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Candida prevalence in saliva before and after oral cancer treatment

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ABSTRACT

Background: Previous studies have shown an increased prevalence of candidiasis in patients receiving radiotherapy for head and neck cancer. However, little is known of the effect the different cancer treatment modalities have on the oral *Candida* status.
Objective and hypothesis: The objective of this study was to investigate the change in salivary *Candida* status of oral squamous cell carcinoma (OSCC) patients undergoing cancer treatment. The hypothesis was that cancer treatments change the oral microbial environment favouring an increase in the prevalence of more pathogenic non-*albicans Candida* (NAC).
Methods: We collected paraffin-stimulated saliva from 44 OSCC patients before surgery and after a minimum of 19 months of follow-up. Chromagar, Bichro-Dupli-test and API ID 32 C were used for identification of different *Candida* species and results were analysed statistically.
Results: At both timepoints, 75% of samples were *Candida* positive with *C. albicans* being the most common yeast. NAC strains were present in 16% of the pre-operative samples and 14% of the follow-up samples. The NAC species found were *C. dubliniensis*, *C. krusei*, *C. guilliermondii* (preoperatively only) and *C. glabrata* (at follow-up only). In 73% of the cases, the salivary *Candida* status remained unchanged. There was an 18% increase in the prevalence of candidiasis. However, the different treatment modalities did not statistically significantly affect the *Candida* status of the patients.
Conclusion: The intraindividual prevalence of salivary *Candida* among OSCC patients seems to be stable and different treatment modalities have little to no effect on the salivary *Candida* status.

Introduction

Oral squamous cell carcinoma (OSCC) is a disease often subject to heavy treatment regimes including either surgery alone or in combination with (chemo)radiotherapy.[1–3] During surgical tumour excision autogenous grafts are often used in wound closure and reconstruction,[1,3] which may introduce new ecologic environments in the oral cavity. This change is anticipated to influence the oral microbiota.

Candida species are commensal, yet opportunistic pathogens found on all epithelial surfaces of the human body. Oral candidiasis is associated with pain, change in taste, dysphagia, and changes in nutritional status.[4] *Candida albicans* is the most common *Candida* species found in the oral cavity;

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thus, commonly a distinction between *C. albicans* and non-*albicans Candida* (NAC) is made. Previous studies have found some NAC species to show more resistance to antifungal medication in comparison to *C. albicans*.[5–7] In addition, some NAC species have been shown to cause further pathological conditions – such as fungemia – aside from oral candidiasis.[8] While harbouring *Candida* in the oral cavity does not seem to link to increased mortality among OSCC patients, oral candidiasis is common in this patient group.[4,9] Indeed, previous studies have shown an increase in the prevalence of *Candida* infections, especially of NAC infections, among OSCC patients receiving radiotherapy.[10–12] This increase in *Candida* has been linked to radiation-induced xerostomia.[13] However, it is not clear whether the different overall treatment modalities affect the salivary *Candida* status of OSCC patients.

In the present study, we examined the prevalence of salivary *Candida* in OSCC patients before and after cancer treatment. We hypothesized that an increase in the amount in *Candida* and a shift towards more virulent NAC species would occur due to radical changes in the oral environment caused by the different treatment modalities.

Materials and methods

Forty-four patients, who were diagnosed with squamous cell carcinoma in the oral cavity and referred for protocol cancer surgery to the Department of Oral and Maxillofacial Diseases, Helsinki University Hospital, Helsinki, Finland, between the years 2011 and 2014, were enrolled in the study. These 44 patients were part of an original group of 100 patients who participated in a study on the prevalence of *Candida* in OSCC patients' saliva preoperatively[9]; however, 66 patients from this original group were lost due to patient-related factors such as moving outside the hospital district, unwillingness to continue participation, or death. The patients gave paraffin stimulated whole saliva samples on the evening before the operation and again after a minimum follow-up period of 19 months.

The inclusion of the patients was based on the diagnosis of OSCC with surgical resection of the tumour. The exclusion criteria were cancers of the lip, tonsils, larynx, and pharynx; cancers other than those of oral squamous cell origin; and the patient's inability to give informed consent.

Paraffin-stimulated whole saliva was collected under standardized conditions (30-s pre-stimulation followed by a 5-min collection by spitting into a test tube while in a forward-leaning sitting position). Salivary flow rates (SFR) were recorded both preoperatively and at follow-up. The patients' medical and dental status at both the preoperative and follow-up stages were recorded per routine treatment protocol and the hospital records were available for analyses. Basic patient characteristics as well as tumour and treatment-related data were collected from medical records.

