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18F-fluoro-2-deoxyglucose-positron emission tomography for the assessment of histopathological response after preoperative chemoradiotherapy in advanced oral squamous cell carcinoma

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Abstract

Background: 18F-fluoro-2-deoxyglucose-positron emission tomography (FDG-PET) is widely used to evaluate tumor metabolic activity. This study aimed to evaluate the usefulness of FDG-PET in assessing the histopathological response to preoperative concurrent chemoradiotherapy (CRT) in oral squamous cell carcinoma (OSCC).

Methods: Forty-five advanced resectable OSCC patients who received preoperative CRT followed by tumor ablative surgery between January 2004 and December 2011 were included. All patients underwent FDG-PET before and after preoperative CRT. The maximum standardized uptake value (SUVmax) before (Pre-SUV) and after preoperative CRT (Post-SUV) and the SUVmax reduction rate (ΔSUV%) were used to evaluate the response to preoperative CRT. Correlations among SUVmax, histopathological response, and expression of Ki-67 and hypoxia-inducible factor-1α (HIF-1α) were analyzed.

Results: Preoperative CRT significantly reduced intratumoral FDG uptake ($P < 0.001$). Pre-SUV and Post-SUV were significantly lower in patients with pathological complete response (pCR) than in those with non-pCR (Pre-SUV: $P = 0.037$; Post-SUV: $P = 0.001$). ΔSUV% was higher in patients with pCR than in those with non-pCR ($P = 0.029$). Pre-SUV was significantly correlated with Ki-67 and HIF-1α expression in pretreatment biopsy specimens (Ki-67: $P = 0.046$, $R = 0.292$; HIF-1α: $P = 0.007$, $R = 0.385$). Ki-67 and HIF-1α expressions were significantly lower in patients with pCR than in those with non-pCR (Ki-67: $P < 0.001$; HIF-1α: $P < 0.001$).

Conclusions: Low Pre-SUV and Post-SUV and high ΔSUV% may predict good histopathological response to preoperative CRT. Ki-67 and HIF-1α expression in pretreatment biopsy specimens were predictors of histopathological response to preoperative CRT.

Keywords: Oral squamous cell carcinoma, FDG-PET, Standardized uptake value, Ki-67, HIF-1α, Chemoradiotherapy

Introduction

Oral cavity cancers account for approximately 1–2% of all malignancies in Japan, with oral squamous cell carcinoma (OSCC) as the most common type [1, 2]. In general, patients with advanced-stage OSCC are treated with combination therapies consisting of surgery, radiotherapy, and/or chemotherapy [3-5]. In our department, preoperative concurrent chemoradiotherapy (CRT) followed by tumor ablative surgery has been performed in patients with advanced OSCC [4]. Many clinical studies have demonstrated that preoperative concurrent CRT is associated with a reduction in distant metastasis and higher long-term survival in patients with resectable locoregionally advanced OSCC. Furthermore, preoperative concurrent CRT increases the possibility of minimally invasive surgery for the preservation of organ function [4, 6-8]. Accurate assessment of the therapeutic response to preoperative concurrent CRT is important in planning subsequent operative procedures such as conventional tumor resection, minimally invasive surgery, and non-surgical organ preservation therapy.

Diagnostic imaging using 18F-fluoro-2-deoxyglucose-positron emission tomography (FDG-PET) has been broadly used to detect primary tumors and lymph node and distant metastasis and recently, to determine therapeutic efficacy. FDG-PET has been used to plan subsequent therapeutic strategies after induction chemotherapy in cancers of the esophagus, larynx, and lung [9-12]. However, the usefulness of FDG-PET to determine the histopathological response to chemotherapy in oral cancer has rarely been investigated. The purpose of this study was to evaluate the usefulness of FDG-PET examination for the clinical and histopathological assessment of preoperative concurrent CRT in advanced OSCC by clarifying the association of the maximum standardized uptake value (SUVmax) of FDG with the pathological and immunohistochemical findings in excised tumor specimens.

Patients and methods

Patients

The study subjects comprised 45 patients with advanced but potentially resectable OSCC who underwent preoperative concurrent CRT at the Department of Oral and Maxillofacial Surgery, Nara Medical University, between January 2004 and December 2011. Of the 45 patients, 28 patients were males, and 17 patients were females. The median age was 61 years (range: 28–68 years). None of the patients had severe complications, including diabetes mellitus. The primary tumor sites were the tongue (n = 29), upper gingiva (n = 6), lower gingiva (n = 4), floor of the mouth (n = 3), and buccal mucosa (n = 3). According to the International Union Against Cancer TNM classification (2002, 6th edition), 25 patients had advanced T2 disease (>30 mm and $\leq$40

mm), 12 patients had T3 disease, and 8 patients had T4 disease before CRT treatment. Most patients (93.3%) had stage III or IV disease (stage II: 3 patients; stage III: 19 patients; stage IV: 23 patients). Three cases of advanced T2cN0 disease (stage II) were poorly differentiated squamous cell carcinoma of the floor of the mouth, suspected to be latent lymph node metastasis. The clinical characteristics of the study population are shown in Table 1. Informed consent was obtained from each patient, and the scientific protocol was approved by the local ethics committee.

Treatment schedule

Before preoperative CRT, computed tomography (CT) or magnetic resonance imaging (MRI) of the head and neck, chest radiography, whole body PET/CT, and histological examination were performed to determine the diagnosis and initial stage of cancer. All patients underwent preoperative concurrent CRT. External beam radiation therapy at a dose of 40 Gy in 2 Gy per day fractions (20 times) was delivered to the primary tumor and lymphatics within 4 weeks. The patients received concurrent carboplatin (70–100 mg/m^2) on days 1–3 and 5-fluorouracil (500–750 mg/day) on days 4–7. Both agents were administered by continuous intra-arterial infusion via the superficial temporal artery for more than 4 hours. In all cases, imaging evaluations (CT, MRI, and PET/CT) and histological examination of biopsy specimens were performed 2–4 weeks after the completion of preoperative concurrent CRT. Biopsy specimens were collected from the central region of the tumor. All patients underwent radical surgery within 4–6 weeks after the completion of preoperative concurrent CRT. Histopathological analysis of resected tumor tissues was routinely performed.

