Optimal sampling site for mucosal candidosis in oral cancer patients is the labial sulcus

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Traditional sampling methods for the diagnosis of oral candidosis in head and neck cancer patients, i.e. saliva collection or tongue scrapings, are often impossible to perform. The aim was to determine the optimal sampling method. Eighteen oral cancer patients and five control subjects were sampled semi-quantitatively from the labial sulcus, dorsum of the tongue, dental plaque and saliva for cultivation of yeasts. The patients were examined prior to all cancer treatment ($n=5$), or 2–4 weeks ($n=5$) or 8–12 weeks ($n=8$) post-operatively. The incidence of *Candida* was found to increase from 40% at the control and pre-operative level up to 73% 8–12 weeks post-operatively. *Candida albicans* was found to be the only species until 4 weeks post-operatively. Thereafter, the incidence of species other than *C. albicans* was 38%. The most sensitive sampling site was found to be the vestibular sulcus, from which all culture-positive cases could be confirmed. Tongue surface scraping was found to be more sensitive than saliva collection in detecting *Candida*. All sampling methods and sites were equally sensitive in detecting the different *Candida* species. Dental plaque was found to have the highest density of *Candida* colonization, and was thus found to be the most significant source of *Candida* infection, which emphasizes the role of dental care in these patients.

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INTRODUCTION

For most people, yeasts are a part of the normal oral flora, and cause infection only if an opportunity appears (Richardson & Warnock, 2003). In health, the amount of yeast is kept under control by specific and non-specific defence mechanisms of the saliva and the oral mucosa, as well as by competition among oral microbes (Calderone, 2002). Thus, oral candidosis is classically a sign of impaired local or systemic defence mechanisms, or altered oral flora. Reduced saliva secretion due to medication or radiotherapy, primary or secondary deficiencies of humoral or cell-mediated immunity, local mucosal diseases, e.g. lichen ruber planus, and the use of wide-spectrum antibiotics, are among well-known predisposing factors.

Candida albicans is the most frequent cause of oral candidosis (in 60–80% of cases) in patients receiving radiotherapy for head and neck cancer, but at least eight other pathogenic species can be found (Redding *et al.*, 1999; Bagg *et al.*, 2003; Belazi *et al.*, 2004). Although all candidal species cause a similar mucositis, there are remarkable differences in the invasiveness and antifungal susceptibilities

among species (Bagg *et al.*, 2003; Belazi *et al.*, 2004). The role of species other than *C. albicans* has become increasingly important, especially in high-risk patients. This underlines the importance of using an appropriate sampling method, in addition to performing accurate microbiological diagnostics, in these patients. Traditional sampling methods for oral yeasts are swabbing the oral mucosa with a cotton swab, scraping with a spatula, suction of saliva, or rinsing the mouth.

The oral cavity goes through radical changes during treatment for oral cancer. Surgical removal of the tumour often results in considerable lack of tissue, and free microvascular flaps of bone, muscle and skin are used for reconstruction. The increase of keratinised surfaces when skin-lined microvascular flaps are used, and subsequent prosthetic rehabilitation with fixed or removable appliances, alter the micro-environment of the oral cavity further. Post-operative radiotherapy to the primary tumour site and regional lymphatics are given to patients with large and aggressive tumours and at risk for metastases. Radiotherapy to a total dose of 50–60 Gy in 2 Gy daily fractions for

5–6 weeks starts as soon as the primary healing of the operation wounds has completed, normally 6–8 weeks post-operatively (Saarilahti *et al.*, 2005). For most patients, radiotherapy results in permanent damage to their salivary glands and lifelong xerostomia.

Lack of saliva and development of surfaces more susceptible to heavy yeast colonization cause a lifelong high risk for oral candidosis for these patients. Traditional sampling methods, i.e. saliva culture, mouth rinses or tongue scrapings, are often impossible to perform, and may result in false-negative results. This is especially problematic, as clinical symptoms may be non-existent due to neural damage and to the considerable decrease in blood flow in the irradiated tissues. The optimal site and method of sampling for oral candidosis in oral cancer patients is not known.

As the first step in a comprehensive follow-up study of the importance of yeast infections in oral cancer patients, we set out to investigate how the sampling method and site of sampling affect the recovery of *Candida* species from the oral cavity.

