An Investigation Into the Conjunctival and Nasal Flora of Patients Receiving Cyclosporine Eye Drop Treatment for Dry Eye

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Research Article

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Abstract

OBJECTIVE

The aim of this study is to analyze the long-term use of cyclosporine drops of anti-inflammatory effect on conjunctival and nasal flora, which are commonly used in dry eye treatment, , to examine antibiotic resistance and to suggest some precautions.

METHODS

The 38 people using cyclosporine drops were classified as Group 1, and the 34 people using preservative-free artificial tear preparations were classified as Group 2. Swabs were taken from the conjunctiva and nasal cavities of the volunteers participating in the study and cultured. Groups 1 and 2 were compared in terms of growing microorganisms and antibiotic susceptibility.

RESULTS

There was no statistically significant difference between Groups 1 and 2 in terms of bacteria growing in the eye and nasal cavities. When it comes to the antibiotic susceptibility statistics for S. epidermidis, low sensitivity to penicillin was observed in particular in both groups, but there was no significant difference between the two groups (p=0.036)

CONCLUSION

The use of cyclosporine or preservative-free artificial tears did not make any difference to the flora or to the antibiotic susceptibility of the flora. It is thought that the use of cyclosporine drops does not predispose to ocular infection.

Introduction

Dry eye is a multifactorial disease that causes damage to the ocular surface through decreased visual quality, eye discomfort, and tear instability. It progresses with tear instability and inflammation of the ocular surface (1). After inflammation develops, inflammation gradually increases with cytokines produced by lymphocytes released from damaged epithelial cells and leaking from dilated conjunctival vessels.. Anatomical barriers, the secretion of mucus and antibacterial substances, and local humoral and cellular immune responses constitute the mixed defense mechanisms of the conjunctiva (2). In the treatment of dry eye, anti-inflammatory agents are preferred. Cyclosporine A is effectively and widely used in drop form due to its inhibitory effect on epithelial apoptosis and cytokine release from activated T cells (3). Having a knowledge of the normal flora of the conjunctiva is of great importance in terms of monitoring changes that may occur in this flora under certain influences and illuminating the pathological conditions that may develop. We know that the normal biological flora of the conjunctiva is formed from birth and that this flora is protective. The microbial flora of the ocular surface is made up of primarily gram-positive microorganisms consisting of Staphylococci and diphtheroids, and the most frequently isolated causative agent is coagulase-negative S. epidermidis (4). Although rare, the presence of gram-negative rods and fungi in the flora is accepted as a temporary ocular microbiota (5). The predominance of S. epidermis prevents the growth of pathogenic microorganisms and thereby protects the ocular surface. The nasal cavity and ocular surface are connected via the nasolacrimal duct for tear drainage. As a matter of fact, it has been proven that the nasal mucosa is exposed to drops applied to the ocular surface (6). Studies have shown that chronic use of some antibiotic drops, antiglaucoma agents, and lubricants containing antiseptic preservatives such as benzalkonium chloride (BAK) change the flora (7–9). It is thought that when these drops are used, the bacteria that cause endophthalmitis or blebitis, which can develop after intraocular surgery, may develop more resistant strains due to the change to the flora (8).

It is thought that when these drops are used, the bacteria that cause endophthalmitis or blebitis, which can develop after intraocular surgery, may develop more resistant strains due to the change to the flora (8). There is no study to date on changes to conjunctival and nasal mucosal flora after the use of cyclosporine, an anti-inflammatory agent used in the treatment of dry eye.

The aim of this study is therefore to analyze the effect of the long-term use of cyclosporine drops on conjunctival and nasal flora, to examine antibiotic resistance, and to suggest some precautions.

