Blood Flow Change Quantification in Cervical Cancer before and during Radiation Therapy Using Perfusion CT

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The purpose of this study was to quantify the changes of tumor blood flow (BF) in cervical cancer after radiation therapy by using perfusion computed tomography (CT), and to examine the difference between maximum slope (MS) and single-input one-compartment model (SOCM) methods. Fourteen consecutive patients who received definitive radiation therapy for cervical cancer from October 2009 to February 2010 were enrolled in this study. Blood flow (BF) analyses were performed using both MS and SOCM methods. Quantitative BF maps were created using Body Perfusion (Toshiba Medical Systems, Co. Tokyo, Japan). Perfusion color maps were successfully created by the two analytical methods. BF of the tumors was clearly higher than that of normal cervix, making it possible to distinguish tumor tissue from normal cervical tissue. BF of the tumors after 20 Gy of radiation therapy calculated by the MS method was significantly larger than that before treatment (126.9 vs. 72.2 ml/min/100 ml, median; p < 0.05). Although BF calculated by the MS and SOCM methods showed a positive linear correlation (p < 0.001, r = 0.981), BF calculated by the MS method was lower than that obtained by the SOCM method (103.7 vs. 115.1 ml/min/100 ml, p < 0.01). The change of tumor BF in cervical cancer before and after radiation therapy can be monitored by conducting blood flow analysis using perfusion CT. BF by the MS method was lower than that by the SOCM method, but the two analytical methods correlated well. Perfusion CT may have potential in noninvasive monitoring of vascular and oxygenation status and for guiding adaptive therapy.

INTRODUCTION

Radiation therapy is one of the most effective treatment modalities for cervical cancer, and tumor oxygenation status has been implicated as a critical factor contributing to tumor control and the curability of cervical cancer.1-6 The local blood flow (BF) influences the oxygenation status of the tumor cells and might affect treatment outcome, so some noninvasive methods to evaluate the tumor perfusion in clinical practice have been proposed.7,8 These reported studies suggest that tumor BF changes assessed by magnetic resonance imaging (MRI) during radiation therapy correlate well with treatment outcome.

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Tumor angiogenesis has become one of the most important themes in oncology. Angiogenesis is an essential process for tumor growth and metastasis, and increasing number of vascular-targeting drugs have been proposed in the treatment of cancer patients. Assessment of tumor microcirculation can provide useful information for monitoring radiation therapy. Angiogenesis is evaluated by microvessel density (MVD) in histopathological studies,5 but the limitation of MVD in clinical use is that it requires the invasive procedure of biopsy, and that it may not reflect the in vivo tumor BF. Therefore, the demand for non-invasive methods of assessing tumor vascularity is increasing.

Due to the recent progress in imaging techniques, computed tomography (CT) has applications not only for anatomical information, but also for functional information such as BF. In reported studies, MRI has been used for evaluating tumor perfusion, but quantitative evaluation with this technique is difficult. Tumor perfusion analysis with dynamic CT can quantify tumor BF, since the concentration of the contrast medium has a linear correlation with the increase in CT number.
Several analytical methods have been proposed to demonstrate BF using perfusion CT, but the standard analytical methods of perfusion CT have not been applied to cervical cancer. The maximum-slope (MS) method of deriving perfusion measurement has been proposed by Miles et al. 9–12 The principle of the MS method is very simple. However, in this method, venous outflow is not taken into account, and high-dose rate bolus injection of contrast material is essential for accurately quantifying the perfusion.

The single-input one-compartment model (SOCM) method has been derived from the classical pharmacokinetic model.13 Materne et al. extended this model to the dual-input one-compartment model (DOCM) method for quantification of liver perfusion.14 With the DOCM method, two inflow vessels are assumed, i.e. hepatic artery and portal vein, while the SOCM method is based on one inflow model. The DOCM method requires a complicated mathematical algorithm to analyze the two input systems, but the SOCM method is simpler and easier to calculate because it has only one input function. The outstanding characteristics of the SOCM and DOCM methods are the consideration of venous outflow and not requiring high-dose rate bolus injection.14–16

The purpose of this study was to quantify the changes of tumor BF in cervical cancer after radiation therapy by using perfusion CT, and to examine the difference between the MS and SOCM methods.

**MATERIALS AND METHODS**

**Patients**

The institutional review board of our hospital approved this interventional study, and written informed consent was obtained from each patient. Between October 2009 and April 2010, fourteen consecutive patients who met the inclusion criteria and agreed to participate in the study were enrolled. Inclusion and exclusion criteria are shown in Fig. 1. Histological conformation was required in all patients that were clinically staged as International Federation of Gynecology and Obstetrics (FIGO) classification IB to IV A. External beam radiation therapy was delivered to the pelvis through anterior and posterior parallel-opposed portals using 10-MV X-rays, with a dose of 2 Gy per fraction, 5 times per week. All patients underwent baseline perfusion CT within 1 week before the beginning of therapy, and followed up with a second perfusion CT study within 1 week after 20 Gy of irradiation.

