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ORIGINAL ARTICLE

Serum and Aqueous Concentrations of Inflammatory Markers in Diabetic Macular Edema

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ABSTRACT

Purpose: To investigate the relationship between the serum and aqueous levels of inflammatory markers and diabetic macular edema (DME).

Methods: The study included four patient groups: the healthy control group ($n = 23$ eyes); the diabetic control group ($n = 22$ eyes); the groups with and without DME ($n = 20$ eyes and $n = 22$ eyes, respectively). The patients were evaluated based on their serum levels of HbA_{1c}, C-reactive protein (CRP) and serum and aqueous levels of tumor necrosis factor-alpha (TNF-α).

Results: Statistically significant differences were present for the serum CRP levels and for the aqueous TNF-α levels between the healthy control group and the group with DME ($p = 0.004$ and $p = 0.03$, respectively); for the serum TNF-α levels between the healthy control group and the groups without and with DME ($p = 0.009$ and $p = 0.001$, respectively).

Conclusions: Increased serum levels of CRP and serum and aqueous levels of TNF-α in DME suggest that inflammation is involved in the pathogenesis of DME.

Keywords: C-reactive protein, diabetic retinopathy, inflammation, macular edema, tumor necrosis factor-alpha

Diabetic retinopathy (DR) is one of the main causes of severe visual loss in developed countries.[1] Hyperglycemia is considered to play an important role in the pathogenesis of diabetic retinopathy by the activation of some molecular and cellular pathways.[2] Diabetic macular edema (DME) is the most common cause leading to visual deterioration in patients with diabetes mellitus.[3,4] DME occurs essentially as a result of disruption of the blood–retinal barrier.[3–5] The fluid leakage from the retinal vessels leads to the accumulation of fluid in the macular area with increased leakage from the macular capillaries observed on fluorescein angiography and fluorophotometry.[3–5]

The concept of diabetes has changed in recent years, with regard to the involvement of chronic, low-grade inflammation in the pathogenesis.[6] Systemic inflammation is nowadays accepted to contribute to the development of clinical diabetes, as well as its complications, including retinopathy.[6]

In persons with impaired glucose tolerance and in recently diagnosed diabetic patients, proinflammatory cytokines, such as C-reactive protein (CRP) and tumor necrosis factor-alpha (TNF-α) were shown to be positively correlated with the degree of insulin resistance.[7]

The role of inflammation in the pathogenesis of DR has been evoked first following the observation of less severe DR in patients receiving high doses of salicylates for rheumatoid arthritis.[8] Several inflammatory molecules (such as TNF-α) have been detected in intraocular tissues and liquids from diabetic

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animals or humans.[6] Characteristic cellular and functional changes of inflammation in diabetic retinopathy are leukostasis, abnormal leukocyte adherence, and increased permeability of the blood retinal barrier.[6] *In vivo* increased production of TNF-α under chronic hyperglycemia may promote the development and progression of diabetic retinopathy through its different actions.[6]

TNF-α is a proinflammatory cytokine known to have a major role in the pathogenesis of various autoimmune diseases. TNF-α is found to be involved in the development of early DR.[9] Moreover, the suppression of TNF-α by non-steroidal anti-inflammatory drugs (NSAIDs) may prevent early diabetic retinopathy and also may partly treat DME.[10] The macular edema secondary to DR could be effectively treated by systemic TNF-α antagonist drugs.[11,12]

In this study, we determined the concentrations of serum CRP and serum and aqueous TNF-α in diabetic patients with non-proliferative diabetic retinopathy (NPDR) with and without DME; in non-diabetic and diabetic control subjects. The aim of the study was to investigate the association of the inflammatory markers (serum CRP levels; serum and aqueous TNF-α levels) with the presence of DME, by comparing the values of the groups.

MATERIALS AND METHODS

Patients

Our study group consisted of 87 eyes of 87 patients (mean age ± SD, 66 ± 9.6 years); 45 women (45 eyes) and 42 men (42 eyes), with relatively clear media allowing the retinal examination. Aqueous samples were collected from these eyes during subsequent cataract extraction.

Enrolled participants in this study were categorized into four groups: individuals without diabetes (healthy control group); patients with diabetes at least for 5 years but without diabetic retinopathy (diabetic control group); patients with diabetes and NPDR without DME (NO-DME group); patients with diabetes and NPDR with DME (DME group). The duration of the study was 1 year.

Exclusion criteria included:

(1) Previous laser photocoagulation within the last 6 months
(2) Previous intravitreal injection of corticosteroid or one of the anti-VEGF agents within last 12 months
(3) Glaucomatous eyes and eyes with pseudoexfoliation
(4) Asymmetric NPDR
(5) Eyes with proliferative DR or with retinal findings of macular and severe retinal ischemia in fluorescein angiography
(6) Associated choroidal, retinal, and macular pathology other than diabetic retinopathy, including vitreomacular interface abnormalities.