Materials And Method

Patients who presented to Medeniyet University's Göztepe Prof. Dr. Süleyman Yalçın City Hospital (Istanbul, Turkey) with a diagnosis of dry eye and used cyclosporine eye drops or artificial tear drops for at least six weeks were included in the study. Approval was obtained from the Institutional Review Board of the University of İstanbul Medeniyet. The decision number of this study is 2020/0085. The study was conducted in compliance with the Declaration of Helsinki. Informed consent was obtained from all study participants.Thirty-eight patients used cyclosporine eye drops for the treatment of dry eye, while the thirty-four patients in the control group used preservative-free tear substitutes. The following inclusion criteria were applied: follow-up with a diagnosis of dry eye, use of preservative-free artificial tears or cyclosporine eye drops for at least six weeks, no previous ocular or nasal surgery, an intact nasolacrimal duct, no bacterial or viral infection in the last six weeks, no allergic conjunctivitis or rhinitis, no chronic corneal or conjunctival disease, no systemic antibiotic use in the form of eye, nose, or ear drops in the last six weeks, no chronic or acute systemic disease, and no previous sinonasal infections. For each patient, one eye and the ipsilateral nasal cavity were included in the study.

After a full ophthalmological examination, a swab sample was taken from the lower conjunctiva using a sterile swab without touching the eyelashes and lids, and without applying local anesthesia in all cases. In addition, samples were taken from the nasal cavity by gently twisting a cotton-tipped applicator.

The samples were inoculated on blood, chocolate, and EMB (eosin methylene blue) media at the head of the patient. The media were delivered to the laboratory within one hour and incubated at 37°C for 24 hours.

The incubation of plaques without growth at 24 hours was extended to 48 hours. All colonies isolated from the media were identified using a VITEK® MS, MALDI-TOF (bioMérieux, France), and antibiotic susceptibility tests were performed using the VITEK® 2 Compact (bioMérieux, France) system in accordance with the manufacturer’s instructions.

Results

The 38 people using cyclosporine drops were classified as Group 1, and the 34 people using preservative-free artificial tear preparations were classified as Group 2. No statistically significant difference was found between Groups 1 and 2 when the bacteria growing in the conjunctiva and nasal cavity were compared. (Table 1)

Bacterial growth in the conjunctiva was observed in 54 (75%) of the 72 patients, while no growth was observed in 25% of patients. While 88.88% of the reproducing microorganisms were S. epidermidis, S. epidermidis growth was detected in 48 (66.66%) of the total patients. While there was no bacterial growth in the nose in two patients, bacterial growth was detected in the other 70 patients (97.22%). Overgrowth of S. epidermidis was detected in 54 (77.14%) of these 70 patients. This rate was found to be 75% across all patients. In general, reproduction was higher in the nose. S. epidermidis was the dominant pathogen in the eyes and nose.

Bacterial growth and antibiotic susceptibility were evaluated by taking separate samples from both the conjunctiva and nasal cavities of 72 patients. Apart from S. epidermidis, in the eye S. viridans was detected in eight patients (11.11%), methicillin-resistant S. aureus (MRSA) in two patients (2.77%), Corynebacterium in two patients (2.77%), and Staph. haemolyticus in two patients (2.77%), while susceptible S. aureus, E. coli, Klebsiella, Staph. hominis, and Proteus were not detected in any patients. In the nose, S. viridans was detected in six patients (8.33%), susceptible S. aureus in 10 patients (13.88%), MRSA in two patients (5.55%), Corynebacterium in 10 patients (13.88%), E. coli in two patients, Klebsiella in two patients (2.77%), Staph. hominis in two patients (2.77%), Proteus in two patients (2.77%), and Staph. haemolyticus in six patients (8.33%). It was observed that a higher rate and different types of pathogens were reproduced in the nasal cavity. There was no statistically significant difference between Groups 1 and 2 in terms of bacteria growing in the eye and nasal cavities (Table 1).

The same microbial agents were generally isolated in the nasal cavity and conjunctiva, and parallelism was observed.

When it comes to the antibiotic susceptibility statistics for S. epidermidis, low sensitivity to penicillin was observed in particular in both groups, but there was no significant difference between the two groups ($p = 0.036$). The strain was found to be most susceptible to trimethoprim-sulfomethoxazole (TMP-SMX) in both Group 1 and Group 2. It was also highly susceptible to gentamicin. Sensitivity to penicillin, cefoxitin, and erythromycin was low in both groups in the conjunctiva and nasal cavity (Table 2).

