学位論文

Doctoral Thesis

Morphological Changes of Salivary Glands in Oral Cancer Patients Treated with Preoperative Chemoradiation Therapy

(口腔癌に対する術前化学放射線療法に伴う
唾液腺の形態学的変化についての検討)

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Contents

Abstract

Purpose:

We evaluated the morphological changes including radiological and histopathological findings of salivary glands in oral cancer patients treated with preoperative chemoradiation therapy (CRT).

Methods:

In the study of Chapter 2, twenty patients with advanced oral squamous cell carcinomas, who treated with preoperative CRT, underwent morphological assessment with Computed Tomography (CT) or magnetic resonance imaging (MRI) and functional assessment with the Saxon test.

In the study of Chapter 3, eligibility criteria were a pathologic diagnosis of oral squamous cell carcinoma, preoperative CRT with a total dose of 30 Gy and oral S-1 (80 mg/m^2/day), the availability of morphological assessments by CT and of functional assessments with the Saxon test before- and 2 weeks after CRT, and the availability of histopathological slides of irradiated parotid and submandibular glands. In the histopathological interpretation, gland structures were divided into acinar-, duct-, and adipose cells and other tissues.

Results:

The post-CRT:pre-CRT parotid volume ratio ranged from 54 - 85% (mean, 71%). There was a correlation between decreased parotid gland volume and decreased saliva production in the patients undergoing CRT. Histopathologically, acinar cell loss is a main contributor to changes in the volume and function of irradiated human parotid and submandibular glands. However, some acinar cells were retained after 30-Gy irradiation.

Conclusion:

In another study, we have demonstrated that the recuperation of morphological and functional changes occur in the course of 2-year follow-up in patients treated with 30-Gy irradiation. To avoid the sequelae of xerosomia, oral cancer patients undergoing CRT should receive oral care for at least 2 years after CRT. If in the course of definitive CRT saliva production is markedly decreased and morphological changes occur in the parotid gland volume, we recommended re-evaluation of the radiation-dose distribution.

List of Publications:

Keiko Teshima , Ryuji Murakami, Etsuji Tomitaka, Tomoko Nomura, Ryo Toya, Akimitsu Hiraki, Hideki Nakayama, Toshinori Hirai, Masanori Shinohara, Natsuo Oya and Yasuyuki Yamashita (2010) Radiation-induced parotid gland changes in oral cancer patients: correlation between parotid volume and saliva production. Jpn J Clin Oncol **40**: 42-46.

Keiko Teshima, Ryuji Murakami, Ryoji Yoshida, Hideki Nakayama, Akimitsu Hiraki, Toshinori Hirai, Yuji Nakaguchi, Naoko Tsujita, Etsushi Tomitaka, Mitsuhiro Furusawa, Yasuyuki Yamashita and Masanori Shinohara1 (2012) Histopathological Changes in Parotid and Submandibular Glands of Patients Treated with Preoperative Chemoradiation Therapy for Oral Cancer: J. Radiat. Res., 53, 492–496

Tomoko Nomura, Ryuji Murakami, Ryo Toya, Keiko Teshima, Aya Nakahara,Toshinori Hirai, Akimitsu Hiraki, Hideki Nakayama, Yoshihiro Yoshitake, Kazutoshi Ota, Takehisa Obayashi, Yasuyuki Yamashita, Natsuo Oya, and Masanori Shinohara (2010) Phase II study of preoperative concurrent chemoradiation therapy with S-1 in

patients with T4 oral squamous cell carcinoma. Int J Radiat Oncol Biol Phys **76**: 1347-1352.

Etsushi Tomitaka, Ryuji Murakami, Keiko Teshima, Tomoko Nomura, Yuji Nakaguchi, Hideki Nakayama,Mika Kitajima, Toshinori Hirai, Yushi Araki, Masanori Shinohara and Yasuyuki Yamashita (2011) Longitudinal Changes over 2 Years in Parotid Glands of Patients Treated with Preoperative 30-Gy Irradiation for Oral Cancer. Jpn J Clin Oncol 2011;41(4)503‐507

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Abbreviations

CRT: chemoradiation therapy

CT: computed tomography

MRI: magnetic resonance imaging

RT: radiation therapy

SCC: squamous cell carcinoma

CCRT: combination of concurrent chemoradiotherapy

IMRT: intensity-modulated radiation therapy

GTV: gross tumor volume

DVH: dose-volume histogram

HU: hounsfield units

mw: molecular weight

Chapter 1

Introduction

1.1 Background

Multimodal therapeutic strategies, i.e., combinations of radiotherapy (RT), chemotherapy, and surgery, are essential in the treatment for advanced head-and-neck squamous cell carcinoma (SCC). Randomized trials have shown that a combination of concurrent chemoradiotherapy (CCRT) and surgery is more effective than surgical resection alone. In patients with advanced oral SCC, combined treatment with preoperative CCRT results in better survival than adjuvant treatment including postoperative radiotherapy. Taking into account a feasible approach to improve overall outcome, we have performed preoperative CCRT with S-1 in patients with advanced oral SCC.

This chapter is organized as in the following. Section 1.2 states the purpose of this thesis. The organization of the dissertation is outlined in Section 1.3.

1.2 Purpose of This Thesis and Overview

In our preoperative CCRT protocol, radiotherapy was started on Day 1 of the study, using 4- or 6-MV photons. A total dose of 30 Gy was delivered at

daily fractions of 2 Gy over the course of 3 weeks using opposed lateral fields. The primary tumor and suspicious metastatic nodal levels were included in the clinical target volume. Concurrent with radiotherapy, S-1 was administered orally twice daily after a meal for 14 consecutive days, followed by the last week of radiotherapy without S-1. The daily S-1 dose (80mg/m^2) was based on reported Phase I studies. Each capsule of S-1 contained 20 or 25mg of tegafur. Individual dose were rounded down to the nearest pill size less than the calculated dose, given the available formulation.

