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FULL PAPER

Dynamic contrast-enhanced MRI of oral squamous cell carcinoma: a preliminary study of the correlations between quantitative parameters and the clinical stage

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Objective: To probe the utility of dynamic contrast-enhanced MRI (DCE-MRI) parameters in assessing the clinical characteristics of oral squamous cell carcinoma.

Methods: A total of 85 tumours were included. We applied the Tofts and Kermode model for the DCE-MRI data and obtained three dependent parameters: the influx forward volume transfer constant into the extravascular extracellular space (EES) from the plasma (K^{trans}), the fractional volume of EES per unit volume of tissue (v_e) and the fractional volume of plasma (v_p). We evaluated the correlations between these parameters and the clinical stages.

Results: The T stage showed a negative correlation with the K^{trans} ($r = -0.2272$; $p = 0.0365$), but it did not show

a significant correlation with the other parameters. The N stage showed a negative correlation with K^{trans} ($r = -0.1948$; $p = 0.0404$), and there were significant differences between N1 and N2+3 (0.119 ± 0.027 vs $0.096 \pm 0.023 \text{ min}^{-1}$; $p = 0.0198$) and between N0 and N2+3 (0.114 ± 0.029 vs $0.096 \pm 0.023 \text{ min}^{-1}$; $p = 0.0288$).

Conclusion: A decrease in the K^{trans} at the primary site was found in advanced N stage cases, which might indicate that the hypoxic status cause a high possibility of the metastasis.

Advances in knowledge: A decrease in the K^{trans} at the primary site suggested the high possibility of an advanced N stage.

Angiogenesis is essential for tumour proliferation and progression. Previous studies have reported that the vessel density correlates with the intensity of vascular endothelial growth factor (VEGF) expression and metastasis in breast, colon and prostate cancer, suggesting that the vessel density may be a prognostic factor. However, this is not completely true in oral squamous cell carcinoma (OSCC). Many groups have demonstrated that there was no association between the vessel density and the T stage, degree of differentiation or cervical lymph node metastasis. Moreover, the vessel density of OSCC with an invasive border (poor prognosis) was smaller than that of OSCC with a well-defined border (good prognosis). Therefore, it has been considered that although angiogenesis is essential for tumour progression, it results in the destruction of the microvessel during tumour growth, especially in the invasive border type.¹⁻³

Several prognostic molecular markers have been identified in head and neck (HN) cancers, such as Ki-67 (reflecting cellular proliferation), VEGF, carbonic anhydrase (CAIX), hypoxia-inducible transcription factor 1 α (HIF-1 α), proliferating cell nuclear antigen and glucose transporter 1. Recently, the hypoxic status has been a focus of many studies because it is associated with lymph node metastasis and a poor prognosis in patients with OSCC.⁴⁻⁹

Many pre-clinical and clinical studies have found that the quantitative parameters obtained by dynamic contrast-enhanced MRI (DCE-MRI) could provide information on the underlying pharmacokinetic nature of the tissue.¹⁰ In the HN region, the pharmacokinetic analysis by DCE-MRI was proven to be useful for a differential diagnosis,¹¹ early prediction of the response and monitoring the effects of chemotherapy and radiotherapy.¹²⁻¹⁵ However, the correlations between the pharmacokinetic parameters of the

primary site and the clinical stage have been assessed in only limited studies. We have found two studies of the correlations between the quantitative parameters and clinical stage of nasopharyngeal cancer; however, the results of these studies were conflicting.^{16,17}

The aim of this study was to probe the utility of the DCE-MRI parameters for assessing the clinical characteristic of OSCC.

METHODS AND MATERIALS

Study population

The institutional ethics review board (Kyushu University Hospital, Fukuoka, Japan) approved this prospective study (approval number 21056). The patients enrolled in the present study were referred to the Department of Oral Surgery and underwent DCE-MRI at our institute, Kyushu University Hospital, between February 2010 and March 2014.

