Inflammation and diabetic retinopathy

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Purpose: To investigate the relationship between inflammation in the vitreous and diabetic retinopathy.
Methods: Vitreous samples from 21 patients with proliferative diabetic retinopathy (PDR), 21 patients with nonproliferative diabetic retinopathy (NPDR), and 21 nondiabetic patients with idiopathic epiretinal membranes (control) were studied. The interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), matrix metalloproteinase (MMP)-2, MMP-9, and adiponectin levels in the vitreous were detected in all samples with enzyme-linked immunosorbent assay (ELISA). Samples were stored at −80 °C until analyzed.
Results: The TNF-α levels in the vitreous were not statistically significant between all groups ($p > 0.005$). The mean IFN-γ levels were statistically significantly higher in patients with PDR (70.98 pg/ml) and patients with NPDR (46.61 pg/ml) than in nondiabetic patients (22.02 pg/ml). There was a difference in the IFN-γ levels in the vitreous between patients with PDR and patients with NPDR ($p < 0.005$). The MMP-2 and MMP-9 concentrations in the vitreous were not different between all groups ($p > 0.05$). There was a correlation between the IFN-γ and TNF-α levels. We investigated the statistically significantly decreased levels of adiponectin in the proliferative ($p < 0.05$) and nonproliferative ($p < 0.05$) diabetic eyes compared to the nondiabetic eyes.
Conclusions: Increased levels of IFN-γ and TNF-α in the vitreous were found in patients with diabetes compared to nondiabetic patients. Decreased levels of adiponectin in the vitreous were found in patients with diabetes compared to nondiabetic patients. The data support the hypothesis that inflammation is associated with diabetic retinopathy.

Diabetic retinopathy is the most common cause of visual loss. There are many pathologic factors that give rise to visual impairment in diabetic retinopathy, such as diabetic macular edema (DME), intraocular neovascularization, and proliferative vitreoretinopathy. Inflammation and microangiopathy play an important role in the pathogenesis of diabetic retinopathy [1].

Tumor necrosis factor-alpha (TNF-α) is a cytokine especially synthesized from T- lymphocytes, monocytes, and macrophages. TNF-α plays a role in inflammation, neovascularization, and apoptosis. Recent studies have shown that inflammation is effective in the development of diabetic retinopathy. The vitreous and serum levels of TNF-α in patients with proliferative diabetic retinopathy (PDR) are statistically significantly higher than the levels in patients with nonproliferative diabetic retinopathy (NPDR). Interferon-gamma (IFN-γ) and TNF-α are inflammatory cytokines. IFN-γ and TNF-α increase the secretion of vascular endothelial growth factor (VEGF) A and C from RPE cells and choroidal fibroblasts. VEGF A is an important proinflammatory molecule that plays a role in the development of diabetic retinopathy. VEGF is a major mediator of angiogenesis. VEGF A causes

breakdown of the blood–retinal barrier and increases vascular permeability. When leukocyte diapedesis occurs in the retina, endothelial cells are activated. This mechanism expresses that endothelial cells are associated matrix metalloproteinases (MMPs). MMP-2 and MMP-9 are elevated in vitreous samples of diabetic retinas [2,3].

Adiponectin (APN), a 30-kDa adipocyte-derived vasoactive peptide, is secreted by adipocytes. APN also has anti-inflammatory and antiatherosclerotic effects on endothelial cells. The development of diabetic retinopathy is linked to oxidative stress and abnormal APN levels [4].

Hyperglycemia causes endothelial dysfunction via increasing oxidative stress and expressing angiogenic factors. Endothelial dysfunction is implicated in the development of diabetic microvascular complications [5-7]. Eventually, inflammation is an important factor in DME and diabetic retinopathy [8]. The epiretinal membrane (ERM) is an avascular fibrocellular membrane that overlies the central macular area between the vitreous and the internal limiting membrane.

