High Concentration of Inflammatory Cytokines in the Tears of Patients with Concomitant Strabismus

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Research Article

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Abstract

BACKGROUND: Our study aimed to determine whether the expression concentration of inflammatory cytokines in ocular surface tears was increased in patients with concomitant strabismus.

METHODS: In this study, the concentration of inflammatory cytokines interleukin-6 (IL-6), interleukin-17A (IL-17A), interleukin-10 (IL-10), interleukin-12p70 (IL-12P70), interferon gamma (INF- γ), and tumor necrosis factor- α (TNF- α) were detected in tears in patients with concomitant strabismus and healthy controls matched by age and gender.

RESULTS: Our results showed that the concentration of IL-6 and TNF- α were significantly higher in the experimental group compared to the normal group. The concentration of IL-17A, IL-10, IL-12p70, and INF- γ in patients with concomitant strabismus were higher than those in the control group, but not reached the statistically significant.

CONCLUSIONS: Our results suggested that most of concomitant strabismus patients have higher concentration of inflammatory cytokines in tears.

Introduction

Strabismus is a condition in which the eyes are skewed by an imbalance of the muscles outside the eyes. The 0.14% of 9,111 surgical strabismus patients met criteria for probable infection at Duke Eye Center from July 1996 to October 2017 and its occurrence may be related to imbalance of immune-related molecules and under 18 years of age[1]. The patients after strabismus surgery (fornix incision, limbal incision) had high dry eye questionnaire scores and shorter tear film breakup time[2]. Tear film instability resulted from goblet cell density depletion and conjunctival sensitivity could be one of the causative factors of ocular irritation symptoms after strabismus surgery[3]. Patients with strabismus had postoperative dry eye symptoms, less goblet cell density, and an inflammatory response. Ocular surface strabismus patients postoperative discomfort may be the result of surgical trauma, however, Many strabismus patients clinically blink frequently, conjunctiva congestion, slit lamp irradiation appears more conjunctival fluorescein staining before surgery. We speculate that long-term strabismus may be one of the important reasons for postoperative dry eye symptoms.

Giannaccare hypothesized that long-term deviation of the eyeball from its primary location could lead to chronic changes in the ocular surface, which could result in dry eyes through two possible mechanisms. Firstly, increased exposure area of conjunctiva may lead to thinner tears film lipid layer and increased tears film instability. Secondly, abnormal anatomical and functional relationships between the eyelids and the eyeball increase mechanical friction to the conjunctival epithelium, leading to microtrauma associated with blinking[4]. Mechanical damage to the conjunctival epithelium caused by tears film instability further activates a series of inflammatory events, and long-term inflammatory cell infiltration lead to further damage to the ocular surface system.

We speculated that the high expression of inflammatory cytokines in tears in patients with concomitant strabismus is one of the important reasons for the poor prognosis of strabismus patients. We have therefore investigated if there are differences about inflammatory cytokines in our cohort of patients with concomitant strabismus and control group.

Materials And Methods

Participants

Inclusion criteria for the experimental group: clinical diagnosis was concomitant exotropia, refractive errors [spherical $\leq \pm 1.00D$ anastigmat or cylindrical ≤ 0.50] were corrected by wearing glasses, and there were no neurological diseases or craniofacial abnormalities. Except for strabismus and refractive errors, the patients had no history of other visual defects and eye diseases, and no family genetic history (including history of strabismus). Inclusion criteria for the control group: refractive errors [spherical $\leq \pm 1.00D$ anastigmat or cylindrical ≤ 0.50] were corrected by wearing glasses, no neurological disease, no craniofacial abnormalities, no history of visual impairment or eye disease, and no family history of hereditary diseases (including strabismus). No related symptoms such as dry eyes. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of The First Affiliated Hospital of Fujian Medical University. Informed consent was obtained from legal guardians.

