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Ocular surface microbiota changes after external dacryocystorhinostomy: a "chicken or egg" problem

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ABSTRACT

Objective: The aim of this study was to evaluate changes in the ocular surface and nasal microbiota after external dacryocystorhinostomy (DCR) in patients with unilateral nasolacrimal duct obstruction (NLDO).

Methods: This prospective study included 68 eyes of 34 patients. Prior to external DCR, nasal and conjunctival smears were obtained from the affected eye and healthy contralateral eye. Results: Ocular surface cultures were positive in 19 (55.9%) of the NLDO eyes and 4 (11.8%) of the healthy eyes ($p<0.001$). The most frequently detected organism was methicillin-sensitive coagulase-negative staphylococci (MSCNS), which accounted for 45.8% of ocular surface isolates and 45.1% of nasal isolates. Pathogenic microorganisms (Citrobacter freundii and Candida krusei) were only detected in 2 NLDO eyes before DCR, and these microorganisms did not grow in any eye at any time after surgery. Although no reproduction was observed in repeated swabs, MSCNS growth was observed in 3 eyes (12.5%) postoperatively. The median postoperative time to ocular surface microbiota normalization was 2 weeks. Microorganisms isolated from ocular and nasal cultures were most resistant to penicillin, with rates of 57.1% and 73.3%, respectively.

Conclusions: Eyes with NLDO showed significantly greater microorganismal growth in ocular surface cultures than the healthy eyes. A safety margin of at least 2 weeks after successful external DCR is required for patients scheduled for intraocular surgery. Our susceptibility data suggest that clinicians should avoid penicillin when selecting antibiotics for patients with NLDO.

Keywords: Conjunctiva, dacryocystorhinostomy, microbiota, nasolacrimal duct obstruction, ocular flora

Introduction

The conjunctiva is a transparent mucous membrane that covers the inner surface of the eyelid and globe surface. It is covered with multilayered non-keratinized epithelial cells and serves as a barrier against infection. Conjunctival flora refers to all microorganisms found on the ocular surface of healthy individuals and does not cause infection under normal circumstances. These microorganisms have an important role in sustaining normal conjunctival functions and preventing ocular infection (1).

Ocular surface flora starts forming at birth and increases throughout an individual's lifetime. From birth, changes in the flora may occur depending on the environment, age, immunity, ocular surface disorders, systemic diseases, climate, region, and general hygienic conditions (2). Ocular surface flora is comprised mostly of gram-positive microorganisms (3). This microbial colonization of the conjunctiva makes it more difficult for potentially pathogenic microorganisms to colonize the ocular surface. Although the flora is essential for defense, it may become pathogenic after surgical procedures or in cases of immune compromise.

Nasolacrimal duct obstruction (NLDO) is a common ophthalmic problem, accounting for 3% of all ophthalmology clinic visits reported in a series (4). Stasis and secondary infection lead to acute or chronic dacryocystitis with epiphora and purulent discharge. NLDO may create an optimal environment for the growth of lacrimal sac flora. An obstructed nasolacrimal duct is treated by making a direct connection between the lacrimal sac and the nasal cavity by dacryocystorhinostomy (DCR).

Treatment of lacrimal system infection is important for preventing complications such as endophthalmitis after cataract and glaucoma surgeries, which are the most common intraoc-

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ular surgeries worldwide. Surgery should be postponed if obvious infection is present in the ocular adnexa or lacrimal system. Lacrimal duct obstruction can sometimes occur without any obvious clinical symptoms. Therefore, detailed history taking is extremely important to reduce the risk of postoperative and posttraumatic endophthalmitis (5). To the best of our knowledge, only few published studies have determined the time required for normalization of conjunctival flora after external DCR.

The primary aim of this study was to identify postoperative changes in the conjunctival microbiota in patients with NLDO after successful external DCR. The secondary aim was to determine the postoperative safety period necessary for conjunctival microbiota normalization after successful external DCR.

