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Detection & analysis of inflammatory cytokines in tears of patients with lacrimal duct obstruction

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***Background & objectives*: Tear proteomic changes can be a candidate etiopathogenesis of lacrimal duct obstruction diseases (LDODs). Studies on proteomics have focused primarily on nasolacrimal duct obstruction, and some specific inflammatory cytokines such as interferon (IFN)-α2a, interleukin (IL)-8 and IL-10, have not been investigated. In addition, differences in inflammatory cytokines in tears according to the LDOD subtype have not been reported. This study aimed to quantitatively compare inflammatory cytokines in tears from patients with LDOD and investigate tear-cytokine differences among different LDOD subtypes.**

***Methods*: Tear samples were collected from both eyes of 30 patients with unilateral LDOD: five patients with prelacrimal obstruction, five with acute dacryocystitis and 20 with chronic dacryocystitis. The contralateral eyes were used as controls. IFN-α2a, IFN-β, IFN-γ, IL-17A, IL-6, IL-8, tumour necrosis factor-alpha (TNF-α), vascular endothelial growth factor (VEGF)-A, induced protein-10 (IP-10) and monocyte chemotactic protein-1 (MCP-1) were quantified in all samples.**

***Results*: The expression of eight cytokines (except for IP-10 and MCP-1) were significantly increased in the affected eyes compared with those in the control eyes. The levels of nine inflammatory cytokines (except for IP-10) in the affected eyes of patients with chronic dacryocystitis were higher than those in the affected eyes of patients with prelacrimal obstruction. In addition, patients with chronic dacryocystitis presented significantly higher IFN-γ level than those with prelacrimal obstruction or acute dacryocystitis.**

***Interpretation & conclusions*: Specific pro-inflammatory cytokines were increased in tears of patients with LDOD compared with those in the controls. The specific cytokine profiles observed in the tears of individuals with different LDOD subtypes may be associated with the unique aetiopathogenesis of these conditions.**

Key words Cytokine - epiphora - inflammation - lacrimal duct obstruction - tear

Lacrimal duct obstruction disease (LDOD) is a common lacrimal drainage disorder that can result in epiphora, mucous and/or purulent secretions, secondary keratitis, severe corneal ulcers, acute dacryocystitis, orbital cellulitis or other complications due to chronic dacryocystitis[1,2]. Numerous factors have been reported

to be associated with the aetiopathogenesis of LDOD, including those within tears. Hence, tear proteomics is a promising tool for further research[3]. Because tears travel from the ocular surface to the nasal cavity through the lacrimal drainage system, any changes in the quality or composition of tears may affect the lacrimal system. Therefore, numerous studies have assessed the quantitative and qualitative changes in tear composition in patients with LDOD[4,5]. The results of such studies have been variable in terms of tear meniscus height, volumetric, osmolarity and cytokine levels in tear fluids[5-8]. To the best of our knowledge, three studies have reported cytokine levels in tears of patients with lacrimal duct obstruction. Matsumura *et al*[9] measured seven cytokines in tears and reported a significant increase in interleukin (IL)-6 levels in the affected eyes of infants with congenital nasolacrimal duct (NLD) obstruction. Lee and Kim[10] examined the level of seven cytokines in tears before and after endoscopic dacryocystorhinostomy. Furthermore, Ali *et al*[5] measured 35 cytokines in tears obtained from patients with primary acquired NLD obstruction. However, specific inflammatory cytokines such as interferon (IFN)-α2a, IL-8 and IL-10 have not been investigated. In addition, differences in inflammatory cytokines in tears according to the LDOD subtype (prelacrimal obstruction, chronic dacryocystitis and acute dacryocystitis) have not been reported. Therefore, we compared the levels of 10 inflammatory cytokines in tears from the eyes of patients with unilateral lacrimal duct obstruction relative to those in the contralateral normal eyes. The results revealed differences in the cytokine levels according to the LDOD subtype.

