

LINEWIDTHS OF PROTON NUCLEAR MAGNETIC RESONANCE PEAKS OBTAINED FOR JAW CYSTS AND ABSCESSSES AT 400 MHz

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We compare the nuclear magnetic resonance (NMR) linewidths of semi-heavy water (HOD) proton peaks in 29 nonhemorrhagic jaw cysts, 8 hemorrhagic cysts, and 19 abscesses. We also investigate the dependence of linewidths on fluid content and radio frequency (RF) application angles. The mixtures used for comparisons were prepared by adding 0.05 mL of each cyst or abscess to 0.95 mL of D₂O. A series of mixtures containing varying amounts of the sample was also prepared. NMR measurements of all mixtures at 400 MHz were acquired using only a 90° RF pulse. The mean linewidths of the cystic groups were quite different ($p = 0.004$) from each other. However, there was a moderate difference between the mean linewidths of nonhemorrhagic cysts and abscesses ($p = 0.048$) and between those of hemorrhagic cysts and abscesses ($p = 0.045$). The linewidth increases linearly with increasing fluid content but decreases with increasing RF application angle. In conclusion, the data suggest that linewidth measurements can distinguish cysts from abscesses. The concentration dependence of the linewidth also suggests that the rapid chemical exchange of protons between free and bound HODs contributes to the broadening of the linewidth.

Keywords: 400-MHz nuclear magnetic resonance, jaw cysts, jaw abscesses, linewidths.

ШИРИНА ЛИНИЙ В СПЕКТРАХ ПРОТОННОГО ЯМР, ПОЛУЧЕННЫХ ДЛЯ КИСТ И АБСЦЕССОВ ЧЕЛЮСТИ НА ЧАСТОТЕ 400 МГц

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Проведено сравнение ширины линий ЯМР протонов полутяжелой воды (HOD) в образцах 29 негеморрагических кист челюсти, 8 геморрагических кист и 19 абсцессов и исследована зависимость ширины линии от содержания жидкости и углов приложения радиочастотного поля (RF). Смеси для сравнения готовили путем добавления 0.05 мл каждой кисты или абсцесса к 0.95 мл D₂O. Приготовлена серия смесей, содержащих различные количества образца. Измерения ЯМР всех смесей на частоте 400 МГц получены с использованием только 90° радиочастотного импульса. Средние значения ширины линий кистозных групп сильно различаются ($p = 0.004$). Однако наблюдается умеренная разница между средними ширинами линий для негеморрагических кист и абсцессов ($p = 0.048$) и геморрагических кист и абсцессов ($p = 0.045$). Ширина линии линейно возрастает с увеличением содержания жидкости, но уменьшается с увеличением RF. Показано, что измерения ширины линии позволяют отличить кисты от абсцессов. Концентрационная зависимость ширины линии также предполагает, что быстрый химический обмен протонов между свободными и связанными молекулами HOD способствует увеличению ширины линии.

Ключевые слова: ЯМР 400 МГц, киста челюсти, абсцесс челюсти, ширина линий.

Introduction. Nuclear magnetic resonance (NMR) relaxation times are used to distinguish jaw cysts from jaw abscesses [1–3]. In addition, magnetic resonance spectroscopy (MRS) is used for spin–lattice (T_1) and spin–spin (T_2) relaxation measurements [4]. NMR and MRS relaxation measurements require the use of inversion recovery (IR) and spin-echo (SE) techniques. These techniques enable the collection of >10 data

points (inversion delays or echo delays) for each of the relaxation times T_1 and T_2 . Therefore, relaxation time measurements are more time-consuming than linewidth measurements.

Since the discovery of NMR, some researchers have investigated the relationship between sample concentration and NMR peak linewidths [5]. However, in recent years, NMR peak linewidth studies have been reported more frequently in [6–18]. These studies have focused on extraction of relaxation times, development of new methods for NMR, and evaluation of line shape analysis and protein dynamics. In other words, studies on NMR peak linewidth have recently attracted many researchers from various fields of NMR.

