The effect of diabetes on vitreous levels of adiponectin and inflammatory cytokines in experimental rat model

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Abstract

Background. Diabetic retinopathy is one of the most common eye diseases faced by diabetic patients. It is a slow-progressing complication that results from damage to the blood vessels of the retina.

Objectives. To investigate the role of adiponectin and inflammatory cytokines in the vitreous of diabetic rats.

Material and methods. The study was conducted in 3–4-month-old male albino Wistar rats (180–240 g). The animals were divided into 2 groups (n = 40 in each group): the diabetes group and the control group. A single dose of streptozotocin (STZ) (45 mg/kg) in citrate buffer (0.1 M; pH 4.5) was intraperitoneally (ip.) injected into the diabetes group rats. A single dose of citrate buffer was injected ip. into the control group rats. All subjects were sacrificed under intramuscular (im.) Na-thiopental (50 mg/kg) anesthesia. The rats' eyelids were opened with an eye speculum and vitreous samples were collected with 20G needles 4 mm posterior to the limbus. The levels of vitreous adiponectin, tumor necrosis factor α (TNF- α), interferon γ (INF- γ), and matrix metalloproteinase (MMP)-2 and -9 were determined using a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA).

Results. The levels of adiponectin, TNF- α , INF- γ , MMP-2, and MMP-9 in the rat vitreous were significantly higher in the diabetes group than in the control group (p < 0.05).

Conclusions. Elevated adiponectin, TNF- α , and INF- γ levels in the vitreous may be diagnostically useful in diabetic retinopathy, and inflammatory cytokines in the vitreous may be pathogenically important in this concentration.

Key words: inflammation, diabetes mellitus, adiponectin, vitreous

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Introduction

Diabetic retinopathy (DR) is one of the most common eye diseases faced by diabetic patients. It is a slow-progressing complication that results from damage to the blood vessels of the retina. In the initial stages of DR, the disease may remain asymptomatic, but eventually, if left untreated, can result in blindness. The development of retinopathy is directly related to the duration of diabetes: within 10 years of diabetes, about 50% of patients will develop it, and within 20–25 years nearly 90% of diabetic patients will have some stage of retinopathy. ^{2,3}

Diabetes is known to display a strong inflammatory component. Circulating levels of endothelial leucocyte adhesion molecule-1 (E-selectin), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) have been demonstrated to be increased in type 2 diabetes patients. The complex pathology of DR involves upregulation of inflammatory molecules, adhesion molecules, and pro-inflammatory cytokines, which play critical roles in the pathogenesis of DR. Increased vitreous levels of certain pro-inflammatory cytokines have been described. The sophisticated inflammatory and chemotactic cascades may be linked to the breakdown of the vascular barrier, which may be one further mechanism contributing to the development of DR.

Adiponectin is a novel protein product of the adipose tissue, sometimes described as adipocytokines. Adiponectin is a plasma protein that was discovered a few years ago. It is produced exclusively and abundantly in adipose tissue and circulates at relatively high concentration. According to current data, adiponectin has powerful metabolic effects, including exacerbation of insulin sensitivity, reduction of hepatic glucose production, and lowered gluconeogenesis. In addition, plasma adiponectin levels were found to be negatively correlated with body mass index (BMI) and fat content, suggesting that fat mass may exert negative feedback on adiponectin production, and thus may negatively modulate the process of atherogenesis.

The study evaluated the effects of inflammatory cytokine and adiponectin in diabetic rat vitreous.

Material and methods

Inducing experimental diabetes

The rats were administered a single dose of 45 mg/kg streptozotocin (STZ) intraperitoneal (ip.) injection, dissolved in 1 mL 0.1 M cold citrate tampon with a pH of $4.5.^{12}$ The rats fasted for 18 h prior to the STZ administration. Fasting blood glucose levels were measured 48 h after the administration. Those with fasting blood glucose levels exceeding 250 mg/dL were considered diabetic and included in the diabetic group. The study was carried out

according to the Dumlupinar University Laboratory Animal Welfare and Ethical Committee Regulations.

Control group (n = 40): A single dose of citrate buffer was injected ip.

