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CHARACTERIZING GRADIENT ECHO SIGNAL DECAYS IN GYNECOLOGIC CANCERS AT 3T USING A GAUSSIAN AUGMENTATION OF THE MONO-EXPONENTIAL (GAME) MODEL

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Abstract

Purpose—To assess whether R_2^* mapping with a standard Mono-Exponential (ME) or a Gaussian Augmentation of the Mono-Exponential (GAME) decay model better characterizes gradient-echo signal decays in gynecological cancers after external beam radiation therapy at 3T, and evaluate implications of modeling for non-invasive identification of intra-tumoral hypoxia.

Materials and Methods—Multi-gradient-echo signals were acquired on 25 consecutive patients with gynecologic cancers and three healthy participants during inhalation of different oxygen concentrations at 3T. Data were fitted with both ME and GAME models. Models were compared using F-tests in tumors and muscles in patients, muscles, cervix and uterus in healthy participants, and across oxygenation levels.

Results—GAME significantly improved fitting over ME ($P < 0.05$): Improvements with GAME covered 34% of tumor regions-of-interest on average, ranging from 6% (of a vaginal tumor) to 68% (of a cervical tumor) in individual tumors. Improvements with GAME were more prominent in areas that would be assumed hypoxic based on ME alone, reaching 90% as ME R_2^* approached 100 Hz. GRE decay parameters at different oxygenation levels were not significantly different ($P = 0.81$).

Conclusion— R_2^* may prove sensitive to hypoxia, however, inaccurate representations of underlying data may limit the success of quantitative assessments. Although the degree to which R_2 or σ values correlate with hypoxia remains unknown, improved characterization with GAME

increases the potential for determining any correlates of fit parameters with biomarkers, such as oxygenation status.

Keywords

Biophysical modeling; Lorentzian; Gaussian; Transverse relaxation; Blood oxygenation; Gynecologic cancers

INTRODUCTION

Hypoxia (reduced oxygen tension) in tumors is associated with increased resistance to radiation treatment, increased metastatic potential and poor patient outcomes (1–6). Among cervical cancers, up to 48% are hypoxic (7) and these are associated with a 2- to 3-fold increase in mortality compared to non-hypoxic tumors (8). An *in-vivo* indicator of tumor hypoxia may have an impact in planning radiation treatment in gynecologic cancers. Treatment of locally advanced cervical cancer using image-based brachytherapy has been shown to be associated with excellent local control (9), reduced toxicity and improved survival (10). Improved targeting of hypoxic areas of tumors with brachytherapy may further improve treatment response (9).

A convenient means for assessing tumor hypoxia is desirable. Polarographic pO₂ needle electrode measurements (11) are the historical “gold standard”, however, these measurements are invasive, are localized with limited sampling capabilities, and suffer from inter-operator variability (12). The difficulty of repeated measurements and the need for construction of 3-D maps prevent their frequent use in therapy planning.

Methods to non-invasively identify hypoxic tumor regions by quantitatively mapping the MRI parameter R_2^* (effective transverse relaxation rate, $R_2^* = 1/T_2^*$) using multiple gradient echo (GRE) imaging have been investigated (13–15). A Mono-Exponential (ME) signal decay model is typically used for R_2^* quantification, and regions of increased R_2^* are assumed indicative of increased deoxyhemoglobin related to hypoxia. In prostate cancer, R_2^* has shown high sensitivity (88%) but low specificity (36%) at 1.5T for depicting hypoxia relative to immunohistochemistry (13); and some trends and correlations were found between R_2^* and pO₂ electrode oxygen tension measurements (14). In cervical cancer at diagnosis, repeated R_2^* weighted imaging and R_2^* mapping showed changes in signal intensity and R_2^* values in various tissues during inhalation of air or oxygen at 3T, and were suggested for detecting hypoxia in cervical cancer (15). In advanced cervical cancer at 3T, baseline R_2^* was a good predictor of concurrent chemoradiotherapy treatment outcome (progression-free and overall survival) (16), which was attributed to high R_2^* reflecting tumor hypoxia and an aggressive tumor phenotype (with sensitivity of 78% and specificity of 68%, at a cutoff of 24Hz).

Accurate characterization of underlying signals is essential for determining reliable MRI biomarkers, such as markers of oxygenation status or tumor aggressiveness. The standard ME model assumes mono-exponential GRE signal decays which result from purely Lorentzian intra-voxel frequency distributions. However, deviations from Lorentzian

behavior were recently found in the brain, and Gaussian fitting, which corresponds to an assumption of Gaussian intra-voxel frequency distributions, performed better than Lorentzian fitting in regions of prominent reversible relaxation (17). Effective relaxation resulting from reversible and irreversible effects, specifically R_2^* from standard ME decay modelling, has been the main mechanism targeted for identifying intra-tumoral hypoxia from GRE signal decays (13–16). Imprecise representations of underlying data due to such potential deviations from Lorentzian behavior may limit the success of quantitative assessments, especially with increasing susceptibility effects at higher fields.

