Oral Cavity as a Source of Atypical Infective Pathogens in Chronic Kidney Disease Patients

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Abstract

Objectives: To investigate the prevalence of pathogens in mouth samples of immunosuppressed patients with Chronic Kidney Disease (CKD). These patients are susceptible to infections due to accumulated uremic toxins and altered leukocyte function. After kidney transplantation anti-rejection therapy further depresses the immune system.

Method and Materials: Eighteen CKD patients were enrolled to this study. Twelve of them were also previously examined at the predialysis stage between 2000 and 2005, and then followed up until 2015. Clinical and radiographic oral examination was performed; Total Dental Index (TDI) and Periodontal Inflammatory Burden Index (PIBI) were calculated to describe the degree of oral inflammation. Subgingival plaque and whole saliva samples were collected for microbial analyses of bacteria. Candida species were detected from oral mucosa.

Results: TDI indicated good oral health in most of the patients. However, culture of bacteria from plaque samples revealed uncommon findings such as Stenotrophomonas maltophilia, Chryseobacterium meningosepticum, and Pseudomonas aeruginosa in 33% (6/18) of the patients. We found Candida (8/18) which was associated with uncommon bacterial findings (5/8) (P<0.05).

Conclusion: Dentists should be aware that a CKD patient’s oral cavity may harbor uncommon immunosuppression related infective pathogens, which are a threat to the patient.

Keywords: Dental plaque; Kidney transplants; Oral health; Saliva

Introduction

The oral cavity is an important source of infections. Over 700 bacterial species have been detected in the oral microbiome, out of which 30-50% is not yet cultivable [1]. Teeth, periodontium, dentures, and mucous membranes of the mouth and nasopharynx have been considered potential reservoirs for pathogens [2-4], particularly in immunocompromised patients [5].

Patients with chronic kidney disease (CKD) are prone to infections because of immune alterations caused by uremia, related conditions such as diabetes, or because of immunosuppressive treatment. Patients with a kidney transplant have permanent immunosuppressive medication to prevent organ rejection. This increases the risk for infections caused both by nosocomial and opportunistic pathogens. Infection is one of the most common causes for hospital admission and death in recipients of solid organ transplants [6-8]. Even through diseases in the oral cavity, such as periodontitis, dental decay, and other manifestations of poor oral health are common in CKD patients, oral infections and inflammation often remain an overlooked problem.

Microbial analyses of subgingival plaque and whole saliva have been used for detection of specific bacterial species such as Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Prevotella intermedia, Campylobacter rectus, and Parvimonas micra, which all...
have been associated with periodontal disease [9]. Bacterial cultures have been used to identify these pathogens. The Polymerase Chain Reaction (PCR) has been used for a direct identification of pathogens in subgingival specimens and also for elucidating the role of specific bacteria. Molecular methods are currently available for the typing and subtyping of bacterial strains in general, but these can vary in efficiency and in the amount of required labor in the laboratory.

Hyposalivation with subsequent xerostomia either due to disease such as diabetes or to the number of drugs used daily may have an impact on oral microbiota especially in the elderly [10,11]. When the host immune defenses are impaired or when the oral microbial flora is disturbed, *Candida albicans* colonization may also be seen in CKD patients [12].

There are only a few longitudinal studies where the aspects of oral health have been followed longitudinally during the course and different treatment phases of CKD [13]. Thus, we have set out to investigate patients from predialysis to dialysis and post-treatment using the aim to study how immunosuppression impacts oral microbiome even after dental infection foci have been eradicated already at the predialysis stage. We hypothesized that immunocompromised CKD patients harbor atypical pathogens in their periodontal pockets in addition to the common bacterial findings.

**Materials and Methods**

**Patient data**

Eighteen patients (13 men, 5 women) with CKD aged 39 to 84 years (mean age 60.6 years) were enrolled to the study. Clinical and radiological oral examination was conducted at the Departments of Nephrology and Oral and Maxillofacial Diseases of the Helsinki University Hospital (HUH), Helsinki, Finland, during the year 2015.
Two samples were taken from each patient and from the same sites, one for cultivation in the hospital laboratory of periodontal pathogenic bacteria Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Tannerella forsythia (Tf), Parvimonas micra (P.m), and Campylobacter rectus (C.r). Presence of live pseudomonas bacteria in pocket samples was analyzed with selective Pseudomonas agar plates (24-48 h at +37°C) supplemented with CFC (cephalothin, fucidin, cetrimide) (Merck Millipore, Billerica, MA, USA). Identification of live bacterial strains were performed with the API20NE method according to the instructions by the manufacturer (BioMerieux, Marcy-l’Étoile, France).

