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An increased expression of oral *Candida* in patients with head and neck cancer during radiotherapy

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Abstract

The patients with head and neck cancer for treating radiotherapy have mucosal damage and dryness in oral cavity and pharynx, which are the risk factors for candidiasis. In this study, it was to assess the relationship between the mount of oral *Candida* colonies and clinical symptoms for oral health condition and function to radiotherapy in the patients with head and neck cancer. Twenty-five patients who received 10-60 Gy of total radiotherapy dose for treating head and neck cancer at Fukuoka University Hospital, from October 2008 through June 2009 were enrolled in this study. Samples for the mycological examination were take a swabbing from buccal mucosa, hard palate, floor of the mouth, tongue region, and gums each patient. Collected samples were incubated immediately, the number of *Candida* colonies was counted after 48 hours. We researched changes of *Candida* species and combined infection caused by cumulative irradiation dose. The Oral Assessment Guide (OAG) was used to evaluate clinical symptoms for oral health condition and function in this study. The Correlation with the number of *Candida* colonies, and cumulative irradiation dose were also investigated by a total score with OAG. *Candida* infectious lesions was detected in all patients before the end of radiotherapy. The most frequently

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isolated yeast was *C. albicans*. The combined infection rate before and the end of radiotherapy were 12% and 40%, respectively (p=0.03). The OAG total score was significantly correlated with the number of *Candida* colonies and the cumulative irradiation, respectively. Although this study could not be concluded that *Candida* directly influences oral clinical conditions, our results suggested that radiotherapy for head and neck cancer alter clinical conditions in the oral cavity and might be involved in growth of *Candida*.

1. Introduction

Candida is a part of the resident floras in the oropharynx and is not clinical symptoms. However, opportunistic infections and microbial substitution lead to a significant increase of candidiasis disease, and sometimes results in oral candidiasis[1]. A decrease in salivary excretion and mucosal inflammation caused by irradiation would be factors for mucosal damage and *Candida* infection[2,3]. Under such the conditions, white milky fur have been gradually scattering on the tongue, gum and inner cheeks. Koc et al reported that the effect of prophylactic treatment of mycotic mucositisin radiotherapy of the patients with head and neck cancer is good. They suggested that an increase of fungus in oral cavity lead clinical symptoms such as taste abnormality, severe pain, and mucosal congestion. However, there is no prior studies directly demonstrated that radiotherapy for head and neck cancer disease induce candidiasis and clinical conditions.

Therefore, this study was developed to evaluate the impact of cumulative radiotherapy on oral clinical conditions by identification and quantification of *Candida* species. If it could be proved, we could consider whether *Candida* affects on clinical symptoms directly.

The objective of this study was to analyze the relationship between the oral *Candida* colonies and clinical symptoms for oral health condition and function to radiotherapy in the patients with head and neck cancer.

2. Materials and methods

2.1. Study population

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Subjects are patients, who were referred to department of Otorhinolaryngology in Fukuoka University Hospital for treating head and neck cancer from October 2008 to June 2009. Patients were selected from those received radiotherapy as an initial treatment strategy, either pre-operative or curative. All patients used the name of Azunol® gargle liquid (4%) during radiation treatment. Patients who had received total parental nutrition, corticosteroids, immunosuppressive drugs, or antibiotics were excluded before radiation [4-6].

Twenty-five patients were eligible in this study. This study was assessed in 25 patients who received a total radiotherapy dose of 10–60 Gy for the sites of head and neck cancer. Oral lesions were included in the radiation field in each patient. The mean age of the 25 patients was 64±8.9 (S.D.) years old, and there were 21 males (range, 46-78 years old) and 4 females (range, 51-70 years old) respectively. Of the 25 patients, 8 had oropharyngel cancer, 5 had cancer of the external acoustic meatus, 4 had hypopharyngeal cancer, 4 had laryngeal cancer, 4 had other solid cancers (floor of the mouth and vocal chords). Eighteen patients were diagnosed at stage 4 and 4 were diagnosed at stage 3 based on a tumor staging system (UICC classification 1998). The mean accumulated radiation dose was 41.1±14.3 Gy. Nineteen patients (76 %) received chemotherapy and 11(44 %) had dental prostheses. The numbers of patients as a function of accumulation irradiation were shown in Table 1.