In this work, we evaluated R_2^* mapping in women with gynecologic cancers after they had received external beam radiation therapy (EBRT) and prior to high-dose-rate (HDR) brachytherapy at 3T; with the purpose of assessing whether GRE signal decays are adequately characterized with a standard ME decay model, or whether they may be better represented with a Gaussian Augmentation of the Mono-Exponential (GAME) decay model.

MATERIALS AND METHODS

Subjects

Twenty-nine women provided written consent and participated in this Institutional Review Board-approved study. One participant was excluded due to an MR technical error (resulting in an acquisition that was not at the scanner isocenter). The remaining twenty-eight included three healthy females (ages: 25 ± 2.5 years, weight: 69 ± 20 kg) and 25 consecutive patients with gynecologic cancers (ages: 55 ± 16 years, weight: 64 ± 19 kg) presenting for high-dose-rate (HDR) brachytherapy following external beam radiation therapy (EBRT).

Seventeen patients had cervical cancer (International Federation of Gynecology stage IIA–IVA (18)), four had vaginal cancer (stages II–III), two had recurrent endometrial cancer at the vaginal cuff (stages IA and IIIB), one had recurrent bladder neck/urethral cancer (stage pT3-4NOMO), and one had primary inoperable uterine cancer with an intact uterus (stage IIIC2).

Data Acquisition

Imaging was performed at 3T (IMRIS Verio, IMRIS, Minnetonka, MN, USA) using a body matrix coil (6 elements) in combination with a spine matrix coil (24 elements total, subset near pelvis used). The MRI protocol included two-dimensional axial, coronal and sagittal T_2 -weighted turbo-spin-echo (T_2 w TSE) imaging with effective echo time (TE)/pulse repetition time (TR) = 102/7740 ms, refocus flip angle = 140° , field of view = 22×22 cm², matrix = 256×320 , in-plane resolution = 0.9×0.7 mm², averages = 2, 30 slices with slice thickness = 4 mm and slice gap = 0.8 mm. Two-dimensional axial, multi-echo spoiled GRE images were acquired at six TE values (3.00, 8.96, 17.92, 26.88, 35.84, and 44.80 ms), TR=187 ms, flip angle = 27° , field of view = 30×30 cm², matrix = 192×192 , in-plane resolution = 1.56×1.56 mm², averages = 2, fat-saturation, slice thickness = 4 mm, and slice gap = 0.8 mm. Scan time was determined by the number of slices necessary to cover the tumor and the maximum breath-hold duration of 36 sec: 12 slices were acquired in 2 min and 24 sec (over 4 breath-holds) or 16–18 slices were acquired in 3 min and 36 sec (over 6 breath-holds). In all cases, shimming was performed in a sub-volume covering the residual

tumor and surrounding tissues to minimize macroscopic magnetic field inhomogeneities. Placement of brachytherapy applicators (needles or tandem and ovoid) was performed under MRI-guidance using axial and sagittal T2-weighted **TSE** imaging with TE/TR = 104/4740 ms, flip angle = 120°, field of view = 28 × 22.4 cm², matrix = 320 × 205, in-plane resolution = 0.9 × 1.1 mm², averages = 1, 36 slices with slice thickness = 3 mm and no slice gap. Brachytherapy applicator placement was followed by three-dimensional T₂w imaging (Space, with TE/TR = 177/3990 ms, flip angle = 120°, field of view = 19.5 × 30 cm², matrix = 207 × 320, in-plane resolution = 0.94 × 0.94 mm², averages = 2, and 120 contiguous slices with slice thickness = 1.6 mm) for contouring of tumor and surrounding organs and by CT imaging for applicator localization, leading to the final radiation treatment plan (19).

Oxygenation

Data were acquired with healthy participants wearing non-rebreather masks, and patients under general anesthesia and intubated for brachytherapy while breathing a mixture of oxygen and medical air. The protocols are summarized in Figure 1. **Protocol N-P** (Normal-Patients): Five patients were imaged without any modifications to the oxygenation level; T₂w and GRE images were acquired at “normal O₂” (an oxygenation level that was not modified from the norm for this procedure, Figure 1a). **Protocol HL-P** (High-Low-Patients): Eighteen patients were imaged at two inhaled O₂ levels; the inhaled O₂ level was set to 100% at the beginning of the study, followed by T₂w imaging, and a single GRE acquisition at this high oxygenation level; the inhaled O₂ level was then lowered (to approximately 36% O₂ through a blend of 20% O₂ and 80% medical air, “low O₂”), followed by another GRE acquisition at this lower oxygenation level (Figure 1b). **Protocol HIL-P** (High-Intermediate-Low-Patients): For two patients the inhaled O₂ level was set to 100% at the beginning of the study, followed by T₂w imaging, and two GRE acquisitions at this high oxygenation level; the inhaled O₂ level was then lowered (to “low O₂”, as defined above), followed by four more GRE acquisitions while the oxygenation level was decreasing (Figure 1c). All patient protocols continued with MR-guided brachytherapy applicator placement at “normal O₂”; followed by high resolution imaging and radiation treatment planning. **Protocol HIL-H** (High-Intermediate-Low-Healthy): For the three healthy participants, the inhaled O₂ level was set to 100% at the beginning of the study, followed by T₂w imaging, and three GRE acquisitions at this high oxygenation level; the inhaled gas was then switched to air (21% O₂) followed by four additional GRE acquisitions while the oxygenation level was decreasing (Figure 1d).