All study subjects underwent a complete ophthalmic examination, including a retinal examination. Macular thickness measurement with Fourier-domain optical coherence tomography (OCT) were carried out routinely for all of patients before or on the first day after cataract surgery, but fluorescein angiography was carried out only for patients with diabetic retinopathy. The diagnosis of DME was made according to the definitions of ETDRS[13] using indirect ophthalmoscopy by macula contact lens.

Sample Collection and Measurements for TNF-α

Fasting blood samples were collected in the early hours of the day before surgery for glucose, HbA_{1c} and CRP tests from all patients. The blood sera were immediately separated and stored at −80 °C until analysis for TNF-α.

Undiluted aqueous humor samples of 0.1–0.2 mL were collected, during cataract surgery, into sterile tubes. The aqueous from each eye was obtained by way of an anterior chamber puncture with a 27G needle of a 1 mL insulin injector, at the beginning of surgery. After collection, the samples were immediately transported and stored at −80 °C.

The concentration of TNF-α was measured by the sandwich ELISA method, using kits for human TNF-α (BD OptEIA™, Human TNF ELISA KIT II; BD Biosciences 10975 San Diego, CA, USA). The linear range of detection was 0–35 pg/mL for the assay.

Methods of quantitative determination for HbA_{1c} and for CRP were turbidimetric inhibition immunoassay and immunoturbidometric assay, respectively.

Ethical Considerations

Informed consent was taken from each patient at least 24 h prior to surgery. The study was approved by the hospital's institutional ethical committee, was conducted strictly following the approved protocol, and conformed to the ethical principles of the Helsinki Declaration.

Statistical Analysis

Groups were compared by Kruskal–Wallis and nonparametric Mann–Whitney tests. Correlations were measured by using nonparametric Spearman correlation test. The confidence interval of the tests was determined as 95%, and $p < 0.05$ was accepted as statistically significant.

TABLE 1. Baseline characteristics of groups.

	Healthy control group ($n=23$)	Diabetic control group ($n=22$)	No-DME group ($n=22$)	DME group ($n=20$)	p value*
Age (years)	67 ± 11	68.8 ± 7.4	65.5 ± 11.6	62.4 ± 6.3	0.165
Sex (male:female)	12:11	10:12	10:12	10:10	NS†
FBG (mg/mL)	104 ± 11.6	176 ± 77.4	164.7 ± 44.2	183.06 ± 60.66	<0.0001
HbA_{1c} (%)	5.3 ± 0.6	7.7 ± 2.2	9.19 ± 2.16	11.1 ± 3.8	<0.0001
CMT (μm)	210 ± 16	213 ± 16.8	224.6 ± 21	361 ± 105	<0.0001

Data presented as mean ± standard deviation. *Kruskal–Wallis test. †Chi-square test. NS, non-significant; FBG, fasting blood glucose; CMT, central macular thickness.

RESULTS

As discussed above, the study included four patient groups: the healthy control group ($n=23$ eyes); the diabetic control group ($n=22$ eyes); the groups with and without DME ($n=20$ eyes and $n=22$ eyes, respectively). Baseline characteristics of the four subject groups, and the serum and aqueous levels of inflammatory markers are summarized in Tables 1 and 2, respectively. The Kruskal–Wallis test showed a statistically significant difference among the serum TNF-α levels of the four groups ($p=0.01$). Serum levels of TNF-α were significantly elevated in the NO-DME group ($p=0.009$) and the DME group ($p=0.001$), respectively compared with the healthy control group (Figure 1). There was no significant difference between the healthy control group vs the diabetic control group; the diabetic control group vs the NO-DME group; the diabetic control group vs the DME group; and the NO-DME group vs the DME group ($p=0.091$, 0.456, 0.272 and 0.55, respectively).

Aqueous levels of TNF-α were significantly elevated in the DME group, compared with the healthy control group ($p=0.03$) and the diabetic control group ($p=0.006$) (Figure 2). Differences in aqueous TNF-α levels between the healthy control group vs the diabetic control group; the healthy group vs the NO-DME group; the diabetic control group vs the NO-DME group; and the NO-DME group vs the DME group did not demonstrate statistical significance after Bonferroni adjustment ($p=0.89$, 0.290, 0.201, and 0.121, respectively). Serum levels of CRP were significantly elevated in the DME group compared with the healthy control group ($p=0.004$) (Figure 3). There was no statistically significant difference in serum CRP between the healthy control group vs the diabetic control group; the healthy group vs the NO-DME group; the diabetic control group vs the NO-DME group; the diabetic control group vs the DME group; and the NO-DME group vs the DME group ($p=0.189$, 0.159, 0.751, 0.16, and 0.085, respectively).