In a mixture containing less amount of the sample (cyst or abscess) and an extremely high amount of D_2O , the H atoms in the H_2O molecules of the cyst or abscess are replaced by the D atoms in D_2O , forming semi-heavy water (HOD) molecules [19–21]. In other words, in such a mixture, HOD molecules replace all H_2O molecules. HOD molecules eliminate radiation damping by reducing the peak height of the water signal and providing a very symmetrical HOD signal [2]. In addition, the narrowing of the high-field $1/T_2$ distribution ranges given in a recent study indicates that the differentiation possibility of pathological fluids containing excessive D_2O is three to four times that of the same fluids without D_2O [2, 3]. This reduces the overlap between different groups [2]. Because $1/T_2$ is directly related to the linewidth, using the linewidths of mixtures instead of T_2 for statistical comparison is practical. Such an approach can provide a simple method for comparing different groups with MRS and NMR and save experimental time.

This study aimed to compare the NMR linewidths of HOD peaks in jaw cysts and abscesses and investigate the dependence of linewidth on the sample fluid content and radio frequency (RF) application angles. A mixture of 0.05-mL (50 μ L) cyst (or abscess) and 0.95-mL D_2O was prepared for each experiment. The NMR spectrum was recorded at 400 MHz. The mean linewidths of cysts and abscesses were compared statistically. The dependences of the linewidth on the fluid content and RF application angles were determined.

Materials and methods. Thirty-seven jaw cysts and 19 jaw abscesses were collected from the Department of Oral and Maxillofacial Surgery at the Faculty of Dentistry, Dicle University, Diyarbakir, Turkey. Every fluid from the lesion of the jaw region was carefully extracted and transferred to a tube. Each sample was centrifuged for 5 min at 2500g for red cell separation. Of the collected samples, eight cysts were naturally bloody, and the red cells of these fluids did not separate. These samples were considered hemorrhagic cysts. The remaining 29 nonhemorrhagic jaw cysts comprised radicular cysts (mostly), dentigerous cysts, residual cysts, keratocysts, globulomaxillary cysts, and nasolabial cysts. Clinical evaluations for the diagnosis of cysts and abscesses were performed through radiological and histological examinations.

After centrifugation, the mixtures were prepared by adding 0.05 mL of each cyst or abscess to 0.95 mL of D_2O . NMR measurements were performed immediately after the preparation of the mixtures. D_2O (99.9%) containing the D atom was purchased from Merck KGaA (Darmstadt, Germany). To understand the effect of the amount of cyst on the linewidth of the HOD signal, the nonhemorrhagic cyst content of the sample was increased from 20 to 60 μ L in increments of 10 μ L. In addition, samples containing 90-, 120-, and 150- μ L cysts were prepared using D_2O . The total volume of each sample was 1 mL. The same set of samples was prepared for abscesses. Three urine specimens from diabetic persons were similarly prepared to check whether the current method was suitable for detecting the water-reduced peaks of other body fluids.

Precipitation was observed in some samples. To eliminate the effect of precipitation on the linewidth of the HOD signal, the homogeneity of each sample was ensured by carefully shaking the sample before being used. The homogeneity was checked after the NMR measurement and was found to be stable.

Special care was taken to achieve the best shimming. Before starting a single-pulse experiment, the NMR tube was fixed in the correct position on the probe head using a white Teflon plate. Tuning and matching were achieved properly. After very careful shimming using the standard mixture (3% $CDCl_3$ in acetone), a resolution of 0.26 Hz was achieved. This corresponds to the best shimming (Avance NMR Systems ZUEP0102, Manuel P/N Z31369, and DWG-No.1077007). The shims were re-adjusted each time a new sample was inserted into the probe. Measurements were performed without spinning the sample. The effects of various factors such as sample concentration, viscosity, solid particles, paramagnetic ions, and salts on the linewidth of the mixture were reduced 19 times by excessively diluting the cysts and abscesses with D_2O (5% sample in 95% D_2O). The HOD signal was symmetrical from the top to the bottom.

The NMR spectrum of the mixtures was obtained with a 400-MHz Bruker Avance NMR spectrometer using only a 90° RF pulse. The sample temperature was maintained at $20 \pm 1^\circ C$ using an automatic temperature controller unit. Linewidth measurements were made after the spectrum was sufficiently extended, and the linewidths were determined using the cursor from two opposite points of the HOD spectrum corresponding to the half-height of the peak maximum. The linewidth measurement was repeated 10 times for each

sample, and the mean value was taken as the linewidth. The error in determining each linewidth was estimated as ± 0.061 Hz.