**Imaging protocol**

All CT images were obtained using a 64 multidetector-row CT (Aquilion 64; Toshiba Medical Systems Co., Tochigi, Japan). The CT parameters and scanning protocol are summarized in Table 1. In perfusion CT, 370 mgI/kg of iopamidol (Iopamiron 370 Inj. Syringe, Bayer Yakuhin, Ltd. Tokyo, Japan) was injected via an 18G needle inserted in the medial cubital vein at the rate of 5.0 ml/s by using an automatic injector (Dual shot; Nemoto Kyorindo Co., Ltd. Tokyo, Japan). Static dynamic CT scanning was initiated simultaneously to contrast medium injection. Image data were obtained every 2 s from the beginning of contrast material administration up to 50 s, and then every 7 s from 50 to 120 s. The patients breathed freely during the scan time. CT images were transferred to the prototype workstation (Toshiba Medical Systems Co., Tochigi, Japan) by DICOM protocol.

**Maximum-slope (MS) method**

The MS method hypothesizes that there is no venous outflow. Under this condition, the CT value of the target tissue increases in proportion to the volume of the transferred contrast medium. On the basis of Fick’s principle, tissue concentration of the contrast medium: Ct(t) corresponds to the value of tissue BF multiplied by the arterial concentration of the contrast medium: Ca(t). Time attenuation curve (TAC) of...
artery and tissue are shown in Fig. 2. Thus, tissue BF is calculated as follows:

$$\text{tissue BF} = \frac{\text{maximum } \frac{dC_t(t)}{dt}}{\text{maximum } \frac{dC_a(t)}{dt}}$$

**Single-input one-compartment model (SOCM)**

The tissue compartment model is shown in Fig. 3. The CM method is summarized by the following equation:

$$\frac{dC_t(t)}{dt} = k_a \cdot C_a(t) - k_v \cdot C_t(t)$$

Ct and Ca represent the concentrations of contrast medium at time t within the tissue and the artery, respectively. The concentrations of contrast medium reflect the CT value derived from TAC of the tissue and artery. The ka and kv indicate arterial inflow and venous outflow constant, respectively. The delay parameter $\tau_a$ represents the transit time from the artery to the tissue. The value of $\tau_a$ is defined as the delay time between the beginning of arterial and tissue enhancement. The value of ka was calculated using the least squares method. The values of ka and kv were converted to the perfusion units by multiplying by 60 s/min and by 100 ml (of blood)/ml (of tissue). As shown in this equation, SOCM method does not need to assume no venous outflow.

**Blood flow measurement**

Perfusion CT analyses were performed in eleven of the 14 patients. Patient characteristics are shown on Table 2. Three cases were excluded from the analyses because of a technical error in contrast media injection, an error in the imaging position, and a tumor size insufficient for selecting a ROI.

In both analytical methods, the external iliac artery was selected as the input function Ca(t). The mean CT value over the maximum section of the tumor was shown as the function C(t). Both parameters were shown as artery and tissue TAC: the green and red line, respectively.
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the maximum section of the tumor was shown as the function \( C(t) \). Both parameters were shown as arterial and tissue TACs. BF was calculated by both methods based on these TACs. In the MS method, the peak gradients of the arterial TAC were selected manually. In the SOCM method, the value of \( \tau_a \) is defined as the delay time between the beginning of artery and tumor enhancement on both TACs.

The CT images were compressed to \( 256 \times 256 \) matrices, and the BFs were calculated pixel by pixel using the two analytical methods (MS and SOCM). After generation of color maps, BF was measured in the slice containing the maximum transverse section of the tumor in the uterine cervix. The regions of interest (ROIs) were contoured to include as much tumor as possible, without including regions directly adjacent to vessels and other organs. The same ROIs were used for both the MS and SOCM methods. We calculated mean BF within the ROIs and investigated the correlation. All measurements were done by a single radiologist (K. S.).

**Statistical analyses**

All statistical analyses were performed using SPSS statistics software (Version 17.0; SPSS Inc., Chicago, IL). Numerical variables are expressed as median (inter-quartile range: IQR). Shapiro-Wilk test was used to test the hypothesis that a given sample was from a normally distributed population. The Wilcoxon signed rank test was used as a non-parametric test for assessing the significance between the two analytical methods. \( p < 0.05 \) was considered statistically significant. The correlation coefficient (R) was calculated using the Spearman’s coefficient correlation method and linear regression.

