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# The expression and significance of inflammatory cytokines and MMP-9 in the tears of the infected eye and contralateral uninfected eye in patients with fungal keratitis

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**Research Article**

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# Abstract

**Background:** We investigated bilateral tear cytokine levels including interleukin (IL)-1β, IL-10, IL-17, tumor necrosis factor (TNF)-α and Matrix metalloproteinase-9 (MMP-9) in patients with fungal keratitis(FK). Meanwhile, we evaluated the relationship between the changes of tear cytokines with corneal perception and pain in infected eyes, and the relationship between tear cytokines and tear film function in contralateral uninfected eyes .

**Methods :** A total of 60(20 FK, 20 contralateral, 20 healthy controls) tear samples were collected prospectively and analyzed by enzyme linked immunosorbent assay(ELISA). Approximately 50 to 60 ul of tear samples in each case were collected. Meanwhile ,we analyzed the changes of visual analogue scale(VAS), tear breakup time (TBUT), Schirmer I test (SIT) and corneal perception compared with healthy controls.

**Results :**The concentrations of IL-1β, IL-10 and IL-17 increased in bilateral eyes compared with healthy controls($P<0.05$). The tear concentrations of MMP-9 , TNF-α only significantly increased in affected eyes ($P <0.05$). Patients with FK showed significant reduction in corneal perception of infected eyes compared with controls($P<0.05$). Corneal perception of the normal eyes in FK patients was slightly lower than that of control group, but there was not statistical difference ($P>0.05$).TBUT and SIT of contralateral uninfected eyes were significantly lower than that of control group($P<0.05$), which were significantly correlated with levels of IL-1β, IL-17($P<0.05$). SIT were also negatively correlated with MMP-9($P<0.05$), while the levels of IL-1β, IL-10, IL-17, TNF-α and MMP-9 in the tears of the healthy control group had no significant correlation with TBUT and SIT indicators($P>0.05$).The corneal perception and VAS score of the affected FK eyes showed correlation with IL-1β, IL-17 and TNF-α($P<0.05$).In addition, concentration of IL-10 inversely was correlated with VAS ($P<0.05$).

**Conclusion:** Proinflammatory tear cytokines are elevated in bilateral eyes with unilateral FK as associated with tear film function ,pain and corneal sensitivity.


# Background

Fungal keratitis(FK) is one of the major vision-threatening disease ,which affects public health worldwide, especially in developing countries[1]. The most common pathogens recognized in FK are Aspergillus spp., Fusarium spp.[2]. Most infections with these organisms are initiated in agricultural environment as a result of ocular surface trauma caused by plant material branches or insects contaminated with fungal spores[3] .These microorganisms will invade the cornea, destruct the protective physical barrier at the ocular surface and initiate an immune or inflammatory response. It has been suggested that immune regulation plays a vital role in the pathogenesis of FK[4]. The recruitment of inflammatory cells induced by microorganisms and inflammation will trigger the release of cytokines and chemokines. In the early stage of this disease, it may be related to the adhesion and invasion of mycotoxin, however ,the further damage may be connected with the strong immune inflammatory. A certain inflammatory response can

remove fungi and promote the repair of tissue. However, long-term overreaction of the host immune response may magnify inflammation, release further attracts more immune cells and lead to corneal opacity, tissue damage, even perforation and vision loss[5, 6].Therefore, studying the inflammatory factors has a great significance to explain the pathogenesis of fungal keratitis, which provides help for FK patients to avoid devastating outcomes. There exists numerous of immunocompetent cells in corneal limbus and conjunctival tissues. Intraocular antigen and mycotoxins will contact the conjunctiva or corneal limbus, enter the systemic lymphatic system and activate the human immune system, which will cause immune inflammation in contralateral eye[7]. Although there are no reports about sympathetic ophthalmia happened in FK patients, some studies have shown related immune changes in contralateral eyes .The collection of intraocular samples such as aqueous humor is invasive, therefore we replaced them with tear samples to reflect the inflammatory response because it has little discomfort and is non-invasive[8]. Nowdays, tear samples are increasingly used to detect biomarkers of normal and diseased ocular surfaces, such as dry eye[9] and human microbial keratitis[10, 11]. In our study, We investigated bilateral tear cytokine levels and MMP-9 to evaluate immune response of bilateral eyes in patients with FK. Meanwhile, we evaluate the influence of these indicators on pain, corneal nerve and tear film changes.

# Methods

### Participants

There were a total of 20 FK patients (12 males,8 females) and 20 healthy controls (11 males,9 females) who had already matched their sex, age, with FK patients in this study. All FK patients were diagnosed in the Ophthalmology Department of the hospital of a comprehensive university. Inclusion criteria of FK patients were in accordance with one of the following three items: (i) fungal hyphae and / or spores were observed under microscope after smeared by corneal scraping; (Fig.1); (ii) fungal hyphae or spores were found under corneal confocal microscope;(iii) the culture results of scraped specimens of fungal lesions were fungi.

Exclusion criteria for FK patients or healthy control subjects (i) systemic immunological or rheumatic diseases;(ii)diabetes;(iii) previously infected with viral or other keratitis;(iv) a history of eye trauma and/or surgery;(v) other ocular surface diseases affecting the secretion of tear cytokines , such as Pterygium or conjunctivitis;(vi) with dry eye symptoms in the past.

This study was approved by the ethics committee of a comprehensive university hospital. All the methods were performed in line with the Declaration of Helsinki. In addition, every patient involved in this study had been informed of the whole study design and signed the informed consent.

### Tear collection

A total of 60(20 FK, 20 contralateral, 20 healthy controls) tear samples were collected .As for healthy controls, the tear samples were always selected from right eyes. Tear samples were collected in the same

time frame(between 7.00 and 8.00 am) without stimulation .By using 10ul glass capillary micropipettes(Drummond, USA),approximately 50 to 60 ul of tear samples in each case was collected. Every time we used a new micropipette sterile tip for each eye. Reserved 50 to 60 ul tear samples were transferred into sterile microfuge tubes and stored at −20 °C until assaying for the cytokine.

### Enzyme -linked immunosorbent assay(ELISA)

IL-1 β, IL-6, TNF- α , IL-10 and MMP-9 kits (R&D systems , Minneapolis, USA were used to detect the expression levels of IL-1 β, IL-10, IL-17, TNF- α and MMP-9 in tears. The operation was according to the instructions of ELISA kit, as follows: (1)Add standard: Set Standard wells, testing sample wells. Add standard 50μl to standard well.(2)Add Sample: Add testing sample 10μl then add 40μl of Sample Diluent to testing sample well; Blank well doesn't add anything. (3) Add 100μl of HRP-conjugate reagent to each well, cover with an adhesive strip and incubate for 60 minutes at 37°C. (4) Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Solution (400μl) using a squirt bottle, manifold dispenser or auto washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting. Invert the plate and blot it against clean paper towels. (5) Add chromogen solution A 50μl and chromogen solution B 50μl to each well. Gently mix and incubate for 15 minutes at 37°C. (7)Add 50μl Stop Solution to each well. The color in the wells should change from blue to yellow. (8)Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

### Visual analogue scale (VAS)

The pain degree of the patients was measured by VAS developed by the National Institutes of Health[12]. The degree of pain on a scale of 0-10: 0 point: no pain; Less than 3 point: mild pain but bearable; 4-6 point: tolerate pain though affecting sleep; 7-10 point: unbearable pain ,which affects appetite and sleep.