Tears collection

We use tears adsorption filter paper (Tianjin Jingming New Technology Development Co., LTD., Tianjin; China Strip (Hereinafter referred to as China Strip)) to collect tears from the subjects. Schirmer. The tears samples were eluted with 200uL PBS and shaken for 5 hour at room temperature, then the samples were stored in a refrigerator at $-80^{\circ}C$ [5].

Measurement of inflammatory cytokines

For SIMOA experimental tears samples were prepared according to the kit specific manufacturers instruction. The levels of inflammatory cytokines were measured using a SIMOA HD-X Cytokine 6-Plex Panel Standards and samples were run utilizing the manufactures assay instructions. The lower limits of quantification (LLOQ) for IL-10, INF- γ , IL-12p70, TNF- α , IL-17A, IL-6 were 0.0660 pg/mL, 0.0710 pg/mL, 0.0290 pg/mL, 0.1270 pg/ml , 0.0320 pg/mL , and 0.1320 pg/mL respectively in the panel 1. The LLOQ for IL-10, INF- γ , IL-12p70, TNF- α , IL-17A, IL-6 were 0.0142 pg/mL, 0.0052 pg/mL, 0.0031pg/mL, 0.0283 pg/ml , 0.0032 pg/mL , and 0.0221 pg/mL respectively in the panel 2. The limit of detection (LOD) for IL-10, INF- γ , IL-12p70, TNF- α , IL-17A, IL-6 were 0.0142 pg/mL, 0.0052 pg/mL, 0.0031 pg/mL, 0.0283 pg/ml, 0.0032pg/mL, and 0.0221pg/mL respectively in the panel 1, and the limit of detection (LOD) for IL-10, INF- γ , IL-12p70, TNF- α , IL-17A, IL-6 were 0.0144 pg/mL, 0.0033pg/mL, 0.0032 pg/mL, 0.0261 pg/ml,

0.0033pg/mL, and 0.0249pg/mL respectively in the panel 2, and concentration below LOD are used the LOD as the concentration of tear.

Statistical analyses

All of the data were analyzed with the SPSS 20.0 program (IBM Corp. SPSS Statistics for Windows, version 20.0, Armonk, NY, USA). All the results were statistically analyzed to compare the differences between groups, and $P < 0.05$ was considered statistically significant.

Results

A total of 22 participants were enrolled, including 11 patients with concomitant strabismus and 11 healthy controls. We collected relevant clinical information, including gender, age, duration of the disease, corneal light reflection test, eye movement, strabismus degree, the dominant eye, and eye symptoms (Table.1). There were no significant difference in the age or gender ratios of participants between the 2 groups.

To investigate the difference of levels in the patients with strabismus and control. We used SIMOA technology to measure the levels of inflammatory cytokines in tears in strabismus and normal patients. Levels of INF- γ , IL-10, IL-12p70, IL-17A, IL-6 and TNF- α were measured. We found that the expression concentration of INF- γ , IL-10, IL-12p70, IL-17A, IL-6 and TNF- α (Fig.1) in tears of strabismus patients, which were significantly higher than those of normal subjects, but only IL-6 and TNF- α were statistically significant (Fig.2).

TNF- α may promote the process of inflammation and apoptosis of goblet cells by through combining with TNFR1 and TNFR2. TNF family of cytokines was a best-characterized inducers of NF- κ B via TNFR1 and cIAP1/2[6]. Major signaling pathway mediated by the TNF- α /TNFR1 include activation of the transcription factor NF- κ B and the mitogen-activated protein kinases (MAPKs)[7]. In the absence of NF- κ B activation, prolonged JNK activation contributes to TNF- α induced apoptosis[8]. Caspase-8 is a cysteine-aspartate specific protease that classically triggers the extrinsic apoptotic pathway, in response to the activation of TNFR[9]. Meanwhile, the density of goblet cells and epithelial cells decreased in conjunctiva of strabismus patients[4]. Based on the above, We speculate that: 1. Activation of the TNF/TNFR signaling pathway leads to upregulation of a large number of inflammatory factors, including IL-6, IL-17A, IL-12p70, and INF- γ ; 2. Activation of TNF/TNFR signaling pathway leads to increased apoptosis of goblet cells and epithelial cells (Fig.3).