Models and Fitting

GRE signals (S) are typically assumed to follow a standard Mono-Exponential (ME) signal decay model with TE :

$$S_{ME} = \rho \exp(-R_2^* TE) \quad \text{Eq. [1]}$$

where ρ is the pseudo-spin density, $R_2^* = R_2 + R_2'$ the apparent transverse relaxation rate, R_2 the irreversible transverse relaxation rate, and R_2' the reversible transverse relaxation rate

corresponding to the half-width-at-half-maximum (*HWHM*) of a Lorentzian intra-voxel frequency distribution (20). On the other hand, if intra-voxel frequency distributions are better characterized with Gaussian rather than Lorentzian functions, *S* vs. *TE* would follow a Gaussian Augmentation of the Mono-Exponential (GAME) decay model instead:

$$S_{\text{GAME}} = \rho \exp(-R_2 TE) \exp(-(\sigma TE)^2/2) \quad \text{Eq. [2]}$$

where the σ is the Gaussian *HWHM* / $\sqrt{2 \log 2}$ (17).

GRE signals were fitted to these two models using Matlab (Mathworks, Natick, MA, USA) as follows: the noise level was estimated from regions of signal void. For each voxel: Data were averaged over an in-plane neighborhood of 3×3 pixels, and results below noise (e.g. regions of complete GRE signal dephasing and signal loss) were excluded from further analysis. For the ME model, ρ and R_2^* were extracted from fits of straight lines to $\log(S)$ vs. *TE*. For the GAME model, ρ , R_2 and σ were extracted from quadratic fits to $\log(S)$ vs. *TE*. Fitting was performed using regress and lsqin functions, with constraints for $R_2 > 0$ and $\sigma^2 > 0$. Residual sum-of-squared errors (SSEs) were calculated for model comparison.

Model Comparison

GAME and ME models were compared using F-tests, which assess model performance by determining whether the additional GAME parameter σ is warranted. Increasing the number of parameters in a model will result in equal or smaller SSEs. F-tests evaluate whether SSEs are reduced beyond what would simply be expected from an increase in the number of parameters. The null hypothesis is that GAME, a three-parameter fit, does not provide a significantly better fit than ME, a two-parameter fit. This null hypothesis was rejected for the desired probability: i.e. between 0 and 1, that improvement of the fit from the addition of σ is due to chance, based on an F-distribution with $(p_{\text{GAME}} - p_{\text{ME}}, n - p_{\text{GAME}})$ degrees of freedom and F-statistic:

$$F = \frac{(SSE_{\text{ME}} - SSE_{\text{GAME}})/(p_{\text{GAME}} - p_{\text{ME}})}{SSE_{\text{GAME}}/(n - p_{\text{GAME}})} \quad \text{Eq. [3]}$$

where $p_{\text{ME}} = 2$ and $p_{\text{GAME}} = 3$ are the number of parameters of each model, and $n = 6$ is the number of echoes. P values of the F-test were calculated at each voxel. Multiple comparison correction was performed to limit the familywise error rate: minimum cluster size thresholds were determined through Monte Carlo simulations (AlphaSim, afni.nimh.gov, 10,000 iterations), and improvements were considered statistically significant for $P < 0.05$. Bonferroni correction, generally considered overly conservative for multiple comparison correction, since it does not consider cluster size or spatial correlation of neighboring voxels (leading to an increase in false negatives while controlling false positives), was also performed.

Regions of interest (ROIs)

Contouring and registration were performed using Aria (Varian Medical Systems, Palo Alto, California, USA) as follows. ROIs were delineated on high-resolution T₂w images (acquired at the beginning of the study prior to GRE imaging). Tumors in all patients, and the cervix and uterus in all healthy participants were delineated (in Aria) by a radiation oncologist with 15 years of MR experience (ANV). Muscle tissues (gluteus maximus and iliopsoas) were delineated in all participants by a post-doctoral fellow. GRE images were registered to T₂w images and then contours were copied from T₂w to GRE images (in Aria). Contours were visually re-verified on GRE images and modified if necessary (e.g., due to motion) by the radiation oncologist. Aria contours were imported into Matlab for analysis of fitting and F-test results within these ROIs.

ROI-Level Statistics

The physical extents of significant improvements using GAME were calculated for four levels of statistical significance (P value cutoffs = 0.05, 0.01, 0.005, and 0.001, from both clustering and Bonferroni methods) for each ROI (tumors, the cervix, uterus, and muscles) as a percentage of the ROI volume, as:

$$100 \times \frac{(\text{volume of ROI where } (P < P \text{ value cutoff}))}{(\text{volume of ROI})} \quad \text{Eq. [4]}$$

Since previous R_2^* mapping studies using the ME model had assumed that regions of increased R_2^* were indicative of hypoxia, the physical extents of significant improvements using GAME were also calculated as a percentage of each sub-volume identified by ME R_2^* values, as:

$$100 \times \frac{(\text{volume of ROI where } (P < 0.05) \text{ and } (\text{ME } R_2^* > R_2^* \text{ cutoff}))}{(\text{volume of ROI where } (\text{ME } R_2^* > R_2^* \text{ cutoff}))} \quad \text{Eq. [5]}$$

for a single statistical significance level ($P < 0.05$, based on clustering) and a range of ME R_2^* cutoffs (0 through 90Hz).