The etiology in the idiopathic epiretinal membrane is unknown. In the secondary epiretinal membrane, there is an anamnesis of ocular pathologies, trauma, or intraocular surgery. Epiretinal membranes contain glial cells, macrophages, fibrocytes, collagen fibers, and RPE cells [9]. We

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TABLE 1. MEAN VITREOUS LEVELS OF THE INF-ɣ, TNF-ᴀ, MMP-2, MMP-9, ADIPONECTIN.			
	Diabetic group		**Control group**
Cytokines	**Nonproliferatif DR (n=21)**	**Proliferatif DR (n=21)**	**Non-diabetic with idiopathic epiretinal membrane (Control) (n=21)**
INF-γ (pg/ml)	46,61±27,38	70,98±44,53	22,02±12,16
TNF-α (pg/ml)	6,58±3,33	7,24±3,75	5,10±1,64
MMP-2 (ng/ml)	220,49±81	193,85±99,68	144,85±93,27
MMP-9 (ng/ml)	12,88±6,19	11,30±2,04	10,74±2,16
Adiponectin (µg/ml)	10,32±7,51	9,32±5,9	19,24±2,87

included nondiabetic patients with idiopathic epiretinal membranes in the control group.

METHODS

Twenty-one patients with PDR, 21 patients with NPDR, and 21 nondiabetic patients (with idiopathic epiretinal membrane) were enrolled. Patients with the following conditions were excluded: a history of ocular trauma, intraocular surgery, and ocular inflammatory diseases or had been receiving topical or systemic steroid treatment. All vitrectomies were performed by one author (NIU). The enrollment criteria for vitrectomy in this study was epiretinal membrane for patients with diabetes and nondiabetic patients.

Undiluted vitreous fluid samples (0.5 ml) from 21 patients with PDR, 21 patients with NPDR, and 21 nondiabetic (control) patients were obtained during pars plana vitrectomy. Samples were stored at −80 °C until analyzed.

IFN-γ, TNF-α, MMP-2, and MMP-9 concentrations in the vitreous samples were analyzed with using commercial 96-well enzyme-linked immunosorbent assay (ELISA) kits. APN levels were also measured with ELISA.

Written informed consent was obtained from all patients. This study was performed in accordance with the Declaration of Helsinki, and the Internal Ethics Committees of the Ankara Numune Education and Research Hospital approved the protocol for the study (E-19–2455).

Statistical analyses were performed using the program SPSS. Kruskal–Wallis and Mann–Whitney U tests were used in statistical analysis. A p value of less than 0.05 was considered statistically significant. Pearson correlations were used to demonstrate the association between all the data. The quantitative data are presented as mean ± standard deviation (SD).

RESULTS

The mean levels of INF-γ, TNF-α, MMP-2, MMP-9, and APN in the vitreous in 63 patients are shown in Table 1. The mean age, gender, duration of diabetes mellitus, and hemoglobulin A1c (HbA1c) levels are presented in Table 2.

In the present study, we investigated the statistically significantly increased levels of INF-γ in proliferative ($p = 0.002$) and nonproliferative ($p = 0.000$) diabetic eyes compared with nondiabetic eyes. The INF-γ levels ($p = 0.049$) were statistically significantly higher in proliferative eyes than in nonproliferative diabetic eyes.

The TNF-α levels in the vitreous were not statistically significant between all groups ($p>0.005$). MMP-2 and MMP-9 concentrations in the vitreous were not different

TABLE 2. DEMOGRAPHICS OF THE OVERALL PATIENTS.			
	Diabetic group		**Control group**
Clinical information	**Nonproliferatif DR (n=21)**	**Proliferatif DR (n=21)**	**Non-diabetic with idiopathic epiretinal membrane (Control) (n=21)**
Female/Male	14/7	13/8	10/11
Age (years)	63,61±7,94	61,26±7,57	62,12±8,18
Diabetes Mellitus duration (years)	8,11±1,74	17,86±2	-
HbA1c (%)	9,03±1,23*	9,76±1,53*	4,5±0,36

* p<0,05 as compared the nondiabetic patients.

between all groups (p>0.05). We investigated the statistically significantly decreased levels of APN in proliferative (p<0.05) and nonproliferative (p<0.05) diabetic eyes compared with nondiabetic eyes. The APN levels in the vitreous were not different between the proliferative and nonproliferative diabetic eyes (p>0.05). There was a very weak positive correlation between the IFN-γ and TNF-α levels in the vitreous (r = 0.258, p<0.05).

