

Vitreous IL-8 and VEGF levels in diabetic macular edema with or without subretinal fluid

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Abstract

Purpose To determine the cytokine levels in vitreous samples of diabetic macular edema (DME) patients in comparison with nondiabetic patients, and to evaluate the effect of subretinal fluid on the cytokine levels of vitreous samples.

Methods In this prospective case–control study, 11 eyes of 11 patients with DME and subretinal fluid, 11 eyes of 11 patients with DME without subretinal fluid, and 14 eyes of 14 patients who had undergone vitreoretinal surgery for the epiretinal membrane or a macular hole (control group) were evaluated. The blood glycosylated hemoglobin (HbA1c) level, vitreous vascular endothelial growth factor (VEGF), and interleukin-8 (IL-8) levels were determined.

Results The vitreous VEGF level of patients in DME groups was significantly higher than the control group ($p < 0.001$) without significant difference between DME patients with and without subretinal fluid ($p = 0.796$). The vitreous IL-8 level of DME patients with subretinal fluid was significantly higher than both control ($p = 0.002$) and DME without subretinal fluid groups ($p = 0.019$). The blood HbA1c level was significantly higher in DME group with subretinal fluid than those without subretinal fluid (8.7 ± 1.32 and $7.1 \pm 1.13\%$, respectively, $p = 0.010$). The only significant correlation was between vitreous VEGF level and blood HbA1c level in DME patients without subretinal fluid ($r = 0.813$, $p = 0.002$).

Conclusions IL-8 level in vitreous samples was higher in DME patients with subretinal fluid than those without subretinal fluid, suggesting that inflammation is an important factor in the progression of DME leading to the subretinal fluid formation in diabetic patients.

Keywords Cystoid macular edema · Intraocular cytokines · Subretinal fluid · Diabetes · Interleukin-8

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Introduction

Diabetic macular edema (DME) is the most frequent cause of visual impairment in patients with diabetic retinopathy [1]. This pathologic condition arises from

the breakdown of the blood-retinal barrier (BRB) and subsequent increase in vascular permeability [2]. Three factors have been found to be related to increased vascular permeability and accumulation of fluid within the intraretinal layers of the retina: the breakdown of cell–cell junctions, pericyte loss, and thickening of the basement membrane [3]. Other factors, such as hypoxia, altered blood flow, retinal ischemia, and inflammation, are also associated with the progression of DME. Inflammatory processes including increased vascular endothelial growth factor (VEGF) levels, endothelial dysfunction, leukocyte adhesion, decreased pigment epithelium-derived factor (PEDF) levels, and increased protein kinase C production are upregulated within the diabetic retinal vasculature [4, 5]. In clinical studies, not only VEGF concentrations were found to be significantly elevated in vitreous and aqueous humor, but ocular concentrations of inflammatory factors such as interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), and interleukin-8 (IL-8) were also found to be increased in DME [6, 7]. A previous study showed that subretinal fluid (SRF) in DME is effectively resolved with intravitreal steroid injection [8, 9].

The development of imaging modalities over the last decade has helped clinicians diagnose and monitor the treatment of DME. Recently, optical coherence tomography (OCT) has enabled the improved visualization and understanding of the disease. OCT could contribute to the classification of DME morphology, such as serous retinal detachment, cystoid macular edema, and sponge-like retinal swelling [8].

We hypothesized that intravitreal inflammation might be related to SRF formation in diabetic patients. The aim of our study was to evaluate the level of intravitreal cytokines in treatment-naïve DME patients and determine if there was a difference between diabetic patients with and without SRF.

Materials and methods

Study population

This was a prospective case–control study. Twenty-two eyes of 22 consecutive patients examined from May 2014 to June 2015 in the Kocaeli University Ophthalmology Clinic Retina Department were included in the study. All of the patients had

treatment-naïve nonproliferative diabetic retinopathy with DME and an indication for intravitreal injection. Due to limited sample size, diabetic retinopathy was not classified according to severity as in the previous studies [10, 11]. The exclusion criteria were glaucoma, ocular hypertension, monocularly, ocular infection, senile macular degeneration, retinal artery or venous occlusion, intravitreal injection of any medication, any kind of laser treatment or signs of proliferative diabetic retinopathy, cataract surgery in the past 6 months, and a debilitating systemic disease. All diagnoses were confirmed by at least two of the authors independently at the time of admission. Study subjects were divided into two groups, each with 11 eyes of 11 patients. The first group included patients with DME with SRF (SDME), and the second group included the patients with DME without SRF (DME).

