**Prediction of Distant Metastases in Patients with Squamous Cell Carcinoma of Head and Neck using DWI and DCE-MRI**

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**Running Title:** Prediction of Distant Metastases in HNSCC

**Keywords:** Squamous cell carcinoma of head and neck; Distant metastases; Dynamic contrast enhanced-MRI; Diffusion weighted imaging; Volume transfer constant; Intracellular water life time

**Abstract**

**Background:** The purpose was to evaluate the prognostic potential of diffusion imaging (DWI) and dynamic contrast enhanced (DCE)-MRI in predicting distant metastases in squamous cell carcinoma of head and neck (HNSCC) patients.

**Methods:** Fifty-six patients underwent pretreatment DWI and DCE-MRI. Patients were divided into groups that subsequently did (n=12) or did not develop distant metastases (n=44). Median values of apparent diffusion coefficient (ADC), volume transfer constant (Ktrans), and mean intracellular water lifetime (τi)**andnodal volume** were computed from metastatic lymph-nodes and were compared between two groups.

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**Results:** Lower but not significantly different Ktrans (0.51±0.15min-1 vs. 0.60±0.05min-1) and τi (0.13±0.03s vs. 0.19±0.02s) were observed in patients who developed distant metastases than those who did not. Additionally, no significant differences in ADC **and nodal volume were** **found**. τi, was the best parameter in discriminating two groups with a sensitivity of 67% and a specificity of 61.4% **and this discriminatory power did not improve significantly even when all imaging parameters were combined with clinical and histological features using a logistical regression analysis.**

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**Conclusion:** Pretreatment DCE-MRI may be useful in predicting distant metastases in HNSCC patients.

**Keywords:** Squamous cell carcinoma of head and neck; Distant metastases; Dynamic contrast enhanced-MRI; Diffusion weighted imaging; Volume transfer constant; Intracellular water life time

**Abbreviation List:**

ADC=Apparent diffusion coefficient

AIF=Arterial inp­ut function

AUC=Area under the ROC curve

CRT=Chemo-radiation therapy

CT=Computer tomography

DCE-MRI=Dynamic contrast enhanced-MRI

DWI = Diffusion weighted imaging

FDG=18F-fluorodeoxyglucose

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**HIF-1α = Hypoxia inducible transcription factor**

HNSCC= Squamous cell carcinomas of head and neck

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**HPV= Human papilloma virus**

Ktrans=Volume transfer constant

MRI=Magnetic resonance imaging

PET=Positron emission tomography

ROC=Receiver operating characteristic curve

ROI= Region of interest

SSM = Shutter speed model

SUV=Standard uptake value

τi = Mean intracellular water molecule life-time

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**VEGF= Vascular endothelial growth factor**

**Introduction**

Head and neck squamous cell carcinomas (HNSCCs) account for approximately 5% of all malignant neoplasms worldwide.1 Radiation therapy and concurrent or induction chemotherapy is the standard of care for patients with non-resectable HNSCCs.2 These therapeutic interventions generally provide effective loco-regional control, however, overall 5-year survival rate of HNSCC patients is only 40-60% suggesting that prognosis of patients with HNSCC remains dismal.3,4 It is widely believed that one of the major causes of poor clinical outcomes is the development of distant metastases (outside the neck lymph nodes) with an incidence rate of 4% to 40%.5-7 Therefore, it would be helpful to identify the subset of patients at initial diagnosis, who are at greater risk for developing distant metastases, so that current treatment strategies could be redefined to more intensified treatment regimen to prolong disease-free survival in these patients.

**Several clinicopathological characteristics and gene signatures such as nodal stage, differentiation grading system, Ki-67 (a cellular proliferation index), vascular endothelial growth factor (VEGF, an endogenous marker of tumor angiogenesis), hypoxia inducible transcription factor (HIF-1α, a biomarker of tumor hypoxia), and human papilloma virus (HPV) burden have been identified as important prognostic factors related to incidence rate of distant metastases in HNSCC.8,9 Despite providing good prognostic indications, a considerable variability in the utility of these biomarkers in predicting distant metastases in HNSCC has been reported.10** Therefore, development of non-invasive prognostic imaging biomarkers that can aid in triaging patients for appropriate treatment and long-term clinical outcomes is warranted.