Between January 2004 and December 2007, we performed preoperative CCRT in 123 eligible patients with Stage III or IV oral SCC. Of these, 46 harbored T4 tumors and were included in a study. All 46 patients underwent radiotherapy as planned; however, the oral administration of S-1 was discontinued in 3 patients who manifested acute toxicity. Although the toxicities of Grade 3 were mucositis (n=9), anorexia (n=4), and neutropenia (n=2), all 46 patients experienced transient mucositis ranging from Grade 1 to 3.

We focused on mucositis which had occurred in the all patients. Mucositis has a strong correlation with saliva production and xerostomia. Xerostomia is a common debilitating adverse effect of CRT in patients with head-and-neck tumors (1-11). The patients with xerostomia should receive oral care to prevent mucositis, dental caries, oral infection, difficulty speaking, and dysphagia (2,3). Although many studies evaluated functional changes in the parotid glands, i.e. subjective symptoms of dry mouth, saliva production, and salivary flow, reports concerning the morphological changes of salivary glands are limited (11-15).

In this thesis, we evaluated a relationship between saliva production and parotid volume. In addition, we investigated histopathological finding of parotid and submandibular glands.

1.3 Organization of This Paper

The organization of the paper is as in the following. In Chapter 2, radiation-induced parotid gland changes in oral cancer patients are discussed. Then histo-pathological changes in parotid and submandibular glands of patients treated with preoperative chemoradiation therapy for oral cancer are presented in Chapter 3. Chapter 4 concludes the paper.

Chapter 2

Radiation-Induced Parotid Gland Changes in Oral Cancer Patients: Correlation between Parotid Volume and Saliva Production

2.1 Abstract

The purpose of this study is to evaluate whether saliva production reflects the parotid volume during the course of radiation therapy (RT) in patients with head-and-neck cancer.

Twenty patients with advanced oral squamous cell carcinomas, who treated with preoperative CRT, underwent morphological assessment with CT or MRI and functional assessment with the Saxon test. For the Saxon test, saliva production was measured by weighing a gauze pad before and 2 min after chewing without swallowing; the low-normal value is 2 g. Saliva production and parotid volumes before and 2 weeks after CRT were compared with the paired t-test, the Spearman rank correlation test, and the Fisher exact test.

After 30-Gy irradiation, mean saliva production was decreased from 4.2-1.0 g ($p<0.01$); the reduction in saliva production ranged from 1.7-5.4 g (mean 3.2 g). The mean parotid volume was decreased from 68.2-47.9 cm3 ($p<0.01$); the post-CRT:pre-CRT parotid volume ratio ranged from 54-85% (mean 71%).

Although the initial parotid volume was correlated with initial saliva production ($r = 0.47$, $p = 0.04$), no significant correlation was noted after CRT ($r = 0.08$, $p = 0.71$) and there were considerable individual variations. The parotid volume ratio was inversely correlated with the saliva-reduction amount ($r=-0.79$, $p<0.01$).

There was a correlation between decreased parotid gland volume and decreased saliva production in patients with head-and-neck cancer undergoing CRT. Parotid volume reduction may predict parotid gland function.

2.2 Introduction

Approximately 60-65% of total saliva is produced by the parotid-, 20-30% by the submandibular-, and 2-5% by the sublingual glands (1). The parotid glands are purely serous and their secretion is watery and albuminous. The submandibular and sublingual glands are composed of mixed serous and mucous acini and their secretions are thicker. The minor salivary glands contain predominantly mucous acini and produce thick, sticky secretions. Radiation injury to the salivary glands primarily results from damage to the serous cells, the mucous cells are less involved (1,2). Xerosomia is explained in Section 1.2.

The Saxon test is a simple, reproducible, and low-cost technique to measure saliva production, which is included in the diagnostic criteria for Sjögren's syndrome (16). The purpose of this study was to evaluate whether saliva production reflects the parotid volume during the course of CRT in patients with head-and-neck cancer.

2.3 Patients and Methods

Written prior informed consent for the routine clinical use of the Saxon tests and for treatments was obtained from all patients. The institutional review board of our hospital approved this retrospective investigation and waived patient informed consent for inclusion in our study.

2.3.1 Patients' Characteristics

Between April 2005 and March 2007, 20 patients with advanced oral squamous cell carcinomas received preoperative chemo-RT and underwent morphological assessment with a single modality either CT or MRI and functional assessment with the Saxon test before and 2 weeks after CRT. In the Saxon test, saliva production is measured by weighing a folded sterile gauze pad before and 2 min after chewing without swallowing; the low-normal value is 2 g (16). There were14 men and 6 women (mean age 71 years; age range 51-87 years) with 2 oral floor-, 3 tongue-, 4 maxillary gingiva-, 5 buccal mucosa-, 6 mandibular gingiva tumors. Clinically, 4 patients had T2-, 5 had T3-, and 11 had T4 tumors; nodal involvement was N0 in 10-, N1 in 3, N2b in 5, and N2c in 2 patients. None of our patients used salivary stimulators or protectors.

In our preoperative CRT protocol, a total dose of 30 Gy was delivered at daily fractions of 2 Gy over the course of 3 weeks using a 4-MV linear accelerator with opposed lateral fields. The primary tumor and suspicious metastatic

nodal levels were included in the clinical target volume. Ipsilateral and contralateral parotid glands were similarly included in irradiated volume. Concurrently, S-1 (Taiho Pharmaceutical Co., Tokyo, Japan) was administered orally at a daily dose of 80 mg/m2 starting on the 1st day of irradiation and continuing for 2 consecutive weeks. S-1 is a novel oral fluoropyrimidine preparation; it is designed to improve the antitumor activity of 5-fluorouracil (5-FU) while reducing gastrointestinal toxicity (17-19). The clinical response 2 weeks after completion of preoperative CRT was clinically and radiologically evaluated, and surgical resection with reconstruction was performed 3 - 4 weeks after the completion of CRT.

