Evaluation of Parotid Gland Function using Equivalent Cross-relaxation Rate Imaging Applied Magnetization Transfer Effect

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Radiotherapy/Function/ECRI/Scintigraphy/Parotid gland.

Safe imaging modalities are needed for evaluating parotid gland function. The aim of this study was to validate the utility of a magnetic resonance imaging (MRI) tool, equivalent cross-relaxation rate imaging (ECRI), as a measurement of parotid gland function after chemoradiotherapy. Subjects comprised 18 patients with head-neck cancer who underwent ECRI and salivary gland scintigraphy. First, we calculated ECR values (signal intensity on ECRI), maximum uptake rate (MUR) and washout rate (WOR) from salivary gland scintigraphy data at the parotid glands. Second, we investigated correlations between ECR values and each parameter of MUR (uptake function) and WOR (secretory function) obtained by salivary gland scintigraphy at the parotid gland. Next, we investigated each dose-response for ECR, MUR and WOR at the parotid gland. A correlation was detected between ECR values and MUR in both the pre- (r = –0.55, p < 0.01) and post-treatment (r = –0.50, p < 0.05) groups. A significant post-treatment correlation was detected between the percentage change in ECR values at 3–5 months after chemoradiotherapy and median dose to the parotid gland (Pearson correlation, r = –0.62, p < 0.05). However, no correlations were detected between median dose to the parotid gland and either MUR or WOR. ECRI is a new imaging tool for evaluating the uptake function of the parotid gland after chemoradiotherapy.

INTRODUCTION

Radiotherapy for head and neck cancers must be performed with care, as various high-risk organs are situated in the surrounding area. Decreasing side effects in these organs is thus problematic. The parotid gland shows high radiosensitivity and inclusion within the irradiation field during radiotherapy for head and neck cancer causes depression of parotid gland function. Evaluation of parotid gland function after radiotherapy has been performed using salivary gland scintigraphy.1–3) This modality can evaluate parotid gland function by observing the movement of radionuclide (99mTcO4–) that accumulates in the parotid gland. However, the use of radionuclides obviously means that radiation exposure for human bodies is unavoidable,4) making this technique unsuitable for regular evaluation of parotid gland depression caused by radiotherapy.

Magnetic resonance imaging (MRI) uses magnetism and electromagnetic waves, representing a noninvasive modality with no exposure to radiation. The apparent diffusion coefficient (ADC) obtained by diffusion-weighted imaging has been reported as a parameter for evaluating parotid function.5–7) However, ADC shows a low correlation coefficient with the function parameter obtained by salivary gland scintigraphy.7)

We selected equivalent cross-relaxation rate imaging (ECRI) applied magnetization transfer effect using MRI.8–12) ECRI can detect minute changes in organization and molecular structure, offering information reflecting interactions with water molecules and biomacromolecules.8)

The aim of the present study was to validate the utility of ECRI for evaluating parotid gland function after chemoradiotherapy. ECRI provides different information for parts irradiated with a single saturation pulse. ECRI can obtain cell-density-weighted images and fiber-density-weighted images by irradiating a saturation pulse close to or far from the center frequency of water, respectively.10,12) The acinar
The cell composing the parotid gland plays a big role to the uptake of saliva. Therefore, we irradiated with a saturation pulse at 7 ppm downfield from the center frequency of water to obtain cell-density-weighted images in this research. We first investigated correlations between ECR values (signal intensity on ECRI) at the parotid gland and parameters (uptake function and secretory function) as obtained by salivary gland scintigraphy. We then investigated each dose-response for ECR and salivary gland scintigraphy parameters in the parotid gland.

**MATERIALS AND METHODS**

**Patients**

Subjects comprised 18 patients with head and neck cancer. Table 1 shows patient characteristics. Disease was staged according to the American Joint Committee on Cancer 1997 clinical staging. All patients received an explanation about the purpose and methods of this research and issues related to the protection of privacy, and informed consent to participate in the study was obtained prior to enrolment. MRI and salivary gland scintigraphy were performed in 6 patients before chemoradiotherapy, 6 patients after chemoradiotherapy and 6 patients both before and after chemoradiotherapy. As a result, 24 series of data were obtained for 48 parotid glands.