In our research plan, we selected the patients, whom an oral surgeon had recommended to undergo enhanced MRI owing to a suspected HN tumour and obtained informed consent for this examination from all patients. Therefore, we were not directly involved in the decision to perform enhanced MRI examinations.

After that, we explained the details of our study to all patients. We told them about the DCE-MRI sequence and the pharmacokinetic analysis. The first purpose of this study was to make a differential diagnosis of the HN region, while the second purpose was to identify any correlations among the parameters obtained by DCE-MRI and the clinical stage, the degree of malignancy (differentiation and mode of invasion) and predicting the effect of pre-operative chemoradiotherapy in patients with OSCC. We explained them to all patients by both verbal instruction and written explanations using the approved documents by our institutional ethics review board. We obtained written informed consent of all patients enrolled in this study. For the second purpose, we evaluated 95 patients with untreated OSCC to evaluate the relationship between the pharmacokinetic parameters and the clinical stage of OSCC in this research.

The location, staging and treatment history of the patients included in this study ($n = 85$; 48 males and 37 females; average age, 61.5 ± 14.2 years) are shown in Table 1. The TNM status and clinical stage in all patients were determined by the consensus of an oral surgeon (with over 15 years' experience in oral surgery) and an oral radiologist (with over 17 years' experience in analysing HN images) with reference to the seventh edition of the International Union against Cancer/American Joint Committee on Cancer (UICC/AJCC) staging system. The carcinomas originating from the basal tongue were excluded, while mobile tongue carcinomas were included. In 75 patients, nodal involvement was interpreted based on the comprehensive interpretation of the findings of CT, ultrasonography and fluorine-18 fludeoxyglucose positron emission tomography (PET), while the nodal status of the remaining 10 patients was interpreted based on the CT/ultrasonography findings.^{18,19} The M stages of all patients were M0.

The number of the patients with T stage 4b was small ($n = 6$); therefore, we combined T4b with T4a into T4, and the simplified

categories were used for the following statistical analyses. Similarly, we combined N2b, N2c and N3 into N2+3. We also merged clinical Stages IVA and IVB into IV.

MRI protocol

All experiments were performed using an Achieva® 1.5-T Nova Dual MR device (Philips Healthcare, Eindhoven, Netherlands) and a 16-channel neurovascular transceiver coil.

A reference pre-contrast scan was acquired immediately prior to the DCE-MRI scan in order to confirm that the scan range covered the tumour. The scan parameters were identical to those of the DCE scan, except for the flip angle, which was 5°. The DCE-MRI used a three-dimensional T_1 fast-field echo sequence. The representative parameters were: repetition time, 6.1 ms;

Table 1. Characteristics of the patients ($n = 85$)

Characteristics	<i>n</i>
Primary site	
Tongue	40
Oral floor	9
Buccal mucosa	8
Maxilla	10
Mandible	18
T stage	
T2	32
T3	13
T4a	34
T4b	6
N stage	
N0	44
N1	16
N2b	20
N2c	4
N3	1
Clinical stage	
II	21
III	17
IVA	41
IVB	6
Treatment	
Surgery	12
CRT → surgery	55
Surgery → CRT	3
CRT	11
Drop out + refusal	4

CRT, chemoradiotherapy.

Table 2. Comparison of the dynamic contrast-enhanced (DCE)-MRI parameters among both primary sites and different extents of differentiation