2.3.2 Parotid Volumes

Considering differences in the volume assessment between different imaging modalities, in individual patients we used a single modality, either CT or MRI, before and after RT. The parotid volumes were consensually assessed by 2 observers, a radiation oncologist (E.T.) and a DDS (T.N.) with 10 and 5 years of experience in diagnosing and treating oral cancers, respectively, blinded to the Saxon test results and the presence or absence of xerostomia. Digital imaging data of the head-and-neck were transferred to a 3D treatment planning system (XiO; CMS Japan K.K., Tokyo, Japan) using the digital imaging and communications in medicine (DICOM) format. The observers manually contoured bilateral parotid glands on each slice of the imaging modality. Then, the parotid volume before and after RT was retrospectively measured and recorded in each pa-

tient, and the post-CRT:pre-CRT parotid volume ratio was calculated as the parotid volume change during the course of CRT.

2.3.3 Statistical Analysis

Saliva production and parotid volumes before and after CRT were compared with the paired Student t-test. The relationship between saliva production and the parotid volume pre- and post-CRT was evaluated with the Spearman rank correlation test and the Fisher exact test. Statistical analyses were carried out using Stat View Version 5.0 (SAS Institute Inc., Cary, NC). Values of $p < 0.05$ were considered to denote significant differences.

2.4 Results

After 30-Gy irradiation, mean saliva production was decreased from 4.2 g (range 2.1 - 7.2 g) to 1.0 g (range 0.1 - 2.1 g) ($p < 0.01$). The post-CRT reduction in saliva production ranged from 1.7 - 5.4g (mean 3.2 g). The mean parotid volume was decreased from 68.2 cm3 (range 29.6 - 89.9 cm3) to 47.9 cm3 (range 24.2 - 70.6 cm3) ($p < 0.01$). The post-RT:pre-RT parotid volume ratio ranged from 54 - 85% (mean, 71%) (Fig. 1).

Although the initial parotid volume was correlated with initial saliva production ($r = 0.47$, $p = 0.04$), no significant correlation was noted after CRT ($r = 0.08$, $p = 0.71$) and there were considerable individual variations (Fig. 2). On the other hand, the post-CRT:pre-CRT parotid volume ratio was inversely correlated

with the post-CRT saliva-reduction amount ($r = -0.79$, $p < 0.01$, Fig. 3). Of 10 patients with a saliva reduction of more than 3 g, 8 (80%) manifested a post-CRT parotid volume less than 70% of the pre-CRT volume. Such marked volume reduction appeared in only 1 (10%) of 10 patients whose saliva production was reduced by less than 3 g ($p < 0.01$). A representative case with marked changes in saliva production and parotid volume is shown in Fig. 4. This 63-year-old man with squamous cell carcinoma of the floor of the mouth (T3N0) was treated with preoperative chemo-RT. His initial saliva production and parotid volume were 5.6 g and 90 cm3, respectively. After 30-Gy irradiation they were 0.9 g and 49 cm3; the changes manifested a post-CRT saliva-reduction amount of 4.7 g and a post-CRT: pre-CRT parotid volume ratio of 54%.

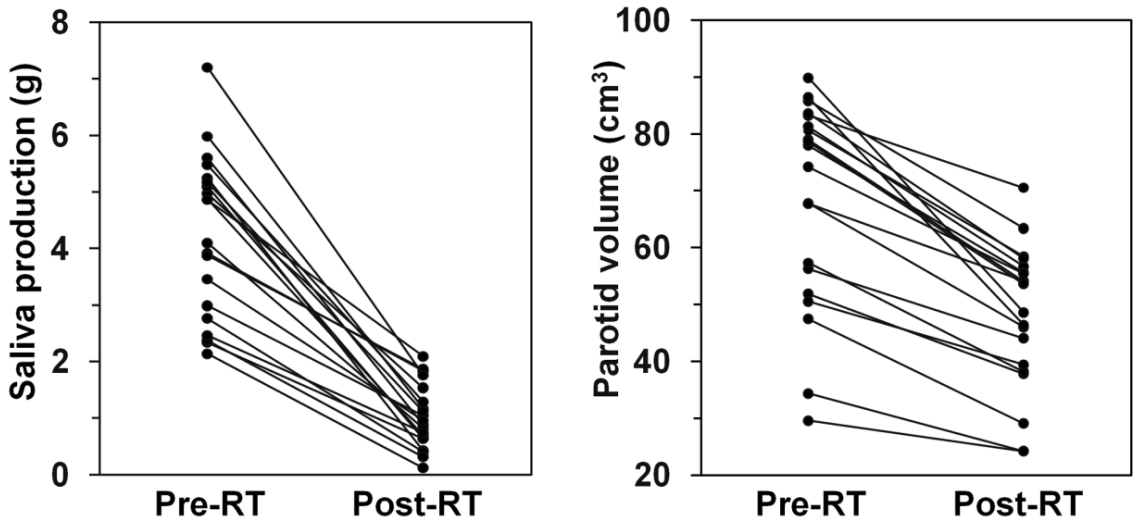

Figure 1. Comparison of saliva production and parotid volume before and after radiation therapy (RT). The post-RT reduction in saliva production ranged from 1.7 - 5.4g (mean 3.2 g). The post-RT:pre-RT parotid volume ratio ranged from 54 to 85% (mean 71%).