Methods

This prospective observational study included adult patients with complaints of unilateral epiphora secondary to NLDO with or without purulent regurgitation. The study was designed as a prospective case-control study and was approved by the ethics committee of the Alaaddin Keykubat University School of Medicine in Alanya, Turkey (1-20/2018). All the patients provided informed consent. The study was conducted in compliance with the Declaration of Helsinki.

All the patients underwent standard irrigation of the lacrimal system and probing to locate the site of the lacrimal obstruction or canalicular stenosis. Endoscopy was performed by the same specialist (C.Ş.) in all patients preoperatively to detect intranasal abnormalities. The exclusion criteria were acute dacryocystitis and any significant eyelid disorders, history of surgery involving the nasolacrimal drainage system, use of topical medications, advanced deviated nasal septum, middle turbinate hypertrophy or concha bullosa, nasal polyps, previous treatment with topical or systemic antibiotics 1 month before undergoing DCR, and systemic immunosuppression. Patients with bilateral NLDO and those who underwent silicone tube placement due to insufficient nasolacrimal anastomosis in surgery were excluded from the study.

All external DCR procedures were performed by the same surgeon (F.A.) using the Dupuy-Dutemps and Bourguet technique. Postoperative systemic antibiotics (a combination of amoxicillin and clavulanic acid 1000 mg every 12 hours for 5 days) and topical antibiotic/steroid fixed combination (0.3% netilmicin/0.1% dexamethasone every 6 hours for 5 days) were prescribed.

One day before surgery, 3 sterile cotton swabs were used to collect the specimens. One swab was thoroughly rubbed over the lower fornix of the operated eyes from the lateral to the medial side (NLDO group). The other swab was used to simultaneously collect specimens from the nonoperated eyes (control group). The third swab was used to obtain a sample from the nasal mucosa of the operated side. All nasal mucosa samples were obtained by the same otolaryngologist from the lateral nasal wall adjacent to the handle of middle turbinate.

Main Points:

- Bacterial growth on the ocular surface was significantly higher in NLDO eyes than in healthy eyes.
- Pathological microorganism growth was detected only in eyes with NLDO.
- Penicillin resistance was observed in almost all reproductive microorganisms.

Conjunctival sampling was performed without a topical anesthetic. Care was taken not to touch the eyelid margin or eyelashes. Postoperatively, conjunctival specimens were obtained from the operated and normal eyes weekly until culture results were negative or the colony count on the operated side was less than that on the normal side. Patency of the lacrimal drainage system was confirmed by irrigation after conjunctival culture.

The specimens were collected and sealed in tubes containing 1-mL thioglycollate medium and sent to the laboratory immediately after collection to obtain bacteriological cultures. After a 24-hour incubation period, blood agar, chocolate agar, and eosin methylene blue agar plates were inoculated with 0.1-mL transport media for aerobic cultures. Following the incubation period, colonies were differentiated and enumerated using standard bacteriological laboratory techniques.

Statistical analyses

As descriptive statistics, categorical data are expressed as number and percentage values, and continuous data are expressed as median and minimum-maximum values. The chi-square test was used to compare categorical data. The concordance between the ocular and nasal culture results was analyzed using kappa analysis. Numerical data were tested for normality of distribution by using the Kolmogorov-Smirnov test. The Mann-Whitney U test was used to compare numerical data that did not fit a normal distribution. Correlations between the 2 sets of numerical data were assessed using the Spearman correlation analysis. A p value of <0.05 was considered statistically significant. Analyses were performed using the Statistical Package for Social Sciences version 20.0 software (IBM Corp.; Armonk, NY, USA).