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Higher pretreatment 18F-fluorodeoxyglucose (FDG) uptake from the metastatic neck nodes usingpositron emission tomography (PET) have been associated with occurrence of distant metastases in HNSCC patients.11-14 However, non-specific and overlapping findings have also been reported with FDG-PET, thus raising doubts about its use as a reliable marker for predicting distant metastases.15,16 Additionally, FDG-PET studies commonly use the standard uptake value (SUV) as a quantitative measure of FDG uptake that has been known to be influenced by various biological factors such as blood glucose level, body size, and breathing patterns.17 Therefore, it is likely that the semi-quantitative nature of SUV may impact the sensitivity of FDG-PET as a prognostic marker. Because of these limitations with FDG-PET, alternative imaging methods should be investigated to predict distant metastases in HNSCC patients.

Magnetic resonance imaging (MRI) techniques such as diffusion-weighted imaging (DWI) and dynamic contrast enhanced (DCE)-MRI have been used extensively for studying tumor microenvironment and for predicting short and long-term treatment response as well as overall survival in HNSCC patients.18-27 DWI derived low baseline apparent diffusion coefficient (ADC)18,21,23-25 and DCE-MRI derived high baseline volume transfer constant (Ktrans)19-22,26,27 as well as high mean intracellular water life time (τi)22 from metastatic neck lymph nodes indicate improved prognosis in patients with HNSCC. However, the prognostic utility of these parameters has not been examined for prediction of distant metastases which is of major clinical importance in the management of HNSCC patients.

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With this objective in mind, the **primary aim of this** study was to investigate the prognostic role of pretreatment ADC, Ktrans τi **and volume** from the neoplastic neck lymph node for predicting development of distant metastases. **Additionally, prognostic utility of HPV status, differentiation grading, nodal staging was also evaluated both in isolation and in combination with MRI parameters.**

R2.1

**Additionally, since strong relationships have been observed between DCE-MRI derived parameters and molecular markers such as Ki-67, VEGF and HIF-1α.28-31  Therefore, a secondary aim of the study was to investigate the differences in pretreatment physiologic MRI parameters on the basis of HPV status, differentiation grade, and nodal stage.**

R2.2

**Materials and Methods**

**Patient Selection**

This retrospective study design was approved by the Institutional Review Board and was compliant with the Health Insurance Portability and Accountability Act. The primary inclusion criteria for patients were as follows: (a) appearance of at least one metastatic neck lymph node measuring >1cm3 on computer tomography (CT)/MRI scans at initial presentation (b) biopsy confirmed diagnosis for lymph node metastasis (c) no history of prior treatment (d) absence of distant metastasis at the time of initial diagnosis as confirmed by whole-body CT and PET/CT scans. Based upon the inclusion criteria, a cohort of 72 newly diagnosed HNSCC patients were recruited. Each patient received appropriate therapy with an intent to cure per the standard guidelines. The multimodality treatment included upfront neck dissection (n=3), concurrent CRT (n=46), or induction chemotherapy followed by CRT (n=23).

**Determination of HPV Status**

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**HPV status was determined from tissue specimens by immunohistochemical evaluation of p16-expression using commercially available monoclonal antibody. Tissue samples were available from only 32 patients and these were divided into two groups [positive (n=21) or negative (n=11)] for p16-expression as per the guidelines reported in an earlier study**.32

**Data Acquisition**

All patients underwent MRI before surgery, radiation, or chemotherapy on 1.5T Sonata scanner (Siemens, Erlangen, Germany) (n=37) or on 3T Magnetom-Trio scanner (Siemens, Erlangen, Germany) (n=35). Structural imaging protocol on both MRI systems included axial T2-weighted and T1-weighted images with and without contrast agent (Omniscan; Nycomed) using standard parameters. Diffusion weighted images were acquired in the axial orientation using a fat-suppressed pulsed spin-echo echo-planar imaging sequence (repetition-time (TR)/echo-time (TE)= 4000/89ms) with three b values of 0, 500, and 1000s/mm2 to generate trace diffusion maps. Other sequence parameters were as follows: bandwidth=1500 Hz/pixel; field of view (FOV)=260×260mm2; matrix size=128×128; number of slices=8; slice thickness=5 mm; interslice gap=0mm; number of excitation (NEX)=4; and acquisition time=1minute 58 seconds.