Material & Methods

Patient selection: This study was approved by the ethics committee of Tongji Hospital (Tongji Medical College, Huazhong University of Science and Technology) Wuhan, P.R. China. Informed consent was obtained from all patients. This was a non-randomized case–control study. The participants were patients with unilateral lacrimal duct obstruction admitted to our hospital from April to October 2018.

Inclusion criteria: (*i*) clinical diagnosis of unilateral lacrimal duct obstruction based on chief patient complaints, symptoms (unilateral epiphora with or without purulent secretion), slit-lamp examination, dye disappearance tests and lacrimal irrigation and probing; (*ii*) patients aged 18-75 yr; and (*iii*) no topical antibiotic or anti-inflammatory eye drop use within one week before sampling. Lacrimal irrigation and probing were implemented after tear collection; however, they were avoided in patients with acute dacryocystitis, which was diagnosed by symptoms and local signs. According to the clinical manifestations and examinations, patients with unilateral LDOD were grouped as, the prelacrimal obstruction (obstruction of the common canaliculus or lacrimal canaliculi), acute dacryocystitis (epiphora and acute inflammation of lacrimal sac) and chronic dacryocystitis groups (lacrimal duct obstruction and secretion from canaliculi upon irrigation). The criteria for the other normal eye were, normal slit-lamp microscopy appearance; Munk score=0; patency of lacrimal irrigation; fluorescein dye disappearance test (-). The contralateral normal eyes were used as the controls.

Exclusion criteria: (*i*) patients with acute conjunctivitis, allergic conjunctivitis, aqueous tear deficiency, glaucoma, iridocyclitis, thyroid-associated ophthalmopathy, ocular fundus disease or other ocular diseases (age-related cataracts was not excluded), and (*ii*) those with a history of ocular trauma or previous eye surgeries, uncontrolled hypertension, diabetes, autoimmune rheumatic diseases, mental disorders and severe systemic diseases.

Tear sample collection: Tear samples were aseptically collected from both eyes of each subject between 10:00 AM and 4:00 PM on the day before clinical treatments, as previously reported[11]. Briefly, a Schirmer test strip, without the fluorescein end, was placed in an Eppendorf tube; then, the tube was weighed. The strip was removed and placed at the inferior conjunctival fornix in the lower eyelid. Both affected and contralateral eyes were tested simultaneously. After five minutes, each test strip was placed back in their respective tubes; which were then re-weighed. Subsequently, 70 µl of tissue protein extraction reagent (Thermo Fisher Scientific, MA, USA) was added to the sample, which was then incubated at 4°C overnight. On the following day, the fluid was absorbed and placed in a second tube pre-cooled to 4°C; then, the sample in the first tube was centrifuged at 15,000 ×*g* for two minutes (4°C). The fluids in the two tubes were combined to obtain the final tear sample, which was stored at −80°C until the quantitative analysis of inflammatory cytokines. The volume of tears in each sample was calculated using the weight of tears and the density of water; the dilution time of tears was recorded and used to calculate the final level of cytokines in tears.

Inflammatory cytokine measurement: The levels of IFN-α2a, IFN-β, IFN-γ, IL-17A, IL-6, IL-8, IFN-γ-induced protein-10 (IP-10), monocyte chemotactic protein-1 (MCP-1), tumour necrosis factor-alpha (TNF-α) and vascular endothelial growth factor-A (VEGF-A) were measured using the meso scale discovery (MSD) multi-spot multiplex immunoassay system (Reagent test kit catalog No: K15067L-1; Machine type: SQ120; Manufacturer: Meso Scale Discovery, Maryland, USA), according to the manufacturer's instructions. The levels of all 10 cytokines were measured using a single reaction mixture of volume 50 µl. Standard curves for each cytokine were generated using the calibration kit provided and samples were assayed without dilution. Data acquisition and analysis were performed using the MSD scale reader and its dedicated software (Discovery Benchmark v.4.0.12).