**Chemoradiotherapy**

All patients were immobilized in a cast, and computed tomography (CT) with 2.5 mm slice thickness was taken for treatment planning. Scans included the target area, regional lymph nodes, and the parotid glands. Target objects and normal structures including both parotid glands were contoured on a Pinnacle workstation (Hitachi Medical Corporation, Tokyo, Japan). Computed tomography (CT) images with the contour objects were transferred to a specific treatment planning system (Tomoprovider; TomoTherapy, Madison, WI).

A dose of 66–70 Gy was prescribed to the primary tumor. Most patients were treated using a fractionation scheme with 2 Gy administered 5 times/week. One patient received 1.8 Gy per fraction. Dose constraints for parotid glands were mean dose < 30 Gy, median < 23 Gy and whole parotid gland volume with < 20 Gy > 20 mm³. Other planning parameters comprised: primary collimator width, 2.5 cm; pitch, 0.3; and modulation factor, 3.0–4.0.

Radiotherapy was performed using a Hi-ART System (TomoTherapy), which is specifically designed for intensity-modulated radiotherapy (IMRT). All patients received daily megavoltage CT (MVCT) acquisitions for setup verification.

Chemotherapy was planned for 16 patients, with only 2 patient undergoing radiotherapy alone, as medical condition was considered insufficient for systemic chemotherapy. Three courses of chemotherapy comprising continuous intravenous administration of 5-fluorouracil at 800 mg/m²/24 h for 5 days (Days 1–5) and nedaplatin (NDP) at 130 mg/m²/6 h for 1 day (Day 6) were administered approximately every 4 weeks in the alternating setting. The details of contents of chemoradiotherapy have been reported in other articles.

**Imaging techniques**

Salivary gland scintigraphy was performed before initial treatment and then 3–5 months after completion of chemoradiotherapy. Salivary gland scintigraphy was performed with the gamma camera from a MillenniumVG system (GE Yokokawa Medical System, Milwaukee, WI). The only restriction before the examination was a dietary restriction. Dynamic imaging was obtained in a 64 × 64 pixel matrix at 15 s per frame for 45 min immediately after intravenous injection of 370 MBq of ⁹⁹ᵐTcO₄⁻. Lemon juice (0.5 ml) was dripped into the oral cavity in 1800 s after intravenous injection as a taste stimulus. The energy window was ±10 % around the 140 keV photopeak of ⁹⁹ᵐTc.

MRI was scheduled before initial treatment and then 3–5 months after completion of chemoradiotherapy. A 1.5-T system (Signa; GE Yokokawa Medical System) was used. Sequences comprised 3-dimensional spoiled gradient recalled acquisition in the steady state (3DSPGR) and saturation-transfer-prepared 3DSPGR (ST-3DSPGR). Single saturation transfer pulse (3.26 μT) frequency was employed at the frequency of 7 ppm downfield from the center frequency of water. Scans included the whole parotid gland. A neurovascular coil was used. Conditions were: repetition time, 40 ms; echo time, 6.9 ms; flip angle, 30°; bandwidth, 15.63 kHz; field of view, 24 cm; slice thickness, 5 mm; overlap locations, 0; locations per slab, 16; acquisition matrix, 512 × 126; and reconstructed matrix size, 512 × 512 (zero-fill interpolation process). The stimulation of the parotid gland by for example lemon juice was not performed as in the scintigraphy protocol during MRI.