For quantification of pre-contrast T1, inversion-recovery prepared T1-weighted images were acquired using inversion times of 60, 200, 400, 800, and 1600ms prior to acquisition of DCE-MRI data. As described previously in detail,19 DCE-MRI was performed by using a rapid 3D-spoiled gradient-echo sequence, modified to acquire 8 angle-interleaved sub-aperture images from the full-echo radial data.Imaging parameters were: TR/TE=5.0/4.2ms, 256 readout points/views, 256 views (32 views/sub-aperture, 8 sub-apertures), FOV=260×260mm2; number of slices=8; slice thickness=5mm. Fat saturation was applied once every 8 excitations. Spatial saturation was applied once every 32 excitations to minimize the flow effect while minimizing acquisition time. This scheme resulted in a temporal resolution of 2.5s for each sub-aperture image with full spatial resolution of 256×256 by using a dynamic k-space-weighted image reconstruction contrast algorithm.33

**Image Processing**

A motion-correction algorithm was used on the raw DWI and DCE-MRI data to correct for any in-plane rotational motion artifacts caused by voluntary and involuntary motion as described previously.18,19,21 All images (T2, T1, post-contrast T1-weighted, DWI and DCE-MRI) were co-registered using a 2-step non-rigid image registration technique. Regions of interest (ROIs) were drawn on solid portion of the largest nodal mass using T2-weighted, T1-weighted, and post-contrast T1-weighted images. Care was taken to avoid necrotic/cystic or hemorrhagic parts as well as surrounding blood vessels on all imaging slices encompassing the metastatic node. Pixel-by-pixel ADC maps were computed by using a weighted linear least-squares fit method. Pharmacokinetic analysis of DCE-MRI data was performed for each voxel in the selected ROIs using shutter speed model (SSM).19,21 Median pretreatment ADC, τi and Ktrans were computed from each patient using only the central 4 slices to avoid erroneous results from wrap-around artifacts in the edge slices.

**Data Analysis**

Three patients who underwent upfront neck dissection and 13 patients who either had corrupted MRI data or insufficient follow-up clinical data were excluded from the study. Final data analysis was performed from the remaining 56 patients. The clinical, **histopathological and immunohistochemical** characteristics from these 56 patients at the time of initial presentation is summarized in **Table 1**. CT chest and whole body FDG-PET scans were used to evaluate the occurrence of distant metastases during a follow-up period of 14 years. Of these 56 patients, distant metastases were observed in 12 patients. In four of these 12 patients, distant metastases were found in two organs. The most common site of distant metastases was lung (n=10) followed by bone (n=3), brain (n=2) and liver (n=1). Based on the follow-up scans, the patients were divided into two groups [patients with distant metastases (n=12) and patients without distant metastases (n=44)]. Mann-Whitney *U* tests were performed on the baseline diagnostic MRI scans to evaluate differences in median values of ADC, Ktrans, τi and nodal volume between these two groups. **For categorical variables [HPV status: HPV positive and HPV negative HNSCCs; differentiation grades: moderately differentiated (MD) and poorly differentiated (PD) HNSCCs; nodal stage: N2 and N3], contingency tables (2 × 2) were constructed and Pearson’s Chi-Square tests were performed to investigate the differences in these categorical variables between two groups of patients (with and without metastases).** A probabilistic (p) **value** of less than 0.05 was considered significant.

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R1.2

Multivariate logistic regression analyses were performed **by incorporating all the continuous (ADC, Ktrans, τi and nodal volume) and categorical variables [HPV status (positive and negative for p16-expression), differentiation grading (moderately and poorly differentiated), and nodal staging (N2 and N3)** to obtain the best combination of parameters in distinguishing the two groups. Receiver operating characteristics (ROC) curve analyses were also performed to determine accuracy (area under the ROC curve, AUC), sensitivity and specificity of all study variables (ADC, Ktrans, τi nodal volume)by using the highest Youden index (Sensitivity + Specificity − 1).

