CLINICAL ORAL IMPLANTS RESEARCH

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Peri-implant parameters in head and neck reconstruction: influence of extraoral skin or intraoral mucosa

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Key words: head and neck reconstruction, irradiation, peri-implant disease, periodontal pathogen, yeast

Abstract

Objective: This study is designed to assess dental implants supporting overdentures in edentulous patients with operated head and neck malignancies using parameters to detect peri-implant disease.

Material and methods: Thirty-four implants supporting overdentures in 34 oral cancer patients were examined. Clinical parameters [plaque index, probing depth, bleeding on probing (BOP), origin of peri-implant soft tissue, and amount of irradiation] were recorded, and microbiological identification of periodontal pathogens was carried out by DNA–DNA hybridization. To identify yeast species, the samples were cultivated on Sabouraud agar plates and subsequently identified by API 20C AUX plates. An implant site showing BOP, probing pocket depth (PPD) $\geq$5 mm and radiographic vertical bone loss was considered to have peri-implant disease.

Results: Colonization by periodontal pathogens was found on 15 implants, while yeast species were found in 14 cases. Using a univariate analysis, none of the investigated parameters (microbiologic sign, detection of yeast, origin of peri-implant soft tissue and irradiation) were significantly correlated to signs of peri-implant disease. In the multivariate analysis, yeast [odds ratio (OR) 12.32, $P=0.033$] and periodontal pathogen (OR 9.88, $P=0.046$) were significant predictor variables for peri-implant disease. Yeasts were less frequently detected around implants placed in re-vascularized skin flaps if irradiation was set as a confounder ($P=0.019$).

Conclusions: With respect to the pilot study nature of the study peri-implant soft tissue origin and irradiation had little influence on the development of peri-implant disease. Yeast and periodontal pathogen were explanatory variables for the development of peri-implant disease. Considering the effect of irradiation on the prevalence of yeast, yeast was less frequently observed in peri-implant soft tissue of the skin. Based on these data, future studies on the role of yeast and soft tissue in peri-implant disease should be encouraged.

Large soft tissue defects can be reconstructed with microvascularized tissue flaps (forearm-free flap, fibula-free flap) including extraoral skin.

Rehabilitation after head and neck cancer therapy is influenced by surgical reconstruction and adverse effects of radiation therapy (Al-Nawas et al. 2006). Reconstructive surgery is not able to fully replace intraoral anatomic structure and function, and hence, frequently fails to support dentures properly. The transplanted graft, which is often too thick, may be unstable and inadequate to support removable dentures (Schliephake et al. 1999). In addition, irradiated oral mucosa

is commonly intolerant to the friction caused by the denture base. Therefore, for the functional rehabilitation of patients after head and neck cancer, implant-supported dentures are clinically well accepted (Gurlek et al. 1998; Mericske-Stern et al. 1999; Schliephake et al. 1999). These treatment modalities for oral cancer can jeopardize the long-term success of dental implants.

Peri-implant microbiology has been well established in many publications (Mombelli et al. 1987; Apse et al. 1989; Mombelli & Mericske-Stern 1990; Alcoforado et al. 1991; Leonhardt et al. 1992; Lee et al. 1999a); however, there are relatively few reports about the microbiologic profiles of dental implants placed in cancer patients who have undergone various treatment modalities (Kwon et al. 2008).

The composition of the peri-implant microbiota around failing implants in healthy patients closely resembles that of the subgingival microbiota associated with advanced periodontal disease (Mombelli et al. 1987; Apse et al. 1989; Quirynen & Listgarten 1990; Papaioannou et al. 1996; Leonhardt et al. 1999; Rutar et al. 2001). Patients with a history of periodontal disease have a high prevalence of anaerobic putative periodontal pathogens, such as *Porphyromonas gingivalis*, *Prevotella intermedia* and *Fusobacterium nucleatum*, 3–6 months after either one- or two-stage implant placement (Mombelli et al. 1995).

Radiation therapy to the head and neck has been reported to increase the proliferation of *Candida* in the oral cavity (Brown et al. 1975; Almstahl & Wikstrom 1999), but the correlation between peri-implant health and the presence of yeast has rarely been considered, although irradiated patients are increasingly being treated with dental implants.