ROIs were subdivided into two sub-regions according to P -values: where the GAME model significantly improved fitting (ROI_{GAME} : regions where GAME improved fit over ME with $P < 0.05$, based on clustering) versus where the GAME model did not significantly improve fitting and simply reduced to the ME model (ROI_{ME} : regions where GAME did not improve fit over ME with $P < 0.05$, but simply fit equally well). Modelling impact was estimated by comparing parameter values between these fit-quality-based subdivisions (ROI_{GAME} versus ROI_{ME}). Changes in parameter values were calculated relative to the appropriate model for each parameter, i.e. for ME R_2^* as:

$$100 \times \frac{(\text{mean in } ROI_{ME}) - (\text{mean in } ROI_{GAME})}{(\text{mean in } ROI_{ME})} \quad \text{Eq. [6a]}$$

and for GAME R_2 and σ as:

$$100 \times \frac{(\text{mean in } ROI_{GAME}) - (\text{mean in } ROI_{ME})}{(\text{mean in } ROI_{GAME})} \quad \text{Eq. [6b]}$$

Means and standard deviations of GRE decay parameters (R_2^* , R_2 and σ) were calculated in each ROI, at each oxygenation level individually, as well as by combining data across oxygenation levels. Multilinear regression was performed for each parameter in each ROI across oxygenation levels using the `regstats` function in Matlab. Trends were considered significant for the P -value of the t -statistic, $p < 0.05$.

Effect of Field Inhomogeneities

Macroscopic background field inhomogeneity levels were estimated from multi-echo spoiled GRE acquisitions, acquired as described above, but where both magnitude and phase data were saved (consecutive patients, ages: 59 ± 12 years, weight: 63 ± 14 kg, $n=5$ in protocol N-P with one stage IIIC2 primary inoperable uterine cancer with an intact uterus, and four cervical cancers of stages IIB, IIIA, IIIB and IVA; $n=1$ in protocol HL-P with stage IIIB cervical cancer). Field maps were estimated by determining the phase accumulation between the first two echoes using Statistical Parametric Mapping (SPM12 `pm_unwrap` (21), www.fil.ion.ucl.ac.uk/spm), and dividing this unwrapped phase by the TE difference:

$$\text{Fieldmap} = \frac{pm_unwrap(|S_2|(\exp(i\angle(S_2))/\exp(i\angle(S_1))))}{(TE_2 - TE_1)} \quad \text{Eq. [7]}$$

where S_1 and S_2 are the complex signals at TE_1 and TE_2 , respectively. The magnitude of the through-plane field gradient (TPG, Hz/mm) across each slice was estimated as:

$$TPG = \frac{\text{abs}(\text{Fieldmap}_{\text{Superior Slice}} - \text{Fieldmap}_{\text{Inferior Slice}})}{2 \times (\text{Slice thickness} + \text{Slice gap})} \quad \text{Eq. [8]}$$

(excluding any areas of low GRE signal with extensive phase accumulation where the field distributions were unknown).

Linear regression analysis was performed to assess the relationship between TPG and σ , using the `fitlm` function in Matlab, for tumor and muscle ROIs. The degree of improvements with GAME (the physical extents of significant improvements using GAME at $P < 0.05$) and σ values were also assessed at various levels of field inhomogeneities, where TPG levels exceeded certain cut-offs (of 0 through 30 Hz/mm):

$$100 \times \frac{(\text{volume of ROI where } (P < 0.05) \text{ and } (TPG > TPG \text{ cutoff}))}{(\text{volume of ROI where } (TPG > TPG \text{ cutoff}))} \quad \text{Eq. [9]}$$

and

$$\text{mean}(\sigma) \text{ over ROI where } ((P < 0.05) \text{ and } (TPG > TPG \text{ cutoff})) \quad \text{Eq. [10]}$$

RESULTS

Models and Fitting

GAME fit GRE signal decays at least as well as ME, resulting in equal or smaller SSEs everywhere, as expected. Figure 2a shows a sample tumor (cervical, stage IVA), and semi-log plots of signal intensity versus TE in and around the tumor. Data are shown overlaid with ME and GAME fits, with the resulting SSEs and fit parameters given below each plot. The $\log(S)$ time course had significant curvature in many voxels ($P < 0.05$), and GAME fits were superior to ME fits, improving SSEs by over 400-fold to over 3000-fold (Figure 2b–d). When the $\log(S)$ time course was linear, i.e. $\sigma \approx 0$, the GAME fit exactly matched the ME fit (Figure 2e).

