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CLINICAL STUDY

Changes in cytokines in tears after endoscopic endonasal dacryocysto-rhinostomy for primary acquired nasolacrimal duct obstruction

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Abstract

Purpose **We attempted to compare the cytokine composition of tears between primary acquired nasolacrimal duct (NLD) obstruction and normal controls.**
We investigated the changes in cytokines in tears after endoscopic endonasal dacryocystorhinostomy (DCR).
Patients and methods **Eighteen patients underwent endonasal DCR, with seven patients undergoing bilateral DCR, resulting in twenty-five DCRs in total. Eleven contralateral un-operated eyes were used as normal controls. Silicone stents were removed 3 months after surgery. Tear samples were collected from all eyes before surgery, and at 1 month, 2 months, 3 months, and 4 months after surgery. The level of interleukin (IL)-1β, IL-2, IL-6, IL-10, transforming growth factor (TGF)-β2, fibroblast growth factor (FGF)-2, and vascular endothelial growth factor (VEGF) in the tears was measured.**
Results **The concentrations of IL-2, IL-6, IL-10, VEGF, and FGF-2 were significantly higher in eyes with NLD obstruction than controls before surgery ($P = 0.006$, 0.018, 0.002, 0.048, and 0.039, respectively). Most inflammatory cytokines (IL-1β, IL-2, IL-6, VEGF, and FGF-2) were higher in the tears of the DCR group compared with the controls during the postoperative follow-up, but then rapidly decreased to the level of the controls after removal of the silicone stent. The recurred eyes showed a higher level of TGF-β2 and FGF-2 in tears compared with the eyes that showed good surgical results ($P < 0.005$ and < 0.005, respectively).**
Conclusion **The tear levels of inflammatory cytokines were higher in eyes with NLD obstruction than controls. The changes in cytokine level during the postoperative period showed the importance of cytokine analysis in understanding wound healing after DCR.**

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Introduction

Primary nasolacrimal duct (NLD) obstruction is a common acquired lacrimal disease, which causes symptoms of continuous tearing, mucous discharge, recurrent conjunctivitis, and ocular surface discomfort. Although the exact mechanisms underlying NLD obstruction are not clear, narrowing of the lacrimal lumen by inflammatory infiltrates and subsequent fibrosis is considered as one of the main processes.[1] Dacryocystorhinostomy (DCR) through transcutaneous incision or by nasal endoscopy has been the treatment of choice for NLD obstruction. In particular, endoscopic endonasal DCR has the advantages of the absence of external scarring, a lower risk of interfering with the lacrimal pump mechanism, and shorter postoperative recovery time.[2,3] Although previous reports have shown lower success rates than transcutaneous DCR, recent advances in technique have resulted in the endoscopic endonasal DCR being comparable to external DCR.[4–6]

The tears contain a variety of cytokines and growth factors required to maintain

homeostasis of the ocular surface. Changes in the cytokines of the tears may reflect an underlying pathophysiologic condition. The analysis of tear cytokines in primary NLD obstruction could offer a better understanding of the mechanisms involved in the disease. In addition, the study of the changes in the cytokines in tears may illuminate the process of wound healing after endoscopic endonasal DCR.[7] Fibroblast growth factor (FGF)-2, transforming growth factor (TGF)-β2, and vascular endothelial growth factor (VEGF) have all been reported to be involved in the wound healing cascade.[8–10] Interleukin (IL)-1β, IL-2, and IL-6 are all well-known inflammatory cytokines that mediate pathophysiologic changes in ocular disease and the wound healing process.[11,12] IL-10 is an anti-inflammatory cytokine present at the ocular surface. Therefore, focusing on the changes in these cytokines could aid in understanding the etiology of and developing treatments for NLD obstruction. Thus far, studies on cytokines in the tears have shown a significant progress in the understanding of some ocular diseases. However, to the best of knowledge, an analysis of the changes in the cytokines in the tears of patients with NLD obstruction has not previously been performed.

In the current study, we examined the tear cytokine level in primary NLD obstruction compared with normal controls to evaluate the possible mechanism of NLD obstruction. We then attempted to determine the changes in cytokines with time after endoscopic endonasal DCR to study the healing process that occurs in response to surgery.