The aim of this study is to investigate the influence of irradiation, soft tissue reconstruction and the microbiologic environment in the presence of peri-implant disease around implants in patients after treatment for head and neck cancer.

Material and methods

Patients

The present study population consisted of 34 patients (19 males, 15 females, mean age: 65.2) who were undergoing periodic check-ups for oral cancer in the clinic for oral and maxillofacial surgery, University Clinic of Mainz. All the patients had been followed up more than 3 years after a major surgery.

The patients had undergone major ablative surgery and implant reconstruction supporting overdentures. The retentive system of the dentures was either a ball attachment or a bar and clip attachment. No specialized items such as magnets were used. They were fully edentulous, and the implants were uncovered at least 6 months before the exam. The patients who had taken antibiotics in the 6 weeks before the exam were excluded, and the patients who had been on steroid therapy or chemotherapy during the last 3 months were also ruled out. Immunocompromised patients such as patients with HIV infections or autoimmune mucosal disease were excluded.

We also excluded partially edentulous patients due to the difficulty in categorizing the patients according to the various individual status, such as the number of remaining teeth and periodontal conditions.

The implant with the deepest pocket was selected in each patient.

Clinical parameters

Modified plaque index (mPI) (Mombelli et al. 1987) and bleeding on probing (BOP) were assessed. To evaluate the influence of the soft tissue type, the origin of peri-implant soft tissue was rated as mucosa or skin. Owing to the major tissue resection, the mucosa around the implants was non-keratinized mucosa. Orthopantomographs of all the subjects were examined to evaluate the bone loss around dental implants.

In order to evaluate the effect of irradiation, patients were classified dichotomously as either having received or not having received irradiation. All patients who had undergone the radiation therapy finished the therapy more than 12 months ago from this study, and so the effects can be referred to as 'late radiation effects' (Cox et al. 1995). None of the patients were treated with bisphosphonates.

Peri-implant disease

The presence of peri-implant disease around the implant was determined based on the definition established by several authors (Karoussis et al. 2004; Ferreira et al. 2006) [probing pocket depth (PPD) $\geq$ 5 mm, positive to BOP and vertical bone loss], and the parameter was expressed as a dichotomous variable ('present' or 'absent'). PPD of the implants was measured at the mesial, distal, lingual and vestibular aspect of the implants using a plastic probe (Plast-O-Probe, de Trey, Germnay) (Sanderink et al. 1983). The PPD was taken by a single experienced clinician in implantology (Y.-D.K.). The maximum PPD of each implant was used for statistical evaluation. Subsequently, a microbial sample was collected using a sterile paper tip inserted into the deepest part of the pocket for 10 s.

Microbiologic assessments

The micro-IDent plus DNA Probe kit (HAIN-lifescience GmbH, 72147 Nehren, Germany) was used to identify *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Treponema denticola*, *P. gingivalis*, *P. intermedia*, *Peptostreptococcus micros*, *F. nucleatum/periodonticum*, *Campylobacter rectus*, *Eubacterium nodatum*, *Eikenella corrodens* and *Capnocytophaga* sp. The paper tips were stored in a dry place and were immediately mailed to the manufacturer's laboratory for the examination of periodontal pathogens using a three-step technique: (1) DNA isolation, (2) amplification with Biotin-marked primers, and (3) reverse hybridization. The data are reported as dichotomous variables; periodontal pathogens are either 'present' or 'absent.'

For the collection of yeast study samples, a sterile paper tip was inserted into the deepest part of pocket and removed after 10 s. Cotton rolls were used to prevent salivary contamination. The paper tip was placed into an Eppendorf tube with 1 ml of sterile water and vortexed for 20 s.

Serial dilutions of the vortexed samples were spread on the SAB agar plates, and the plates were incubated for 24 and 48 h. Following incubation, the colonies were counted and the yeast sample taken from the agar plate was processed with Suspension medium® and C-Medium® (Biomerieux, Marcy-l'Etoile, France). Then, the samples were inoculated in API-20C AUX (Biomerieux) for 48–72 h to identify the type of the yeast. The data are given dichotomously as either yeast 'present' or 'absent.'