Classification	Case number	AUGC (mM × min)	K^{trans} (min^{-1})	v_e	v_p
Primary site					
Tongue	40	1.37 ± 0.32	0.110 ± 0.025	0.32 ± 0.09	0.03 ± 0.01
Gingiva	28	1.36 ± 0.35	0.106 ± 0.027	0.32 ± 0.12	0.03 ± 0.01
Oral floor	9	1.44 ± 0.35	0.117 ± 0.040	0.36 ± 0.08	0.03 ± 0.01
Buccal mucosa	8	1.34 ± 0.26	0.107 ± 0.034	0.33 ± 0.07	0.03 ± 0.01
Differentiation ^a					
Well	63	1.37 ± 0.31	0.108 ± 0.025	0.33 ± 0.10	0.03 ± 0.01
Moderate	17	1.31 ± 0.35	0.107 ± 0.032	0.30 ± 0.11	0.03 ± 0.02
Poor	1	1.20	0.079	0.34	0.03

AUGC, area under the gadolinium concentration curve; K^{trans} , the influx forward volume transfer constant; v_e , fractional volume of the extravascular extracellular space; v_p , fractional volume of plasma.

Neither the location of the primary site nor the differentiation of the primary site showed any significant differences in any of the DCE-MRI-derived parameters (the Steel-Dwass test).

^aIn four cases, we could not evaluate the differentiation because the histological examination was performed in other institutes.

echo time, 4.6 ms; two different flip angles (5° for reference pre-contrast scan, 15° for dynamic scan); field of view (FOV), 220 mm; rectangular FOV, 87.5%; slice thickness, 5 mm; sensitivity encoding (SENSE) factor, 1.8; acquisition voxel (mm), $1.72 \times 1.82 \times 5.00$; and reconstruction voxel (mm), $0.86 \times 0.86 \times 5.00$. 30 s after the start of data acquisition, the operator initiated the injection of contrast medium (CM).

We obtained 80 phases with a temporal resolution of 3.5 s. A single injection of gadopentetate dimeglumine (Magnevist®; Bayer Schering Pharma, Osaka, Japan) was administered at a dose of 0.1 mmol kg^{-1} through the antecubital vein at a rate of 2 ml s^{-1} via a mechanical injector, immediately followed by a 20-ml saline flush.

Before performing DCE-MRI, T_2 weighted (T2W) and T_1 weighted (T1W) axial images, coronal T2W images, sagittal T1W images and axial diffusion-weighted images were acquired. After the DCE-MR examination, routine T1W images were obtained.

Tofts and Kermode model analysis

The MR image data (Philips PAR/REC format) were transferred to a personal computer and processed using a proprietary

software program in the PAR/REC format (PRIDE software; Philips Healthcare) according to the interface data language. The realignment of the time series of DCE-MRI was performed using non-linear registration methods.

The procedure used in this manuscript was described in a previous article,^{15,20,21} and its main principles were as follows: the first procedure was to estimate the pre-contrast T_1 map using image data with two different flip angles²¹ and to estimate the dynamic T_1 maps using the methods described by Daldrup et al.²² The second procedure was to convert the difference of $1/T_1$ into the concentration of the CM by assuming a linear relationship between them. In this study, we set the r_1 relaxivity of the CM to 4.5 mM s^{-1} .^{23,24} The third procedure was to apply the pharmacokinetic analysis (two-compartment model) based on the extended Tofts model.

The subsequent output map was calculated automatically. The three dependent parameters [K^{trans} , the influx forward volume transfer constant into the extravascular extracellular space (EES) from the plasma; v_e , the fractional volume of EES; v_p , the fractional volume of plasma per unit volume of tissue] were obtained. In addition, the total area under the gadolinium

Table 3. Correlation of dynamic contrast-enhanced (DCE)-MR parameters and clinical staging

Pharmacokinetic parameters	International Union against Cancer stage		T stage		N stage	
	Coefficient	<i>p</i> -value	Coefficient	<i>p</i> -value	Coefficient	<i>p</i> -value
K^{trans}	-0.1425	0.1933	-0.2272	0.0365 ^a	-0.1948	0.0404 ^a
v_e	-0.2041	0.0609	-0.1974	0.0702	-0.1774	0.1043
v_p	-0.1381	0.2074	-0.111	0.312	-0.236	0.0297 ^a
AUGC	-0.1222	0.2652	-0.1744	0.1105	-0.2158	0.0473 ^a

AUGC, area under the gadolinium concentration curve; K^{trans} , the influx forward volume transfer constant; v_e , fractional volume of the extravascular extracellular space; v_p , fractional volume of plasma.