Materials and methods

The protocol and consent forms for the study were approved by the Institutional Review Board of Chung-Ang University Hospital, Seoul, Korea. This was a prospective interventional study in which all procedures were performed under the tenets of the Helsinki Declaration, and informed consent was obtained from all patients.

Between March 2011 and February 2012, 25 eyes of 18 patients who were scheduled to undergo endoscopic endonasal DCR for the treatment of NLD obstruction as evidenced by complete obstruction upon irrigation and probing were recruited for this study. Eleven contralateral un-operated eyes were used as controls. Patients with a history of previous ocular surgery, facial bone fracture, dry eye syndrome, allergic conjunctivitis, lid or cilia abnormality such as trichiasis, distichiasis, entropion, or ectropion, or who received medications including anti-glaucoma drugs and rheumatoid arthritis drugs were excluded from the study. All patients underwent ophthalmic examination including slit-lamp examination, irrigation of nasolacrimal drainage systems, fluorescein dye disappearance test, and intranasal examination preoperatively.

The surgery was done by one author (LEE) using the same technique with the patients under general anesthesia. Before surgery, the nasal mucosa was packed with gauze soaked with a mixture of lidocaine and epinephrine for 30 min to decrease bleeding. After localizing the lacrimal sac, local anesthetics were injected into the nasal mucosa. The nasal mucosa and underlying bone were removed enblock by kerrison punch. After exposure of lacrimal sac, the sac was tented using a lacrimal probe and incised using a sickle knife. A bicanalicular silicone stent (Lacrimal Intubation Set; Beaver-Visitec International Ltd., Abingdon, UK) was inserted through the punctum into the nasal cavity and secured by 5-0 prolene. Merocel was inserted into the nasal cavity and removed at postoperative day 3. After surgery, the eyes received topical neomycin-polymyxin B sulfate-dexamethasone eye drops (Maxitrol; Alcon, Ft. Worth, TX, USA) two times daily for 1 month.

Follow-up visits were done at 7 days and 1 month after surgery, and then every month for 4 months. Success was defined as improvement of subjective symptoms, patency of irrigation, and the absence of complications. The silicone stent was removed at postoperative 3 months. Any complications observed during the study period were recorded and managed appropriately.

Tear samples were collected from all patients presurgery and at 1 month, 2 months, 3 months, and 4 months after surgery. Tear samples were collected from the inferior tear meniscus after topical anesthesia (Alcaine; Alcon) to minimize irritation using 75 mm/75 μl Hematocrit-capillary tubes (Code No. 910-02-75). Tears were collected from both eyes, and 20 μl of tears was obtained in each eye. For the experiment, tear samples were diluted 10 times with assay diluent. The tubes were placed into the end of a micropipette tip and centrifuged at 10 000 r.p.m. for 5 min. IL-1β, IL-2, IL-6, IL-10, VEGF, and FGF-2 were measured by using a MILLIPLEX MAP Human Cytokine/Chemokine Panel (MPXCYTO-60k; Millipore, St Charles, MO, USA) which standard curve range is 3.2–10 000 pg/ml, and TGF-β2 was measured by using a MILLIPLEX MAP TGF-β2 Singleplex (TGFb-64k-01; Millipore) which standard curve range is 9.8–2500 pg/ml. Tear samples were incubated with antibody-coated capture beads overnight at 4 °C. Washed beads were further incubated with biotin-labeled anti-human cytokine antibodies, followed by streptavidin–phycoerythrin incubation. Standard curves of known concentrations of recombinant human cytokines/soluble receptors were used to convert fluorescence units to concentrations (pg/ml or ng/ml). To calculate molecular concentrations in tear samples,

we analyzed the median fluorescent intensity (MFI) data using a 5-parameter logistic or spline curve-fitting method.

Data are expressed as mean ± SD. Statistical analyses were performed using the SPSS statistical software (version 18.0; SPSS, Inc., Chicago, IL, USA). *A priori* sample size determination was not possible as we had no reliable estimates of the difference in cytokine measurements, and *post hoc* power estimation was performed to detect a 10% difference. Differences in cytokine concentration between eyes with NLD obstruction and controls were analyzed using the Mann–Whitney test. Repeated-measures analysis of variance (RMANOVA) with pairwise comparison was used to compare changes in cytokine level on the day before surgery and at 1 month, 2 months, 3 months, and 4 months after surgery. A *P*-value of <0.05 was considered to be statically significant.

