

Analysis of TNF, IL2 & IL6 in the serum and aqueous humour of patient with cataract in Basra/Iraq

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Abstract—The expression patterns and function of cytokines during the development of inflammatory cataract remain unknown, so this study was aimed to detect the presence of cytokines in aqueous humor and serum of patients with cataract, in order to detect the involvement of these cytokines in development of cataract. Two types of clinical specimens were collected from seventy vision impairment patients who have a various types of cataract, who underwent cataract surgery in Basra General Hospital. One hundred and forty samples were collected (seventy of aqueous humor and seventy of blood) for serological detection of both TNF- α , IL-2, IL-6 by ELISA, the procedure were followed according to the instructions of the manufacturers. The level of TNF- α was positive in (73.3%) of serum and aqueous humor together, while (32.5%) is negative in both of them. The level of IL-2 was positive in serum only in (22.9%) of patients while (77.1%) of patients was negative, (62.2%) of these patients the IL-2 is positive in aqueous humor. In the present study the frequency of cataract patient who were positive for IL-6 in was (61.4%) aqueous humor and 23.3%, in both serum and aqueous humor. In conclusion :this study revealed that high percentage of cataract patients had a positive levels of TNF α ,IL2 and IL6 in aqueous humor than in the serum may indicate that these interleukins may play important role in the pathogenesis of cataract.

Keywords — Cataract, TNF, IL2, IL6

1 INTRODUCTION

A cataract is a cloudiness or opacity in the normally transparent crystalline eyes lens. This cloudiness can lead to a decrease in vision and may prompt possible visual impairment. [1].

There are many types of cataracts. Some cataracts are faint and do not interfere with vision. Other cataracts are dense and cause severe vision loss [2]. Cataract are the most widely recognized eye disease that may prompt visual impairment and extreme visual disability among adults aged 50 years or older [3].

Now a day, the frequency of postoperative complexities is diminishing with the improvement of phacoemulsification and posterior chamber intraocular lens implantation.

Cytokines are of most extreme significance for physiology and pathophysiology of the retina and retinal issue. Cytokines such as tumor necrosis factor α (TNF- α) and interleukins are extremely connected with inflammatory disturbances in the eyes [4].

TNF α is approved as pro-inflammatory cytokine participated in the natural immune reaction [5], It has pleiotropic impacts on various cell types and plays a basic role in the pathogenesis of chronic inflammatory diseases,

The increase of TNF α levels had a close relationship with presence of the intraocular lens itself which affects the discharge of TNF α . This suggested that TNF α as the principal mediators of immunological and inflammatory responses, so that may play critical role in anterior ocular inflammatory response after traumatic cataract extraction or intraocular lens implantation [6].

Interleukin-6 (IL-6) is an endogenous substance which is effective

in inflammation, and also in B cell maturation. It has been recognized that, although in general regarded as a pro-inflammatory cytokine, IL-6 also has many regenerative or anti-inflammatory actions [7]. It was associated significantly with prevalent nuclear cataract [8] and its level was significantly higher in Age-Related cataract patients, revealed that IL-6 was significantly correlated with the inflammation index [9]. IL-6 may play an essential part in the event of inflammation after cataract surgery, and might prompt new therapeutic ways to deal with this issue [10].

After cataract surgery, IL-6 may be produced in vivo by residual lens epithelial cells, causing postoperative inflammation and lens epithelial cell proliferation [11].

Interleukin-2 (IL-2) is a pleiotropic cytokine that drives T-cell development, increases NK cytolytic activity, prompts regulatory T cells differentiation, also mediates activation-induced cell death [12]. It has essential biological functions as immunotherapeutic agent against renal cell carcinoma therapy and for metastatic melanoma [13]. Levels of IL-2 in aqueous humor and peripheral blood from patients with uveitis were significantly higher, compared with those in the controls [14].

The severity of eye diseases, and specifically their complications, is identified with the intraocular concentration of these cytokines [15]. The expressions of interleukin-2 (IL-2), from the three types of cataract patients, show there were no significant differences among the three groups in either the AqH or the peripheral blood; this result was most likely due to the subjects being in the inactive period because previous studies have found notably increased levels of IL-2 in active patients [9]. In plasma of vernal and atopic keratoconjunctivitis pa-

tients, levels of interleukin-2 receptor was significantly increased compared with control.

Understanding of the role of cytokines in inflammatory eye disease has the potential to lead to the development of therapies to abrogate the effects of these important mediators of the inflammatory response [16]. Along these lines, in this study, we utilized the ELISA immunoassay to

Detect the contribution of proinflammatory cytokines in aqueous humor and serum of patients with cataract at the beginning of cataract surgery and appearance levels to the advancement of disease.