Comparison of the healthy control group (23 eyes) with the overall two NPDR groups (42 eyes) showed that patients with NPDR had significantly higher levels of CRP ($p=0.016$) and serum TNF-α ($p<0.0001$) than healthy control patients (CRP 4.98 ± 2.8 vs 3.6 ± 2.6 and serum TNF-α 28.34 ± 12.22 pg/mL vs 17.08 ± 6.97, respectively). Correlation analysis showed that in the overall NPDR groups (42 eyes), HbA_{1c} levels were positively correlated with serum levels of CRP ($p=0.016$). In the DME group, the serum and aqueous levels of TNF-α were found to be positively correlated ($p=0.047$) and also aqueous TNF-α was correlated with age ($p=0.045$). No other correlations were detected.

DISCUSSION

The present study shows that serum levels of the inflammatory markers (CRP and TNF-α) and aqueous levels of TNF-α were significantly elevated in the DME group compared with the healthy control group. The pathogenesis of diabetic retinopathy is related to hyperglycemia-induced biochemical alterations.[2] A worse hyperglycemic history in diabetic patients is strongly associated with retinopathy development and progression.[2,14] Advanced glycosylation end-products (AGEs) circulating at high levels in diabetic persons are considered to increase oxidative stress and inflammation.[14] Increased serum concentration of AGEs in diabetic patients is associated with high serum CRP and TNF-α levels.[15,16] It was also shown that the levels of TNF-α were correlated with the levels of HbA_{1c}.[17]

TNF-α mediated retinal leukostasis and apoptosis contribute to the progressive breakdown of the blood–retinal barrier (BRB) in DR.[18] TNF-α has also been shown to increase retinal vascular permeability by the alterations in the endothelial tight junction complex.[19] Etanercept, a soluble TNFα receptor inhibitor, has been reported to reduce vascular leukocyte adherence and blood–retinal barrier breakdown in the diabetic retina.[9]

The systemic biomarkers such as CRP and vascular cell adhesion molecules, are known to be associated with DR factors and considered to help in the prediction and progression of diabetic retinopathy.[20] It has been shown that under hyperglycemic conditions, endothelial cells synthesize and secrete

TABLE 2. The serum and aqueous levels of inflammatory markers of groups.

	Healthy control group ($n = 23$)	Diabetic control group ($n = 22$)	No-DME group ($n = 22$)	DME group ($n = 20$)	p value*
CRP (mg/mL)	3.61 ± 2.6	4.2 ± 2.4	4.1 ± 1.78	6.07 ± 3.46	0.039
S-TNF (pg/mL)	17.08 ± 6.97	23 ± 13.1	26.76 ± 10.76	30.21 ± 13.88	0.01
A-TNF (pg/mL)	9.3 ± 4.04	9.8 ± 1.45	10.07 ± 3.47	12.53 ± 3.22	0.04

Data presented as mean ± standard deviation. *Kruskal–Wallis test. CRP, C-reactive protein; S-TNF, serum level of TNF-α; A-TNF, aqueous level of TNF-α.

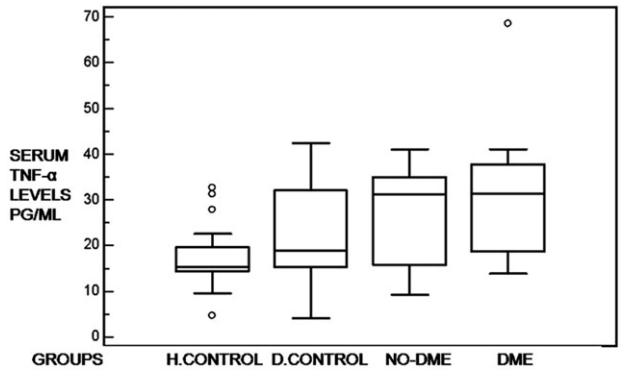

FIGURE 1. Graphical representation of the serum levels TNF-α in the four patient groups. This graph compares the values of serum TNF-α levels from group to group. Serum levels of TNF-α are significantly higher in the NO-DME and the DME groups than in the age-matched healthy control group.