Results

Eighteen patents underwent endonasal DCR, with seven patients undergoing bilateral DCR, resulting in twenty-five DCRs being performed in total. Eleven contralateral un-operated eyes were used as normal controls. Age and sex ratios are shown in Table 1, but a significant difference was not observed. Twenty-three eyes in the DCR group had improved symptoms, with patency of the drainage system, while two eyes in this group were noted to have recurrent epiphora, with one patient complaining of recurrence of epiphora 2 months after surgery and another complaining of recurrence 3 months after surgery. Neither recurred patients showed improvement after silicone stent removal, at postoperative 3 months, and revisional endoscopic DCR was performed in both patients 1 month after stent removal. The data of these two recurred eyes were excluded in the analysis of changes in cytokine with time after surgery, but instead were collected separately and were compared with data from good surgical results.

Before surgery, the concentrations of IL-2, IL-6, IL-10, VEGF, and FGF-2 in the tears of eyes with NLD obstruction were significantly higher than in the controls. IL-10 was not detected in the controls. IL-1β and TGF-β2 were also highly measured in the tears of eyes with NLD obstruction compared with the controls, but did not reach statistical significance (Table 2).

During the follow-up period, most cytokines (IL-1β, IL-2, IL-6, VEGF, and FGF-2) were higher in the tears of eyes that underwent DCR than in the normal controls. The operated eyes showed a sharp decrease compared with the levels of the controls after silicone stent removal (Figure 1). The expression of IL-1β was 5.16 ± 1.41 pg/ml preoperative, decreased slightly to 4.39 ± 0.86 pg/ml 1 month after surgery, and was 3.22 ± 0.60 pg/ml 2 months after surgery. The concentration of IL-1β significantly decreased to 1.95 ± 0.30 pg/ml at 4 months postoperatively after silicone stent removal, which was the same level as the normal controls. However, IL-2 levels significantly increased from 1.95 ± 0.30 pg/ml preoperatively to 3.72 ± 0.90 pg/ml at 1 month postoperatively, and remained higher than preoperative levels, being 3.20 ± 0.73 pg/ml at 2 months after surgery, and 3.30 ± 0.07 pg/ml 3 months after surgery. IL-2 expression markedly decreased to 0.96 ± 0.53 pg/ml at 4 months postoperatively, which was similar to the level of the controls (Table 3).

IL-6 initially significantly decreased preoperatively from 17.05 ± 6.86 pg/ml to 4.08 ± 3.01 at 1 month postoperatively. At 2 months and 3 months postoperatively, IL-6 levels increased significantly compared with preoperative levels, and then decreased to the level of the normal controls at 4 months postoperatively. The level of IL-10 rapidly decreased from 12.3 ± 2.54 pg/ml to 7.48 ± 1.48 pg/ml at 1 month postoperatively, and was not detected at 2 months after surgery. IL-10 was not detected in the normal controls during the study period (Table 3).

The concentration of VEGF decreased continually over the follow-up period without statistical significance. VEGF expression was higher in eyes that underwent DCR than in the normal controls during the study

Table 1 Patient age and sex distribution

	DCR	*Controls*	P-*value*
Number of eyes	25	11	
Age (mean ± SD)	63.1 ± 7.4	59.6 ± 8.7	0.47[a]
Sex (M:F)	2 : 16	1 : 10	1.00[b]

Abbreviations: DCR, dacryocystorhinostomy; F, female; M, male.
[a] Mann–Whitney *U*-test.
[b] Fisher's exact test.

Table 2 Comparison of cytokines expression in eyes with NLD obstruction and control

Cytokine	*Control (pg/ml)*	*NLD obstruction (pg/ml)*	P-*value*[a]
IL-1β	3.66 ± 1.53	5.16 ± 3.07	0.08
IL-2	0.21 ± 0.48	1.92 ± 0.25	0.006
IL-6	4.12 ± 4.63	17.05 ± 6.86	0.018
IL-10	0	12.3 ± 2.54	0.002
VEGF	20.6 ± 46.06	117.2 ± 40.5	0.048
FGF-2	16.88 ± 15.07	40.3 ± 4.38	0.039
TGFβ-2	1192 ± 504.3	2901.44 ± 507.76	0.065

Abbreviations: FGF, fibroblast growth factor; IL, interleukin; NLD, nasolacrimal duct; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.
[a] Mann–Whitney *U*-test.

