research article

Assessment of hyperbaric oxygenation treatment response in parotid glands by T_2 mapping following radiotherapy for head and neck tumours

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Radiol Oncol 2022; 56(1): 60-68.

Received 23 September 2021
Accepted 10 December 2021

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Disclosure: No potential conflicts of interest were disclosed.

Background. The study was designed to evaluate the influence of hyperbaric oxygenation therapy (HBOT) on the parotid gland in patients following radiotherapy for head and neck tumours.

Patients and methods. HBOT response was monitored by 3T magnetic resonance imaging (MRI) using T_2 mapping and subsequent measurement of mean T_2 and T_2 variability as well as by salivary tests (salivary flow, buffer capacity, and pH). Eighteen patients previously treated with irradiation doses between 50 and 80 Gy as well as 18 healthy gender and age matched controls were enrolled. MRI was performed prior to HBOT (40.2 ± 20 months after radiotherapy) and after 20 daily HBOT at 2.5 ATA (absolute atmosphere). Each HBOT consisted of breathing 100% oxygen for 90 minutes.

Results. Significant differences in mean T_2 prior to HBOT were observed between the ipsilateral irradiated (121 ± 20 ms), contralateral parotids (107 ± 21) and control group (96 ± 12 ms). A positive correlation in patients between T_2 variability and irradiation dose was detected in contralateral parotids before HBOT ($R = 0.489$, $p = 0.0287$). In addition, negative correlations were observed between mean T_2 in the ipsilateral as well as the contralateral gland and salivary flow before and after HBOT. Negative correlations between mean T_2, T_2 variability and pH of unstimulated saliva were also observed in the sides of parotid before and after HBOT.

Conclusions. The study confirmed that T_2 mapping had a potential for monitoring the differences between irradiated and normal parotid glands. It could also be useful in the assessment of the glandular tissue response to HBOT.

Key words: salivary glands; MRI; T_2 mapping; hyperbaric oxygenation therapy

Introduction

The main method of treatment of the malignant head and neck tumours is surgical removal and/or radiation therapy with therapeutic doses between 50 and 70 Gy.[1,2] Doses above 40 Gy results in irreversible changes in the salivary glands – atrophy and necrosis[3,4] – which leads to the reduction of the flow of saliva and the development of xerostomia when salivary glands are in the field of irradiation[4-6]; the latter is probably due to the apoptosis of salivary tissue, as observed in other

tissues under radiation therapy.[7-9] Consequently, both stimulated and unstimulated salivary flow, salivary pH and buffer capacity are reduced[6,10] and the ion composition of saliva is also changed.[11,12] These changes cause the accumulation of plaque and an increased number of microorganisms in the saliva[13-15] which may result in rapidly progressing radiation caries.[16,17] In addition, the reduced excretion of saliva causes complaints associated with oral dryness in patients. It effects the use of oral prostheses as well as speech and taste. The quality of life is also compromised.[16,17] Effective treatments of radiation-induced xerostomia are warranted.[18,19]

Hyperbaric oxygenation therapy (HBOT) is an acceptable method of treatment for the prevention of osteoradionecrosis and soft tissue necrosis in the oral cavity.[20] It improves blood circulation in post-ischemic tissues[21], reduces oedema formation[22], increases diffusion of oxygen in the tissues[23], and accelerates activation of stem cells.[24] The likely cause of the beneficial effect of HBOT against the long-term negative effects of radiotherapy is the accelerated angiogenesis and revascularization of tissues.[25] Until now, there has been no studies that objectively measured the impact of HBOT on salivary gland tissue.