R2.3

**Additionally, Mann-Whitney *U* tests were performed on MRI parameters divided into two groups based upon dichotomous HPV status, differentiation grade and nodal stage.** All statistical analyses were performed by using a statistical package, SPSS for Windows (version 18.0; SPSS Inc., Chicago, IL, USA).

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**Results**

Representative pretreatment anatomic MR images, ADC map, and color-coded parametric Ktrans, and τi maps from a patient who developed distant metastasis are shown in **Figure 1**. In general, the metastatic lymph node volume was greater in patients with distant metastases (mean ± standard error [SE]= 89.31 ± 27.5mm3) compared to those exhibiting no distant metastases (68.53 ± 9.4mm3), although these differences were not significant (p>0.05).

No significant differences in ADC (1.06 ± 0.10 x 10-3mm2/s vs. 1.02 ± 0.04 x 10-3mm2/s, p>0.05) were observed between patients with and without distant metastases. Additionally, trends of lower Ktrans (0.51 ± 0.15min-1 vs 0.60 ± 0.05min-1) and τi (0.13 ± 0.03s vs 0.19 ± 0.02s) were observed in patients with distant metastases. However, none of these parameters were significantly different between the two groups (p > 0.05). **Figure 2** shows the distributions of ADC, Ktrans, τi and nodal volume, from metastatic lymph node between two groups of patients.

**Notably, a greater number of HPV positive patients (n=18 vs. 3) were associated with an absence of distant metastases. However, no significant difference was observed between patients with and without distant metastases on the basis of HPV status (p=0.16). Similarly, differentiation grading and nodal staging did not differ significantly (p>0.05) between the two groups of patients.**

R2.3

When ROC analyses were performed, τi was noted to be the single best predictor for distinguishing patients with and without distant metastases with an AUC of 0.66, a sensitivity of 67% and a specificity of 61.4% at a threshold value of 0.12s (**Fig. 3**). A summary of sensitivity and specificity for all individual continuous parameters in distinguishing patients with and without distant metastases is presented in **Table 2**. **When all continuous (volume, ADC, Ktrans, and τi) and categorical variables (HPV status, differentiation grades and nodal stages) were incorporated in multivariate logistic regression analyses, none of the variables was selected in a backward stepwise method and ROC analyses revealed no improvement in the overall prognostic performance for combination of parameters.**

R2.3

**When Mann-Whitney *U* tests were performed to ascertain the differences in MRI parameters based on HPV status, a trend towards higher τi was observed in HPV positive patients than thosewith HPV negative (0.20 ± 0.05s vs. 0.11 ± 0.04s, p=0.06). Moreover, higher Ktrans (0.54 ± 0.09min-1 vs. 0.43 ± 0.07min-1) and lower ADC (1.06 ± 0.06 x 10-3mm2/s vs. 1.10 ± 0.15 x 10-3mm2/s) were found in HPV positive compared to HPV negative patients****. However, these parameters (Ktrans andADC) were not significantly different (p>0.05) between these two sub-groups of patients. The distributions of physiologic MRI parameters between HPV positive and HPV negative patients are shown in Figure 4.**

R2.2

**A trend towards higher Ktrans (0.67 ± 0.07min-1 vs. 0.46 ± 0.07 min-1, p=0.08) was also observed in poorly differentiated HNSCCs than those with moderately differentiated HNSCCs (Figure 4). However, there were no significant differences (p>0.05) in parameters (τi and ADC) between these two sub-groups of patients. Similarly, as shown in Figure 4, no significant differences (p>0.05) in MRI parameters were obtained on the basis of nodal stage.**

