# Bacterial flora on the surface of oral squamous cell carcinoma

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## SUMMARY

**Background:** *The aim of the study was to determine the microbial presence and type in the biofilms present on the surface of oral squamous cell carcinomas and benign lesions of oral mucosa.*

**Methods:** *Thirty new (untreated) patients with oral squamous cell carcinoma were examined. The control group consisted of thirty subjects with benign lesions of oral mucosa. Swab samples were taken from the superficial layer of the carcinoma and benign lesions for bacteriological examination. Swabs were transported within 2 hours and immediately sown and incubated. Oral health index was assessed in all patients.*

**Results:** *The prevalence of pathological bacterial microflora was significantly higher in cancer patients ($p<0.01$). No significant associations were found between bacterial presence and gender, localization, cigarette smoking, alcohol consumption and denture wearing ($p>0.05$). Poor oral hygiene was also registered in cancer patients.*

**Conclusion:** *Possibility of microflora retention on irregular oral carcinoma surface possibly contributes to chronic inflammation often observed on the surface of oral carcinomas.*

**Key words:** *Mouth Neoplasms; Carcinoma, Squamous Cell; Bacteria; Oral Hygiene Index; Streptococcus*

## INTRODUCTION

In about 15% of oral cancer patients without known risk factors, possible etiology can be attributed to viral or fungal presence (human papillomavirus, Epstein-Barr virus, *Candida albicans)* (1). Despite the knowledge about relationship between microflora and oral cancer is still uncertain, a few assumptions and theories have been made. It is considered that different bacteria can induce carcinogenesis either through chronic inflammation or by metabolism of potentially carcinogenic substances such as acetaldehydes (1-3), or nitrosamines and nitrosodiethylamines (NDEA) (4).

The other theory says that changes in microflora on oral mucosa begin after cancerous alteration, due to changes of pH, irregularity of lesion surface and broken defense mechanism of the oral mucosa. Changes in microflora on oral carcinoma surfaces may lead both to local and systemic infections because of weakened immune system by chemotherapy, irradiation, and surgery (5, 6). It remains unclear whether higher presence of bacteria on the surface of oral squamous cell carcinoma (OSCC) is caused by patients' weakened immune system, chronic inflammation, easy bacterial retention on irregular surface, or just neglecting of oral hygiene.

The aim of the study was to determine the microbial presence and type in the biofilms present on the surface of OSCC and benign lesions of oral mucosa, and to determine any associations between bacterial presence and gender, localization, cigarette smoking, alcohol consumption and denture wearing.

## MATERIALS AND PATIENTS

The research was conducted as a clinical prospective study on the Clinic for Maxillofacial and Oral Surgery, Clinical Centre of Vojvodina, from June 2007 to August 2008. Thirty treatment-naïve patients (24 men and 6 women; median age 61.47 year (range 41-81 years)), with clinically and histopathologically diagnosed OSCC were examined. The control group consisted of 30 patients (16 men and 14 women) with median age of 54.53 years (range 16-83 years). All patients had clinically visible and histologically confirmed benign lesions of oral mucous tissues. We found mucosal fibroma in 8 patients (26.6%), pyogenic granuloma in 4 patients (13.3%), mucocele in 4 patients (13.3%), capillary mucous haemangioma in 3 patients (10.0%), mucosal polyp in 3 patients (10.0%), chronic nonspecific sialadenitis in 3 patients (10.0%), mucosal papilloma in 2 patients (6.7%), retention cyst in 2 patients (6.7%), and subacute nonspecific inflammation in 1 patient (3.3%).

In cancer group of patients, there were 24 smokers, 20 patients who consume alcohol on daily bases, and 9 denture-wearing patients. Of all patients, 3 were only smokers, 1 only consumed alcohol, 1 only wore dentures, 13 were smokers, alcohol consumers, 2 were smokers and denture wearers, and 6 of them had dentures, smoked and consumed alcohol. The control group consisted of 7 smokers, 6 alcohol consumers on daily bases and 12 patients with dentures (4 were just smokers, 1 only consumed alcohol, 5 were denture wearers, 2 were smokers and had dentures, 4 consumed alcohol and wore dentures and 1 was smoker who consumed alcohol and had denture). The patients of both groups were selected by the order of applying for the treatment. The information about the contents of the investigation and the purpose of the usage of obtained results were distributed to the patients in the written form. After that, the patients gave their written consent. The investigation was approved by the Ethical committee of Medical faculty in Novi Sad.