Results

The study included data from 68 eyes of 34 patients, including 7 men (20.6%) and 27 women (79.4%). The median age was 50.5 years (20.0-75.0 years). Preoperative purulent regurgitation was detected in 14 eyes (41.2%) treated with external DCR. The median operative time was 36.0 minutes (25.0-46.0 minutes), and the median symptom duration was 8.5 months (3.0-18.0 months). The patients had a mean of 1.0 (0.0-3.0) preoperative acute dacryocystitis episode. The demographic properties of the patients are shown in Table 1. Positive ocular cultures were significantly more frequent in the NLDO eyes than in the healthy eyes, whereas the positivity rates of the nasal cultures were similar (p=0.161; Table 2). The ocular cultures yielded at least one organism in 55.9% and 11.8% of the eyes in the NLDO and control groups, respectively ($p<0.001$). Analysis of the concordance between the nasal and ipsilateral ocular culture isolates revealed no statistically significant correlation in the NLDO or entire study group.

Table 1. Demographic and clinical characteristics of the study group

		n	(%)
Sex	Male	14	(20.6)
	Female	54	(79.4)
Age (years)*		49.2	± 15.8
Operative time (min)*		35.5	± 5.9
Number of acute dacryocystitis attacks*		1.2	± 1.2
Symptom duration (months)*		8.2	± 3.6
Affected side	Right	34	(50.0)
	Left	34	(50.0)
Purulent regurgitation	No	20	(58.8)
	Yes	14	(41.2)

*Expressed as mean ± standard deviation.
**Expressed as median (range) values.

Table 2. Comparison of positive culture rates among the study groups

		Patient		Control		
		n	(%)	n	(%)	p
Positive ocular surface culture	No	15	(44.1)	30	(88.2)	<0.001
	Yes	19	(55.9)	4	(11.8)	
Positive ipsilateral nasal culture	No	6	(17.6)	11	(32.4)	0.161
	Yes	28	(82.4)	23	(67.6)	

Chi-square test

Table 3. Distribution of microorganisms isolated from ocular surface and nasal cultures

		n	(%)
Ocular surface culture isolates	*Streptococcus pneumoniae*	3	(12.5)
	MSSA	2	(8.3)
	MSCNS	11	(45.8)
	MRCNS	3	(12.5)
	Citrobacter freundii	1	(4.1)
	Candida krusei	1	(4.1)
	Multiple strains	3	(12.5)
Nasal culture isolates	*Streptococcus pneumoniae*	2	(3.9)
	MSSA	3	(5.9)
	MSCNS	23	(45.1)
	MRCNS	12	(23.5)
	Multiple strains	11	(21.6)

MSSA: Methicillin-sensitive *Staphylococcus aureus*; MSCNS: Methicillin-sensitive coagulase-negative staphylococci; MRCNS: Methicillin-resistant coagulase-negative staphylococci

When the distributions of microorganisms isolated from the ocular surface and nasal cultures were analyzed, the most common organism was methicillin-sensitive coagulase-negative staphylococci (MSCNS) in both cultures (45.8% and 45.1%, respectively; Table 3). Gram-negative bacteria (Citrobacter freundii) were detected in 1 patient (4.1%), and fungal growth (Candida krusei) was detected in 1 patient (4.1%). The median number of colonies was 35 (10-2000) in the ocular cultures and 350 (10-2000) in the nasal cultures. Analysis of microorganism distributions in the ocular surface and nasal cultures revealed a significant weak to moderate correlation. For ocular surface cultures yielding *Streptococcus pneumoniae*, methicillin-sensitive *Staphylococcus aureus* (MSSA), MSCNS, and methicillin-resistant coagulase-negative staphylococci (MRCNS), the same organisms were detected in the ipsilateral nasal cultures at rates of 33.3%, 50.0%, 58.3%, and 66.7%, respectively (κ=0.336, p=0.001; Table 4). Low colony MSCNS growth was observed in the postoperative second week swab in only three (12.5%) of the eyes showing culture positivity before DCR. Culture positivity was not detected in the repeated swab samples from these eyes.

In the mean 2 weeks after DCR, either culture negativity or colony count in the control eyes was reached in the NLDO eyes. No pathological microorganism reproduction was detected in all the eyes in any postoperative period. Time to flora normalization was not associated with sex or the presence of purulent regurgitation. In addition, no significant correlation was detected between normalization time and age, operative time, symptom duration, number of episodes, or number of colonies in the cultures (Table 5). No statistically significant difference was found between the strains isolated from the ocular cultures and time to normalization (p=0.333). Although not statistically significant, normalization time was longer in the eyes with multiple microorganism growth before surgery.