**Table 1. Patient characteristics**

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<tr>
<td>N</td>
<td>18</td>
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<tr>
<td>Male/female</td>
<td>15/3</td>
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<tr>
<td>Median age (range)</td>
<td>53 (16–74)</td>
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<tr>
<td>Tumor site</td>
<td></td>
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<tr>
<td>Nasopharynx</td>
<td>16</td>
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<tr>
<td>Oropharynx</td>
<td>2</td>
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<tr>
<td>Stage</td>
<td></td>
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<tr>
<td>I</td>
<td>1 (6%)</td>
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<tr>
<td>II</td>
<td>2 (11%)</td>
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<td>III</td>
<td>8 (44%)</td>
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<td>IV</td>
<td>7 (39%)</td>
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Regions of interest (ROIs) were located for bilateral parotid glands and a frontal sinus on the planar image obtained by salivary gland scintigraphy (Fig. 1a), using the count for the frontal sinus as the background signal. A time-activity curve (TAC) for the parotid gland was created by subtracting the background from the count for the parotid gland (Fig. 1b). Maximum uptake rate (MUR) was calculated for bilateral parotid glands according to Equation 1.

\[
MUR = \left(1 - \frac{C_{vpp}}{C_{max}}\right) \times 100\% \quad (1)
\]

where \(C_{vpp}\) is the count on TAC at 60 s after administration of \(99mTcO_4^-\) (reflecting blood flow, capillary permeability and secretion rate in the parotid gland) and \(C_{max}\) is the maximum count on the TAC (reflecting capacities of blood vessel lumens and intercellular spaces in the parotid gland). MUR was used as the parameter indicating uptake function.

Washout rate (WOR) was calculated for bilateral parotid glands according to Equation 2.

\[
WOR = \left(1 - \frac{C_{min}}{C_{max}}\right) \times 100\% \quad (2)
\]

where \(C_{min}\) is the minimum counts after taste stimulation. WOR thus shows the secretion of \(99mTcO_4^-\) per capacities of blood vessel lumens and intercellular spaces in the parotid gland. WOR was used as the parameter indicating secretory function.

ECRI was obtained using Equation 3.

\[
ECR = \left(\frac{M_0}{M_s} - 1\right) \times 100\% \quad (3)
\]

where \(M_s\) and \(M_0\) represent signal intensities in 3DSPGR and ST-3DSPGR images, respectively. A ROI was located for the parotid gland on ECRI, and ECR at the parotid gland was measured.

We investigated correlations between ECR and both MUR and WOR at the parotid gland before and after chemoradiotherapy. Next, we investigated simple linear correlations between percentage changes in ECR, MUR and WOR at 3–5 months after chemoradiotherapy and median dose to the parotid gland.

### Statistical analysis

R2.5.1 statistical software (www.r-project.org/) was used to perform all analyses. Student’s t test was used to compare differences in patient groups. Pearson’s correlation was used to evaluate correlations between ECR and MUR and between ECR and WOR at the parotid gland, and between percentage changes in ECR, MUR and WOR at 3–5 months after chemoradiotherapy and median dose to the parotid gland. The level of significance was set at 5%, and all p values were based on two-tailed tests.

### RESULTS

**Findings from salivary gland scintigraphy**

Planar images taken by salivary gland scintigraphy were obtained in all cases without any acquisition failure. MUR and WOR could be obtained from all parotid glands on planar images. Table 2a shows changes in MUR and WOR before and after chemoradiotherapy.
between pre- and post-treatment groups. MUR was lower in the post-treatment group than in the pre-treatment group (Student’s t test, p < 0.001). WOR was also lower in the post-treatment group than in the pre-treatment group (Student’s t test, p < 0.001). Losses of uptake and secretory function in the parotid gland were thus confirmed by salivary gland scintigraphy.

Findings from ECRI

Both 3DSPGR and MT-3DSPGR images were obtained in all cases without any acquisition failure. ECR images were obtained using Equation 3. Figure 2 shows examples of 3DSPGR, MT-3DSPGR and ECR images before and after chemoradiotherapy. A clear reduction in parotid gland size after chemoradiotherapy was detected in this representative case (Fig. 2, arrowhead). ECR images were expressed in a graded color diagram of ECR values ranging from 0 to 120, with red indicating areas of high ECR, black showing areas of low ECR, and white representing areas with ECR > 120. Mean ECR values at parotid glands (± standard deviation) were 14.2 ± 0.72% and 34.3 ± 0.70% before and after chemoradiotherapy, respectively (Fig. 2). ECR values were higher in the post-treatment group than in the pre-treatment group (Table 2b; Student’s t test, p < 0.001).