Table 1 – Basic characteristics of the OSCC patients in comparison with their salivary *Candida* findings pre-operatively and at follow-up. Percentages shown are calculated from 44 patients.

	N	Pre-treatment, N (%)				Follow-up, N (%)			
		C. albicans	NAC	both	neither	*C. albicans*	NAC	both	neither
Female	24	12 (27.3)	2 (4.5)	3 (6.8)	7 (15.9)	12 (27.3)	2 (4.5)	2 (4.5)	8 (18.2)
Male	20	14 (31.8)	1 (2.3)	1 (2.3)	4 (9.1)	15 (34.1)	2 (4.5)	0	3 (6.8)
Location of tumour according to ICD10 classification[a]									
C02	17	9 (20.5)	2 (4.5)	1 (2.3)	5 (11.4)	10 (22.7)	0	1 (2.3)	6 (13.6)
C03	8	5 (11.4)	0	1 (2.3)	2 (4.5)	6 (13.6)	1 (2.3)	0	1 (2.3)
C04	9	4 (9.1)	1 (2.3)	1 (2.3)	3 (6.8)	5 (11.4)	1 (2.3)	0	3 (6.8)
C05	4	3 (6.8)	0	0	1 (2.3)	1 (2.3)	1 (2.3)	1 (2.3)	1 (2.3)
C06	6	5 (11.4)	0	1 (2.3)	0	5 (11.4)	1 (2.3)	0	0
Extent of primary tumour according to TNM classification[b]									
T1	26	13 (29.5)	3 (6.8)	2 (4.5)	8 (18.2)	16 (36.4)	2 (4.5)	1 (2.3)	7 (15.9)
T2	7	5 (11.4)	0	1 (2.3)	1 (2.3)	5 (11.4)	1 (2.3)	0	1 (2.3)
T3	3	2 (4.5)	0	0	1 (2.3)	2 (4.5)	0	0	1 (2.3)
T4	8	6 (13.6)	0	1 (2.3)	1 (2.3)	4 (9.1)	1 (2.3)	1 (2.3)	2 (4.5)
Stage I	22	12 (27.3)	3 (6.8)	1 (2.3)	6 (13.6)	15 (34.1)	1 (2.3)	1 (2.3)	5 (11.4)
Stage II	6	5 (11.4)	0	0	1 (2.3)	5 (11.4)	0	0	1 (2.3)
Stage III	1	0	0	0	1 (2.3)	0	1 (2.3)	0	0
Stage IV	15	9 (20.5)	0	3 (6.8)	3 (6.8)	7 (15.9)	2 (4.5)	1 (2.3)	5 (11.4)
OLP[c]	16	10 (22.7)	0	1 (2.3)	5 (11.4)	11 (25.0)	1 (2.3)	0	4 (9.1)
Smokers	25	13 (29.5)	3 (6.8)	4 (9.1)	5 (11.4)	14 (31.8)	4 (9.1)	2 (4.5)	5 (11.4)
Non-smokers	19	13 (29.5)	0	0	6 (13.6)	13 (29.5)	0	0	6 (13.6)
Alcohol users	34	21 (47.7)	2 (4.5)	4 (9.1)	7 (15.9)	23 (52.3)	3 (6.8)	2 (4.5)	6 (13.6)
Alcohol non-users	10	5 (11.4)	1 (2.3)	0	4 (9.1)	4 (9.1)	1 (2.3)	0	5 (11.4)
Removable prosthetics between samples	11	9 (20.5)	0	2 (4.5)	0	7 (15.9)	1 (2.3)	0	3 (6.8)

[a] ICD10 = World Health Organization's International Classification of Diseases 10th Revision.
[b] TNM = TNM Classification of Malignant Tumours 7th Edition.
[c] OLP = Oral lichen planus.

Table 2 – The *Candida* species found preoperatively and at follow-up.

	Pre-operative		Follow-up	
	N	%	N	%
C. albicans	30	68.2 %[a]	29	65.9 %[a]
C. dubliniensis	5	11.4%	2	2.5%
C. krusei	1	2.3%	3	6.8%
C. guilliermondii	1	2.3%	0	0%
C. glabrata	0	0%	1	2.3%
No *Candida*	11	25.0%	11	25%

[a] Four patients pre-operatively and 2 patients at follow-up had 2 different *Candida* species growing in samples. In all cases the other species was *C. albicans*.

