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ORIGINAL ARTICLE

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Prospective study of the long term change of the oral flora after radiation therapy

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Abstract *Objectives:* The aim of this prospective study was to evaluate the long term change in oral pathogens following radiation therapy. *Methods:* Twenty-two patients with planned radiation therapy (>30 Gy) of head and neck squamous cell carcinoma were included. Before radiation therapy, after 3, 6, and 12 months samples from the deepest periodontal pocket were drawn. Five major periodontal pathogens were studied using DNA probes (*Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Bacteriodes forsythus, Treponema denticola*). Stimulated saliva was sampled for the microbiologic study of caries pathogens (*streptococci and lactobacilli*). Plaque index and decayed, missing, filled tooth surfaces (DMF-S) were recorded. *Results:* A normalisation of the caries bacteria is not found correlating to a significant increase in the number of affected teeth (DMF-S) from 80.7 to 88.5 after 12 months. The plaque index remained unchanged. The incidence of periodontal pathogens did not significantly change during the follow up. *Conclusion:* In contrast to radiation caries there seems to be no microbiological evidence for "radiation periodontitis". Despite of the intensive oral hygiene no reduction of the high number of caries pathogens is found, which leads to a high risk of tooth decay even 12 months after radiation.

Keywords Caries pathogens · Mucositis · Periodontal pathogens · Radiation caries

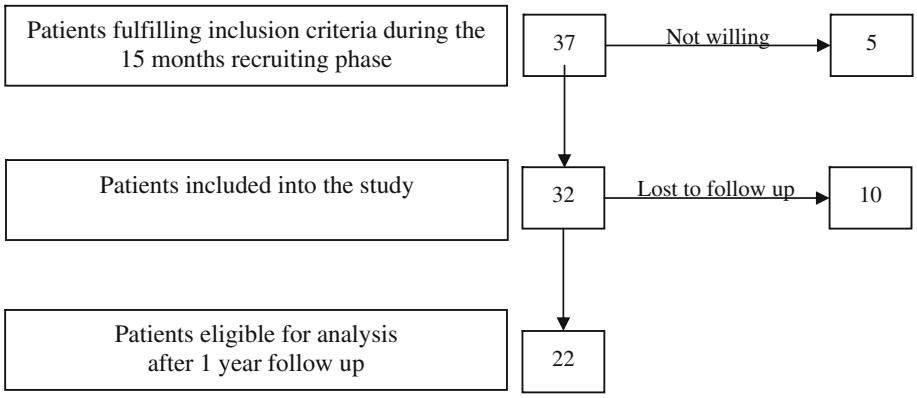
Introduction

Delayed wound healing of soft tissue and bone wounds after radiation therapy can cause major clinical problems even years after radiation therapy. Special surgical techniques for all dental and maxillofacial surgical procedures is required to lower the risk of the development of ("infectious") osteoradionecrosis [1]. The current concept of tissue damage mainly relies on the theory of hypocellular, hypovascular and hypoxic tissue after radiation [2, 3]. Besides these change in functional parameters, a short term change in the oral flora is widely accepted [4–7]. Some clinical concepts of prophylaxis of mucositis during radiotherapy include *Candida* species [8]. Together with a colonisation with yeast an increased bacterial colonisation is presumed. Early studies from the seventies and eighties emphasize on a correlation between the reduction of the amount of saliva, lower pH and acidophilic organisms, which can indirectly lead to radiation caries [9–12]. Due to better diagnostic techniques, using the wide spread use of molecular biological techniques, the pathogenesis of periodontal disease and caries has been related to major pathogens. Only few data are found on a possible change in the incidence and number of these major pathogens in patients under radiation therapy. One recent clinical study focuses on *lactobacilli* and *streptococci* as primary criteria for the effect of a fluoride gel in patients who underwent radiation therapy [11]. A clinical intervention study with a selective reduction of Gram-negative bacteria however showed little effect in the reduction of mucositis [12]. Regarding the major periodontal pathogens to our knowledge no data for radiotherapy patients is found. In contrast to the well documented short term change in oral flora after radiotherapy, a long term normalisation of the oral flora after radiation therapy is only presumed.

Patients and methods

The aim of this prospective study is the long term evaluation of the incidence and number of periodontal and caries pathogens in patients who underwent radiotherapy

Table 1 Flow chart of patient recruiting and follow up



for head and neck squamous cell carcinoma. Intraindividual controls were used in this one arm longitudinal study. The data is related to scintigraphic salivary flow rate, *Candida* colonisation and mucositis scoring which were reported earlier [13]. Inclusion criteria were: (1) Planned radiation therapy of the head and neck area. (2) Oral cavity and the salivary glands covered by the radiation field. (3) Radiation dose >30 Gy. (4) After surgical removal of all teeth with poor prognosis prior to radiation, at least four teeth had to remain within the radiation field.

