ORIGINAL ARTICLE

Interleukin-6 and tumor necrosis factor-α levels in tear film of Keratoconus patients

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Background: It is hypothesized that increased inflammatory markers in keratoconus (KC) may be one of the causes of corneal damage. The aim of our study was to the measurement of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL)-6 in tear of patients with KC and investigate their relationship with the severity of KC. **Materials and Methods:** The current study was performed on KC patients and healthy controls with a case-control setting. Tear levels of TNF-α and IL-6 were measured after collecting the tears from the tear lake using a filter paper via Schirmer I method without anesthesia. **Results:** Eighty-one KC patients (mean age 29.45 ± 5.06 years) and 85 controls (mean age 28.01 ± 5.14 years) were enrolled. The mean levels of IL-6 and TNF-α were 26.77 ± 8.16, and 34.58 ± 9.82 pg/ml in the healthy group and 103.22 ± 51.94, and 183.76 ± 54.61 pg/ml in the KC group, respectively ($P < 0.001$). There was a significant relationship between the severity of the KC and the mean levels of IL-6 TNF-α in the case group ($P < 0.001$). **Conclusion:** Our results indicated that the mean levels of IL-6 and TNF-α are significantly higher in KC than the healthy group, and the disease severity was significantly associated with TNF-α and IL-6.

Key words: Inflammation, interleukin, keratoconus, tumor necrosis factor

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INTRODUCTION

Keratoconus (KC) is characterized by progressive, noninflammatory stromal collagen changes and the central and paracentral corneal alternation.[1,2] On one hand, there is an association of KC with atopic disease, allergies, and allergic rhinitis[3-5] and on the other hand, allergic conditions including atopic diastasis have considered as one of the important related factors associated with KC.[4-6]

The atopic situation may accelerate the course of KC[5,7] because atopic conditions is usually associated with eye rubbings that is an important factor in KC and their progression.[8,9]

KC has been considered a noninflammatory disease for many years, but recent investigations have shown inflammatory pathways in the mechanism of KC.[3,6,10,11] Tear is an important and accessible source for the detection of biomarkers. Detection of some inflammatory cytokines including interleukin-1 (IL)-1 β, IL-6, interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α) in tear of patients with KC have been shown in several studies.[10,12,13]

In one study evaluating the amount of cytokines in tear of KC patients, researchers showed that cytokines such as matrix metallopeptidase 9 (MMP-9) and IL-6 are overexpressed in tears of KC patients and that the chronic inflammatory events in the eye may be the cause of KC progression.[14] Recently, there are clinical

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and experimental evidence that supports the role of proinflammatory cytokines in KC.[14-16]

The aim of the current study was to assess levels of TNF-α and IL-6 tears of patients with KC and healthy individuals and determine their relationship with disease severity.

MATERIALS AND METHODS

The current study was performed on patients with KC and healthy controls with normal corneal topography in a case-control design. The cases included KC patients (based on imaging findings) aged between 18 and 50 years. The control group was healthy age- and gender-matched individuals that were candidates for refractive surgery without any corneal diseases. Exclusion criteria were other corneal diseases (such as herpetic corneal inflammation and corneal opacity), pregnancy, breastfeeding, uveitis, and malignancy before or during the study. Written informed consent was obtained from each subject before the initiation of the study. The study was approved by the local ethical committee before the initiation (Project NO.: IR.MUI.MED.REC.1397.920).

Completed ophthalmological examination performed for all participants. One eye of each subject was randomly selected for inclusion in the study. Tears of the subjects were collected from the tear lake through the Schirmer I method via Schirmer tear test strip without anesthesia. The tear levels IL-6 and TNF-α were measured at baseline. The amount of tear obtained was calculated considering 1 mm of wet Schirmer strip containing 1 μL of tear. The levels of tear markers were measured through the highly sensitive method of enzyme-linked immunosorbent assay (ELISA). The limits of detection for the cytokine kits were 2 pg/ml.

Data were analyzed using SPSS (SPSS Inc., Chicago, Illinois, USA, Ver 21). Numerical variables were reported as mean ± standard deviation (SD) and median (mid-quartile range) while categorical variables were reported as frequency (percentage). The Spearman correlation coefficient and *t*-test were used to compare the two groups in terms of quantitative variables and disease severity. We used the Chi-square test to compare the two groups. Results were considered as statistically significant when $P \leq 0.05$.

RESULTS

Eighty-one patients with KC and 85 healthy individuals were included study. The mean ± SD age of the patients was 29.45 ± 5.06 years in the control group and 28.01 ± 5.14 years in the case group [Table 1].

The mean levels of IL-6 were 26.77 ± 8.16 pg/ml in the control group and 103.22 ± 51.94 pg/ml in the healthy controls and the mean levels of TNF-α were 34.58 ± 9.82 pg/ml in the control group and 183.76 ± 54.41 pg/ml in the case group. The effect of gender variable was considered constant using the mixed model analysis (by controlling the effect of gender variable) and the mean of IL-6, TNF-α, and keratometry of the individuals in the groups were examined. After modifying the amounts, there is significant differences between the groups regarding the level of IL-6 and TNF-α, and keratometry ($P < 0.001$) [Table 2].

