• Clinical Research •

Evaluation of conjunctival flora in congenital ptosis

Mehmet Balbaba[1], Fatih Ulaş[2], Mehmet Canleblebici[3], Ozan Güven[1], Zülal Aşcı Toraman[4], Hakan Yıldırım[1], Murat Erdağ[1]

[1]Department of Ophthalmology, Faculty of Medicine, Fırat University, Elazığ 23119, Türkiye
[2]Department of Ophthalmology, Faculty of Medicine, Abant Izzet Baysal University, Bolu 14030, Türkiye
[3]Department of Ophthalmology, Kayseri State Hospital, Kayseri 38080, Türkiye
[4]Fırat University, Faculty of Medicine, Department of Microbiology, Elazığ 23119, Türkiye
Correspondence to: Mehmet Canleblebici. Department of Ophthalmology, Kayseri State Hospital, Kayseri 38080, Türkiye. mehmetcl@hotmail.com
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Abstract

● **AIM:** To assess and compare the conjunctival bacterial flora in patients with congenital ptosis (CP) to that of healthy individuals.

● **METHODS:** The study included 38 patients with CP and 42 healthy control subjects. Conjunctival cultures were collected using a cotton-tipped swab applied to the inferior conjunctival fornix. The samples were inoculated into blood agar, eosin methylene blue agar, chocolate agar for bacteria, and Sabouraud dextrose agar medium for fungi.

● **RESULTS:** The culture positivity rate was 68.4% in CP and 47.6% in the control group (P<0.001). Additionally, there was a significant difference in culture positivity between the eyes of patients with unilateral ptosis (P=0.039). In the CP group, the most common found microorganism was *Staphylococcus epidermidis* at 23.67%, followed by *Haemophilus species* at 21.04%, and *Corynebacterium species* at 15.78%. In the control group, *Staphylococcus epidermidis* and *Corynebacterium species* were both the most commonly isolated microorganisms, each accounting for 19.04%. More than one species of bacteria was grown in the cultures of 36.84% and 23.80% of the CP and control subjects, respectively. Gram-negative bacteria were more common in CP than in control subjects (P=0.031).

● **CONCLUSION:** Culture positivity is significantly higher in the ptotic eyes of CP. Potentially pathogenic microorganisms are more frequently isolated from the CP group compared with the healthy control group.

● **KEYWORDS:** congenital ptosis; conjunctival flora; bacteria
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INTRODUCTION

The conjunctival flora is acquired at birth and persists throughout an individual's lifetime[1]. The composition of conjunctival flora can vary based on several factors, including environment, age, immune status, presence of ocular surface diseases, systemic diseases, climate, geographical region, and overall hygiene conditions[2-4]. The normal bacterial flora of the conjunctiva is similar to that of the skin, primarily composed of organisms such as *Staphylococcus epidermidis, diphtheroids, Streptococcus species,* and *Haemophilus species*. It has also been reported that the isolation rates of different bacterial species can vary across different age groups[5]. Anaerobic bacteria and fungal species are also part of the normal conjunctival flora, though they are less commonly found compared to aerobic bacteria.

It is known that bacterial species in the conjunctival flora cause postoperative infectious endophthalmitis. Molecular analysis studies have demonstrated a close relationship between the bacteria found on the ocular surface and those that cause serious ocular infections[6]. Therefore, knowing the ocular surface flora is critical to prevent resistant ocular infections. In previous studies, conjunctival flora was evaluated in many diseases, such as Behçet's disease, Parkinson's disease (PD) and diabetes mellitus (DM) and significant changes were detected when compared with healthy control group[7-9].

In this study, we aimed to evaluate and compare the conjunctival bacterial flora in patients with congenital ptosis (CP) to that of healthy individuals, marking the first investigation of its kind in the literature, to our knowledge. In addition, we investigated whether there was a relationship between levator function (LF) and conjunctival flora in CP.

PARTICIPANTS AND METHODS

Ethical Approval The study was conducted in compliance with the principles of the Declaration of Helsinki, and all of the procedures were approved by the Ethics Committee of Firat University Faculty of Medicine (Approval No: 2024/0-11). Informed consent was taken from all participants.