Statistical analysis: Results were expressed as mean±standard deviation. All statistical analyses were performed using the SPSS software (v.21.0; IBM Corp., Armonk, NY, USA). The Wilcoxon signed-rank sum test was performed to compare inflammatory cytokines in tear samples between the affected and control eyes. The Mann–Whitney rank sum test was used to compare between the groups. The results with a $P<0.05$ were considered as statistically significant.

Results

Patient characteristics: The age of the 30 patients enrolled in the study was 18–75 yr (mean: 47.6±13.2 yr). Group A was comprised of five patients (1 male and 4 females; mean age: 49.8±5.8 yr) diagnosed with pre-lacrimal obstruction (obstruction of the common canaliculus or lacrimal canaliculi). Group B was comprised of five patients (5 females; mean age: 50.0±9.9 yr) diagnosed with acute dacryocystitis and Group C was comprised of 20 patients (2 males and 18 females; mean age: 46.5±15.3 yr) diagnosed with chronic dacryocystitis. All 30 patients received lacrimal catheterization and/or transnasal dacryocystorhinostomy according to their specific condition.

Overall comparison: In patients with unilateral LDOD, the levels of the following cytokines in the affected eyes were significantly higher than those in the control eyes: IFN-α2a ($P<0.001$), IFN-β ($P<0.001$), IFN-γ ($P<0.001$), IL-17A ($P<0.001$), IL-6 ($P<0.001$), IL-8 ($P<0.001$), TNF-α ($P<0.001$) and VEGF-A ($P=0.001$). However, there were no significant differences in the levels of IP-10 ($P=0.465$) and MCP-1 ($P=0.299$) between the affected and contralateral eyes (Table I).

Table I. Comparisons of cytokine expression in tears from the affected and contralateral eyes of patients with lacrimal duct obstruction disease

Cytokines	Affected eyes (pg/ml) n=30	Contralateral eyes (pg/ml) n=30
IFN-α2a	2509.45±1657.53	876.98±463.15***
IFN-β	1258.48±1036.94	375.33±195.89***
IFN-γ	2332.80±907.77	1212.26±484.54***
IL-17A	778.96±436.44	327.22±128.10***
IL-6	469.89±542.06	111.63±61.22***
IL-8	42856.10±60382.51	3983.26±6176.27***
IP-10	93021.50±63355.13	81254.19±37928.52
MCP-1	1720.07±1633.82	1949.33±2753.99
TNF-α	736.01±502.55	273.44±119.19***
VEGF-A	12307.66±9188.09	7811.03±3844.73**

Data are shown as mean±SD. *P* values were determined using Wilcoxon signed rank test (*P* **<0.01, ***<0.001). IFN, interferon; IL, interleukin; IP-10, IFN-γ-induced protein-10; MCP-1, monocyte chemoattractant protein-1; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor

Comparison within the groups: In Group A, there was a significant increase in the levels of IFN-α2a, IFN-β, IFN-γ, IL-17A, IL-6 and TNF-α in the affected eyes compared with those in the control eyes. However, there were no significant differences in the levels of IL-8, IP-10, MCP-1, and VEGF-A. In Group B, the levels of IFN-α2a, IFN-β, IL-17A, IL-6 and TNF-α were significantly higher in the affected eyes than in the control eyes, whereas the levels of IFN-γ, IL-8, IP-10, MCP-1 and VEGF-A did not present a significant difference. In Group C, the levels of IFN-α2a, IFN-β, IFN-γ, IL-17A, IL-6, IL-8, TNF-α and VEGF-A were significantly higher in the affected eyes than in the control eyes, whereas the levels of IP-10 and MCP-1 did not show a significant difference (Table II).