Multiparametric MRI is already a common diagnostic tool for the parotid gland tumours[26, 27], since it enables the optimization of contrast among various soft tissues based on the values of T_1 and T_2 relaxation times of various tissues and organs. T_2 mapping is specifically a magnetic resonance imaging technique used to calculate the T_2 relaxation times of the specific tissues and displaying them voxel-vice on a parametric map. It has been used for tissue characterization in various types of tissue (e.g. myocardium).[28] The T_2 relaxation time, also referred to as the spin-spin or transverse relaxation, is a time constant for the decay of transverse magnetization and is tissue-specific with regards to its ability to differentiate the abnormal tissues from the normal ones. T_2 values reflect water content in the respective tissue and are mainly used for the evaluation of oedema, e.g., in myocardial inflammation or infarction as in other pathologies. In addition, T_2 mapping and mDIXON Quant imaging proved to be useful for non-invasive evaluation of the radiation-induced parotid damage.[29] Consequently, the mapping of the transversal relaxation time (T_2-mapping) enables good differentiation among different stages of soft tissue inflammation without the need of contrast medium and would be an appropriate method for the early detection of salivary gland tissue changes due to radiation and HBOT.

In the present *in vivo* study, the patients with head and neck tumours who had undergone radiation therapy were scanned by the T_2 mapping MRI technique before and after HBOT. The obtained T2 maps were further correlated with the standard clinical salivary tests (salivary flow, pH, and buffering capacity).

Patients and methods

Patient group

The study was carried out on 18 patients (2 females and 16 males) with the head and neck tumours previously treated with radiotherapy. The mean age of the patients was 60.9 ± 11.7 years. Patients had been diagnosed with different types of tumours, the majority of which were located in the oral cavity. In all patients, the salivary glands were in the radiation field. The patients received irradiation doses from 50 to 80 Gy (mean irradiation dose 64.3 ± 6.3 Gy). Each patient included in the study received 20 daily HBOT in a hyperbaric chamber at 2.5 ATA (absolute atmosphere) where patients breathed 100% oxygen for 90 minutes each day. Patients were examined by MRI as well as salivary function testing twice; the first MRI examination was performed at baseline before the first HBO therapy (40.2 ± 20 months after radiation therapy) and the second 3 to 7 days after the last HBOT to avoid possible acute effects of higher oxygen levels. All patients were able to perform routine daily activities prior to each MRI examination.

Contraindications to MRI such as an implanted pacemaker constituted exclusion criteria. All participants provided written informed consent approved by the Ethical Committee of the National Ministry of Health (Approval number 0120-41/2017/13) to participate in this protocol and conformed to the STROBE guidelines. The clinical study was undertaken with the understanding and written consent of each subject according to the Declaration of Helsinki (version 2008).

Control group

The control group was composed of 18 healthy gender- and age-matched participants (mean age: 56.7 ± 11.8 years; 2 females and 16 males), who were enrolled in the study as volunteers and did not receive any HBOT. All the measurements in the control group (MRI measurements as well as salivary

