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CLINICAL STUDY

# Aqueous humor pentraxin-3 levels in patients with diabetes mellitus

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**Abstract**

*Purpose* **To evaluate aqueous humor (AH) pentraxin-3 (PTX3) levels in diabetic patients with and without diabetic retinopathy (DR).**

*Methods* **In this prospective study, patients undergoing cataract surgery were enrolled. The study group was composed of 26 type-2 diabetic patients without DR (group 1), 32 diabetic patients with DR (group 2) and 29 age-matched subjects without any systemic disease (group 3). Fifteen proliferative DR (PDR) and 17 non-proliferative DR (NPDR) patients were enrolled in Group 2. HbA1c levels and duration of diabetes were noted. AH samples were obtained from anterior chamber at the beginning of cataract surgery and PTX3 levels were analyzed with Elisa kit.**

*Results* **Baseline demographic characteristics were similar between groups. The mean duration of diabetes was $11.9 \pm 7.9$ years in group 1 and $15.8 \pm 7.8$ years in group 2 ($P = 0.11$). The mean plasma HbA1c levels in group 1 was $9.1 \pm 2.6$ and $8.2 \pm 2.4$ in group 2 ($P = 0.36$). PTX3 levels were $5.75 \pm 0.41$ in group 1, $6.11 \pm 1.47$ in group 2 and $4.93 \pm 0.84$ ng/ml in group 3 ($P = 0.01$). PTX3 levels in group 2 were higher than in group 1 and 3 ($P = 0.06$ and $P = 0.01$, respectively). There was no correlation between HbA1c and PTX3 levels ($P = 0.06$ $r = 0.57$, $P = 0.19$ $r = 0.3$, respectively). The mean PTX3 was $6.6 \pm 0.3$ in PDR group and $5.6 \pm 0.5$ ng/ml in NPDR group ($P = 0.04$).**

*Conclusions* **PTX3 is an important marker especially for vascular endothelial damage. Since diabetic vascular changes are dependent on endothelial cell damage, high levels of AH PTX3 of DR patients may indicate the importance of PTX3 protein in the pathogenesis of DR.**

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## Introduction

The prevalence of diabetes mellitus (DM) is increasing worldwide and becoming a global health problem.[1] One of the microvascular complication of DM is diabetic retinopathy (DR), which can be a vision threatening complication. The pathogenesis of DR is still under investigation. Recent studies are especially based on the role of ocular and systemic inflammation in the DR development. Hyperglycemia-induced vascular damage is the initial mechanism of the retinopathy.[2] Vascular endothelial injury and permeability alteration trigger the inflammatory process.[3] Increased levels of inflammatory cytokines and proteins in the vitreous,[4] tear,[5] aqueous humor,[6] and plasma samples[7] of the DR patients have been shown in previous studies.

Pentraxin protein superfamily is composed of acute phase proteins; C-reactive protein (CRP), serum amyloid protein (SAP), and pentraxin-3 (PTX3). Unlike hepatic production of CRP and SAP, PTX3 is synthesized by several peripheral tissues.[8,9] It has been suggested that PTX3 expression is increased under the conditions of vascular endothelial dysfunction.[10,11] Microvascular damage in DR and ocular inflammation are related reciprocally. Inflammatory status in DR patients has been supported by increased serum CRP and high sensitive CRP (hsCRP) levels.[12,13] In addition, the level of aqueous humor inflammatory cytokines and their correlation with DR development, severity and prognosis has been reported.[14]

Accordingly, the aim of the present study was to evaluate the relationship between aqueous PTX3 level and DR pathogenesis and progression.

## Materials and methods

### *Subjects*

This prospective study was approved by the local institutional ethics committee. Informed

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consent was obtained from all participants before cataract surgery. Patients undergoing cataract surgery in Ataturk Training and Research Hospital between December 2015 and June 2016 were enrolled in the study. We obtained AH samples from 26 type-2 diabetic patients without DR (group 1), 32 type-2 diabetic patients with DR (group 2) and 29 age-sex matched controls (group 3). Group 2 was composed of 15 patients with proliferative DR (PDR) and 17 patients with non-proliferative DR (NPDR). Before cataract surgery all participants had detailed ocular examination. The severity of DR was graded according to the modified Early Treatment Diabetic Retinopathy Study (ETDRS) retinopathy severity scale.[15,16] Patients with glaucoma, pseudoexfoliation, active ocular inflammation, history of ocular surgery and any retinal pathologies rather than DR were excluded. Other ocular exclusion criteria for DR group were having previous intravitreal corticosteroid or anti vascular endothelial growth factor (anti-VEGF) injections and argon laser focal, grid or panretinal photocoagulation within last 6 months. The stage of DR, duration of DM and serum HbA1c levels were noted. Preoperative systemic examination and routine blood tests including HbA1c level were performed for all participants within 1 week before cataract surgery. Group 3, as control group, had no systemic or ocular disease apart from cataract. Cerebrovascular or cardiovascular disease, chronic renal or hepatic failure, autoimmune disease, malignancy, uncontrolled hypertension presence were systemic exclusion criteria for the study groups.

