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Comparative Lid Flora in Anterior Blepharitis

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Abstract: The microbiologic lid flora evaluations of 117 patients with anterior blepharitis were compared with those of 52 age- and sex-matched healthy controls. The sampled bacterial cultures were identified according to standard conventional techniques and fatty acid profiles by the Microbial Identification System (MIS). Chi-square and difference between two percentage Z tests were used for the statistical analyses. The most commonly isolated organisms were *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Propionibacterium acnes* and mixed skin flora (diphtheroid rods, *Neisseria* spp. and *Streptococcus viridans*). The variability of aerobic bacteria between the patients and the control group was found to be statistically significant ($P<0.001$). *Prevotella* spp., *Escherichia. coli*, *Proteus mirabilis*, *Neisseria sicca*, *Enterobacter aerogenes*, *Pseudomonas auriginosa*, *Fusobacterium* spp., *Bacteroides* spp., *Staphylococcus lugdunensis*, *Neisseria elongata* and *Corynebacterium diphtheriae* were found in decreasing order. *P. acnes*, not found in the control group, was the most commonly isolated anaerobic bacteria ($P<0.05$). The *Corynebacterium* spp., *Acinetobacter*, and *Moraxella* spp. reported in the literature were not observed in our samples. The differences between the studies may be due to the nature of the endemic flora and to the number of patients studied. Patients with anterior blepharitis are more likely to have normal skin flora on their lids and in greater quantity than the controls. Therefore, the presence of these species may contribute to the occurrence of blepharitis.

Key Words: Blepharitis, lid flora.

Introduction

Blepharitis is the general term used to refer to inflammations involving the eyelids and particularly to those involving the lid margins. They are typically chronic and bilateral (1). Chronic blepharitis is a very common and often difficult problem for the general ophthalmologist as well as for the patient.

There are different classification modalities of chronic blepharitis which are helpful in approaching therapy. One classification approach divides inflammation into anterior (staphylococcal) and posterior (meibomian gland dysfunction) lid disease, distinguishing the disease involving the anterior lid margin and lashes from that involving the meibomius glands (2). These forms have similar symptoms including chronic irritation, a burning sensation, mild redness, and occasional itching of the lid margins. Another approach to classification is reported by McCulley et al., who categorized chronic blepharitis into staphylococcal, seborrheic alone, mixed seborrheic/staphylococcal, seborrheic with secondary meibomianitis, seborrheic with meibomian seborrhea, and primary meibomianitis and others as atopic, psoriatic and fungal (3).

The eyelids display signs of inflammation quite readily because of the overlying thin skin and a subcutaneous layer composed of loose strands of connective tissue without fat. Bacteria are the principle pathogens responsible for chronic blepharitis, with *Staphylococcus* spp. being the most common cause (4). Other bacteria less frequently implicated in anterior blepharitis include *Pseudomonas* spp. (5), *Proteus mirabilis* (6) and *Capnocytophaga ochracea* (7).

Serious ocular complications may result from microbial infections of the ocular adnexa. Prevention of complications requires early recognition of distinctive clinical features and prompt microbiological investigation. We carried out the present study to identify lid flora in cases of anterior blepharitis and to compare the results with those from an age- and sex-matched population of normal individuals free of any ocular or lid diseases.

Materials and Methods

Selection of Patients

Between October 1999 and May 2000, 117 patients with chronic blepharitis who had been admitted to the Department of Ophthalmology at Atatürk University, School of Medicine and 52 healthy sex- and age-matched controls were included in the study. After complete ophthalmologic examinations, the patients were diagnosed with anterior blepharitis according to relatively inflamed lids, with crusting of mixed characteristics, and with significant exacerbations (2). They had had symptoms for at least three months and had received no therapy for at least one month. The patients with posterior blepharitis characterized by inflammations centered around all the meibomian glands with concomittant plugging were excluded.