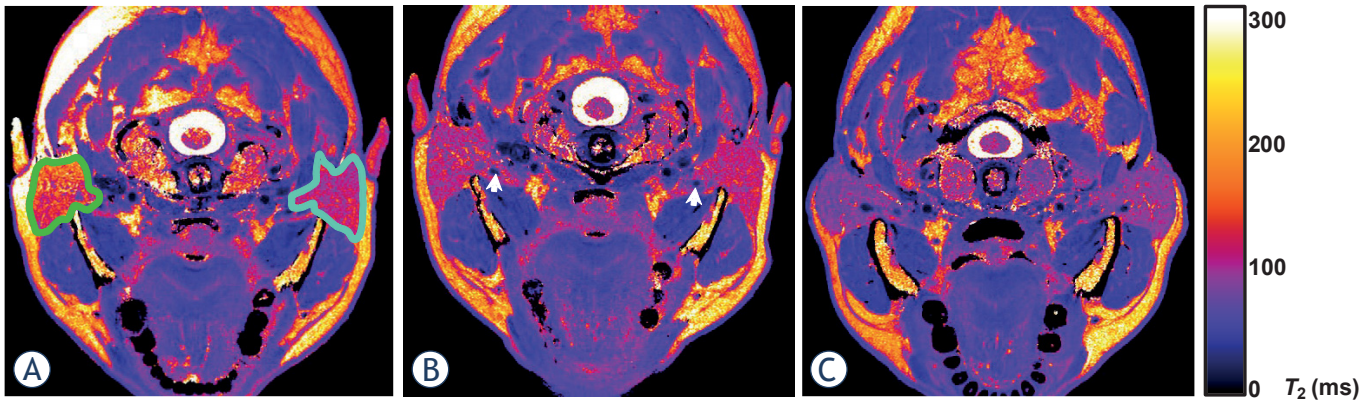

FIGURE 1. Representative T_2 maps of parotid glands in a single transversal slice in a patient following radiotherapy for head and neck tumour before **(A)** and after hyperbaric oxygenation therapy (HBOT) **(B)** and in healthy control **(C)**. Region of interest (ROI) on the ipsilateral side is encircled by green and on the contralateral side by the white-blue colour. Retromandibular veins (white arrows in B) were always carefully omitted from the ROI.

function testing) were performed only once in the same manner as in the patient group. Specifically, analysis of the MRI measurements was performed in both parotid glands. The results of the control group were used as a reference.

MR image acquisition

The MR imaging of the parotid glands was performed on a 3T MRI system (TX Achieva, Philips, Netherlands) with a maximum gradient strength of 80 mT/m and use of a 32-channel receive head coil. The MR images were acquired using a multi-spin-echo (MSE) MRI sequence with parameters: TR = 2000 ms; TE = 7.8, 16, 24, 32, 40, 47 ms; field of view (FOV) 160 × 160 mm^2; slice thickness 2 mm; image acquisition/reconstruction matrix 380 × 311/560 × 560; acquisition/reconstruction voxel size 0.42 × 0.51 × 2.5/0.29 × 0.29 × 2.5 mm^3; single slice; bandwidth 290 Hz/pixel; no signal acquisition acceleration; and acquisition time for all 6 echoes was equal to 10 min 24 s. The imaging plane was oriented so that it contained most of the parotid glad, *i.e.*, in the transversal orientation.

MR data analysis

A central slice in the transversal plane, covering the largest area of the parotid gland tissue, was used for T_2 mapping analysis. T_2 map was calculated using pixel-wise least-square fitting analysis of a set of T_2-weighted images with TE values as specified above. The fitting analysis implemented in the MRI for the calculation of T_2 maps used Analysis Calculator plugin (ImageJ, National Institutes of Health, USA) that utilizes a mono-exponential T_2 signal decay function $S_{i,j}(TE) = S_{0\ i,j} \exp(-TE / T_{2\ i,j})$ as the model function for the analysis and the pair (i,j) denotes the pixel coordinate. From the calculated T_2 maps, mean and variability of T_2 values in the region of interest (encircled in Figure 1) were determined in the ipsilateral as well as the contralateral parotid gland of the subjects in the study. The variability of T_2 values was used for a quantitative assessment of tissue heterogeneity.

Salivary function testing

Tests for the evaluation of the functioning of the salivary glands were always performed between 11-12 a.m. The patients were instructed to clean their teeth in the morning and not to drink, eat or smoke for two hours before the measurements. Unstimulated salivary flow was determined by a 5-minute saliva collection. Saliva production was then stimulated with a 5-minute chewing of a paraffin block. The paraffin blocks (each weighting 1g and with a melting point of 48 °C) were part of the CRT buffer test provided by Ivoclar Vivadent (Liechtenstein). During this time, patients were not allowed to swallow saliva. After the stimulation, the salivary flow was determined. The buffering capacity was determined only in stimulated saliva with a CRT buffer (Ivoclar Vivadent, Liechtenstein) due to the negligible amount of unstimulated saliva. After five minutes, the colour of the pad was compared with the colour chart. The salivary pH was determined by a pH-meter (Iskra, Slovenia). In order to exclude the influence of saliva enhancement and disinfection procedures on