FDG-PET/CT imaging

Before FDG injection, all patients underwent at least a 5-hour fast to ensure that blood glucose was <180 mg/dl. Sixty minutes after an intravenous injection of 3 MBq/kg FDG, whole-body FDG-PET/CT from the head to thigh was performed at a rate of 120 seconds per frame using a PET/CT scanner (Discovery LS; GE Healthcare, Milwaukee, WI, USA). PET data were collected in 2-dimensional acquisition imaging mode and reconstructed using an ordered-subset expectation maximization algorithm (2 iterations, 28 subsets). The low-dose unenhanced CT data on PET/CT (120 Kv and 40–200 mA under a free breath hold) were used for attenuation correction and anatomical localization.

PET/CT scans were obtained before and 2–4 weeks after preoperative CRT in patients with oral mucositis grade 2 or less according to the Common Terminology Criteria for Adverse Events (CTCAE) ver. 4.0. PET/CT data were visually interpreted by an independent nuclear medicine physician. Tumor SUV was calculated using the following formula: SUV = [decay corrected activity per unit volume of tissue (Bq/ml)] / [dose of injected

tracer (Bq) / body weight (g)]. A region of interest (ROI) was placed on the SUV image over the area that included the site of maximum FDG accumulation in the lesion. SUV max in ROI was defined as tumor SUV. The reduction rate of SUVmax (ΔSUV%) was calculated using SUVmax before CRT (Pre-SUV) and SUVmax after CRT (Post-CRT) as follows: ΔSUV% = [(Pre-SUV) - (Post-SUV)/Pre-SUV] × 100 (%).

Histopathological response

The regression grading system proposed by Shimosato et al. [13] was used to define the histopathological response to preoperative concurrent CRT in semi-serial sections of whole resected specimens. Patients with grade III and IV tumors were considered to have had a pathological complete response (pCR) because of the low number or absence of viable tumor cells.

Immunohistochemistry

Pretreatment biopsy specimens of primary OSCCs were formalin-fixed, paraffin-embedded, and cut into consecutive 4-μm sections. Immunohistochemistry was performed using an immunoperoxidase technique as described previously [14]. Antigen retrieval was performed using microwave treatment (95°C) in citrate buffer (pH 6.0) for 45 minutes. Specimens were incubated in 3% H_2O_2-methanol for 15 minutes to block endogenous peroxidase and then rinsed with phosphate-buffered saline (PBS) three times. The primary antibodies used were anti-Ki-67 antibody (DAKO, Carpinteria, CA, USA) and anti-hypoxia-inducible factor-1α (HIF-1α) antibody (Thermo Fisher Scientific Inc., Rockford, IL, USA). Primary antibodies were diluted to a final concentration of 1 μg/ml. After 2-hour incubation at room temperature, the specimens were rinsed with PBS three times and incubated with biotinylated anti-mouse secondary antibody (DAKO) at room temperature for 1 hour. A catalyzed signal amplification system (CSA-Kit, Dako) based on a streptavidin-biotin-peroxidase reaction was used for signal amplification and visualization according to the manufacturer's instructions. The specimens were rinsed with PBS three times, and immunostaining was developed using diaminobenzidine (DAB) solution (DAKO). After washing, the specimens were counterstained using Mayer's hematoxylin (Sigma Chemical Co., St. Louis, MO, USA). Immunostaining of all samples was performed under the same antibody reaction and DAB exposure conditions. For evaluation of Ki-67 and HIF1-α immunostaining, 5 fields of 500–1000 cells with maximum immunostaining ('hot-spots') were selected, and tumor cells with positive nuclei were counted. The percentage of tumor cells with positive nuclei were calculated, and the mean values were obtained. The mean value was defined as the labeling index (LI). Ki-67 and HIF-1α LIs above and below the mean value were considered high and low expression, respectively. High expression of Ki-67 and HIF-1α was noted in the nuclei of OSCC cells (Fig. 1).

Statistical analysis

The Mann-Whitney *U*-test or Student's paired *t*-test was used to compare quantitative variables between the two SUVmax groups. Spearman's rank correlation was used to determine the correlation between SUVmax and immunohistochemical expression of Ki-67 and HIF-1α. A P value <0.05 was considered statistically significant. All analyses were performed using JMP (version 8) (SAS Institute Inc., Cary, NC, USA).

Results

Pre-SUV, post- SUV, and ΔSUV%

The mean Post-SUV (SUVmax after CRT) was significantly lower than the mean Pre-SUV (SUVmax before CRT; $P < 0.001$) (Fig. 2). Mean Pre-SUV and Post-SUV were 11.4 ± 4.1 (range: 4.1–21.2) and 5.5 ± 2.4 (range: 2.1–13.3), respectively. Mean ΔSUV% in all patients was 47.9 ± 21.2% (range: 0.9–82.9%). Pre-SUV, Post-SUV, and ΔSUV% according to T category and clinical stage are shown in Table 2. Pre-SUV, Post-SUV, and ΔSUV% were not significantly different according to T category (Pre-SUV: $P = 0.115$; Post-SUV: $P = 0.679$; ΔSUV%: $P = 0.481$) or clinical stage (Pre-SUV: $P = 0.070$; Post-SUV: $P = 0.083$; ΔSUV%: $P = 0.778$). In addition, Pre-SUV, Post-SUV, and ΔSUV% were not significantly associated with age, gender, or primary lesion.