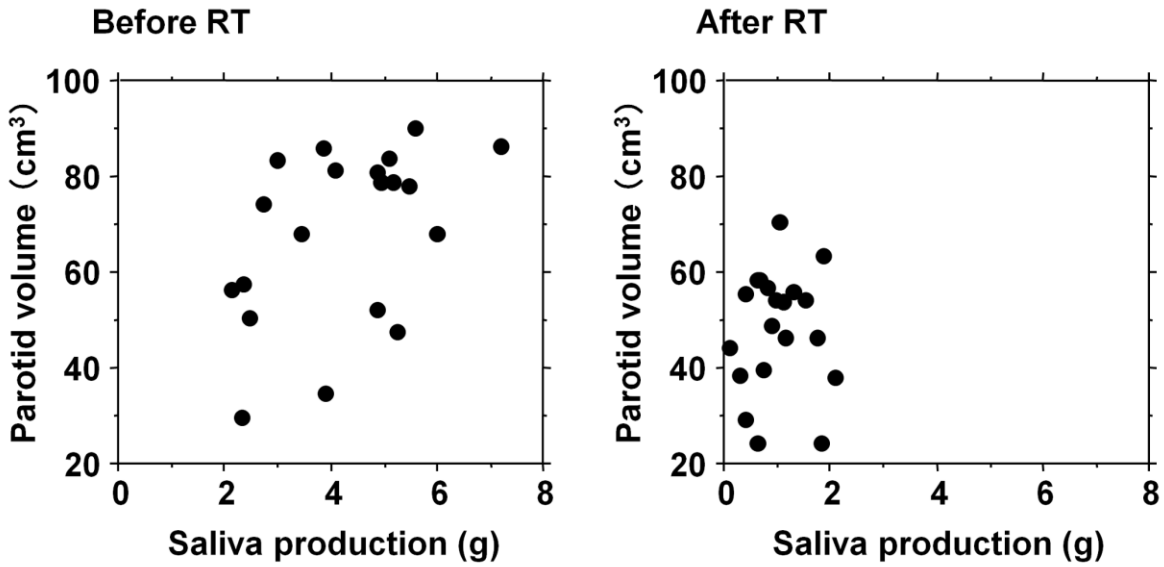

Figure 2. Relationship between saliva production and parotid volume before and after radiation therapy (RT). The Spearman rank correlation coefficients before and after RT were 0.47 ($p = 0.04$) and 0.08 ($p = 0.71$), respectively.

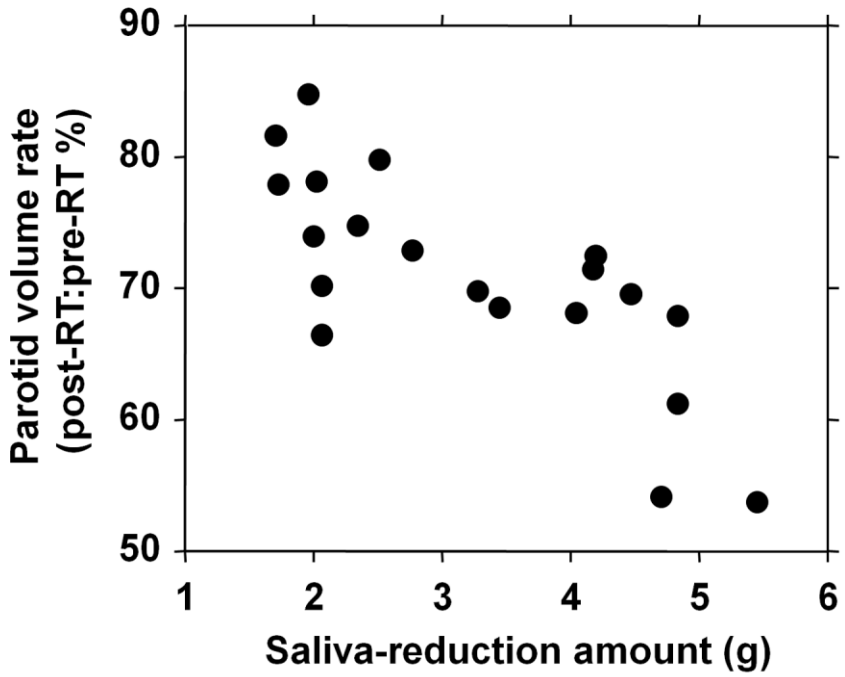

Figure 3. Relationship between the saliva-reduction amount and the parotid volume ratio (post-RT:pre-RT). The Spearman rank correlation coefficient was -0.79 ($p < 0.01$).

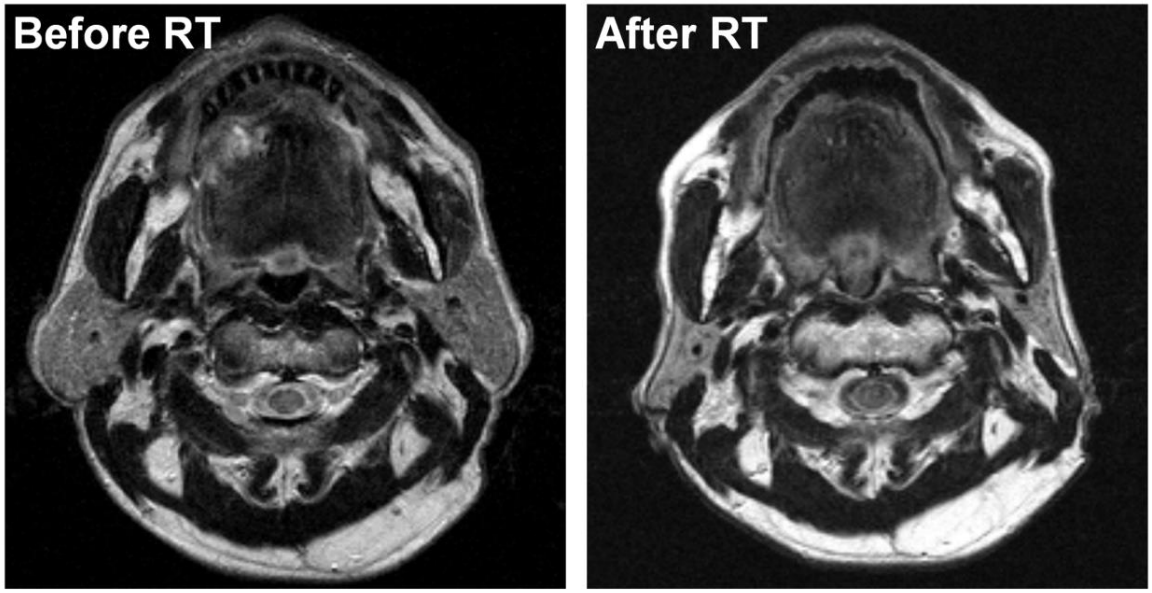

Figure 4. This 63-year-old man with squamous cell carcinoma of the floor of the mouth (T3N0) was treated with preoperative CRT. T2-weighted (3700/92) MR images obtained before and after CRT demonstrated morphological changes of the parotid glands. After 30-Gy irradiation, the parotid volume was decreased from 90 cm3 to 49 cm^3; the changes manifested a post-CRT:pre-CRT parotid volume ratio of 54%.