The protocol was approved by the local ethics committee (837.089.98) and all patients gave written informed consent.

All patients with carcinoma of the head and neck who were seen for pre-radiotherapeutic dental care at the Oral and Maxillofacial Surgery of the J. Gutenberg University Mainz in a 15-month time interval were screened for possible inclusion into this study. Before start of the radiotherapy intensive oral hygiene instructions and professional plaque removal were performed due to established clinical standards [14]. During the radiotherapy all patients were administered a prophylaxis protocol for mucositis consisting of 5% Dexpanthenol oral rinse (Bepanthen Roche Lösung, Roche Nocholas, Germany), 100,000 I.E. Nystatin (Nystaderm S Suspension, Dermapharm, Germany) and 1 g Sulcralfat (Ulcogant Suspension, Merck, Germany) each thrice daily during the radiation period. A 1.25% flouride gel (Elmex Gelée, GABA Germany) and 1% chlorhexidine gel (Corsodyl Gel, GlaxoSmithKline Consumer Healthcare Germany) was administered in an altering mode two times daily using individual splints [13]. Oral prophylaxes were performed directly following each examination. The first examination (U0) was done before start of the radiation therapy together with the plaque removal and the oral hygiene instruction. The following examinations were done 3 (U3), 6 (U6) and 12 (U12) months following U0 (start of radiotherapy). Plaque index according to Sillness and Loe was recorded. The dental status was recorded using the DMF-S index, which counts the decayed, missing, filled tooth surfaces [15]. In 12 of the 22 patients a sterile paper tip was used to draw a sample from the clinically deepest periodontal pocket. The qualitative test for five periodontal pathogens was preformed using a commercially available DNA probe (MicroDent, Hain Diagnostika, Nehren Germany. The PCR f[16] or the following major periodontal pathogens was performed by the manufacturer: *Actinobacillus actinomycetemcomitans* (AAC), *Porphyromonas gingivalis*, *Bacteroideus forsythus*, *Prevotella intermedia,Treponema denticola*. Major caries pathogens (*streptococci* and *lactobacilli*) were cultivated on commercially available selective agar (CarioCheck plus; Hain Diagnostika, Nehren Germany) using the dip slide method [16]. For this study 1 ml stimulated saliva was inoculated on each side of the medium. Together with a CO_2 tablet the set is cultivated for 48 h at 37°C. The numbers of pathogens is given semiquantitatively after the visual exclusion of contamination by *S. faecalis*, which, in contrast to the convex colonies of *S. mutans*, shows as flat and light-blue colonies. *S. mutans* and *lactobacilli* were counted by visual examination. The test has an upper reading limit of 1,000,000 cfu/ml. A descriptive statistical evaluation was done. For the in-

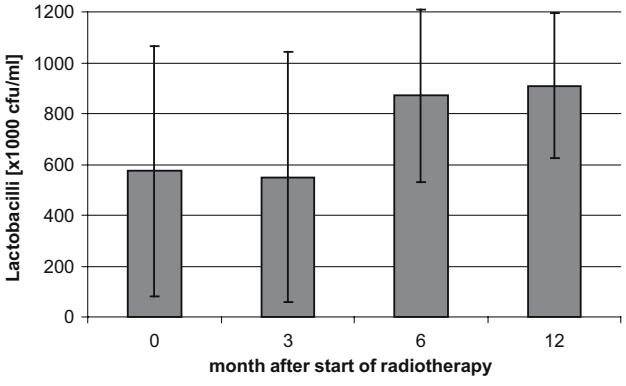


Fig. 1 Mean *Lactobacilli* counts in saliva for all 22 patients following radiation therapy of head and neck cancer

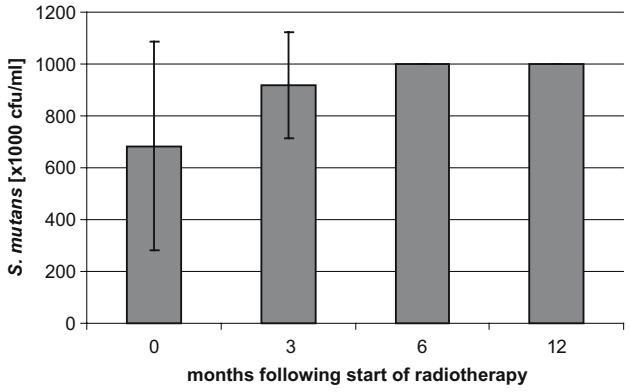


Fig. 2 Mean *S. mutans* counts in saliva for all 22 patients following radiation therapy of head and neck cancer