Correlation between histopathological response and SUVs

Histological examination of the biopsy specimens after preoperative CRT and surgical resection specimens showed that 28 and 24 of the 45 patients had achieved pCR, respectively. This difference in the number of patients who achieved pCR between preoperative and postoperative histological examination was not significantly different ($P = 0.468$). The correlation between histopathological response and SUVs is summarized in Fig. 3. Pre-SUV was significantly lower in patients with pCR (10.2 ± 4.2) than in those with non-pCR (12.7 ± 3.6; $P = 0.037$). Post-SUV was also significantly lower in patients with pCR (4.5 ± 1.1) than in those with non-pCR (6.7 ± 2.9; $P = 0.001$) (Table 2). Moreover, in 11 patients with a Post-SUV of 6.5 or higher, viable tumor cells were present in the resected specimens (Fig. 4). ΔSUV% was higher in patients with pCR (54.8 ± 17.4%) than in those with non-pCR (40.7 ± 23.1%; $P = 0.029$). Therefore, patients with low Pre-SUV and Post-SUV and high ΔSUV% had a greater potential to achieve pCR after preoperative CRT.

Immunohistochemical expression of Ki-67 and HIF-1α

LIs of Ki-67 and HIF-1α in all patients before preoperative CRT ranged from 11.2% to 74.4% (mean: 43.0%) and from 0% to 84.4% (mean: 39.0%), respectively. The association of Ki-67 and HIF-1α LIs in primary

OSCCs with clinical characteristics is summarized in Table 3. Immunohistochemical expression of Ki-67 and HIF-1α were not significantly associated with age, gender, primary lesion, tumor size, or stage.

Correlation between immunohistochemical expression of Ki-67 and HIF-1α and histopathological response

The association of Ki-67 and HIF-1α LIs in primary OSCCs with histopathological response in resected specimens is summarized in Table 3. The mean LIs of Ki-67 and HIF-1α were significantly lower in patients with pCR (36.6 ± 15.0% and 27.1 ± 18.2%, respectively) than in those with non-pCR (50.3 ± 17.2% and 52.5 ± 21.7%, respectively; $P < 0.001$). This result suggested that low expression of Ki-67 and HIF-1α was indicative of a higher preoperative CRT response.

Association between immunohistochemical expression of Ki-67 and HIF-1α and Pre-SUV

The correlation between Pre-SUV and LIs of Ki-67 and HIF-1α is shown in Fig. 5. LIs of Ki-67 and HIF-1α in all patients before preoperative CRT ranged from 11.2% to 74.4% (mean: 43.0%) and from 0% to 84.4% (mean: 39.0%), respectively. Ki-67 and HIF-1α LIs were significantly positively correlated with Pre-SUV (Ki-67: $P = 0.046$, $R = 0.292$; HIF-1α: $P = 0.007$, $R = 0.385$).

Correlation between immunohistochemical expression of Ki-67 and HIF-1α and SUVs

The correlation between SUVs and immunohistochemical expression of Ki-67 and HIF-1α in pretreatment biopsy specimens is shown in Table 4. Mean Ki-67 and HIF-1α LIs were 43.0% and 39.0%, respectively. Ki-67 and HIF-1α LIs above and below the mean value were considered high and low expression, respectively. High Ki-67 expression and low Ki-67 expression were present in 22 (48.9%) and 23 (51.1%) patients, respectively. Pre-SUV and Post-SUV were higher in patients with high Ki-67 expression (12.6 ± 3.8 and 6.9 ± 2.6, respectively) than in those with low Ki-67 expression (10.0 ± 4.1 and 4.1 ± 1.2, respectively; $P = 0.036$ and $P < 0.001$, respectively). ΔSUV% was lower in patients with high Ki-67 expression than in those with low Ki-67 expression (41.5 ± 22.2% vs. 54.7 ± 18.1%; $P = 0.034$).

High and low HIF-1α expression was present in 21 (46.7%) and 24 (53.3%) patients, respectively. Pre-SUV and Post-SUV were significantly higher in patients with high HIF-1α expression (12.8 ± 3.6 and 7.0 ± 2.7, respectively) than in those with low HIF-1α expression (10.2 ± 4.2 and 4.3 ± 1.2, respectively; $P = 0.030$ and $P < 0.001$, respectively). ΔSUV% was significantly lower in patients with high HIF-1α expression (41.1 ± 22.4%) than in those with low HIF-1α expression (53.9 ± 18.5%; $P = 0.043$). High expression of Ki-67 and HIF-1α was associated with high Pre-SUV and Post-SUV and low ΔSUV%.

Discussion

Preoperative CRT is associated with high histopathological response and survival rates in OSCC as described in our previous reports [5, 8]. Morphological changes and visible tumor reduction rate on CT and/or MRI have been used to evaluate the therapeutic response after preoperative CRT. However, normal tissue changes after CRT such as edema, fibrosis, and necrosis present as mass lesions on CT and MRI, which are based on anatomic parameters. Histopathological examination of biopsy specimens after preoperative CRT is considered the gold standard for the direct assessment of therapeutic response. However, accurate evaluation of biopsy specimens after preoperative CRT may not be feasible depending on the sampling site. Therefore, the use of CT and MRI findings or histopathological examination alone is not sufficient to precisely evaluate the therapeutic response to preoperative CRT [15]. On the other hand, FDG-PET imaging, which is based on glucose levels, can provide information regarding the metabolic activity in the entire tumor. It has been suggested that reduced tumor metabolism assessed by FDG-PET may precede the tumor volume reduction on CT and MRI findings after CRT in head and neck squamous cell carcinoma [16]. Furthermore, FDG-PET findings have a high sensitivity and accuracy compared with CT/MRI [17].