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**Discussion**

In this study, we evaluated the prognostic utility of pretreatment DWI and DCE-MRI from metastatic lymph nodes in predicting distant metastases in HNSCC. **Additionally, we also assessed the potential utility of HPV status, differentiation grading, and nodal staging in predicting distant metastases in HNSCC.** Among all variables studied, DCE-MRI derived τi was the best parameter in predicting distant metastases with a sensitivity of 67% and a specificity of 61.4%. While some studies11-14 have reported significant association of elevated FDG uptake from neck lymph nodes with the development of distant metastases in HNSCC patients, some other studies15,16 found no significant differences in FDG-uptake between patients with and without distant metastases. Moreover, there has been a growing concern over the radiation exposure of patients from undergoing FDG-PET. In view of these shortcomings, clinical use of FDG-PET in HNSCC patients for predicting distant metastases may not be tenable. On the other hand, physiologic MR techniques such as DCE-MRI may be more appropriate for its use in predicting prognosis in HNSCC patients given low cost, less complexity of operation and reduced patient discomfort compared with FDG-PET.

R2.3

HNSCC is considered as a systemic disease and hematogenous dissemination of neoplastic cells can occur in the regional neck lymph nodes or to a distant site. The incidence of distant metastasis in HNSCC is low in comparison to other types of cancers and is variable8-11 depending on the location of the primary tumor, TN staging, and rate of loco-regional failure. In the present study, only 12/56 (21.4%) patients developed distant metastases during a follow-up period of 14 years. The low incidence rate of distant metastases in our cohort of patients may be attributed to relatively high success rate of loco-regional control as reported previously by our group.18,19,21 While there has been some success in achieving better loco-regional control by other groups also,34-36 effective control of distant metastases remains elusive.37 At the time of initial diagnosis, it is particularly important to identify and stratify the subgroup of patients who are at a greater risk of developing distant metastases so that those patients can be offered alternative/aggressive therapies. Using concurrent cetuximab, cisplatin, and concomitant boost radiotherapy, a pilot phase II trial reported a reduction in the occurrence of distant metastases.38 Some other studies39,40 have also demonstrated that patients receiving induction chemotherapy followed by CRT had lower propensity to develop distant metastases compared to those receiving CRT alone. Using a treatment regimen comprising of induction therapy with chemo and/or check point inhibitors, a recent ongoing clinical trial41 has shown promising initial results in controlling the dissemination of distant metastases in HNSCC, thus further raising the hope that incident rate of developing distant metastases in these patients may be substantially controlled if treated appropriately at an early stage. Since only a subset of HNSCC patients will eventually develop distant metastasis, it is not feasible or ethical to subject all HSNCC **patients to these** expensive therapeutic regimens. Hence, availability of imaging methods, that can non-invasively identify the patients likely to develop distant metastasis will be highly beneficial.

R1.3

In the present study, patients who developed distant metastases exhibited lower pretreatment Ktrans, and τi from the metastatic lymph node compared to those in whom distant metastases did not occur. Higher pretreatment Ktrans has been reported to predict treatment response to CRT,19,21,23 induction therapy,26 and overall survival20,22,27 in patients with HNSCC indicating that higher pretreatment Ktrans is associated with better prognosis. Our results are in line with these prior findings as patients with higher pretreatment Ktrans also did not develop distant metastases. **As Ktrans reflects a combination of tumor perfusion and microvascular permeability,27 our results and those of earlier published reports19-22,26,27 support the notion that tumor vascularity may be an important predictor of disease control in HNSCC. Along with tumor perfusion, hypoxia in tumor tissues is also considered as an important prognostic indicator of response to CRT in HNSCC.42 While some studies43,44 have reported an inverse relationship between DCE-MRI derived Ktrans and 18F-fluoromisonidazole (18F-FMISO)- positron emission tomography (PET) derived standard uptake value (SUV) indicating that hypoxic nodes are poorly perfused compared to non-hypoxic nodes, the relationship between tumor perfusion and hypoxia is generally more complicated. It has also been demonstrated that hypoxic nodes are associated with a more asymmetric distribution of Ktrans than nodes without hypoxia indicating heterogeneous regions in HNSCCs are characterized by various perfusion-hypoxia relationships.43 These regions may be associated with high perfusion with very little visible hypoxia, or with poor perfusion with high levels of hypoxia, and even with good tumor perfusion along with hypoxia.44 An existence30,31 of a direct positive relationship between heterogeneity measures of Ktrans andHIF-1α factor also provides an evidence for the hypothesis that tumors with high perfusion can be associated with functionally hypoxic regions. It has also been reported45 that head and neck cancer patients harboring high perfusion-hypoxic tumor regions are sensitive to show improved clinical outcomes secondary to developing a successful radiation therapy-induced reoxygenated tumor microenvironment.**