Discussion

We have shown that the concentration of tears mediators increased obviously in concomitant strabismus patients, and the IL-6 and TNF- α could be an important mediators of inflammation. Significant increases in six inflammatory cytokines, including 1 anti-inflammatory factor (IL-10) and 5 pro-inflammatory factors (INF- γ , IL-12p70, IL-17A, IL-6 and TNF- α), were found in tears of asymptomatic concomitant strabismus.

We used the SIMOA technology to detect the concentration of inflammatory factors in tears while ELISA could not effectively detect the concentration of inflammatory cytokines in tears. By introducing the concept of inflammatory factors into the ocular surface of strabismus patients, early intervention in these patients can avoid long-term high concentration of inflammatory factors and chronic histological changes.

Dry eyes is a common ocular disease characterized by reducing tears production on both sides and unstable tears film. Dry eyes syndromes have obvious clinical symptoms including eyes irritation, congestion, glare, eyes fatigue, and blurred vision. Goblet cell loss was directly related to surface cell apoptosis after chronic inflammatory injury, resulting in further tears film instability/imbalance[10]. Significant differences in the concentration of cytokines (IL-1 α , IL-1 β , IL-1ra, IL-4, IL-6, IL-8, IL-10, IL-13, monocyte chemoattractant protein-1 (MCP-1), TNF- α) were detected in tears samples collected from patients with dry eyes using Schirmer[11]. Pro-inflammatory forms of IL-1 (IL-1 α and mature IL-1 α) are increased in tears of patients with dry eyes[12]. The concentration of IL-6, IL-10, IL-17A, GM-CSF, E-selectin, P-selectin and S-ICAM in the ocular surface disease group were significantly higher than those in the normal control group[13]. Compared with the non-dry eyes control group, the mRNA expression levels of various inflammatory cytokines encoding TNF- α , IL-1 and, IL-6, IL-8, IL-10 and TGF- β 1 in the conjunctival epithelium of sjogren's syndrome patients were increased[14].

Local application of Latanprost can promote the activation of conjunctival P38/NF- κ B signal and the production of TNF- α and IL-1, and lead to the damage of dry eyes surface in mice. [15] Local application of tumor necrosis factor- α blocker effectively inhibits lacrimal gland and corneal inflammation by inhibiting interferon- γ , IL-21, and IL-6 [16]. Tumor necrosis factor- α blocker(infliximab) was also effective in reducing angiogenesis and lymphangiogenesis in different animal models of corneal neovascularization, which reduced local inflammatory responses[17]. NF- κ B activation was an important regulator of TNF- α mediated apoptosis of corneal fibroblasts [18]. Endogenous TNF- α not only inhibited the expression of TGF- β 1 and VEGF in fibroblasts, but also antagonized the induction of TGF- β 1 and VEGF in vascular endothelial cells and may block angiogenesis [19].

The study has several limitations. Firstly, the number of samples included in this study was small, because we need to conduct rigorous screening of the subjects included in the study, and ,it should be tested as soon as possible to prevent long-term storage of samples. Secondly, the experimental results of this study were not repeated for many times, and the test operation process was standardized by professional technicians without any information about sample type. Thirdly, some cytokine concentration in the research were lower than LLOQ, and we treated this part of data in accordance with the LLOQ of the corresponding Panel, and this alternative result was reasonable (The concentration of this part was mainly in the control group).