FDG-PET has been used to evaluate therapeutic response after induction chemotherapy in cancers of the esophagus, larynx, and lung [9-12]. In the present study, we found that Pre-SUV, Post-SUV, and the reduction rate of SUVmax (ΔSUV%) were associated with the efficacy and histopathological response of preoperative CRT in advanced OSCC. SUVmax of FDG-PET was remarkably decreased by preoperative CRT in primary OSCC tumors. The SUVmax after CRT was very low and ΔSUV% was high in the resected specimens of pCR cases. These findings suggested that it may be possible to decrease the extent of primary tumor resection or shift to organ preservation therapy depending on FDG-PET findings. T category and clinical stage have been reported to be associated with SUVmax [9, 27, 31]; however, we found no significant association between these parameters and SUVmax in our study. The lack of statistical significance of these parameters might be due to the fact that our study included patients with advanced but potentially resectable OSCC, which limited the evaluation of tumor size. Residual tumor was present in the resected specimens of patients with SUVmax values of 6.5 or higher after CRT; therefore, we suggest that conventional surgery should be performed in these cases. However, 50% of non-pCR patients exhibited SUVmax values of less than 6.5 after CRT. Therefore, it is difficult to distinguish pCR from non-pCR patients based solely on SUVmax values after CRT.

Tumor proliferative potential and hypoxia within the tumor microenvironment are associated with resistance to chemotherapy and radiotherapy in solid tumors [18, 19] and therefore, they may influence the therapeutic response to preoperative CRT. Ki-67 is expressed in all phases of the cell cycle (G1, S, G2, and M)

except the resting phase. Ki-67 is extensively used to assess the proliferation rate of neoplasms. High expression of Ki-67 has been reported to be a poor prognostic factor in lung and breast cancers [20-22]. HIF-1α, a hypoxia-sensitive transcription factor, is thought to induce therapeutic resistance by increasing the expression of various proteins associated with glucose metabolism, vascularization, invasion, and metastasis [23, 24]. The expression of Ki-67 and HIF-1α has been correlated with the SUVmax of FDG-PET before preoperative CRT in breast cancer and squamous cell carcinoma [25, 26]. Furthermore, high SUVmax before CRT was associated with therapeutic resistance and poor prognosis in cancers of the esophagus, pharynx, lung, and head and neck [27-29]. Our findings are consistent with these previous reports. We found that high immunohistochemical expression of Ki-67 and HIF-1α was frequently observed in the pretreatment biopsy specimens of non-pCR patients. Furthermore, the expression rates of Ki-67 and HIF-1α were correlated with SUVmax before preoperative CRT. Thus, our results indicate that high expression of Ki-67 and HIF-1α is associated with resistance to preoperative CRT.

In our study, SUVmax after preoperative CRT was significantly different between pCR and non-pCR cases; however, some overlaps were present. SUV measurement conditions such as tumor size, inflammation, and timing of PET images have been associated with the overlaps [17, 30, 31]. Oral mucositis, a toxic event associated with CRT, can obstruct PET finings. Inflammatory tissues are known to increase FDG uptake. In fact, CRT-induced esophagitis militates against the diagnosis of esophageal cancer obtained from FDG-PET findings [15, 30, 32]. Maintaining diagnostic precision under inflammatory conditions remains a problem with PET use. In an attempt to resolve this problem, we performed PET imaging only in patients with oral mucositis grade 2 or less according to the CTCAE v. 4.0. Furthermore, FDG-PET evaluation was limited to a period within 2–4 weeks from CRT completion to surgical operation. Our results suggest that FDG-PET examination provides an accurate evaluation of therapeutic response after preoperative CRT under these conditions. To further improve the precision of FDG-PET in evaluating the therapeutic response after preoperative CRT, further studies with larger numbers of patients are necessary to clarify the criteria to evaluate CRT response, cut-off points of SUVmax, timing of PET imaging after preoperative CRT, and prognostic value of PET.

In our study, FDG-PET findings were significantly correlated with therapeutic response to preoperative CRT and immunohistochemical findings in advanced OSCC. Our results suggest that FDG-PET is a comprehensive and reliable examination that can be used not only for tumor detection but also for therapeutic response evaluation in advanced OSCC. Therefore, FDG-PET may be used as a substitute for conventional examinations to assess therapeutic response, such as CT and MRI.

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Figure legends

Fig. 1 Ki-67 and hypoxia-inducible factor-1α (HIF-1α) expression in oral squamous cell carcinoma

High expression of Ki-67 and HIF-1α was noted in the nuclei of oral squamous cell carcinoma.

Bar = 100 mm

Fig. 2 Maximum standardized uptake (SUVmax) values before and after preoperative concurrent chemoradiotherapy (CRT)

The mean Post-SUV (SUVmax after CRT) was significantly lower than the mean Pre-SUV (SUVmax before CRT).

Fig. 3 Maximum standardized uptake (SUVmax) values according to histopathological response in resected specimens

