–15.24)****	16.60 (14.48–19.79)*	18.91 (15.69–21.66)***	21.35 (16.74–23.41)****	<0.001

\* P<0.05 vs. control; \*\* P<0.05 vs. diabetic patients without DR; \*\*\* P<0.05 vs. NPDR patients.

compared with patients without DR. However, no significant differences in serum visfatin levels were found between NPDR patients and patients without DR.

## Discussion

This study provides the first report of the association of serum and vitreous visfatin levels with the presence and severity of DR. The results indicate that serum and vitreous visfatin levels in PDR patients were significantly elevated compared with those in control subjects, diabetic patients without DR, and NPDR patients. NPDR patients showed elevated vitreous visfatin levels compared to patients without DR.

Visfatin exerted insulin-mimetic effects in cultured cells and lowered plasma glucose levels by binding to and activating the insulin receptor in mice. Mice heterozygous for a targeted mutation in the visfatin gene had modestly higher levels of plasma glucose relative to wild-type littermates [8]. Visfatin has been shown to be associated with the development of diabetes. Serum visfatin levels were found to be elevated in patients

with type 2 diabetes [8], type 1 diabetes [9], and gestational diabetes mellitus [10]. In addition, a polymorphism in the promoter of visfatin is associated with the presence of type 2 diabetes. All these results point to the role of visfatin in the mechanism of diabetes.

Adipose tissue is no longer considered to be an inactive organ that only stores lipids and serves as an energy reservoir. Numerous studies have shown that it is an active endocrine organ and secretes many substances called adipokines, including tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), adiponectin, leptin, resistin, and apelin, which are involved in the regulation of several metabolic and physiologic processes [11]. Serum and vitreous levels of several adipokines such as leptin, resistin, and apelin were found to be higher in patients with PDR compared with controls [12–14]. On the other hand, serum adiponectin concentrations in patients with PDR or NPDR were significantly lower than those in patients without diabetic retinopathy [15], indicating that adipokines play an important role in the pathogenesis of DR. Our results showed that serum and vitreous visfatin levels were significantly elevated in patients with PDR and NPDR compared with controls. PDR patients showed

significantly higher levels of serum and vitreous visfatin compared with NPDR patients. This suggests that serum and vitreous visfatin may serve as a biomarker to predict the presence and severity of DR in order to evaluate the risk of developing DR in diabetic patients and then to target strategies to prevent DR for patients with diabetes.

Angiogenesis, the process by which new vascular networks develop from preexisting vessels, is a traditional characteristic of PDR and often leads to catastrophic loss of vision due to vitreous hemorrhage and/or traction retinal detachment. Angiogenesis is regulated by a dynamic balance between angiogenic stimulators and inhibitors [16]. A recent study showed that visfatin potently stimulates neovascularization in chick chorioallantoic membrane and mouse Matrigel plug [17]. It also activates migration, invasion, and tube formation in human umbilical vein endothelial cells (HUVECs) [17]. Moreover, visfatin evokes activation of the extracellular signal-regulated kinase 1/2 (ERK1/2) in endothelial cells, which is closely linked to angiogenesis [17]. In another study, visfatin was

found to concentration- and time-dependently enhance cell migration and tube formation, indicating the angiogenic capability of HUVECs via regulating VEGF, which is an important regulator for the angiogenesis of DR [18]. Therefore, visfatin may be involved in the pathogenesis of DR by promoting the angiogenesis process.

This study has several potential limitations. First, the sample size was not large enough to reach definitive conclusions. Further studies with larger numbers of subjects are warranted. Second, our study had a cross-sectional design and the causative relationship must be confirmed by longitudinal studies.

## Conclusions

Serum and vitreous visfatin levels were correlated with the presence and severity of DR. Serum and vitreous visfatin levels may serve as new biomarkers in addition to the traditional methods for assessing the risk of DR.

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