Correlations between ECR value and salivary gland scintigraphy parameters

To determine whether ECR values can be used to evaluate parotid gland function, we investigated correlations between ECR value and salivary gland scintigraphy parameters at the parotid gland. The correlation coefficient between ECR and MUR was –0.55 in the pre-treatment group (Pearson correlation, p < 0.01) and –0.50 in the post-treatment group (Pearson correlation, p < 0.05) (Fig. 3a). The correlation coefficients between ECR and WOR were –0.32 in the pre-treatment group (Pearson correlation, p = 0.12) and –0.06 in the post-treatment group (Pearson correlation, p = 0.79) (Fig. 3b).

Dose response

The 6 patients who underwent salivary gland scintigraphy and MRI both before and after chemoradiotherapy received a median dose of 19.8–26.5 Gy to the parotid glands (Table 3). The doses (several cGy per a MVCT acquisition) from the MVCT imaging were not contained in the median dose. Figure 4 shows the correlation between percentage change in parameters and median dose to the parotid gland. A significant correlation was identified between percentage change in ECR value at 3–5 months after chemoradiotherapy and median dose to the parotid gland (Pearson correlation, r = –0.62, p < 0.05). The correlation between percentage change

![Example images](image_url)
in MUR at 3–5 months after chemoradiotherapy and median dose to the parotid gland was not significant (Pearson correlation, \( r = 0.11 \), \( p = 0.74 \)). The correlation between percentage change in WOR at 3–5 months after chemoradiotherapy and median dose to the parotid gland was also not significant (Pearson correlation, \( r = -0.31 \), \( p = 0.33 \)).

**DISCUSSION**

Matsushima et al. previously reported ECRI as a potentially useful method for evaluating the efficacy of sentinel lymph node biopsy\(^5,\text{10}\) and for cellular density imaging of axillary lymph nodes.\(^\text{11}\) Yuen et al. reported ECRI as a feasible imaging technique for demonstrating breast cancer.\(^\text{12}\) ECRI can thus detect minute changes in molecular and organizational structure.\(^\text{8}\)

The present study represents the first trial of evaluating parotid gland function after chemoradiotherapy using ECRI. Parotid gland evaluation by MRI has been reported using ADC, which detects the motion of water molecules and microcirculatory blood flow.\(^\text{5–7}\) Theony et al. reported that ADC value decreased immediately after taste stimulation, then increased until static state.\(^\text{5}\) Dirix et al. reported that ADC value decreased significantly after irradiation.\(^\text{6}\) Likewise, Lin et al. reported that ADC value decreased significantly after irradiation, and correlated with parameters obtained by salivary scintigraphy (uptake rate; \( r = 0.36 \), \( p < 0.01 \), MUR; \( r = 0.33 \), \( p < 0.01 \)).\(^\text{7}\) In our results, correlation coefficients between ECR and MUR were \(-0.55 \) (\( p < 0.01 \)) in the pre-treatment group and \(-0.50 \) (\( p < 0.05 \)) in the post-treatment group. The correlation coefficient between ECR and MUR was higher than that between ADC and MUR in past studies.\(^\text{7}\) The reason why ECR correlated with MUR before chemoradiotherapy is as follows. When capacities of blood vessel lumens and intercellular spaces are large, \( C_{\text{vpp}} / C_{\text{max}} \) is low, and MUR (defined as \( 1 - C_{\text{vpp}} / C_{\text{max}} \)) is high. Conversely, cell densities are relatively decreased, and the ECR (expressing cell density) is thus low. ECR thus showed a negative correlation with MUR. Moreover, ECR showed a correlation with MUR in the post-treatment group for the following reasons. Animal experiments have identified shrinkage of irradiated parotid glands.\(^\text{17}\) Likewise, the parotid gland after chemoradiotherapy shrank in the present study (Fig. 2). ECR shows a high value due to the rise in cell density, while MUR was low due to decreased free water and the narrowness of the free water division, with shrinkage of gland tissues. ECR therefore shows a negative correlation with MUR in the post-treatment group. This relationship suggests that ECR value can be used to evaluate uptake function of the parotid gland after chemoradiotherapy without exposure to radiation. In addition, as ECRI can provide