2.5 Discussion

Many head-and-neck cancer patients treated with CRT experience xerostomia (1-11) and some manifest a volume reduction in the parotid glands (11-15), although there are individual variations in the functional and morphological changes of the parotid glands throughout the course of CRT. Nömayr et al. (12), who assessed the MRI appearance of radiation-induced changes in normal cervical tissues, reported that the parotid volume was reduced by an average of 26% following 30- and by approximately 40% after 70-Gy irradiation. Barker et al. (13) suggested that the median parotid volume loss during conventional CRT was 0.2 cm3/day (range 0.04 - 0.84) or 0.6%/day (range 0.2 - 1.8%) of the initial volume. Robar et al. (14) demonstrated that in patients subjected to IMRT, the lateral aspects of both parotid glands showed a medial translation of 0.85 mm/wk; the glands shrank by 4.9%/wk. Our mean parotid volume ratio of 71% after 30-Gy irradiation was compatible with previous reports..

Although xerostomia is a subjective symptom, objective functional assessments can be used (3-9). Salivary flow rate measurement, most often applied to assess parotid gland function, is an invasive technique. Recently, the ability of Diffusion-weighted (DW) MRI or MRI sialography to depict radiation-induced parotid changes has been demonstrated (10). The Saxon test, a simple, low-cost technique, can objectively measure saliva production (16). However, some patients, especially older ones, may find it difficult to chew a gauze pad for 2 min. Our result, the correlation between saliva production and parotid volume, suggests that parotid volume reduction may predict parotid gland function.

To spare glandular function, the recommended dose to the parotid gland is less than 25 - 30 Gy (6-8). Patients treated with intensity-modulated radiation therapy (IMRT) experienced less severe xerostomia and similar treatment outcomes as those subjected to conventional RT (20,21). On the other hand, advances in high-precision RT techniques, notably three-dimensional conformal radiation therapy (3D-CRT) and IMRT, render the precise delineation of both the gross tumor volume (GTV) and organs at risk essential for RT planning (13-15). As patients receiving RT experience geometric changes including shrinkage of the GTV and changes in body habitus (12), the radiation-dose distribution should be re-evaluated in patients manifesting these changes in the course of RT. If in the course of RT saliva production is markedly decreased and morphological changes occur in the parotid gland volume, we recommend re-evaluation of the radiation-dose distribution.

In our preoperative CRT protocol we administered S-1, a novel oral fluorouracil derivative. This anti-tumor drug combines contains tegafur (a prodrug of 5-FU), gimeracil (which inhibits the 5-FU degeneration enzyme dihydropyrimidine dehydrogenase), and oteracil (which reduces the gastrointestinal toxicity of 5-FU). The advantages of chemotherapy and of CRT with S-1 have been demonstrated in patients with head-and-neck-, lung-, gastric-, rectal-, and pancreatic cancer (17-19). To our knowledge, there have been no reports suggestive of a relationship between S-1 and salivary dysfunction after completion of treatment.

In our retrospective study we could not ignore the influence of different imaging modalities, i.e., CT and MRI, on the assessment of parotid volume.

Therefore, in individual patients we used a single modality, either CT or MRI, before and after CRT, and considered the post-CRT:pre-CRT parotid volume ratio to reflect parotid volume changes. In terms of other study limitations, we should consider radiation-induced changes of the submandibular and sublingual glands within radiation fields, although radiation injury to the salivary glands primarily results from damage to the parotid glands (1,2). Furthermore, we have no data on morphological and functional changes in parotid glands irradiated with doses other than 30 Gy. Our patients underwent preoperative CRT with 30 Gy and surgical resection. It is necessary to validate our results by means of a large prospective study, especially in 3D-CRT and IMRT. Efforts are underway in our hospital to collect longitudinal data on changes in both the parotid volume and saliva production in patients treated with definitive RT.

2.6 Conclusion

After 30-Gy irradiation, parotid volume was decreased. There was a correlation between decreased parotid gland volume and decreased saliva production in patients with head-and-neck cancer undergoing CRT. Parotid volume reduction may predict parotid gland function. If in the course of CRT saliva production is markedly decreased and morphological changes occur in the parotid gland volume, we recommend re-evaluation of the radiation-dose distribution.

Chapter 3

Histopathological Changes in Parotid and Submandibular Glands of Patients Treated with Preoperative Chemoradiation Therapy for Oral Cancer

3.1 Abstract

We retrospectively evaluated the relationship between computed tomography (CT)- and histopathological findings of parotid and submandibular glands in six patients treated for advanced oral cancer. Eligibility criteria were a pathologic diagnosis of oral squamous cell carcinoma, preoperative chemoradiation therapy (CRT) with a total dose of 30 Gy and oral S-1 (80 mg/m2/day), the availability of morphological assessments by CT and of functional assessments with the Saxon test before- and 2 weeks after CRT, and the availability of histopathological slides of irradiated parotid and submandibular glands. In the histopathological interpretation, gland structures were divided into acinar-, duct-, and adipose cells and other tissues. The Mann-Whitney test and the Spearman rank correlation test were used to determine histopathological changes. After 30-Gy irradiation, saliva production and parotid and submandibular volumes were significantly decreased ($P < 0.05$ each). Histopathological analysis demonstrated that 30-Gy irradiation resulted in a loss of acinar cells although acinar cells in the submandibular gland were relatively retained; the median acinar rate in the parotid

and submandibular glands was 1.1% and 19.0%, respectively. The CT values after CRT were inversely correlated with adipose ratios ($r = -0.98$, $P < 0.01$) and there was a strong correlation between CT values before and after CRT ($r = 0.97$, $P < 0.01$). Our results suggested that acinar cell loss is a main contributor to changes in the volume and function of irradiated human parotid and submandibular glands. The CT value may reflect the adipose ratio rather than salivary function.