### Tear film examination

The examination of the liquid was mainly carried out from two items: tear breakup time(TBUT) and Schirmer I test(SIT).(1)TBUT: TBUT reflects the stability of tear film. Fluorescein sodium strips(Tianjin Jingming New Technological Development Co., Ltd.) was placed in the lower eyelid conjunctival sac for about 1-2 seconds, After taking out the test paper , we told patient to blink several times, then look straightly ahead and try to prolong opening time of the eye. We observed the tear film under slit lamp cobalt blue light, and calculated time from the last blink after natural opening eyes until appearing the first dry spot on the corneal surface. After repeated measurement for 3 times in each eye, the average value was taken to record the results.(2)SIT: Schirmer I test reflected the basal secretion of tears. We folded the Schirmer tear test strips of 5mmx30mm standard(Tianjin Jingming New Technological Development Co., Ltd.) from the opening of the notch for 5 mm. Then we placed it at the junction of the conjunctival sac of the lower eyelid (gently, minimize stimulation), and told the patient to look down and close his eyes gently. 5 minutes later, we took out the filter strip and recorded the soaking length.

### Corneal perception examination

The Coehet-Bonnet keratometer ( Luneau SA company ,France) was used to detect the corneal sensitivity ,of which the diameter of the fiber was 0.12 mm, and the longest length was 60 mm. After telling the patient to sit upright, and look straight forward, we contacted the center of the cornea vertically with the fiber endings. The examiner saw the fiber bending with the naked eye. The positive feedback was that the patient felt the sensation of foreign body in the cornea. Starting from 60 mm, the length was reduced by 5 mm each time until the patient showed a positive reaction, and the average value was taken 3 times. All examinations were performed by the same physician.

### Statistical analysis

Statistical analyses were performed by SPSS software(version 22, SPSS Inc., Chicago, Illinois, USA). The basic information (age, weight) between the patient and the healthy control group was compared by independent sample t-test, while gender differences were by Fisher's accurate test .The differences among the three groups were compared by Kruskal-Wallis H test, further comparison between groups using Nemenyi test, and the differences of BUT between the two groups were compared by Mann-Whitner U test. We used prism for Windows version 7(GraphPad Software, Inc., San Diego, CA, USA) to draw statistical figures. Spearman's correlation analysis was used to evaluate the correlation between VAS, TBUT , SIT, corneal Perception and cytokine level. For each test, differences were considered statistically significant when P <0.05 and data were presented as mean ± SD.

## Results

### *Participants*

There were no marked differences in weight (P = 0.677), age (P = 0.982) and sex(P=1.000) between the FK group and healthy group. More details are showen in Table 1.Clinical data of FK patients are presented in Table 2. We collected 20 fungal keratitis patients, we found the most frequent causative microorganism of fungal keratitis was Fussarium spp.; The duration of the illness ranged from 3 to 60 days. The most common cause was damage from plants or insects contaminated by fungal spores. Fig. 1 shows the slit-lamp photographs of infected eye and contralateral uninfected eye from the FK patient.

**Table1** Demographics and clinical measurements of FK and HC groups

| | FK | HCs | *t* | *P** |
|---|---|---|---|---|
| Male/Female | 12/8 | 11/9 | N/A | 1.000# |
| age（Year） | 53.65±14.64 | 52.98±14.29 | 0.273 | 0.982* |
| weight（Kg） | 60.25±6.43 | 61.05±7.08 | -0.374 | 0.677* |

Notes:N/A means data is not available, * represents independent sample t-test, # represents Fisher's exact test.When $P > 0.05$,the difference is not significant.

**Table 2** Demographic ,clinical aspects in fungal keratitis patients

| No. | Age(Years) | Gender | Duration (days) | Pathogeny | Microbial culture | Ulcer（mm×mm） |
|---|---|---|---|---|---|---|
| 1 | 76 | Female | 3 | None | Fusarium | 5×6 |
| 2 | 70 | Male | 15 | Foreign body | Fusarium | 6×7 |
| 3 | 56 | Male | 15 | Plant injury | Colletotrichum | 3×4 |
| 4 | 25 | Male | 7 | Insect trauma | Aspergillus fumigatus | 3×3 |
| 5 | 42 | Female | 30 | None | Fusarium | 4×5 |
| 6 | 64 | Male | 14 | Plant injury | Fungal hyphae | 3×4 |
| 7 | 54 | Female | 30 | Plant injury | Fungal hyphae | 3×2 |
| 8 | 47 | Female | 12 | Plant injury | Fusarium | 2×2 |
| 9 | 68 | Female | 14 | Plant injury | Curvularia lunata | 6×4 |
| 10 | 61 | Male | 60 | Plant injury | Fusarium | 4×4 |
| 11 | 52 | Male | 7 | None | Fusarium | 5×4 |
| 12 | 44 | Male | 30 | Foreign body | Aspergillus fumigatus | 4×4 |
| 13 | 26 | Female | 5 | None | Fusarium | 2×4 |
| 14 | 56 | Male | 2 | None | Fusarium | 4×5 |
| 15 | 67 | Female | 7 | Plant injury | Aspergillus fumigatus | 3×3 |
| 16 | 32 | Male | 3 | Plant injury | Fusarium | 4×3 |
| 17 | 58 | Male | 15 | Plant injury | Curvularia lunata | 2×3 |
| 18 | 71 | Female | 5 | None | Fungal hyphae | 5×4 |
| 19 | 59 | Male | 15 | Plant injury | Fungal hyphae | 2×2 |
| 20 | 45 | Male | 28 | Plant injury | Fusarium | 3×3 |

## Tear Cytokine Concentrations

Compared with healthy controls, the concentrations of IL-1β,IL-10 and IL-17 in tears from FK patients increased in bilateral eyes($P<0.05$), and the levels of IL-10 in tears of the contralateral uninfected eye were higher than those in infected eyes, the concentrations of TNF-α and MMP-9 only increased in affected FK eyes($P<0.05$), but not contralateral eyes ($P > 0.05$). The detailed tear cytokine concentrations measured by ELISA are shown in Fig. 2 and Table 3.