Statistical analysis. Samples were grouped as nonhemorrhagic cysts, hemorrhagic cysts, and abscesses. The results of statistical analysis are presented as mean \pm standard deviation (SD). A one-way analysis of variance (one-way ANOVA) and Games–Howel post hoc tests (not assuming equal variance) were used for multiple comparisons with 95% confidence. The mean difference is significant at the 0.05 level ($p \leq 0.05$).

Results and discussion. There was some overlap between the linewidths of the groups. The mean values and SDs of the linewidths of the groups and statistical differences between the groups are listed in Table 1. The mean linewidth of the nonhemorrhagic jaw cysts was the least, that of the hemorrhagic cysts was the largest, and that of the abscesses was between those of the former two. There was a significant difference between the mean linewidths of nonhemorrhagic cysts and hemorrhagic cysts ($p = 0.004$). A moderate difference was found between the mean linewidths of abscesses and hemorrhagic cysts ($p = 0.045$) and between those of the nonhemorrhagic cysts and abscesses ($p = 0.048$). This comparison shows that cysts can be distinguished from abscesses by measuring the linewidth.

TABLE 1. Comparison of the Mean Linewidths of Jaw Cysts and Abscesses

Groups	Samples	Mean \pm SD $\Delta v_{1/2}$	P values		
			1-2	1-3	2-3
Non-hemorrhagic jaw cysts	29	10.26 \pm 0.59	0.048	0.004	0.045
Abscesses	19	10.69 \pm 0.60			
Hemorrhagic jaw cysts	8	11.50 \pm 0.73			

Note. $\Delta v_{1/2}$ denotes the half-height linewidth of groups.

The relationship between linewidth and the nonhemorrhagic cyst content of a sample is shown in Fig. 1. A similar relationship was obtained for abscesses and is not shown here to avoid repetition. The linewidth is linearly proportional to the sample content up to 90 μL , and it deviates from linearity as the content rises above 90 μL . This deviation should be related to the effect of radiation damping on relaxation times and linewidth [22].

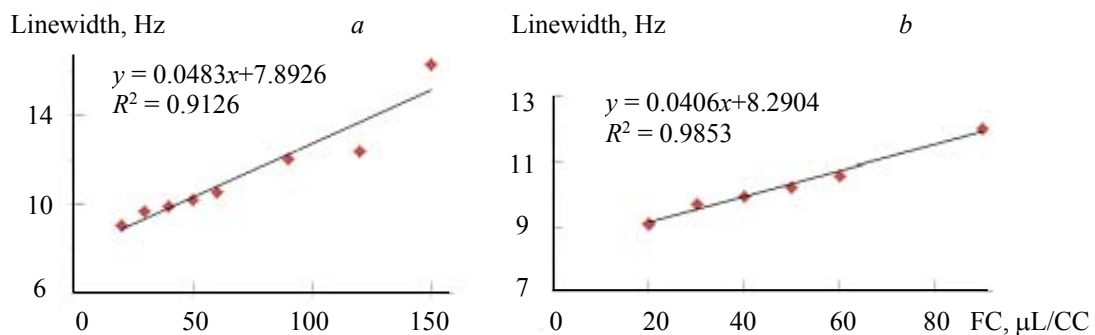


Fig. 1. The least-squares fit of the linewidth versus cystic fluid content FC in a sample containing D_2O (a) and linear part of the relationship between linewidth and cystic fluid content FC (b).

The linear portion in Fig. 1a is shown in Fig. 1b. The least-squares data fit provides the best fit with a correlation coefficient of 0.985. This indicates the rapid chemical exchange of protons between free and bound HOD molecules [23].

The relationship between the linewidth and RF application angle is shown in Fig. 2. As the application angle increases, the linewidth decreases. It is the narrowest at 90° . Therefore, the linewidth directly related to T_2 is achieved by applying a 90° RF pulse.

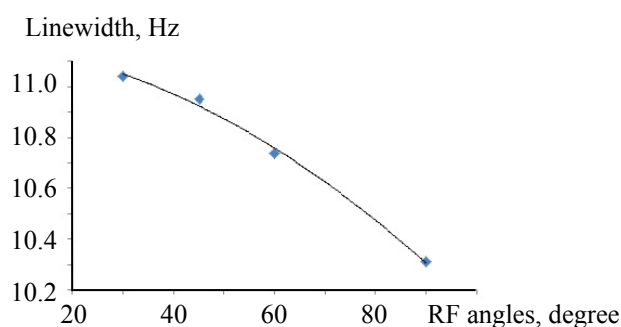


Fig. 2. Best fit for the dependence of changes in linewidth on radio frequency (RF) application angles.