One hundred microliters of undiluted sample were cultivated on CHROMagar® Candida –medium (CHROMagar, Paris, France) and incubated for 2–3 days at 37 °C. After incubation, yeast growth was recorded by registering the number and appearance of colonies. Candidiasis was determined according to Epstein et al.[14] *i.e.* the amount of *Candida* found in the sample was over 400 colony-forming units per millilitre saliva (CFU/ml). All green colonies were subjected to latex agglutination test (Bichro-Dubli Fumouze®, Fumouze Diagnostics, Levallois-Perret, France) to distinguish between *C. albicans* and *Candida dubliniensis*. Pure cultures of the colonies were cultivated on Sabouraud –medium and incubated at 37 °C for 1–2 days. Further identification of the pure-cultured NAC colonies was performed using an API ID 32C yeast identification kit (bioMérieux, Lyon, France) with visual reading of results and supplementary tests when necessary. The isolates were stored in 20% skim milk at −80 °C. IBM SPSS Statistics version 25 was used for statistical analysis.

The Research Ethics Committee of the Hospital District of Helsinki and Uusimaa approved the study design (ethical permit No. 525/E6/2003 and HUS/3054/2017), and institutional study permission was granted. All patients signed an informed consent form for participation.

Results

Basic characteristics of the patients with respect to their salivary *Candida* status at the pre- and post-treatment stage are given in Table 1. Twenty-four women (54.5%) and 20 men (mean age at the pre-treatment stage 66 years; range 31–83; S.D. 9) participated in the study. The average follow-up time was 48.1 months (median 44.5; range 19–80).

Candida was present in 75% of the samples both pre-operatively and at follow-up with *C. albicans* being the most common species in both cases (N = 30 pre-operatively, N = 29 at follow-up). As seen in Table 2, the NAC species identified were *C. dubliniensis*, *Candida krusei*, *Candida guilliermondii*, and *Candida glabrata*. More than one species was present in four samples pre-operatively and two samples at follow-up. In all these cases the total number of different species per sample was two and in all cases *C. albicans* was found to be the other species identified. The mean amount of *Candida* was 2004 CFU/ml pre-operatively and 3119 CFU/ml at follow-up. The mean change in the amount of *Candida* in samples was an increase of 892 CFU/ml (S.D. 7040.7). Candidiasis was found in 19 patients pre-operatively and in 27 patients at follow-up.

Change in the prevalence of different *Candida* species between the first and second saliva samples occurred in 12 cases as shown in Fig. 1. The change was in favour of NAC in only two cases (4.5%). Two patients went from harbouring both *C. dubliniensis* and *C. albicans* in their saliva to

Fig. 1 – Changes in *Candida* prevalence between pre-operative state and follow-up. NAC = non-*albicans Candida*. N = number of samples.