Swab samples were taken from the superficial layer of the carcinoma for bacteriological examination, while in the control group swab was taken from the surface of clinically benign lesions. Swabs were transported within 2 hours to the Department of microbiology, Institute for public health of Vojvodina, where they were immediately sown on blood agar, MacConkey agar and tioglicolate surfaces with dextrose, and were

incubated under aerobic conditions on 37°C for 24/48 hours. Review of sown nutrition surfaces after 48 hours was performed in order to identify clinically significant species of bacteria.

Using dental probe and mirror, oral health index was assessed and numerical values were allocated based on the following criteria:

0 points - dental plaque and supragingival calculus do not exist;

1 point - dental plaque and supragingival calculus are localized only in the gingival third of the clinical crown of the tooth;

2 points - dental plaque and supragingival calculus cover more than one and less of two-thirds of clinical crown of the tooth;

3 points - dental plaque and supragingival calculus cover more than two-thirds of the clinical crown of the tooth.

The final oral hygiene index value was given to each patient after all teeth were checked, and highest found score was taken as definite. In patients who had no teeth, oral hygiene index was not assessed.

For the statistic processing of data, commercial statistical program *SPSS 14 for Windows* was used. Attributive characteristics data were shown as absolute and relative numbers; mean values and measures of variability were used for numeric characteristics. Student's t-test was applied for testing of the differences between the two groups, and $\chi^2$ test or $\chi^2$ test (Yates correction) for attributive characteristics. The value $p<0.05$ was accepted as statistically significant.

## RESULTS

In the OSCC group, male to female ratio was 4:1; in the control group, sex distribution was almost the same. Differences in gender prevalence had statistical significance ($p<0.05$). Comparison of average age in both groups did not show statistical significance ($p>0.05$). Table 1 shows that OSCC was most frequently localized on the floor of the mouth (33.3%), mandibular gingiva (20%) and tongue (13.3%). In the control group, lesions were most frequent on the tongue (43.3%) and the lower lip (33.3%).

**Table 1. Localisation of oral alterations in OSCC and control group**

| Localization | OSCC group | | Control group | |
|---|---|---|---|---|
| | n | % | n | % |
| Tongue | 4 | 13.3 | 13 | 43.3 |
| Bucal mucosa | 2 | 6.7 | 3 | 10 |
| Lower lip | 1 | 3.3 | 10 | 33.3 |
| Upper lip | 0 | 0 | 1 | 3.3 |
| Hard and soft palate | 2 | 6.7 | 1 | 3.3 |
| Mandibular gingiva | 6 | 20 | 1 | 3.3 |
| Pterygomandibular region | 2 | 6.7 | 1 | 3.3 |
| Floor of the mouth | 10 | 33.3 | 0 | 0 |
| Oropharynx | 3 | 10 | 0 | 0 |
| Total | 30 | 100 | 30 | 100 |

OSCC - oral squamous cell carcinoma
n - number

Pathological bacterial microflora was found in 17 (56.7%) OSCC patients and in 4 (13.3%) control patients, which was statistically significant ($p<0.01$). Most frequently isolated bacteria on cancer sites was *Strepotococcus alpha-haemoliticus* in 33.3% of cases (13.3% *Streptococcus viridans*), while control group had 13.3% of positive bacterial findings but without predominant type (Table 2). Using $\chi^2$ test (Yates correction) in both groups, no statistically significant associations were found between bacterial presence and gender ($p>0.05$), localization ($p>0.05$), cigarette smoking ($p>0.05$), alcohol consumption ($p>0.05$) and denture wearing ($p>0.05$).

**Table 2. Bacterial type representation in OSCC and control group**

| Bacterial type | OSCC group | | Control group | |
|---|---|---|---|---|
| | n | % | n | % |
| Normal bacterial microflora | 13 | 43.3 | 26 | 86.7 |
| Pathological bacterial microflora | 17 | 56.7 | 4 | 13,3 * |
| Escherichia coli | 0 | 0.0 | 1 | 3,3 |
| Streptococcus beta haemoliticus | 1 | 3.3 | 1 | 3.3 |
| Streptococcus pyogenes | 0 | 0.0 | 1 | 3.3 |
| Staphylococcus aureus | 1 | 3.3 | 1 | 3.3 |
| Streptococcus viridans | 4 | 13.3 | 0 | 0.0 |
| Enterococcus | 1 | 3.3 | 0 | 0.0 |
| Pseudomonas aeruginosa | 2 | 6.7 | 0 | 0.0 |
| Streptococcus alfa haemoliticus | 6 | 20.0 | 0 | 0.0 |
| Clebsiela pneumonie | 1 | 3.3 | 0 | 0.0 |
| Enterobacter spp. | 1 | 3.3 | 0 | 0.0 |
| Total | 30 | 100.0 | 30 | 100.0 |

* $p< 0.01$

Considering oral hygiene index, 40% of patients in the OSCC group had score 3 (dental plaque and supragingival calculus present on more than 2/3 of tooth crown surface), and 53.3% of patients in the control group had score 1 (Table 3). A large number of Index categories and a small patient sample made statistical analysis unachievable.