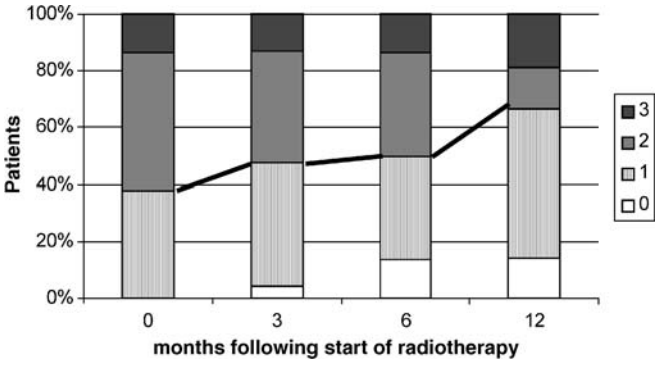


Fig. 4 Plaque index in patients following radiation therapy of head and neck cancer

traindividual testing of differences between U0 and U12 the Wilcoxon test was used for paired continuous variables (bacterial counts, dmfs values) and the Friedman test was used for the comparison of non-continuous variables.

Results

The flow chart of patient recruiting is given in Table 1. Beyond the prophylaxis protocol, no anti-infective therapy was administered during the study time. Seven of the 22 patients were female, with a mean age of 47.9 years for all patients and a mean dose of radiation of 52.9 Gy ±15.76 in fractions of 2±0.2 Gy per day. Antifungal prophylaxis and dexpanthenol rinsing was performed by all patients during the radiation phase. Six patients had stopped administration of fluoride and chlorhexidine rinsing in the second half of their radiation therapy due to severe mucositis.

The mean values of lactobacilli counts are given in Fig. 1 and indicate an increase from a mean value of 575 cfu/ml at U0 to 910 cfu/ml at U12. The results of the *S. mutans* counts are given in Fig. 2. Before start of the radiation therapy a mean number of 680 cfu/ml is found. After the radiation this number increased to 920 cfu/ml at U3 and later reached the upper detection limit of 1,000 cfu/ml. The intraindividual change in lactobacilli and streptococcal counts is given in Fig. 3. At U0–U3 no significant intraindividual change is seen. At U0–U6 and U0–U12 an increase in mean intraindividual counts of caries bacteria is seen. The changes U0–U6 showed borderline statistical significance for streptococci (p=0.102) and were not statistically significant for lactobacilli (p=0.257). The changes U0–U12 showed borderline statistical significance for both streptococci and lactobacilli (p=0.059 and 0.102).

Results of the periodontal probe analysis revealed at the start of the study (U0) in 50% of the patients positive results

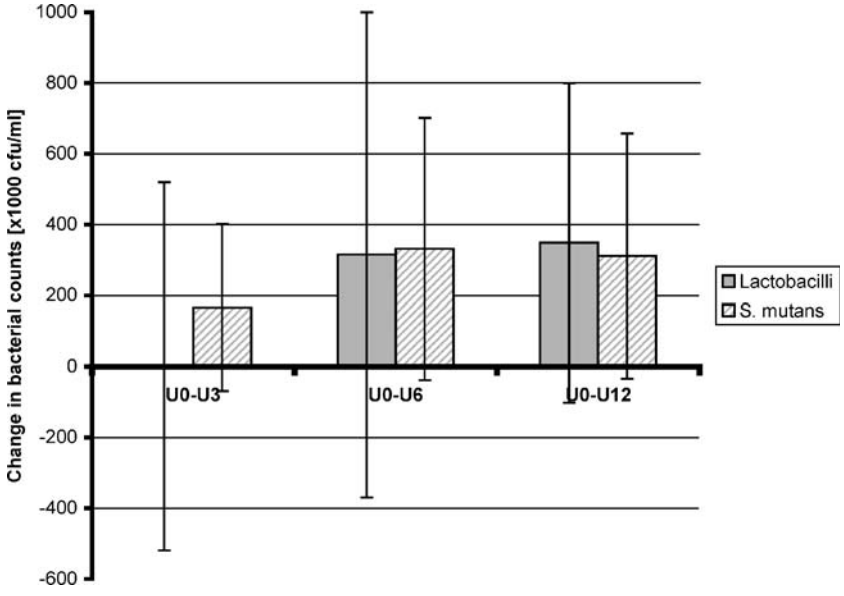


Fig. 3 Mean intraindividual (longitudinal) changes of lactobacilli and *S. mutans* counts between the time points