Figure 2f shows another sample tumor (cervical, stage IIB) with ROI depictions and the corresponding ME and GAME parameter maps overlaid on anatomical images, with the (log) linear relaxation rate (apparent R_2^* , i.e. ME R_2^*) estimates from ME fits in Figure 2g, (log) linear (i.e. GAME R_2) and non-linear (i.e. GAME σ) relaxation rate contributions identified by GAME fits in Figures 2h and 2i. On the right side of the tumor (white arrows) R_2^* , R_2 and σ were quite small and uniform. In the center of the tumor (gray arrows), most of the high R_2^* values could be attributed to high R_2 , while σ was fairly small. Conversely, on the left anterior and right posterior (black arrows) of the tumor, R_2 was smaller and most of the high R_2^* values could be attributed to higher σ values.

Oxygenation

No significant trends were observed in R_2^* , R_2 or σ values across acquisitions at different oxygenation levels in healthy participants or in patients ($P > 0.05$ in all cases, $P = 0.81$ on average, the strongest trends, i.e. the smallest P values were 0.098 and 0.153 in volunteers and patients, respectively). Data combined across oxygenation levels were used in further processing.

Model Comparison, Significance and Extent

Improvements using the GAME model were statistically significant in large portions of the ROIs ($P < 0.05$). Figures 3a and 3b show quantitative comparison of GAME versus ME models, in the sample tumors of Figure 2. P values indicate the probabilities that improvements using GAME are due to chance, with $P = 0$ indicating certainty that the new

parameter is warranted (pink). Only regions with $P < 0.05$ are overlaid in color, and demonstrate the prevalence of areas where GAME performs significantly better than ME.

The statistical significance and physical extent of improvements with GAME are summarized in Figure 3c for each ROI in patients and healthy participants. Statistically significant improvements using GAME (Eq. [4] with $P < 0.05$, based on clustering) were found in 34% of tumor ROIs on average (37% of patient muscles; 36%, 27% and 25% of healthy-subject cervix, uterus and muscles, respectively). Supporting the robustness of the GAME approach, improvements using GAME persisted at higher levels of significance, covering 24%, 21% and 16% of tumor ROIs, at $P < 0.01$, $P < 0.005$, and even $P < 0.001$ based on clustering, and 13%–12% of tumor ROIs based on Bonferroni correction, respectively. By tumor type, more extensive improvements with GAME were seen in recurrent endometrial tumors (41%), followed by cervical (37%), vaginal (25%), recurrent bladder neck/urethral (18%), and primary inoperable uterine (17%) tumors. The extent of improvements with GAME for individual tumors ranged from as little as 6% (of a vaginal tumor) to as much as 68% (of a cervical tumor) at $P < 0.05$.

The extent of improvements with GAME over ME was higher in areas that would be assumed indicative of hypoxia using the ME model alone. Figure 3d shows the physical extent of improvements with GAME over ME (Eq. [5] with $P < 0.05$) as a percentage of regions identified by ME as having higher R_2^* , i.e. more “hypoxic” according to ME. As the R_2^* found by ME increased, improvements with GAME over ME became more prevalent (R_2^* cutoff > 0 in Figure 3d). GAME performed significantly better in over 50% of tumor ROIs when ME found $R_2^* > \sim 30\text{Hz}$, and up to 90% of ROIs as the ME R_2^* approached 100 Hz ($P < 0.05$).

Model Comparison, Impact on Parameter Values

The effect of modeling differences on resulting parameters was estimated by comparing parameter values between fit-quality-based subdivisions of each ROI: ROI_{GAME} (Eq. [6a]) versus ROI_{ME} (Eq. [6b]). Figure 4 shows average parameters in tumors and muscles of patients. Using an appropriate model for the underlying data resulted in large differences in calculated parameters: ME R_2^* was lower in ROI_{ME} (by 20% in tumors, $P = 0.0022$, and by 7% in muscles, $P = 0.0523$). Similarly, GAME R_2 was lower in ROI_{GAME} (by 25% in tumors, $P = 0.0067$, and by 27% in muscles, $P < 0.00001$). GAME σ was always higher in ROI_{GAME} (by 65% in muscles and by 76% in tumors, $P < 0.00001$). Remaining results are presented from regions where models matched the underlying data, i.e. ME R_2^* values from ROI_{ME} , and GAME R_2 and σ values from ROI_{GAME} . GRE decay parameters for each participant, tissue, and tumor type and the number of participants in each group are summarized in Table 1.

Effect of Field Inhomogeneities

Macroscopic background inhomogeneity levels, estimated from multiple GRE phase data, helped assess whether or not high σ values corresponded with high TPGs. Sample field map estimates and TPGs are shown for two consecutive slices including a tumor in Figure 5. The corresponding GAME σ values are also shown where GAME statistically significantly