3.2 Introduction

Approximately 90% of total saliva is produced by the parotid and submandibular glands; sublingual and minor salivary glands together produce only 10% of the saliva volume.1, 22) The parotid glands are purely serous and their secretion is watery. The submandibular and the sublingual glands are mixed; serous units predominate in the submandibular- and mucinous units in the sublingual glands. Clinical data on radiation therapy (RT) indicate that saliva production is reduced after the delivery of 10 - 15 Gy to the parotid gland and although functional recuperation over time is possible after irradiation with 40 - 50 Gy, higher doses produce irreversible and permanent xerostomia.4,5,23,24) To spare glandular function, the recommended dose to parotid glands is less than 25 - 30 Gy.7,8,25) Histopathologically, radiation injury to salivary glands primarily results from damage to the serous cells; the mucinous cells are less involved.1,26) Therefore, the submandibular gland is thought to be less radiosensitive than the parotid gland.27,28) Although the histopathological effects of radiation on salivary

glands have been reported in animal models,26-29) there is little information on these radiation-induced changes in humans.30)

While normal parotid glands contain abundant adipose cells, adipose tissue is not a significant component of submandibular glands.31-33) As the presence of xerostomia in patients with Sjogren Syndrome suggests a correlation between decreased computed tomography (CT) values and impaired saliva production, CT values may represent functional changes in salivary glands.33,34) Elsewhere we documented radiation-induced changes in the parotid volume and saliva production of patients who had received 30-Gy chemoradiation therapy (CRT) before radical neck dissection. Here we retrospectively evaluated histopathological data from these patients to elucidate the relationship between CT- and histopathological findings after 30-Gy irradiation to human parotid and submandibular glands.

3.3 Patients and Methods

3.3.1 Patients' Characteristics

Histopathological slides of the parotid gland were obtained from 6 patients (5 men, 1 woman, mean age 73 years, range 59-78 years) who had undergone neck dissection after CRT for advanced oral cancer, as part of an institutional review board-approved study. Eligibility criteria were a pathologic diagnosis of squamous cell carcinoma, preoperative CRT with a total dose of 30 Gy, the availability of morphological assessments by CT and functional assessments with the Saxon test before- and 2 weeks after CRT, and the availability of histopatholog-

ical specimens of the irradiated parotid gland. In 5 patients, we also evaluated histopathological slides of the submandibular gland. The Saxon test measures saliva production by weighing a folded sterile gauze pad before- and 2 min after chewing without swallowing; the low-normal value is 2 g.

Our preoperative CRT protocol delivers a total dose of 30 Gy at daily fractions of 2 Gy over the course of 3 weeks using a 4 MV linear accelerator with opposed lateral fields. The primary tumor and nodal levels suspected of metastatic involvement were included in the clinical target volume; ipsi- and contralateral parotid glands were included in the irradiated volume. Based on dose-volume histogram (DVH) analysis in the 6 patients, the radiation dose (mean ± SD) to the ipsi- and contralateral parotid glands was 29.6 ± 1.1 and 29.2 ± 1.2 Gy, respectively; the ipsi- and contralateral submandibular doses were 31.1 ± 0.5 and 31.0 ± 0.5 Gy. S-1 (Taiho Pharmaceutical Co., Tokyo, Japan), a novel oral fluoropyrimidine preparation designed to improve the antitumor activity of 5-fluorouracil (5-FU) while reducing gastrointestinal toxicity, was orally administered at a daily dose of 80 mg/m^2 starting on the 1st day of irradiation and continuing for 2 consecutive weeks. The response 2 weeks after completion of preoperative CRT was evaluated clinically and radiologically; surgical resection with reconstruction was performed 3 - 4 weeks after CRT. Radical en bloc resection was based on the degree of tumor extension determined before CRT. The involved area, including a planned safety margin, was marked by an ink tattoo. Neck dissection was performed depending on nodal staging. In all patients, the ipsilateral submandibular gland and a part of the ipsilateral parotid gland

were resected. In one patient we were unable to obtain a submandibular gland specimen.

For histopathological comparisons, 10 control subjects were selected from a group of non-pre-treated patients who had undergone neck dissection for head- and neck cancer. Their mean age was 73 years (range 56-83 years) and they were age-matched to the CRT group, creating a control group for parotid and submandibular glands.

3.3.2 Radiological Interpretation

The parotid and submandibular CT findings were assessed by one of the authors (R.M.) who had 17 years of experience in the CT diagnosis of oral cancer. The ipsi- and contralateral parotid and submandibular volumes before- and 2 weeks after CRT were retrospectively measured and recorded for each patient. The glandular CT values expressed in Hounsfield Units (HU) were recorded for comparisons between the CT values and the histopathological structures.

3.3.3 Histopathological Interpretation

For histopathological comparisons between the CRT- and control group hematoxylin and eosin-stained slides were consensually analyzed by 2 investigators (M.S. and K.T.); they had 30 and 7 years of experience in the pathological diagnosis of oral cancer and were blinded to the CT findings. The parotid and submandibular structures were divided into acinar-, duct-, and adipose cells and

other tissues consisting of connective and mesenchymal tissues. In the submandibular glands, acinar cells included some mucinous cells. Morphometry was performed by using an ocular with a 10 × 10 grid mesh and counting hits at the cross-points.28,29) We counted 100 points per microscopic field and used averaged values from counts of 3 random fields as the structural ratios.

3.3.4 Statistical Analysis

Saliva production and parotid volumes before and after CRT were compared with the Wilcoxon signed rank test. The Mann-Whitney test was used for histopathological comparisons between control and CRT groups. The relationship between the CT value and the histopathological structures was evaluated with the Spearman rank correlation test. The difference between CT values before and after CRT was assessed by using the Bland-Altman method. Statistical analyses were carried out with the MedCalc program (version 9.2.1.0; MedCalc Software, Mariakerke, Belgium). For all analyses, values of $p < 0.05$ were considered to denote significant differences.

3.4 Results

After CRT, the median saliva production was decreased from 3.0 g (range 2.1-5.2 g) to 1.3 g (range 0.8-3.3 g) ($P = 0.0312$) and the parotid and submandibular volumes were also significantly decreased (Fig. 5). When original post-CRT data were converted to fractions of pre-CRT values (normalized parotid and sub-

mandibular volumes) because there were considerable individual variations, the median ipsi- and contralateral parotid volumes were 78.0 and 74.5%, respectively; the corresponding submandibular volumes were 68.1 and 71.0%, respectively.