Antibiotic susceptibility testing revealed that penicillin resistance was most common among the ocular surface culture isolates, while piperacillin resistance was most common among the nasal culture isolates. The antibiotic resistance rates of the microorganisms isolated in the ocular and nasal cultures are shown in Table 6.

Discussion

For years, the ocular surface was thought to be sterile owing to the presence of lysozyme, antimicrobial peptides, immunoglobulin A complement, and other substances. By using an animal model, a group led by Rachel Caspi at the US National Eye Institute found that local bacteria on the ocular surface maintain ocular immunity and that transient disruption of bacteria by antibiotic therapy results in a reduction in immune-related mechanisms (6).

Microbiological changes in the ocular surface and nasal passages of patients with NLDO have been an area of interest for researchers in recent years. The conjunctival flora of healthy individuals includes *S. epidermidis*, *S. aureus*, and diphtheroids, while the nasal flora includes coagulase-negative staphylococci (CNS), aerobic diphtheroids, *S. aureus*, *S. viridans*, meningococci, enterococci, Moraxella, peptostreptococci, and Bacteroides species (7-9).

Table 4. Analysis of positive culture rates and concordance between microorganisms isolated from ocular surface and nasal cultures

	Ocular surface isolates										
	Streptococcus pneumoniae		MSSA		MSCNS		MRCNS		*Candida krusei*		
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	p
Nasal cultures											
Negative	0	(0.0)	2	(50.0)	2	(16.7)	0	(0.0)	1	(100.0)	
Streptococcus pneumoniae	1	(33.3)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	κ=0.336 p=0.001
MSSA	0	(0.0)	2	(50.0)	2	(16.7)	0	(0.0)	0	(0.0)	
MSCNS	2	(66.7)	0	(0.0)	7	(58.3)	1	(33.3)	0	(0.0)	
MRCNS	0	(0.0)	0	(0.0)	1	(8.3)	2	(66.7)	0	(0.0)	

Kappa agreement analysis
MSSA: Methicillin-sensitive *Staphylococcus aureus*; MSCNS: Methicillin-sensitive coagulase-negative staphylococci; MRCNS: Methicillin-resistant coagulase-negative staphylococci

Table 5. Correlation analysis between ocular surface flora normalization time and patient age, operative time, symptom duration, number of episodes, or number of colonies in cultures

	Normalization time (weeks)	
	Rho	p
Age (years)	0.066	0.790
Operative time (min)	0.165	0.500
Number of acute dacryocystitis attacks	0.079	0.749
Symptom duration (months)	−0.106	0.667
Number of colonies in ocular surface culture	−0.101	0.682
Number of colonies in nasal culture	0.247	0.356

Spearman correlation analysis

Ocular surface bacteriology and alterations are essential concepts in ophthalmology. Studies have shown that the determinants of normal conjunctival flora are related to age, geographical region, climate, and occupation (10). Alterations in flora composition are determined by numerous factors, but only few studies have described microbiological changes in patients who underwent surgery for dacryostenosis (11, 12).

Timely and effective treatment of infections involving the lacrimal system is undeniably important for the prevention of endophthalmia after cataract and glaucoma surgeries, which are the most common intraocular surgeries. Chronic dacryocystitis increases in frequency after the age of 40 years but may not cause clinical complaints of epiphora because tear production decreases with age. Therefore, a thorough history taking must be performed preoperatively (13). Conjunctival flora is known to be the primary source of contamination in postoperative endophthalmia (14). CNS and diphtheroids are the main elements of conjunctival flora in healthy individuals. Pathogenic microorganisms such as *S. aureus*, *S. pneumoniae*, *Haemophilus influenzae*, *Pseudomonas species*, and enteric bacteria are isolated less frequently (15).