Comparison between the groups: To determine the influence of different obstructive sites on the levels of inflammatory cytokines in tears, their levels in the affected eyes between Groups A and C (obstruction of common canaliculus or lacrimal canaliculi *vs.* chronic dacryocystitis) were compared. The levels of IFN-α2a ($P=0.003$), IFN-β ($P=0.001$), IFN-γ ($P=0.003$), IL-17A ($P=0.003$), IL-6 ($P=0.002$), IL-8 ($P=0.007$), MCP-1 ($P=0.021$), TNF-α ($P=0.002$) and VEGF-A ($P=0.035$) in the affected eyes were significantly lower in Group A

Table II. Intragroup comparisons of cytokine expression in tears from the affected and contralateral eyes of patients with lacrimal duct obstruction disease

Cytokines	Affected eyes (pg/ml)	Contralateral eyes (pg/ml)
Group A (n=5)		
IFN-α2a	920.01±208.09	510.52±209.32*
IFN-β	378.95±108.47	219.62±88.68*
IFN-γ	1321.18±254.51	785.63±175.95*
IL-17A	340.01±69.86	216.43±46.81*
IL-6	105.26±49.91	59.18±30.57*
IL-8	1893.43±1240.71	1741.38±2057.47
IP-10	87790.28±22,480.75	69401.86±11,974.61
MCP-1	757.30±527.13	786.06±873.67
TNF-α	288.93±54.54	178.13±42.42*
VEGF-A	6264.84±2587.45	5764.72±3068.24
Group B (n=5)		
IFN-α2a	1837.03±403.21	1002.92±516.50*
IFN-β	870.43±344.58	476.93±281.07*
IFN-γ	1786.44±370.94	1262.55±527.70
IL-17A	620.12±151.39	351.78±144.72*
IL-6	373.83±268.90	143.36±79.35*
IL-8	25449.98±20,975.39	7881.76±12,336.84
IP-10	74655.83±68,869.03	58476.28±8420.88
MCP-1	2976.06±3514.64	2958.69±4072.09
TNF-α	553.59±128.09	322.07±160.94*
VEGF-A	11732.10±7010.18	8837.27±4347.36
Group C (n=20)		
IFN-α2a	3074.92±1740.60	937.10±467.66***
IFN-β	1575.37±1124.63	388.85±176.05***
IFN-γ	2722.30±841.19	1306.34±485.10***
IL-17A	928.41±451.68	348.78±127.66***
IL-6	585.07±617.54	116.81±56.41***
IL-8	57448.30±68,750.55	3569.11±4448.34***
IP-10	98920.73±69,920.89	89911.75±43,568.32
MCP-1	1646.76±866.34	1987.81±2701.65
TNF-α	893.38±542.19	285.11±112.67***
VEGF-A	13962.25±10,223.47	8066.05±3892.77**

Data are shown as mean±SD. *P* values were determined using Wilcoxon signed rank test (*P* *<0.05, **<0.01, ***<0.001). Group A, pre-lacrimal obstruction; Group B, acute dacryocystitis; and Group C, chronic dacryocystitis

than in Group C (Table III), whereas the level of IP-10 was not significantly different (*P*=0.786). In addition, the levels of IFN-α2a, IFN-β, IFN-γ, IL-17A, IL-6 and TNF-α in the contralateral eyes were also significantly lower in Group A than in Group C. However, there were no significant differences in the levels of IL-8, IP-10, MCP-1 and VEGF-A in the contralateral eyes between the two groups (Table III).

A comparison between Groups B and C (acute dacryocystitis *vs.* chronic dacryocystitis) revealed that the IFN-γ level in the affected eyes was significantly lower in Group B than in Group C (*P*=0.014; Table III). Moreover, there were no significant differences in the levels of inflammatory cytokines in the contralateral eyes between Groups B and C (Table III).