**Table 3** Cytokine levels in tears from fungal keratitis patients and controls（`X±SD）

| | Group A (n=20) | Group B (n=20) | Group C (n=20) | A vs B | | A vs C | | B vs C | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | c2 | P | c2 | P | c2 | P |
| IL-β (pg/ml) | 72.65±13.44 | 23.38±5.14 | 9.62±3.66 | 13.59 | <0.01 | 53.74 | <0.01 | 13.28 | <0.01 |
| IL-10 (pg/ml) | 32.63±5.53 | 42.88±6.34 | 14.70±3.72 | 7.29 | <0.05 | 16.45 | <0.01 | 45.62 | <0.01 |
| IL-17 (pg/ml) | 39.74±13.44 | 20.92±4.65 | 12.83±5.10 | 10.52 | <0.05 | 39.50 | <0.01 | 9.25 | 0.01 |
| TNF-α (pg/ml) | 54.34±15.33 | 19.58±8.90 | 14.17±6.09 | 17.07 | <0.01 | 41.00 | <0.01 | 5.16 | >0.05 |
| MMP-9 (ng/ml) | 10.41±1.82 | 4.53±1.37 | 4.37±1.50 | 28.56 | <0.01 | 30.43 | <0.01 | 0.03 | >0.05 |

Notes: Group A represents the infected eye group, group B represents the contralateral uninfected eye group, and group C represents the healthy control group; The differences among the three groups were compared by Kruskal-Wallis H test, further comparison between groups using Nemenyi test, differences were considered statistically significant at P <0.05.
Abbreviations : IL, Interleukin;MMP-9, Matrix metalloproteinase-9; TNF, Tumor necrosis factor.

## Corneal perception and tear film function

The corneal perception in affected FK eye was significantly lower than that of the healthy controls, and the corneal perception of the contralateral uninfected eye was slightly lower than that of the control group, but there was not statistical difference (P>0.05), The TBUT and Schirmer strip of the contralateral uninfected eye were significantly lower than that of the healthy control group, and the difference was statistically significant (P<0.05). The Schirmer strip of the affected FK eyes were significantly longer than healthy controls (P<0.05). More details are shown in Fig.3 ,Table 4.

**Table 4** Cytokine levels in tears from fungal keratitis patients and controls (`X±SD)

| | Group A (n=20) | Group B (n=20) | Group C (n=20) | A vs B | | A vs C | | B vs C | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | c2 | P | c2 | P | c2 | P |
| IL-β (pg/ml) | 72.65±13.44 | 23.38±5.14 | 9.62±3.66 | 13.59 | <0.01 | 53.74 | <0.01 | 13.28 | <0.01 |
| IL-10 (pg/ml) | 32.63±5.53 | 42.88±6.34 | 14.70±3.72 | 7.29 | <0.05 | 16.45 | <0.01 | 45.62 | <0.01 |
| IL-17 (pg/ml) | 39.74±13.44 | 20.92±4.65 | 12.83±5.10 | 10.52 | <0.05 | 39.50 | <0.01 | 9.25 | 0.01 |
| TNF-α (pg/ml) | 54.34±15.33 | 19.58±8.90 | 14.17±6.09 | 17.07 | <0.01 | 41.00 | <0.01 | 5.16 | >0.05 |
| MMP-9 (ng/ml) | 10.41±1.82 | 4.53±1.37 | 4.37±1.50 | 28.56 | <0.01 | 30.43 | <0.01 | 0.03 | >0.05 |

Notes: Group A represents the infected eye group, group B represents the contralateral uninfected eye group, and group C represents the healthy control group; The differences among the three groups were compared by Kruskal-Wallis H test, further comparison between groups using Nemenyi test, differences were considered statistically significant at P <0.05.
Abbreviations : IL, Interleukin;MMP-9, Matrix metalloproteinase-9; TNF, Tumor necrosis factor;.

## Correlation analysis

### Correlation of inflammatory cytokines in tears with corneal perception and VAS

Tear concentrations of IL-1 β, IL-17 and TNF- α were positively correlated with VAS (R=0.873, P<0.001; R=0.748, P<0.001, R=0.809, P<0.001, respectively;Fig.4A-B.D).While IL-10 was correlated inversely with VAS(R=-0.668,P=0.001,Fig.4C), tear concentrations of IL-1 β, IL-17 and TNF- α were correlated inversely with corneal perception (R=-0.492, P<0.001; R=-0.686, P=0.001; R=-0.598, P<0.05, respectively; Fig.4E-F.H).IL-10 was not correlated with corneal perception significantly (P>0.05, Fig.4G). Tear concentrations of MMP-9 was also not correlated with VAS and corneal perception significantly (P>0.05).

### Correlation between inflammatory cytokines and tear film function in the contralateral uninfected tear

The levels of IL-1 β, IL-17 and MMP-9 were inversely correlated with Schirmer strip(R=-0.822, P<0.001, R=-0.811, P<0.001, R=-0.508, P<0.05, respectively; Fig.5A-C). The tear concentrations of IL-1 β, IL-17 were also inversely correlated with TBUT(R=-0.619, P<0.05; R=-0.551, respectively; Fig.5D-F). MMP-9 was did not correlated with TBUT significantly(P=0.058, Fig.5F). There were no significant correlations between other cytokines, and Schirmer strip and ,TBUT (P > 0.05).

### Correlation between tear inflammatory cytokines and tear film function in healthy controls

There was no significant correlation between tear film function and the levels of IL-1 β, IL-10, IL-17, TNF- α and MMP-9 in normal control group (fig. 6, Fig. 7) (r =- 0.323-0.134, P > 0.05) .

# Discussion

When fungi invades the body, it will trigger a series of inflammatory events, including infiltration of inflammatory cells and factors. IL-1β is produced by immune cells and mucosal epithelial cells of the ocular surface, it is one of the crucial proinflammatory and inflammation mediator[13] involving in fungal-induced corneal injury[11]. TNF-α is a crucial proinflammatory mediator in a variety of corneal diseases, TNF-α can disrupt the barrier function of human corneal epithelial cells and promote ocular inflammation [14]. MMP-9, known as gelatinase B. is distributed in corneal stroma as associated with epithelial repair and remodeling in physiological environment. Under pathological conditions, it is actively secreted by neutrophils in the process of injury and inflammation, and excessive MMP-9 is expressed in corneal epithelial cells, which will cause severe tissue damage [15].IL-10 is a key negative regulator of inflammation and has immunosuppressive activity[16]. It can regulate innate and adaptive immune cells and prevent the pathological development of immunity in different ways[17]. In the fungal keratitis model, the expression of IL-10 in the cornea increased at first and then decreased after a period of time [18]. IL-17 is secreted by Thl7 cells, neutrophils and glial cells can also secrete a few [19]. A large amount of IL-17 expression was found in human cornea with filamentous fungal keratitis[20]. IL-17 can alleviate the severity of fungal keratitis by the expression of CX43 in corneal peripheral vascular endothelial cells[21]. Therefore, it suggests that low levels of IL-10 ,IL-17 was useful to enhance the corneal defense system which is to protect against disease onset, whereas such an episode, a high level of IL-10,IL-17 assists in dampening the inflammatory response ,and minimizing tissue destruction and scarring[22].Therefore ,the increase of anti-inflammatory factors represents the ability to buffer inflammatory response and limit collateral damage to the host [23].