Histopathological quantitative analysis comparing the control- and CRT group demonstrated that 30-Gy irradiation resulted in a loss of acinar cells especially in the parotid glands. In the submandibular glands serous acinar cells with secretory granules were relatively retained; the median acinar rate in the parotid and submandibular glands was 1.1% and 19.0%, respectively (Table 1). Although duct cells were relatively increased in the submandibular glands, there was no significant difference in the duct and adipose ratios between the control- and CRT group.

Although there were considerable individual variations in the post-CRT CT values of the submandibular- and especially the parotid glands (median 21.9 HU, range -50.6 - 51.8 HU), the CT values were inversely correlated with adipose ratios ($r = -0.982$, $P < 0.0001$). There was also a strong correlation between the CT values before and after CRT ($r = 0.965$, $P < 0.0001$). Bland-Altman analysis indicated that the 95% limit of agreement between the CT values before and after CRT ranged from -6.7 to 9.1 HU; the difference level within ±10 HU was not clinically significant.

3.5 Discussions

Studies on the parotid glands of rats after fractionated irradiation revealed a dose-dependent decrease in serous acinar cells followed by replacement with fi-

brotic tissue; duct cells, on the other hand, were not affected.28,29) In rat submandibular glands that harbor only mucinous acinar cells, acinar cell loss was not as prominent as in parotid glands. These structural changes observed in animal models were compatible with our human data although serous units predominate in human submandibular glands. The remaining acinar cells may provide the basis for morphological and functional recuperation.

We found that loss of acinar cells was severe in the human parotid gland irradiated with 30 Gy and that these cells were relatively retained in the irradiated submandibular gland; median acinar rates were 1.1% and 19.0%, respectively. Parotid glands are purely serous; they contribute most of the stimulated saliva volume. In the mixed submandibular glands serous units predominate; they contribute approximately two-thirds of the unstimulated saliva volume.1, 22,26). It is generally agreed that human saliva contains 2 major-, i.e. high- and low molecular weight (mw), mucinous components.35) The high-mw component is produced and localized in mucinous cells while the low-mw component is present in both mucous and serous cells of the submandibular and sublingual glands, but not in parotid serous cells. Additional biochemical and immunohistological studies are needed to test our hypothesis that the functional diversity among serous cells of the parotid and submandibular glands explains their different radiosensitivity.

Typically, the normal parotid gland harbors abundant adipose cells while adipose tissue is not a significant component of submandibular glands.22, 31-33) Although there were individual variations, in our control group, the median adipose ratio of parotid and submandibular glands was 41.9% and 15.5%, re-

spectively. In contrast, the median acinar ratio of parotid- was lower than of submandibular glands. These structural ratios are compatible with previous histopathological findings of Scott et al.31,32) who demonstrated that the adipose ratios tended to increase with age and in chronic alcohol abuse. Radiologically, the parotid glands are of a relatively fatty appearance while the submandibular glands appear more cellular. We demonstrate that the CT values were inversely correlated with adipose ratios. Although 30-Gy irradiation did not alter the CT values in our small series, further investigations are necessary to elucidate the effects of different radiation doses and late-phase effects.

According to Nömayr et al.,12) the parotid volume was reduced by approximately 26% and 40% after conventional 30- and 70-Gy irradiation, respectively. Reported results and our studies suggest that parotid and submandibular volumes after 30-Gy irradiation were approximately 70% of the baseline values.

Our study has some limitations. Under our preoperative CRT protocol we administered S-1, a novel oral fluorouracil derivative. Although no relationship between S-1 and salivary dysfunction has been documented, we must consider the possibility that the radiosensitizer acts not only on tumors but also on normal tissues.30) We recommend the routine Saxon test during and after treatment in patients undergoing RT for head-and-neck tumors; this simple low-cost test can objectively measure saliva production, a factor included in the diagnostic criteria for Sjogren's syndrome. However, some patients with oral tumors, especially older individuals, may find it difficult to chew a gauze pad for 2 min. In histopathological analyses, evaluated slides should not represent whole glands because there may be heterogeneous RT dose distribution in parotid and subman-

dibular glands. Our results must be validated by prospective studies on large populations, by using various radiation doses, and by long-term follow-up. Efforts are underway at our hospital to collect morphological and functional data on parotid and submandibular glands in patients treated with RT including intensity modulated RT.

3.6 Conclusion

Acinar cell loss is a main contributor to functional and volume changes in irradiated human salivary glands. We postulate that the submandibular- in contrast to the parotid gland, is less radiosensitive because it is a mixed gland that contains serous and mucinous cells and because its serous cells exhibit functional diversity. The CT value may reflect the adipose ratio in the salivary gland rather than saliva production.

Table 1. Histopathological quantitative analysis in the control- and CRT groups

Parameter	Control group	CRT group	P value*
Parotid gland	(n=10)	(n=6)	
Acinar cells (%)	31.5 (17.7-49.0)	1.1 (0.3-2.2)	0.0011
Duct cells (%)	4.5 (2.3-7.7)	5.8 (3.3-7.0)	0.0875
Adipose cells (%)	41.9 (26.0-63.3)	49.7 (14.7-80.3)	0.6374
Other tissues (%)	21.1 (12.1-31.6)	43.5 (13.5-77.4)	0.0509
Submandibular gland	(n=10)	(n=5)	
Acinar cells (%)	43.3 (34.7-49.7)	19.0 (11.0-22.3)	0.0022
Duct cells (%)	9.3 (5.7-16.3)	13.3 (8.7-18.3)	0.0576
Adipose cells (%)	15.5 (10.0-41.7)	13.0 (8.7-20.0)	0.1984
Other tissues (%)	27.3 (10.3-42.3)	55.3 (47.0-68.0)	0.0022

Abbreviation: CRT = chemoradiation therapy.

Data are median values. Numbers in parentheses are ranges of the parameters.

*Mann-Whitney test.