Diabetes group (n = 40): Diabetes was induced by a single dose of STZ (45 mg/kg) injected ip. Following diabetes inducement, fasting blood glucose levels were periodically monitored for 2 weeks. Diabetes stabilization was anticipated.

All subjects were sacrificed under intramuscular (im.) Na-thiopental (50 mg/kg) anesthesia. The rats' eyelids were opened with an eye speculum. Vitreous samples were collected with 20G needles 4 mm posterior to the limbus. The vitreous samples were stored at -80°C until the analyses were carried out.

Biochemical analyses

Rat vitreous tumor necrosis factor α (TNF- α), interferon γ (INF- γ), matrix metalloproteinase (MMP)-2, and MMP-9 were determined using a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

The data was presented as mean \pm standard deviation (SD). Statistical analyses were carried out using the Kruskal–Wallis test and the Mann–Whitney U test (SPSS for Windows v. 15.0; SPSS Inc., Chicago, USA). Values of p < 0.05 were taken to be significant.

Results

The study was conducted in 3-4-month-old male albino Wistar rats (180–240 g). The animals were divided into 2 groups (n = 40 in each group): The diabetes group and the control group.

The levels of adiponectin, TNF- α , INF- γ , MMP-2, and MMP-9 in the vitreous were significantly higher in the diabetes group than in the control group (p < 0.05; Table 1).

Table 1. Adiponectin, TNF- α , INF- γ , MMP-2, and MMP-9 levels in the vitreous of the diabetic and control groups (mean \pm SD)

Groups	Diabetic group (n = 40)	Control group (n = 40)
Adiponectin [μg/mL]	17.32 ±5.39*	10.54 ±6.43
TNF-α [pg/mL]	11.69 ±3.83*	7.06 ±3.27
INF-γ [pg/mL]	86.18 ±35.19*	57.61 ±27.62
MMP-2 [ng/mL]	135.81 ±48.02*	95.40 ±46.19
MMP-9 [ng/mL]	14.18 ±4.80*	11.24 ±2.17

TNF- α – tumor necrosis factor α ; INF- γ – interferon γ ; MMP-2 – metalloproteinase; MMP-9 – metalloproteinase 9; *p < 0.05, as compared to the control group.

Discussion

Diabetic retinopathy is a retinal neovascular disease and a significant diabetic complication, whose development is strongly linked with hyperglycemia, dyslipedimia, 13 and mitochondrial dysfunction accompanied by induced oxidative stress¹⁴ and associated with abnormal adiponectin levels. Clinically, diabetic retinopathy can be classified into non-proliferative retinopathy (phase 1) and proliferative retinopathy (phase 2), similar to retinopathy of prematurity. Hyperglycemia through metabolic changes leads to retinal vascular loss (phase 1). The incompletely vascularized retina is deprived of nutrition and oxygen, inducing pathological angiogenesis (phase 2). Adiponectin modifies these primary drivers of diabetic retinopathy. Abnormalities in the adiponectin pathway result in increased insulin resistance, 15 and adiponectin gene polymorphisms are associated with retinopathy in diabetic patients.¹⁶

Adiponectin is a regulator of energy homeostasis and is widely recognized for its antidiabetic, anti-inflammatory, antiangiogenic, antiatherogenic, antihypertensive, and cardioprotective effects. 17,18 Yilmaz et al. demonstrated that circulating levels of adiponectin are lower in both obesity and type 2 diabetes mellitus (T2DM).¹⁹ Moreover, T2DM patients with DR (proliferative as well as non-proliferative), show lower levels of adiponectin than matched patients without retinopathy. Additionally, hypoadiponectinemia is positively correlated with the severity of retinopathy in T2DM.²⁰ Recently, Costagliola et al. and Mao et al. analyzed the levels of vascular endothelial growth factor (VEGF) and adiponectin in the aqueous humor of patients with proliferative diabetic retinopathy (PDR), T2DM, and macular edema and found that they were significantly higher than those recorded in the control subjects.^{21,22}

Ziez et al. have shown elevated adiponectin levels in the serum of patients with type 2 diabetes and PDR. ¹⁶ Hong et al. have shown that adiponectin levels are higher in obese and non-obese patients with proliferative retinopathy than in those without apparent retinopathy. ²³ Kato et al. demonstrated that blood levels of adiponectin are elevated in patients with DR as well as a positive correlation with the severity of the disease. ²⁴ Danna et al. showed that adiponectin levels in the aqueous humor are higher in PDR. ²⁵ These elevated levels may be a protective mechanism in PDR.