In our study, IL-1β, IL-6 and TNF-α showed a positive correlation with VAS, while IL-10 showed a negative correlation with VAS. IL-1β had been shown to induce additional proinflammatory mediators, such as IL-6, NO, prostaglandin E2(PGE-2), and cyclooxgenase-2(COX-2)[24]. COX-2 and PGE-2 are crucial pain factors. Meanwhile,IL-1β can modulate neuronal intracellular calcium signaling and activates astrocytes ,which can further enhance and prolong neuroinflammation-induced chronic pain[25]. IL-17 can promote astrocyte proliferation and proinflammatory cytokines secretion ,which are related to the neuropathic pain[26]. TNF- α is a crucial regulator of inflammatory response ,which participates in the production of pain[27]. As an anti-inflammatory factor, IL-10 plays an essential role in neuropathic pain, which can reduce the pain response by inhibiting the release of pro-inflammatory factors[28]. This is consistent with our study that pain scores decrease with the increase of anti-inflammatory factors. In addition, a new study shows that pain behavior can be reduced by injecting recombinant IL-10 into the trigeminal ganglion[29], which provides ideas for trigeminal neuralgia of keratitis patients in future. Besides, these inflammatory cytokines are inversely proportional to corneal perception, it may be related to mycotoxin

and inflammatory cytokine-mediated damage, and may lead to the damage of trigeminal nerve. However, pain sensitivity may decrease with post infection corneal ulcer aggravation, which may be caused by decreasing corneal nerve density in FK patients[30].

The expression of a large number of inflammatory cytokines can directly damage the corneal barrier and lead to changes in tear film function .Moreover ,the interaction between dry eye and inflammation will further lead to the activation of ocular surface cascade immune inflammation [31, 32]. These may influence the tear film function of contralateral uninfected eyes. Study have showed that there are relations between tear inflammatory cytokines and dry eye [33]. The increase of IL-10 was also found in tear and conjunctival with dry eye patients [34]. TFOS DED II guilds showed IL-1 β, IL-17, TNF- α and MMPs played a vital role in the pathogenesis of dry eye[35]. IL-17 may be involved in the immune pathogenesis of xerophthalmia and destroy the corneal epithelial barrier to promote the occurrence of dry eye. Meanwhile, drying stress can induce Th1 inflammatory cytokines on the ocular surface and destroy the corneal epithelial barrier associated with Th17[36]. This is consistent with our research. This indicates further that the increase of related inflammatory factors in the contralateral eyes will cause corresponding changes in tear film function. Due to stimulation such as injury, toxin and immunity, which may stimulate the lacrimal gland nerves, the tear secretion of the affected eyes will be increased reflexively. Therefore, We did not measure TBUT of the affected eye.

Study has shown that when unilateral eye is infected or damaged, the nerve growth factor[37], inflammatory cytokines[10], dendritic cell[38], the nerve density began to decrease [39] and leukocyte exudation phenomenon can be detected in the contralateral uninfected eyes [40], which further indicates that there is a connection in bilateral eye, and the corresponding immune changes will also occur in the contralateral uninfected eye. In the tear cytokine study of keratitis [10], it was found that the expression level of IL-10,IL-17 in the contralateral eye increased; The increase of IL-10,IL-17 may be a preventive mechanism to avoid infection in the contralateral eye. Furthermore, In the study of cataract surgery, IL-1β was elevated in contralateral eye, which may be associated with pain[41]. This manifests that pain and immunity are also interrelated. In the herpes simplex virus study, it was observed that when monocular infectious keratitis occurs, the tear film indexes such as SIT, TBUT will decrease, the tear osmotic pressure increased, and the nerve density will decrease[42], which is consistent with our study. The peripheral nervous system can regulate the activation, deployment and homeostasis of the immune system, and initiate adaptive immunity, which indicates that there is also a connection between the corneal nerve and immune system[43]. Patients with depression has an increase in tear inflammatory cytokines and shows dry eye symptoms [9]. As we all know ,low sleep quality and discomfort of eye caused by vision loss, pain makes the FK patients in a state of anxiety, which increases the probability to develop dry eye syndrome in contralateral uninfected eyes. Although the patient does not have obvious subjective feelings, this may be covered by pain of infected eye.

# Conclusion

Our study demonstrated that unilateral FK can result in bilateral inflammatory tear inflammatory cytokines alterations, which indicates that the immune response links between bilateral eyes. Meanwhile, these inflammatory factors appear to be related to pain and corneal perception, which indicates that immune-nerve-pain is interactive and interrelated. The long-term uncured fungal infection will potentially influence the contralateral uninfected ocular surface environment in patients with unilateral FK. They are more likely to suffer from dry eye than healthy people in future. This also indicates researchers should be careful to use the contralateral uninfected eyes as a healthy control.

# Declarations

### Ethics approval and consent to participate

The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Human Ethics Committee of The First Affiliated Hospital of Nanchang University. Written informed consent was obtained from individual or guardian participants.

### Consent for publication

All data  generated or analyzed during this study from patients are included in this published article.

Every patient involved in this study had been informed of the whole study design and signed the informed consent. Patients were consent to publish these pictures.

### Availability of data and materials

The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

### Competing interests

This was not an industry supported study. The authors report no conflicts of interest in this work.


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### Authors' contributions

Shuang Zhang and Gui-Ping Gao analyzed and interpreted the patient data regarding the hematological disease and the transplant. Shuang Zhang, Hui-Min Wu and Xiang-Ni Cao performed the histological examination of the kidney, and Shuang Zhang was a major contributor in writing the manuscript. Xian-Qi Zhang was responsible for polishing the article .All authors read and approved the final manuscript.