Bacteriologic Evaluation

Both eyes of 52 healthy controls and 117 patients with anterior blepharitis were sampled by bacterial culture. One side of sterile, double cotton-tipped applicators moistened with brain heart infusion broth (BHI) was used for aerobic cultures and the other side for anaerobic cultures. The applicator was rubbed twice along the lid margin from punctum to lateral canthus. Aerobic and anaerobic cultures were inoculated to sterile BHI and chopped meat broths respectively. The swabs rolled onto BHI broth were incubated at 35ºC for three hours and then subcultured onto 5% sheep blood agar, chocolate agar and eosine methylene blue plates. Aerobic cultures were incubated at 35ºC in 5-10% CO_2, 5% sheep blood agar and eosine methylene blue plates. Anaerobic cultures were examined after 7 days of incubation in chopped meat broth and then each swab was also rolled onto another brucella blood agar plate enriched with hemin, vitamin K and yeast extract (Baltimore Biological Laboratories, BBL) and BHIBLA medium (REMEL # 01-158). Then anaerobic cultures were placed into GasPek Jars (AnaeroGen-Oxoid) for 48 hours. The bacteria in plates with bacterial growth were identified according to the standard conventional techniques and bacterial fatty acid profiles.

Saponification, methylation and extraction techniques were applied to the bacterial colonies in the sheep blood agar, chocolate agar, eosine methylene blue and BHIBLA plates for bacterial fatty acid identification. The results were evaluated by the Microbial Identification System (MIS)(8,9).

Statistical Analysis

Chi-square and difference between two percentages Z-tests were used.

Results

Fifty male and sixty-seven female (total 117) patients with ages ranging from 8 to 68 years (mean 27±13.50) were investigated. The results were compared with the control group including 25 male, 27 female (total 52) healthy individuals with ages ranging from 9 to 61 years (mean 28.80±13.91).

A general evaluation of bacteriological results sampled from patients with anterior blepharitis according to the presence of bacterial growth and their variability is given in Table 1. There was no growth in 4 (3.4%) samples. Pure and mixed aerobic bacterial growth was commonly observed in the cultures of 55 (47%) and 19 (16%) samples respectively. The combined presence of multiple organisms including aerobic and anaerobic bacterial species occurred in 16 (13.7%) samples.

Table 1. A general evaluation of bacteriological findings in patients with anterior blepharitis.

Total number of samples	117
No growth	4 (3.4%)
Pure aerobic growth	55 (47%)
Mixed aerobic growth	19 (16%)
Mixed aerobic and anaerobic growth	16 (13.7%)
Pure anaerobic growth	20 (17%)
Mixed anaerobic growth	3 (2.6%)

Table 2 lists the frequency of the most commonly isolated organisms in the control group. Only 15.4% of

Table 2. The evaluation of bacteriological findings in the healthy control group.

Total number of samples	52
No growth	8 (15.4%)
S. aureus	3 (5.8%)
S. epidermidis	6 (11.5%)
S. auerus + *S. epidermidis*	11 (21.2%)
S. epidermidis + diphteroid rods	7 (13.5%)
Mixed skin flora (diphtheroid rods, *Neisseria* spp., *Strep. viridans*)	17 (32.7%)
Mixed skin flora + *P. acnes*	6 (11.5%)*

* Mixed skin flora includes this value

all lid cultures in healthy persons were sterile. Mixed skin flora was found to be 32.7%. However, 35.29% of these cultures included *P. acnes*.

The aerobic bacteria isolated from the patients with anterior blepharitis were identified at species level. These species compared with the control group are listed in Table 3. The most commonly isolated aerobic organisms from lids with anterior blepharitis were *Staphylococcus epidermidis* 19.7%, *Staphylococouc aureus* 11%, mixed skin flora (diphtheroid rods, *Neisseria* spp., *Streptococcus viridans*) 9.4%, *S. aureus* with *S. epidermidis* 4.3%, *S. epidermidis* with diphtheroid rods 2.6%. The same organisms isolated from the healthy control group were: 11.5%, 5.8%, 32.7%, 21.2% and 13.5% respectively. Other aerobic organisms isolated relatively infrequently from anterior blepharitis included *Staphylococcus lugdunensis*, *Streptococcus simulans*, *Staphylococcus bovis*, *Neisseria sicca*, *Neisseria elongata*, *Corynebacterium diphtheriae*, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas auriginosa* and *Proteus mirabilis*. These species were not seen in the cultures of healthy persons' lids. In statistical analysis, except for the infrequent organisms listed above, there was a statistically significant difference between the results of the anterior blepharitis group and the control group (Chi-square=34.26, P< 0.001).