A significant relationship existed between the disease severity (mean keratometry) and the levels of TNF-α and IL-6 in the case group ($P < 0.001$) [Table 3].

DISCUSSION

The current study indicated that the mean tear levels of TNF-α and IL-6 were significantly increased in KC patients compare to control groups. Mean keratometry in patients with KC was significantly associated with the levels of IL-6 and TNF-α.

Despite multiple studies conducted on the etiology of KC, its underlying cause still is unknown. It is proposed that increased inflammatory markers in patients with

Table 1: Mean and frequency of demographic characteristics of individuals in the study groups

	Control	KC	*P*
Age, mean±SD	29.45±5.06	28.01±5.14	0.072*
Gender, *n* (%)			
Female	60 (70.6)	40 (49.4)	0.007**
Male	25 (29.4)	44 (50.6)	
Eye			
Right	43 (50.6)	41 (50.6)	0.997**
Left	42 (49.4)	40 (49.4)	

*t-test; **Chi-square. KC=Keratoconus; SD=Standard deviation

Table 2: Mean levels of interleukin-6, tumor necrosis factor-α, and keratometry in the study groups

Main variables	Mean±SD		*P*
	Control	KC	
IL-6	26.77±8.16	103.22±51.94	<0.001
TNF-α	34.58±9.82	183.76±54.41	<0.001
Keratometry	45.3±2.20	52.31±5.66	<0.001

KC=Keratoconus; IL=Interleukin; TNF=Tumor necrosis factor; SD=Standard deviation

Table 3: Relationship of keratometry with interleukin-6 and tumor necrosis factor-α in the case group

Pearson correlation coefficient	IL-6	TNF-α
Mean keratometry		
Correlation coefficient	0.632	0.650
P	0.000	0.000
n	81	81

IL=Interleukin; TNF=Tumor necrosis factor; SD=Standard deviation

KC may be one of the causes of corneal damage and its progression.[10,15,17]

The presence of a vicious cycle between inflammatory cytokines and proteases and their inhibitors, as well as excessive oxidative stress, result in increased apoptosis.[14,18,19]

The results of our study showed a significant increase of IL-6 and TNF-α and the disease severity (mean keratometry) in tears of KC. In this regard, a study was performed aiming to determine the levels of IL-4, IL-6, IL-10, RANTES, INF-γ, and TNF-α in tears of 48 patients with KC and their first-degree family members. The researchers showed that the levels of inflammatory markers such as INF-γ, IL-10, IL-1 β, IL-4, IL-6, and TNF-α significantly increased in patients with KC and their first-degree family members compared to the control group.[12]

Sorkhabi *et al.* determined the level of inflammatory markers in tear of 42 KC patients and 30 controls. Similar to our study, Sorkhabi *et al*. demonstrated that there is a statistically significant difference in the levels of inflammatory markers such as IL-6, IL-10, IL-1 β, and IFN-γ in tears of patients with KC versus control group.[16]

While in a review study aimed at answering the question "is KC an inflammatory disorder?" Galvis *et al.* demonstrated that the increased levels of IL-6, TNF-α, and MMP-9 in the lacrimal fluid of KC patients do not meet all the classical criteria of inflammatory disease.[14] This can be attributed to the limited number of studies performed by one kit, while the majority of them use the standard ELISA with less sensitivity to multiplex polymerase chain reaction. In their review study, Ionescu *et al.* suggested that there are some hypotheses about potential and different pathways in the expression of inflammatory markers in patients with KC.[15]

Although overexpression of cytokines may be a cause of inflammation in KC and overexpression of inflammatory biomarkers may be a complementary risk factor, the present study showed a significant relationship between disease severity and IL-6 and TNF-α expression in the KC group. Similarly, Ionescu *et al.* found a statistically significant relationship between lacrimal expression of IL-6 and disease severity in the KC group.[12]

Similar to our finding, Balasubramanian *et al.* reported that the expression of IL-4, IL-5, TNF-α, IL-10, and IL-6 increased in the tears of patients with KC. Therefore, KC can be classified as inflammatory conditions. Moreover, there was a positive correlation between cytokines and keratometry.[20]

Although our study had several limitations, larger sample size compared to other studies was the strength of the study. Results of our study provide promising horizons about the feasibility of inflammatory response modulators in the control of the progression of disease. Our study had some limitations. We have evaluated only two main proinflammatory cytokines, IL-6 and TNF-a in the tears of patients with KC. Another limitation was the lack of evaluating the level of these cytokines in blood samples for assessment of systemic involvement in KC disease.

CONCLUSION

The current study showed that the levels of IL-6 and TNF-α increased in KC patients compared to healthy individuals. Furthermore, change of level of IL-6 and TNF-α significantly associated with increasing disease severity. Biochemical findings might help diagnose and determine the severity and progression of KC.

Availability of data and materials

All datasets related to the current study are available from the corresponding author.

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Conflicts of interest

There are no conflicts of interest.

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