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# Figures

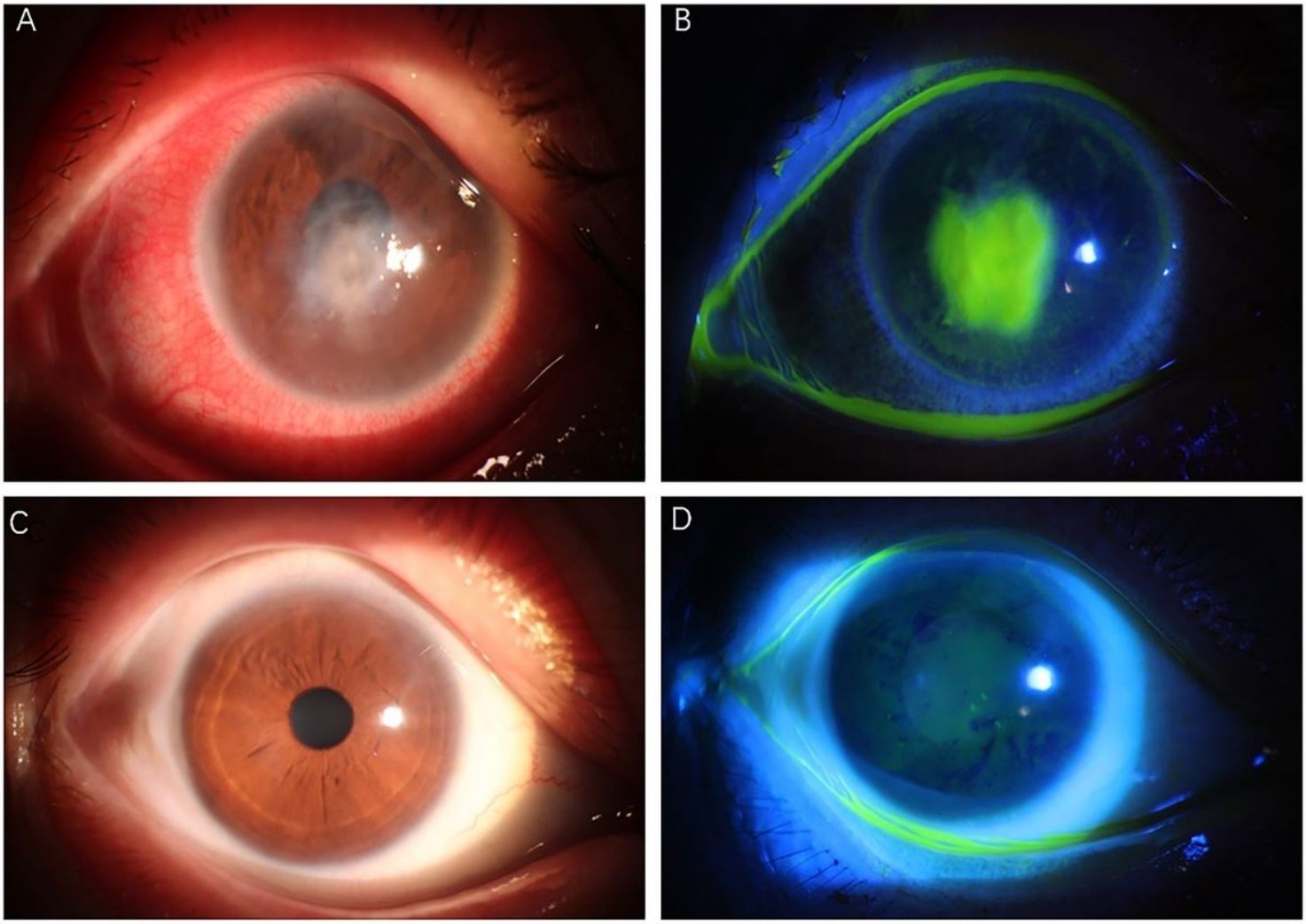
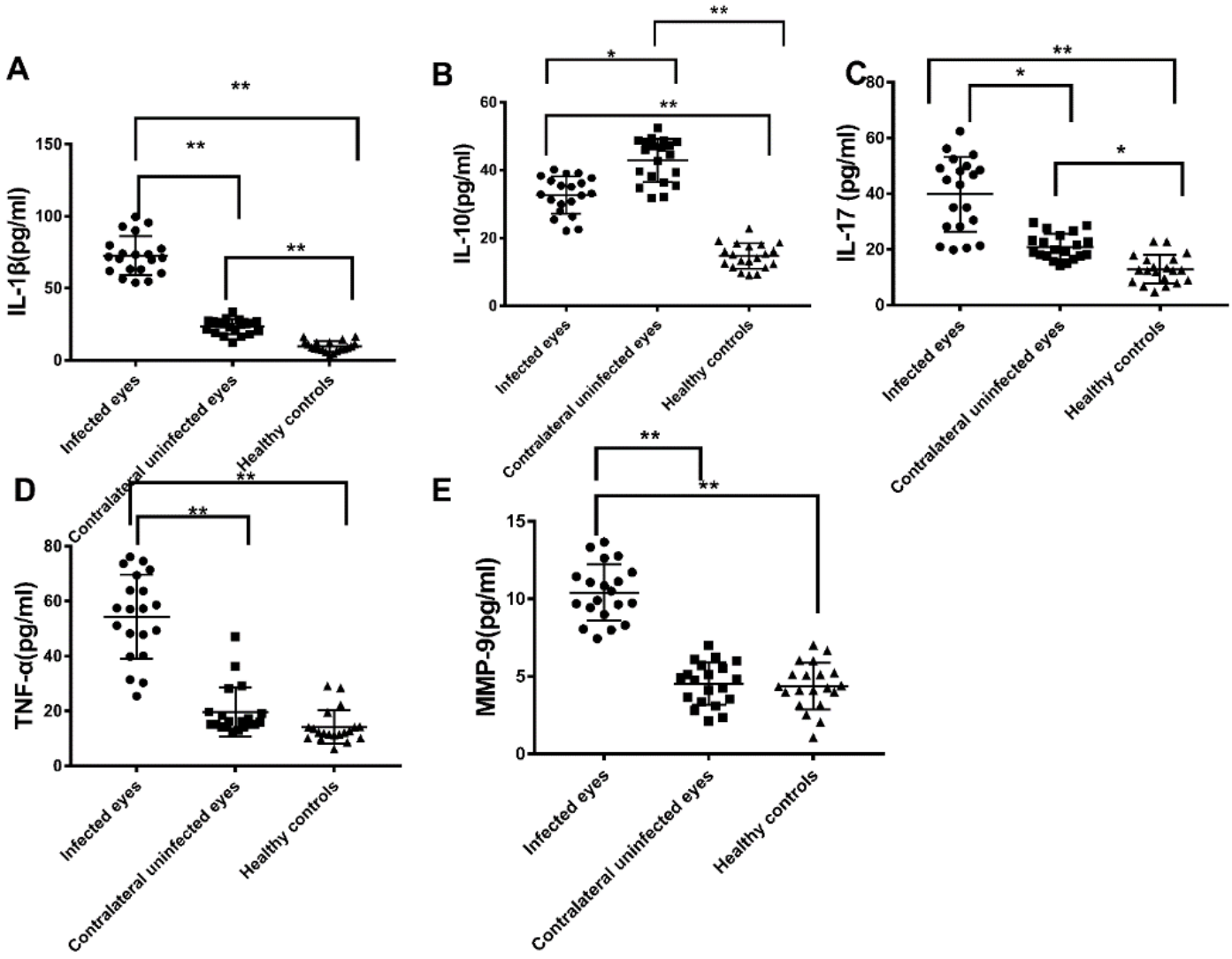



**Figure 1**

Slit lamp images from the fungal keratitis patient Figure 1A: We can see toothpaste-like appearance, pseudopodia and immune ring and other typical fungal infections symptoms .Figure 1B: The image of the infected eye after fluorescent staining, which shows a large of stained cornea area. Figure 1C: The image of contralateral uninfected eye without any obvious abnormality. While Figure 1D shows that cornea is slightly stained after fluorescent staining, and tear film is instability.