Statistical analysis

The dichotomous variables 'irradiation,' 'soft tissue origin,' 'detection of yeast' and 'periodontal pathogen' were descriptively analyzed using crosstabs with 'peri-implant disease' as a dependent variable, and χ^2 with Fisher's exact was used for the univariate analysis. Owing to the 'pilot study nature' of these data, no adjustment for multiple testing was performed, resulting in local statistical significance. To identify independent predictive variables for peri-implant disease, a multivariate analysis was performed. These clinical and microbiological data were collected once per a patient.

Results

Transplanted skin tissue was observed around nine implants and oral mucosa was surrounding the remaining implants ($n = 25$). Colonization of periodontal pathogenic bacteria that could be identified by micro-IDent plus was observed in 44.1% (15 patients out of 34 patients). *P. gingivalis* was found on one implant and *T. forsythia* was found on three implants; in one implant, both *P. gingivalis* and *T. forsythia* were found. Ten of these were from a peri-implant pocket surrounded by mucosa, and five were surrounded by the transplanted skin counterpart. In 14 cases (44.1%), yeasts were identified, with four cases of *C.albicans*, nine cases of *Candida glabrata* and one case of *C. tropicalis*.

Sixteen patients were positive for signs of peri-implant disease. All the implants were still osseointegrated and none of those were mobile. In the crosstable analysis, the presence or absence of 'irradiation,' 'soft tissue origin,' 'yeast' and 'periodontal pathogens' were correlated to the presence or absence of 'peri-implant disease' (Table 1). The prevalence of peri-implant disease at the mucosa and soft skin tissue was similar. Irradiation, yeast and periodontal pathogens showed a tendency to be higher at implants with peri-implant disease, although this trend showed no statistical significance in the univariate analysis.

In the multivariate analysis (Table 2), two parameters, periodontal pathogen [odds ratio (OR) 9.918, $P = 0.046$] and yeast (OR 12.76, $P = 0.033$), were assumed to be potential explanatory variables for the development of peri-implant disease.

Table 1. **χ^2 and Fisher's exact tests for the four parameters based on clinical signs of peri-implant disease**

Parameters	Group	Clinical signs of peri-implant disease		*P*-value
		Negative	Positive	
Irradiation	No	11 (68.8%)	5 (31.3%)	0.082
	Yes	7 (38.9%)	11 (61.1%)	
Soft tissue origin	Mucosa	13 (52%)	12 (48%)	1
	Skin	5 (56%)	4 (44%)	
Detection of yeast	Negative	13 (65%)	7 (35%)	0.163
	Positive	5 (36%)	9 (64%)	
Periodontal pathogen	Negative	12 (63.2%)	7 (36.8%)	0.179
	Positive	6 (40%)	9 (60%)	

Table 2. **Statistical analysis for the four parameters based on clinical signs of peri-implant disease**

Parameters	Odds ratio	95% confidence interval	Significance
Irradiation	0.854	0.065–11.264	0.904
Soft tissue origin	1.145	0.176–7.311	0.887
Detection of yeast	12.76	1.179–138.068	0.036
Detection of periodontal pathogens	9.918	1.045–94.113	0.046

Table 3. **Distribution of yeasts according to soft tissue and irradiation ($P = 0.019$*)**

Irradiation	Soft tissue	Yeast		*P*-value**
		Negative	Positive	
No	Mucosa	12	2	1
	Skin	2	0	
Yes	Mucosa	1	10	0.013
	Skin	5	2	

*calculated by a Mantel–Haenszel common odds estimate and
**were calculated by Fisher's exact tests.

We paid special attention to the parameter of soft tissue origin. Although a low incidence of yeast was noted in the peri-implant soft tissue originating from the transplanted extraoral skin, the result was not statistically significant ($P > 0.05$). In order to investigate the interaction effect, χ^2 with Fisher's exact tests were carried out according to the presence and absence of irradiation. In the non-irradiated group, the result was not significant ($P = 1$) but there was a significant result ($P = 0.013$) in the irradiated group (Table 3). After designating the parameter 'irradiation' as a confounder (Table 3), we saw significantly more frequent yeast at the irradiated mucosa than at the irradiated skin (Mantel–Haenszel χ^2, $P = 0.019$).

