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Microbial colonization and antimicrobial susceptibility of the nose and external ear canal in hemodialysis patients

Hemodiyaliz hastalarında dış kulak yolu ve nazal kavitede mikrobiyal kolonizasyon ve antimikrobiyal duyarlılık

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Abstract

Aim: The effect of normal bacterial flora on the immune system is to make it more effective against harmful micro-organisms and to maintain it in readiness for a more rapid response. The purpose of this study was to determine the bacterial flora in the external ear canal and nasal cavities in hemodialysis patients, to compare this with the normal population and to show the microorganisms' antibiotic susceptibilities.

Materials and Methods: Patients receiving hemodialysis therapy for at least 3 years were included in the study. A control group (n=62) was established consisting of patients with no immune-suppressive condition and presenting to hospital with non-ear symptoms. Swab specimens were taken from all subjects' external ear canals and nasal cavities. Results of the cultures were recorded and antimicrobial susceptibility test was performed.

Results: The most commonly isolated micro-organisms in the study group were MRCoNS (41%), followed by MSCoNS (15.5%) and diphtheroids (9.8%). The micro-organism most commonly isolated from the nasal cavity in the study group was CoNS (52.7%), of which MRCoNS constituted 69.2%.

Conclusion: We think that infections that play a major role in mortality and morbidity in hemodialysis patients may originate from the external ear canal as much as the nasal cavity, for this reason it is important for micro-organisms colonizing these regions to be eliminated with appropriate treatment on the basis of antibiotic susceptibility tests.

Key words: Hemodialysis, external ear, nasal cavity, immune suppression, antimicrobial susceptibility.

Öz

Amaç: *Normal bakteriyel floranın bağışıklık üzerindeki etkisi onu mikroorganizmaların zararlı etkilerine karşı daha etkili hale getirmek ve hızlı bir cevap oluşturacak şekilde hazır tutmaktır. Bu çalışmanın amacı hemodiyaliz hastalarında nazal kavite ve dış kulak yolundaki bakteriyel florayı tespit etmek, bunu normal populasyonla karşılaştırmak ve mikroorganizmaların antimikrobiyal duyarlılığını ortaya koymaktı.*

Gereç ve Yöntem: *Çalışmaya en az 3 yıldır hemodiyaliz alan hastalar dâhil edildi. Herhangi bir immunsupresyon durumu olmayan ve kulak hastalıkları dışı sebeplerle hastaneye başvuran hastalardan da kontrol grubu oluşturuldu (n=62). Tüm katılımcıların nazal kavite ve dış kulak yollarından kültür çubuğu ile kültür örnekleri alındı. Kültür sonuçları ile antimikrobiyal duyarlılık test sonuçları kaydedildi.*

Bulgular: *Çalışma grubunda dış kulak yolundan en çok izole edilen bakteri MRCoNS (%41) iken bunu MSCoNS (%15,5) ve Difteroidler (%9,8) izlemekteydi. Nazal kaviteden ise en sık izole edilen bakteri CoNS olup (%52,7) bunun %69,2'sini MRCoNS oluşturmaktaydı.*

Sonuç: *Hemodiyaliz hastalarında mortalite ve morbiditede önemli rol oynayan enfeksiyonların nazal kavite kadar dış kulak yolundan da kaynaklanabileceğini, bu nedenle antimikrobiyal duyarlılık testleri ile belirlenen uygun antibiyoterapi ile bu bölgedeki mikroorganizmaların da elimine edilmesinin önemli olduğunu düşünmekteyiz.*

Anahtar Sözcükler: *Hemodiyaliz, dış kulak yolu, nazal kavite, immün süpresyon, antimikrabiyal duyarlılık.*

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Introduction

The effect of normal bacterial flora on the immune system is to make it more effective against harmful micro-organisms and to maintain it in readiness for a more rapid response. The micro-organisms isolated from normal bacterial flora of the external ear canal and nasal cavity are staphylococcus species and diphtheroid bacilli. The micro-organism most commonly isolated from the nasal cavity is *Staphylococcus aureus*, while *Staphylococcus auricularis* is the form most frequently isolated in the external ear canal (1,2). This normal bacterial flora may exhibit changes in conditions in which the immune system is depressed, such as dialysis treatment, AIDS and intravenous drug dependence.