## Figure 2

Inflammatory cytokines and MMP-9 levels in the tears of FK bilateral eyes and healthy control eyes
Notes:** represents P<0.01, * represents P<0.05, A P value of less than 0.05 was considered statistically significant. Abbreviations : MMP-9,Matrix metalloproteinase-9; FK ,Fungal keratitis.

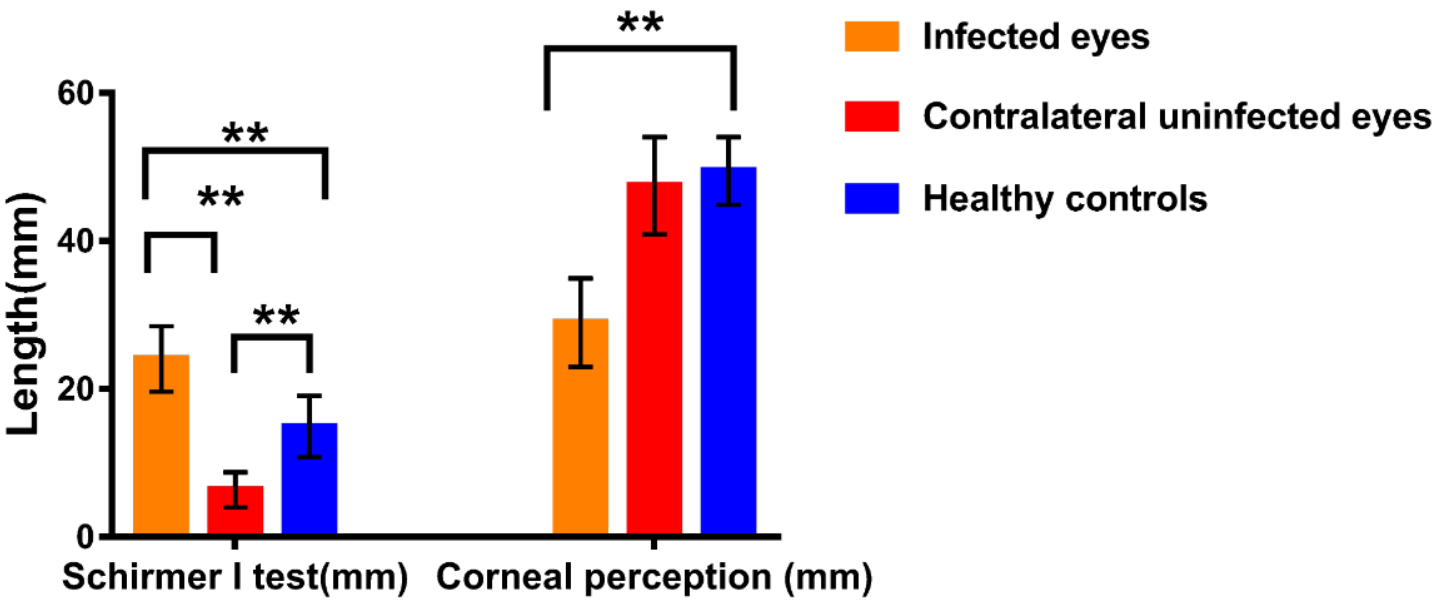



Figure 3

The comparison of Schirmer Strip, corneal perception ,tear breakup time between FK group and healthy group Notes: ** represents P<0.01, A P value of less than 0.05 was considered statistically significant. Abbreviations :TBUT, tear film rupture time; FK ,Fungal keratitis.

