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# Cariogenic Activity in Saliva of Korean Head and Neck Cancer Patients

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**The purpose of this study is to evaluate salivary flow rate, salivary pH, and cariogenic activity using unstimulated saliva of the head and neck cancer patients. Twenty three cancer patients (19 males, 4 females) who had undergone chemotherapy and radiation therapy and twenty four healthy volunteers (14 males, 10 females) as a control were included. Salivary flow rate, salivary pH, and cariogenic activity using unstimulated saliva were examined. Compared to saliva of the control group, salivary flow rate ($p<0.001$) and salivary pH ($p<0.001$) were significantly lower in head and neck cancer patients. The colony counts of *Lactobacilli* was higher in head and neck cancer patients ($p<0.05$) than in control group. These salivary factors and cariogenic activity can increase the prevalence of dental caries in head and neck cancer patients.**

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**Key words: head and neck cancer patients, cariogenic activity, salivary pH, salivary flow rate**

## Introduction

According to the Korean National Cancer Statistics, more than 200,000 new patients were diagnosed with cancer in 2010. In korean cancer patients, 2.2% patients (approximately 3,700 patients) are newly diagnosed to head and neck cancers each year (http://ncc.re.kr/english/infor/kccr. jsp.).

Malignancies of head and neck occur in the oral cavity, pharynx, paranasal sinuses, larynx, thyroid gland, salivary glands, bronchial tubes, and esophagus. Histologically, squamous cell carcinoma is the most frequent malignant followed by other types, such as an undifferentiated carcinoma, sarcoma, and melanoma [1].

The primary risk factors are smoking and alcohol. The incidence rates are 5 to 25 times greater in smokers than in non-smokers. In addition, poor oral hygiene and chronic inflammations of respiratory organs are high-risk factors [2,3]. The incidence of head and neck tumors increase with age. Tumors of the upper aerodigestive tract occur mostly in the fifth and sixth decades of life [2,3]. The present aging population has comparatively higher number of natural teeth as compared to those of the past. For the last 20 years, the number of patients visiting dental offices for caries has decreased in the pediatric population, but has increased for those who were 40-to-50 years old [4]. Thus, there is a need for different ways of prevention and management of tooth decay for different age groups.

The management of cancer patients depends on the type of cancer, metastasis, and age. Head and neck cancers are treated with a combination of surgery, radiation therapy, and chemotherapy to improve survival rate [5]. These treatments cause physical, emotional, and social side effects. Chemotherapy and radiation therapy destroy and weaken not only cancer cells but also non-cancerous cells, which produce intra-oral complications such as stomatitis, xerostomia, the change of taste, pain, and bleeding [6-9]. With the radiation therapy, salivary glands are irreversibly damaged, and resulted in xerostomia. Reduced salivation increase the occurrence of rampant caries [6-9]. As for the children with cancer, radiation therapy affect the developing teeth and cause oligodontia, microdontia, abnormal root growth, and hypocalcification [10]. Deterioration of quality of life due to dental complications has been unavoidable. In particular, head and neck cancer patients experience serious worsening in vocalization, deglutition, and physical appearance, which deteriorates the quality of life [6-9].

Therefore, management head and neck cancer should not only include the treatment of the disease itself but also the minimizing functional and aesthetic complications. In order to do so, oral examination should be performed to find out oral diseases and proper dental treatment prior to cancer therapy. These efforts should be made during and/or after anticancer treatments for preventing oral complications.

There have been researches on anticancer treatment-related oral complications [6-10]. However, few studies have investigated the characteristics of saliva and caries activity in these patients. Thus, researches about the characteristics of saliva and caries activity in this population are urgently needed.

In this study, salivary flow rate, salivary pH, salivary buffering capacity, and caries activities were compared between healthy subjects and head and neck cancer patients. An accurate caries risk assessment can identify patients at high caries risk for preventive therapies and improved treatment effectiveness. This research was performed to obtain a baseline data for proper oral hygiene management for individuals.