Cotton swabs were used to sample the dorsal surface of the tongue or cheek mucosa for detecting yeasts. The Transpocult® dip-slide method was used for cultivation (Copan Diagnostics Inc., Murrieta, CA, USA). Resting saliva was collected for 5 min using a free-flow method as previously described [18]. Stimulated saliva samples were collected for 5 min by giving the patients a 1 g piece of paraffin to chew. Salivary flow rates were measured as milliliter per minute.

Statistical analyses

Associations of uncommon bacterial findings of pathogens and Candida were analyzed by cross-tabulation and chi-square tests. TDI and PIBI were reported by median values with an interquartile range (IQR) and P-values obtained from a Wilcoxon matched-paired signed-rank test. Median distribution with IQR (Interquartile range) values were analyzed by cross-tabulation and chi-square tests. P-values obtained from the Wilcoxon matched-paired signed-rank test were reported by median values with an interquartile range (IQR) and P-values obtained from a Wilcoxon matched-paired signed-rank test.

Results

The eighteen patients had the following etiology of their CKD: diabetic nephropathy (N=8), IgA nephropathy (N=5), polycystic kidney disease (N=3), interstitial nephritis (N=1) and MPO vasculitis (N=1). In twelve patients, all oral infection foci had been treated at the predialysis stage in our hospital between the years 2000-2005.

By the follow-up, sixteen patients had received a kidney transplant, one patient was in hemodialysis since the year 2008 (patient #6), and one patient was still at the predialysis stage (patient number 18) (Table 2). All patients were under immunosuppressive treatment with the exception of two cases (patient’s number 6 and number 18). In the patient number 18 with MPO vasculitis the immunosuppression had been stopped after previous examination. The immunosuppressive treatment included a calcineurin inhibitor (either cyclosporine A or tacrolimus), an antitumoristabolite (mofetil mycophenolate [N=17] or azathioprine [N=1]), and steroids (N=1) (Table 2).

At the predialysis stage, the median TDI score was 3 (IQR 2.0 - 3.0) and the median PIBI score was 6 (IQR 2.0 - 13.0). The median scores decreased to 2 (IQR 0.0 –3.0) for TDI and to 0 (IQR 0.0 –1.25) for PIBI, indicating good oral health at the predialysis stage in most of the patients (P=0.028 and P=0.016) whose oral infection foci had been treated at the predialysis stage. Both stimulated and unstimulated salivary flow-rates decreased at the post-transplantation stage in these patients (P= 0.025 and P= 0.031) (Table 3).

We further analyzed pooled subgingival plaque samples from the periodontal pockets of 17 patients. In one patient, we were unable to recover enough biological material. The result of the culture was: Prevotella intermedia (13/17; 76%) > Parvimonas micra (7/17; 41%) > Porphyromonas gingivalis (5/17; 29%) > Campylobacter rectus (3/17; 18%) > Tannerella forsythia (2/17; 12%) > Aggregatibacter actinomycetemcomitans (0/17; 0%).

Culture screening of the Pseudomonas selective agar plates revealed uncommon bacterial findings in 33% (6/18) of the patients. S. maltophilia (Sm) was detected in three patients, C. meningosepticum (Cm) in two patients, and P. aeruginosa (Pa) in one patient. Candida species, C. albicans was the most commonly detected (N=7), while C. kruisi was found in one patient. All six patients with uncommon

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**Table 3: Comparison of TDI and PIBI scores and salivary flow rates of patients at the predialysis and post-transplantation stage.**

<table>
<thead>
<tr>
<th>Pre-dialysis stage (N=12)</th>
<th>Post-transplantation stage (N=18)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDI**†</td>
<td>3.0 (2.0-3.0)</td>
<td>2.0 (0.0-3.0)</td>
</tr>
<tr>
<td>PIBI*</td>
<td>6.0 (2.0-13.0)</td>
<td>0.0 (0.0-1.25)</td>
</tr>
<tr>
<td>Stimulated salivary flow-rate (&gt;0.1 ml/min)</td>
<td>1.20 (0.78-1.18)</td>
<td>0.61 (0.22-0.91)</td>
</tr>
<tr>
<td>Unstimulated salivary flow-rate (&gt;0.7 ml/min)</td>
<td>0.40 (0.17-0.80)</td>
<td>0.21 (0.85-0.63)</td>
</tr>
</tbody>
</table>