Discussion

Various studies have determined that the conjunctival flora can change in response to the age of a person, contact lens use, drug use, dry eye, immunosuppression, and environmental factors (4).

Conjunctival culture positivity ranges between 30.0–93.0% (4),(10). Staphylococcus epidermidis is known to be the most densely colonized bacterium in the conjunctival flora (11). In their study in the US, Singer et al. stated that S. epidermidis was the most common bacteria, making up 40% of growth in the conjunctival flora, while S. aureus could make up 3%. The same investigators stated that diphtheroid rods are the second most common bacteria after S. epidermidis, making up 25% of growth in the conjunctival flora (12). Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus spp., Moraxella spp., Neisseria spp., Bacillus spp., and gram-negative rods have been isolated, but this is usually temporary colonization (13). In our study, the conjunctival growth rate was found to be 75%. In addition, in accordance with the literature, the bacteria that reproduced in the conjunctiva was predominantly S. epidermidis (88.88%).

The type of medium used also plays an important role in the growth of microorganisms. Coşkun et al. used three types of media (blood agar, chocolate agar, EMB agar) and found a conjunctival growth rate of 93.6% (4). In this present study, all three types of media were used to achieve the highest growth rate. While the reproduction rate in the conjunctival samples analyzed was 75%, this was 97.22% in the nasal cavity samples. In a study showing that local anesthetic drops affect the flora during sample collection, the CNS growth rate was found to be 52.8% in samples taken before anesthesia, while this rate was found to be 33.3% in samples taken after local anesthetic drops were applied (14). In our study, anesthetic drops were avoided with the aim of increasing the reproduction rate.

In one study, the sensitivity rate of S. epidermidis to penicillin G was found to be 14% in normal conjunctival flora (4). In a recent large series study, CNSs in normal conjunctival flora were shown to be 90.8% resistant to penicillin, 84.4% resistant to erythromycin, and 6.9% resistant to gentamicin (15). In our samples, S. epidermidis had similar rates of resistance. Unlike previous studies evaluating normal conjunctival flora, the use of cyclosporine or preservative-free artificial tears did not make any difference to the flora or to the antibiotic susceptibility of the flora.

The ocular surface and nasal cavity are connected via the nasolacrimal canal. Ocular drops are known to reach the nasal mucosa and be absorbed from there (16). Forty percent of the standard 50-microliter eye drops reach the nasal mucosa through the nasolacrimal duct where they are then absorbed (6). The nasal mucosa acts as a reservoir of bacteria for the ocular surface. In one study, it was shown that mupirocin drops applied to the nasal cavity reduce the conjunctival flora (17). Benzalkonium chloride (BAK), which is frequently used as a preservative in eye drops, has been shown to have an antibacterial effect on the nasal mucosa (7). In another study worth noting, it was found that bacteria isolated in an endophthalmitis case series were genetically similar to bacteria taken from the nose (18). In our study, no difference was observed between the eyes and noses of the patients in terms of bacterial growth and antibiotic susceptibility.

Bacteria that make up the normal flora of the conjunctiva protect the eye from diseases by preventing the colonization of resistant and pathogenic species (19). In this phenomenon known as “competitive exclusion”, S. epidermidis, which makes up the majority of the normal flora of the conjunctiva, functions as a probiotic and provides protection against a more pathogenic species, S. aureus (20). Despite the protective effect of S. epidermis, it is the most frequently isolated opportunistic bacteria in ocular surface and intraocular infections. In addition, these resistant strains can develop resistance to antibiotics rapidly by making changes in their biofilms (21). In “The Antibiotic Resistance of Conjunctiva and Nasopharynx Evaluation” study (ARCANE), which is the most comprehensive study to have examined antibiotic resistance developing in the conjunctiva and nasal mucosa after repeated use of topical antibiotics, colonization of resistant strains was observed (16). In another study, it was found that resistant strains of S. aureus colonized rapidly after repeated use of macrolide and fluoroquinolone antibiotic eye drops (9). Many studies have shown that BAK, which has antiseptic properties and is used as a preservative in eye drops, causes changes in the conjunctival flora (16),(22).

