Original Article

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Intraoperative Assessment of Interleukin-2, Interleukin-6 and Tumor Necrosis Factor-α in Aqueous Humor of Diabetic and Non-diabetic Patients Undergoing Phacoemulsification Surgery

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ABSTRACT

Background: Cataract is a condition where the clear lens of the eye becomes dull. Diabetes mellitus is well known disease that accelerate cataract progression. Cytokines are low molecular weight proteins that mediate the immune response. Low-grade chronic inflammation and activation of the innate immune system are strongly related to the diabetes mellitus pathogenesis. Interleukin-2 (IL-2), Interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and certain other chemokines are inflammatory mediators are influenced in the development of diabetes and its complications.

Objective: To evaluate and correlate the levels of inflammatory cytokines IL-2, IL-6 and TNF-α in the aqueous humor of eyes of diabetic and non-diabetic patients undergoing cataract surgery.

Patients and Methods: Seventy cataract patients (51 males and 19 females) were included, their ages ranging from 21 to 95 years. Intracameral cytokines IL-2, IL-6 and TNF-α were detected using ELISA.

Results: TNF-α significantly increased in the aqueous humor of diabetic patients more than in that of the non-diabetic ones (p-value < 0.05). The aqueous humor contained detectable IL-2 levels more frequently in diabetic patients. This difference was not statistically significant (p > 0.05). On the other hand, IL-6 was more frequently detectable in the eyes of the non-diabetic patients.

Conclusions: The distribution of cytokines in the aqueous sampling shows that TNF-α levels were higher in diabetic patients than in non-diabetic patients. On the other hand, IL-6 levels were higher in non-diabetic patients than in diabetic patients. With regard to IL-2, there was no significant difference.

Keywords: Diabetic retinopathy, cataract, IL-2, IL-6, TNF-α

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INTRODUCTION

Cataract is a condition where the crystalline lens clarity is lost causing the vision to be blurry, cloudy and distorted (1). Generally, cataract progression is believed to be age-related process with gradual lens nucleus sclerosis. In addition, other factors such as drugs, excessive sun exposure, intraocular surgery and many other conditions contribute to the development of cataract (2).

Diabetes mellitus is well-known to accelerates cataract progression in adults, which is believed to be the leading cause of deterioration of vision in diabetics. The aqueous humor nourishes the lens and supply it with the dissolved oxygen. In diabetic patients, the level of glucose in the lens and the aqueous humor will increase in correspondence to its rise in the blood; this leads to lens swelling (3). Furthermore, collection of sorbitol in the lens may affect the aqueous humor, making the lens opaque and cataractous (4).

Inflammatory cytokines does not pass freely to the eye due to the blood-aqueous barrier which causes difference in their concentration between the blood plasma and the aqueous humor. This explains the need for aqueous sampling rather than blood sampling to evaluate the inflammatory mediators (5).

Cytokines are low-molecular-weight proteins that mediate the immune response. They regulate the inflammatory process through a complex pathway and trigger the inflammatory response in any lesion site. Low-grade chronic inflammation and activation of the innate immune system are known to be strongly related to the diabetes mellitus pathogenesis (6).

Interleukin-2 (IL-2), IL-6, Tumor Necrosis Factor - alpha (TNF-α) and certain other chemokines are inflammatory mediators that are involved in the development of diabetes and its complications (7). It has previously been shown that the suppression of some cytokines plays a protective role against the pathological change of retinal capillaries in animal models (8).

PATIENTS AND METHODS

A total of 70 cataract patients (51 males and 19 females) were enrolled in this study from April 2016 to May 2016. Their ages ranged from 21 to 95 years. The general characteristics of the participants are summarized in Table 1.

All the patients underwent uneventful phacoemulsification cataract surgery at the Basra Teaching Hospital in South Iraq.

The exclusion criteria were as follows:

1. Traumatic cataract
2. Congenital cataract
3. Secondary cataract (to another ophthalmic disorder)
4. Toxic cataract (side effect of drug intake)

The diabetic patients had been diagnosed (preoperatively) by expert physicians, and they possessed documents of their diagnosis and type of treatment.

Table 1: Demographic characteristics of study participants.

Characteristics	No.	%
Age (years)		
20 ≤	2	2.9%
35 ≤	7	10%
50 ≤	29	41.4%
65 ≤	29	41.4%
80–95	3	4.3%
Sex		
Male	51	72.9%
Female	19	27.1%
Diabetic	29	41.4%
Non-diabetic	41	58.6%

Hemoglobin A1c (HbA1c) had been performed on every patient in this study to further confirm their diabetic status. The analysis was done using a Roche analyzer machine.

Aqueous Humor Collection:

Through anterior chamber paracentesis, approximately 0.1 ml of aqueous humor was aspirated into sterile plastic tubes and kept frozen at -70°C (deep freeze) to avoid denaturation until the serological examination was performed using the ELISA test.

Aqueous humor was then diluted with a sample diluent before use. It was divided into three parts, each emptied into a disposable tube, ready to be used to measure the following:

1. TNF-α – using EIA-TNF-α (Immunotech, a Beckman Coulter Company, France);
2. Human IL-6 – using the ELISA kit (Cusabio Biotech Co., P.R. China)
3. Human IL-2 – using the ELISA kit (Cusabio Biotech Co., P.R. China).

All the procedures were performed according to the manufacturers' instructions and the results revealed detectable or non-detectable quantity of the cytokines (positive or negative readings).