The control group included 14 eyes of 14 patients. Patients in the control group had undergone vitreoretinal surgery for the epiretinal membrane or a macular hole. None of the patients in this group had diabetes mellitus. Additional exclusion criteria for the control group were uveitis, glaucoma, senile macular degeneration, retinal artery or venous occlusion and cataract surgery in the past 6 months.

Written informed consent was obtained from all patients before treatment, and all the procedures were performed at the Kocaeli University Hospital. The study protocol was approved by the Ethics Committee of the Kocaeli University Hospital (Kocaeli, Turkey). The study was conducted in accordance with the latest version of Declaration of Helsinki.

Clinical assessment

The clinical histories of all patients were obtained from their medical records. All patients underwent visual acuity examination, Goldmann applanation tonometry, slit-lamp biomicroscopy and funduscopy. Fundus fluorescein angiography examinations were used to evaluate DME, retinal ischemia, and neovascularization. Central macular thickness (CMT) was also measured. The patients in SDME and DME groups had a visual acuity under 0.8. We did not observe retinal neovascularization and macular ischemia in fundus fluorescein angiography. They had diffuse or focal leakage in the macular area in fluorescein angiography and macular thickening

higher than 300 μm , including the fovea, in the OCT examination.

The history of diabetes and hypertension was recorded. The blood level of glycated hemoglobin (HbA1c) was measured and expressed in %.

Techniques for sample collection and injection

All samples were collected by the same surgeon. In the study groups, topical proparacaine hydrochloride was used for topical anesthesia. The eyelids and periorcular area were wiped with a 10% povidine iodine-soaked gauze swab. The surgical area was covered with a sterile drape, and a sterile eyelid retractor was placed. Then, the ocular surface and conjunctival fornix were washed with 5% povidine iodine. After 3 min, the ocular surface was washed with a sterile isotonic solution. Before injection of the indicated intravitreal medication, a 0.4–0.5 mL vitreous was aspirated. Wounds were controlled for leakage. Then, an intravitreal injection of 0.5 mg ranibizumab was performed as planned. After the injection, the injection site was compressed by cotton swab, and leakage was controlled. The day after injection, patients were controlled for Snellen visual acuity, slit-lamp biomicroscopy, Goldmann applanation tonometry, and funduscopy examinations.

In the control group, after retrobulbar (lidocaine 50% + bupivacaine 50%) anesthesia, the eyelids, and periorcular area were wiped with a 10% povidine and iodine-soaked gauze swab. The surgical area was covered with a sterile drape, and a sterile eyelid retractor was placed. Then, the ocular surface and conjunctival fornix were washed with 5% povidine iodine. After 3 min, the ocular surface was washed with a sterile isotonic solution. A 23-gauge transconjunctival trocar was used for sclerotomy at the 10 o'clock position in the right eye and the 2 o'clock position in the left eye. Approximately 0.4–0.5 mL of a vitreous sample was aspirated. After the sample was taken, the rest of the 23-gauge trocars were located, and the patient underwent vitrectomy for epiretinal membrane or macular hole.

Samples were injected into sterile Eppendorf tubes and frozen in $-80\text{ }^{\circ}\text{C}$ liquid nitrogen tank and kept in a $-80\text{ }^{\circ}\text{C}$ refrigerator.

ELISA assay measurements

The levels of VEGF and IL-8 in vitreous samples were measured with a commercial human VEGF ELISA kit (Life Technologies, Carlsbad, USA) and a human IL-8 ELISA kit (Life Technologies, Carlsbad, USA).

Statistical analysis

All the statistical analyses were performed using SPSS for Windows (Statistical Package for Social Sciences, version 20.0, SPSS Inc., Chicago, IL, USA) software. A Kolmogorov–Smirnov test was used to assess the assumption of normality. Normally distributed continuous variables were expressed as the mean \pm standard deviation, whereas continuous variables without a normal distribution were expressed as the median (interquartile range). The categorical variables were summarized as counts (percentages). Comparisons of normally distributed continuous variables between groups were performed using Student's *t* test and one-way analysis of variance (ANOVA). For nonnormally distributed continuous variables, differences between groups were tested using the Mann–Whitney *U* test and Kruskal–Wallis test. The associations between categorical variables were determined by Chi-square analysis, and associations between continuous variables were determined with Pearson (*r*) and Spearman (*r_s*) correlation analyses. A two-sided $p < 0.05$ was considered statistically significant.