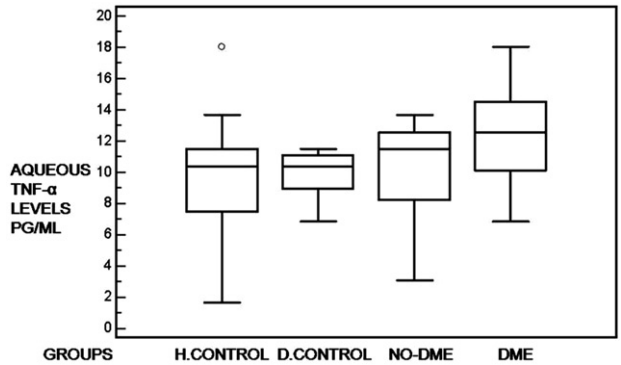

FIGURE 2. Graphical representation of the aqueous levels TNF-α of the four patient groups. This graph compares the values of aqueous TNF-α levels from group to group. Aqueous levels of TNF-α are significantly higher in the DME group than in the age-matched healthy and diabetic control groups.

increased levels of CRP, promoting endothelial dysfunction and inflammation.[15]

Elevated serum concentrations of TNF-α and VEGF have been identified to correlate with the presence and severity of DR.[3,21] Levels of inflammatory markers in the serum[22] and vitreous[21,23] were found to be correlated with the serum levels of HbA_{1c}. The serum and vitreous levels of proinflammatory molecules were significantly elevated in patients with the more severe retinopathy and proliferative retinopathy.[23–27]

In our study, the levels of both serum inflammatory markers (CRP and TNF-α) were significantly elevated in the patients with DME compared with healthy control patients but although the serum levels of these inflammatory markers were higher in the DME group, they did not demonstrate a statistically significant difference from those of the diabetic patients without DR and with NPDR without DME.

In a study by Oh et al., the aqueous levels of IL-6 were positively correlated with the macular volume and thickness,[28] but another study failed to demonstrate a correlation between serum cytokine and vascular endothelial growth factor (VEGF) levels and macular edema.[29]

Funatsu et al. showed elevated vitreous inflammatory factors and VEGF in eyes with DME.[30] In another study, aqueous levels of various inflammatory and angiogenic molecules were measured but only the IL-6 and VEGF levels were shown to be significantly higher in diabetic patients with retinopathy compared with diabetic patients without retinopathy.[31] Jonas et al. measured the concentrations of various cytokines in aqueous humor of eyes with DME and found elevated levels of cytokines in DME and also an association with the amount of DME.[32]

In this study, we found significantly elevated aqueous levels of TNF-α in the DME group compared with both the healthy and diabetic control groups but not with the NO-DME group, however a correlation between inflammatory markers (serum CRP and TNF-α; aqueous TNF-α) and central macular thickness was not detected in eyes with DME. However, we should bear in mind that the pathogenesis of DME is multifactorial; various vasoactive and inflammatory molecules, biochemical mechanisms, vitreoretinal interface abnormalities, are collectively involved in the genesis of this condition.[3,4]

In the present study, elevated levels of CRP and TNF-α in the sera of patients with DME may not be directly linked to retinal status but may suggest a contributing low-grade systemic inflammatory state in these patients. Whereas the significant elevation of TNF-α in the anterior chamber of eyes with DME may reflect, partly, the breakdown of the blood–retina barrier and somewhat the increased levels of local inflammatory factors into the retina due to the passage thorough vitreous. The importance of local

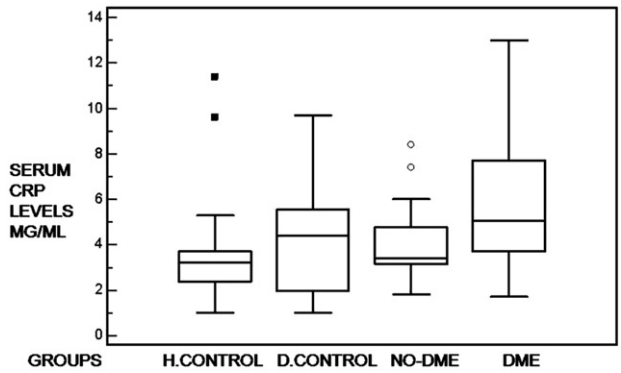

FIGURE 3. Graphical representation of the serum levels of CRP in the four patient groups. This graph compares the values of serum CRP levels from group to group. Serum CRP levels-α are significantly higher in the DME group than in the age-matched healthy control group.

production of the cytokines has been suggested as the cause of the lack of correlation between serum and intraocular concentrations.[33]

The limitations of our study include the relatively small sample size of patient groups and the measurement of only two inflammatory markers.

CONCLUSION

The results of our study show that inflammatory markers are considerably elevated in the serum and aqueous humor in eyes with DME. These results imply that there may be some relationship between inflammatory markers and DME. However, it is not clear whether inflammatory markers cause DME or vice-versa, given that DME is a multifactorial condition and that the mechanisms between this pathology and inflammatory markers could be complex. Despite the obvious evidence about the role of inflammation, further clinical and laboratory research is needed for a better understanding of the role of local and systemic inflammatory mediators in the pathogenesis of DME.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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