Discussion

LDOD is a multifactorial disorder of unknown aetiology; however, hormonal microenvironments, antimicrobial defenses, vascular factors, tear proteomics and tear duct-associated lymphoid tissues are proposed to play crucial roles[3]. Moreover, these etiologic factors may be interdependent; for example, prolactin (PRL) has significant immunomodulatory effects with its receptors expressed on lymphocytes, macrophages and fibroblasts, all of which are likely to play a role in the aetiopathogenesis of LDOD[12]. With this complex interconnected network, it is challenging to determine the primary etiologic factors or to characterize the underlying mechanisms. Nevertheless, LDOD is characterized by progressive inflammation, fibrosis and the subsequent obstruction of the NLD[13,14]. Moreover, as tears flow from the ocular surface to the nasal cavity through the lacrimal drainage system, tear proteomics, especially with regard to inflammation and fibrosis, may be altered in patients with lacrimal diseases.

Previous studies on tear proteomics in lacrimal diseases have focused primarily on NLD obstruction. For instance, Lee and Kim[10] found that the levels of IL-2, IL-6, IL-10, VEGF and FGF-2 were higher in the tears from the affected eyes of patients with primary NLD compared with those from the contralateral eyes. Similarly, Ali *et al*[5] reported a significant upregulation in 10 pro-inflammatory factors, including matrix metalloproteinase 9, serpin E1, IL-6, hepatocyte growth factor, VEGF-A, VEGF-R2, platelet-endothelial cell adhesion molecule-1, C-reactive protein, chemokine ligand 2 and platelet-derived growth factor-AA, in tears from the affected eyes of patients with primary NLD compared with those in the non-diseased control. However, these studies did not indicate the specific

Table III. Inter-group comparisons (*P*-values) of cytokine expression in tears from the affected and contralateral eyes of patients with lacrimal duct obstruction disease

Cytokines	Affected eyes	Contralateral eyes
Group A (n=5) *vs.* Group C (n=20)		
IFN-α2a	0.003**	0.035*
IFN-β	0.001**	0.042*
IFN-γ	0.003**	0.014*
IL-17A	0.003**	0.025*
IL-6	0.002**	0.025*
IL-8	0.007**	0.197
IP-10	0.786	0.541
MCP-1	0.021*	0.118
TNF-α	0.002**	0.030*
VEGF-A	0.035*	0.154
Group B (n=5) *vs.* Group C (n=20)		
IFN-α2a	0.154	0.786
IFN-β	0.248	0.634
IFN-γ	0.014*	0.946
IL-17A	0.118	0.892
IL-6	0.415	0.377
IL-8	0.634	0.634
IP-10	0.248	0.057
MCP-1	0.786	0.684
TNF-α	0.308	0.497
VEGF-A	0.734	0.455

P values were determined using the Wilcoxon signed rank test (*P* *<0.05, **<0.01). Group A, pre-lacrimal obstruction; Group B, acute dacryocystitis; and Group C, chronic dacryocystitis

time points associated with tear collection, which may have affected the results, given that cyclical diurnal variations in the levels of inflammatory factors have been observed. For example, a significant increase in the levels of IL-1β, IL-6, IL-10, IL-12p70 and TNF-α has been observed in the early morning and at night[11]. Hence, to avoid the effects of diurnal fluctuations in inflammatory cytokine levels, in the present study, the collection time of tears was set between 10:00 AM and 4:00 PM, the period when smaller fluctuations in inflammatory cytokine levels have been reported[11].

Although the NLD is the primary site of obstruction, the canaliculi can also be blocked in some patients, making treatment more difficult. Therefore, it is of interest to examine the potential differences between these sites of obstruction. To this end, in the present study, the inflammatory cytokine levels were compared in tears from 30 patients with LDOD and differences in the cytokine profiles of tears among different LDOD subtypes (prelacrimal obstruction, acute dacryocystitis and chronic dacryocystitis) were investigated. Although, expectedly several inflammatory cytokines were significantly higher in the affected eyes than in the contralateral eyes (Tables I and II), the results also showed that the levels of IFN-α2a, IFN-β, IFN-γ, IL-17A, IL-6, IL-8, MCP-1, TNF-α and VEGF-A were higher in the affected eyes of patients with chronic dacryocystitis than those with prelacrimal obstruction (Table III).