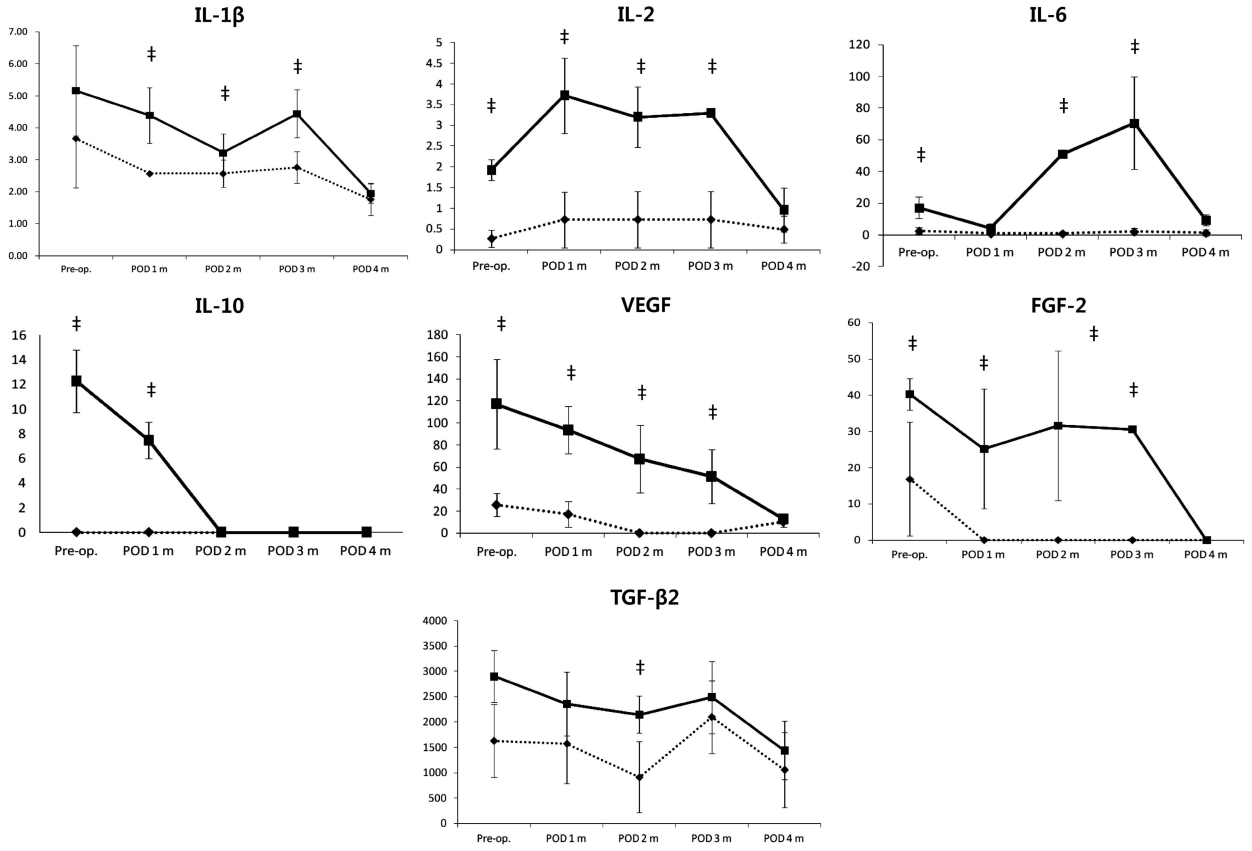

Figure 1 Comparison of cytokine level between the DCR group and controls. During the follow-up period, most cytokines (IL-1β, IL-2, IL-6, VEGF, and FGF-2) were higher in the tears of eyes that underwent DCR than normal controls, and showed a sharp decrease in the level of normal controls after silicone stent removal.