Table 3. Aerobic bacteria isolated from the patients with anterior blepharitis and the healthy control group.

Bacteria	Blepharitis		Control	
	Number	%	Number	%
S. aureus	13	11	3	5.8
S. epidermidis	23	19.7	6	11.5
S .lugdunensis	1	0.9	-	-
S. simulans	3	2.6	-	-
S. bovis	1	0.9	-	-
N. sicca	2	1.7	-	-
N. elongata	1	0.9	-	-
C. diphteriae	1	0.9	-	-
E. coli	3	2.6	-	-
E. aerogenes	2	1.7	-	-
P. auriginosa	2	1.7	-	-
P. mirabilis	3	2.6	-	-
S. epidermidis + diphteroid rods	3	2.6	7	13.5
S. aureus + *S. epidermidis*	5	4.3	11	21.2
Mixed skin flora (diphtheroid rods, *Neisseria* spp., *Strep. viridans*)	11	9.4	17	32.7
Total	74	63	44	84.6

The anaerobic bacteria isolated from the lids with anterior blepharitis are listed in Table 4. The most common anaerobic bacteria was *Propionibacterium acnes*, 10.3%. It was also found statistically significant that *P. acnes* was the most commonly isolated anaerobic bacteria (P< 0.05; Z-test). The listed bacteria were not found in the control group.

Table 4. Anaerobic bacteria isolated from the patients with anterior blepharitis.

Bacteria	Number	%
P. acnes	12	10.3
Prevotella spp.	4	3.4
Fusobacterium spp.	2	1.7
Bacteroides spp.	2	1.7
P. acnes + *Bacteroides* spp.	2	1.7
Prevotella spp. + *Bacteroides* spp.	1	0.9
Total	23	19.7

The combined aerobic and anaerobic bacteria isolated from the patients with anterior blepharitis compared with the control group are shown in Table 5. As expected, mixed skin flora with *P. acnes* was seen in both of the groups. The other identified bacteria were not found in the controls.

Discussion

Colonization of the eyelids and the conjunctiva by normal microbial flora contributes to the defense against ocular disease. Indigenous bacterial flora inhibit the

Table 5. Combined aerobic and anaerobic bacteria isolated from the patients with anterior blepharitis and the healthy control group.

Bacteria	Blepharitis		Control	
	Number	%	Number	%
Mixed skin flora + *P. acnes*	6	5.1	6	11.5
S. epidermidis + *Prevotella* spp.	4	3.4	-	-
S. aureus + *Bacteroides* spp.	3	2.6	-	-
S. epidermidis + *Bacteroides* spp.	2	1.7	-	-
S. aureus + *Prevotella* spp.	1	3.4	-	-
Total	16	13.7	6	11.5

establishment for foreign, potentially pathogenic bacteria by elaborating antibacterial substances and by competing for space and nutrients. The predominant isolates recovered from the eyelids and conjunctiva are *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Corynebacterium* spp. and *Propionibacterium acnes*. The topical use of antimicrobials, local immunosuppression caused by topical corticosteroids, or the presence of ocular surface diseases as such blepharitis can alter the normal resident flora and promote the growth of increased pathogenicity (10).

In our clinically isolated samples from the healthy control group, normal lid flora consisted of *S. aureus*, *S. epidermidis*, diphtheroid rods and mixed skin flora (*Neisseria* spp., diphtheroid rods and *Streptococcus viridans*) with *P. acnes* (Tables 3,5). Dougerty and McCulley compared the microbiological findings of conjunctiva and the lids, claiming that the flora of conjunctiva was relatively similar to that of the lids (11). Manav et al. reported that *S. epidermidis*, diphtheroid rods, *Streptococcus* spp., and *Hemophylus* were the most commonly isolated organisms of normal conjunctival flora and these flora can change according to the environment (12).