Results

The clinical and demographic findings of the study and control groups are summarized in Table 1.

The mean vitreous VEGF level of patients in SDME ($131.7 \pm 61.5\text{ pg/mL}$) and DME groups ($110.6 \pm 35.3\text{ pg/mL}$) was significantly higher than the control group ($75.9 \pm 5.2\text{ pg/mL}$, $p < 0.001$) without significant difference between SDME and DME groups ($p = 0.792$) (Fig. 1, Table 2). The vitreous IL-8 level of SDME group was significantly higher than the control group (8.45 ± 0.40 and $8.08 \pm 0.23\text{ pg/mL}$, respectively, $p = 0.002$). Furthermore, the vitreous IL-8 level was significantly higher in patients with SRF (SDME group) than those without SRF (DME group) ($p = 0.019$) (Fig. 2, Table 2). However, there was no statistically

Table 1 Demographic and clinical findings of the study groups

	SDME group (<i>n</i> = 11)	DME group (<i>n</i> = 11)	Control group (<i>n</i> = 14)	<i>p</i>
Age (years)	56.82 ± 10.32	60.00 ± 12.71	66.36 ± 6.39	0.060
Sex				0.603
Male	5 (45.5%)	7 (63.6%)	7 (50%)	
Female	6 (54.5%)	4 (36.4%)	7 (50%)	
Hypertension	3 (27.3%)	3 (27.3%)	6 (35.3%)	0.828
Diabetes duration (years)	11.27 ± 4.71	16.64 ± 6.02	–	0.171
Lens status				0.111
Phacic	10 (90.9%)	7 (63.6%)	6 (42.9%)	
Pseudophacic	1 (9.1%)	4 (36.4%)	8 (57.1%)	
CMT (µm)	544.55 ± 142.69	450.90 ± 112.66	435.29 ± 74.53	0.048 ^a

Data are presented as mean ± standard deviation or *n* (%)

SDME, diabetic macular edema with subretinal fluid; DME, diabetic macular edema without subretinal fluid; CMT, central macular thickness

^aOn multiple comparisons, *p* = 0.131 for SDME versus DME; *p* = 0.037 for SMDE versus control; *p* = 0.917 for DME versus control

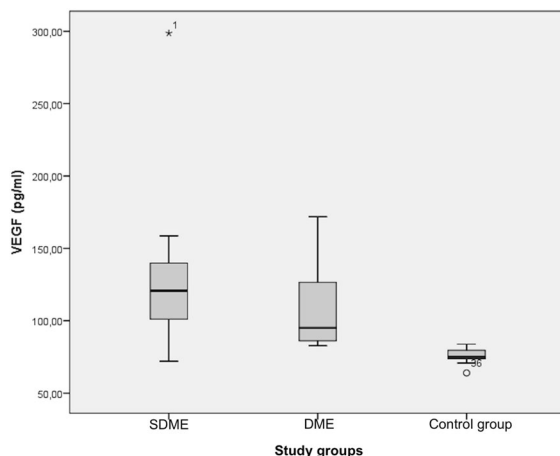


Fig. 1 The box-and-whisker plot showing the vitreous VEGF levels of two study groups (SDME and DME) and control group. The vitreous VEGF level of patients in SDME and DME groups was significantly higher than the control group (*p* < 0.001) without significant difference between SDME and DME groups (*p* = 0.796). The horizontal line within the box indicates the median, boundaries of the box indicate the 25th and 75th percentile, and the whiskers indicate the highest and lowest values of the results. The mild outliers are marked with open circles (o) and extreme outliers with asterisks (*)

significant difference between the DME and control groups in terms of IL-8 levels (*p* = 0.558). Although CMT was significantly higher in SDME group compared to control group, CMT was not significantly correlated with vitreous levels of IL-8 ($r_s = 0.118$,

p = 0.729 in SDME group; $r = -0.225$, *p* = 0.506 in DME group; $r = -0.145$, *p* = 0.620 in control group) and VEGF ($r_s = -0.250$, *p* = 0.459 in SDME group; $r_s = -0.382$, *p* = 0.247 in DME group; $r = 0.212$, *p* = 0.467 in control group).