Make Comparison of cytokines level between the aqueous humor and serum of same patient. To discover whether these cytokines play major role in evolution of disease (cataract)

2 MATERIALS AND METHODS

This study was carried out from April 2015 till May 2016. A total of 70 patients (51 male & 19 females) were included, their age ranged from (21-95 years old).

Exclusion criteria: traumatic cataract, congenital cataract, secondary cataract (to another ophthalmic disorder), toxic cataract (side effect of drug intake). All patients underwent cataract surgery in Basra General Hospital.

Blood sample collection: By using a sterile disposable syringe, 3ml of fasting venous blood was drawn from each patient emptied into disposable tube which was left standing for 1hour at room temperature allowed to clot and then centrifuged at (3000 rpm) for 5 minutes, serum drawn by clean pipette into 3 sterile plastic tubes and kept frozen at -70C ° (deep freeze) until the serological examination was performed for detection of cytokins (IL-2, IL-6, TNF-a) in serum by ELISA test. No serum sample was freezeed and thawed more than twice.

Collection of Aqueous Humor: Aqueous humor was collected through anterior chamber paracentesis before cataract surgery began.

Aqueous Humor was suctioned utilizing a small syringe connected to a 30-gauge needle which entered the peripheral anterior chamber (AC) at the limbus, on a plane approximately parallel with the long axis of the elliptical lens. Approximately 0.1 ml Aqueous Humor were obtained was considered to have physical properties similar to saline at 37°C ,drawn into sterile plastic tubes and kept frozen at -70 °C (deep freeze) until the serological examination was performed for detection of cytokins (IL-2,IL-6, TNF-a) in Aqueous Humor by ELISA test, (Immunotech TNF-a enzyme immunoassay IMMUNOTECH A BECKMAN COULTER COMPANY, Human Interleukin 6 (IL-6) ELISA Kit (CUSABIO), Human Interleukin 2 (IL-2) ELISA Kit CUSABIO The procedures were performed according to manufacturer's instructions.

Statistical Analysis: Statistical Package for Social Science (SPSS) version22 was used to analyze the data. Chi-square (X2) test, and mean value were used to assess the significance

of differences between groups. P. value less than 0.05 was considered as statistically significant and P- value less than 0.01considered as highly significant.

3 RESULTS

The general characteristic of the patients participated in this study are summarized in Table 1.

Table 1 General Characteristics of the Study population

| Characters | 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% |

Analysis of data was done to determine the relation of TNF alpha in aqueous humor and the serum of the study population (Table 2). 73.3% of the cataract patients had elevated level of TNF alpha in both Aqueous humor and the serum, while 67.5% of those with positive TNF in Aqueous humor were negative in the serum. These differences were statistically not significant (P>0.05).

Table 2 The relation of TNF alpha in aqueous humor and the serum of the study population

| Level of TNF in the serum | Level of TNF in the aqueous humor | | Total |
|---------------------------|-----------------------------------|-------------|-------|
| | Positive | Negative | |
| Positive | 22 73.3% | 8 26.7% | 30 |
| Negative | 27 67.5% | 13 32.5% | 40 |
| Total | 49 | 21 | 70 |

$$X^2=0.278 \quad df=1 \quad p>0.05 \quad (NS)$$

The distribution of TNF in Aqueous humor according to sex among study population was summarized in Table 3. It shows that, although there was no significant effect of sex on the distribution TNF in Aqueous humor genes among cases (P>0.05) but it tend to occur more in the male than in female.

Table 3 The distribution of TNF in Aqueous humor according to the gender

| Sex | TNF in Aqueous humor | | Total |
|--------|----------------------|--------------|-------------|
| | Positive | Negative | |
| Male | 35 71.4% | 16 76.20% | 51 72.9% |
| Female | 14 28.6% | 5 23.8% | 19 27.1% |
| Total | 49 100% | 21 100% | 70 100% |

$\chi^2=0.169$ $df=1$ $p>0.05$

The relation of IL2 in aqueous humor and the serum of the study population summarized in Table 4 which show that 37.8% of cataract patients had elevated level of IL-2 in both serum and a aqueous humor. While 62.2% of them had elevated level of IL-2 in the aqueous humor only. This difference was statistically significance ($P<0.05$).

Table 4 The relation of IL2 in aqueous humor and the serum of the study population

| Level of IL2 in the serum | Level of IL2 in the aqueous humor | | Total |
|---------------------------|-----------------------------------|-------------|-------------|
| | Positive | Negative | |
| Positive | 14 37.8% | 2 6.1% | 16 22.9% |
| Negative | 23 62.2% | 31 93.9% | 54 77.1% |
| Total | 37 100% | 33 100% | 70 100% |

$\chi^2=9.989$ $df=1$ $p<0.05$

Although the highest percentage of cataract patients with positive IL-2 in aqueous humor were male 27(73%), but still there was no significant effect of sex on the level of IL-2 in aqueous humor $p>0.05$.

