



ORIGINAL PAPER

Wojciech Domka ¹(ABDFG), Łukasz Ożóg ²(ABDFG), Dorota Bartusik-Aebisher ³(ABDFG),
David Aebisher ²(ABDFG)

The larynx cancer in vitro study by MRI relaxation time of water

¹ Department of Otolaryngology, Medical College of Rzeszów University, Rzeszów, Poland

² Department of Photomedicine and Physical Chemistry, Medical College of Rzeszów University, Rzeszów, Poland

³ Department of Biochemistry and General Chemistry, Medical College of Rzeszów University, Rzeszów, Poland

ABSTRACT

Introduction. Squamous cell carcinoma (SCC) of the larynx accounts for a significant percentage of all head and neck cancers. **Aim.** In this paper we determine the differences in magnetic resonance relaxation time (MRI) of water in cancerous and healthy larynx tissues.

Material and methods. This study is aimed on T₂ MRI modalities for monitoring morphology of larynx tissue.

Results. Our results showed that T₂ MRI relaxation time measured in larynx tissue can be used to assess early cancer condition of larynx tissues. The changes of T₂ MRI correspond to tumor growth within normal tissue.

Conclusion. The study showed potential of MRI for the non-invasive monitoring of larynx condition.

Keywords. larynx tissue, magnetic resonance imaging, T₂ mapping, T₂ relaxation time

Introduction

The use of MRI to assess and characterize pathology and normal tissue has been extensively described in the literature. By using the differences in tissue relaxation behavior, we are able to show differences between pathological tissue and normal tissue.^{1,2} However, this requires proper preparation of both the equipment (appropriate calibration) and the sequences themselves (optimization of the test protocol) so that the results are reliable.^{3,4} An important role in the characterization of tissues is played by tissue heterogeneity, especially those pathological. The use of T₁ and T₂ relaxation times and mapping techniques available in MRI systems gives the

opportunity to evaluate tissues on the basis of their relaxation properties.

One of the more interesting MRI imaging techniques that uses differences in tissue relaxation is the T₂ mapping technique. T₂ mapping sequence and processing is technique acquires multiple echoes at different echo times (TE) at each slice location that represent different T₂ weighting. Data obtained using this technique can be processed to produce T₂ color maps which demonstrate more subtle changes in tissues structure that are not visible on gray scale MR images. The changes in T₂ values correlate with the variations in water and oxygen content. T₂ is a tissue-specific time parameter

Corresponding author: David Aebisher, e-mail: daebisher@ur.edu.pl

Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 1.05.2020 | Accepted: 1.06.2020

Publication date: September 2020

that differs between tissues. There is no precise value for the specific tissue but is constant for a given magnetic field strength. Abnormal tissues, on the other hand, tends to have a higher T_2 -value than normal tissue. The value of T_2 depends on the tissue type, the structure of the environment, and the mobility of protons.^{5,6}

Aim

This study aimed to presents the results of MRI mapping in larynx tissue.

Material and methods

Larynx Tissue

Freshly excised samples (N=11) of normal and cancerous laryngeal tissue and normal and cancerous thyroid tissue were obtained from the Frederic Chopin Clinical Regional Hospital No. 1 in Rzeszów, Poland. The human tissue studies were approved by the Bioethical Commission of the District Medical Chamber in Rzeszów (Resolution number 105/B/2017). Once received, the tissues were stored in 15 mL polypropylene graduated conical test tubes fitted with a screw tight cap (Kartell Labware, Milano, Italy) at 5°C. The average size of the excised tissues was 10 mm×4 mm×5 mm, respectively.

T_2 Mapping

These experiments were performed using Magnetic Resonance Imaging (MRI) 1.5 Tesla field. The laryngeal tissues were taken to the MR imaging plant to determine the T_2 time. A total of 6 larynx cancer tissues and 5 normal larynx tissues were treated and imaged in this study. Immediately after surgery to remove the tumor, high-resolution MR images were acquired from each tissue to determine T_2 relaxation times and T_2 maps. The tissues MR images were acquired using a 1.5 Tesla field MR scanner (Optima MR360 Advance, General Electric Healthcare, USA). A 3 inch surface coil was used for the image acquisitions. Images with varying echo times (TEs) were then obtained using a commercial T_2 MAPS sequence to enable T_2 calculation. MR images were obtained at eight different echo-times (9.2, 18.4, 27.6, 36.9, 46.0, 55.2, 64.4 and 73.6 milliseconds) in one single acquisition (TR=650 milliseconds, field of view 5.00 cm×5.00 cm, matrix size 224×224, slice thickness 2.0 mm, receiver bandwidth 31.25 kHz, NEX = 3). Typically, one coronal slice was acquired to cover all tissue through the middle. The total scan time to simultaneously acquire the eight T_2 -weighted images was 7 minutes 20 seconds. These MR parameters were used for all tissues.

Statistical Analysis

The data was analyzed using Statistica 13.1 software (StatSoft Polska Sp.z o.o., Krakow, Poland). The data were analyzed using the dependent samples *t*-test to

check the differences in the T_2 time results between the individual steps of the experiment. Values were considered significantly different when the *p*-value was less than 0.05.

Results

We used the software package READY View on workstation AV4.6 (General Electric Healthcare) to compute the T_2 maps. Second, we manually chose a layer that cuts the center of the tissue on which the T_2 maps were generated. On the T_2 -weighted MR images, the tissues appeared as a bright region. One author manually drew the boundary of the tissue on the image and then saved the object map of the tissue. The T_2 value for each voxel was determined within the tissue region (and the average value for the area was calculated). We compared the mean T_2 values for the cancer tissues and normal tissues of larynx. MR relaxation times measurements *in vitro* is reproducible, completely stable and readily available. T_2 maps obtained during measurements are presented below. T_2 maps were generated by single exponential fitting from multiecho (0–90 ms) pulse sequences with intervals of 8.2 ms, a time repetition of 650 ms and a field of view of 5 cm over a 224×224 matrix (Figure 1).

