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RESEARCH ARTICLE

Floral Differences of Conjunctiva in Patients with Renal Diseases

Lokman Aslan[1], Sumeyra Kocturk[2], Mustafa Gül[2], Murat Aslankurt[1], Orcun Altunören[3]

[1]*Department of Ophthalmology, KSU, School of Medicine, Kahramanmaras, Turkey*
[2]*Department of Microbiology, KSU, School of Medicine, Kahramanmaras, Turkey*
[3]*Department of Internal Medicine, Nephrology Unit, KSU, School of Medicine, Kahramanmaras, Turkey*

ABSTRACT

Objectives: This study aimed to evaluate whether conjunctival flora is different in patients with chronic renal disease (CR) among its subgroups and healthy subjects.

Methods: A total of 105 adult CR patients; 35 hemodialysis (HD), 35 diabetic hemodialysis (DHD), 35 renal transplantation (RT), and 40 healthy subjects were enrolled. After ophthalmologic examination, a swab was taken from the lower fornix of the right eye using a cotton-tipped applicator and directly sub-cultured on 5% sheep's blood agar, chocolate agar, and eosin methylene blue agar. Differences in both bacterial growth and flora diversity of the groups were compared.

Results: The order of bacterial growth rates in the groups are RT 65%), DHD (57%), HD (56%), and control (48%). The RT group had a statistically significant difference from the control group ($p<0.05$), but there is no difference among other groups. There is also no correlation between the duration of diabetes, hemodialysis, or transplantation with bacterial growth. The most frequently isolated bacteria are *Staphylococcus hominis* (23%) and *Kocuria rosea* (11%) in HD; *Staphylococcus epidermidis* (20 %) and *S. hominis* (15%) in DHD; *K. rosea* (17%) and *S. hominis* (14%) in RT and *S. epidermidis* (22%) and *K. rosea* (10%) in controls.

Conclusion: The most prominently isolated bacteria in the RT group and overall were coagulase-negative staphylococcus species. Unlike previous reports, S aureus, a more pathogenic microorganism, was less isolated, but *Kocuria* species were detected as one of the most prevalent types in this study. The study suggests the condition of hemodialysis and diabetes have little effect on bacterial flora, and the RT group receiving immunosuppressive drugs significantly differed in both bacterial growth and flora diversity. *J Microbiol Infect Dis 2023; 13(3):63-68.*

Keywords: *Ocular flora, Chronic renal disease, Ocular surface; Diabetes, Renal Transplantation*

INTRODUCTION

The external ocular surface acquires a microbial flora at birth and continues throughout life. Characteristics of this flora vary according to age, living condition, immune status, and the presence of local and systemic disease [1]. Some of the commensal flora may become resident in the conjunctiva and eyelids, potentially becoming pathogenic [2]. Ocular microbial flora is a significant source of ocular surface disorders and intraocular surgical contamination [3,4]. According to previous studies, bacterial contamination of aqueous humor samples taken from the anterior chamber in cataract surgery varies from 30 % to 50%. Most of them are gram-positive bacteria arising from ocular flora [5-8]. Additionally, in acute postoperative and posttraumatic bacterial endophthalmitis cases, the positive vitreous culture constitutes the majority of bacterial flora; the rate is 64% to 82% [9,10].

Several studies reported that the ocular surface integrity and tear function are impaired in patients with chronic renal disease (CR) [11,12]. They can alter the characteristics of

Correspondence: Dr. Lokman Aslan, Department of Ophthalmology, KSU, School of Medicine, Kahramanmaras, Turkey
E-mail: lokaslan46@yahoo.com
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flora and facilitate settlement here of the bacteria, making them susceptible to bacterial infections. In addition, side effects of immunosuppressive medication and steroid use emphasize that patients became more vulnerable to colonization and leading problems requiring surgical procedures than the general population [11]. These patients also have a high risk for immune system dysfunction and postoperative bacterial infections [1,10]. However, the causative agents in postoperative endophthalmitis can't be isolated in all the cases, and the positive bacterial culture rate in vitreous samples remains at about 60-70 % [10]. Therefore, previously well-known ocular flora may provide an advantage for empirical treatment of ocular bacterial infections and prevention of postoperative endophthalmitis. In these cases, immediate diagnosis and treatment are essential to avoid vision-threatening outcomes. Some changes in ocular flora of patients with CR in relation to the duration of hemodialysis were reported in a previous study [13]. However, diabetic hemodialysis and renal transplant patients include a significant majority of the CR and there is no sufficient study describing ocular flora in the CR including hemodialysis (HD) without/with diabetes and renal transplant (RT) patients. The aim of this study was to investigate the conjunctival flora in the mentioned subgroups of CR and to compare it with healthy controls.

METHODS

The study was adherent to the tenets of the Declaration of Helsinki. Ethical approval for the study was obtained from the Research Committee of the Hospital of Kahramanmaraş Sütçü İmam University, and consent was obtained from all participants. A total of 105 adult CR (35 non-diabetic, 35 diabetics, and 35 transplant patients) and age-sex matched 40 healthy subjects were enrolled in the study. The study group was composed of patients with CR undergoing hemodialysis and applied for renal transplants in our University nephrology clinic and a private dialysis center. The patients having renal transplantation and applying hemodialysis for at least one year were included in the study.