Table 5 The distribution of IL-2 in Aqueous humor according to the gender

| Sex | IL-2 in Aqueous humor | | Total |
|--------|-----------------------|-------------|-------------|
| | Positive | Negative | |
| Male | 27 73.0% | 24 72.7% | 51 72.9% |
| Female | 10 27.0% | 9 27.3% | 19 27.1% |
| Total | 37 100% | 33 100% | 70 100% |

$\chi^2=0.01$ $df=1$ $p>0.05$

The data summarized in Table 6 show that out of 70 patient

with cataract, 43 (61.4%) patients had positive Level of IL6 in the aqueous humor. Out of the 43 IL-6 positive patients: 23.3% had elevated level of IL6 in both serum and aqueous, while 76.7% had elevated Level of IL6 in the aqueous humor only. The difference was (Statistically not significant ($p>0.05$)).

Table 6 The relation of IL6 in aqueous humor and the serum of the study population

| Level of IL6 in the serum | Level of IL6 in the aqueous humor | | Total |
|---------------------------|-----------------------------------|--------------|-------------|
| | Positive | Negative | |
| Positive | 10 23.3.8% | 9 33.3% | 19 22.9% |
| Negative | 33 76.7% | 18 66.7% | 51 77.1% |
| Total | 43 61.4% | 27 38.57% | 70 100% |

$\chi^2=8.52$ $df=1$ $p>0.05$

Table 7 show the distribution of IL-6 in Aqueous humor according to the sex. Although high percentage of IL6 positive patients were male (74.4%) and only 25.6% were female but the result was not significant at $p < 0.05$.

Table 7 The distribution of IL-6 in Aqueous humor according to the sex.

| Sex | IL-6 in Aqueous humor | | Total |
|--------|-----------------------|-------------|-------------|
| | Positive | Negative | |
| Male | 32 74.4% | 19 70.4% | 51 72.9% |
| Female | 11 25.6% | 8 29.6% | 19 27.1% |
| Total | 43 100% | 27 100% | 70 100% |

$\chi^2=0.137$ $df=1$ $p>0.05$

4 DISCUSSION

Until now, little was known about the function of cytokines in the development of cataracts Cytokines are crucial in the regulation of normal human immune responses and related diseases [17]. The level of TNF-a was positive in (73.3%) of serum and aqueous humor together, while (32.5%) is negative in both of them ,this result was statistically not significant ($P > 0.05$) due to of small sample size , but may gave a hint that the majority of cataract patient in this study had high level of aqueous TNF. In fact this result was in agreement with Zhao-hui Z et al (1999) study who found that the TNF levels are significantly higher ($P<0.05$) and he suggested that TNF as the principal mediators of immunological and inflammatory responses, so that may play critical role in anterior ocular in-

inflammatory response after traumatic cataract extraction and intraocular lens implantation [6]. The level of IL-2 was positive in serum only in (22.9%) of patients while (77.1%) of patients was negative, (62.2%) of these patients the IL-2 is positive in aqueous humor besides (37.8%) of patients is positive in serum and aqueous humor. the positive result in aqueous humor is more than in serum. Scheinberg MA et al also found that the patients with uveitis had an increased level of aqueous humor, he suggested that IL-2 had an immunoregulatory function in acute uveitis and raises the opportunity that its identification might be valuable in treatment [18]. Interleukin-2 is understood for the advancement of immune and inflammatory reactions, furthermore have vital immunosuppressive capacities [19]. In the present study the frequency of cataract patient who were positive for IL-6 in was (61.4%) aqueous humor and 23.3%, in both serum and aqueous humor: with mean IL-6 concentration among positive cases was 9.38/5.84 of IL-6 in aqueous humor and serum respectively. The means ratio was (9.38/5.84) more than 1 which indicate intraocular synthesis of IL-6. The difference was statistically non significance due to small sample size.

It is surely understood that IL-6 is a multifunctional cytokine that has angiogenic capacities and proinflammatory through the affectation of VEGF [20]. IL-6 may assume a crucial role in the advancement of postoperative inflammation after cataract surgery, since it has been appeared to stimulate collagen synthesis and may therefore be included in corneal wound healing: therefore those post operative patients who had positive aqueous IL-6 should considered as risky patient to develop post operative complication like macular edema [21].

5 CONCLUSION

This study revealed that high percentage of cataract patients had a positive levels of TNF alph, IL2 and IL6 in aqueous humor than in the serum may indicate that these interleukins may play an important role in the pathogenesis of cataract.

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