R2.5

R2.7

The usefulness of τi as a prognostic imaging biomarker in cancers of different anatomical sites has been reported.46-51 In a recent study,22 we reported that HNSCC patients exhibiting higher baseline τi had better overall survival than patients with lower τi. Similar to those findings, we observed in the present study that patients with higher τi were less frequently associated with the development of distant metastases. Koch *et al.*52 reported an inverse correlation between τi and hypoxia, suggesting that tumors with low τi are likely to be hypoxic and hence more aggressive, resistant to CRT with poor survival and even a likelihood of demonstrating distant metastases, as noted in our study. An earlier study53 reported that tumors with higher blood flow and reduced hypoxia are related to increased oxygenation levels and that these tumors were radiosensitive and accessible to chemotherapeutic agents. Collectively, these studies and our findings suggest that tumors with higher τi may harbor a favorable microenvironment for CRT to exert its optimal therapeutic effects, thereby reducing the likelihood of developing distant metastases.

**DWI derived ADC parameter depends upon the nature of cellular packing, presence of intracellular organelles, cell membranes, and macromolecules in various tissue compartments.54 Variation in ADC values also reflects the alteration and redistribution of water molecules between intracellular and extracellular compartments of a tissue.54 Several previous studies18, 21, 23-25 have successfully demonstrated the potential of ADC as a prognostic imaging parameter in HNSCC. These studies have reported that tumors harboring higher pretreatment ADC values may be associated with higher degree of micronecrosis and, consequently, of increased resistance to treatment and poor treatment outcomes. Despite these encouraging findings, usefulness of ADC in predicting distant metastases in HNSCC patients has not been widely reported. In a recent study,55 no significant difference in ADC was reported between patients with distant metastasis and those without it. The data analysis in the present study also did not reveal any significant difference in ADC between the two groups of patients. However, a lower pretreatment ADC was observed from metastatic lymph nodes in patients who did not develop distant metastasis in comparison to those who developed distant metastases further highlighting the fact that tumors harboring lower pretreatment ADC may be associated with better prognosis. Histologically, HNSCCs are very heterogeneous in nature with marked variations in various tissue components such as cellular density, nuclear pleomorphism, micronecrosis, microhemorrhages and keratinization.56 We believe that a nonsignificant difference in ADC values between the two groups of patients in our study might be because of high intratumor heterogeneity.** **The investigators of previously mentioned study55 found moderate sensitivities (range: 75%-78%) and specificities (range: 68%-76%) for slow diffusion coefficient and diffusion kurtosis parameters in predicting distant metastases in HNSCC. Taken together, these findings suggest that diffusion MR imaging techniques may have a limited role in stratifying high-risk patients at an early stage who may develop distant metastases in the future.**

R2.8

R2.3

**HPV/p16 gene is known as a key etiological factor in HNSCC with distinct epidemiologic, clinical and molecular characteristics.57 Patients harboring p16-gene are generally more sensitive to CRT and are associated with improved prognosis and prolonged survival.58,59Congruently, we also observed significantly longer survival for HPV positive patients in comparison to HPV negative patients in our prior study.22 Nevertheless, development of distant metastases in HPV positive patients has also been recognized. This important observation indicates that HPV positive patients may not always be associated with good clinical outcomes.60 In the present study, a greater number of patients harboring positive p16-expression were associated with negative development of distant metastases. However, no significant difference in HPV status was observed between patients who developed and who did not develop distant metastases. Despite non-significant finding, a trend towards better prognosis for p16-positive patients as observed in the present study emphasizes the importance of HPV status in predicting occurrence of distant metastases in HNSCC.**