While the groups were evaluated, it was checked whether there was a correlation between the parameters and age or gender. There was no correlation between the IFN-y, TNF-a, MMP-2, MMP-9, and adiponectin levels in the vitreous and age or gender.

DISCUSSION

In the present study, we determined the effect of decreased APN levels in the vitreous and increased INF-γ levels in the vitreous in diabetic eyes. APN is a cytokine secreted by adipocytes. APN is increased in the vitreous of patients with microangiopathy and chronic inflammation. APN regulates lipid-glucose metabolism and anti-inflammatory effect. Cholesterol mediates the activation of the acid sphingomyelinase that disrupts RPE autography. APN may modulate retinal lipid metabolism by removing excess cholesterol, reducing acid sphingomyelinase activation, or converting ceramide to sphingosine-phosphate. Decreased APN levels are associated with diabetic retinopathy. APN levels in the blood are positively correlated with retina blood flow in patients with diabetes [10]. Liao et al. reported decreased APN levels in the serum in type 2 patients with diabetes [4]. El Dayem et al. found increased adiponectin levels in the serum in adolescent girls with type 1 diabetes [11]. Pradeepa et al. reported that APN levels in the serum increased with the severity of diabetic retinopathy [12].

INF-γ is a part of the helper T-cell cytokine response, which activates proinflammatory macrophages and B cells. INF-γ is a proinflammatory cytokine. We know that high vitreous levels of INF-γ increase inflammation, and this is important in the pathogenesis of diabetic retinopathy. Tsai et al. reported INF-γ upregulation in patients with diabetes, but the researchers did not categorize the patients as with PDR or NPDR [13]. Wakabayashi et al. reported high levels of INF-γ in the vitreous in diabetic retinopathy [14].

Oh et al. indicated that IL-6 may play a role in the development of diabetic macular edema. They also suggested that ocular levels of IL-6 are not correlated with the severity of diabetic retinopathy [15]. IL-6 is a multifunctional cytokine that plays a role in the regulation of the immune process and

angiogenesis. Increased levels of IL-6 in ocular fluids were reported in PDR [8,16].

Retinal Müller glial cells produce VEGF and TNF-α due to inflammation and hypoxia. TNF-α stimulates VEGF secretion in retinal vascular cells [16]. In the present study, the TNF-α levels in the vitreous were not statistically significant between all groups. Studies have investigated increased TNF-α levels in the plasma in patients with diabetes [17]. Chen et al. investigated increased VEGF and decreased TNF-α levels in the plasma in patients with diabetes [8].

MMPs may play role in the pathogenesis of PDR. Increased levels of MMP-9 and MMP-2 contribute to activating retinal neovascularization. MMP-9 is an important molecule in inflammatory cells for the initial phase of inflammation and the late phase of tissue remodeling. MMPs are key factors to switch in acute and chronic inflammation [18,19]. Jin and coworkers reported increased MMP-9 levels in the vitreous in patients with diabetes. However, the authors did not detect a statistically significant change in the MMP-2 levels in the vitreous [3]. MMP-2 and MMP-9 concentrations in the vitreous were not different between all groups in the present study. MMP levels in the vitreous may not be direct indicators of tissue levels.

Increased levels of proinflammatory cytokines in diabetic eyes may directly induce vessel development via affectionally endothelial cells, by inducing endothelial cells to produce proangiogenic mediators. Endothelial cells are sensitive to cytokines, especially TNF-α and INF-γ [20]. We also investigated whether INF-γ levels are statistically significantly higher in proliferative eyes than in nonproliferative diabetic eyes. There was a very weak positive correlation between IFN-γ and TNF-α levels in the present study.

Additional studies are needed to understand the molecular mechanisms underlying ocular inflammation in diabetic retinopathy. In the future, it will be important to prevent inflammation in the treatment of diabetic retinopathy.

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