In the present study, it was determined that the vitreous adiponectin levels were significantly higher in the diabetes group than in the control group. One possible explanation of this finding may be attributed to the increased blood retinal barrier permeability documented among patients with diabetes. Another possible explanation could be the local reparative response to endothelial dysfunction; in fact, adiponectin induces endothelial nitric oxide production in vitro.

Proliferative retinopathy is associated with elevated intravitreous concentrations of certain cytokines. Although

inflammatory cytokines are thought to play an important role in the pathogenesis of DR, the precise pathophysiological mechanisms have not yet been totally explained.²⁶

Tumor necrosis factor alpha is the primary pro-inflammatory cytokine. It is expressed by mast cells, fibroblasts, and endothelial cells in addition to neutrophils and macrophages; it has autocrine, paracrine, and endocrine effects on the target cells. This cytokine plays an important role in many biological processes, such as infection control, preparation of tissue for repair, increasing phagocytic activities, stimulation of keratinocyte migration to the wound, phagocytic activity, fibroblast proliferation and chemotaxis, and degradation of the extracellular matrix proteins.²⁷

Interferon gamma plays an important role in inflammation. It is an important immune regulator that has been shown to inhibit collagen synthesis by fibroblasts, resulting in delayed healing in incision wounds.²⁸

It was demonstrated that vitreous TNF- α levels are significantly higher in patients with proliferative retinopathy than in those without retinopathy, after adjusting for covariates. ²³ In the study by Costagliola et al., TNF- α levels in tears were found to be highly correlated with the severity of retinopathy, which suggested that local TNF- α production has greater clinical significance. ²⁹

It was demonstrated that TNF- α and IFN- γ levels were also lower in the vitreous than in the plasma in patients with DR. 30

In the present study, it was determined that vitreous TNF- α and IFN- γ levels were significantly higher in the diabetes group than in the control group.

Matrix metalloproteinases (MMPs) are a group of enzymes involved in physiological and pathogenic processes associated with extracellular matrix (ECM) remodeling. They play a central role in organ development and subsequent tissue remodeling as well as in inflammation and injury. Several studies have pointed out that MMPs may be involved in the pathogenesis of PDR and other vitreoretinal diseases, and that MMPs may play a role in the development of postoperative proliferative vitreoretinopathy.³¹

Previous studies have shown that MMP-2 and MMP-9 are present in the vitreous samples of patients with PDR, and that MMP-9 – but not MMP-2 – is elevated in PDR. $^{32-34}$

It has been demonstrated that among the 7 different MMPs examined, concentrations of only MMP-2 and MMP-9 were significantly higher in vitreous samples from PDR-affected eyes compared to those from nondiabetic eyes.³⁵

Previous studies have shown increased activity of both MMP-2 and MMP-9 in the epiretinal neovasculature membrane of patients with PDR. 36,37

Patients with DR and animal models have demonstrated elevated MMP-2 and MMP-9 levels in the retina and the vitreous. Recent research has demonstrated that MMPs have a dual role in the development of DR: In the early

period of the disease (pre-neovascularization), MMP-2 and MMP-9 facilitate the apoptosis of retinal capillary cells, possibly via damaging the mitochondria, and in the later period, they help in neovascularization.³¹

In the present study, it was determined that vitreous MMP-2 and MMP-9 levels were significantly higher in the diabetes group than in the control group. In the study, we only used diabetic rat model so additional prospective studies and, possibly, randomized clinical trials may be helpful in confirming the results and hypotheses.

Conclusions

The results of this study suggest that the inflammatory-immune process, adiponectin, and MMPs play an important role in PDR pathogenesis and vessel damage. Moreover, the levels of various inflammatory biomarkers may add clinically relevant, predictive information to existing, well-established risk factors for PDR.

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