Table 3 The change of cytokine expression in tear with time after endoscopic endonasal dacryocystorhinostomy during the postoperative follow-up

Cytokine	*Pre-op.*	*POD 1 month*	p^a	*POD 2 months*	P^b	*POD 3 months*	P^c	*POD 4 months*	P^d
IL-1β	5.16 ± 1.41	4.39 ± 0.86	0.461	3.22 ± 0.60	0.039	4.43 ± 0.75	0.380	1.95 ± 0.3	0.003
IL-2	1.92 ± 0.25	3.72 ± 0.90	0.034	3.20 ± 0.73	0.006	3.30 ± 0.07	0.011	0.96 ± 0.53	0.038
IL-6	17.05 ± 6.86	4.08 ± 3.01	0.003	51 ± 0.85	0.048	70.6 ± 29.14	0.031	9.26 ± 3.58	0.020
IL-10	12.30 ± 2.54	7.48 ± 1.48	0.039	0	0.006	0	0.006	0	0.006
VEGF	117.2 ± 40.5	93.9 ± 21.3	0.350	67.43 ± 30.50	0.398	51.40 ± 24.44	0.905	13.17 ± 4.32	0.010
FGF-2	40.3 ± 4.38	25.22 ± 16.60	0.685	31.65 ± 20.68	0.461	30.6 ± 3.84	0.667	0	0.039
TGF-β2	2901.44 ± 507.76	2357.77 ± 625.10	0.535	2148.89 ± 365.06	0.531	2488.93 ± 710.37	0.279	1441.94 ± 580.28	0.011

Abbreviations: FGF, fibroblast growth factor; IL, interleukin; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.
p^a: pre-op. *vs* pod 1 mon, p^b: pre-op. *vs* pod 2 month, p^c: pre-op. *vs* pod 3 month, p^d: pre-op. *vs* pod 4 month.

period, and decreased to the level of the controls 1 month after silicone stent removal. The concentration of FGF-2 and TGF-β2 did not show a significant change over the follow-up period. After removal of the silicone stent, the concentration of FGF-2 and TGF-β2 decreased to the level of the controls at 4 months postoperatively (Table 3).

Post hoc power analysis indicated out study had 95% power to detect a 10% difference in cytokine level between DCR groups and controls and 95% power to detect a 10% difference in cytokine change with time after surgery (two-sided analysis and an alpha value of 0.05) (Tables 2 and 3).

The two recurred eyes showed higher concentrations of FGF-2 and TGF-β2 in comparison with eyes with good surgical results at 4 months postoperatively (Figure 2). However, the concentrations of IL-1β, IL-2,

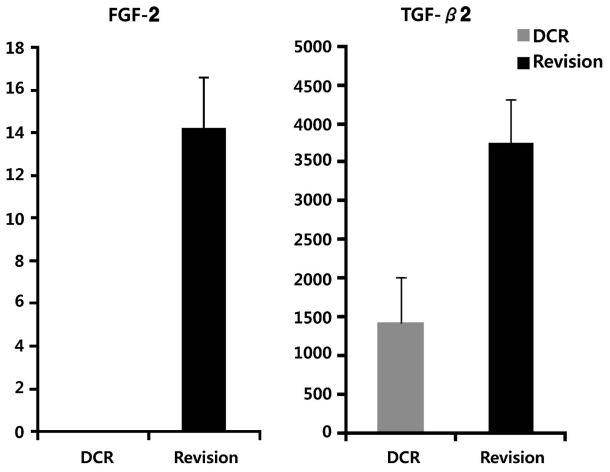

Figure 2 Comparison of cytokine level between eyes with good surgical results and eyes with recurrence. The two recurred eyes showed higher expression of FGF-2 and TGF-β2 in comparison with eyes with good surgical results 4 months postoperatively.

IL-10, and VEGF did not show a significant difference between recurred eyes and the eyes with good surgical results.