TABLE 1. Mean T_2 and T_2 variability values of parotid glands in patients following radiotherapy for head and neck tumours before and after hyperbaric oxygenation therapy (HBOT) and in healthy controls

	Ipsilateral side (N = 18)		Contralateral side (N = 18)		Controls (N = 18)
	before HBOT	after HBOT	before HBOT	after HBOT	
MEAN T_2 (ms)	121 ± 20†	113 ± 16†*	107 ± 21**	103 ± 14	96 ± 12
T_2 VARIABILITY (ms)	30 ± 8	25 ± 8*	21 ± 8	19 ± 6	16 ± 4

†-statistically significant difference with healthy controls

*-statistically significant change in response to HBOT

**-statistically significant difference between ipsilateral and contralateral side

the results of the study, instructions regarding oral hygiene and saliva flow enhancement were also provided after the saliva evaluation at the end of HBOT.

Protocol for HBOT

Patients were treated in a multi-place hyperbaric chamber (Kovinarska P&P, Slovenia). For each patient, 20 dives were held consecutively on each working day of the week. Each individual dive in the hyperbaric chamber filled with air at a pressure of 2.5 ATA (absolute atmosphere) lasted 90 min. The patients breathed 100-percent oxygen through a mask at a pressure of 2.5 ATA. For each patient, MRI as well as saliva tests before the start and 3 to 7 days after the last HBOT were performed.

Statistical analysis

The results were expressed as mean and standard deviation in the case of a passed Shapiro-Wilk test or as the median value and the interquartile range (IQR) in the case of a failed Shapiro-Wilk test, both with the criterion of significance at $p < 0.05$.

The mean $T_2$and T_2 variability values of the patients' parotid glands were compared by Analysis of Variance (ANOVA) with repeated measures using 19 degrees of freedom and a Bonferroni's post-hoc test. The obtained mean T_2 and T_2 variability values in the patient group (ipsilateral and contralateral gland respectively) were compared with values obtained in healthy controls with a Student t-test. The values of the salivary flow and pH of unstimulated as well as stimulated saliva in patients before and after the end of HBOT were compared with a paired t-test. To assess the magnitude and direction of change in the buffer capacity at the beginning and at the end of HBOT, the Wilcoxon signed-rank test was used.

Correlation tests between mean T_2 and T_2 variability values from the ipsilateral as well as the contralateral gland and saliva test parameters (i.e. salivary flow, pH and buffering capacity) and with irradiation doses in patients were made by a linear regression (Pearson correlation coefficient).

Results

MRI analysis

At the beginning of HBO therapy, significantly higher mean T_2 value was observed in the examined slice of the ipsilateral parotid when compared to the mean T_2 value on the contralateral gland ($p = 0.007$, Table 1). Furthermore, significant difference was observed between mean T_2 values in the parotid glands of healthy controls and in ipsilateral parotid glands of patients before HBO therapy ($p = 0.0004$). In contrast, no significant difference was observed between mean T_2 in the contralateral parotid glands of patients and healthy controls. In addition, no significant differences in T_2 variability of parotid glands of patients before HBOT and healthy controls were observed.

A significant higher mean T_2 on the ipsilateral gland was found in patients after the end of HBOT when compared to healthy controls ($p = 0.002$). In contrast, no difference was observed on the contralateral side. On the ipsilateral side, statistically significant decrease in mean T_2 and T_2 variability was observed in patient as a response to HBOT. In contrast, no significant change in mean T_2 and T_2 variability of contralateral parotid glands was observed in patients in response to HBOT.