Discussion

Four parameters (irradiation, soft tissue origin, detection of yeast and identification of periodontal pathogen) were evaluated as risk factors for peri-implant disease. It was believed that the elimination of the subgingival environment by extracting all natural teeth probably initiated the disappearance of periodontopathogenic bacteria such as *A. actinomycetemcomitans* (Kononen et al. 1991; Danser et al. 1995, 1997). However, owing to DNA probe technology, periodontal pathogens belonging to red and orange complexes have been found even in fully edentulous patients with a past history of periodontitis (Lee et al. 1999b; Leonhardt et al. 1999). Nevertheless, the unfavorable ecology associated with complete edentulism still decreased the incidence of periodontopathogens such as *P. gingivalis* (Leonhardt et al. 1999). In our study on fully edentulous cancer patients, orange or red complex periodontopathogens were found in only three patients. Owing to the loss of major bacterial reservoirs, the implants are less likely to be infected by severe peri-implant disease (Lang 2008). This is true despite the frequent presence of deep pockets, which may

be attributed to the thick bulk of the microvasularized flap and the vestibular position of implants due to the altered anatomy following the reconstruction. One of the major contributing factors to the establishment of peri-implant microbiota is the past history of advanced periodontitis (Mombelli et al. 1995).

The effect of radiation therapy on oral microflora has been mentioned in several reports (Weischer et al. 1996; Al-Nawas & Grotz 2006), in which a high number of cariogenic pathogens were found in irradiated patients in spite of extensive oral hygiene. In accordance with other studies, the general profile of the periodontal pathogens seemed to have no correlation with radiation therapy in this study.

Yeasts in peri-implant tissue have not been well described so far, but they can cause mucosal inflammation. Although *Candida albicans* is a frequent post irradiation yeast species, *C. glabrata,* which is a recently emerging cause of oropharyngeal candidiasis after radiation therapy (Redding et al. 2004), was the most frequently observed yeast in our study. In the multivariate analysis, yeast was a potential explanatory variable in the development of peri-implant disease in this study. Because of the special nature of the subjects in this study, the number of the samples was relatively small for the multivariate test; therefore, further studies involving several referring clinics should be encouraged to support this preliminary result of these research.

BOP was established as a parameter to predict the activation of periodontal diseases (Lang et al. 1990). In a prospective study, BOP was available as a diagnostic measure in dental implants (Luterbacher et al. 2000). In the third ITI consensus conference, a periodic check of BOP is recommended to monitor peri-implant soft tissue conditions (Salvi & Lang 2004) and this was clinically validated during the sixth European workshop on periodontology (Lindhe & Meyle 2008). However, in irradiated patients, BOP might not be a reliable diagnostic parameter because the typical side effects of irradiation are radiation-induced mucositis and proliferation of *Candida* developing *Candida*-induced mucositis.

It is clear that therapeutic radiation can increase *Candida* prevalence; therefore, we should take irradiation into account in order to evaluate the effect of peri-implant soft tissue origin on *Candida* presence. Regarding the origin of peri-implant soft tissue (mucosa or skin), which has not been studied so far, it was believed that a transplanted skin flap was mucosalized. However, it was assumed that the free flap, although clinically resembling mucosa, retains the histological features of the skin (Beahm et al. 1997; Khan et al. 2001). They attempted to show a correlation between the intraoral skin flap and the presence of *Candida*, but they failed to do so. When the possible effects of different soft tissue origin were properly investigated with 'irradiation' designated as a confounder, we may assume that yeast prevalence is lower in skin-originating peri-implant soft tissue than in the mucosa-originating counterpart, but a larger subject study will still be necessary to support our results. The clear correlation of the presence of yeast to peri-implant disease should be subject to further studies on non-irradiated and partially edentulous patients to elucidate the possible pathophysiological role of yeast in peri-implant disease.

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