The immune systems of hemodialysis patients are weak and susceptible to various infections. *Staphylococcus species* represent a significant part of dialysis-related infections (3). We think that the nasal cavity or external ear canal may be the source of these infections.

The human external ear canal and nasal cavity contain a commensal bacterial flora. Previous studies have investigated the bacterial flora of the external ear canal in healthy individuals (1), The pediatric age group (4), swimmers and divers (5) and in subjects with diabetes (6). However, only one previous publication has examined the flora of the external ear canal in hemodialysis patients. Our study differs from that research in that it involved a control group and an antibiotic susceptibility test.

The purpose of this study was to determine the bacterial flora in the external ear canal and nasal cavities, to compare this with the normal population and to show the microorganisms' antibiotic susceptibilities.

Materials and Methods

Subjects

Patients receiving hemodialysis therapy at the Hemodialysis Center for at least 3 years were included in the study. A control group (n:62) was established consisting of patients with no immune-suppressive condition and presenting to hospital with non-ear symptoms. Study group was consisted of sixty-one patients. Mean duration of dialysis was 44±6 months. Local ethical approval was granted. The patients were informed about procedures. Any subjects with a history of ear infection or ear surgery, receiving antibiotic therapy for any reason or with upper respiratory tract infection were excluded.

Sample collection

Swab specimens were taken from all subjects' external ear canals and nasal cavities. All swab specimens were inoculated on 5% Sheep Blood Agar (BA) medium plates (Difco laboratories, Detroit, Mich. and BBL, MD), Eosin Methylene Blue (EMB) medium (Difco laboratories, Detroit, Mich. and BBL, MD) and Chocolate Blood Agar (CBA) (Difco laboratories, Detroit, Mich. and BBL, MD). Cultures were then incubated at 37^{o}C for 24-72 h. The cultures were evaluated by conventional microbiological methods (colony morphology, hemolysis on BA, gram staining characteristics, catalase, oxidase and tube coagulase tests, DNase production, ability to produce H_2S and gas and effect on Three Sugar Iron Agar). Strains that could not be identified using conventional methods were analyzed using a VITEK 2 (bioMeriux, USA) automated bacterial identification system.

Antibiogram

The Antimicrobial Susceptibility Test (AST) (disc diffusion on Mueller-Hintonagar) against *Staphylococcus species* among the identified strains were assessed on the basis of European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (7). The AST plates were incubated at 35±1^{o}C for 18±2h. The disks used for antibiotic susceptibility test (Oxoid, Hampshire, UK) included cephazolin, cephaclor, trimetoprim+sulfa-methoxazole, amoxicillin, oxacillin, clindamycin, erythro-mycin, tetracycline, ampicillin+sulbactam, amoxicillin+ clavulanic acid. Some of the Minimal Inhibitory Concentration (MIC) susceptibility break points used for the antibiotics tested were as follows: ampicillin ≤8 mg/L, amoxicillin/clavulanate ≤8/2 mg/L, trimethoprim/sulfa-methoxazole ≤2/38 mg/L. Most staphylococci are penicillinase producers, which are resistant to benzylpenicillin, phenoxymethylpenicillin, ampicillin, amoxicillin, piperacillin and ticarcillin. Isolates negative for penicillinase and susceptible to methicillin can be reported susceptible to these agents. Isolates positive for penicillinase and methicillin susceptible are susceptible to beta-lactamase inhibitor combinations and isoxazolyl penicillins (oxacillin, cloxacillin, dicloxacillin and flucloxacillin) (7).

Statistical analysis

All data were analyzed with Microsoft Excel software, STATA Version 13 (College station, Texas) and SPSS (SPSS 12. Data Analysis Basics) programs. Mean and standard deviation were used for quantitative values while percentage values were used for categorical data. Normal distribution of the data was analyzed with Kolmogorow Smirnov test and when $p>0.05$, it is thought that the distribution is normal. As the study was performed with two independent groups, the chi square test was used to analyze the categorical values and t-test was used to analyze the quantitative values. $p<0.05$ was regarded as statistically significant for all tests.

Results

Gender and age distribution

Sixty-one patients aged between 27 and 84 (58±9) were enrolled, 34 men and 27 women. Mean duration of dialysis was 44±6 months. A control group was established consisting of 62 patients, 27 men and 35

women, aged 18-74 (54±12.02). No statistically significant difference was determined between the groups in terms of age and gender (p=0.078, p=0.092, respectively).