^aSignificant difference ($p < 0.05$) (Spearman' rank correlation test).

Figure 1. A 71-year-old male with buccal mucosa squamous cell carcinoma with TNM stage T4bN0M0 disease. The tumour region of interest is outlined (a). The mean area under the gadolinium concentration curve was 1.42 mM min^{-1} (b), the influx forward volume transfer constant (K^{trans}) was 0.127 min^{-1} (c) and the mean fractional volume of the extravascular extracellular space (v_e) was 0.283 (d).

concentration curve (AUGC) after injection, which was not a non-model parameter, was also estimated.

All data obtained from the pharmacokinetic analysis of the PAR/REC format were converted to the Analyze format using the MRIcro software program (<http://www.cabiatl.com/mri-cro/>); the subsequent measurement of parameters within the volumes of interest (VOIs) were carried out using the ImageJ software program (<http://rsb.info.nih.gov/ij/>). We drew the contours of the primary site of the tumour in all slices including the tumour and set the VOIs. The necrotic areas were excluded from the VOIs based on the AUGC map, and we also referred to the dynamic image and axial T2W image. This procedure was performed by consensus between the two authors (with 17 years' experience and 5 years' experience in HN radiology, respectively).

Statistical analysis

The correlations between the MRI quantitative parameters (K^{trans} , v_e , v_p and AUGC) of the primary site and clinical stage (T stage, N stage and UICC stage) were examined by using Spearman's rank correlation test. The Steel-Dwass test was performed for comparisons among three or more categories (JMP v. 9.0.2; SAS Institute, Cary, NC).

RESULTS

Of the 95 patients, 10 were excluded from this study owing to poor images (severe metal artefacts, 4; severe motion artefacts, 6); therefore, 85 patients were evaluated in the present study.

Neither the location of the primary site nor the differentiation of the primary site showed significant differences in any of the DCE-MRI-derived parameters (Table 2). The relationships between the MR quantitative parameters and clinical features, including the UICC stage, T stage and N stage are detailed in Table 3. The T stage showed a negative correlation with the K^{trans} ($r = -0.2272$; $p = 0.0365$) but showed no significant correlation with the other three parameters (Table 3). The N stage showed a negative correlation with three parameters (K^{trans} , $r = -0.1948$; $p = 0.00404$; v_p , $r = -0.2360$; $p = 0.0297$; AUGC, $r = -0.2158$; $p = 0.0473$).

Figures 1 and 2 show representative images of patients with N0 disease and N2b disease, respectively. For the total of 85 cases, the cases with advanced N stage disease showed decreases in the AUGC and K^{trans} , and there were significant differences in between both N0 and N2+3 (1.44 ± 0.031 vs $1.25 \pm 0.030 \text{ mM min}^{-1}$; $p = 0.047$) in AUGC, and between both N1 and N2+3 (0.119 ± 0.027 vs $0.096 \pm 0.023 \text{ min}^{-1}$; $p = 0.0198$) and

Figure 2. A 46-year-old male with tongue squamous cell carcinoma with TNM stage T4aN2bM0 disease. The tumour region of interest is outlined (a). The mean area under the gadolinium concentration curve was $0.867 \text{ mM min}^{-1}$ (b), the influx forward volume transfer constant (K^{trans}) was 0.0688 min^{-1} (c) and the mean fractional volume of the extravascular extracellular space (v_e) was 0.182 (d).

between N0 and N2+3 (0.114 ± 0.029 vs $0.096 \pm 0.023 \text{ min}^{-1}$; $p = 0.0288$) in K^{trans} . However, neither the clinical stage nor the T stage showed any significant differences between any of the pairs of each stage in any of the parameters (Table 4).