**RESULTS**

**Blood flow changes of cervical cancer before and after radiation therapy**

Perfusion color maps for the remaining 11 patients were successfully created by the two analytical methods from dynamic CT data. BF of the tumors was obviously higher than that of normal cervix, making it possible to distinguish tumor tissue from normal cervical tissue. The heterogeneity of BF in tumor tissues was clearly visualized by perfusion CT, as shown in Fig. 4.

Baseline BFs were 72.2 (median; IQR 46.7–132.2) ml/min/100 ml by the MS method and 103.0 (median; IQR 50.9–129.5) ml/min/100 ml by the SOCM method. The BF of the tumors after 20 Gy of radiation therapy calculated by the MS method was significantly larger than that before treatment (126.9 vs. 72.2 ml/min/100 ml, median; \( p = 0.016 \)). However, the difference between tumor BF before and after 20 Gy of radiation therapy calculated by the

**Fig. 4.** A 48-year-old female with FIGO Ib2 uterine cervical cancer. Images in each row are from each evaluation point. (a, b, c: before radiation therapy d, e, f: after 20 Gy irradiation). (a, h) Contrast enhanced CT (CECT) images show the mass in the uterine cervix. The BFs were measured in the slice containing the maximum transverse section of the tumor. The regions of interest (ROIs) were contoured to include as much tumor as possible, without including regions directly adjacent to vessels and other organs. (b, c) The mean BF calculated by the MS method before and after 20 Gy irradiation are 38.4 and 152.1 ml/min/100 ml, respectively. (c, d) The mean BFs calculated by the SOCM method before and after 20 Gy irradiation are 57.7 and 240.5 ml/min/100 ml, respectively. The same ROIs were used for both the MS and SOCM methods.
The initial treatment response at the end of radiation therapy consisted of complete response (CR: n = 9) and partial response (PR: n = 2) by Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1). Significant difference was not observed between CR and PR groups in tumor BFs or BF changes during radiation therapy. Local control was accomplished in all cases during the follow-up period (505 days median; range 62–687), and local recurrence was absent.

**Comparison of the maximum slope method and single-input one-compartment model method**

The results of the BF of the tumor evaluated by the two analytical methods are summarized in Table 3. In 22 analyses of 11 patients, the BF values as calculated by the MS method were lower than that obtained by the SOCM method (103.7 vs. 115.1 ml/min/100 ml, median; p = 0.002) (Table 3). BF values calculated by the MS and SOCM methods showed a positive linear correlation (p < 0.001, R = 0.981) (Fig. 6).
DISCUSSION

In this study, BF in the tumor tissues were successfully visualized on perfusion CT using both analytical methods, and could be expressed as numerical values. The BF of the tumors were clearly higher than that of normal uterine tissue, and the tumor and normal tissues were easily distinguished. It is well known that intratumoral oxygenation is improved by fractionated irradiation, since the treatment decreases intratumoral cell density, which in turn increases BF. 17,18 In our study, perfusion CT successfully confirmed increased BF after 20 Gy of radiation therapy. Our results demonstrated that there is individual variation in tumor BF and changes in BF in response to radiation therapy. Two cases of adenocarcinoma showed minimal changes in BF before and after 20 Gy of radiation therapy. Additional studies with a larger number of cases are necessary, but BF changes due to radiation therapy may indeed vary between histologic type. It is well known that radiation response of adenocarcinoma is slower than that of squamous cell carcinoma. It has also been reported that the proliferative compartment as growth fraction of adenocarcinoma did not significantly increase during radiation therapy, whereas that of squamous cell carcinoma increased significantly. 19 Taking these findings into consideration, there may be histological differences in the oxygen effect between adenocarcinoma and squamous cell carcinoma.

Most perfusion studies have been performed on MRI, and there are few studies using CT. Mayr et al. 7 reported in a study which used MRI that pre-therapy high perfusion and pre-therapy low perfusion with subsequent improvement during radiation therapy were associated with better outcome in cervical cancer. The blood vessels are one of the most important targets in radiation therapy, and changes in tumor perfusion as well as oxygenation during irradiation have been revealed experimentally. 20,21 Lyng et al. 22,23 demonstrated a correlation between changes in tumor perfusion and oxygenation during fractionated radiation therapy in patients with cervical cancer by using MRI. Fractionated radiation therapy is thought to improve the blood supply and oxygenation status of the tumor cells by reduction of cell density and modulating the tumor microenvironment, resulting in a substantial reduction of radiation-resistant hypoxic tumor cells. Thus, it is reasonable that tumor perfusion is associated with the effect of radiation on cervical cancer. We can take advantage of these parameters for optimizing treatment if we can predict clinical outcome. To our knowledge, most prior studies have focused on dynamic contrast MRI and there has been only one published study regarding perfusion CT of cervical cancer. 17 The study reported moderate correlation between pretreatment perfusion parameters and partial pressure of oxygen in tumor tissues, but it did not investigate BF changes during treatment. Our study is the first to demonstrate BF changes during treatment by perfusion CT. Dynamic contrast MRI in an attempt to measure tumor perfusion has the advantages of no radiation exposure, minimal invasiveness, and ease of availability, but it has some disadvantages in quantification of tissue BF: the correlation between gadolinium and signal intensity is nonlinear, and obtaining an accurate arterial input function is made difficult by inflow phenomenon and susceptibility. Hence, perfusion CT is regarded to be a more accurate detector of BF than MRI.