These studies show the conditions caused by drugs that suppress the flora. However, as far as we know, no studies have investigated the effect of cyclosporine – an immunosuppressive drug – on flora. In this present study, culture results of samples obtained from the conjunctival and nasal mucosal swabs of a group using only artificial tears for the treatment of dry eye were compared with those of a group using cyclosporine drops.

It has been reported that at a dose of 0.05%, cyclosporine A is effective at suppressing ocular inflammation by blocking TH2 lymphocyte proliferation and interleukin 2 (IL-2) production (23). Cyclosporine A is effectively and widely used because of its inhibitory effect on epithelial apoptosis and cytokine release from activated T cells (24). In a study that observed changes to flora when cyclosporine is applied to human skin grafts, after six days of application S. aureus and coagulase-negative Streptococci were replaced by the more pathogenic organisms E. coli, E. faecium, Micrococcus, and Pseudomonas (25). Pathogenic microorganisms kept under control by the immune system may become dominant in the case of immunosuppression (25).

In this present study, the effect of cyclosporine on the flora as a result of its anti-inflammatory properties was investigated based on the changes that topical antibiotics and antiseptics had on the conjunctival and nasal flora. No such effect was seen in this study, which was conducted with the hypothesis that when inflammatory cells and mediators are suppressed on the conjunctival surface, they will increase the number of species of pathogens or help develop resistance to antibiotics. This situation can be explained by the fact that cyclosporine inhibits an excessive inflammatory response, does not affect the normal inflammatory response, and plays a stabilizing role in the protection of the normal flora.

In the nasal cavity, although the presence of different and more pathogenic microorganisms in Group 1 compared to Group 2 was worth noting, no significant difference was found. This can be interpreted as indicating that the use of cyclosporine does not create a tendency to infection. This result is especially important in terms of assessing whether patients who use cyclosporine for a long time and are set to undergo intraocular surgery are at increased risk of infection. However, studies with a larger patient series will provide more precise information on this subject and will form the basis for studies on the potential to reduce the prevalence of postoperative endophthalmitis.

The limitations of our study are that MIC data were not collected, meaning that the bacteria were classified only as “resistant” or “susceptible”.

In conclusion, according to the findings obtained in this study, cyclosporine drops with anti-inflammatory properties do not have any effect on conjunctival and nasal flora and do not affect antibiotic resistance. Larger studies are needed for more precise results.

Declarations

Declaration of conflicting interests: The Author(s) declare(s) that there is no conflict of interest

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Tables

Table 1: Comparison of the groups in terms of bacteria growing in the conjunctiva and nasal cavity

Conjunctiva	Group 1	Group 2	p
s.epidermidis	%73,6	%58,82	0,139
s.viridans	%15,78	%5,88	0,169
MRSA	%5,26	%0	0,275
corynebacterium	%0	%5,88	0,219
staf. heamaliticus	%0	%5,88	0,219

Nasal cavity	Group 1	Group 2	p
s.epidermidis	%68,42	%82,35	0,138
s.viridans	%10,52	%5,88	0,392
s.aerus	%15,78	%11,76	0,442
MRSA	%5,26	%5,88	0,649
corynebacterium	%21,06	%5,88	0,062
e.coli	%5,26	%0	0,275
klebsiella	%5,26	%0	0,275
staf.hominis	%5,26	%0	0,275
proteus	%0	%5,88	0,219
citrobacter	%5,26	%0	0,275

Table 2: Comparison of the groups in terms of S.epidermidis’ antibiotic susceptibility

Antibiotic susceptibility (conjunctiva)	Group 1	Group 2	p
penisilin	%29,41	%0	0,036
sefoksitin	%58,82	%57,14	0,606
eritromisin	%47,05	%35,71	0,394
gentamisin	%76,47	%92,85	0,233
TMP-SMX	%94,11	%92,85	0,708

Antibiotic susceptibility (nasal cavity)	Group 1	Group 2	p
penisilin	%25	%14,28	0,342
sefoksitin	%64,7	%85,71	0,129
eritromisin	%41,17	%47,61	0,473
gentamisin	%81,25	%100	0,072
TMP-SMX	%100	%95	0,541