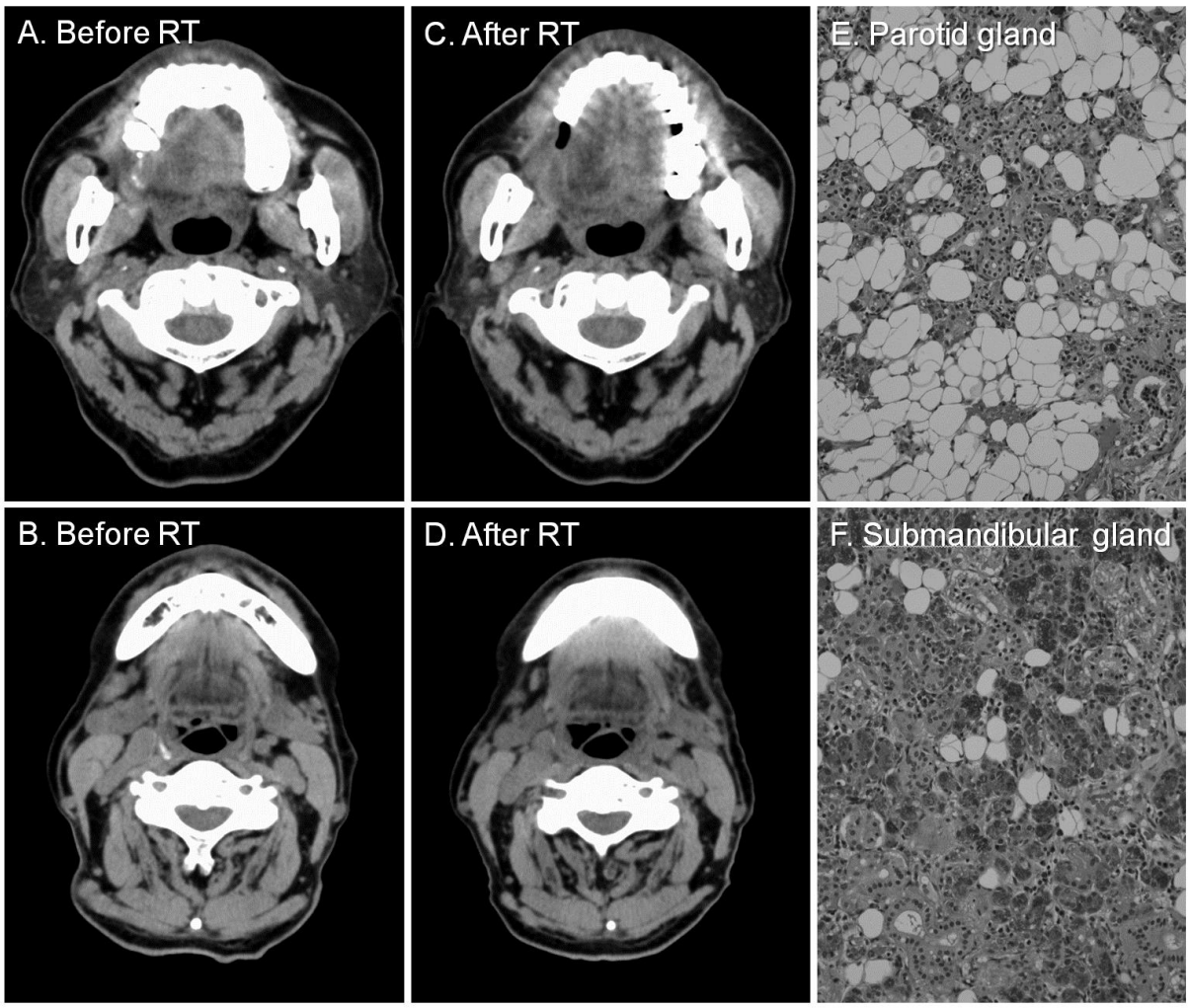

Fig. 5. A 75-year-old man with squamous cell carcinoma of the right buccal mucosa (T3N2b) was treated with preoperative chemoradiation therapy (CRT). Computed tomography (CT) images obtained before and after CRT demonstrated morphological changes in the parotid and submandibular glands (A, parotid glands before CRT; B, submandibular glands before CRT; C, parotid glands after CRT; D, submandibular glands after CRT). After 30-Gy irradiation, the ipsi- and contralateral parotid volumes were decreased to 79.6 and 78.3%, respectively; the ipsi- and contralateral submandibular volumes were decreased to 60.8 and 56.8%, respectively. Histopathological slides (H&E, ×100) of the irradiated

parotid (E) and submandibular (F) glands suggest replacement of acinar cells by ductal metaplasia, periductal fibrosis, and inflammatory cell infiltration. Although serous acinar cells with secretory granules were relatively retained in the submandibular gland, acinar cell loss was severe in the parotid gland. Compared to submandibular glands, parotid glands with abundant adipose cells exhibited lower density on CT images obtained before and after CRT.

Chapter 4

Conclusions

4.1 Summary of This Thesis

Xerostomia, a common debilitating adverse effect of CRT in patients with oral cancer, requires oral care to prevent mucositis, dental caries, oral infection, difficulty speaking, and dysphagia. The parotid volume and saliva production were decreased after 30-Gy irradiation. .There was a correlation between decreased parotid volume and decreased saliva production in patients with oral cancer undergoing CRT. Despite individual variations, parotid volume reduction may predict parotid gland function. If in the course of CRT saliva production is markedly decreased and morphological changes occur in the parotid gland volume, we recommend re-evaluation of the radiation-dose distribution.

Histopathological findings suggested that acinar cell loss is a main contributor to functional and volume changes in irradiated human salivary glands. The remaining acinar cells may provide the basis for morphological and functional recuperation. We postulate that the submandibular- in contrast to the parotid gland, is less radiosensitive because it is a mixed gland that contains serous and mucinous cells and because its serous cells exhibit functional diversity.

We have demonstrated elsewhere that the recuperation of morphological and functional changes occur in the course of 2-year follow-up in patients treated with 30-Gy irradiation. To prevent the serious mucositis, these patients should receive oral care for at least 2 years after CRT.

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