Discussion

Our study demonstrated that concentrations of most cytokines increased in tears of patients with NLD obstruction as compared with normal controls. Previous reports have shown that levels of inflammatory cytokines increased in the tear fluid from eyes with various ocular surface diseases such as dry eye, allergic conjunctivitis, corneal neovascularization, and thyroid-associated ophthalmopathy.[13–16] Currently, the mechanism of the increase in cytokine expression in the tears of patients with NLD obstruction is not fully understood. The delayed tear clearance due to lacrimal insufficiency may be an important mechanism for the increase in cytokines in the tears of patients with NLD obstruction.[17–19] The increases in all of the studied cytokines including the pro-inflammatory cytokines (ie, IL-2, IL-6, VEGF, and FGF-2) and the single anti-inflammatory cytokine (IL-10) indirectly support the possibility of accumulation of cytokines in the tears due to drainage failure. Otherwise, the lining epithelium or the fibroblasts of the lacrimal drainage system might be able to produce cytokines in an inflammatory state. Though the flow of tears through the lacrimal drainage is a one-way system governed by a pressure gradient, locally produced cytokines might diffuse back into the tear film and influence the cytokine profile. In addition, it is possible that the ocular surface tissues might be responsible for the overexpression of cytokines, resulting in NLD obstruction. The pathogenesis of NLD obstruction is as of yet unknown, and thus the role of these cytokines in the pathogenesis of NLD obstruction deserves further study. Furthermore, increases in inflammatory cytokines in the tears might be responsible for the irritation and ocular surface diseases that are common in NLD obstruction.

During clinical follow-up, we observed that the inflammatory signs that occur on the ocular surfaces of patients with NLD obstruction usually subside in the early postoperative period after successful endoscopic endonasal DCR. Nevertheless, most inflammatory cytokines (IL-1β, IL-2, IL-6, VEGF, and FGF-2) were at higher levels in the tears of eyes that underwent DCR than in the normal controls over the follow-up period. More surprisingly, the expression of cytokines showed a marked decrease after removal of the silicone stent, suggesting that inflammation associated with the silicone stent as a possible reason for the cytokine change over time. The necessity of a silicone stent after DCR is still controversial, even though it is common in practice. Several studies have reported that silicone stents offer no significant advantage in primary DCR, and that stents may even contribute to failure by inciting granuloma formation.[20–22] In contrast, other studies have reported higher success rates with the use of silicone stents in DCR, especially in patients with high-risk factors for failure.[23,24] Other studies have also shown no significant histopathologic changes with the use of silicone stents in the canalicular epithelium and the lacrimal sac after DCR.[25,26] In this study, endoscopic DCR with silicone stent showed a high success rate of 88.9%. However, the prolonged expression of inflammatory cytokines during the follow-up period raised concerns about the actual benefits of silicone stents and the timing of silicone stent removal after surgery. Though there is debate regarding the recommended time period for silicone stent removal, our study showed that a longer duration of silicone stent placement can result in a higher expression of inflammatory cytokine. Thus, the removal of the silicone stent at no later than 3 months after surgery in consideration of wound healing may not affect the surgical outcome.

IL-1β, IL-2, and IL-6 are inflammatory cytokines. IL-1β and IL-2 are important mediators of the inflammatory response and are involved in various cellular activities, especially in the growth and differentiation of cytotoxic T lymphocytes.[27] IL-6 is an inflammatory cytokine that stimulates the wound healing process, likely by a fibroblast-dependent mechanism.[28] IL-6 has been reported to be a key molecule in dry eye disease.[29,30] These cytokines are usually increased in inflammation

and early wound healing. They are released by ocular surface cells, monocytes, and T lymphocytes during the inflammatory cascade. A previous study reported that mucosa-associated lymphoid tissue (MALT) extends from conjunctival epithelium to the nasolacrimal drainage system.[31] These inflammatory cytokines and T lymphocytes have highly interrelated activity and are involved in regulating wound healing.[32] Our results, which showed higher inflammatory cytokine expression during the postoperative period, suggest involvement of lymphoid activity in MALT system in the wound healing process after DCR surgery. MALT system in ocular surface is connected with nasal cavity-associated lymphoid tissue *via* NLD,[33] so cytokine level in tear might reflect the wound healing process after DCR regardless of cellular origin of these cytokines. However, sustained expression of these inflammatory cytokines for longer than 2 months after surgery may suggest the presence of another stimulating factor causing the chronic inflammation, such as the silicone stent.