**Table 3. Representation of oral hygiene index values in OSCC and control group**

| Oral hygiene index | OSCC group | | Control group | |
|---|---|---|---|---|
| | n | % | n | % |
| 0 | 1 | 3.3 | 4 | 13.3 |
| 1 | 2 | 6.7 | 16 | 53.3 |
| 2 | 6 | 20.0 | 2 | 6.7 |
| 3 | 12 | 40.0 | 1 | 3.3 |
| Index is not measurable | 9 | 30.0 | 7 | 23.3 |
| Total | 30 | 100.0 | 30 | 100.0 |

## DISCUSSION

The male to female ratio reported in this study was 4:1 in OSCC group, which differs comparing to 2.3-2.4:1 ratio shown in previous studies (7, 8). In the present study, most frequent sites of malignancy were floor of the mouth (33.3%), mandibular gingiva (20%), and tongue (13%). Bhurgi et al. (9) reported that in oral malignancy, buccal mucosa was the most frequently involved site (55.9%), followed by the tongue (28.4%). Another study described the tongue (67.4%) as first frequent site, followed by buccal mucosa (7.7%) (7).

We found significant difference in microbial flora present in patients with OSCC comparing to patients with benign oral lesions, same as in previous studies where microbial content from the carcinoma site was compared

to healthy oral mucosa (5). This indicates that possible retention of bacteria on irregular carcinoma surface does not have a significant role in bacterial adherence.

Comparing the healthy sites in oral cancer patients and health patients, in terms of microbial profile, Mager et al. (10) found that healthy sites in oral cancer subjects were more similar to oral lesions than to healthy sites in cancer-free subjects. The reason for this finding is unclear.

Streptococcus species was predominant and present in 39.9% of OSCC cases, mainly *Streptococcus alpha-haemoliticus* (including *Streptococcus viridans*), but also *Streptococcus beta-haemoliticus* and *Enterococcus*. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were sporadically isolated in OSCC patients. Recently, Byakodi et al. (11) obtained similar finding. Nagy et al. (5) reported Haemophilus, Enterobacteriaceae and Streptococcus spp. as microbial types isolated in increased numbers at tumor sites. Hooper et al. (12) analyzed different microbial types in superficial layer of OSCC and according to their results, *Streptococcus viridans* was reported in 73.7% of OSCC cases. Examining acetaldehyde production by different streptococci from viridians group, Kurkivuori et al. (13) reported that *Streptococcus salivarius*, *Streptococcus intermedius* and *Streptococcus mitis* had largest capability to produce a carcinogenic substances, such as acetaldehyde.

Dentition, tooth loss, poor dental status and oral hygiene habits, that we also found in OSCC group, have frequently been associated with risks for oral cavity cancer, and it is generally agreed that the influence of poor oral hygiene as a risk factor is much less compelling than alcohol and smoking (14). Poor condition of the mouth, poor dentition, lack of toothbrush use, and never having a dental check-up are risk factors for head and neck cancers, independent of tobacco use and alcohol consumption (15). In previous study, we found presence of *Pseudomonas aeruginosa* and *Escherichia coli* in healthy young smokers, which also indicated lower oral hygiene level in smokers (16).

We found no significant associations between bacterial presence and gender, age, localization of OSCC, cigarette and alcohol consumption and denture wearing. Earlier studies reported microbial changes in oral microflora of alcoholics as less intensively described, but that no bacterial species have been associated with high alcohol consumption (14, 17). Cigarette smoking and OSCC are associated with an alteration of the oral microflora that could modulate oral cancer risk by activating or degrading some cigarette carcinogens (18).

However, associations between gender, localization, cigarette and alcohol consumption, denture wearing and presence of pathological bacteria on oral cancer surface were not find in this study, but high Oral Hygiene Index shows that oral squamous cell carcinoma patients have low and unsatisfying oral hygiene level. Nevertheless, it cannot be decisively asserted whether oral hygiene level was low before the occurrence of the OSCC or if it is the consequence of the OSCC presence.

**Conflicts of Interest**

We declare no conflicts of interest.

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