pCR: pathological complete response

Pre-SUV: SUVmax before chemoradiotherapy

Post-SUV: SUVmax after chemoradiotherapy

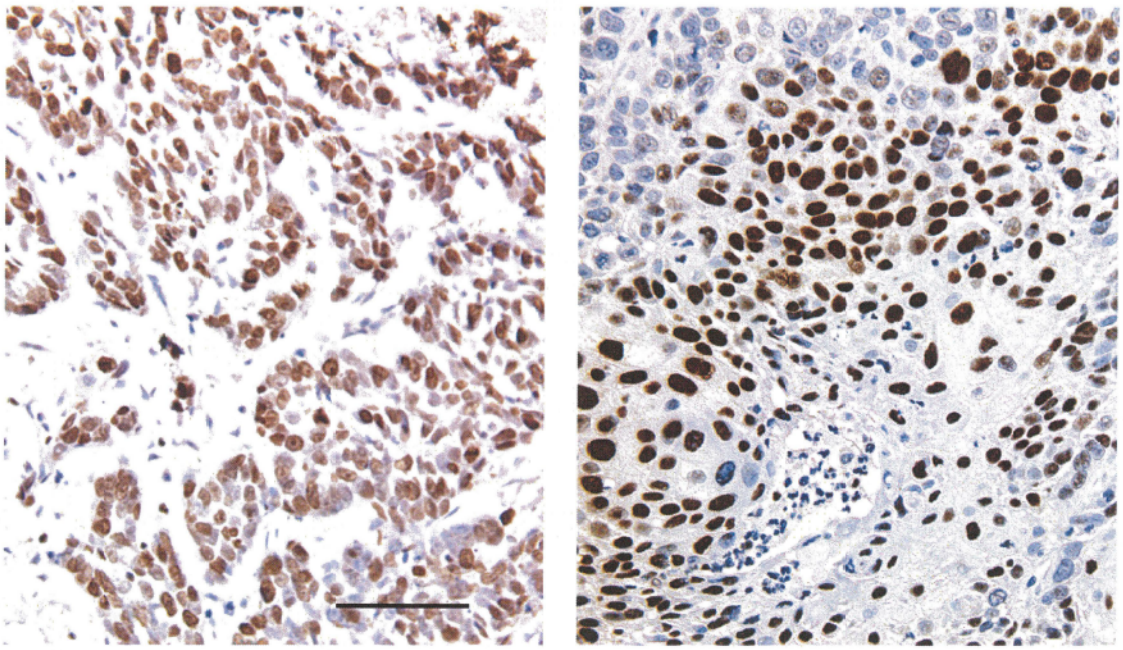
ΔSUV%: SUVmax reduction

Fig. 4 Association between SUVmax before chemoradiotherapy (Post-SUV) and histopathological regression in resected specimens

Viable tumor cells were present in the resected specimens of patients with a Post-SUV of 6.5 or higher.