improved fitting ($P < 0.05$). GAME σ tended to be large when TPGs were large, however, GAME σ could also be large in the absence of large TPGs, and field-map estimates derived from the same acquisition helped differentiate between these cases. Quantitative analysis of interactions between σ and field inhomogeneity are shown in Figure 6. Scatter plots for each subject are overlaid with regression lines and adjusted coefficients of determination (Figure 6a): Despite shimming over a small volume covering each tumor, most voxels experienced TPGs of up to ~ 15 Hz/mm, with slightly larger TPGs found in tumors relative to muscles. σ had non-zero intercept values in all cases, it tended to increase slightly with TPG in tumors but did not tend to change as much with TPG in muscles. Figure 6b and 6c show the physical extent of statistically significant improvements with GAME over ME and σ values (across all patients with fieldmaps, Eq. [9]), as a function of TPG levels. TPG cutoff = 0 Hz/mm indicates that entire ROIs (tumor or muscle) were included in the analysis, while increasing TPG cutoffs indicate that only parts of tumor or muscle ROIs with TPG levels exceeding these cutoffs were included in the analysis. The extent of improvements with GAME in tumors increased as a function of TPG up to ~ 15 Hz/mm (which covers TPGs experienced by most ROIs, as seen in Figure 6a, then decreased for regions of larger TPG), while the extent of improvements with GAME in muscles was fairly stable across TPG levels. Mean σ increased by around 30% with increasing TPG cutoffs (Figure 6c, Eq. [10]).

DISCUSSION

Intra-tumoral hypoxia is widespread in gynecologic cancers and affects their response to treatment. R_2^* may prove sensitive to hypoxia, however, inaccurate representations of the underlying GRE signal decays may limit the success of quantitative assessments. Here we have demonstrated that GRE signals generally do not obey the simple ME decay model, and a GAME decay model performs substantially better in much of the female pelvis at 3T, as previously also observed in the brain (17).

GAME fits were especially valuable where ME fits returned high R_2^* values, which are regions previously assumed to indicate hypoxia. The ME model appears to assign erroneously large values to R_2^* in the presence of Gaussian decay behavior. Non-exponential signal decay may reflect an interaction between macroscopic gradients (due to air/tissue and bone/tissue interfaces, which lead to higher susceptibility effects at higher field strengths) and actual slice profiles (22) which may be far closer in reality to Gaussian than the ideal, rectangular profiles that are often assumed (23). Indeed, we have observed higher GAME σ in regions with large TPGs. However, this mechanism fails to completely explain the observed non-exponential GRE decays, given that higher GAME σ was also observed in the absence of large TPGs.

Mesoscopic gradients due to susceptibility differences between distinct glandular or stromal subcomponents could also contribute to greater signal curvature: while the Lorentzian line shape has traditionally been assumed to underlie FID signal decay, Gaussian signal modification has been theoretically predicted in the static dephasing regime (24) for objects that could be modeled as having certain shapes and distributions (e.g., spheres and cylinders) in the short-time regime. The static dephasing regime dominates at higher external field strengths, e.g., covering mid-to-large scale vessels at 1.5T, expanding to cover smaller

vasculature at 3T, eventually covering the entire blood vessel network at 4T and beyond. The rather complicated behavior of NMR signal evolution has been demonstrated to potentially deviate from standard exponential decay, showing Gaussian time dependence (25) in the short-time regime for more accurate descriptions of real biological objects as well (e.g., prolate and oblate spheroids), with signal behavior and the region of the short-time regime depending on the shape and distribution of these objects. Empirical evidence of Gaussian behavior was also seen in in vitro human blood samples (26) to a degree dependent on the oxygenation status (26). If such shapes provide reasonable approximations of real biological objects, the current observations might indeed reflect underlying biological phenomena.

The GAME R_2 and ME R_2^* values we observed were generally in good agreement with the prior literature. For example, we found GAME R_2 in muscle of 27–36 Hz as compared to reported values of 20–35 Hz (27–29), and GAME R_2 in the uterus of 16–17 Hz as compared to reported values of 17 Hz in the endometrium and 13 Hz in the myometrium (28). GAME R_2 in the cervix of 25–26 Hz was higher than the reported value of 12 Hz (28), however, these observations were consistent; whereas our ROI covered the entire cervix, the previous study used a small circular ROI only in the cervical myometrium, excluding areas of large R_2 near the cervical os and resulting in a smaller R_2 . The ME R_2^* in muscle of 36–45 Hz we observed at 3T was slightly larger than a previous report of 28 Hz at 1.5T (13), in line with an expected increase in the underlying irreversible relaxation rate component with field strength. ME R_2^* compared well to values reported at 3T, e.g., 25 Hz in the healthy uterus as compared to reported values of 24.5 – 28 Hz (15), 34 Hz in the healthy cervix compared to a reported value of 39 Hz (15), and 33 Hz in cervical tumors (17 stage IIA to IVA tumors) as compared to reported values of 16–33 Hz (4 stage IIA or IIB tumors) (15).