Table 6. Antibiotic resistance of microorganisms isolated in ocular surface and nasal cultures

	Ocular surface culture n (%)		Nasal culture n (%)	
	Sensitive	Resistant	Sensitive	Resistant
Ampicillin	3 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)
Amikacin	20 (95.2)	1 (4.8)	47 (100.0)	0 (0.0)
Ciprofloxacin	18 (81.8)	4 (18.2)	41 (85.4)	7 (14.6)
Gentamicin	20 (87.0)	3 (13.0)	47 (95.9)	2 (4.1)
Erythromycin	17 (70.8)	7 (29.2)	37 (75.5)	12 (24.5)
Cefepime	19 (100.0)	0 (0.0)	39 (100.0)	0 (0.0)
Cefoxitin	18 (85.7)	3 (14.3)	30 (66.7)	15 (33.3)
Levofloxacin	3 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)
Linezolid	23 (100.0)	0 (0.0)	46 (93.9)	3 (6.1)
Moxifloxacin	20 (90.9)	2 (9.1)	44 (91.7)	4 (8.3)
Ofloxacin	2 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)
Optochin	3 (75.0)	1 (25.0)	2 (100.0)	0 (0.0)
Piperacillin	NR	NR	0 (0.0)	2 (100.0)
Penicillin	9 (42.9)	12 (57.1)	12 (26.7)	33 (73.3)
Trimethoprim-Sulfamethoxazole	16 (69.6)	7 (30.4)	39 (79.6)	10 (20.4)
Tetracycline	18 (75.0)	6 (25.0)	37 (77.1)	11 (22.9)
Teicoplanin	2 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)
Vancomycin	3 (100.0)	0 (0.0)	1 (100)	0 (0.0)

NR: Not reviewed

NLDO is one of the local risk factors of postoperative and posttraumatic endophthalmia, and if clinicians cannot intervene with the duct obstruction for any reason, they should opt for a powerful and intensive antibiotic therapy to manage the condition. The few studies in patients with dacryocystitis and NLDO have shown that most microorganisms are gram-positive bacteria (16). Consistent with the literature, we isolat-

ed mostly gram-positive bacteria in ocular cultures obtained before external DCR. At this point, we and other researchers have been perplexed by the classic "chicken or egg" problem of the vicious cycle; that is, do changes in the microbiological spectrum underlie NLDO, or does microorganismal proliferation occur as a result of NLDO? Bacterial isolation from the conjunctiva of healthy controls has been reported at rates of 15-100% (17).

Balıkoğlu-Yılmaz et al. (18) reported positive culture rates of 46.9% and 34.4% in eyes with NLDO and healthy eyes, respectively. Coden et al. (19) reported a 52% positive culture rate in a dacryocystitis case series. On the other hand, Başal et al. (20), who investigated ocular nasal flora changes after endoscopic DCR, reported 1-year results of surgical cases combined with silicone tube intubation. The researchers, who achieved 90% anatomical patency within 1 year, found preoperative bacterial culture growth rates of 45% and 20% in conjunctiva with and without NLDO, respectively (p=0.177). In our study, cultures were positive in 55.9% of the eyes with dacryostenosis and in only 11.8% of the healthy eyes (p<0.001). The main reason for this difference is the fact that patients use more systemic and topical antibiotics depending on the mean patient complaint duration.

In their study, Owji and Khalili (21) compared samples obtained from the conjunctiva prior to DCR and from the lacrimal duct during the procedure and found that 90% yielded the same organisms. They isolated mostly gram-positive bacteria such as Staphylococci and Streptococci. In our study, MSCNS was the most commonly isolated organism in ocular surface and nasal cultures (45.8% and 45.1%, respectively). Gram-negative bacteria (*C. freundii*) and fungus (*C. krusei*) were isolated in one patient each. These rates may be due to the relatively small number of patients in our study group, and the geographical and cultural differences.