Analysis of salivary tests

The salivary flow and pH value of unstimulated and stimulated saliva were significantly lower in

TABLE 2. Salivary flow, pH, and buffer capacity in patients following radiotherapy for head and neck tumours before and after hyperbaric oxygenation therapy (HBOT) and in healthy controls

	before HBOT (N = 18)	after HBOT (N = 18)	Controls (N = 18)
Unstimulated salivary flow (mL/min) (median and IQR)	0.22 (0.04-0.54) †	0.32 (0.08-0.70)*†	0.61 (0.49-0.99)
pH of unstimulated saliva (mean ± SD)	6.61± 0.69†	6.72 ± 0.71†	7.56 ± 0.53
Stimulated salivary flow (mL/min) (mean ± SD)	0.82 ± 0.60†	0.90 ± 0.64†	2.04 ± 0.91
pH of stimulated saliva (mean ± SD)	7.38 ± 0.74†	7.48 ± 0.51†	8.00 ± 0.28
Buffering capacity of stimulated saliva (median and IQR)	2.00 (1.75-3.00)	3.00 (2.00-3.00)*	3.00 (2.00-3.00)

†-statistically significant difference with healthy controls

*-statistically significant change in response to HBOT

IQR = interquartile range; SD = standard deviation

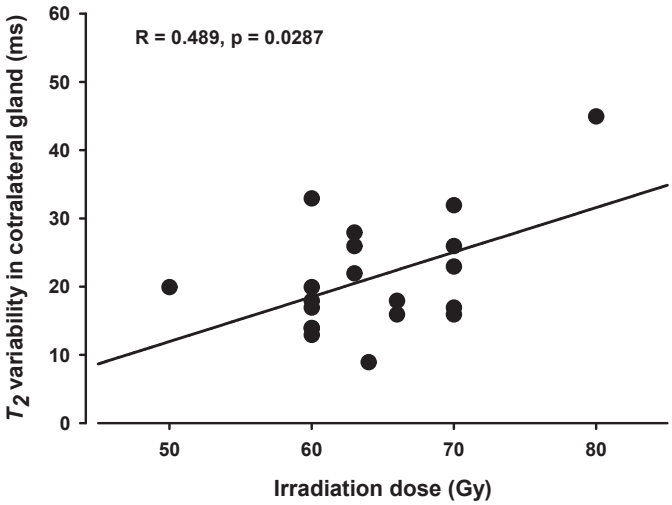

FIGURE 2. A correlation between an irradiation dose and variability of T_2 values in contralateral parotid glands before hyperbaric oxygenation therapy (HBOT).

patients prior to and after the end of HBOT when compared to the values obtained in healthy controls (Table 2, $p < 0.01$). In contrast, no significant difference in the buffering capacity of stimulated saliva was observed between patients and controls. Statistically significant increase in the unstimulated salivary flow as well as in the buffering capacity of stimulated saliva was observed in patients in response to HBOT. In contrast, no significant change was found in other measured salivary parameters.

Correlation between MRI parameters, irradiation dose and salivary tests

A significant positive correlation between T_2 variability of the contralateral parotid gland and the irradiation dose was observed before HBOT (Figure 2). In contrast, no significant correlation between mean T_2 on either ipsilateral or contralateral gland or T_2 variability in ipsilateral parotid glands before HBOT and the irradiation dose was found.

On the ipsilateral side, a significant negative correlation was observed between mean T_2 and stimulated salivary flow before HBOT (Figure 3A) and between mean T_2 and unstimulated salivary flow after HBOT (Figure 3B). On the contralateral side a negative correlation between mean T_2 and unstimulated (Figure 4A) as well as stimulated salivary flow (Figure 4B) was observed after the HBOT.

In addition, significant negative correlations between mean T_2 and T_2 variability and pH of unstimulated saliva were observed in ipsilateral parotid glands in patients before and after HBOT (Table 3). On the contralateral side negative correlations were also observed except for the correlation between T_2 variability and pH of unstimulated saliva before HBOT. No correlations were found between mean T_2 or T_2 variability and pH of stimulated saliva.