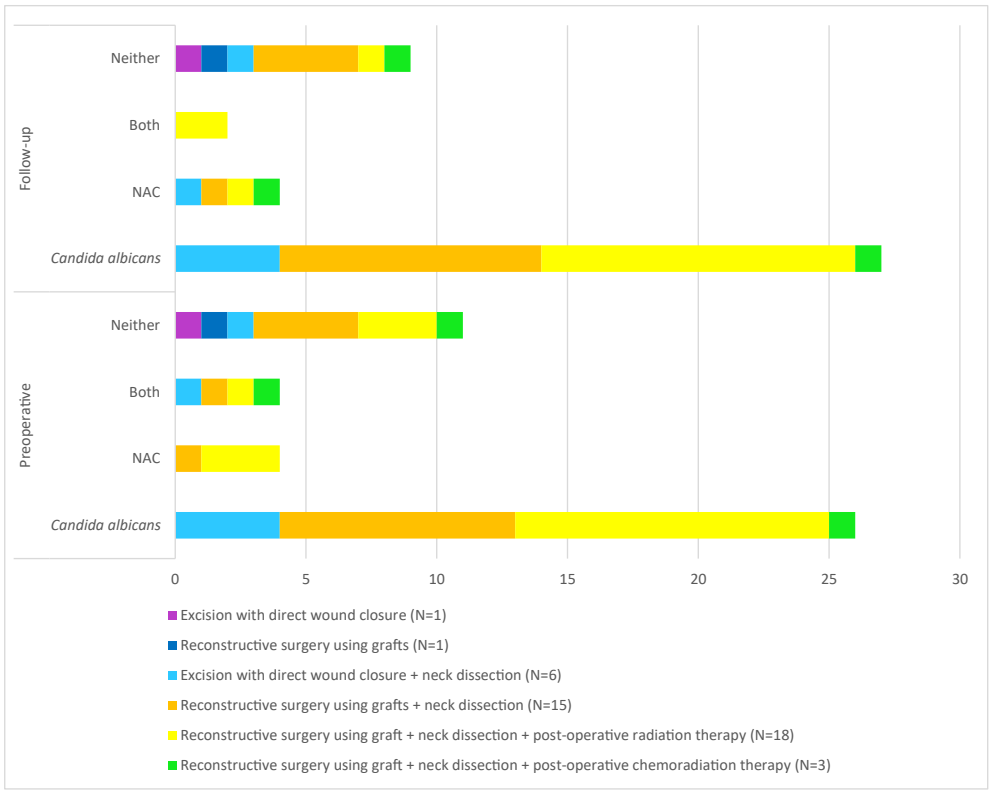

Fig. 2 – Preoperative and follow-up *Candida* prevalence of patients with regard to treatment modality. NAC = non-*albicans* *Candida*.

harbouring only *C. dubliniensis* at follow-up, whereas in one preoperatively similar case the patient harboured only *C. albicans* at follow-up. In three cases no *Candida* was found in the pre-operative sample but, in the follow-up sample, one of these patients harboured *C. krusei* and the other two harboured *C. albicans*. In two cases, a change in species was observed: one patient switched from having *C. dubliniensis* preoperatively to harbouring *C. albicans* at follow-up and another patient harboured *C. dubliniensis* preoperatively and both *C. albicans* and *C. krusei* at follow-up. Three patients had been using antifungal oral rinse (nystatin) within 90 days before giving the follow-up sample. However, these patients showed no change in their salivary *Candida* status between the first and second sampling. Two of these three patients harboured *Candida* in their saliva.

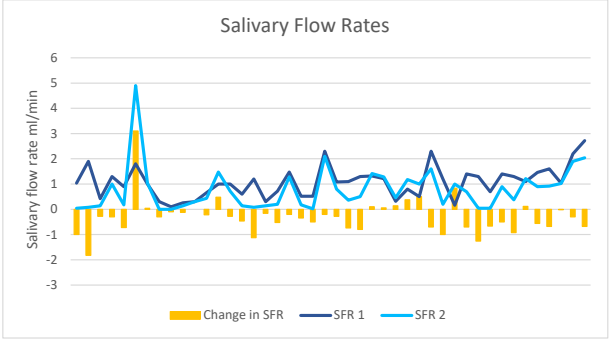

Fig. 3 – The pre- (SFR 1) and post-treatment (SFR 2) salivary flow rate measured in ml/min and the changes in the salivary flow rate. In most cases the change in flow rate was negative; however, some patients experienced an increase in the SFR.

Figure 2 shows the pre- and post-treatment *Candida* prevalence of patients with regard to the different treatment modalities. Thirty-four patients underwent reconstructive surgery using a fascio-cutaneous microvascular transfer, two patients had reconstructive surgery using full-thickness skin graft and in one case a facial artery musculo-mucosal flap was used. Seven patients underwent tumour excision with direct wound closure. Neck dissection was also done in 42 cases to eradicate any possible metastatic lymph nodes. No change in *Candida* status occurred in the two cases without neck dissection. Twenty-one patients underwent adjuvant radiotherapy as part of the treatment regime including three patients who received chemoradiotherapy with cisplatin. No difference in the prevalence of salivary *Candida* occurred in two-thirds of these patients. In two cases the patients' salivary *Candida* status changed from "no *Candida* present" to "*Candida* positive", while another two patients moved in the opposite direction. Similarly, one patient's pre-operative sample showed growth of two different *Candida* species while in the follow-up sample only one species was detected, whereas the opposite occurred in other patients' (N = 2) samples. The salivary *Candida* prevalence remained mostly unaffected in both radiotherapy-treated and not-treated patient groups and no significant difference between the groups was detected.

Salivary flow rates (SFR) were recorded both pre-operatively and at follow-up and the results are presented

in Fig. 3. Mean SFR was pre-operatively 1.1 ml/min (S.D. 0.6) and at follow-up 0.8 ml/min (S.D. 0.9). Eight patients preoperatively and 20 patients at follow-up had an SFR less than 0.5 ml/min which is considered the cut-off point for hyposalivation.[15] Thirty-three patients presented a lower SFR at follow-up out of which two patients were unable to produce any paraffin stimulated saliva. A change from normal salivary flow to hyposalivation occurred in nine patients receiving (chemo)radiotherapy and in five patients who did not receive (chemo)radiotherapy; this difference was, however, not significant. The mean change in SFR was −0.3 ml/min (S.D. 0.7). However, 10 patients presented an increase in the SFR, with one patient having an increase of 3.1 ml/min in salivary flow. While the change in SFR was statistically significant ($p = 0.011$), it did not correlate with the change in oral *Candida* status. However, hyposalivation did correlate with candidiasis both preoperatively ($p = 0.044$) and at follow-up ($p = 0.002$).