The study by Owji and Khalili (21) included 40 adult patients with NLDO, and the mean conjunctival flora normalization time after external DCR was 4.5 weeks (range, 3-8 weeks). They also reported negative cultures in only 67.5% of the patients in the study group at 4 weeks after the DCR procedure. Studies suggest a significant association between conjunctival flora normalization time and the species and colony numbers isolated. This relationship has not been reported for the duration of epiphora or number of past acute dacryocystitis episodes.

Eshraghi et al. (22) determined a mean conjunctival flora normalization time of 3.8 weeks (range, 1-7 weeks) and 2.6 weeks (1-5 weeks) in patients with and without preoperative purulent regurgitation, respectively. Silicone tube placement was needed in one-quarter of the DCR procedures, and the mean normalization times for the patients with and without silicone tubes were 6.2 and 3.9 weeks, respectively. They detected significant correlations between normalization time and high number of colonies in the preoperative culture, silicone tube placement, and purulent regurgitation. The authors indicated that 7 weeks is a safe interval between DCR and cataract surgery for patients with concurrent NLDO and cataracts. In their study that compared different surgical techniques, Balıkoğlu-Yılmaz et al. (18) found that the mean normalization time after external DCR was 1.47 weeks (range, 1-5 weeks) and recommended a safe interval of 5 weeks due to the risk of endophthalmia.

In this study, the postoperative normalization time for the conjunctival microbiota was 2 weeks (range, 2-5 weeks). Unlike other studies, our study showed no correlation between normalization time and factors such as age, sex, operative time, number of acute episodes, presence of purulent regurgitation, or microorganisms isolated and number of colonies in ocular surface and nasal cultures. Patients who required silicone tube placement due to insufficient anastomosis were excluded from the study.

On the basis of antibiogram results, Pinar-Sueiro et al. (23) detected penicillin resistance in 83.3% of 697 patients who underwent external DCR. The authors reported 100% sensitivity to gentamicin, co-trimoxazole, cefuroximaxetil, chloramphenicol, tetracycline, rifampicin, tobramycin, mupirocin, fusidic acid, and cephalotin. Pradeep et al. (24) isolated CNS in 85% of the samples they collected from lacrimal duct contents obtained during external DCR in 44 patients and reported vancomycin as the most effective antibiotic (100% sensitivity), followed by erythromycin and penicillin (75% and 72% sensitivity, respectively). They recommended third-generation cephalosporin and amoxicillin/clavulanic acid for the treatment of chronic dacryocystitis. In advanced cases, parenteral amikacin and vancomycin therapies are recommended.

In our study, the positive culture rate in the eyes treated with external DCR was 55.9%, with MSCNS isolated most frequently (45.8%). No pathological microorganism growth was detected in any of the operated and control eyes after DCR. Antibiotic susceptibility tests revealed 100% sensitivity to amikacin, cefepime, levofloxacin, ofloxacin, teicoplanin, and vancomycin, whereas the highest resistance rates were to penicillin and erythromycin (57.1% and 29.2%, respectively). The discrepancy between these rates and those reported in the literature reflect differences in laboratory techniques, symptom duration, and history of antibiotic use, and may be related to the number of acute dacryocystitis episodes.

The main limitations of our study were that we only studied aerobic bacteria and fungi, and our study sample was relatively small. In addition, we investigated viruses or conducted anaerobic cultures. Therefore, it must be kept in mind that anaerobic bacteria and/or viruses may have been etiological factors in patients with negative cultures. The strengths of our study were that for each patient, we performed culture antibiogram for both the ocular surface and nasal passage, obtained nasal mucosa samples, and conducted weekly follow-up of the healthy eyes.

Pathogenic microorganisms were isolated at a higher frequency from the ocular surface microbiota in the eyes with NLDO than from the healthy eyes. Conjunctival flora normalization was achieved at a mean of 2 weeks after external DCR. This safety period should be considered when scheduling intraocular surgeries to prevent endophthalmia.

Ethics Committee Approval: Ethics committee approval was received for this study from Alanya Alaaddin Keykubat University School of Medicine Local Ethics Committee (Approval No: 1-20/2018).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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