The increased levels of several cytokines may be reflective of tear accumulation because of abnormal drainage or increased production of inflammatory factors within the lacrimal passages. Alternatively, the ocular surface may be the source of these cytokines, which may subsequently promote LDOD. It is well documented that significant anatomical differences exist between the canaliculus and NLD. For example, the vascular plexus are located around the lacrimal sac and NLD[15]. Moreover, thicker mucosa-associated lymphoid tissue has been observed in the sac and NLD than in the canaliculi[16]. Alternatively, the retention of tears in the NLD and lacrimal sac of the eyes affected with chronic dacryocystitis may increase the interaction between the immune system and antigens capable of promoting a cellular or humoral immune response. In addition, the levels of six inflammatory cytokines were higher in the contralateral eyes of patients with chronic dacryocystitis compared with those in the eyes of patients with prelacrimal obstruction (Table III). This difference may have been caused by the small sample size. A previous study reported that there were no significant differences in cytokine expression in tears from the control eyes between patients with NLD obstruction and the healthy subjects[5]. However, it cannot be ruled out that the contralateral eyes of patients with chronic dacryocystitis may have been in a sub-health status, given that patients with unilateral chronic dacryocystitis often present in clinical practice, and it gradually develops into bilateral chronic dacryocystitis. These findings raise interesting points, including whether the contralateral eyes of patients with chronic dacryocystitis represent an early stage of this disease and whether the increased inflammatory

cytokine levels of the affected eyes also affect the contralateral eyes.

Further comparison between the groups revealed that the IFN-γ level in the affected eyes of Group B was lower than that of Group C; however, no difference was observed in inflammatory cytokine levels in the contralateral eyes between these groups (Table III). Notably, IFN-γ may be involved in the regulatory process of chronic fibrosis following recurrent inflammation[17,18]. Specifically, although the regulation of fibrosis during inflammation is a complex process involving numerous inflammatory cytokines, the increased level of IFN-γ observed in the present study may be related to progressive chronic fibrosis associated with dacryocystitis.

There were certain limitations to this study. First, tears flow continuously, allowing a basic level of secretion to be constantly maintained. Hence, tears collected from the conjunctival fornix may partially reflect the tears in the lacrimal duct. However, the findings would be more direct if tears in the lacrimal sac or the NLD proximal to the obstructed site could be obtained, which would enable comparison with tears collected at the fornix. Second, the limited number of patients with prelacrimal obstruction and acute dacryocystitis made it difficult to draw definitive conclusions. Third, this study was a single-point observational study with no follow up comparisons performed in the same patients at different times of onset and post-treatment. These aspects should be considered in the design of future studies.

Overall, the levels of several cytokines in the tears of the affected eyes were significantly higher than those in the tears of the control eyes. Therefore, the dysregulation of certain cytokines related to inflammation, angiogenesis and fibrosis contributes to the aetiopathogenesis of LDOD. It is also interesting that the cytokine expression profiles in tears differed among the clinical subtypes of LDOD; however, additional cases from different subtypes and a larger panel of cytokines are needed to characterize the contributions of these cytokine alterations in the aetiopathogenesis of LDOD.