The study included 38 patients with CP, who were either under follow-up or recently diagnosed at the Ophthalmology Department of Firat University Faculty of Medicine, along with 42 healthy control subjects matched by sex and age. Subjects aging between 12-27y were included in the study to alleviate the effect of age on conjunctival flora. Patients who received systemic antibiotic and corticosteroid treatments or used topical ocular medications within the last 8wk, and patients with systemic disease (DM, hyperthyroidism, renal failure), adnexal or systemic infection, blepharitis, conjunctivitis, chronic dacryocystitis, dry eye, contact lens use, and previous ocular surgery were excluded from the study.

After a medical history was obtained, a detailed ophthalmologic examination, including best corrected visual acuity, slit-lamp biomicroscopy, and binocular indirect ophthalmoscopy was performed. In patients with CP lid crease, LF, palpebral fissure height, and margin reflex distance (MRD) were evaluated. Patients with severe CP (MRD 0-2 mm) were included in the study. LF was evaluated as poor (0-4 mm), moderate (5-11 mm) and good (12 mm and above).

For conjunctival culture sampling, a cotton-tipped sterile dry swab was applied to the fornix of the inferior conjunctiva. Anesthetic drops were not applied during the sampling process, as they can inhibit the growth of some bacteria[10-11]. The samples were immediately inoculated into culture media. Aerobic cultures were initially placed in brain heart infusion liquid medium. Samples that showed growth were then transferred to eosin methylene blue agar, 5% sheep blood agar, and chocolate agar solid media. These aerobic cultures were incubated at 37℃ for 24 to 48h. For fungal isolation, Sabouraud dextrose agar medium was used, with samples incubated at 26℃ and 37℃ for a duration of 1 to 3wk. The identification of culture-positive microorganisms was performed using standard microbiological techniques.

Statistical Analysis The data was conducted using SPSS software (Version 29, SPSS Inc., Chicago, Illinois, USA). Parameters were reported as mean, standard deviation or percentage, as appropriate. Parametric data were analyzed with the independent-samples *t*-test, while nonparametric data were analyzed using the Chi-square test. Pearson correlation or point biserial correlation analysis was applied to quantify correlations between parameters. The threshold for statistical significance was set at $P<0.05$.

RESULTS

The current study included 38 CP [20 (52.63%) males and 18 (47.36%) females] and 42 healthy control subjects [24 (57.14%) males and 18 (42.85%) females]. The average age was 20.74±5.89y (range 12-27y) in the CP group, and 21.67±5.62y (range 12-27y) in the control group. There were no significant differences in demographic characteristics between the groups, with a *P*-value of 0.766. In the CP group, 26 (68.38%) had unilateral ptosis and 12 (31.56%) had bilateral ptosis. LF was measured as good in 10 (26.30%), moderate in 20 (52.6%), and poor in 8 (21.04%) patients. In the control group, LF was measured as good in 30 (71.40%) and moderate in 12 (28.56%) subjects.

The study found significant differences in the culture positivity rates between the CP and the control groups, with rates of 68.4% and 47.6% respectively ($P<0.001$). Among unilateral ptosis patients, the culture positivity rates were 46.15% for the non-ptotic eye and 84.62% for the ptotic eye, showing a significant difference ($P=0.039$). In the CP group, the most frequently isolated microorganism was *Staphylococcus epidermidis* (23.67%), followed by *Haemophilus species* (21.04%), and *Corynebacterium species* (15.78%). In the control group, *Staphylococcus epidermidis* and *Corynebacterium species* were both the most commonly isolated microorganisms, each accounting for 19.04%. For the fungal growth only a patient in the CP group had *Candida albicans* (2.63%). The colonization rates for *Streptococcus pneumoniae, Staphylococcus aureus, Moraxella catarrhalis,* and notably *Haemophilus species* were higher in the CP group compared to the control group. Table 1 displays the isolates from the conjunctival flora of both CP and control subjects. Additionally, more than one species of bacteria was grown in the cultures of 14 (36.84%) CP, and 10 (23.80%) control subjects; however, this difference was not statistically significant ($P=0.361$). Gram-negative bacteria were grown in the cultures of 10 (26.30%) and 3 (7.14%) of the CP and control subjects, respectively. Gram-negative bacteria were more common in CP than in control subjects ($P=0.031$). As expected, due to sampling of cases, there was a very weak, non-significant correlation between culture positivity and age/ptosis duration ($r=0.133$, $P=0.415$). There was a very weak, non-significant correlation between culture positivity and LF ($r=-0.131$, $P=0.421$). There was a moderate significant correlation between Gram-negative staining and age/ptosis duration ($r=0.325$, $P=0.040$) and a fair non-significant correlation between Gram-negative staining and age/ptosis duration ($r=0.290$, $P=0.069$).