The blood HbA1c level was significantly higher in SDME group than DME group (8.7 ± 1.32 and $7.1 \pm 1.13\%$, respectively, *p* = 0.010). We correlated HbA1c levels with VEGF and IL-8 levels in each group. There was no correlation between HbA1c level and vitreous VEGF level ($r_s = -0.143$, *p* = 0.713) and IL-8 level ($r = 0.412$, *p* = 0.270) in the SDME group. However, in the DME group, although there was no correlation between blood HbA1c level and vitreous IL-8 level ($r = -0.175$, *p* = 0.629), there was a positive significant correlation between blood HbA1c level and vitreous VEGF levels ($r_s = 0.813$, *p* = 0.002) (Fig. 3).

Discussion

In this case–control study, we primarily found that DME patients with SRF had significantly higher vitreous IL-8 levels than DME patients without SRF.

Serous macular detachment may be present in 15–30% in patients with diabetic retinopathy [12]. Various studies were carried out to investigate the

Table 2 Vitreous levels of IL-8 and VEGF, and blood HbA1c of the study groups

	SDME group (<i>n</i> = 11)	DME group (<i>n</i> = 11)	Control group (<i>n</i> = 14)	<i>p</i>
Vitreous VEGF (pg/mL)	131.7 ± 61.5	110.6 ± 35.3	75.9 ± 5.2	< 0.001 ^a
Vitreous IL-8 (pg/mL)	8.45 ± 0.40	8.08 ± 0.25	8.08 ± 0.23	0.005 ^b
Blood HbA1c (%)	8.7 ± 1.32	7.1 ± 1.13	–	0.010

Data are presented as mean ± standard deviation

SDME, diabetic macular edema with subretinal fluid; DME, diabetic macular edema without subretinal fluid

^aPairwise comparison results: *p* = 0.002 for SDME versus control; *p* = 0.558 for DME versus control; *p* = 0.019 for SDME versus DME

^bPairwise comparison results: *p* < 0.001 for SDME versus control; *p* < 0.001 for DME versus control; *p* = 0.792 for SDME versus DME

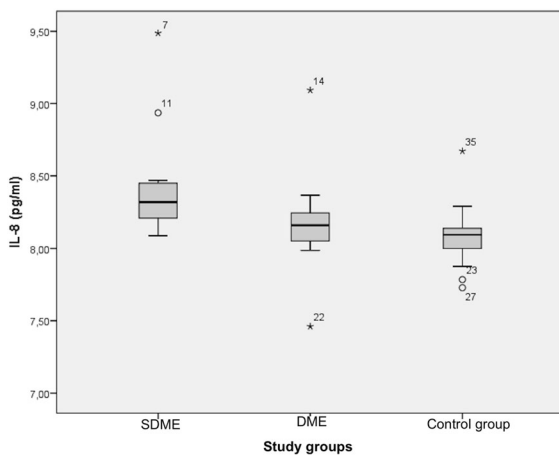


Fig. 2 The box-and-whisker plot showing the vitreous IL-8 levels of two study groups (SDME and DME) and control group. The vitreous IL-8 level of SDME group was significantly higher than both control (*p* = 0.002) and DME groups (*p* = 0.019). The horizontal line within the box indicates the median, boundaries of the box indicate the 25th and 75th percentile, and the whiskers indicate the highest and lowest values of the results. The mild outliers are marked with open circles (o) and extreme outliers with asterisks (*)

cause of SRF in DME. DME is mainly caused by the breakdown of the internal BRB, which results from the loss of anchor proteins in tight junctions and transendothelial vesicular transport in capillary endothelial cells [13]. This damage leads to an increase in the passive leakage of water and electrolytes into the extracellular spaces of the retina and to retinal thickening [12]. However, the mechanism for SRF accumulation is not clearly elucidated. Normally, the external limiting membrane is not permeable to fluid and albumin [14]. Kang et al. reported that with the disruption of the inner BRB,

excessive fluid might reach the subretinal space in large amounts and cannot be removed properly due to subretinal detachment of the retinal pigment epithelium. It was also reported that the foveal detachment on many occasions leads to cystoid foveal changes [15]. For this reason, SRF accumulation might cause additional intraretinal fluid accumulation in diabetic retinopathy. Gaucher et al. suggested that SRF accumulation can also occur without massive macular edema in the early stages of diabetic retinopathy, so SRF in diabetic retinopathy might not be accepted as a sign of progression of diabetic retinopathy [12].