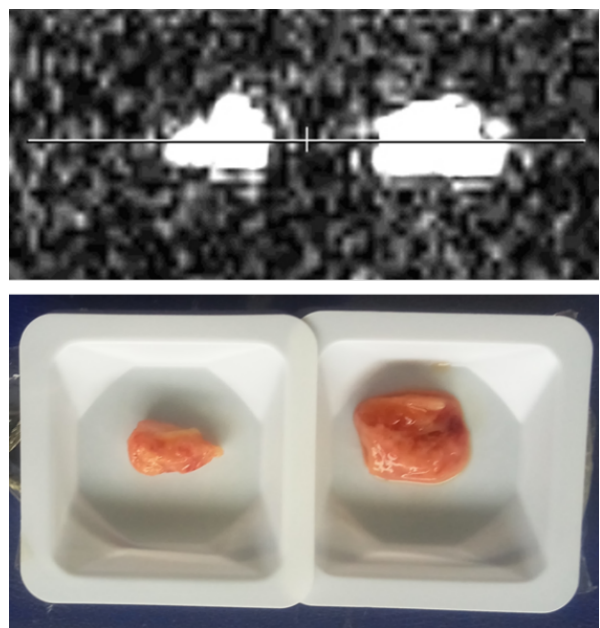


Fig. 1. The MR image of the calibration sequence presenting the method of planning the examination and the selection of the scanning layer (layer thickness 2mm) and the same cancer tissue and health tissue of larynx on real photo

Measured and then determined T_2 values of cancer tissues (mean=102±7 ms; n=6) were significantly higher (*p*=0.031; *p*<0.05) than normal tissues (mean=90±9,6 ms; n=5). The central rectangle shows the first quartile to the third quartile (interquartile range - IQR).

Table 1. T₂ values for cancerous (C1-C6) and healthy (H1-H5) tissues

	C1	C2	C3	C4	C5	C6
T ₂ (ms)	93 ± 4	98 ± 5	100 ± 5	104 ± 5	102 ± 5	114 ± 6
	H1	H2	H3	H4	H5	
	79 ± 4	80 ± 4	92 ± 5	102 ± 5	92 ± 5	

A segment inside the rectangle (horizontal line) shows the median and “whiskers” above and below the box show the locations of the minimum and maximum measured T₂ time. This result confirms that the T₂ maps technique makes it possible to distinguish between normal and cancerous tissue.⁷⁻¹¹

Discussion

The method to study tissue condition is one of the most important aspects in individual treatment. MRI can offer very sophisticated analysis of diseased and healthy larynx tissues. Squamous cell carcinoma (SCC) of the larynx showed decrease in water content in *in vitro* samples. Thus, MR parameters such as T₂ relaxation time measured *in vitro* is useful as a basis for correlation with clinical noninvasive measurement. Additionally, MR relaxation times measured in cancer cell cultures can be a predictor of cellular changes during treatment. In MRI, the signal is strongly depending on the change of TE and TR parameters; choosing these parameters on the basis of the obtained data we are able to plot relaxation curves and determine times T₂ (Table 1).

Conclusion

Quantitative T₂ relaxation times allows to distinguish between healthy and cancerous tissue. T₂ mapping allows to monitor changes in water concentration *in vitro*, and thus allows to characterized tissue condition. The measurements of healthy tissue show lower values of T₂ than cancerous tissue. MRI noninvasive and repetitive measurements is very attractive tool in the pre- and clinical context in otolaryngology.

References

1. Filippi M, Charil A, Rovaris M, et al. Insights from magnetic resonance imaging. *Handb. Clin Neurol.* 2014;122:115-149.
2. Young RJ, Knopp EA, J. Young RJ, et al. Brain MRI: tumor evaluation. *Magn Reson Imaging.* 2006;24:709-724.
3. Bottomley PA, Hardy CJ, Argersinger RE. A review of 1H NMR relaxation in pathology: are T1 and T2 diagnostic? *Med Phys.* 1987;1:1-37.
4. Bottomley PA, Foster TH, Argersinger RE, A review of normal tissue ... species, excision, and age. *Med Phys.* 1984;11:425.
5. Allisy-Roberts P. *Farr's Physics for Medical Imaging.* Eng. 2. ed. Edinburgh, New York: Saunders, 2007.
6. Welsch GH, Hennig FF, Krinner S. T2 and T2* Mapping. *Curr Radiol Rep.* 2014;2:60.
7. Cavaliere C, Romeo V, Aiello M, et al. Multiparametric evaluation by simultaneous PET-MRI examination in patients with histologically proven laryngeal cancer. *Eur J Radiol.* 2017;88:47-54.
8. Allegra E, Ferrise P, Trapasso S, et al. Early Glottic Cancer: Role of MRI in the Preoperative Staging. *Biomed Res Int.* 2014;890385.
9. Blitz AM, Aygun N. Otolaryngol. Radiologic evaluation of larynx cancer. *Clin North Am.* 2008;41:697.
10. Agnello F, Cupido F, Sparacia G, et al. Computerised tomography and magnetic resonance imaging of laryngeal squamous cell carcinoma: a practical approach *Neuroradiol J.* 2017;30: 197-205.
11. Jager EA, Ligtenberg H, Caldas-Magalhaes J, et al. Validated guidelines for tumor delineation on magnetic resonance imaging for laryngeal and hypopharyngeal cancer. *Acta Oncol.* 2016;55:1305 -1311.