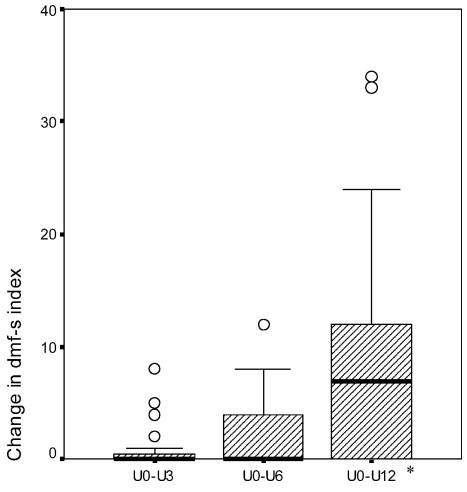


Fig. 5 Box plot of intraindividual (longitudinal) change in DMF-S in patients following radiation therapy of head and neck cancer (outliers are marked by *dots*)

for at least one periodontal pathogen. This rate increased to 80% at U3, 71% at U6 and 64% at U12. This difference between the different time points was not statistically significant. At U0 the periodontopathogenic flora consisted of *B. forsythus* in a high percentage of positive findings, followed by *T. denticola* and *P. gingivalis*. Only few patients showed a positive reaction for AAC or *P. intermedia.* During the follow up a reduction of the incidence of AAC, *P. gingivalis, B. forsythus* and *T. denticola* and a relative increase of *P. intermedia* without statistical significance was found.

The plaque index values are shown in Fig. 4. Despite the oral hygiene instruction no significant intraindividual change in plaque indices is seen (p=0.76). However a clinically relevant increase in patients with good and moderate oral hygiene can be noted (Plaque index 0 and 1). The DMF-S values as given in Fig. 5 show a significant increase at U0–U12 (p=0.001).

Discussion

In comparison to epidemiologic studies the patients in this study are representative for head and neck cancer [17]. Radio-xerostomia and radiation caries represent the major chronic side effects of head and neck irradiation [18]. Both the high consumption of short chain carbohydrates and reduced oral hygiene are known as promoting factors for radiation caries [19].

Despite of the strict concept of care including professional plaque removal our study revealed no decrease in plaque indices and caries pathogens. Even the administration of chlorhexedine gel seems not to be able to prevent the high colonisation with *streptococci* and *lactobacilli* in a significant number of patients. This is supported by the different individual development of the dmf-s index. The third German Study on Oral Hygiene (DMS III) 1997 gives an average dmf-s value of 54.7 for adults patients of 35 to 44 years [20]. These results, together with direct radiogenic damage to odontoblast viability [21], limit the hope for an effective reduction of radiation caries based on conventional concepts.

Comparable to a normal collective of non-oncologic patients half of the patients showed at least one periodontal pathogen prior to radiation. At the first post radiation visit (U3) about 6 weeks following radiation end 80% of the patients show at least one periodontal pathogen, which suggests a clinically relevant change. In contrast to the persistently high numbers of caries bacteria and *Candida* colonisation [13] our study shows a continuing normalisation of the periodontal pathogens. This supports the clinical suggestion, that in contrast to a radiation caries no long-term "radiation periodontitis" is known. Results from patients with hyposalivation of non-radiotherapeutic aetiology support our findings: In a case-control study higher numbers of caries bacteria in cases with hyposalivation are found. The incidence of periodontal pathogens remained unchanged [22]. Moreover classical microbiological data of the periimplant pocket in radiotherapy and non-radiotherapy patients revealed no differences in quality [23].

It should however be mentioned that local chlorhexidine prophylaxis might have influenced our microbiological results [24, 25]. Our microbiologic data is contrasted by clinical data from one earlier [26] and one recent clinical study [27]. Both authors focussed on clinical parameters of periodontitis and found a periodontal attachment loss of teeth within the radiation field.

With respect to the existing data on periodontitis there seems to be a direct damage to the periodontium rather than a long term microbiologic changes of the whole mouth. Changes in oral pathogens are greatly affected by the changed saliva parameters as seen for radiotherapy as well as other non-radiotherapy reason. The increase of the cariogenic microflora in contrast to the nearly unaffected incidence of periodontopathogenic pathogens might explain the better long term survival of dental implants [28] compared to natural teeth [29] after radiotherapy. This requires broad efforts in the social and orofacial rehabilitation of oral cancer patients.

Conclusion

In contrast to the well known radiation caries there seems to be no microbiological evidence for "radiation periodontitis". On the other hand there still is a need for long term data on the periodontal status of patients following radiotherapy. In the multifactor pathogenesis of radiation

caries this study reveals that the high rate of caries pathogens highlights the relevance of intensive oral hygiene care. Clinical efforts for these patients should focus on the prophylaxis of radioxerostomia [30].

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