Ethical Aspect:

All the patients signed an informed consent form and confirmed understanding the aim of the study.

Statistical Analysis:

Version 22 of Statistical Package for Social Science (SPSS) was used in the data analysis. The Chi-square test of the mean value was used in the assessment of the significance of differences between groups. A p-value of less than 0.05 was considered statistically significant.

RESULTS

This study shows that 25 (86.20%) of the 29 diabetic patients had detectable amounts of aqueous TNF-α, while 58.50% of the non-diabetic patients had non-detectable amounts. This variation is statistically significant ($p < 0.05$; see Table 2).

The overall distribution of aqueous IL-2 in diabetic and non-diabetic patients is summarized in Table 3. It shows that the presence of detectable amounts of IL-2 was statistically non-significant between the two groups ($p > 0.05$).

Table 4 shows that out of 41 non-diabetic patients, 32 (78%) had detectable amounts of IL-6 in the aqueous humor. On the other hand, out of 29 diabetic patients, only 11 (37.9%) had

detectable amounts of IL-6, making the difference statistically significant between the two groups ($p < 0.05$).

Table 2: Aqueous humor TNF-α in diabetic and non-diabetic patients

TNF-α in aqueous humor	Study group		Total
	Diabetic	Non-diabetic	
Positive	25 86.2%	24 58.5%	49 70%
Negative	4 13.8%	17 41.5%	21 30.0%
Total	29 100%	41 100%	70 100%

X2 = 6.19 df = 1 $p < 0.05$

Table 3: Aqueous humor IL-2 in diabetic and non-diabetic patients

IL-2 in aqueous humor	Study group		Total
	Diabetic	Non-diabetic	
Positive	18 62.1%	19 46.3%	37 52.9%
Negative	11 37.9%	22 53.7%	33 47.1%
Total	29 100%	41 100%	70 100%

Table 4: Aqueous humor IL-6 in diabetic and non-diabetic patients

IL-6 in aqueous humor	Study group		Total
	Diabetic	Non-diabetic	
Positive	11 37.9%	32 78.0%	43 61.4%
Negative	18 62.1%	9 22.0%	27 38.6%
Total	29 100%	41 100%	70 100%

X2 = 11.53 df = 1 $p < 0.05$

DISCUSSION

This study showed that, more than the non-diabetic group, the diabetic cataract patients had a statistically significant increase in aqueous TNF-α. This is in agreement with Zuo CZ who found that the aqueous concentration of TNF-α in patients with cataract and diabetic retinopathy increased significantly (9).

Regarding IL-2, diabetic patients with cataract were positive to IL-2 levels in aqueous humor more than the non-diabetic group. This appears statistically insignificant like in the study by Cheung et al. who found that the level of IL-2 decreased in the diabetic group, unlike in the non-diabetic control. They hypothesized that low concentrations of these cytokines in non-diabetic patients' eyes provide protection against retinopathy (10). The disruption of such immune homeostasis is likely to be responsible for some of the pathological changes seen in diabetic patients' eyes (10). Higher vitreous levels of IL-2 and IL-6 were found in subjects with wet age-related macular retrogression, proliferative diabetic retinopathy and central retinal vein occlusion (11).

Oh et al. showed that, compared to normal controls, the serum level of inflammatory cytokines was increased in diabetic mellitus patients (12). In the current study, higher levels of serum and aqueous humor IL-2 was found in the diabetic group, approximate to that found in the non-diabetic group. This coincides with previous studies, considering the fact that several inflammatory cytokines were reported to have increased in both the serum (13) and the aqueous humor (14) of diabetes mellitus subjects. Chen et al., in their study on three types of cataracts (cataracts secondary to Behcet's disease, age-related cataracts, and Vogt-Koyanagi-Harada disease), revealed non-significant changes in the inflammatory cytokines between the peripheral blood and aqueous humor

sampling. They suggested that this result was most likely due to the subjects being in the inactive period, as previous studies have found notably increased levels of IL-2 in active patients (15).

Yang et al. suggested that the changes in the cellular immune system in patients with normal tension and open angle glaucoma may assume a vital role in the start and/or sustainment of glaucoma (16).

Interleukin-6 is an inflammatory cytokine linked to the development of type II diabetes mellitus. In the current study, the levels of IL-6 in aqueous sampling were higher in diabetic patients with cataract. This suggests that IL-6 plays a role in the pathogenesis of diabetes mellitus and may be a strong predictor of postoperative complications such as postoperative macular edema (17).

Dong et al. suggested that IL-6 levels might be potential indicators of the development of macular edema and macular thickness in diabetic patients after uncomplicated cataract surgery (18). The significant increase of IL-6 in the aqueous sampling from diabetic patients' eyes plays an important role in the development of diabetic retinopathy (19). IL-6 may also be helpful in analyzing diabetic retinopathy pathogenesis and anticipating disease course (20).

Aqueous humor sampling was needed as plasma levels differ significantly; it has been previously suggested that increased IL-6 in aqueous humor may originate intraocularly, rather than in systemic circulation (21).

Diabetic retinopathy patients showed increasing levels of inflammatory cytokines corresponding to the increasing neovascularization (22), reflecting that the measurement of inflammatory cytokines may be a predictor of diabetic retinopathy progression.

CONCLUSIONS

The distribution of cytokines in aqueous sampling shows that TNF-α levels are higher in diabetic patients than in non-diabetic patients. On the other hand, IL-6 levels are higher in non-diabetic patients. With regard to IL-2, there is no significant difference between the two populations.

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