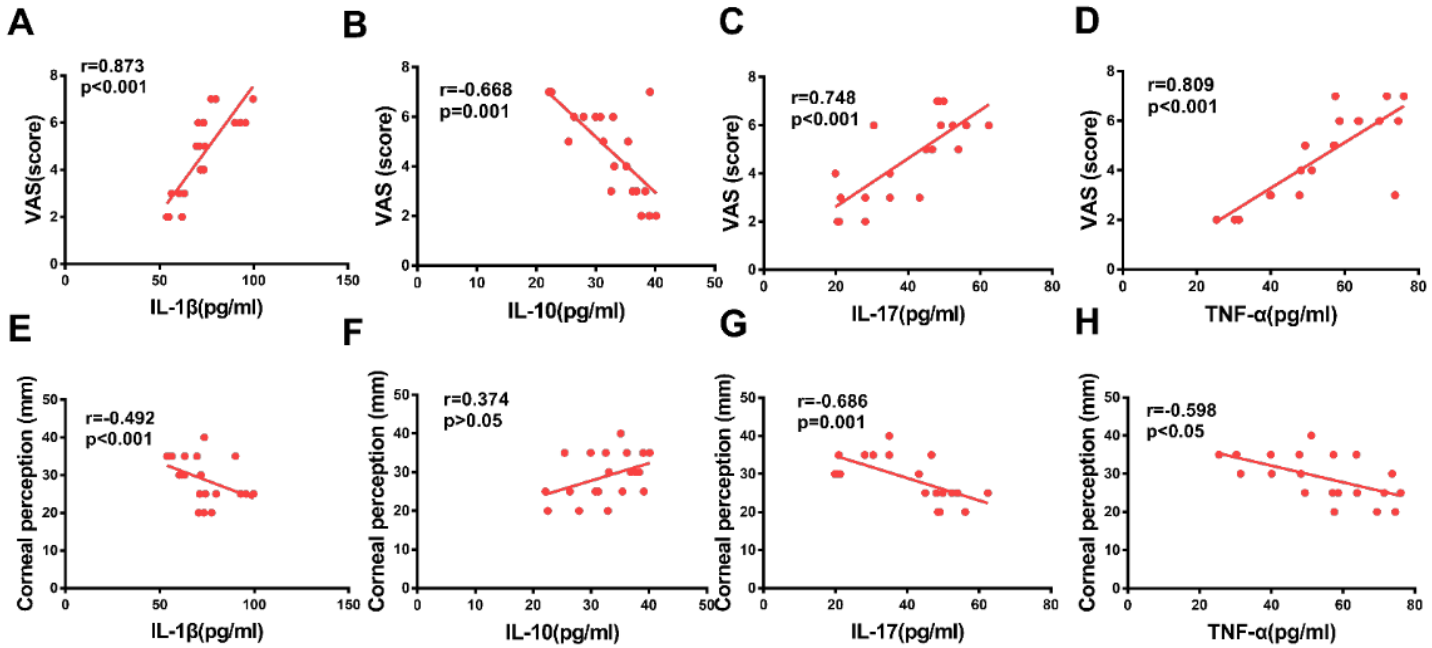
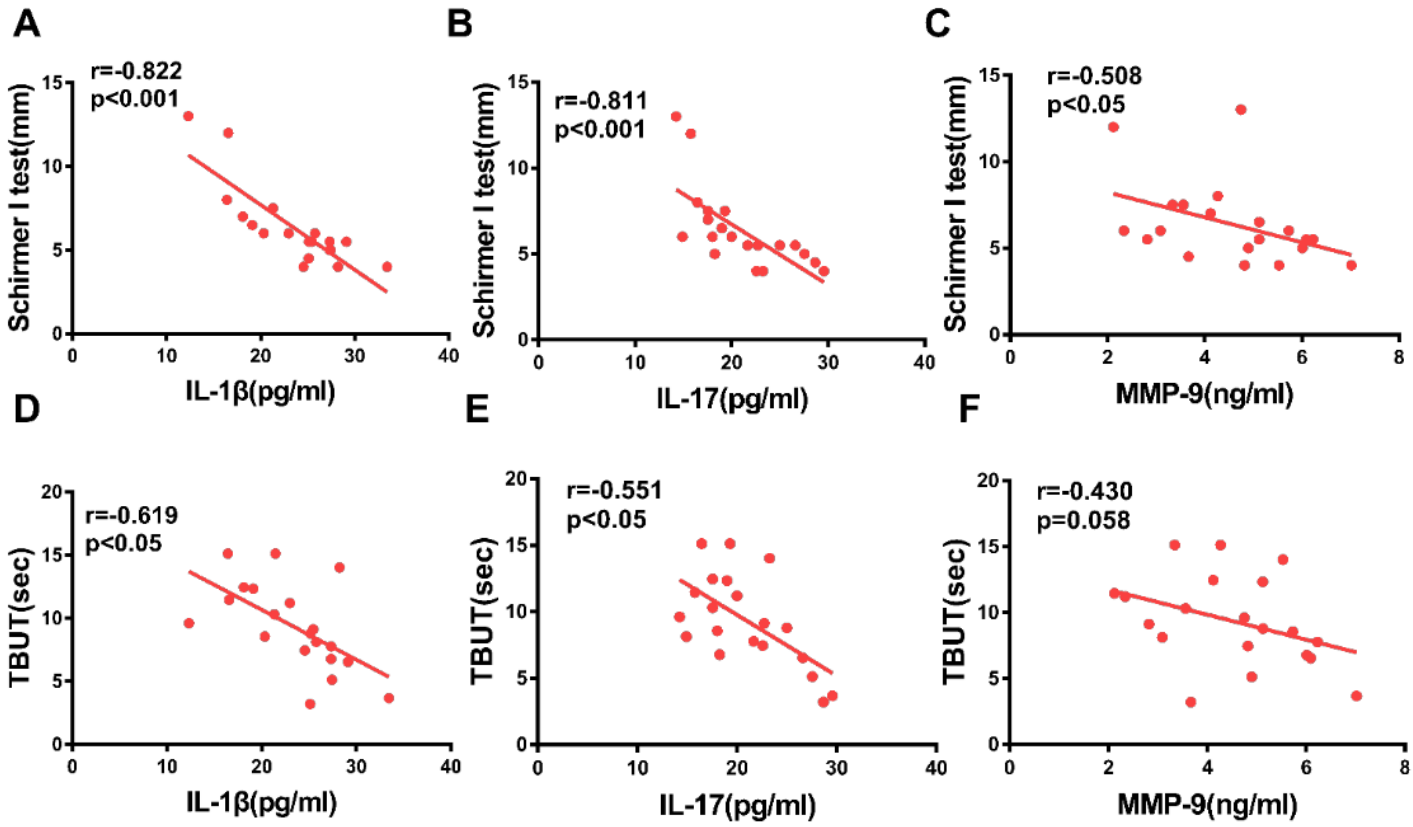