Many investigators have studied the role of lid flora in blepharitis for years. In early studies, topical application of *S. aureus* filtrate caused conjunctivitis in rabbits and human volunteers (13,14). In the following years there were similar findings with subconjunctival injections in rabbits (15). Recently it was found that *Staphylococcus* keratitis in the rabbit causes acute inflammation in the overlying eyelid (16). *S. epidermidis* injections also resulted in conjunctivitis but a more severe reaction was found when the filtrates of epidermidis isolated from lids with blepharitis were injected (17). In further studies it was concluded that *S. epidermidis* may play a role in chronic blepharitis. Because of the common pathway of lipolytic exoenzyme production, *S. epidermidis*, Corynebacteria and *P. acnes* may be involved in chronic blepharitis (18).

The most frequently isolated bacteria in this study are compared with those in the literature in Table 6. As in the series of Dougerty, McCulley and Groden et al., *S. epidermidis*, *P. acnes*, *S. aureus* and *Corynebacterium* spp. were the organisms isolated most commonly (11,18). Acinetobacter was found in the study of Groden et al. but was not reported in other studies (18). Seal et al. also found *S. epidermidis* as the most frequent organism; in addition, mixed skin flora and *Moraxella* spp. were listed (19). Kuğu et al. and Karakaş et al. reported the most common bacteria to be *S. epidermidis* and *S. aureus* (20,21). In our study, *S. epidermidis*, *S. aureus*, *P. acnes*, mixed skin flora (diphtheroid rods, *Neisseria* spp. and *Streptococcus viridans*), *Prevotella* spp., *E. coli, P. mirabilis*, *N. sicca*, *E. aerogenes, P. auriginosa, Fusobacterium* spp., *Bacteroides* spp., *S. lugdunensis, N. elongata* and *C. diphtheriae* were found in decreasing order. *Corynebacterium* spp., *Acinetobacter* and *Moraxella* spp., were not observed in our samples. The differences between the studies may be due to the nature of the endemic flora and to the number of patients studied.

There has been much confusion about the etiology of chronic blepharitis (11). Therefore, the probable biochemical, microbiological and histopathological bases of blepharitis still need to be investigated.

Table 6. The most frequently isolated bacteria compared with other studies.

	Groden et al. (18)	Dougerty, McCulley (11)	Seal et al. (19)	Kuğu et al. (20)	Karakaş et al. (21)	This study
No growth	*	2%	6%	35%	*	3.4%
S. epidermidis	95.8%	95.55%	74%	20%	57.89%	26.6%**
S. aureus	10.5%	37.77%	8%	36.25%	42.10%	15.3%**
P. acnes	92.8%	95.55%	*	-	-	10.35%
Mixed skin flora	*	*	13%	-	-	9.4%
Corynebacterium spp.	77.3%	31.11%	*	-	-	-
Acinetobacter spp.	11.4%	*	*	-	-	-
Moraxella spp.	*	*	2%	-	-	-

*no data shown

**pure combined with mixed culture

In this study, clinical isolates were identified by standard clinical microbiological practices and by MIS. The first technique included growth and colony morphology on different media. In the second technique, the variations in the fatty acids present in bacterial cells were used for bacterial classification and identification of strains. In addition to classical identification methods based mainly on demonstrations of differences in the metabolic pathways of different species, methods have been developed which identify bacteria according to chemical compounds present in the bacterial cells. The main advantage of these methods is increased speed of analysis, since there is no need to cultivate the bacteria that are no longer viable. In this respect, fatty acid analysis by MIS seems to be one of the most promising methods, in particular to study clinical bacteria (8,9).

There is not sufficient scientific data supporting a pathogenic role of any single organism in all types of blepharitis. Much emphasis has been focused on the role of *S. aureus* and *S. epidermidis* as etiological agents. Although it appears that *P. acnes* represents one of the main components of normal skin and lid flora, the role of this organism in chronic blepharitis has been thought to play an adjuvant role and promote hypersensitivity to *S. epidermidis* (11,18).

In this study we concluded that *S. epidermidis*, *S. aureus*, mixed skin flora and *P. acnes* were significantly greater in blepharitis patients. Patients with blepharitis are more likely to have normal skin flora on their lids and in greater quantity than the healthy controls. We suggest that these bacteria may contribute to the occurrence of blepharitis.

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