Discussion

In the present study, the structural and functional response to HBOT in parotid glands of the patients after the radiotherapy of head and neck tumours was monitored by T_2 mapping and functional salivary test. Two of the MRI parameters obtained from the T_2 maps, *i.e.* mean T_2 and T_2 variability were used for the assessment of tissue structure after radiotherapy, prior to and after HBOT. Mean T_2 was used to assess tissue oedema and T_2 variability

TABLE 3. Correlations between mean T_2 or T_2variability and pH of unstimulated saliva before and after hyperbaric oxygenation therapy (HBOT) (R-correlation coefficients and p-values)

	Ipsilateral side (N = 18)				Contralateral side (N = 18)			
	before HBOT		after HBOT		before HBOT		after HBOT	
	R	p	R	p	R	p	R	p
Mean T_2 (ms)	-0.647	0.0037	-0.571	0.0133	-0.557	0.0164	-0.675	0.0021
T_2 variability (ms)	-0.595	0.0092	-0.506	0.0323	-0.130	0.607	-0.588	0.0133

for the assessment of structural changes in the tissue, *e.g.*, tissue heterogeneity. Mean T_2 is strongly dependent on the free water content and its mobility in the tissue; however, it lacks more detailed information about the tissue structure heterogeneity, otherwise visualized on the T_2 maps of the examined slice. Therefore, mean T_2 values were complemented with T_2 variability.

Prior to HBOT, consistent high mean T_2 values were observed in the examined slices with the parotid gland on the ipsilateral side compared to the values obtained from the glands on the contralateral side of the same patients as well as healthy controls. The most plausible explanation for this phenomenon is the onset of radiation induced parenchymal changes in the affected parotid glands in patients following radiotherapy.[26,29] Therefore, the augmented T_2 values in the parotid glands on the side of radiation can be explained by the prolonged effect of the inflammation along with glandular oedema. T_2 variability values in parotid glands are also the highest in the ipsilateral irradiated parotid glands and somewhat lower on the contralateral side in patients before HBOT when compared to the control group. This can be explained by a different structural tolerance of parotid glands to the received radiation. Due to the proximity of the ipsilateral side to the radiation source, a relatively high radiation dose was accumulated, causing scarring and narrowing of the blood vessels with more severe parenchymal atrophy.[30] The latter resulted in a more heterogeneous structure, as seen in T_2 maps of the parotid glands on the ipsilateral side, and consequently in a relatively high T_2 variability. It should be emphasized that the MRI assessment of parotid glands performed at the late time prior to HBOT might represent late radiation effects (LRE) resulting in tissue oedema as well as chronic tissue changes.[31]

After the HBOT, a decrease in mean T_2 and T_2 variability in the patients' ipsilateral gland was observed. This can be explained by HBOT effects on LRE through a complex series of changes in the affected tissues. Tissue oedema is probably improved through an osmotic effect of oxygen while the onset of a steep oxygen gradient across an irradiated tissue margin is a powerful stimulus for