Discussion

In this study, we wanted to see if OSCC patients' oral *Candida* status is affected by cancer treatment. The hypothesis was that the heavy treatment methods and the changed oral environment of these patients would favour the enrichment of more pathogenic non-*albicans Candida* in the oral cavity and thus also in the saliva. However, contrary to the hypothesis this did not seem to be the case.

Most significantly, the changes in the patients' salivary *Candida* status were scarce and in only 4.5% of the cases was the change in favour of the more pathogenic NAC species. In 72.7% of the cases, the salivary *Candida* prevalence remained unchanged as seen in Fig. 1 and the different treatment modalities of the patients had no significant effect on the salivary *Candida* status. Indeed, even radiotherapy with or without combined chemotherapy seemed to not make a statistically significant difference to the patients' salivary *Candida* status as would have been expected in light of some previous studies.[5,6,11,16] When looking at the change in the prevalence of different *Candida* species together with the change in the prevalence of candidiasis, the overall change in the salivary *Candida* status occurred in 24 patients (45.5%). However, the presence of *Candida* and candidiasis in the pre-treatment stage explained the presence of candidiasis in the follow-up stage of these patients ($p = 0.0001$ and $p = 0.001$, respectively).

As expected, the cancer treatment affected the patients' salivary flow most commonly negatively (77% of patients) decreasing the SFR on average by almost 0.3 ml/min. However, the change in the SFR did not statistically significantly correlate with either the change in the amount of *Candida* found or with the change in the *Candida* prevalence of the patient. Some studies have reported similar findings; for example, Rhodus et al.[17] found that the amount of *Candida* present seems not to be relative to SFR among patients with Sjögren's syndrome. However, our findings do contradict many of the previous studies in which a significant correlation was found between the decrease in SFR and *Candida* colonisation among OSCC patients treated with radiotherapy.[16,18,19]

As in previous studies,[20,21] *Candida* was very common among the patients since 75% of our patients harboured *Candida* both at the pre- and post-treatment states. In most cases, the patients were *C. albicans* positive with NAC species present in 16% of the samples pre-operatively and 14% at follow-up. Curiously, both pre-operatively and at follow-up, the NAC-positive patients were all smokers. The non-smokers showed very little shift in *Candida* status, with only two patients moving between *C. albicans* positive and *Candida* negative groups. However, the differences between smokers' and non-smokers' *Candida* status were not statistically significant.

Interestingly and despite the changes in the oral cavity environment, our study seems to fall in line with a Portuguese study made among healthy young dentistry students, which showed that the intraindividual stability of commensal fungi remained consistent during a follow-up period of 30 weeks.[22] According to our study, the OSCC patients did harbour *Candida* in their saliva, but even surgical cancer resection combined with chemoradiotherapy had little to no effect in favour of the more pathogenic *Candida* species in saliva suggesting a high level of intraindividual stability also among the OSCC patients. Our findings further suggest, that the OSCC patients' preoperative salivary *Candida* status almost certainly affects the patients' post-treatment salivary *Candida* status, presentation of oral candidiasis and, perhaps, even the consequent need for antifungal medication. It may, therefore, be warranted that the salivary *Candida* status be included in the preliminary testing of a patient in the beginning of their treatment path for OSCC.

To the authors' knowledge, this study is the first to show the effect of different OSCC treatment modalities on oral *Candida* status. The group of OSCC patients studied was smaller than anticipated at the beginning of this study because many patients were lost during follow-up. This contributed to the heterogeneity of the study population, which may have affected the lack of statistical significance found. Nevertheless, a trend can be seen in which the pre-treatment salivary *Candida* status does affect the post-treatment status of the OSCC patients. However, larger studies with more patients per different treatment modalities are needed for a definitive conclusion. Finally, whole saliva was chosen as study material due to it being in contact with all the surfaces of the oral cavity and thus giving an idea of the state of the whole mouth environment. While it is well known that stimulated saliva is somewhat diluted in comparison to unstimulated saliva, the patients were expected to suffer from hyposalivation especially at the post-treatment stage and thus paraffin stimulation was planned to ensure a sufficient sample from most patients.

To further increase our knowledge on the possible changes in oral microbiology due to cancer treatments, studies including the full microbiome should be done as it might even affect the prognosis of the disease.

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Declaration of competing interest

The authors declare no conflicts of interest regarding this study.

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