Recently, image guided radiotherapy and adaptive brachytherapy have become widely available. 24-26 We can take advantage of perfusion parameters obtained by perfusion CT for optimizing treatment if they can be used to predict the effect of radiation. However, we did not analyze the correlation between BF and clinical endpoints such as treatment response, local control and disease free survival, because of the short follow up period. Perfusion CT could help the future development of individualized approaches in radiation therapy of cervical cancer, providing aggressive treatment for the cases with poor prognosis, and to minimize adverse events for the cases with good-prognosis. The clinical value of perfusion CT and such targeted approaches require additional study.

A few prior studies have suggested that tumor perfusion after 20 Gy irradiation assessed by MRI was a predictor of treatment outcome. 3 Based on these findings, we performed perfusion CT before treatment and after 20 Gy radiation therapy. However, it was impossible to investigate the association between BF and the local control in this study, because local control was accomplished in all cases during the follow up period. The obvious correlation between BF and initial treatment effect was not observed due to the small number of cases. We intend to increase the number of cases and the length of the follow up periods and report the relationship between the perfusion parameters and clinical endpoints following the present study.

To the best of our knowledge, this was the first report comparing different analytical methods in perfusion CT of cervical cancer. In this study, the BF calculated by the MS and SOCM methods showed a highly significant positive linear correlation. The MS method has been widely accepted in perfusion CT of the body, since it is simple, convenient and clinically applicable. However, the MS method requires a high bolus injection of the contrast medium, due to the assumption that venous outflow does not occur before the maximum slope of the tumor TAC. On the other hand, the SOCM method is based on the classical pharmacokinetic model (12), although both MS and SOCM methods are categorized as compartmental analysis. The SOCM method has the advantage of being less influenced by the bolus infusion rate, since it takes venous outflow into account. Moreover, in compare to the DOCM method used in liver perfusion CT based on some pharmacokinetic model, which requires a
complicated mathematical algorithm to analyze the two input systems, the SOCM method is simple and easy to calculate because it has only one input function. The BFs calculated by the SOCM method correlated well with those by the MS method, and it may replace the MS method.

Furthermore, our results suggest that the BF values are lower with the MS method than with the SOCM method. It remains controversial which analytical method reflects BF more accurately. However, when considering the theoretical background, the MS method, which does not hypothesize venous outflow, may underestimate BF. It is clinically difficult to completely fulfill the conditions the MS method requires, even if contrast media is given rapidly. Presence of intratumoral arteriovenous shunting is considered another reason. It is well known that intratumoral vasculature is often functionally and structurally abnormal. Arteriovenous shunts theoretically induce early venous drainage of the contrast medium, and reduce the gradient of the maximum slope.

This study had some limitations. First, we used 370 mgI/ml contrast medium at 5 ml/s in the present study. According to Miles et al., the recommended bolus injection is 40–50 ml contrast medium (300 mgI/ml) at 7 ml/s for the MS method. However, some patients cannot tolerate a bolus injection that high. Second, the version of the software used for this study limited us to measurement of BF only, but perfusion parameters include BF, blood volume, permeability, mean transit time, and so on. These additional parameters might provide better results for analyzing the hemodynamics of tumor microcirculation in detail. Thirdly, our results were not correlated with any gold standards, since there were no other practical methods to evaluate tissue perfusion in vivo. Recently, various analytical models for perfusion CT have been developed, such as some non-compartmental analysis methods: the deconvolution method or the Patlak plot model. These new methods may also help in obtaining information about the reliability of these perfusion analytical methods in cervical cancer.

In conclusion, the changes of tumor BF in cervical cancer before and after radiation therapy could be monitored using perfusion CT by conducting blood flow analysis. BF by the MS method was lower than that by the SOCM method, but the two analytical methods correlated well. It is hoped that the perfusion study protocol can eventually be helpful in providing valuable information for monitoring vascular modulation by radiation therapy or anti-angiogenesis drugs.

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