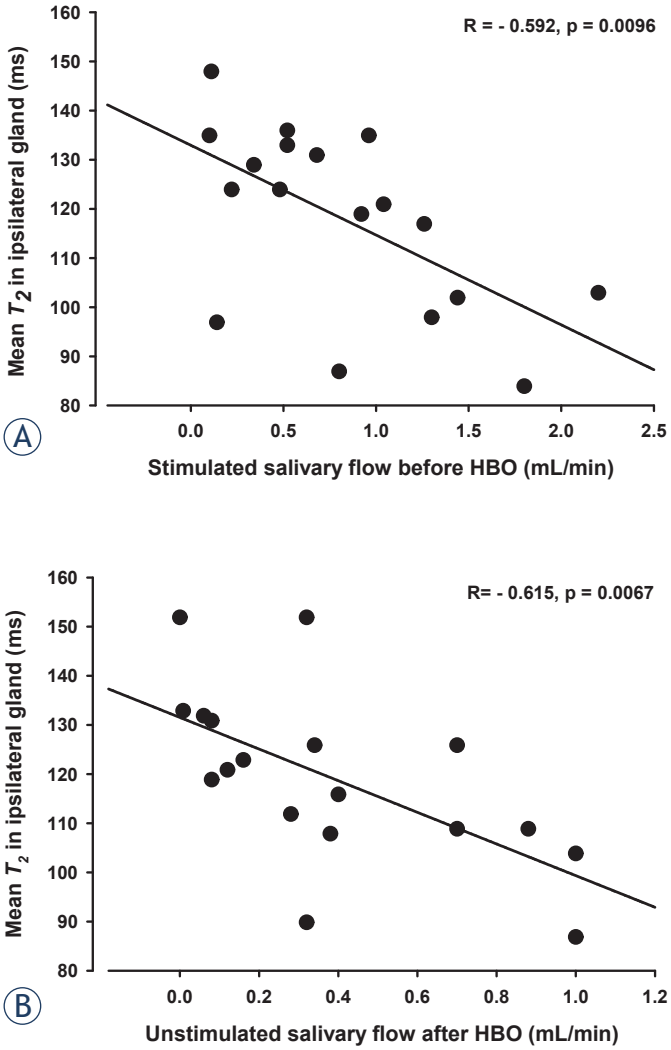

FIGURE 3. A correlation between the mean T_2 values in ipsilateral parotid glands and stimulated salivary flow before hyperbaric oxygenation therapy (HBOT) **(A)** and unstimulated salivary flow after HBOT **(B)**.

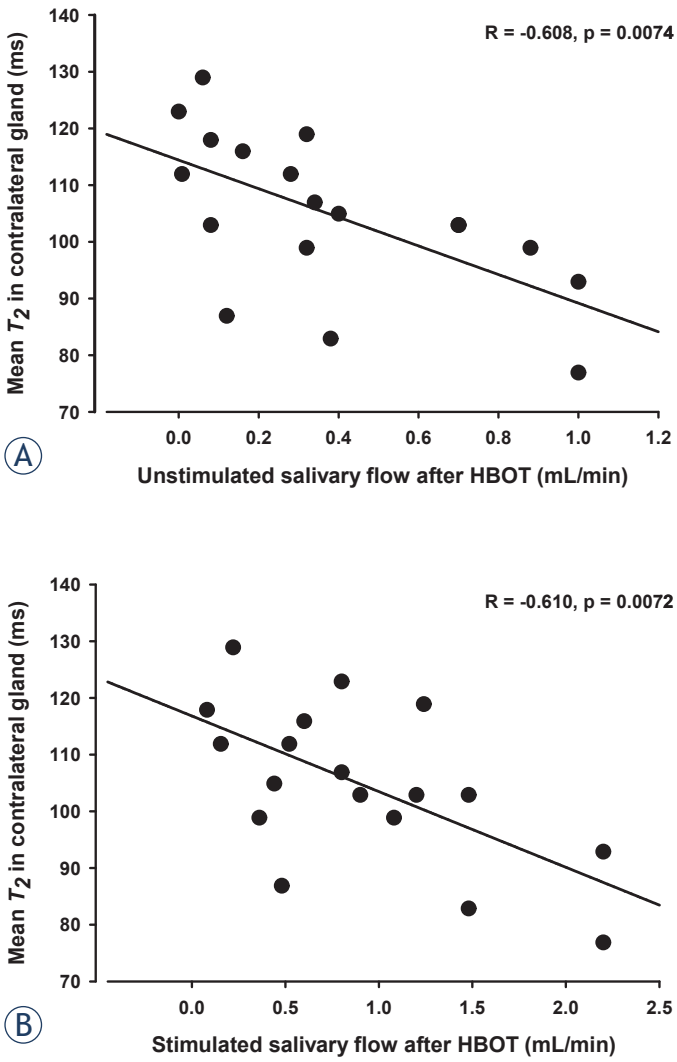

FIGURE 4. A correlation between the mean T_2 values in contralateral parotid glands and unstimulated **(A)** and stimulated salivary flow **(B)** after hyperbaric oxygenation therapy (HBOT).

the growth of new blood vessels and subsequent tissue neovascularisation. In addition, an increase in oxygen levels, improves white cell and fibroblast function, thus enabling further enhancement of wound healing and tissue quality improvement.[32] The effect of HBOT was slightly more pronounced on the ipsilateral side due to the more severe structural changes.