DISCUSSION

The eyelid and conjunctival flora serve as protective mechanisms, helping to prevent the colonization of possible pathogenic microorganisms. While the conjunctiva may be sterile in some individuals, over time, a varied flora develops that is influenced by factors such as environment, aging, season, immune resistance, and overall hygiene conditions.

Table 1 Growth in conjunctival cultures from congenital ptosis, and healthy control group *n* (%)

Bacteria	Congenital ptosis group	Control group	Blood agar medium	EMB agar medium	Chocolate agar medium	SDA medium
Gram-positive						
Staphylococcus epidermidis	9 (23.67)	8 (19.04)	17	-	17	-
Corynebacterium species	6 (15.78)	8 (19.04)	14	-	14	-
Streptococcus pneumoniae	5 (13.15)	3 (7.14)	8	-	8	-
Staphylococcus aureus	4 (10.52)	2 (4.76)	6	-	6	-
α-hemolytic Streptococcus	-	1 (2.38)	1	-	1	-
Gram-negative						
Haemophilus species	8 (21.04)	1 (2.38)	9	-	9	-
Bacillus species	4 (10.52)	3 (7.14)	7	7	7	-
Moraxella catarrhalis	2 (5.26)	-	2	-	2	-
Neisseria species	2 (5.26)	2 (4.76)	4	-	4	-
Fungi						
Candida albicans	1 (2.63)	-	1	1	1	1

Blood agar medium: 5% sheep with blood; EMB medium: Eosin methylene blue medium; Chocolate agar medium: 5% sheep with blood; SDA medium: Sabouraud dextrose agar medium.

In a previous study, Sakisaka *et al*[12] evaluated the changes in the conjunctival flora with age and found that the number of isolated bacteria increased. In addition, they reported that *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, and *Corynebacterium species* were more common with increasing age. It is hypothesized that the increased diversity of species in the conjunctival flora may be associated with higher expression of Toll-like receptor 2 in the corneal epithelial cells of older patients. This receptor plays a crucial role in recognizing bacterial components, suggesting that aging might enhance the eye's immune responsiveness to microbial presence[13]. Increased comorbidities and dry eye conditions also influence the conjunctival flora in elderly patients[14]. In our study, we analyzed similar age groups in the CP and healthy control subjects.

The bacterial composition of the conjunctival flora resembles that of the skin and nasal flora. While the microbial community on the ocular surface predominantly consists of gram-positive organisms, such as *Staphylococci* and *Diphtheroids*, the rate of positive cultures and the variety of microorganisms that are cultured can vary significantly[15]. *Staphylococcus aureus*, a potentially pathogenic bacterium, is frequently isolated from ocular infections and can also be found in the normal conjunctival flora, with isolation rates ranging from 3% to 15%[16]. In the current study, *Staphylococcus aureus* was isolated in 10.52% of the CP group and 4.76% of the control group. Additionally, according to Watanabe *et al*[17], bacterial growth was observed in 87.2% of conjunctival cultures from patients who did not exhibit signs of ocular infection, 30.8% by Ugimori *et al*[18], and 56% by Kato and Hayasaka[19]; and the most frequent organism was *Staphylococcus epidermidis*. In this study, bacterial growth was observed in 47.6% of the healthy control group, and the most commonly isolated microorganism was *Staphylococcus epidermidis* in both the CP and control groups, accounting for 23.67% and 19.04% of isolates, respectively. This finding is consistent with the typical prevalence of *Staphylococcus epidermidis* as a common member of the conjunctival flora.