### *Sample collection and measurements for PTX3*

Undiluted AH samples were collected at the beginning of phacoemulsification surgery through anterior chamber paracentesis with a 27G needle of tuberculin injector. After collection of 0.1–0.2 ml of AH, samples were immediately transported and frozen at − 80 °C until biochemical analysis.

The concentration of PTX3 was measured by kit for human PTX3 (R&D Systems, Inc., Minneapolis, MN, USA) using sandwich ELISA method and measurements were blinded to patient's status. The assay was conducted based on the manufacturer's instructions.

### *Statistical analysis*

All statistical analysis was performed using SPSS software for Windows version 20.0 (Chicago, IL, USA). The normality of the data was confirmed using the Shappiro-Wilk test. Results were given as a mean ± standard error. One-way variance analysis (ANOVA) followed Tukey's *post hoc* test was used to determine the statistical significance of the comparisons between groups. The correlation between variables was evaluated by Pearson correlation test. A *P*-value less than 0.05 was assumed to be statistically significant.

## Results

Demographical characteristics were shown in Table 1. The mean age was 75.1 ± 9.5 in group 1, 65.6 ± 7.2 in group 2 and 63.6 ± 11.9 in group 3 ($P = 0.26$). There was no significant difference between groups in terms of gender ($P = 0.61$). Duration of DM in group 1 was 11.9 ± 7.9 years and 15.8 ± 7.8 years in group 2 ($P = 0.11$). Serum HbA1c levels were 9.1 ± 2.6 in group 1 and 8.2 ± 2.4 in group 2 ($P = 0.36$; Table 2).

The mean PTX3 levels were 5.75 ± 0.41 (5.09–8.65) in group 1, 6.11 ± 1.47 (4.93–9.57) in group 2 and 4.93 ± 0.84 (4.01–6.78) ng/mL in group 3 (Figure 1). The difference in PTX3 levels between three groups was statistically significant by using ANOVA test ($P = 0.01$). In DR group, the mean PTX3 level was significantly higher than in control group ($P = 0.01$). The mean PTX3 level of diabetic patients without DR was lower than that in DR group, whereas higher than that in control group ($P = 0.06$, $P = 0.78$, respectively). Two group comparisons (group 1 *vs* 2 and 1 *vs* 3) did not show any significant difference by post hoc analysis of Tukey's method.

The mean PTX3 levels in NPDR cases ($n = 17$ eyes) was 5.6 ± 0.5, in PDR cases ($n = 15$ eyes) 6.6 ± 0.3 ng/ml which was significantly higher ($P = 0.04$).

There was no significant correlation between serum HbA1c and PTX3 levels in both group 1 and 2 ($P = 0.06$, $r = 0.57/P = 0.19$, r = 0.3, respectively).

## Discussion

There is an increasing trend in evaluating different inflammatory molecules in the pathogenesis of DR.

**Table 1** Demographics of the groups

| | *Group 1 (non DR)* n = *26* | *Group 2 (DR)* n = *32* | *Group 3 (non DM)* n = *29* | P-*value* |
|---|---|---|---|---|
| *Gender* | | | | |
| Female/male | 14/12 | 16/16 | 16/13 | 0.61 |
| Age (mean ± SD, range) | 75.1 ± 9.5 (45–86) | 65.6 ± 7.2 (56–83) | 63.6 ± 11.9 (29–77) | 0.26 |

Abbreviations: DR, diabetic retinopathy; DM, diabetes mellitus; n, number of patients.

**Table 2** Mean HbA1c level and duration of DM

| | *Group 1 (non DR)* n = *26* | *Group 2 (DR)* n = *32* | P-*value* |
|---|---|---|---|
| HbA1c (mean ± SD, range) | 9.1 ± 2.6 (5.5–13.2) | 8.2 ± 2.4 (4.7–13.6) | 0.36 |
| Duration of DM (years, mean ± SD, range) | 11.9 ± 7.9 (2–25) | 15.8 ± 7.8 (3–30) | 0.11 |

Abbreviations: DR, diabetic retinopathy; DM, diabetes mellitus; n, number of patients.