**Differentiation grading system has been proposed as an important pathological prognostic factor in HNSCC. While a few studies61,62 have reported that poorly differentiated tumors are associated with higher recurrence rates and a shorter survival compared to well and moderately differentiated tumors, some other studies63,64 have found that grading system may not confer a prognostic benefit for determining the disease outcomes in HNSCC patients. We also did not find significant difference in grading system on prediction of distant metastases. Taken together, our result and earlier published findings63,64 provide a notion that differentiation grading system may not be a reliable independent prognostic biomarker in predicting the status of distant metastases in HNSCC.**

R2.3

**In the present study, we also sought to determine the differences in physiologic MRI parameters between HPV positive and negative patients, differentiation grades and nodal stages. We believe that investigation of differences in these biologic behaviors as determined by the use of various imaging parameters will help us in better understanding the tumor microenvironment of HNSCC. When such data analyses were performed, we found a trend towards higher τi in HPV positive patients compared to HPV negative patients. Additionally,we also observed non-significantly higherKtrans in HPVpositivepatients incomparison to HPV negative patients. While an earlier study,28 did not find significant difference in Ktrans between two groups of patients based on HPV status,another study65 reported significantly greater percentage of pixels of higher Ktrans in HPV positive group than those in HPV negative group. These results are consistent with the hypothesis that HPV positive tumors are associated with elevated blood flow and increased vascular permeability. This hemodynamic behavior of HPV positive HNSCCs may be attributed to the presence of high vascular endothelial growth factor (VEGF, one of the potent proangiogenic factors) mRNA levels in HPV positive tumors.66 It is widely accepted that VEGF promotes tumor angiogenesis leading to improvement in blood flow within the tumor bed besides inducing vascular permeability.67 Importantly, we also observed a trend towards higher Ktrans inpoorly differentiated HNSCCs compared to moderately differentiated tumors in the present study. In an earlier study,68 significantly higher Ktrans was found from poorly differentiatedHNSCCs than from well-differentiated HNSCCs. However, it should be noted that morphological characteristics of well-differentiated HNSCCs generally remain indistinguishable from that of normal tissues on histopathology.69 Elevated levels of Ktrans from poorly differentiated HNSCCs compared to moderately or well-differentiated HNSCCs may be ascribed to the existence of more immature endothelial cells along with inadequate number of pericytes and smooth muscle fibers in poorly differentiated HNSCCs.70 Collectively, these histopathological characteristics may confer a greater degree of vascular permeability in poorly differentiated HNSCCs.**

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Our study was limited by the relatively small sample size as we had to exclude 16 patients because of reasons mentioned in the methods section. As **the** majority of our patients responded positively to CRT with good loco-regional control,15,16,18 most of these patients did not develop distant metastases. This fact may introduce some unintentional bias in terms of unequal distribution of patients in the two groups (with and without distant metastases) in the present study. However, it should be noted that the relative proportion of patients with distant metastases in our study is in line with reported incidence rates of distant metastases in HNSCC.5-7 Another limitation of the study was that we analyzed imaging data only from metastatic neck lymph nodes. We realize that analyzing imaging parameters from primary tumors would also be important; however, artifacts induced by physiological motion precluded us from analyzing primary tumors in the present study. **We did not have information about the status of p16-expression from some patients, as these individuals were enrolled between the years 2004-2009 when HPV status was not determined routinely at our institution. Another limitation of our study was that we could not evaluate the relationships among MRI parameters and molecular markers such as Ki-67, HIF-1α, and VEGF because of non-availability of immunohistochemical data from these biomarkers.**

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In conclusion, our findings indicate that DCE-MRI derived parameters (Ktrans, and τi)may be helpful in predicting the development of distant metastases in HNSCC patients. Future studies on a larger patient cohort are required to validate our initial findings. **Future studies will also be useful to investigate the potential utility of a radiomics based prediction model by incorporating both structural and physiologic MRI parameters in predicting distant metastases in HNSCC.**

R1.5

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R2.10

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**Legends**

**Figure 1**

Representative images from a patient who developed distant metastases after the end of CRT. Axial T2-weighted image demonstrates a heterogeneous hyperintense metastatic left level IIb lymph node (arrow). It appears hypointense on co-registered T1 weighted image with heterogeneous enhancement on post-contrast T1-weighted image. DCE-MRI-derived τi, and Ktrans maps overlaid on post-contrast T1-weighted images demonstrating low τi, (0.025s) and Ktrans (0.14min-1) from the node.

