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Rhinology

Comparison of the bacterial flora of the nasal vestibule and cavity in haemodialysis patients

Confronto della flora batterica tra vestibolo e cavità nasale nei pazienti dializzati

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SUMMARY

Staphylococcal infections are the major causes of morbidity in haemodialysis patients. The source of the staphylococci is the anterior nares. Elimination of nasal carriage of staphylococci could result in a remarkable decrease in the infection rate. The aim of this study was to investigate if there was a difference in the bacterial flora between the nasal vestibule and cavity as well as their antibiotic susceptibility in haemodialysis. Swab samples obtained from 35 haemodialysis patients were subjected to conventional microbiological methods. The antimicrobial susceptibility test was performed for *Staphylococcus spp.* using cephazolin, cephaclor, trimetoprim + sulfamethoxazole, amoxicillin, oxacillin, clindamycin, erythromycin, tetracycline, ampicillin + sulbactam and amoxicillin + clavulanic acid. *Staphylococcus spp.* was found more often in the vestibule than in the cavity (88.5 *vs*. 77.1%). The effectiveness of clindamycin, erythromycin and tetracycline was particularly striking for the methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative Staphylococci isolates. In conclusion, existence of difference in bacterial flora between the nasal cavity and vestibule and their responsiveness to antibacterial agents may require reconsideration of elimination of secondary infections in haemodialysis patients.

KEY WORDS: Nose • Bacterial flora • Antibiogram • Haemodialysis

RIASSUNTO

Le infezioni stafilococciche rappresentano la maggiore causa di morbidità nei pazienti in emodialisi. La fonte dell'infezione Stafilococcica è la porzione anteriore del naso. L'eliminazione del trasporto nasale di Stafilococchi potrebbe risultare in una significativa diminuzione nel tasso di infezione. Lo scopo di questo studio è stato di valutare la presenza di una differenza nella flora batterica tra il vestibolo e la cavità nasale e la sua vulnerabilità antibiotica nel paziente dializzato. Il tampone ottenuto da 35 pazienti dializzati è stato sottoposto ai test convenzionali microbiologici. L'antibiogramma è stato eseguito per lo Stafilococco spp. *testando il cephazolin, il cephaclor, il trimetoprim + sulfametossazolo, l'amoxicillina, l'oxacillina, la clindamicina, l'eritromicina, la tetraciclina, l'ampicillina + sulbactam e l'amoxicillina + acido clavulanico. Lo* Stafilococco spp. *è stato trovato più frequentemente nel vestibolo che nella cavità nasale (88,5 contro 77,1%). L'efficacia di clindamicina, eritromicina e tetraciclina riguardava particolarmente lo* Stafilococco aureus *meticillino-resistente e gli Stafilococchi meticillino-resistenti coagulasi-negativi. In conclusione, l'esistenza di una differenza nella flora batterica tra la cavità nasale ed il vestibolo e la sua diversa sensibilità agli agenti antibatterici potrebbe far riconsiderare la possibilità di ridurre le infezioni secondarie nei pazienti dializzati.*

PAROLE CHIAVE: *Naso • Flora batterica • Antibiogramma • Dialisi*

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Introduction

The bacterial flora of the nasal cavity cover potential bacterial pathogens (PBPs) including *Staphylococcus aureus*. Several factors, such as diabetes mellitus, intravenous drug abuse, and dialysis treatment, increase the rate of colonization [1 2]. The reservoir for *Staphylococcus aureus* skin carriage is the anterior nares [3]. *Staphylococcus aureus* is considered to be the most important nasal PBP and has been found in 20% to 25% of healthy American adults. It was estimated that 30% of these patients were infected with *Staphylococcus aureus* and that the patient's nose was its place of origin. *Neisseria meningitidis, Moraxella catarrhalis, Streptococcus pneumonia, Haemophilus influenzae* are the other PBPs found in the nasal cavity. Carriers of nasal methicillin resistant *Staphylococcus aureus* (MRSA) are particularly problematic [4]. Nasal MRSA was detected in 46% of patients with liver cirrhosis [5].