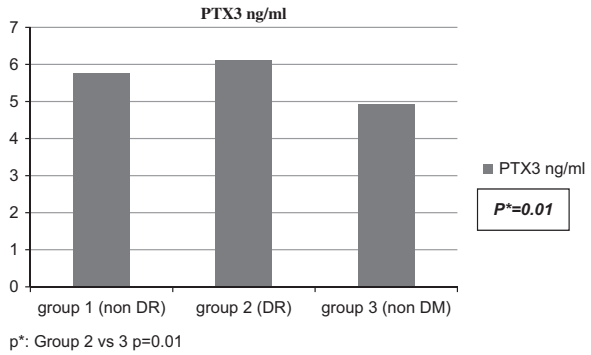


**Figure 1** Aqueous humor PTX3 levels. *P**: Group 2 vs 3 $P = 0.01$.

Recent studies in DR have demonstrated the inflammatory basis of the disease via serum or ocular fluid inflammatory cytokine levels. Elevated serum interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), VEGF, IL-8 levels and their correlation with retinopathy severity have been identified by clinical studies.[17–19] IL-6, IL-8, IL-10, IL-13, VEGF and monocyte chemoattractant protein-1 (MCP-1) are some of the studied local factors measured from vitreous or aqueous samples related with progression of DR.[20–23] These inflammatory biomarkers have a crucial role in DR pathogenesis and treatment modalities.

Pentraxin protein superfamily is composed of structurally different acute phase proteins; C-reactive protein (CRP) is classical short pentraxin whereas PTX3 is a member of long pentraxin family.[8,9] The synthesis of PTX3 protein is induced by IL-1 or TNF-α[24,25] and released by several cell types such as endothelial cells, fibroblasts, mononuclear phagocytes in response to inflammatory circumstances.[26,27] Woo *et al*[28] reported that human retinal pigment epithelial cells may be a major source of PTX3 production in the presence of proinflammatory cytokines IL-1 and TNF-α according to their cell culture study. Therefore PTX3 could be a novel protein for inflammatory response in the retinal pathologies.

Serum levels of proteins, cytokines and chemokines reflecting inflammatory status may be affected by many systemic conditions and this lead to conflicting results in the literature. For instance, while some studies showed increased serum CRP levels in DR[12,13] others showed no significant or inverse relation between CRP and DR.[29–31] Therefore AH and vitreous sampling seem to be better option for close reflection of retinal pathological changes. Both samples can be obtained during cataract or retinal surgeries. Generally pars plana vitrectomy (PPV) is not indicated in patients with non-PDR. Vitreous sampling through PPV has some risks like retinal tears or vitreous hemorrhage. In addition, significant relationship between the levels of cytokines from aqueous and vitreous samples has been shown by Funatsu *et al*.[32]

As an acute phase-reactant PTX3 has a key role in leukocyte recruitment during the inflammatory process.[33] This leukocyte-endothelial interactions lead to vascular permeability increment as seen in DR. Yang *et al*[34] have reported elevated levels of plasma PTX3 in patients with DR and its positive association with DR severity. In the same study they suggested that, the correlation between DR severity and plasma high sensitive CRP (hsCRP) levels were not significant. To the best of our knowledge, this is the first study to show AH PTX3 levels in diabetic patients.

In the current study we compared the AH levels of PTX3 protein between diabetic patients with and without retinopathy. The level of PTX3 in DR was significantly higher than in control group. Patients with PDR had the highest levels of PTX3 among the cases. This may indicate the importance of PTX3 in pathogenesis as well as in progression of DR. Although diabetic patients without retinopathy had higher PTX3 levels than control patients, the difference was not statistically significant. It may indicate that endothelial cell damage is not significant in the earlier phases of diabetes when DR was not manifest. DM duration and serum HbA1c levels were statistically similar among diabetic patients. Although systemic status of diabetes were similar, local PTX3 level difference might have a critical role in retinopathy pathogenesis. According to our study there was no correlation between HbA1c and PTX3 levels. However, serum[35] and vitreous[36,37] levels of inflammatory markers were found positively correlated with the serum HbA1c levels in some studies. In our study this correlation was absent. It might be because of small sample size or local production of PTX3 protein might be independent of current diabetic control.

One of the limitations of our study is the lack of serum samples of the study groups. Serum and AH PTX3 levels correlation could have been done in order to demonstrate

the presence of relationship between systemic and local levels of PTX3. But at the same time it has been shown that diabetic vascular complications apart from DR also affect the serum levels of PTX3 protein.[38–40] Another limitation of our study was its relatively small sample size. It was because of PTX3 levels might be affected by several systemic diseases such as cardiovascular or cerebrovascular diseases, chronic kidney and liver failure, autoimmune disorders, irregular systemic hypertension. For this reason we could include a limited number of patients.

In conclusion, significantly increased AH levels of PTX3 in DR patients indicate its importance in DR pathogenesis. Further prospective studies with a larger number of participants will be needed to confirm our results moreover, to clarify its benefit as a prognostic biomarker.

**Summary**

**What was known before**

- PTX3 synthesis is increased in the vascular endothelial damage PTX3 serum levels are high in diabetic patients.

**What this study adds**

- Aqueous humor PTX3 levels are high in patients with DR.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgements

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