A complete ophthalmologic examination was performed by the same ophthalmologist on all patients in order to exclude clinical abnormality before sample collection. Ocular and systemic histories were also obtained for each participant undergoing conjunctival culture. Patients with active inflammation or red eye, previous intraocular surgery, use of contact lenses, taking topical medications, and a history of antibiotic medication within three months were excluded from the study.

The same ophthalmologist examined all patients for ophthalmologic findings to exclude clinical abnormality before sample collection. Ocular and systemic anamnesis were obtained for each participant undergoing conjunctival culture. Patients with active inflammation or red eye, previous intraocular surgery, use of contact lenses, topical medications, and a history of antibiotic medication within three months were excluded from the study.

The conjunctival swab was obtained using a sterile strip from the lower lid fornix of the right eye, and an effort was made to minimize contamination from the lashes and skin. The application was performed without a topical anesthetic by a trained medical doctor and a field technician. Swabs were sent to the microbiology laboratory in culture tubes containing 0.5 ml of modified Stuart's transport media. The swabs were directly sub-cultured on 5 % sheep's blood agar, chocolate agar media, and eosin methylene blue agar and incubated for 18-24 hours at 37°C. Chocolate agar plates were then incubated for 72 h in an atmosphere containing 5% CO2 at 37°C. We viewed the colony growth on agar for the first time 18 to 24 h after starting the culture and then made an observation once per day. The sample with no colony growth on the agar 72 h after culture was regarded as negative. Some specimens were also cultured with thioglycolate broth. After this enrichment, these specimens grew bacteria. Microbiological identification was made using standard microbiological techniques, and swabs were identified using either Vitek-2 (bioMerieux, France) automatized system or conventional methods.

The bacterial growth and flora diversity of the groups were compared to the controls

Statistical Analysis

The SPSS 16.0 software (SPSS, Chicago, IL) was used for the statistical evaluation of our study data. Categorical variables were identified with numbers and percentages. Descriptive statistics such as median and the smallest and biggest values were used for numerical variables. The Kruskal Vallis test was used in the comparisons between the groups in terms of categorical variables while the One Way ANOVA test was used in the comparisons in terms of numerical variables. A p-value <0.05 was accepted as statistically significant.

RESULTS

The demographic data of all participants are summarized in Table 1. There was no difference in the gender distribution and no relation between the sex and bacterial growth in the groups (p>0.05, Spearman). While the mean age distribution of the HD, RT, and control groups was compatible with each other, diabetic hemodialysis (DHD) patients had a statistically significant difference from other groups (p<0.05). However, there was no correlation between bacterial growth and the mean age of groups (p>0.05, Spearman). Bacterial growth rates in the groups (DHD, HD, RT HS) were summarized in the Figures. Although the duration of diabetes mellitus, renal transplantation, and hemodialysis did not affect the bacterial growth, there is a significant correlation with the duration of CRF (r= 0,422, p<0.05 Pearson). Also, Kocuria spp. was also the dominant one detected in this group. After detection antibiotic susceptibility tests were performed by the disk diffusion method on Mueller-Hinton agar with 24 h of incubation at 35 ^{0}C. The results were expressed as susceptible, intermediate, or resistant according to the criteria of the National Committee for Clinical Laboratory Standards for the modified Kirby-Bauer method [14].

Table 1: Demographic features of the subjects.

Demographic	HD	HD & DM	RT	Controls
Age, years (mean ± SD)	47.8 ± 16	59.4 ± 10**	41.7± 14	44.6±8
Male/female	19/16	17/18	18/17	20/20
HD *	4.9 ± 4.1	9.0±3.8	-	-
CR *	6.8 ± 3.7	9.1 ± 3.9	8.3 ± 4.4	-
RT *	-	-	3.9 ± 1.6	-
DM *	-	18.5±5.3	-	-

HD: Hemodialysis, CR: Chronic renal disase, RT: Renal Transplant, DM: Diabetes Mellitus *: Duration mean years (SD), ** p<0.05 (oneway ANOVA).

DISCUSSION

Hemodialysis and renal transplantation are essential for the survival of patients suffering from chronic renal failure, and Diabetes Mellitus (DM) is a significant cause of renal failure. However, both uremia and hemodialysis lead to various ocular surface disorders, including calcium deposition in the cornea and conjunctiva, squamous metaplasia of the conjunctival epithelium, and decreased tear secretion so that they can influence conjunctival flora [11,15,16]. In addition, previous studies reported that DM and administration of immunosuppressive agents altered ocular bacterial flora [17]. In animal models was also shown that diabetes caused alterations of bacterial flora in the bulbar conjunctiva in rats, with some bacterial species disappearing and others emerging [18]. Moreover, the differences in conjunctival flora were identified in some systemic conditions such as Behcet's disease, Parkinson's disease, chronic alcoholism, and steroid use [19,20]. Still, it is unclear whether this diversity of bacterial species and a high bacterial growth rate in such a disease come from either the disease itself or its treatment modality. Otherwise, in patients receiving immune suppressive therapy for bone marrow transplantation and AIDS, the bacteria in the conjunctival flora are similar to healthy subjects [21,22].