METHODS

Patients and controls. Eighteen previously untreated patients with primary oral cancer (mean age 60 years, range 42–81 years, male:female ratio 11:7) were enrolled in the study (Table 1). All patients were hospitalized due to cancer treatment courses in 2004–2005. Five healthy, non-medicated members of the hospital personnel were included as controls (mean age 42 years, range 28–54 years, male:female ratio 3:2). For this study, the patients

were examined prior to all cancer treatment ($n=5$), or 2–4 weeks ($n=5$) or 8–12 weeks ($n=8$) after the primary surgical treatment. Eleven of the 13 patients who had undergone operations (85%) had received conventionally fractionated radiotherapy to a mean total dose of 55 Gy (range 20–76 Gy) post-operatively. Two of these (18%) had received chemoradiotherapy. The primary sites of the oral cancer were as follows: tongue, six cases; mandible, six cases; maxilla, two cases; buccal mucosa, one case. In three cases, metastasis had been identified. The general status of the dentition, dental status (recorded in the WHO Diseased Missing Filled Index) and oral hygiene (examiner-assessed subjective scale 1–3), as well as the use of antifungals, was recorded.

The study protocol was approved by the ethical committee of the Helsinki University Central Hospital (ethical permit number 525/E6/2003 28-01-2004). All patients signed an informed consent.

Sampling and cultivation of yeasts. Eighteen oral cancer patients and five control subjects were sampled once semi-quantitatively from the labial sulcus, dorsum of the tongue, dental plaque and saliva for cultivation of yeasts. All samples were taken non-invasively with sterile instruments. Particular care was taken to perform the sampling in a standardized way and to avoid contamination from adjacent areas. The precise site of sampling varied a little from patient to patient, depending on the dentate status and anatomical circumstances in the mouth. Saliva samples and samples from the upper labial sulcus were taken with sterile cotton swabs. For the saliva sample, the swab was placed into a moist area in the floor of the mouth for 10 s. For the labial sulcus sample, each sulcus was gently swabbed with single wipes. For the plaque sample, dental plaque of the labial surface of one lower molar tooth was collected with an explorer. Samples from the dorsum of the tongue were taken with one gentle scrape using a spatula.

The samples were collected into sterile tubes containing 0.5 ml sterile saline. The samples were immediately transported to the laboratory,

Table 1. Patient demographics

Characteristic	Number of patients				Number of controls
	Pre-operative	2–4 weeks post-operative	8–12 weeks post-operative	Total	
<i>n</i>	5	5	8	18	5
Sex					
Female	1	2	4	7 (39%)	2 (40%)
Male	4	3	4	11 (61%)	3 (60%)
Age (years)					
Mean	61	57	62	60	42
Range	—	—	—	42–81	28–54
Location of cancer					
Tongue	1	0	4	5	0
Floor of mouth	1	3	3	7	0
Maxilla	0	1	1	2	0
Cheek mucosa	1	0	0	1	0
Metastasis	2	1	0	3	0
Treatment					
Surgery	0	5	8	13	0
Radiotherapy	0	3	8	11 (61%)	0
Mean dose	—	—	—	54 Gy	—
Dose range	—	—	—	20–76 Gy	—
Chemotherapy	0	1	1	2 (11%)	0

Fig. 1. Incidence of *Candida* species in oral cancer patients at different stages of treatment, and in control subjects. Black bars, *C. albicans*; grey bars, non-*albicans*; white bars, *C. albicans* + non-*albicans*; wk, week; post-op., post-operatively.

and 100 µl of the saline was plated onto Sabouraud dextrose plates (HUSLAB) after careful mixing, and incubated at 37 °C for 48 h. Thereafter, colonies were counted and cultivated further on CHROMagar *Candida* medium (CHROMagar) for the presumptive identification of *Candida* species. The Bichro-Dubli latex co-agglutination test (Fumouze Diagnostics) was used to differentiate between *C. albicans* and *Candida dubliniensis*. Species other than *C. albicans* and *C. dubliniensis* were identified by API 32C auxanographic strips (bioMérieux). Multiple colonies were tested at every identification step.

RESULTS

The incidence of positive *Candida* growth in the control and pre-operative groups was 40 % (Fig. 1). After the beginning of cancer treatment, the incidence was found to increase rapidly (69 %, 9/13, post-operatively). Of the patients who had undergone operations, 73 % (6/8) were positive for *Candida* 8–12 weeks post-operatively (Table 2). In addition to the increase in the incidence of *Candida*, the colony

Fig. 2. The density of *Candida* in colonized oral cancer patients at different stages of treatment, and in control subjects. The values represent the means of the total number of colonies isolated from each patient. Abbreviations: wk, week; post-op., post-operatively.

density also increased after the beginning of the cancer treatment (Fig. 2). In addition, although the incidence of *Candida* was found to be the same in the control and pre-operative groups, the colony density was already found to be markedly higher (15–500-fold) in the oral cancer patient group before the beginning of cancer treatment (Table 3).

Table 2 presents the percentages of samples positive for yeast growth with respect to the site of sampling. The site-specific samples that were positive most often had been taken from the labial sulcus (100 %) and from the dorsum of the tongue (91 %). However, the number of c.f.u. was highest in the dental plaque samples. The most sensitive sampling site was found to be the vestibular sulcus, from which all culture-positive cases could be confirmed. In addition, a tongue surface scraping was found to be more sensitive than saliva in detecting *Candida* in the patients. All sampling methods were equally sensitive in detecting the different species of *Candida*.