*Six patients were not attended in the predialysis examination
*Six patients were not attended in the predialysis examination
*P-values obtained from the Wilcoxon matched-paired signed-rank test
†TDI value missing from one patient in the predialysis stage, the median obtained from 11 patients

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**Table 4: Cultivation of VMGA subgingival dental plaque samples from 6 patients at the post-transplantation stage with uncommon findings of S.maltophilia*, *Cryseobacterium meningosepticum** and *Pseudomonas aeruginosa***.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Patient number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1*</td>
</tr>
<tr>
<td>A.a</td>
<td>-</td>
</tr>
<tr>
<td>P.gingivalis</td>
<td>0.10%</td>
</tr>
<tr>
<td>T.forsythia</td>
<td>-</td>
</tr>
<tr>
<td>P.intermedia</td>
<td>-</td>
</tr>
<tr>
<td>P.micra</td>
<td>1%</td>
</tr>
<tr>
<td>C.rectus</td>
<td>+</td>
</tr>
</tbody>
</table>

*CFU: Colony Forming Unit.

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"Cotton swabs were used to sample the dorsal surface of the tongue or cheek mucosa for detecting yeasts. The Transpocult® dip-slide method was used for cultivation (Copan Diagnostics Inc., Murrieta, CA, USA). Resting saliva was collected for 5 min using a free-flow method as previously described [18]. Stimulated saliva samples were collected for 5 min by giving the patients a 1 g piece of paraffin to chew. Salivary flow rates were measured as milliliter per minute.

Statistical analyses

Associations of uncommon bacterial findings of pathogens and Candida were analyzed by cross-tabulation and chi-square tests. TDI and PIBI were reported by median values with an interquartile range (IQR) and P-values obtained from a Wilcoxon matched-paired signed-rank test. Statistical software program SPSS version 22 was used for analyses (Chicago, Illinois, USA).

Results

The eighteen patients had the following etiology of their CKD: diabetic nephropathy (N=8), IgA nephropathy (N=5), polycystic kidney disease (N=3), interstitial nephritis (N=1) and MPO vasculitis (N=1). In twelve patients, all oral infection foci had been treated at the predialysis stage in our hospital between the years 2000-2005."
bacterial findings were dentate but three of them had partial dentures. No association was found between the periodontal bacteria and S. maltophilia, C. meningosepticum, or P. aeruginosa.

No statistical difference between uncommon bacterial findings and the stimulated or unstimulated salivary flow rate and the total number of daily drugs could be found.

The patients with uncommon bacterial findings are described in more detail below and in Table 4-6.

**Patients with S. maltophilia**

All three patients with S. maltophilia (numbers 1, 2 and 3 in Tables 2,4 and 6) had IgA nephropathy diagnosis. After peritoneal dialysis (PD) treatment, they had received kidney transplant 7, 9 and 11 years before the last oral examination. Patient #1 suffered from recurrent Herpes zoster lumbar infection and had previously had four peritonitis episodes during a PD treatment period. Peritonitis had not been caused by oral bacteria but by Micrococcus and Enterobacter cloaceae. After transplantation, patient 2 had had one infection caused by Staphylococcus sp. Patient #3 had had three infections, caused by Campylobacter, Legionella, and one infection that could not be specified. Patients 1 and 3 had partial dentures with good condition and had no clinical signs of yeast or bacterial infections. However, both these patients had positive oral culture of C. albicans and reduced salivary flow (hyposalivation).

**Patients with C. meningosepticum**

Patient #6 (Tables 2,4 and 6) with C. meningosepticum found in subgingival sample had polycystic kidney disease and was in hemodialysis treatment for seven years. He also had severe atherosclerosis and due to poor general health was not considered a candidate for kidney transplantation. He also had a history of C. albicans and C. krusei. The other patient #9, (Tables 2,4 and 6) with C. meningosepticum was negative. Since 2011, he had also suffered from P. aeruginosa infection and had hyposalivation. Culture for C. albicans was positive.