Conclusion

In summary, we found high expression levels of inflammatory factors (IL-17A, IL-10, IL-12p70, INF- γ , IL-6, and TNF- α) in tears of patients with concomitant strabismus, IL-6 and TNF- α were statistically significant differences in expression level. Higher levels of inflammatory cytokines can be detected in the tears of patients with concomitant strabismus using SIMOA techniques, and the inflammatory cytokines may lead to the development of dry eyes symptoms and wound infection after strabismus surgery. Further studies are needed to determine the ocular surface inflammation in patients with strabismus, and whether the early intervention of ocular surface inflammation is beneficial to the prognosis of patients with concomitant strabismus.

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of The First Affiliated Hospital of Fujian Medical University. Informed consent was obtained from legal guardians.

Consent for publication

As the first author, I approve on behalf of all the authors in the study.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files.

Declaration of competing interest

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Authors' contributions

Design of the study (Xiaoping Hong, Weidong Zheng); conduct of the study (Fadian Ding, Shirong Huang); collection and management of data (Xiaoping Hong, Wei Lian); analysis and interpretation of data (Shirong Huang); writing of manuscript (Xiaoping Hong, Fadian Ding); and review or approval of manuscript (Weidong Zheng).

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Tables

Table.1: The Characteristics of included concomitant strabismus and healthy controls.

	Strabismus group (n=11)	Control group (n=11)	p
Gender (male: female)	6:5	6:5	>0.05
Age (year)	6 $\pm$ 0.4264	6 $\pm$ 0.4045	>0.05
Time (year)	1.25 $\pm$ 0.217	-	
Corneal light reflection test	-20.42 $^{\circ}$ $\pm$ 1.9604	-	
Movement (unrestricted:restricted)	6:14	-	
Strabismus degree	-42.5 Δ $\pm$ 3.5906	-	
The dominant eye OD OS	6:5	-	
Symptoms foreign-body sensation, pain, irritation, ocular fatigue and eye redness	2	-	

Figures

	INF- γ Conc. (pg/ml)	IL-10 Conc. (pg/ml)	IL-12p70 Conc. (pg/ml)	IL-17A Conc. (pg/ml)	IL-6 Conc. (pg/ml)	TNF- α Conc. (pg/ml)
strabismus	0.0284 $\pm$ 0.0125	0.3358 $\pm$ 0.1872	0.0253 $\pm$ 0.0078	0.1551 $\pm$ 0.0437	4.683 $\pm$ 1.329	0.2095 $\pm$ 0.0703
control	0.0090 $\pm$ 0.0024	0.0513 $\pm$ 0.0149	0.0099 $\pm$ 0.0023	0.0793 $\pm$ 0.0313	1.455 $\pm$ 0.3917	0.05131 $\pm$ 0.0149

Figure 1

The level of inflammatory cytokines in tears of patients with concomitant strabismus.

	INF- γ Conc. (pg/ml)	IL-10 Conc. (pg/ml)	IL-12p70 Conc. (pg/ml)	IL-17A Conc. (pg/ml)	IL-6 Conc. (pg/ml)	TNF- α Conc. (pg/ml)
strabismus	0.0284 $\pm$ 0.0125	0.3358 $\pm$ 0.1872	0.0253 $\pm$ 0.0078	0.1551 $\pm$ 0.0437	4.683 $\pm$ 1.329	0.2095 $\pm$ 0.0703
control	0.0090 $\pm$ 0.0024	0.0513 $\pm$ 0.0149	0.0099 $\pm$ 0.0023	0.0793 $\pm$ 0.0313	1.455 $\pm$ 0.3917	0.05131 $\pm$ 0.0149

Figure 1

The level of inflammatory cytokines in tears of patients with concomitant strabismus.

Figure 2

The statistical analysis of inflammatory cytokines in tears of patients with concomitant strabismus.

Figure 2

The statistical analysis of inflammatory cytokines in tears of patients with concomitant strabismus.

Figure 3

The role of TNF- α and IL-10 in the ocular inflammation and dry eyes.

Figure 3

The role of TNF- α and IL-10 in the ocular inflammation and dry eyes.