Fig. 5 Correlation between SUVmax before chemoradiotherapy (Pre-SUV) and immunohistochemical expression of Ki-67 and hypoxia-inducible factor-1α (HIF-1α) in pretreatment biopsy specimens

Pre-SUV was significantly correlated with Ki-67 and HIF-1α labeling indexes.



Ki-67 (×200)

HIF-1α (×200)

Figure 1

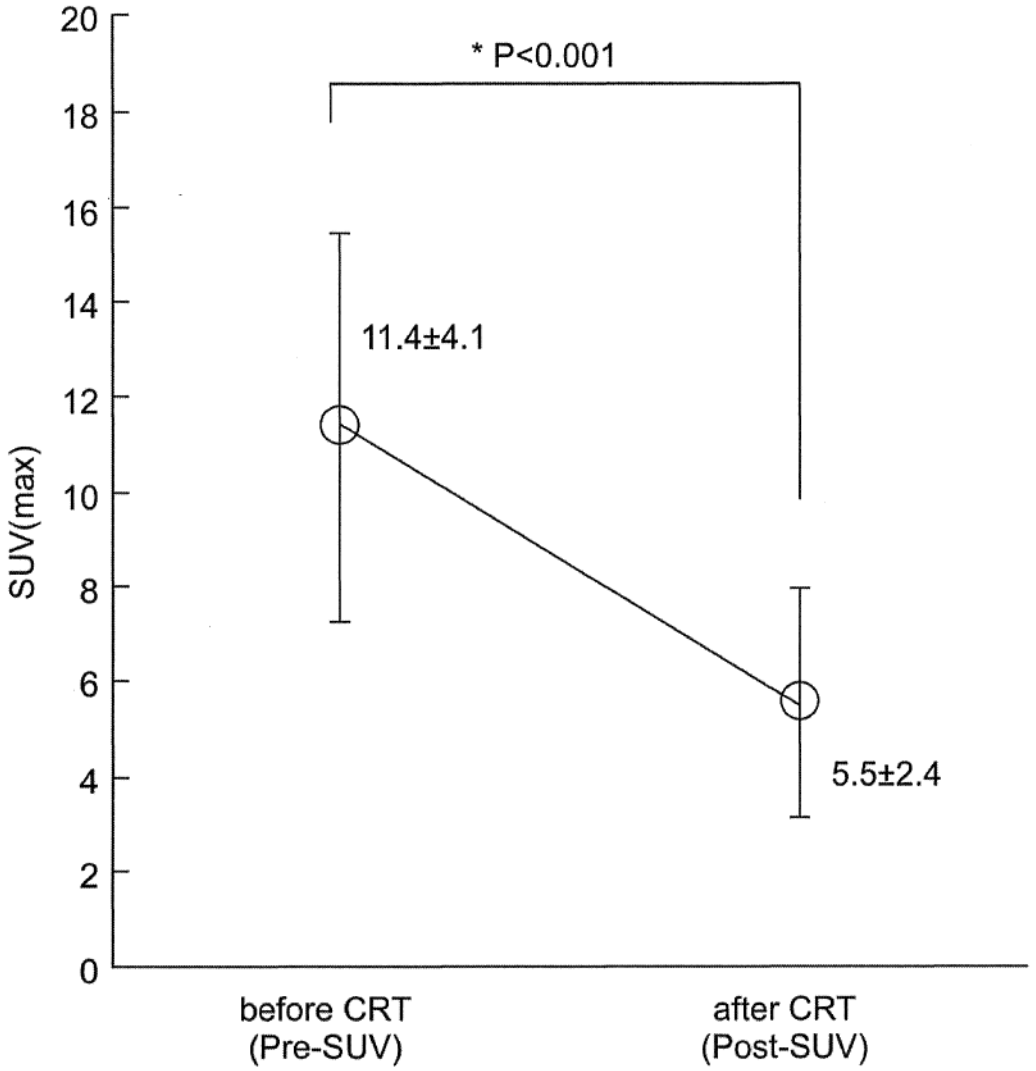
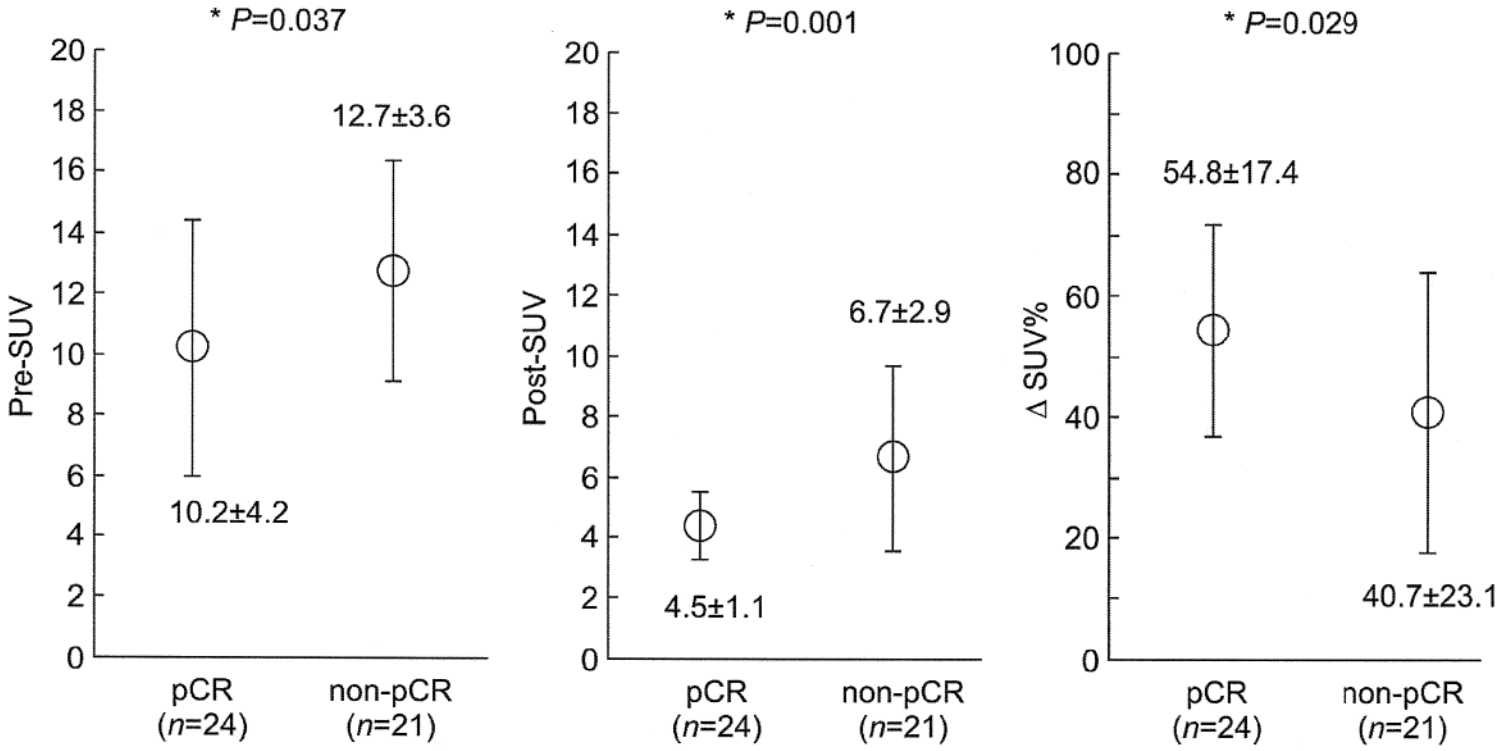