## Materials and Methods

### The subjects

The study recruited patients visiting the dental clinic of the Chonnam National University Hwasun Hospital (Hwasun, Korea). Subjects with systemic diseases were excluded, save the history of head and neck cancer for the first group. 23 head and neck cancer patients (19 males and 4 females) with a mean age of 57.3±8.7 years participated. All patients had undergone chemotherapy and radiation therapy. At least three months after therapeutic termination, all patients were enrolled. The control group included 23 healthy subjects (14 males and 9 females) with a mean age of 51.1±19.2. The age difference between the cancer patients and control group was not significant. None of them had received antibiotics. All subjects in both patient and control groups were advised of the purpose of the study and signed a consent form. This study was approved by the research ethics committee at the Chonnam National University Hwasun Hospital (HCRI 09 032-3).

### The salivary flow rate of unstimulated saliva

All saliva collections were performed at least 1 hour after a meal. The unstimulated saliva was collected by spitting methods in 50 ml conical tubes (SPL, South Korea) for 5 minutes [11,12].

The amount of saliva was measured and the saliva was transferred to 1 ml tubes (Axygen, California, USA).

### Measurement of unstimulated salivary acidity

After calibrating the pH meter (pH-200L; iStek, Seoul, Korea), salivary pH value was evaluated immediately.

### Caries activity tests

(1) Test for salivary buffering capacity

The buffering capacity of saliva was tested with Dentobuff strip (Orion Diagnostica, Espoo, Finland). A drop of each sample was applied onto the strips, and developed for 5 minutes. A color of the strip was read as the color index. The the degree of salivary buffering capacity was rated according to the developed color:

1 (low): yellow, the final pH value is equal to 4 or smaller,
2 (medium): green, the final pH value is equal to 4.5 or greater, but smaller than 5.5,
3 (high): blue, the final pH value is equal to 6 or greater.

(2) Measurement of the number of *Streptococci mutans*

The number of Streptococcus mutans (S. mutans) was measured with Dentocult SM (Orion Diagnostica, Espoo, Finland). Dentocult SM is composed of two strips. The 'screening strip' for the number of *S. mutans* in saliva was applied against the tongue. The 'site strip' is used to measure that in dental plaques. A sterilized cotton swab was rubbed on inter-proximal sites of posterior teeth for plaque inoculation. The inoculated sample was spread on the strip. Strips were incubated for two days and evaluated according to the density criteria :

0 : < $10^4$ CFU/ml (CFU : colony-forming unit),
1 : < $10^5$ CFU/ml,
2 : $10^5$~$10^6$ CFU/ml,
3 : > $10^6$ CFU/ml.

(3) Measurement of the number of *Lactobacilli*

The number of *Lactobacilli* was measured with Dentocult LB (Orion Diagnostica, Espoo, Finland). The saliva samples were poured onto the culture slide and incubated for four days at 35~37°C to be evaluated as the density criteria :

0 : nearly none to $10^3$ CFU/ml of saliva,
1 : $10^3$~$10^4$ CFU/ml,
2 : $10^4$~$10^5$ CFU/ml,
3 : $10^5$ CFU/ml or more.

### Statistic analysis

The Mann-Whitney test was used to compare the salivary flow rate, pH, and caries activity of unstimulated saliva between healthy subjects and cancer patients. SPSS (Statistical Packages for Social Science 19.0, Chicago, IL, USA) was used for the analyses, with the significance level of 0.05.

## Results

### Comparison of salivary flow rate and pH between head and neck cancer patients and healthy subjects

The salivary flow rate was 1.12±0.87 ml/5min for head and neck cancer patients and 2.59±1.36 ml/5min for healthy subjects. The difference of salivary flow rate was statistically significant (p<0.001) (Table 1).

The unstimulated saliva pH was 6.46±0.45 for head and neck cancer patients and 6.78±0.34 for healthy subjects. The difference of salivary pH values was statistically significant (p<0.01) (Table 1).

### Comparison of caries activities between head and neck cancer patients and healthy subjects

The degree of saliva buffering capacity was lower for cancer patients (1.65±0.78) than for healthy subjects (2.00±0.59), but this difference was not statistically significant.

The density of *S. mutans* colony counts was 1.13±0.92 in saliva for head and neck patients and 0.63±0.82 for healthy subjects. The density of *S. mutans* colony counts in plaque was 1.78±0.93 for head and neck patients and 1.50±0.67 for healthy subjects. Differences of the density of *S. mutans* were not significant in saliva and in plaque.

The density of *Lactobacilli* colony counts was significantly higher for head and neck cancer patients (1.30±1.18) than that for healthy subjects (0.58±0.88) (p<0.05) (Table 2).