Staphylococcus aureus carriage, in the nose and on the skin, has been shown to be more common in patients receiving chronic haemodialysis than in the general popula-

tion [6-8]. Prophylactic administration, with agents shown to be effective in eliminating the nasal carrier state, should lead to a significant decrease in the frequency of staphylococcal infections in haemodialysis patients. Oral antimicrobial agents have proved to be only marginally successful in eliminating staphylococcal nasal carriage. Therefore, generally topical application is performed at the level of the anterior nares [3].

Few studies have evaluated the bacterial profile of the nasal vestibule and cavity simultaneously [4 9]. Kakinohana et al. investigated the incidence of *Staphylococcus aureus* from the nasal vestibulum and the throat in children. They showed that *Staphylococcus aureus* was isolated more frequently from the throat than from the nasal vestibulum [10]. Therefore, they suggested that surveying *Staphylococcus aureus* only in the nasal vestibulum was not sufficient to control nasocomial infection. This could also be imperative in haemodialysis patients. The aim of this study was to determine whether the bacterial status of the vestibule and cavity and their antibacterial status differ among haemodialysis patients.

Subjects and Methods

Subjects

Overall, 16 males and 19 females, age range 19-62 years (mean: 49.2 ± 14.8) on haemodialysis at the Dialysis Unit of the Research and Application Hospital, Atatürk University, were examined for bacterial flora in the upper respiratory tract. Mean duration on dialysis was 39.6 ± 35.4 months. The patients were informed about the nature of the experimental procedures and asked for their consent. They were free of infectious diseases in the upper respiratory tract and had received no antimicrobial agents during the preceding four weeks.

Sample collection

Cultures were taken both from the vestibules and the posterior parts of both nasal cavities with cotton swabs (Sterile Transport Swab, COPAN, Brescia, Italy) as described by Glück and Gebbers [4]. First, swabs were taken from the vestibules. Second, cotton swabs were passed through the posterior nasal cavities after placing a sterilized Killian nasal speculum. Samples were then put into the tubes containing 2 ml brain-heart infusion broth medium and incubated at 37°C for 6-8 hours. They were then transferred to eosin methylene blue medium, chocolate blood Agar (BA), MacConkey's agar and 5% sheep blood agar medium plates (Difco Laboratories, Detroit, MI, USA) and incubated at 37°C in a 5% CO_2 atmosphere condition for 24-48 hours. The cultures were evaluated by standard microbiological and biochemical methods, including colony morphology, haemolysis on BA, gram staining peculiarity, catalase test, oxidase test, tube coagulase test, DNAse production, its ability to create H_2S and gas production and its influence on three sugar iron agar.

Antibiogram

The Antimicrobial Susceptibility Test (Disc diffusion on Mueller-Hinton agar) against *Staphylococcus spp*. was carried out as recommended by the Clinical and Laboratory Standards Institute [11]. The disks used for antibiotic susceptibility test (Oxoid, Hampshire, UK) included cephazolin, cephaclor, trimetoprim + sulfamethoxazole, amoxicillin, oxacillin, clindamycin, erythromycin, tetracycline, ampicillin + sulbactam, amoxicillin + clavulanic acid. Data were subjected to the Univariate and Means Procedures (SPSS, Version 10.0 Windows Release, Chicago, IL, USA) and expressed as a percentage of totals. Moreover, nasal site differences and responses to antibiogram were attained by Chi-square test. Statistical significance was declared at a probability p value ≤ 0.05.

Results

Bacterial population

Microflora in the right and left nasal cavities (31/35, 88.5%) and vestibules (28/35, 80.0%) were the same in all patients. Potential bacterial pathogens were identified in 32 of the 35 haemodialysis patients (91.4%). When the nasal side was considered, PBPs were isolated from 54 of the 70 nasal cavities (77.1%) and 62 of the 70 vestibules (88.5%).