IL-10 is an anti-inflammatory cytokine that downregulates Th1 immunization.[34] Interestingly, the expression of IL-10 was detected only in eyes with NLD obstruction, and rapidly decreased to an undetectable level 2 months after surgery. IL-10 was not detected in control eyes over the entire study period. A previous study reported the expression of IL-10 in healthy volunteers.[35] Our result that IL-10 was undetectable in normal controls might be due to the different cytokine standards or the antibody kit. However, it is noteworthy that the expression of IL-10 showed a specific alteration pattern compared with other inflammatory cytokines, suggesting that IL-10 may have a potential role as a bio-indicator for lacrimal passage obstruction. At present, there is no data that could elucidate the mechanism underlying this change. It is widely known that IL-10 is an anti-inflammatory cytokine, and its increase in eyes with NLD obstruction could compensate for the chronic inflammation caused by NLD obstruction. However, considering the higher expression of some inflammatory cytokines (IL-2 and IL-6) in contrast to the abrupt decrease in IL-10 over the postoperative follow-up, it is unlikely that the role of IL-10 is that of a simple compensation mechanism for chronic inflammation. We assume that the specific expression of IL-10 might be a possible mechanism for the pathogenesis of NLD obstruction, and a further study is needed to corroborate the role of IL-10 in NLD obstruction.

The most common cause of surgical failure after endoscopic DCR has been reported to be membranous obstruction attributed to soft tissue scarring at the intranasal ostium.[36] The wound healing process of nasal ostium created by endoscopic DCR is dependent on reciprocal interactions between various cytokines and the different cell types lining the lacrimal sac and nasal mucosa. The tears from the two recurred eyes had significantly increased TGF-β2 and FGF-2 levels in comparison with eyes with good surgical results. TGF-β2 has various roles in cell growth, maturation, proliferation, and production of the extracellular matrix. FGF-2 is known to be an important contributor to postsurgical fibrosis.[37,38] Neither the expression of TGF-β2 nor FGF-2 showed a significant change with time in eyes with good surgical results. Compared with the higher expression of FGF-2 in the tears of operated eyes after surgery, TGF-β2 did not show a significant difference between operated eyes and control eyes over the study period except at 2 months postoperatively. Postoperative fibrosis is usually significant 2 months after surgery, which might reflect the role of TGF-β2 in wound maturation. Though it is difficult to clarify the exact role of TGF-β2 in this study due to the low rate of recurrence, it is possible that the concentration of TGF-β2 in tears could be used as an indicator of poor surgical results. In addition, good surgical results might be obtained if the expression of TGF-β2 could be reduced during the healing stage.

Our study has several limitations. The relatively small number of subjects in this study made it difficult to draw definitive conclusions. During the study, eyes that were operated on were treated with eye drops containing a mix of steroid and antibiotics. Previous studies demonstrated that topical steroids significantly decrease the expression of inflammatory cytokines.[39,40] In addition, the preservatives in eye drops can influence the expression of cytokines. Therefore, we used the topical steroid for only 1 month postoperatively to minimize the possible effects on cytokine expression. Taking together the overall higher expression of cytokines in tears form eyes with NDL obstruction compared with normal control, and the comparable changes in cytokines over the entire postoperative follow-up period, it did not appear that the use of topical eye drops resulted in significant changes opposite to current results.

In conclusion, we have shown that eyes with NLD obstruction have higher concentrations of cytokines than controls. Furthermore, inflammatory cytokines in the tears of eyes with NLD obstruction maintained levels higher than those of controls over the follow-up period, even in case of successful DCR surgery. Cytokine levels returned to normal after removal of the silicone stents. The higher concentrations of FGF-2 and TGF-β2 in the tears of recurred eyes suggest that inflammatory cytokines have a significant role in the pathogenesis of NLD obstruction. Further research could clarify the roles of these cytokines in NLD obstruction.

Summary

What was known before:

- Studies on cytokines in the tears have shown a significant progress in the understanding of some ocular diseases, but an analysis of the changes in the cytokines in the tears of patients with nasolacrimal duct (NLD) obstruction has not previously been performed.
- Dacryocystorhinostomy has been the treatments of choice for NLD obstruction, but the role of silicone stent and wound healing process after surgery has some controversy.

What this study adds:

- Eyes with NLD obstruction have higher concentrations of inflammatory cytokines than normal controls.
- Inflammatory cytokines in the tears of eyes with NDL obstruction maintained higher than those of controls after dacryocystorhinostomy, and returned to normal levels after removal of silicone stent.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

JKL and THK were involved in design and conduct of study and analysis and interpretation of data; THK was involved in data collection and management; and JKL was involved in preparation, review, and approval of manuscript.

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