Table 2. Sensitivity of the sampling site in finding subjects positive for *Candida*

Sampling site	Positive patients/total patients (%)				Positive controls/total controls (%)
	Total	Pre-operative	2–4 weeks post-operative	8–12 weeks post-operative	
All sites	11/18*	2/5	3/5	6/8	2/5 (40)
Tooth surface	8/9† (89)	2/2	1/1	5/6	1/2
Tongue	10/11 (91)	2/2	2/3	6/6	1/2
Sulcus	11/11 (100)	2/2	3/3	6/6	1/2
Saliva	8/11 (73)	1/2	2/3	5/6	0/2

*The number of the study subjects positive for *Candida* as a proportion of the total number of patients sampled.

†The number of subjects positive for *Candida* at this sampling site as a proportion of the total number of positive subjects.

Table 3. Colony density of *Candida* (c.f.u. ml⁻¹) at various sampling sites at different stages of treatment for oral cancer, and for control subjects

Sampling site	Patients				Controls
	Pre-operative	2–4 weeks post-operative	8–12 weeks post-operative	Total	
Tooth	730	260	185 240	186 000	10
Tongue	140	10 015	18 660	29 000	5
Sulcus	380	7830	30 000	38 000	25
Saliva	500	7035	67 420	75 000	0

Candida albicans was found to be the predominant species. It was the only yeast detected in the control group as well as in the patient groups up to 2–4 weeks post-operatively (Fig. 1). At 8–12 weeks post-operatively in three of the six *Candida*-positive patients, a species other than *C. albicans* strain was identified. Of all the *Candida*-positive patients who underwent operations, 66 % had *Candida albicans*, while the rest were species other than *C. albicans*. *C. dubliniensis* was not found in any of the patient samples. Antifungal prophylaxis, mainly fluconazole 100 mg p.o. or 150 mg i.v. daily, had been given to 44 % of patients. Of the patients with negative yeast growth, 57 % were receiving antifungal treatment. About half of the patients who received antifungal drugs still had positive yeast growth, mainly of species other than *C. albicans*. Of the patients undergoing radiotherapy, 67 % had positive yeast growth, although five out of eight were receiving antifungal treatment. All patients receiving chemoradiotherapy had positive yeast growth. Of the patients who smoked, 70 % had positive yeast growth.

The general status of the dentition and oral hygiene of the patients was recorded. Of the patients, 56 % were smokers. All patients had been seen by a hospital dentist pre-operatively and had no cavities, but had a higher number of missing teeth in the post-operative phase of cancer treatment. Otherwise, the general status of the dentition did not differ markedly between the groups. The oral hygiene of the patients improved during the first months of their cancer treatment.

DISCUSSION

The sampling of oral cancer patients for candidosis is problematic. The oral cavity goes through radical changes during treatment for oral cancer. Surgical treatment is normally followed by radiotherapy, and occasionally chemoradiotherapy, causing temporary or permanent damage to the salivary glands and a lifelong high risk of oral candidosis for these patients. Traditional sampling methods, i.e. saliva culture, mouth rinses and tongue scrapings, are often impossible to perform, and may result in false-negative results. This is especially problematic, as clinical symptoms may be non-existent due to neural damage and to the considerable decrease of blood flow in the irradiated tissues.

The results of our study showed that the site of sampling for oral yeast cultivation is crucial for a correct diagnosis. Positive growth was most often detected in samples taken from the labial sulcus and upper aspect of the tongue. Dental plaque on tooth surfaces was found to have the highest density of *Candida* colonization of all sites sampled. It was thus found to be the most significant source of *Candida* infection, which emphasizes the role of dental care in these patients. However, there is a risk of overdiagnosis and treatment when sampling the tooth surfaces, as the amount of yeast cultured is used for treatment decisions in these patients. The labial sulci and dorsum of the tongue are often more relevant sampling sites in oral cancer patients, in whom surgical operations may have deformed other anatomical areas of the oral cavity. The optimal sampling site for *Candida* was found to be the vestibular sulcus.

Our findings of an increase in the incidence of *Candida* and a shift from *C. albicans* to other *Candida* species is in accordance with the findings of others (Bagg *et al.*, 2003; Redding *et al.*, 2004). These findings have been thought to be a result of the disease and its treatment, and of the use of azole antifungals. Our findings confirm that the choice of antifungals and the mode of drug administration need careful attention in these patients. The use of systemic antifungals to control superficial infection at a site of decreased blood flow and lack of saliva results in suboptimal drug concentrations and a high risk of the development and selection of resistant strains. The use of systemic drugs cannot be sanctioned unless the patient is unable to apply topical drugs or is immunocompromised.

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