A positive correlation between T_2 variability and the irradiation dose was observed only in contralateral parotid glands prior to HBOT. This confirms that the contralateral side could also be affected with higher doses of radiation. In contrast, absence of any significant correlation on the ipsilateral side could be attributed to rather comparable cumulative radiation doses between patients, *i.e.*, most of them were exposed to doses between 60 to 70 Gy. Furthermore, mean T_2 and T_2 variability were also relatively high on the ipsilateral side and would probably require enrolment of more patients with doses ranging from relatively low (~50 Gy) to relatively high (~80 Gy) to obtain a significant correlation.

Previous studies on the effects of radiation on the function of salivary glands have shown that the reduction of salivary gland activity depends on the dose of radiation and the volume of irradiated tissues.[33] Namely, doses above 60 Gy result in a dramatic decrease in salivary flow rate.[4] The latter is in agreement with the results of functional salivary tests in our patient group prior to HBOT. Since the radiation doses were nearly the same in all patients, we could not find any correlation between the radiation doses received and salivary flow. As a response to HBOT, a significant improvement of salivary gland function was observed in all measured salivary parameters. The results confirm the findings of previous studies demonstrating a subjective reduction of problems related to swallowing, taste sensation and saliva quantity.[34]

A negative correlation between mean T_2 and T_2 variability in the examined slice of ipsilateral parotid glands and unstimulated saliva pH as well as stimulated salivary flow was observed in patients prior to and after HBOT. These correlations can be attributed to the fact that structural changes in glandular tissues influence the function of all gland and subsequent cumulative saliva secretion. In addition, these results are also in agreement with another MRI study showing an increase in apparent diffusion coefficient (ADC) of salivary glands due to radiation injury as well as a correlation between ADC, stimulated salivary flow and xerostomia questionnaire scores.[35]

The present study has several limitations, mainly due to MRI scanning time as well as the comparison of MRI results with the functional salivary tests. Firstly, the achievable resolution in our experimental setup was limited by a reasonable MRI scanning time, *e.g.*, approximately ten minutes per scan for T_2 mapping, therefore allowing only T_2 mapping in the central slice of the parotid gland. Consequently, only a T_2 map of the slice with the largest proportion of glandular tissue was measured and analysed. For the purpose of more in-depth analysis of the parotid structure, the whole area of the parotid gland should be scanned for T_2 mapping; however, this would require unreasonably prolonged scanning time. Secondly, we

analysed parotid glands on both sides. Because of limitations in the experimental setup, allowing only single slice T_2 mapping and clinically applicable salivary tests, only single-sided T_2 values from ipsilateral and contralateral parotid gland were correlated with the salivary tests in each patient. Such analysis does not enable accurate correlation between a single-sided T_2 values with functional salivary testing, which includes the cumulative function of all glands. Namely, in the case of hypofunction of one gland, its function deficit may be compensated by the glands on the contralateral side. Since structural changes observed in T_2 maps on both glandular sides were proportional prior to and after HBOT, this is less likely, suggesting that the function of both glands was affected to some extent and our approach seems still reasonable. Ideally, this could be avoided by using advanced MRI methods, combing T_2 mapping or even ADC mapping with MR functional salivary flow imaging (MR dynamic sialography). However, such complex scanning results in excessively long scanning time.[36]

Conclusions

The results of the present study confirm that T_2 mapping has a potential for the evaluation of the differences between irradiated and normal parotid glandular tissue. In this study, it is shown that T_2 mapping could also be useful in the evaluation of the glandular tissue response to HBOT.

Acknowledgement

This work was supported by Grant No.: P3-0019, Ministry of Higher Education, Science and Technology, Slovenia.

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