We observed no significant changes in GRE decay parameters with changes in the inhaled O_2 level ($P = 0.81$). Our observations are in excellent agreement with previous reports in the renal cortex or medulla (30), and in the spleen, liver, skeletal muscle, subcutaneous fat, or renal cortex (31). However, they are in disagreement with one report (15) of R_2^* changes with inhaled O_2 level ($+0.6 \pm 4\%$ to $-2.5 \pm 3.5\%$ in cervical tumors, $-1.7 \pm 2.2\%$ to $-7.1 \pm 4.1\%$ in the uterus of patients, $-9.6 \pm 3.3\%$ in the healthy cervix, and $-12.2 \pm 4.2\%$ in the healthy uterus). A major difference in our study is that patients were imaged post-EBRT (vs. at diagnosis pre-EBRT), and pelvic irradiation has previously been shown to significantly affect uterine anatomy, metabolism and perfusion (32). Another difference was multi-slice coverage of ROIs in our study (vs. single-slice acquisitions). R_2^* is expected to vary by 31–46% across different regions of the uterus and across the menstrual cycle (33). R_2^* changes with inhaled O_2 level reported in (15) were modest relative to these expected variations, and covering slightly different portions of the uterus in each single-slice acquisition might have also contributed to some of the observed changes. Theoretically, breathing oxygen should have negligible effect on R_2^* under normal conditions (i.e. in the absence of pulmonary disease, hyperbaric chamber etc.): an inhaled oxygen level of 21% (in room air) results in near full arterial hemoglobin oxygen saturation ($\sim 98\%$), such that increasing the inhaled oxygen level from 21% to 100% by breathing oxygen can at most increase arterial hemoglobin oxygen saturation from $\sim 98\%$ to 100%, and oxygen dissolved in plasma has a negligible effect on susceptibility (26). Although a modest increase in venous hemoglobin

saturation may be achieved, this effect is expected to be counteracted by reduced organ perfusion mediated by oxygen-induced vasoconstriction (34,35). The cumulative effect of these processes may account for negligible and/or opposing responses seen across different organs and participants.

Interesting extensions to the present work may involve the Voigt profile (36) as well as acquisition strategies such as GESFIDE (Gradient Echo Sampling of FID and Echo) (20) or GESSE (Gradient Echo Sampling of the Spin Echo). These sample signals while both reversible and irreversible transverse relaxation dephase the spin system, as well as while reversible processes rephase the spin system and work against irreversible processes. The Voigt profile is the convolution of Lorentzian and Gaussian profiles, where R_2' and σ characterize the Lorentzian and Gaussian components of reversible transverse relaxation, respectively. While one would not be able to separate R_2' from R_2 using GRE data alone, more sophisticated acquisition strategies such as GESFIDE or GESSE parameterized with the Voigt profile, may enable simultaneous estimation of R_2 , R_2' and σ . Identifying components contributing to apparent transverse relaxation could further improve tissue characterization in gynecologic cancers.

Our study demonstrates the need for improved modeling; however, without a gold standard of hypoxia available and a corresponding ROC analysis, whether more accurately determined parameters correlate with hypoxia or improve clinically-relevant tissue characterization remains to be determined in further investigations (e.g. with acquisition of immunohistochemistry, pO₂ microelectrode, or possibly PET data). Entire tumors were delineated in our study, however, some of the other factors besides oxygenation that can influence GRE signal decays in the pelvis (e.g., air/tissue and air/bone interfaces, blood products, calcifications, temperature, etc.) could be excluded based on careful comparisons across anatomical, diffusion and contrast enhanced images. Underlying processes (e.g., hypoxia) may have chronic components as well as acute fluctuating components (i.e., due to diffusion or perfusion limitations) which may require dynamic multi-phase imaging for characterization. Our patients were imaged at one time point, however a larger cohort and a longitudinal study following progression from diagnosis through outcomes would be desirable. Finally, generalizability to other cancers may be limited as most patients in our study had cervical cancers.

In conclusion, non-invasive MRI assessment of tumor aggressiveness, as expressed by hypoxia may have significant impact on development of personalized treatment strategies for gynecologic cancers, and may ultimately lead to improvement of patient outcomes. We have shown that modeling with GAME, as opposed to ME, improved characterization of GRE signals in post EBRT gynecologic cancers at 3T; GRE decay parameters did not change with inhalation of oxygen; high ME R_2^* may not necessarily represent hypoxia; and that existence of TPGs contribute to but are not the sole reason for Gaussian deviations. Although the degree to which R_2 or σ values correlate with hypoxia remains unknown, improved characterization of underlying signals with GAME, along with more sophisticated acquisition strategies such as GESFIDE or GESSE, increases the potential for determining

any correlates of fit parameters with biomarkers, such as oxygenation status or other physiological variables.

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Figure 1.

Summary of patient and healthy participant protocols: (a) N-P = Normal in patients (n=5), (b) HL-P = High-Low in patients (n=18), (c) HIL-P = High-Intermediate-Low in patients (n=2); and (d) HIL-H: High-Intermediate-Low in healthy participants (n=3) (“...” denotes other imaging, unused in this study, of 5–7 minute duration).

Figure 2.

Sample fits and parameters (a) Anatomy (T2W TSE) with tumor contour, on Patient X (b–e) Semi log plots of multi-echo GRE signal (arbitrary units) over time (TE: 3, 8.96, 17.92, 26.88, 35.84, and 44.80 ms) at the red voxel locations (b–d) log(Signal) vs. TE shows significant curvature ($P < 0.05$), and GAME outperforms ME improving SSEs by over 400-fold to over 3000-fold (e) As $\sigma \rightarrow 0$, log(Signal) vs. TE becomes linear, and the models become equivalent. (f) Anatomy (T2W TSE) with tumor (red) and muscle (green, yellow) contours, on Patient Y (g) ME R_2^* (h) GAME R_2 , and (i) GAME σ , parameter maps (black-

white checkered regions in (g) demonstrate areas within the tumor with complete signal loss where fitting was not performed; white arrows point to areas of low R_2^* , R_2 and σ ; gray arrows point to areas of high R_2^* and R_2 , with low σ ; black arrows point to areas of high R_2^* and σ , with low R_2).