Considering the PBPs, the difference between the bacterial spectra of the vestibule and the cavity was considerable, in 20 (28.5%) of the 70 nasal sides. The most common bacteria isolated from both the cavity and vestibule, in descending order of frequency, were methicillin-sensitive *Staphylococcus aureus* (MSSA) (20.2 *vs*. 20.6%), methicillin-sensitive coagulase-negative staphylococci (MSCNS) (18.9 *vs*. 19.5%), methicillin-resistant *Staphylococcus aureus* (MRSA) (17.7 *vs*. 16.3%) and methicillin-resistant coagulase-negative staphylococci (MRCNS) (6.3 *vs*. 8.6%), and there were no differences in frequencies of these bacterial counts by the site (Table I).

Diphteroids spp. were also present at a high frequency in the nasal vestibule and cavity (13.0 *vs*. 15.1%; Table I). Other species were present in both regions at less than 5%. *Escherichia coli* was absent in the nasal cavity, whereas *Pneumococci spp.* was absent in the nasal vestibule.

Antibiotic sensitivity

Antibiotic susceptibility results of *Staphylococcus spp*. isolated from the vestibule and cavity are listed in Table II. Clindamycin and erythromycin were 100% effective on MRCNS ($p < 0.05$), oxacillin, ampicillin + sulbactam and amoxicillin + clavulanic acid on MSCNS ($p < 0.05$) and cephazolin and oxacillin on MSSA ($p < 0.05$), respectively. However, no antibiotics were 100% effective on MRSA.

Table I. Aerobic bacteria cultured from nasal cavity and vestibules in haemodialysis patients*.

Bacteria**	Nasal cavity	Vestibule
MRCNS	5 (6.3)	8 (8.6)
MRSA	14 (17.7)	15 (16.3)
MSCNS	15 (18.9)	18 (19.5)
MSSA	16 (20.2)	19 (20.6)
Diphteroids spp.	12 (15.1)	12 (13.0)
Acinetobacter spp.	3 (3.7)	3 (3.2)
Escherichia coli	–	2 (2.1)
Proteus mirabilis	2 (2.5)	2 (2.1)
Pseudomonas aeruginosa	1 (1.2)	2 (2.1)
Haemophilus influenzae	4 (5.0)	4 (4.3)
Proteus vulgaris	2 (2.5)	2 (2.1)
Neisseria spp.	3 (3.7)	5 (5.4)
Streptococcus pneumoniae	2 (2.5)	–
Total	79	92

** Values are n (%). There was no nasal site effect on bacterial flora. ** MRCNS: methicillin-resistant coagulase-negative staphylococci; MRSA: methicillin-resistant* Staphylococcus aureus*; MSCNS: methicillin-sensitive coagulase-negative staphylococci; MSSA: methicillin-sensitive* Staphylococcus aureus.

cci. The reservoir for *Staphylococcus aureus* skin carriage is the anterior nares [3]. In a study by Tulloch, staphylococci isolated from skin lesions in patients with chronic staphylococcal skin infection were of the same phage type as staphylococci isolated from the anterior nares in 88% of instances in which the staphylococci were typable [6].

A few microbiological studies have been undertaken to differentiate bacteriology of the vestibule and cavity [4 9]. Not unlike in the present study (28.5%; Table I), Glück and Gebbers reported about one third of all subjects with proven PBPs, with a significant difference in the bacterial spectra of the vestibule and cavity in healthy men [4]. Moreover, in disagreement with our results, Glück and Gebbers reported a higher prevalence of *Staphylococcus aureus* in the cavity than in the vestibule [4]. The microbiological results in both cavities were similar in 88.5% (31/35) of all patients with PBPs and similar in both vestibules in 80.0% (28/35) of the cases. Glück and Gebbers reported these results as 71% for cavity and as 89% for vestibule, in healthy men [4].

Examination of normal bacterial flora is important in the clinical treatment and prevention of disease [15]. An antiseptic regimen is also imperative for infection control in patients

Table II. Susceptibility profile of staphylococcal isolates recovered from nasal cavity and vestibule of haemodialysis patients*.