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References

1. Vinciguerra A, Nonis A, Giordano Resti A, Bussi M, Trimarchi M. Best treatments available for distal acquired lacrimal obstruction: A systematic review and meta-analysis. *Clin Otolaryngol* 2020; *45* : 545-57.
2. Das AV, Rath S, Naik MN, Ali MJ. The incidence of lacrimal drainage disorders across a tertiary eye care network: Customization of an indigenously developed electronic medical record system-eyeSmart. *Ophthalmic Plast Reconstr Surg* 2019; *35* : 354-6.
3. Ali MJ, Paulsen F. Etiopathogenesis of primary acquired nasolacrimal duct obstruction: What we know and what we need to know. *Ophthalmic Plast Reconstr Surg* 2019; *35* : 426-33.
4. Yaginuma S, Konno K, Shigeyasu C, Yamada M. Tear protein analysis in patients with primary acquired nasolacrimal duct obstruction treated with lacrimal passage intubation. *Jpn J Ophthalmol* 2021; *65* : 409-15.
5. Ali MJ, Patnaik S, Kelkar N, Ali MH, Kaur I. Alteration of tear cytokine expressions in primary acquired nasolacrimal duct obstruction – Potential insights into the etiopathogenesis. *Curr Eye Res* 2020; *45* : 435-9.
6. Roh JH, Chi MJ. Efficacy of dye disappearance test and tear meniscus height in diagnosis and postoperative assessment of nasolacrimal duct obstruction. *Acta Ophthalmol* 2010; *88* : e73-7.
7. Ohtomo K, Ueta T, Fukuda R, Usui T, Miyai T, Shirakawa R, *et al*. Tear meniscus volume changes in dacryocystorhinostomy evaluated with quantitative measurement using anterior segment optical coherence tomography. *Invest Ophthalmol Vis Sci* 2014; *55* : 2057-61.
8. Yuksel N, Akcay E, Ayan B, Duru N. Tear-Film osmolarity changes following dacryocystorhinostomy in primary acquired nasolacrimal duct obstruction. *Curr Eye Res* 2017; *42* : 348-50.
9. Matsumura N, Goto S, Uchio E, Nakajima K, Fujita T, Kadonosono K. Cytokine profiles of tear fluid from patients with pediatric lacrimal duct obstruction. *Invest Ophthalmol Vis Sci* 2017; *58* : 252-6.
10. Lee JK, Kim TH. Changes in cytokines in tears after endoscopic endonasal dacryocystorhinostomy for primary acquired nasolacrimal duct obstruction. *Eye (Lond)* 2014; *28* : 600-7.
11. Uchino E, Sonoda S, Kinukawa N, Sakamoto T. Alteration pattern of tear cytokines during the course of a day: Diurnal rhythm analyzed by multicytokine assay. *Cytokine* 2006; *33* : 36-40.
12. Ali MJ, Paulsen F. Prolactin and Prolactin-inducible protein (PIP) in the pathogenesis of primary acquired nasolacrimal duct obstruction (PANDO). *Med Hypotheses* 2019; *125* : 137-8.
13. Mauriello JA, Jr., Palydowycz S, Deluca J. Clinicopathologic study of lacrimal sac and nasal mucosa in 44 patients with complete acquired nasolacrimal duct obstruction. *Ophthalmic Plast Reconstr Surg* 1992; *8* : 13-21.
14. Kashkouli MB, Sadeghipour A, Kaghazkanani R, Bayat A, Pakdel F, Aghai GH. Pathogenesis of primary acquired nasolacrimal duct obstruction. *Orbit* 2010; *29* : 11-5.
15. Ayub M, Thale AB, Hedderich J, Tillmann BN, Paulsen FP. The cavernous body of the human efferent tear ducts contributes to regulation of tear outflow. *Invest Ophthalmol Vis Sci* 2003; *44* : 4900-7.

16. Knop E, Knop N. Lacrimal drainage-associated lymphoid tissue (LDALT): A part of the human mucosal immune system. *Invest Ophthalmol Vis Sci* 2001; *42* : 566-74.
17. Fielding CA, Jones GW, McLoughlin RM, McLeod L, Hammond VJ, Uceda J, *et al*. Interleukin-6 signaling drives fibrosis in unresolved inflammation. *Immunity* 2014; *40* : 40-50.
18. Yao Y, Li L, Yang SH, Gao CY, Liao LH, Xie YQ, *et al*. CD8+ T cells and IFN-γ induce autoimmune myelofibrosis in mice. *J Autoimmun* 2018; *89* : 101-11.

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