Figure 3.

(a–b) Highly significant improvements are seen with GAME over ME in tumors ($P < 0.05$), P -values are shown for Patients X and Y (of Figure 2) (c) The physical extent of improvements with GAME over ME, for all patient and healthy participant ROIs (as a percentage of the entire ROI volume, Eq. [4]). Improvements with GAME cover 34% of tumor ROIs on average at $P < 0.05$ (based on clustering), and persist at increasing levels of statistical significance (P_c : P value based on clustering; P_b : P value based on Bonferroni correction) (d) The extent of statistically significant improvements with GAME over ME increases in areas that would be assumed indicative of hypoxia based on ME fits alone (areas of high R_2^* according to the ME model). ME R_2^* cutoff = 0 corresponds to calculating the extent of improvements with GAME as a percentage of the entire ROI volume (irrespective of the ME R_2^* estimates within the ROI, Eq. [4], as in Figure 3c). ME R_2^* cutoff > 0 corresponds to selecting high R_2^* sub-ROIs within each ROI, where using the ME model alone would have indicated hypoxia, and calculating the extent of improvements with

GAME as a percentage of these smaller ROIs (Eq. [5]) (shown for muscle and tumor ROIs at $P < 0.05$, combined across all patients; circles depict ROIs of decreasing size with increasing ME R_2^* cutoff; red-blue stripes indicate part of these ROIs that improve significantly with GAME).

Figure 4.

ME and GAME fit parameters (mean $\pm$ SD, Hz) in tumors and muscles of patients, in regions where GAME significantly improved fitting ($P < 0.05$, ROI_{GAME}) versus where GAME did not significantly improve fitting and simply reduced to the ME model (ROI_{ME}) (Eqs. [6]). ME R_2^* was lower in ROI_{ME} , while GAME R_2 was lower and GAME σ was higher in ROI_{GAME} .

Figure 5.

Macroscopic background inhomogeneities estimated from multi-echo GRE data helped identify regions where high σ did or did not overlap with high TPGs (a, b) Consecutive slices (T2w TSE with tumor outlined); (c, d) Multi-echo GRE field map estimates (Eq. [7], Hz); (e, f) Magnitude of the TPG across each slice (Eq. [8], Hz/mm); (g, h) GAME σ (Hz), shown in areas where GAME statistically significantly improved blue color coded portions within each circle indicate the approximate fraction of each ROI that improves significantly with GAME and where σ values were calculated, for tumors and muscles, respectively).

Figure 6.

σ and TPG (a) Scatter plots, regression lines and adjusted coefficients of determination, (for each subject with a fieldmap). σ increases with TPG in most tumors, but in few muscles (b) The extent of improvements with GAME over ME as a function of TPG ($P < 0.05$, across all patients with fieldmaps): increases then decreased in tumors, but is stable in muscles (c) Mean σ increases with increasing TPG cutoffs. TPG cutoff = 0 corresponds to using the entire ROI volume (irrespective of the field inhomogeneity within the ROI, Eq. [4]). TPG cutoff > 0 corresponds to selecting high TPG sub-ROIs within each ROI, and calculating the extent of improvements with GAME as a percentage of (Eq. [9]) and mean σ values within (Eq. [10]) these smaller ROIs (Sizes of the circles within each graph indicate the approximate sizes of the ROIs under consideration: ROIs of decreasing size with increasing TPG cutoff. Sizes of the red and blue color coded portions within each circle indicate the approximate fraction of each ROI that improves significantly with GAME and where σ values were calculated, for tumors and muscles, respectively).

Table 1

GRE decay parameters for each participant, tissue, and tumor type

Participant Type	Tissue	R_2^* (Hz)	R_2 (Hz)	σ (Hz)	n	
Healthy	Muscle - Gluteus Maximus	44 ± 2	36 ± 1	29 ± 7	3	
	Muscle - Iliopsoas	39 ± 1	33 ± 3	20 ± 2	3	
	Uterus	25 ± 1	17 ± 3	21 ± 3	3	
	Cervix	34 ± 6	26 ± 5	30 ± 8	3	
Patients Post-EBRT	Non-Tumor	Muscle - Gluteus Maximus	45 ± 5	33 ± 5	33 ± 8	25
		Muscle - Iliopsoas	36 ± 6	27 ± 6	26 ± 7	25
	Tumor	Cervical Tumor	33 ± 5	25 ± 5	30 ± 6	17
		Vaginal Tumor	36 ± 6	29 ± 7	31 ± 10	4
		Endometrial Tumor	46 ± 7	35 ± 10	40 ± 0	2
		Urethra-Bladder Tumor	35	28	30	1
		Uterine Tumor	23	16	22	1

mean $\pm$ standard deviation, n: number of cases