**Discussion**

Overall, chronic kidney disease patients of the present study had fairly good oral health at the post-transplantation stage as shown by the TDI and PIBI scores, and by lack of deep periodontal pockets. This finding was not surprising since all CKD patients in our hospital go through a careful oral examination with subsequent treatment of infections before entering dialysis and before they are put on the list for organ transplantation. In spite of this, however, uncommon bacterial findings were found from the patients, namely P. aeruginosa in one, C. meningosepticum in two and S. maltophilia in three patients. Candida was also prevalent and hyposalivation was a common observation. In addition, many patients had cultivable periodontal bacteria: P. intermedia in 13/17 patients, M. micro in 7/17, P. gingivalis in 5/17, C. rectus in 3/17, and T. forsythia in 2/17. These periodontal bacterial findings were not associated with the uncommon bacteria, however.
P. aeruginosa is a respiratory pathogen found in immunocompromised patients such as patients with cystic fibrosis or HIV infection [1,5]. Before colonizing lungs it may cross different anatomical sites such as the nose, the paranasal sinuses, and the oral cavity. The source is unclear. It has been reported that the patients are initially colonized by a single environmental strain that persists for several years [1,5].

P. aeruginosa with periodontal bacteria P. gingivalis, F. nucleatum, A. actinomycetemcomitans, T. forsythia, T. denticola, P. intermedia, E. corrodens, C. rectus, and P. micra has previously been detected in subgingival plaque samples and saliva from patients with cystic fibrosis or with periodontal disease pointing to the possible role of oral cavity as reservoir for P. aeruginosa [1,19]. P. aeruginosa has also been found in plaque samples from removable dentures of elderly patients further indicating that oral cavity can harbor it [4]. These reports are in line with findings of the present study. P. aeruginosa is resistant to antibiotics and difficult to eradicate as was the case also in our patient who had persistent lower limb infections colonized by P. aeruginosa for several years.

S. maltophilia (previously known as Pseudomonas maltophilia, Xanthomonas maltophilia) is a nosocomial pathogen causing infections in immunocompromised patients. It forms biofilms and is resistant to antibiotics [20]. It has been an etiologic agent of catheter-related infections [21]. Patients with type 1 diabetes have been reported to have higher frequencies of S. maltophilia DNA fragments in their sera compared with non-diabetic subjects. In patients with diabetes, levels of serum IgA antibodies against pseudomonas bacteria correlated significantly with the serum, C-reactive protein [22]. S. maltophilia was detected in the oral cavity of two of our CKD patients. S. maltophilia is an important nosocomial pathogen among immunocompromised patients and it may be life-threatening especially in hospitalized patients [21]. The increase in S. maltophilia detection rates may be associated with the changes in antibiotic use and changing nature of hospital patient population in general [23]. Therefore, it is important to prevent colonization of these bacteria and note that it can also be harbored in the mouth.

Chryseobacterium spp. are organisms of low virulence and therefore their presence usually indicates colonization but not infection, except for C. meningosepticum, formerly known as Flavobacterium meningosepticum. This microorganism is known to cause a variety of infections in immunocompromised patients, such as dialysis and kidney transplant patients, or in patients with diabetes, those on immunosuppressive medications, or with neutropenia. Since C. meningosepticum is inherently resistant to most antibiotics it can be a potential nosocomial pathogen [24,25].

In our study, the uncommon bacterial findings were associated with positive culture of Candida species. Candida albicans may be part of the commensal microbial flora, but when the host immune defenses are impaired recurrent infections may occur, as was seen in our study. Hyposalivation increases the risk for Candida colonization and patients taking multiple prescribed medications daily have significantly lower stimulated or unstimulated saliva flow rates than those with less daily medications [10,11]. However, in our study where saliva flow rates were seen to be decreased in the post-transplantation stage this was not the case in two patients with uncommon bacterial findings.

Finally, it needs to be pointed out that P. aeruginosa, S. maltophilia, and Chryseobacterium spp. are uncommon pathogens mainly found in immunocompromised patients and that these bacteria cannot be found by routinely used conventional culture methods for periodontal pathogens. This was also the case in the present study.

Conclusion

Our study hypothesis was confirmed since the results showed that CKD patients’ oral cavity indeed harbored both nosocomial and opportunistic pathogens probably due to immunosuppressive treatment or the disease itself. The often encountered concomitant Candida infection might also alter the oral microbiota. Furthermore, even though TDI and PIBI indexes were low at the post-transplantation stage indicating good oral health in most of our patients, the present results emphasize the role of the oral cavity as a source of potentially dangerous pathogens in immunocompromised patients thus challenging the oral health care personnel and diagnosis.

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Competing Financial Interests

P-H.G. has received lecture honoraria from Abbot, Boehringer Ingelheim, Cebix, Eli Lilly, Genzyme, Novartis, Novo Nordisk, and MSD, and research grants from Eli Lilly and Roche. P-H.G. is an advisory board member for Boehringer Ingelheim, Novartis, and Medscape.

References