As we mentioned, the main reason for the occurrence of DME might be the breakdown of the inner BRB. The inner BRB is a biological unit formed primarily by tight junctional complexes between retinal vascular endothelial cells (RVE) and well-differentiated networks of glial cells (astrocytes and Müller cells) that operates to maintain a low permeability environment [16]. The mechanism of the BRB breakdown is multifactorial. It is secondary to changes in tight junctions, pericyte loss, retinal vessel leukostasis, upregulation of vesicular transport, increased permeability of the surface membranes of RVE and retinal pigment epithelium cells, activation of advanced glycation end product receptors, downregulation of glial cell-derived neurotrophic factor, retinal vessel dilation and vitreoretinal traction [4]. Vasoactive factors are also important for the development and progression of DME. Hypoxia due to diabetes-related vasoconstriction and capillary loss leads to upregulation in the expression of VEGF and increases vascular permeability [17–19]. VEGF induces phosphorylation of tight junction proteins,

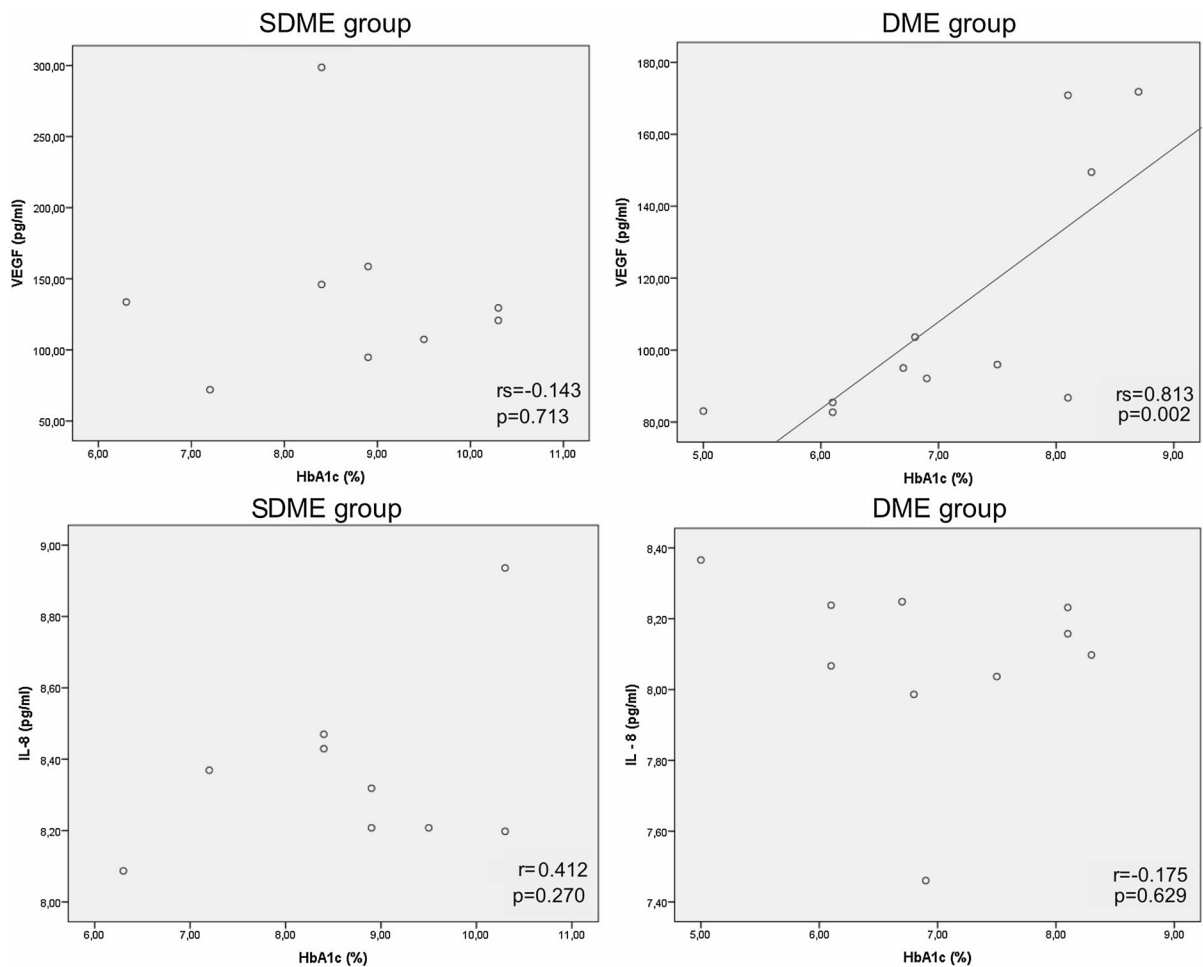


Fig. 3 The scattered diagrams showing the correlation between blood HbA1c level and vitreous VEGF and IL-8 levels in SDME and DME groups. r_s , Spearman's rho correlation coefficient; r , Pearson's correlation coefficient