20
18
16
14
12
10
8
6
4
2
0
SUV(max)
* P<0.001
11.4±4.1
5.5±2.4
before CRT
(Pre-SUV)
after CRT
(Post-SUV)

Figure 2



*pCR : histopathologic complete response

Figure 3

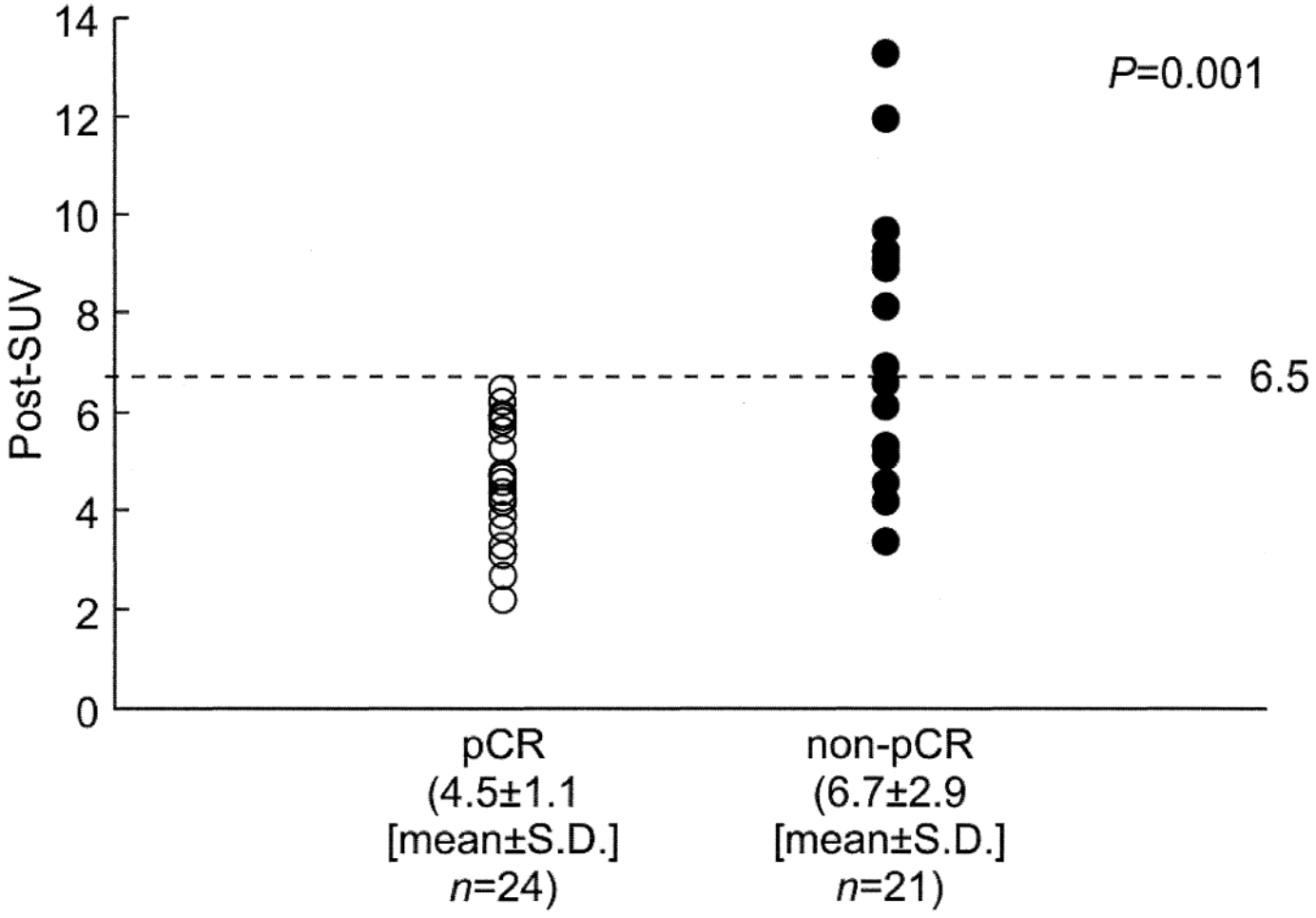

14
12
10
8
6
4
2
0
Post-SUV
P=0.001
6.5
pCR
(4.5±1.1
[mean±S.D.]
n=24)
non-pCR
(6.7±2.9
[mean±S.D.]
n=21)

Figure 4

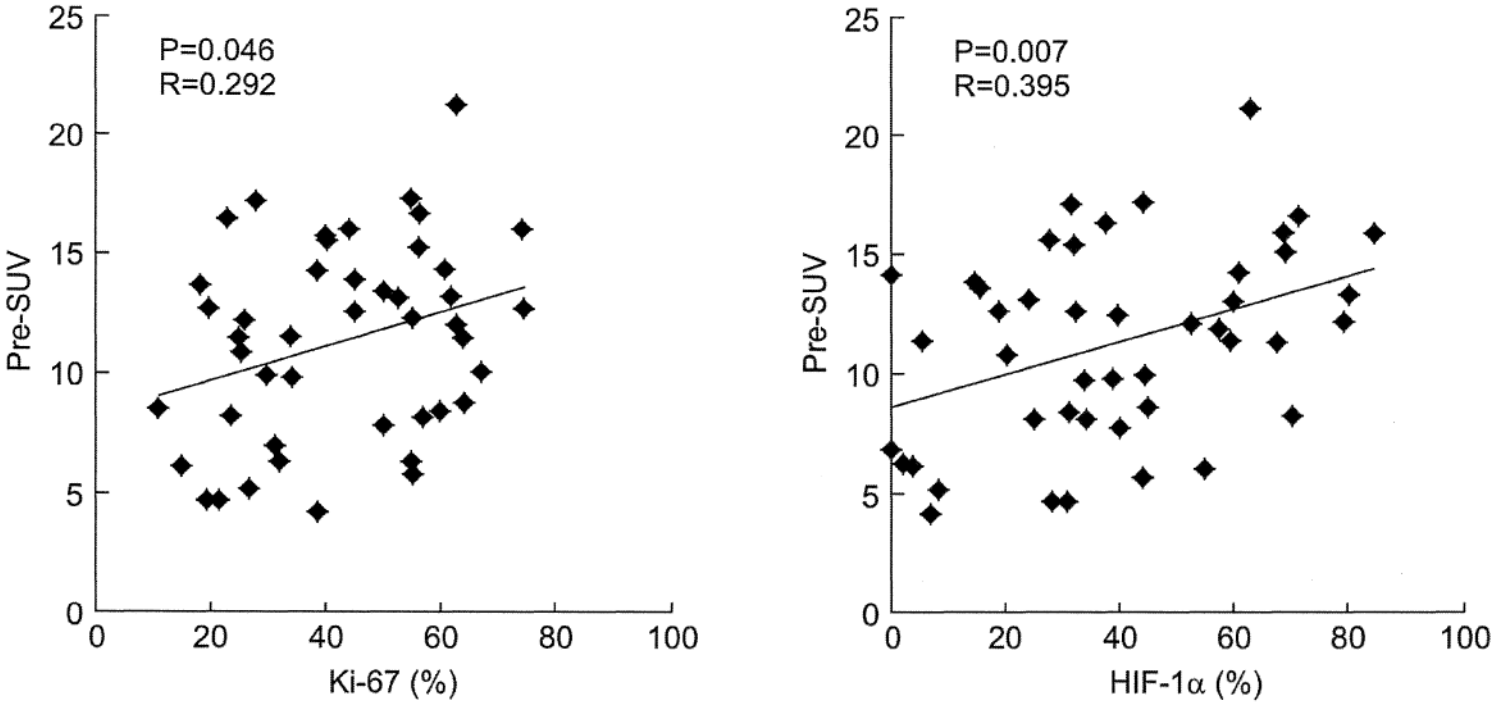

P=0.046
R=0.292
Pre-SUV
Ki-67 (%)
0
5
10
15
20
25
0
20
40
60
80
100
P=0.007
R=0.395
Pre-SUV
HIF-1α (%)