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<th>Table 3. Median dose to parotid glands [Gy]</th>
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Fig. 3. Relationships between ECR value and salivary gland scintigraphy parameters. (a) MUR; (b) WOR.

Fig. 4. Correlations between percentage change in parameters from baseline and median dose to parotid glands.
a 2-dimensional color map (Fig. 2), areas of weak uptake function in the parotid gland can be identified visually. The details of mechanism for the uptake of saliva are unknown. Therefore, the visualization of uptake function may contribute for the clarification of the loss part of uptake function of saliva. The correlation coefficients between ECR and WOR were –0.32 in the pre-treatment group (Pearson correlation, \( p = 0.12 \)) and –0.06 in the post-treatment group (Pearson correlation, \( p = 0.79 \)) (Fig. 3b). However, as the stimulation of the parotid gland by for example lemon juice was not performed as in the scintigraphy protocol during MRI, both exams could not be compared.

On salivary scintigraphy, dose-response with parotid gland function has been studied by other investigators.\(^1\)–\(^{3,18–20}\) Roesink et al. found a significant correlation between salivary excretion factor (defined as the percentage of activity in the parotid gland that disappeared within 15 min following administration of carbachol) and mean radiation dose to the parotid glands.\(^3\) However, in our research, WOR did not show a linear correlation with radiation dose to the parotid glands (Fig. 4). This lack of correlation may be due to low number of patients and differences in dose ranges applied in this study. Moreover, the difference between median and mean doses might be involved. On the other hand, calculations of secretory functions (such as salivary excretion factor and WOR) and uptake functions (such as MUR) have been widely recognized for salivary scintigraphy. However, dose-response for uptake function has not been reported. In our research, the percentage change in MUR at 3–5 months after chemoradiotherapy did not show a linear correlation with median radiation dose to the parotid gland (Pearson correlation, \( r = 0.11, p = 0.74 \)). Conversely, the percentage change in ECR values at 3–5 months after chemoradiotherapy showed a linear correlation with median radiation dose to the parotid gland (Pearson correlation, \( r = –0.62, p < 0.05 \)). ECR thus showed a linear correlation with median radiation dose to parotid glands in the range of 19.8–26.5 Gy. The reason why ECR correlates with median radiation dose to the parotid gland can be described as follows from the consideration of cell density. In this research, cell-density-weighted images were obtained by irradiating the saturation pulse at a frequency 7 ppm downfield from the center frequency of water. Matsushima et al. reported that ECR correlated with cell density in clinical situations.\(^11\) In addition, the number of acinar cells is known to be decreased in irradiated salivary gland.\(^{21–27}\) Li et al. reported that the number of acinar cells in irradiated parotid glands was decreased at 16 weeks after radiotherapy.\(^{23}\) This duration after radiotherapy is similar to that used in our research. Loss of acinar cells is markedly increased with increasing dose to the parotid gland.\(^{26,27}\) The percentage change in ECR values at 3–5 months after chemoradiotherapy thus shows a clear inverse correlation with median radiation dose to the parotid gland. This suggests that ECR value can be used to predict uptake function of the parotid gland after chemoradiotherapy.

In conclusion, we verified that ECRI is useful for evaluating parotid gland function after chemoradiotherapy. ECRI allowed visual evaluation of uptake function in the parotid gland without exposure to radiation.

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