occludin and ZO-1, which leads to increased vascular permeability [20]. Hypoxia also promotes expression of other cytokines such as insulin-like growth factor, interleukin-6 (IL-6), and protein kinase C-beta. Additionally, leukostasis causes the apoptosis of pericytes and endothelial cells, vascular obstruction, nonperfusion and the release of cytokines, which increases vascular permeability [21]. These cytokines enhance the expression of VEGF [22, 23]. As a result, VEGF and other cytokines work together to help create macular edema and potentiate each other's effects. In our study, we found that VEGF and IL-8 were both elevated in patients with or without SRF compared to nondiabetic control subjects. However, IL-8 elevation was significantly higher in patients with SRF. This finding might lead us to hypothesize that inflammatory

cytokines might be more effective in SRF formation in DME. In our clinical practice, we have observed that SRF resolves more quickly with intravitreal steroids than with intravitreal anti-VEGF drugs. Some reports show that intravitreal steroids provide effective treatment for SRF [24]. This might be related to the effect of steroids on cytokine load of patients with SRF.

IL-8 is a proinflammatory and angiogenic cytokine released from the endothelial and glial cells of the retina [25]. IL-8 elevation has been shown in proliferative diabetic retinopathy. It was reported that IL-8 and VEGF synthesized by glial cells was related to retinal neovascularization [26]. Petrovic et al. found that IL-8 elevation in patients with proliferative diabetic retinopathy was associated with gliotic large vessel occlusion [27]. Kim et al. evaluated cytokines

in aqueous humor and found that IL-8 levels were elevated in patients with DME, but they could not find a difference between patients with SRF and patients without [28]. However, unlike our study, they obtained the aqueous humor from the anterior chamber. In our study, we analyzed vitreous samples considering that diabetic retinopathy initially affects retina and cytokines first accumulates in vitreous. It is known that IL-8 synthesis is provoked by hypoxia. We found that IL-8 is also elevated in patients with nonproliferative diabetic retinopathy. As we mentioned before, this elevation was higher in patients with SRF formation.

Getting the blood glucose level close to a normal blood glucose level as safely as possible is very important in diabetic patients. This can prevent or slow the progress of disease complications and organ damage. The American Diabetes Association recommends keeping HbA1c levels at less than 7% [29]. A strong correlation has been shown between the level of HbA1c and development of macular edema [30]. In a clinical study, HbA1c levels were compared in patients with DME with or without SRF. It was found that HbA1c was significantly higher in patients with SRF [31]. In our study, we also found that the HbA1c was significantly higher in the SDME group than the DME group. We suggest that HbA1c increases in patients with poor metabolic control, which might be related to SRF formation. However, HbA1c was found to be correlated only with VEGF in the DME group, but not in the SDME group. We think that the limited sample size complicates significance of and commenting on correlation analysis.

The main limitation of the study was its small sample size, which precludes us from reaching a more definitive conclusion on the relation between vitreous cytokines and DME and SRF. Furthermore, we could not evaluate the probable relation of other inflammatory factors with macular edema and SRF, particularly those which have been shown to be elevated in vitreous and aqueous humor in DME [6, 7]. Although we initially planned to measure vitreous MCP-1 and IP-10, but, we were not able to obtain laboratory results due to technical problems. Our findings should be supported by further large-scale studies, which will consider other inflammatory factors.

In conclusion, IL-8 level in vitreous samples was higher in DME patients with SRF than those without SRF. We suggest that inflammation is an important

factor in the progression of DME and might also cause SRF formation in diabetic patients. We also think that in order to treat DME, we should aim to treat all factors that cause the pathology. Both intravitreal steroids and intravitreal anti-VEGF drugs are used for the treatment of DME and are both effective. We believe that a combination therapy might address different mechanisms in this pathology and provide a more effective and long-standing treatment.

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Compliance with ethical standards

Conflict of interest All the authors declared that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional ethical committee with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Institutional Review Board Approval Number: KOÜ KAİK 2014/155.

Informed consent Informed consent was obtained from all individual participants included in the study.

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