Figure 5

Table 1. Clinical characteristics of the study patients

Characteristics	No. of patients
Gender	
Male	28 (62.2%)
Female	17 (37.8%)
Age (years)	
Median (range)	61 (28-68)
Primary sites	
Tongue	29 (64.4%)
Upper gingiva	6 (13.3%)
Lower gingiva	4 (8.9%)
Floor of mouth	3 (6.7%)
Buccal mucosa	3 (6.7%)
T classifications	
Advanced T2	25 (55.5%)
T3	12 (26.7%)
T4	8 (17.8%)
Stage	
Advanced II	3 (6.7%)
III	19 (42.2%)
IV	23 (51.1%)
Radiotherapy of 40 Gy	45 (100 %)
Chemotherapy regimen	
CBDCA + 5FU	45 (100 %)

CBDCA, carboplatin; 5FU, 5-fluorouracil

Table 2. Standardized uptake values (SUVs) according to patient characteristics and histopathological response as observed in resected specimens

	No.	Pre-SUV [a]		Post-SUV [b]		⊿SUV% [c]	
		Mean	P value	Mean	P value	Mean	P value
No. of patients	45	11.4 ± 4.1		5.5 ± 2.4		47.9 ± 21.2	
Age							
≤Median	23	10.7 ± 3.7	0.272	5.6 ± 2.9	0.783	45.3 ± 20.9	0.409
>Median	22	12.1 ± 4.3		5.4 ± 1.9		50.6 ± 21.6	
Gender							
Male	28	11.8 ± 4.0	0.359	5.7 ± 2.5	0.543	44.8 ± 22.0	0.209
Female	17	10.7 ± 4.2		5.2 ± 2.3		53.1 ± 19.2	
Primary sites							
Tongue	29	10.7 ± 3.8	0.116	5.1 ± 1.7	0.127	50.8 ± 17.9	0.221
Other	16	12.7 ± 4.4		6.3 ± 3.3		42.7 ± 25.9	
T category							
Advanced T2	25	10.5 ± 3.8	0.115	5.4 ± 2.5	0.679	45.9 ± 21.5	0.481
T3＋T4	20	12.5 ± 4.2		5.7 ± 2.5		50.5 ± 21.0	
Clinical stage							
II + III	22	10.3 ± 4.0	0.070	4.9 ± 1.7	0.083	47.0 ± 20.9	0.778
IV	23	12.5 ± 3.9		6.1 ± 2.9		48.8 ± 21.8	
Histopathological response							
pCR	24	10.2 ± 4.2	0.037	4.5 ± 1.1	0.001	54.8 ± 17.4	0.029
non-pCR	21	12.7 ± 3.6		6.7 ± 2.9		40.7 ± 23.1	

pCR, pathological complete response

[a] Pre-SUV: SUVmax before preoperative CRT

[b] Post-SUV: SUVmax after preoperative CRT

[c] ΔSUV% (SUVmax reduction rate) = [(Pre-SUV − Post-SUV)/Pre-SUV] × 100 (%)

Table 3. Immunohistochemical expression of Ki-67 and hypoxia-inducible factor-1α (HIF-1α) in pretreatment biopsy specimens according to patient characteristics and histopathological response as observed in in resected specimens

	No.	Ki-67		HIF-1α	
		% Positivity	P value	% Positivity	P value
No. of patients	45	43.0 ± 17.3		39.0 ± 23.5	
Age					
≤Median	23	42.1 ± 16.4	0.729	34.6 ± 24.6	0.204
>Median	22	43.9 ± 18.6		43.5 ± 21.9	
Gender					
Male	28	44.9 ± 16.3	0.346	40.1 ± 24.3	0.668
Female	17	39.8 ± 18.9		37.0 ± 22.7	
Primary sites					
Tongue	29	40.3 ± 17.6	0.160	35.6 ± 21.7	0.206
Other	16	47.9 ± 16.2		44.9 ± 26.3	
T category					
Advanced T2	25	43.6 ± 18.0	0.787	4.2 ± 24.4	0.130
T3＋T4	20	42.2 ± 16.8		44.9 ± 21.4	
Clinical stage					
II + III	22	41.1 ± 14.4	0.682	35.2 ± 25.4	0.296
IV	23	41.9 ± 20.0		42.6 ± 21.4	
Histopathological response					
pCR	24	36.6 ± 15.0	<0.001	27.1 ± 18.2	<0.001
non-pCR	21	50.3 ± 17.2		52.5 ± 21.7	

pCR, pathological complete response

Table 4. Correlation between Ki-67 and hypoxia-inducible factor-1α (HIF-1α) expression levels in pretreatment biopsy specimens and standardized uptake values (SUVs)

Ki-67 (average: 43.0%)

	High expression[d] (n = 22)	Low expression[d] (n = 23)	P value
Pre-SUV[a]	12.6 ± 3.8	10.0 ± 4.1	0.036
Post-SUV[b]	6.9 ± 2.6	4.1 ± 1.2	<0.001
ΔSUV%[c]	41.5 ± 22.2	54.7 ± 18.1	0.034

HIF-1α (average: 39.0%)

	High expression[d] (n = 21)	Low expression[d] (n = 24)	P value
Pre-SUV[a]	12.8 ± 3.6	10.2 ± 4.2	0.030
Post-SUV[b]	7.0 ± 2.7	4.3 ± 1.2	<0.001
ΔSUV%[c]	41.1 ± 22.4	53.9 ± 18.5	0.043

[a] Pre-SUV: SUVmax before preoperative CRT

[b] Post-SUV: SUVmax after preoperative CRT

[c] ΔSUV% (SUVmax reduction rate) = [(Pre-SUV − Post-SUV)/Pre-SUV] × 100 (%)

[d] High expression: ≥ average, Low expression: < average