**Table 1.** Salivary Flow Rate of the Study Groups

| | Patient group (N=23) (ml/5min) | Control group (N=23) (ml/5min) | *P*-value |
|---|---|---|---|
| Salivary secretion | 1.12±0.87 | 2.59±1.36 | 0.000*** |

Values are Mean±SD.
*** : $P < 0.001$, by Mann-Whitney test.

**Table 2.** Salivary pH of the Study Groups

| | Patient group (N=23) | Control group (N=23) | *P*-value |
|---|---|---|---|
| Salivary pH | 6.46±0.45 | 6.78±0.34 | 0.009** |

Values are Mean±SD.
** : $P < 0.01$, by Mann-Whitney test.

**Table 3.** Comparision of Cariogenic Activity of the Study Groups

| | | Patient group (N=23) | Control group (N=23) | *P*-value |
|---|---|---|---|---|
| Dentocult Buffer | | 1.65±0.78 | 2.00±0.59 | 0.063 |
| Dentocult SM | Dentocult screening strip | 1.13±0.92 | 0.63±0.82 | 0.057 |
| | Dentocult site strip | 1.78±0.93 | 1.50±0.67 | 0.118 |
| Dentocult LB | | 1.30±1.18 | 0.58±0.88 | 0.027* |

Values are Mean±SD.
* : $P<0.05$, by Mann-Whitney test.
(1) Dentocult Buffer
degree of salivary buffering capacity ; 1: the final pH value is equal to 4 or smaller, 2: equal to 4.5 or greater, but smaller than 5.5, 3: equal to 6 or greater.
(2) Dentocult SM : the number of *Streptococci mutans*
The 'screening strip' measuring the number of *S. mutans* in saliva was applied against the tongue. The 'site strip' is used to measure that in dental plaques. The density criteria; 0: < $10^4$ CFU/ml (CFU: colony-forming unit), 1: < $10^5$ CFU/ml, 2: $10^5$~$10^6$ CFU/ml, 3: > $10^6$ CFU/ml.
(3) Dentocult LB : the number of *Lactobacilli*
The density criteria; 0: nearly none to $10^3$ CFU/ml of saliva, 1: $10^3$~$10^4$ CFU/ml, 2: $10^4$~$10^5$ CFU/ml, 3: $10^5$ CFU/ml or more.

## Discussion

Anticancer treatments on head and neck cancers cause complications such as stomatitis, infections, hemorrhage, xerostomia, dysmasesis, dysphagia, taste change, and trismus. These complication aggravate the quality of life [6-9].

In this study, the salivary flow rate, salivary acidity, and caries activity were compared between head and neck patients and healthy subjects. Caries activity test was composed of salivary buffering capacity and the numbers of *S. mutans* and *Lactobacilli*.

Dental caries is the results of a complex interaction between acid-producing bacteria and fermentable carbohydrate, and host factors [13]. Caries activity test is predicting the risk of caries in considering pathogenic, host, and environmental factors [14]. Saliva is an important host factor acting as mechanical washing, antimicrobial function, remineralization of teeth surface and regulating oral pH [13].

In general, the patients undergoing chemotherapy exhibited reversible reductions in salivation and changes of salivary composition [6-9]. Radiation therapy in the head and neck region leads to a rapid and predictable the reduction of salivation. It is known that, on irradiation, the serous cells of salivary glands are damaged by acute inflammatory reactions, and this change produces less viscous saliva due to the markedly reduced amount of secretion. The amount of salivation began to decrease three days after irradiation, was at the minimum after four weeks, and thereafter returned to normal levels after a year [15,16]. In the present study, salivary flow rate was significantly lower for head and neck cancer patients than for healthy subjects. This finding agree with previous studies.

Normal salivary flow rate is important to a protective effect against dental caries. With normal salivary flow rate, the half time for saliva clearance is much shorter than the time required for oral bacterial cell division. Therefore, these bacteria cannot survive in the mouth unless they have the ability to bind to teeth or the oral mucosa [17]. As Kaur A1 *et al*'s study, 90% children in caries-free group had a stimulated salivary flow rate greater than 1 ml/min as compared to 33.3% in caries-active group. The salivary flow rate was 0.1-2.2ml/5min (mean 1.12±0.87 ml/5min) for head and neck patients in the present study. It is considered a potential risk factor when the unstimulated salivary flow rate is lower than 0.30 ml/min [18].