Table 1.　Number of patients in accumulative irradiation

Cumulative irradiation (Gy)	0	5	10	15	20	25	30
No. of patients	25	25	25	24	23	21	21
Cumulative irradiation (Gy)	35	40	45	50			
No. of patients	14	13	13	10			

2.2. Study design

This study design used a prospective method.

2.3. Collection method

Samples were collected for the mycological examination by firmly swabbing the buccal mucosa, hard palate, floor of the mouth, tongue region, and gums. The samples were collected before breakfast and then inoculated in the broth and 100 μL of bacterial liquid were plated on BD CHROMager® Candida（NIPPON BECTON DICKINSON CO., LTD）agar with the spiral plating machine EDDY JET (SPIRAL SYSTEM INSTRUMENTS, INC.). After incubation at 35℃ for 48hrs, colonies on the plate were counted. The colony count was divided by the liquid volume corresponding to the lesions over which the colony count was obtained. A direct cytological examination was also performed with the same samples.

2.4. Assessment

We introduced the Oral Assessment Guide (OAG) to our clinical observations. OAG is a tool for evaluating clinical symptoms for oral health condition and function[7,8]. Moreover, OAG is clinically useful in assessing oral cavity changes in patients who have received radiation and anti-cancer therapies. We evaluated oral findings as three grades between 1 and 3 in accordance to OAG such as voice, swallowing, color and dryness of lips and tongue, saliva, color and state of mucous membranes, gingiva and teeth. OAG of each patient was scored twice per a week. The total for each category was marked as OAG-total. Minimal and maximal number for grade was 1 and 3 for OAG, respectively. Therefore, OAG-total was calculated in a range from a score of 8 (score 1 × 8 categories), indicating a healthy oral cavity, to a score of 24 (score 3 × 8 categories), indicating severe mucositis.

2.5. Statistics

The statistical analysis was performed using the Mann-Whitney test to evaluate the combined infection rate before and the end of radiotherapy. The relationship

between the number of *Candida* species colonies and OAG total score was performed using the Pearson's correlation coefficient to evaluate. The OAG analysis according to category was performed using mutiple regression analysis. Analyses were performed using　Dr.SPSS (SPSS Japan, Tokyo, Japan). All statistical tests were performed at an alpha level of 0.05.

2.6. Ethics

The Study protocol was submitted to and approved by the Fukuoka University institutional ethics committee of each participating center prior to starting any study-related procedure. Written informed consent was obtained from each participant.

3. Results

Isolated *Candidas* are shown in Table 2. *Candida* was detected 12 out of 25 patients before radiotherapy, whereas it was detected in all patients at 10.4±13.5 Gy (range 0-40 Gy). Before irradiation, *Candida albicans* (n=8) was indentified in 32 % (8 of 25). At the end of radiotherapy, *C. albicans* (n=19) was most frequently detected, followed by *C. parapsilosis* (n=4), *C. dubliniensis* (n=4), *C. glabrata* (n=4), *C. tropicalis* (n=3), *C. guilliermondii* (n=2), and *C. krusei* (n=1), and *Candida spp* (n=2). The combined infection rate before and the end of radiotherapy were 12 % and 40 %, respectively. It significantly increased by irradiation (3 patients vs. 10 patients, $p = 0.03$).

Before and end of radiotherapy, the number of patients who were detected 3 *Candida* species were 1 and 4. Figure 1 shows the relationship between the number of *Candida* colonies and the OAG total score. The number of *Candida* colonies significantly correlated with the OAG total score ($r^2 = 0.31$, $p < 0.0001$). Table 3 shows the multiple regression analysis. There were a significant correlation between number of *Candida* colonies and cumulative irradiation dose, as explanatory variable, and OAG total score, as objective variable.（$r^2 = 0.63$, $p < 0.0001$）.

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Table 2.　Candidas isolated from swab　　n = 25

Candida species	Radiation	
	Before (No. of patients)	After (No. of patients)
Candida albicans	8	19
Candida parapsilosis	—	4
Candida dubliniensis	4	4
Candida glabrata	2	4
Candida tropicalis	—	3
Candida guilliermondii	1	2
Candida krusei	1	1
Candida spp	—	2

Twelve and 25 patients were *Candida*-positive among 25 patients, and 16 and 39 of *Candida* species were identified before and after radiotherapy, respectively.