The current study is comprised of patients with CR, including different conditions such as hemodialysis, diabetes, and the use of immunosuppressive agents, and each of them could separately affect the ocular flora. However, in our culture results, coagulase-negative staphylococcus is dominant in the conjunctival flora of CR patients, similar to the control group as previously confirmed. Surprisingly, Kocuria species had greater frequency, especially in the transplant group. The mean age of this group was also the lowest among others. Recently Kocuria sp. was reported as a member of conjunctival flora of different aged healthy individuals. *K. varians* was detected in conjunctival flora of the younger age group, but *K. rosea* was seen in elder subjects in the same study [23]. In previous studies, higher growth rates were seen in the elderly, but we couldn't find any significant relationship between age and growth rates. However, Kocuria spp. was seen in younger transplant patients receiving immunosuppressive drugs, and higher growth rates were also seen. Although previous reports were limited to human infections, *Kocuria* species were responsible for some human conditions, mainly in compromised hosts with severe underlying diseases. Due to the usage of better identification methods, there will be an increased incidence of different types of *Kocuria* infections reported.

We used thioglycolate broth, a multipurpose, enriched, differential medium used primarily to determine the oxygen requirements of microorganisms. After this enrichment, these specimens grew bacteria, especially *Kocuria spp.* grew after an enrichment. *Kocuria spp.* is gram-positive cocci occurring in tetrads belonging to the family Micrococcaceae. They were nonhemolytic, catalase-positive, strictly aerobic, nonmotile, and unable to reduce nitrate to nitrites or hydrolyze gelatin, arginine, and esculin. The bacterial isolates produced acid from trehalose, glucose, fructose, mannose, glycerol, and saccharose but not from mannitol, raffinose, arabinose, lactose, or ribose [23]. Colonies seem smooth or rough, slightly convex; orange, red, or pink [24]. Peritonitis and bacteremia episodes were described by Kaya et al. [25] and Altuntas et al. [26] respectively, were presumably caused by *K. rosea*, but genome-based confirmation was not provided in either case. In our study, we couldn't use a molecular assay, which was a limitation of our study. As well as Kocuria species being misidentified by phenotypic tests, misidentification of coagulase-negative staphylococcus as Kocuria species (*K. varians*/*K. rosea*) using the Vitek 2 system has been reported in the literature. Therefore, 16S rRNA gene sequencing has been evoked in this case to eliminate confusion arising from biochemical variation of clinical strains. Furthermore, the identification of CoNS isolates as Kocuria species by Vitek 2 is considered frequent [27]. However, recent studies, including ours, correctly identified Kocuria using the Vitek-2 ID-GPC gram-positive identification card, perhaps due to the recently introduced more extensive database that allows the identification of additional taxa [28]. Based on this knowledge, we can say that approving our results with molecular-based tests would show different results. Most Kocuria isolates were reported to be susceptible to many of the first- and second-line drugs, except ampicillin and norfloxacin.

Normal ocular flora was identified through multiple studies, and the bacterial growth in the conjunctival culture ranged from 39.2% to 86.2 % [17,29]. Consistent with prior reports, the vast majority of isolates identified in the present study were Gram-positive organisms, specifically *coagulase-negative Staphylococcus*. It has usually been considered to contribute to normal ocular flora. It may have a probiotic function by directly preventing colonization of the host by S aureus, a more severe pathogen. Despite the possibility of a protective role, S. epidermidis can cause ocular infections as an opportunistic pathogen. In healthy subjects, *S. aureus* was isolated from the conjunctival sac at a rate of 3 % to 15 % as temporary colonization [1,4] but not detected in the controls of the present study. It just was isolated in diabetic hemodialysis patients in all groups.

A similar study was conducted by Balbaba et al. [13] only dealt with chronic renal failure who are undergoing hemodialysis, and renal transplantation patients and diabetic patients were not distributed of the isolated bacterial species and their relation to the hemodialysis duration. We obtained fewer numbers of pathogenic microorganisms such as *S. aureus*, but the more prevalent type was *Kocuria* species despite the mentioned study.

The study's limitations are the small sample size, diversity of age distribution, and drug usage in the patient groups. To shed light on further studies, widening this study in larger groups may reveal more valuable data.

In conclusion, although current experiments show conjunctival colonization with microorganisms frequently seen were S. epidermidis, *Corynebacterium spp.*, and gram-positive rods, our culture results showed some other bacteria prominently as *Kocuria rosea* as a member of ocular flora. On the other hand, *S. aureus*, the most pathogenic cocci seen mostly in blepharitis cases; typically causes ocular infection was less isolated (in only one patient) than in previous studies, and S. pneumonia was the same. Moreover, in our research, DHD patients and the non-diabetic group showed similar culture results, but different types of bacteria like Kocuria spp. were detected in transplantation. Because these patients were exposed to immunosuppressive drugs such as Tacrolimus, mycophenolate mofetil, azathioprine, and low dose prednisolone, this condition may inhibit some frequent bacteria.

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