In previous studies, conjunctival flora was evaluated in many diseases. Gunduz *et al*[7] evaluated the conjunctival flora of Behçet's disease patients and found that *Staphylococcus aureus*, *Streptococcus species*, and *Moraxella species* colonization were higher than the normal conjunctival flora. In another study[8], the conjunctival flora of patients with DM was compared with the normal healthy control group. In the study, while there was no significant difference in the overall frequency of bacterial growth between the diabetic group and the normal group, the diabetic group did yield a higher proportion of gram-negative bacterial cultures. This suggests a variation in the type of bacteria prevalent among diabetics, despite similar growth rates. Kusbeci *et al*[9] evaluated the conjunctival flora of PD patients and found that *coagulase-negative Staphylococcus, Staphylococcus aureus*, and *Corynebacterium species* were the most frequently found bacterial species. In the study, *Staphylococcus aureus* was the only bacterium found to be significantly more prevalent in the conjunctival flora of patients with PD compared to the control group. Additionally, the occurrence of cultures with two or more bacterial species was higher in the PD group than in those in the healthy group, indicating a more diverse bacterial profile in this patient population. They speculated that reduced blinking rate and immunological changes in eye tears in PD patients affect the conjunctival flora[20-23]. To our knowledge, and literature search, this is the first study in the literature that evaluated the conjunctival bacterial flora of patients with CP and compared it to that of healthy subjects. Our findings revealed that the rates of culture positivity, presence of gram-negative bacteria, and growth of more than one bacterial species were significantly higher in the conjunctival flora of CP.

In this study, it was determined that culture positivity was significantly higher in ptotic eyes of the CP when compared with control subjects and non-ptotic eyes of unilateral ptosis patients. In addition, potentially pathogenic *Streptococcus pneumoniae, Moraxella catarrhalis, Staphylococcus aureus,* and especially *Hemophilus species* were more commonly isolated from ptotic eyes of the CP. A higher incidence of potentially pathogenic microorganisms in the CP group might cause morbidity. It is difficult to explain the difference in culture results of healthy controls and the CP group due to scarce literature about the conjunctival flora of ptosis patients, and this might limit appropriate measures against occasional morbidities. Variation of culture positivity and isolated microorganisms in different age groups, geoographical and climatic conditions might limit evaluation of such studies. However, we included a group of subjects with similar ages, geoographical and climatic conditions, which might further enlighten the evaluation of the results of the current study. Therefore, we could speculate that the difference in culture results of the healthy controls and the CP could be due to narrowed palpebral fissure and decreased LF, which might limit maintenance of local homeostasis and well-being, such as conservation of conjunctival flora and limiting inflammation.

Our study has a few limitations. We could evaluate only patients with CP within a limited age range. Additionally, this is not a multi-center study, which limits the generalizability of the findings. The small sample size and the geographic restriction to a specific region also constrain the broader applicability of our results. Bacterial growth is revealed in approximately 50% of cases in traditional ocular surface culture, as in the current study, and often fails to demonstrate a complex network of bacterial species[24]. Additionally, we did not evaluate the anaerobic bacterial flora and did not perform antibiogram sensitivity testing.

In conclusion, the isolation of potentially pathogenic microorganisms was higher in the conjunctival flora of the patients with CP compared to the control group. This suggests that CP may have an altered conjunctival flora that could influence their susceptibility to ocular infections. There is a need for further studies that require evaluation of cases with other types of ptosis, like aponeurotic ptosis, which is more common in older patients where intraocular surgery is required more frequently, especially using techniques that reveal the culturable microbiome of the conjunctiva.

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Availability of Data and Materials: The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

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