**Figure 2.**

Box-and-whisker plots demonstrating the distributions of ADC (x10-3mm2/s), Ktrans (min-1), τi, (s) and volume (mm3) from metastatic neck nodes of HNSCC patients who were positive for distant metastases (white, n=12) and negative for distant metastases (shaded, n=44). The bottom and top edges of boxes represent the 25th percentile, and the 75th percentile values. The bands within the boxes represents 50th percentile (median) values. Whiskers display the range of data distribution. Outliers are marked with open circles (values 1.5 box length from the 75th/25th percentiles).

**Figure 3**

Receiver operating characteristic (ROC) curves for ADC, Ktrans, τi, and nodal volume in distinguishing patients who developed distant metastases from those who did not. Using univariate logistic regression model, τi, provided the best area under the ROC curve (AUC) of 0.66 in discriminating two groups of patients with a sensitivity of 67% and a specificity of 61.4% with a threshold value of 0.12s.

**Figure 4.**

**Box-and-whisker plots demonstrating the distributions of ADC (x10-3mm2/s), Ktrans (min-1), and τi, (s) from metastatic neck nodes of patients who were HPV positive (n=21) and negative (n=11, top row); harbored moderately differentiated (n=18) and poorly differentiated (n=34) HNSCCs (middle row) and had N2 (n=48) and N3 (n=6) nodal stages (bottom row). The bottom and top edges of boxes represent the 25th percentile, and the 75th percentile values. The bands within the boxes represents 50th percentile (median) values. Whiskers display the range of data distribution. Outliers are marked with open circles (values 1.5 box length from the 75th/25th percentiles). A trend (p=0.06) towards higher τi was observed in HPV positive patients than in HPV negative patients. Similarly, a trend (p=0.08) towards higher Ktranswas observed in poorly differentiated patients than thosewith moderately differentiated patients.**

R2.9

**Table 1: Clinical characteristics of patients at the time of recruitment**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| | **Characteristics** | | --- |  |  | | --- | | | |
| **Number of patients** | 56 | |
| Mean-age | 61.12 ± 8.74years | |
| Gender | | |
| Male | 46 | |
| Female | 10 | |
| **Primary Tumor site** | | |
| Base of tongue | | 22 |
| Tonsil | | 14 |
| Larynx | | 6 |
| Less common/unknown sites | | 14 |
| **T staging** | |  |
| Tx | | 12 |
| T0 | | 2 |
| T1 | | 2 |
| T2 | | 13 |
| T3 | | 9 |
| T4 | | 18 |
| **N staging** | |  |
| N1 | | 2 |
| N2 | | 48 |
| N3 | | 6 |
| **M staging** | |  |
| M0 | | 56 |
| **P16-expression** | | |
| **Positive** | | **21** |
| **Negative** | | **11** |
| **Unknown** | | **24** |
| **Differentiation Grading** | |  |
| **Moderately differentiated** | | **18** |
| **Poorly differentiated** | | **34** |
| **Undifferentiated** | | **4** |

R2.2.

**Table 2: Area under receiver operating curves (AUC) and associated sensitivity and specificity of nodal volume, DWI and DCE-MRI parameters using logistic regression analyses.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter (Region)** | **AUC** | **Threshold Value** | **Sensitivity (%)** | **Specificity (%)** |
| ADC | 0.57 | 1.05×10-3mm2/s | 58.3 | 54.5 |
| Ktrans | 0.63 | 0.31min-1 | 50.0 | 61.4 |
| τi | 0.66 | 0.12s | 67.0 | 61.4 |
| Nodal Volume | 0.55 | 58.8mm3 | 75.0 | 50.0 |