Maintenance of physiologic hydrogen ion concentration at the mucosal epithelial cell surface and the tooth surface is an critical function of salivary buffers. The quantitative assessment of resistance to pH changes is referred to as buffer capacity. High salivary buffering capacity protects the tooth from dental caries [19]. The salivary pH is determined by [HCO3-]/[H2CO3]. When the amount of salivation is greater, the saliva is weakly alkaline.

Conversely, when the amount is smaller, it is slightly acidic [20]. Fast-flowing saliva is alkaline reaching pH values of 7.5-8.0 and is vitally important in raising the pH of dental plaque previously lowered by exposure to carbohydrates [18]. Low buffering capacity is usually associated with caries development because of its impaired neutralization of plaque acids and reduced remineralization of early enamel lesions [21]. Studies have shown that patient with low or no caries activity had a resting salivary pH of around 7.0. Those with extreme caries activity had a resting pH below critical pH 5.5 [18]. In the present study, the pH and salivary flow rate were significantly lower for head and neck cancer patients than for healthy subjects. But, the difference of saliva buffering capacity was not statistically significant. More studies are necessary to determine saliva buffering capacity of head and neck patients.

Radiation therapy-related caries especially affects cervical areas of teeth and spreads throughout the entire dental arches. This type of caries does not directly result from the radiation itself; rather, it is a consequence of decreased salivation and subsequent increases in number of cariogenic bacteria [9,22]. Saliva serves as an oral circulating fluid for bacterial transmission and a reservoir for bacterial colonization. The oral cavity harbors over 700 species of bacteria that contribute to the health and physiological status of oral cavity. There are about $10^8$ to $10^9$ CFU/ml oral microorganisms in saliva. These salivary microbial species reflect the health and disease status of the oral cavity [23-25].

It is known that, on irradiation, the serous cells of parotid glands are damaged by acute inflammatory reactions, and this change produces less viscous saliva due to the markedly reduced amount of secretion [15,16]. Decreases in salivation rate is associated with decreased pH and buffering capacity. In turn, this promotes acidification of saliva at the teeth surface and increases the risk dental caries [19,21]. Therefore, the changes of salivation rate and salivary composition may increase the proportions of acidophilic microbes in the oral flora, such as *S. mutans*, *Lactobacilli*, and yeast [25,26]. Decreased salivation is very closely associated with *Lactobacilli* [27]. *Lactobacilli* are highly acidogenic, but do not play significant role in the initiation of caries. However, once a lesion has been established, its proportions were seen to increase [28]. Therefore, salivary *lactobacilli* level could be indirectly related to caries progression. In a study on salivary bacterial composition after radiation therapy among oral cancer patients, the number of acidophilic bacteria, *Candida, Staphylococcus, Lactobacillus*, and *S. mutans* were found to be significantly increased after irradiation. In this same study, the *S. mutans* numbers increased immediately upon completion of irradiation but did not increase thereafter [25].

Tong *et al.* observed changes in the numbers of intra-oral microbes in patients with reduced salivation from anti-cancer drugs and/or radiation therapy. They reported that the number of *S. mutans* was decreased before the radiation therapy but was not detected right after the therapy, and that under low pH, *Lactobacillus*, *Streptococcus mitis*, and *Streptococcus salivarius* were primarily detected [29]. Our findings are in agreement with this report. In this study, the number of *Lactobacilli* in the cancer patients was significantly greater for than that in the healthy subject. While the differences of the *S. mutans* numbers between the two groups was not significant.

In conclusion, salivary flow rate and pH were lower for the head and neck cancer patients than for the control group. The number of *Lactobacilli* increased significantly in the patient group. These results indicate that an individualized education for oral hygiene is essential to promote a patient's ability of managing oral health. In order to improve a patient's self-cleansing capacity, salivary substitutes and oral lubricants should be prescribed. Individuals with reduced salivary flow should be advised to maintain acceptable patterns of oral hygiene and to reduce the frequency of sugar ingestion. For patients undergoing radiation therapy on head-and-neck area, dental examination and fluorine coating should be carried out regularly. Professional oral health care program and systematical management should be applied on a regular basis as long as possible. Further studies are needed to understand the change of caries activities before, during, and after anticancer treatments.

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## Conflict of interest

The authors declare that they have no competing interest.

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