	Bacteria**			
Antibiotic	MRCNS (n = 13)	MRSA (n = 29)	MSCNS (n = 33)	MSSA (n = 35)
Cephazolin	0 (0) [c]	0 (0) [c]	27 (82) [b]	35 (100) [a]
Cephaclor	0 (0) [b]	0 (0) [b]	25 (76) [a]	25 (71) [a]
Trimethoprim+sulfamethoxazole	0 (0) [b]	4 (13) [b]	30 (91) [a]	5 (14) [b]
Amoxicillin	0 (0) [c]	0 (0) [c]	10 (30) [b]	17 (49) [a]
Oxacillin	0 (0) [b]	0 (0) [b]	30 (100) [a]	35 (100) [a]
Clindamycin	13 (100) [b]	14 (48) [b]	28 (85) [a]	30 (86) [a]
Erythromycin	13 (100) [a]	9 (31) [b]	0 (0) [c]	6 (17) [b]
Tetracycline	11 (85) [b]	10 (34) [b]	30 (91) [a]	31 (89) [a]
Ampicillin + sulbactam	0 (0) [b]	0 (0) [b]	33 (100) [a]	30 (86) [a]
Amoxicillin + clavulanic acid	0 (0) [b]	0 (0) [b]	33 (100) [a]	30 (86) [a]

** Values are n (%). Different superscripts within same rows differ (p < 0.05). ** MRCNS: methicillin-resistant coagulase-negative staphylococci; MRSA: methicillin-resistant* Staphylococcus aureus*; MSCNS: methicillin-sensitive coagulase-negative staphylococci; MSSA: methicillin-sensitive* Staphylococcus aureus.

Discussion

Staphylococcus aureus infections remain a major cause of morbidity and mortality in haemodialysis patients. The most common organism infecting the vascular access site is *Staphylococcus aureus*, accounting for 70-92% of these infections [3 12]. Staphylococcal bacteraemia is a natural consequence of staphylococcal vascular site infection, with a mortality rate of about 10% in two studies involving haemodialysis patients [13 14]. These patients are more vulnerable to staphylococcal infections because of their depressed immunity and increased skin colonization by staphylococci-carrying nasal PBPs after major operations, or when suffering from injuries of the head and nasal sinus area [4 16 17]. Such regimens may also be pertinent to diabetics and patients on haemodialysis or other causes with impaired immunity [3 4]. Athough previously regarded as harmless commensal bacteria, the coagulase-negative staphylococci (CNS) are increasing in importance as the cause of hospital acquired infections, generally associated with the use of medical devices in seriously ill and immunocompromised patients. Given the excessive use of antibiotics, multi-drug resistant coagulase-negative staphylococci have emerged, including methicillin-

resistant isolates [18]. Methicillin-resistant coagulase-negative staphylococci are cross resistant to all β-lactam antibiotics, even though some isolates might be susceptible to certain β-lactam agents by *in vitro* testing [19]. de Mattos et al. investigated MRCNS isolates from patients undergoing continuous ambulatory peritoneal dialysis [20], taking samples from various parts of their bodies, including their nasal cavities. These Authors found that 30 of the 79 (38%) CNS isolates were resistant to methicillin (MRCNS). In the present study, 13 (28.2%) of the 46 isolates were MRCNS (Table II). Chow and Yu compared the ability of ofloxacin, cephalexin, erythromycin and clindamycin to eradicate staphylococcal nasal carriage [3]. In this study (Table II), ofloxacin and clindamycin appeared to warrant more extensive evaluation for their ability to eliminate staphylococcal nasal carriage. Moreover, the effectiveness of clindamycin, tetracycline and erythromycin was particularly striking when the MRSA and MRCNS isolates were taken into consideration, in the present study.

In conclusion, *Staphylococcus aureus* and other bacteria were abundant in the posterior cavity, suggesting that the topical medicine should be applied to the whole nasal cavity. In this way, it is probable that duration of recolonisation emerging after prophylactic administration could be extended. Also, knowing antibiotic susceptibility (*e.g.*, ofloxacin and clindamycin, in the present study) could save time and money and improve well-being preventing the probable infections.

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