Bacterial population

Analysis of the external ear canal culture results revealed that the most commonly isolated micro-organisms in the control group were Methicillin sensitive, coagulase negavite staphylococci (MSCoNS) (50%) and diphtheroids (35%). These were followed by low levels of Methicillin resistant. coagulase negative staphylococci (MRCoNS) and Methicillin sensitive staphylococcus aereus (MSSA). In the study group, the most commonly isolated micro-organism was MRCoNS (41%), followed by MSCoNS (15.5%) and diphtheroids (9.8%). *Pseudomonas aeruginosa* was isolated in two patients in the study group.

The micro-organism most commonly isolated from the nasal cavity in the control group was MSSA (72.5%). This was followed by low levels of diphtheroids and *Escherichia coli*. In the study group, the most commonly isolated micro-organism was Coagulase negative staphylococci (CoNS) (52.7%), of which MRCoNS constituted 69.2%. In addition, MSSA was isolated at a level of 13.5% and Methicillin resistant staphylococcus aereus (MRSA) in two patients. Micro-organisms isolated from subjects' external ear canals and nasal cavities are summarized in Table-1.

Table-1. Culture Results of Nasal Cavity and External Ear.

Micro-organisms	Nasal Cavity	Ext. ear	Nasal Cavity	Ext ear
MSCoNS		33	12	11
MRCoNS		3	27	29
MSSA	45	4	10	
MRSA			2	
Diphteroids	3	23	5	7
E.coli	2			
P.auroginosa				2
P.mirabilis			1	
C.diversus			1	
K.pneumonia			1	
S.pneumoniae			1	
Candida			1	
Aspergillus				1
Mucor			1	1
No breeding	12	3	12	20
Total	62	66*	74**	***71

*Multiple breeding was detected at 5 cultures.
**Multiple breeding was detected at 13 cultures. MSCoNS and MRCoNS were found together in 4 cultures.
***Multiple breeding was detected at 10 cultures. MSCoNS and MRCoNS were found together in 2 cultures.
n=The number of breeding.

When the antibiotic susceptibility of micro-organisms isolated from the external ear canal and nasal cavity was investigated, as there was no MRSA isolated from control group, a comparison could not be performed in terms of antibiotic susceptibility of MRSA isolates of control and study groups. The highest resistance was observed against penicillin G, co-trimoxazole and erythromycin in control group, while there was no resistance against vancomycine in both control and study groups. A total number of 36 strains of CoNS were observed in control group. As only 3 of them were MRCoNS, no statistical comparison could be performed. Highly erythromycin and Penicillin G resistance were observed in MSCoNS isolates. 3 MRCoNS strains isolated in the control group were sensitive to vancomycin and ciprofloxacin but highly resistant to other antibiotics. A total number of 91 staphylococcus strains were isolated from study group. Of the 12 *Staphylococcus aureus* strains, only 2 were resistant to methicillin. As the number of strains resistant to methicillin is lower, no statistical comparison could be performed. Two isolated MRSA strains were 50% susceptible to ciprofloxacin and gentamycin, 100% susceptible to vancomycin, and 100% resistant to all the other antibiotics studied. The highest resistance in MSCoNS strains was against Penicillin G, while MRCoNS strains were highly resistant to penicillin G, co-trimoxasole and erythromycin. Again, the number of isolated strains was lower in control group to establish a statistical comparison. Antibiotic susceptibility test results for the micro-organisms isolated are shown in Table II. While no significant resistance profile was observed in the gram negative micro-organisms isolated, piperacillin-resistance was observed in one of the two pseudomonas strains.

Discussion

The bacterium most frequently isolated from the external ear canal in our control group was MSCoNS, followed by diphtheroids; the most frequently isolated bacterium in the study group was MRCoNS, followed by MSCoNS. MSSA was most frequently isolated in nasal cultures in the control group, and MRCoNS in the study group. In addition, MRSA was isolated in nasal cultures from two patients in the study group. Vancomycin was 100% effective against both MRSA and MRCoNS strains at antibiotic susceptibility tests.