DISCUSSION

In our study, we showed the negative correlation between T stages; however, there were no significant differences among the T stages. Several previous studies about DCE-MRI have demonstrated that the K^{trans} was negatively correlated with hypoxia in both animal models and *in vivo*.^{25,26} Gulliksrud et al²⁵ implanted human melanoma xenografts in adult female mice and investigated whether DCE-MRI could differentiate between hypoxic and non-hypoxic experimental tumours. They immunohistochemically evaluated the hypoxic status by pimonidazole staining, and demonstrated that the hypoxic area had a smaller K^{trans} . Sathy et al²⁷ evaluated both DCE-MRI parameters and the tumour volume, and found that the increase of the K^{trans} occurred before the marked tumour growth in an animal experiment. They concluded that there was angiogenic switching prior to tumour growth. In the present study, Spearman's rank correlation test showed that there was a significant correlation between the K^{trans} and T stage; however, there were no significant

differences among the T2–4 categories. The progression of the T stage caused increased angiogenesis, and the appearance of the hypoxic area occurred at the same time; therefore, we considered that the correlation between the K^{trans} and T stage was complicated and likely to be owing to several factors.

The K^{trans} decreased significantly with the progression of the N stage. Øvrebo et al performed animal experiments using R-18 melanoma xenografts growing in mice. They clarified that the mice with metastatic tumours had significantly lower K^{trans} values than did the metastasis-negative mice, consistent with the hypothesis that hypoxia promotes lymph node metastasis.²⁸ They also demonstrated that the K^{trans} , but not the v_e , was associated with the metastatic potential of the tumour.²⁸ We found that the progression of N stage was associated with a decrease of the K^{trans} , consistent with the results obtained in animal experiments.

In humans, hypoxia-inducible factor one is an essential element associated with changing the transcriptional response of tumours under hypoxia, which is a common feature of many tumours. In OSCC, the overexpression of HIF-1h has been found to correlate with lymph node metastasis and the TNM

Table 4. Comparison of the dynamic contrast-enhanced (DCE)-MRI parameters among the different stages

Classification	Case number	AUGC (mM × min)	K^{trans} (min^{-1})	v_e	v_p
Clinical stage					
II	21	1.45 ± 0.29	0.113 ± 0.030	0.36 ± 0.08	0.031 ± 0.008
III	17	1.36 ± 0.35	0.117 ± 0.028	0.31 ± 0.07	0.031 ± 0.012
IV	47	1.34 ± 0.33	0.105 ± 0.027	0.32 ± 0.11	0.029 ± 0.012
P (II vs III)		0.74	0.95	0.062	0.74
P (III vs IV)		0.98	0.32	0.97	0.98
P (II vs IV)		0.43	0.60	0.13	0.43
T stage					
T2	32	1.44 ± 0.35	0.118 ± 0.031	0.35 ± 0.09	0.029 ± 0.009
T3	13	1.35 ± 0.23	0.108 ± 0.018	0.28 ± 0.04	0.033 ± 0.009
T4	40	1.33 ± 0.32	0.103 ± 0.027	0.32 ± 0.11	0.028 ± 0.012
P (T2 vs T3)		0.35	0.63	0.0031 ^a	0.47
P (T3 vs T4)		1.00	0.63	0.63	0.24
P (T2 vs T4)		0.25	0.11	0.19	0.60
N stage					
N0	44	1.44 ± 0.31	0.114 ± 0.029	0.34 ± 0.10	0.031 ± 0.012
N1	16	1.38 ± 0.36	0.119 ± 0.027	0.32 ± 0.08	0.029 ± 0.010
N2	25	1.25 ± 0.30	0.096 ± 0.023	0.30 ± 0.10	0.026 ± 0.008
P (N0 vs N1)		0.85	0.86	0.46	0.99
P (N1 vs N2+3)		0.40	0.0198 ^a	0.59	0.24
P (N0 vs N2+3)		0.047 ^a	0.0288 ^a	0.094	0.10

AUGC, area under the gadolinium concentration curve; K^{trans} , the influx forward volume transfer constant; P, *p*-value; v_e , fractional volume of the extravascular extracellular space; v_p , fractional volume of plasma.

