

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

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### Radiation therapy/Xerostomia/Salivary glands/Computed tomography/Histology.

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/m<sup>2</sup>/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.

### 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.<sup>1,2)</sup> 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.<sup>3–6)</sup> To spare glandular function, the recommended dose to parotid glands is less than 25–30 Gy.<sup>7–9)</sup> Histopathologically, radiation injury to salivary glands primarily results from damage to the serous cells; the mucinous cells are less involved.<sup>2,10)</sup> Therefore, the submandibular gland is thought to be less radiosensitive than the parotid gland.<sup>11,12)</sup> Although the histopathological effects of radiation on salivary glands have been reported in animal models,<sup>10–13)</sup> there is little information on these radiation-induced changes in humans.<sup>14)</sup>

While normal parotid glands contain abundant adipose cells, adipose tissue is not a significant component of submandibular glands.<sup>15–17)</sup> 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 func-

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doi:10.1269/jrr.11115

tional changes in salivary glands.<sup>17,18)</sup> 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.<sup>19,20)</sup> 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.

## MATERIALS AND METHODS

### *Patient 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 histopathological 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.<sup>19,20)</sup>

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  $\pm$  SD) to the ipsi- and contralateral parotid glands was  $29.6 \pm 1.1$  and  $29.2 \pm 1.2$  Gy, respectively; the ipsi- and contralateral submandibular doses were  $31.1 \pm 0.5$  and  $31.0 \pm 0.5$  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  $80 \text{ mg/m}^2$  starting on the 1st day of irradiation and continuing for 2 consecutive weeks.<sup>21)</sup> 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.

### *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.

### *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  $10 \times 10$  grid mesh and counting hits at the cross-points.<sup>12,13)</sup> We counted 100 points per microscopic field and used averaged values from counts of 3 random fields as the structural ratios.

### *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.

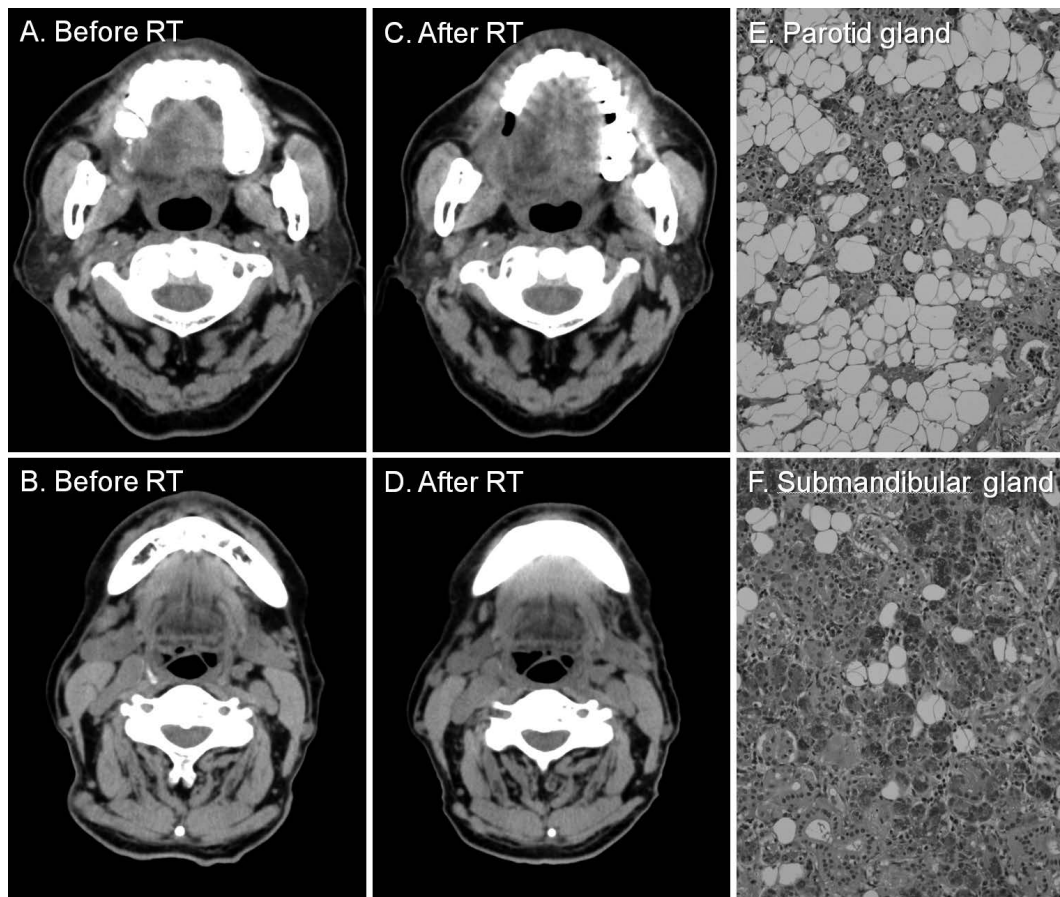
## RESULTS AND DISCUSSION

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. 1). When original post-CRT data were converted to fractions of pre-CRT values

(normalized parotid and submandibular volumes) because there were considerable individual variations,<sup>19,20</sup> 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. According to Nömayr *et al.*,<sup>22</sup> the parotid volume was reduced by approximately 26% and 40% after conventional 30- and 70-Gy irradiation, respectively. These results suggest that parotid and submandibular volumes after 30-Gy irradiation were approximately 70% of the baseline values.

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 aci-

nar 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. Our histopathological findings suggested that acinar cell loss played a role in the functional and volume changes observed in the salivary glands. Studies on the parotid glands of rats after fractionated irradiation revealed a dose-dependent decrease in serous acinar cells followed by replacement with fibrotic tissue; duct cells, on the other hand, were not affected.<sup>12,13</sup> In rat submandibular glands that harbor only mucinous acinar cells, acinar cell loss was not as prominent as in parotid glands.<sup>12</sup> These structural changes observed in animal models were compatible with our human data although serous



**Fig. 1.** 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,  $\times 100$ ) 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.

**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.

units predominate in human submandibular glands.

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.<sup>1,2,10</sup> It is generally agreed that human saliva contains 2 major-, i.e. high- and low molecular weight (mw), mucinous components.<sup>23</sup> 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.

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  $\pm 10$  HU was not clinically significant. Our results suggest that the CT value may reflect the adipose ratio in the parotid and submandibular glands although 30-Gy irradiation did not affect the CT value. Typically, the normal parotid gland harbors abundant adipose cells while adipose tissue is not a significant component of submandibular glands.<sup>1,15–17</sup> On CT, the parotid glands are of a relatively fatty appearance while the submandibular glands appear more cellular. Although there were individual variations, in our control group, the median adipose ratio of parotid and submandibular glands was 41.9% and 15.5%, respectively. In contrast, the median acinar ratio of parotid- was lower than of sub-

mandibular glands (31.5% and 43.3%, respectively). These structural ratios are compatible with previous histopathological findings of Scott *et al.*<sup>15,16</sup> who demonstrated that the adipose ratios tended to increase with age and in chronic alcohol abuse. 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.

Our study has some limitations. Under our preoperative CRT protocol we administered S-1, a novel oral fluorouracil derivative.<sup>21</sup> 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.<sup>14</sup> 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 submandibular 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.

In summary, 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.

### ACKNOWLEDGEMENT

This work was supported by Grant-in-Aid for Scientific Research (22591388) from Japan Society for the Promotion of Science.

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Received on July 2, 2011

Revision received on October 30, 2011

Accepted on February 28, 2012

J-STAGE Advance Publication Date: April 9, 2012