The bacteria most commonly isolated from the external ear canal of healthy individuals are Staphylococcus spp. and diphtheroids. Stroman et al. studied normal external ear canal flora in children and adults. Staphylococcus spp. was identified as the most common isolate. *S. auricularis* was the most common isolate, at a level of 21%, followed by S. epidermidis among the staphylococcus species. *Coryneform bacteria* (diphtheroids) were the next most commonly isolated organisms (1). Salit et al. (6) investigated bacterial flora in diabetic and non-diabetic subjects. The most common isolates determined in the two groups were *S. epidermidis*, diphtheroids and *Bacillus spp*.

Table-2. Antimicrobial Susceptibility Test Results.

	Control				Study			
	MSSA (nT=49)	**MRSA (nT=0)**	**MSCoNS (nT=33)**	**MRCoNS (nT= 3)**	**MSSA (nT=10)**	**MRSA (nT=2)**	**MSCoNS (nT=23)**	**MRCoNS (nT=56)**
Tetracyclin	15	-	2	2	3	2	11	14
Ciprofloxacin	12	-	13	1	0	1	2	10
Penicillin G	37	-	23	3	4	2	19	54
Oxacillin	8	-	13	3	1	2	1	2
Erythromycin	28	-	23	3	3	2	7	43
Cephalothin	1	-	12	2	1	2	1	8
Ceftriaxone	16	-	13	2	0	2	1	8
Co-trimoxazole	32	-	13	2	3	2	8	48
Vancomycine	0	-	0	0	0	0	0	0

The given numbers are the total number of breeding that is resistant to the stated antimicrobial agent.
nT: Total number of isolated microorganisms

Brook et al. (4) investigated the flora of the external ear canal in children and identified S. epidermidis as most common bacterium and diphtheroids as the second most frequently isolated organisms. We also isolated CoNS (54.5%) most frequently from the normal population external ear canal in our study, followed by diphtheroids (34.8%).

Pathogenic organisms including *P. aeruginosa* and *S. aureus* have been reported to colonize the external ear canal in hospitalized patients and subjects receiving antimicrobial therapy (8). Hemodialysis can create an opportunity for compromise of normal flora and pathogenic micro-organism colonization by weakening the immune system. Studies have shown that *S. aureus* infections are one of the main causes of morbidity and mortality in hemodialysis patients (9). Alçelik et al. (10) reported CoNS followed by diphtheroids as the most commonly isolated bacteria in the external ear canal in hemodialysis patients. CoNS was also the major pathogen in the external ear canal in hemodialysis patients in our study. However, MSCoNS (50%) was the major micro-organism in the control group, but this was isolated at a level of 15.4% in the study group. MRCoNS was observed at a level of 4.5% in our control group and at 40.8% in the study group. *P. aeruginosa* was also isolated in two patients in our study group. MRCoNS isolates in our study group were highly resistant to several antibiotics, particularly penicillin and oxacillin, but developed no resistance to vancomycin.

The most frequently isolated bacteria from the nasal cavity in normal healthy individuals were *Staphylococcus spp.*, present at a level of 20-25% in healthy individuals. Nasal *S. aureus* carriage plays a key role in the development, not only of community-acquired infections but also of infections in dialysis units. Lai et al. determined that nasal MRSA carriage is associated with high morbidity and mortality in hemodialysis patients (11). Levels of nasal *S. aureus* carriage in dialysis patients range between 24% and 76% in studies from various hemodialysis centers (12,13). Ucuncu et al. (2) also reported that *Staphylococcus spp.* was most commonly colonized from the nasal cavity in the nasal vestibule in hemodialysis patients (2). Alçelik et al. (10) observed that nasal cavity and external ear canal flora in hemodialysis patients exhibited similar characteristics. In our study, MSSA was most frequently isolated from the nasal cavity in the control group, while the most commonly isolated bacterium in hemodialysis patients was MRCoNS. MRSA was also isolated in the nasal cavity in two patients in our study group. This species was resistant to all antibiotics, with the exception of vancomycin, at antibiotic susceptibility tests.

Conclusion

Significantly high levels of different pathogen colonies were observed in external ear canal and nasal cavity flora of hemodialysis patients compared to the control group. We think that infections that play a major role in mortality and morbidity in hemodialysis patients may originate from the external ear canal as much as the nasal cavity, for which reason it is important for micro-organisms colonizing these regions to be eliminated with appropriate treatment on the basis of antibiotic susceptibility tests.

Conflict of interest: All authors declare that they have no conflict of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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