^aSignificant difference ($p < 0.05$) (the Steel-Dwass test).

stage, but not with the primary site or histological grade (differentiation).^{4–9} Many groups have demonstrated no association to exist between the vessel density and cervical lymph node metastasis. Furthermore, an association between the lymphatic vessel density, rather than the vessel density, and lymph node metastasis in OSCC has been elucidated. We speculated that the reduction of the blood flow caused the hypoxic state and resulted in the high possibility of lymph node metastasis. This trend was also similar to the results for nasopharyngeal carcinoma obtained by Zheng *et al.*¹⁷ Further studies to compare the K^{trans} obtained by DCE-MRI and an immunohistological evaluation of the excised tissue will be needed.

The present study was associated with several potential limitations. The first limitation was that the TNM stage was determined clinically, not by histopathology, because many patients underwent chemoradiotherapy, and the number of the patients who underwent the neck dissection was small. Moreover, the N stage was interpreted by either CT/ultrasonography or CT/ultrasonography/PET-CT. However, in our previous study, our image-based diagnosis of the lymph nodes was quite high, and the diagnostic accuracy of CT/ultrasonography was

similar to that of PET-CT.^{18,19} Therefore, we considered that CT/ultrasonography was sufficient and did not have a significant impact on the present results.

The second limitation is that we used the model arterial input function (AIF) value in the analysis, because the measurement of individual AIF values was quite difficult. Especially in the HN region, the previously reported parameters obtained by individual AIF values might be somewhat unreliable because of the in-flow effect owing to the high blood velocity, which reduces the concentration of CM in the plasma and can hamper the accurate estimation of the AIF.^{29,30}

Yuan *et al.*²⁹ pointed out that the previously reported parameters, which did not sufficiently compensate for the effect of the in-flow phenomenon of the arteries, were found to be quite different from the true parameters. Recently, the AIF measurement using phase information has been found to be a potentially useful method to accurately estimate the AIF, because it is unaffected by any in-flow phenomena,^{31,32} however, both the reproducibility and the robustness of the phase AIF in the HN region were not sufficient in our preliminary study (data not shown). Therefore, we used the model AIF in this study instead

of the individual AIF.³³ In future studies, other methods, such as arterial spin labelling might be needed to evaluate the relationship between the blood flow and the TNM stage.

The third limitation included potential pre-contrast T_1 mapping errors owing to the dual flip angle. Although, a more accurate measurement, such as the look-locker sequence, would have been preferable, we used the dual flip angle method in order to decrease the examination time. To reduce the errors, we selected flip angles because these values were close to the Ernst angle of many soft tissues, and the two angles were on opposite sides of the Ernst angle.²¹

CONCLUSION

Neither the location nor differentiation resulted in significant difference in any pharmacokinetic parameters. A decrease of the K^{trans} was associated with the progression of the N stage.

CONFLICTS OF INTEREST

We used a proprietary software program (PRIDE software; Philips Healthcare, Eindhoven, Netherlands) using the interface data language (IDL, RSL). However, one of the authors belongs to Philips Electronics Japan, Tokyo, Japan. This study was performed as a collaborative research between Kyushu University (Fukuoka, Japan) and Philips. 32 out of 85 patients have been used for a previous study, which demonstrated the usefulness of the pharmacokinetic analysis for differentiating head and neck cancer. However, in this research, the patients with oral squamous cell cancer ($n = 85$) were included, and we evaluated the correlation between the parameters obtained by dynamic MRI and the clinical stage.

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