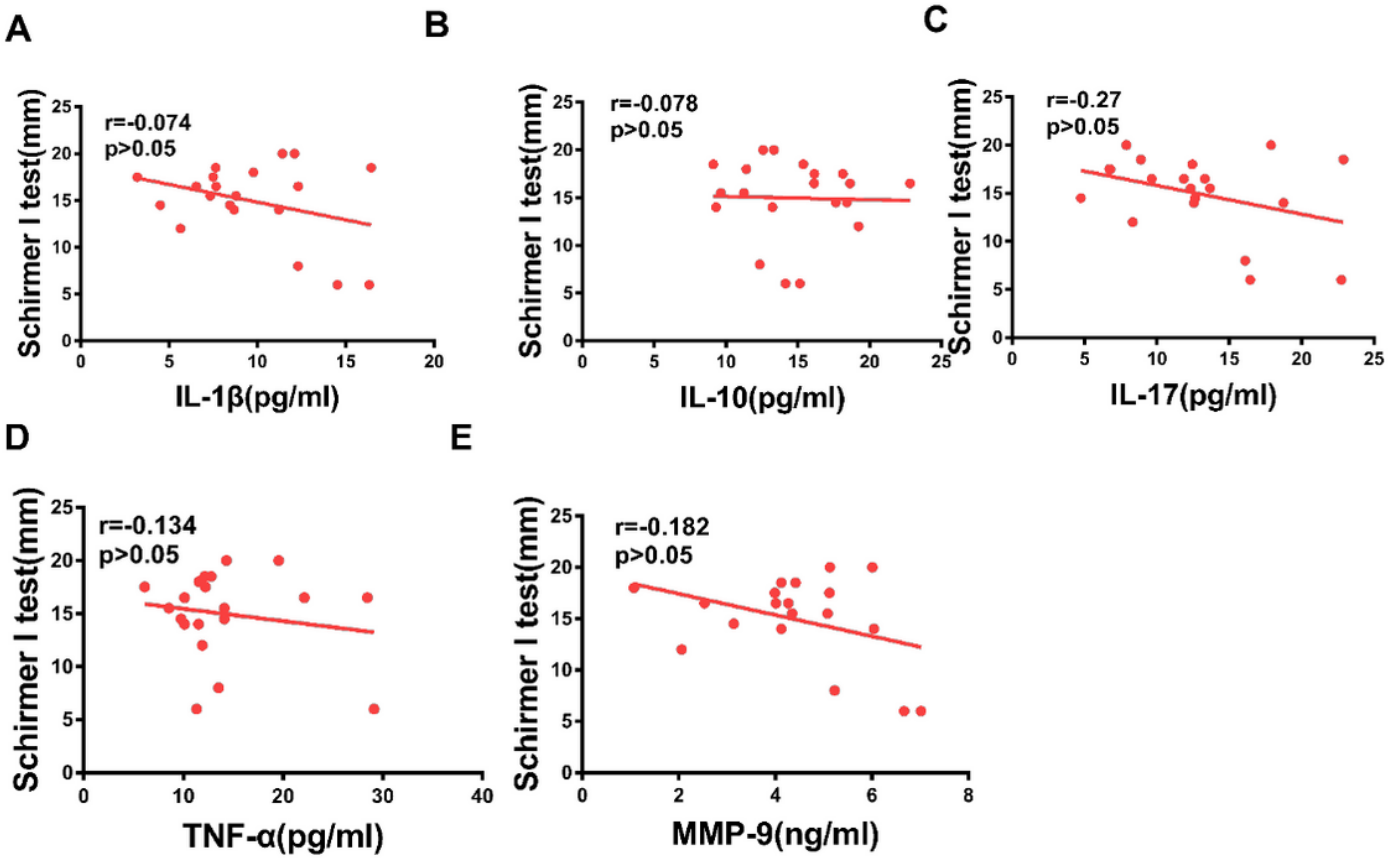

Figure 4

Correlation of IL-1β(Fig.4A),IL-10(Fig.4B), IL-17(Fig.4C),TNF -α (Fig.4D) with VAS scores; Correlation of IL-1β(Fig.4E),IL-10(Fig.4F), IL-17(Fig.4G),TNF -α (Fig.4H) with corneal perception. Notes: The relationship between corneal perception and VAS and inflammatory cytokines in FK-affected eyes are all analyzed by Spearman correlation. A P value of less than 0.05 was considered statistically significant. Abbreviations :IL, Interleukin; TNF, Tumor necrosis factor; VAS, Visual analogue scale.



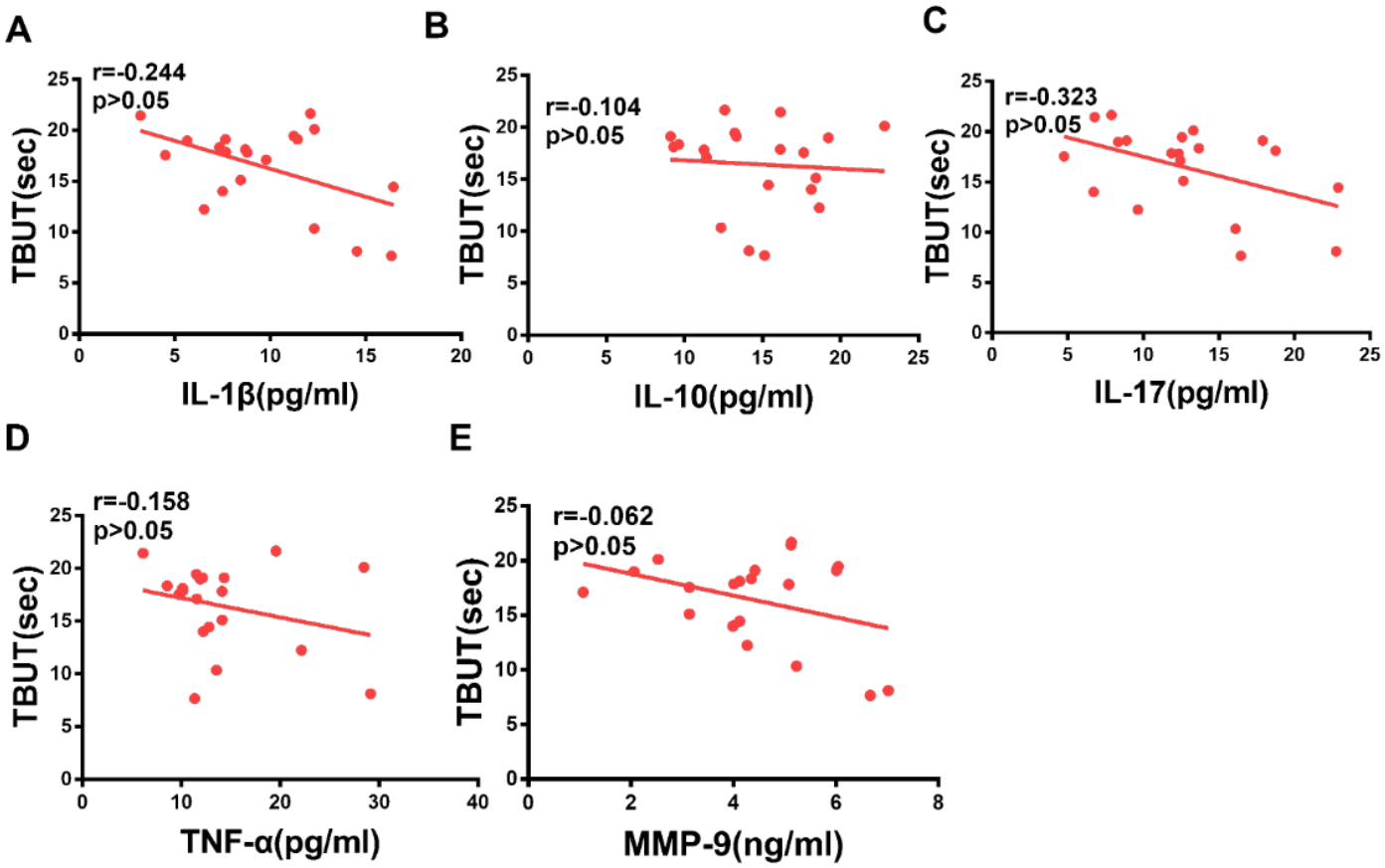

## Figure 5

Correlation of IL-1β(Fig.5A), IL-17(Fig.5B),MMP-9 (Fig.5C) with Schirmer I test; Correlation of IL-1β(Fig.5D), IL-17(Fig.5E),MMP-9 (Fig.5F) with TBUT. Notes: The relationship between tear film function and inflammatory cytokines on the contralateral side of FK was analyzed by Spearman correlation. A P value of less than 0.05 was considered statistically significant. Abbreviations :IL, Interleukin;MMP-9, Matrix metalloproteinase-9; TBUT, tear breakup time.




## Figure 6

Correlation of tear cytokines with Schirmer Strip in healthy controls Notes: The relationship between filter paper infiltration length and inflammatory cytokines in the healthy control group was analyzed by Spearman correlation. A P value of less than 0.05 was considered statistically significant. Abbreviations :IL, Interleukin;MMP-9, Matrix metalloproteinase-9; TNF, Tumor necrosis factor.




## Figure 7

Correlation of tear cytokines with TBUT in healthy controls Notes: The relationship between filter paper infiltration length and inflammatory cytokines in the healthy control group was analyzed by Spearman correlation. A P value of less than 0.05 was considered statistically significant. Abbreviations : IL, Interleukin;MMP-9, Matrix metalloproteinase-9; TNF, Tumor necrosis factor; TBUT, tear breakup time.