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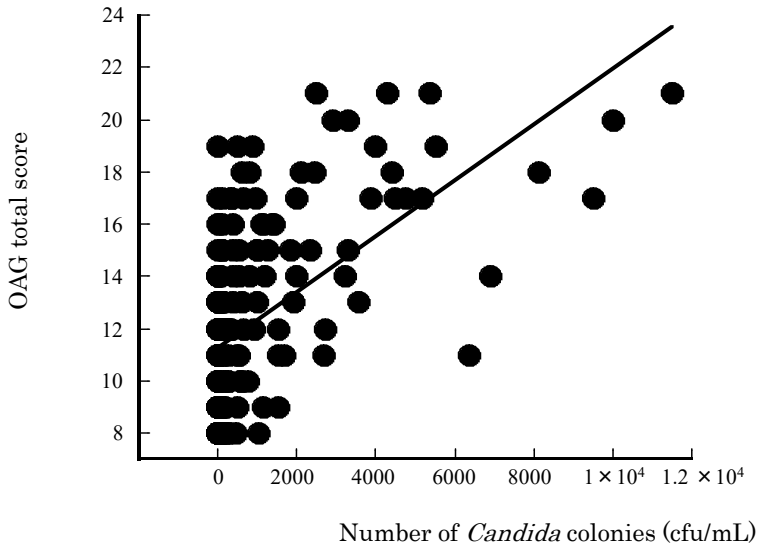

Fig. 1. Relationship between oral Assessment Guide total score and the number of *Candida* colonies

The Oral Assessment Guide (OAG) is a tool for evaluating clinical symptoms for oral health condition and function. OAG-total was calculated as the total for each category including voice, swallowing, color and dryness of lips and tongue, saliva, color and state of mucous membranes, gingiva and teeth.

Table 3. Coefficient of determination, significant p value by multiple regressions

n=25

	Multiple regression analysis (vs OAG total score)	
	95% CI	*p* value
Cumulative irradiation	0.107 - 0.143	< .0001

4. Discussion

The detection rate of *C. albicans* is reported approximately 30 % in the patients with head and neck cancer[9,10], which was similar to our study data. *C.albicans* has more potent intrinsic attachment capability than *C. non-albicans*. In addition, *C. albicans* which is more pathogenic than *C. non-albicans* has a high production capacity of a hydrolase, *Candida* Acid Proteinase (CAP)[11,12]. On the other hand, *C. non-albicans* such as *C.glabrata*, which produces very little CAP has also been identified as the cause of oral candidiasis[13].

Of 16 cases of *Candida* detected before radiotherapy, 8 were those of *C. albicans* (50 %), and 8 of *C. non-albicans* (50 %). The number of detection of *Candida* increased from 16 to 39 cases. Combined infection rate increased and *C. non-albicans* were emerged. A combined infection tends to occur under conditions in which biological defense decreases due to radiation[14].

In our study, *Candida* was detected in all patients at the end of radiation. Even in patients with negative *Candida* testing before radiotherapy, favorable condition for *Candida* growth in the oral cavity might be created by radiotherapy. Mucosal inflammation induced by irradiation damages the host tissues, which facilitated *Candida* colony formation to increase the amount of *Candida*.

We employed OAG score to investigate the relationships between *Candida* and clinical conditions. It was thought that the radiotherapy related to the growth factor of *Candida* which affected oral condition and that the therapy was the confounding variable which caused oral dryness disease, mucosal inflammations. It was the clear fact that radiotherapy caused damages to oral mucosa, which had a profound influence to the OAG score. In addition, we found out that the number of *Candida* colony also was significantly correlated with the OAG score; the increase of the *Candida* colony influenced the clinical symptoms. *Candida* is generally normal inhabitant and it does not make any negative effects to a host even though that fungus colonizes[15]. However, there are some reports that tissue damages caused by Candida changed the oral resident flora[16,17] and opened the host defense against fungal infection[18].

Thus this study might be indicated that the radiotherapy was a main factor which leaded to infection and that Candida also worked as a secondary factor. Our results suggested that both *Candida* species and combined infection increased by

cumulative irradiation dose. In other words, it was assumed that *Candida* which inhabited in vivo grows when ability to defend against infection declines, which influenced the variation factor of the OAG score.

Consequently, *Candida* distribution indicated a high value of the OAG score, so that it might reflect the trends of the adverse effect on clinical findings. It would be supposed to spread to opportunistic infection such as candidiasis.

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