The relationship between the half-height linewidth $\Delta\nu_{1/2}$ and $1/T_2$ is given as follows [24]:

$$1/T_2 = \pi\Delta\nu_{1/2} \text{ or } \Delta\nu_{1/2} = 1/\pi T_2. \quad (1)$$

As shown in Eq. (1), the linewidth is directly proportional to $1/T_2$. Therefore, the molecular information in an NMR relaxation is also available in the NMR linewidth. However, experimental linewidth measurements are influenced by magnetic-field inhomogeneity associated with the NMR machine and the sample-related inhomogeneity. In the present study, the broadening of the linewidth caused by machine-related factors was minimized by adopting the best shimming and tuning in our high-field NMR machine. As stated in the materials and methods section, the effects of sample-related factors such as sample concentration, viscosity, and the presence of solid particles, paramagnetic ions, and salts on the linewidth of the mixture were reduced by 19 times by extremely diluting the cysts and abscesses with D_2O . Therefore, the linewidth broadening of high-field NMR peaks is significantly related to factors such as the lifetime of the excited state, dipolar coupling of the spins, and chemical exchange of the spins in two sites [25, 26]. Hence, prior comparisons of relaxation times between cysts and abscesses can also be made using linewidth measurements.

In the late 1980s, the half-linewidths of the NMR methyl and methylene peaks of plasma samples from healthy persons and patients suffering from cancer and other illnesses were measured and compared for diagnostic purposes [27, 28]. These studies revealed a significant difference between the mean linewidths of the groups and led to the widespread use of the water-suppression pulse techniques. The significant difference between the mean linewidths of cysts and abscesses listed in Table 1 is in agreement with these pioneering studies and is also consistent with previous T_2 studies that showed a significant difference between the mean $1/T_2$ ratios of cysts and abscesses [2, 3]. Statistically significant differences between the mean linewidths of different patient groups found by previous NMR researchers indicate the availability of linewidth measurements for group comparisons [27, 28]. In addition, using only the 90° RF pulse and avoiding time-consuming IR and SE techniques are some of the advantages of linewidth measurements. Moreover, the dependence of relaxation times on frequency showed that the relaxation rates of body fluids decreased significantly at frequencies higher than 60 MHz [29]. Therefore, comparisons of high-field NMR T_1 and T_2 measurements in normal and diseased groups are rare. However, using a mixture containing excessive amounts of D_2O results in narrower relaxation distribution ranges of pathological groups at 400 MHz [2]. Such a narrowing reduces the overlap and increases the likelihood of different patient groups being separated by high-field NMR T_1 and T_2 measurements. This encourages the use of high-field NMR to compare relaxation times in mixtures prepared from normal and diseased body fluids and excessive D_2O . Because the linewidth is proportional to $1/T_2$, high-field NMR and *in vitro* MRS studies are very attractive methods for comparing the HOD linewidths of different mixtures.

Because of radiation-damping effects, the high-field NMR and MRS spectra of biological fluids cannot be obtained using only the 90° pulse for the H_2O proton [22]. Water-suppression techniques are required to obtain a proper water signal and other signals that are suppressed by water [30]. The current HOD spectrum obtained from urine samples of diabetic persons is shown in Fig. 3. This figure shows the resulting signals after removal of radiation damping effects by excessive D_2O dilution. The elimination of radiation damping affecting the water signal and the emergence of urine signals suppressed by water (Fig. 3) demonstrate the ability of the extremely high D_2O -dilution method to detect the NMR and *in vitro* MRS spectra. In addition, relaxation times cannot be measured using the previous techniques used for water suppression and spectrum detection. Additional advanced techniques including J-resolved PRESS and data-processing systems are required to measure T_1 and T_2 under a high field *in vivo* [4]. The current extremely high D_2O -dilution approach

provides a very suitable water signal. Moreover, it shows suppressed peaks in the spectrum (Fig. 3) and reveals that the relaxation times (particularly HOD relaxations) can be measured using traditional methods such as IR and Carr–Purcell–Meiboom–Gill (CPMG) pulse sequences [2].

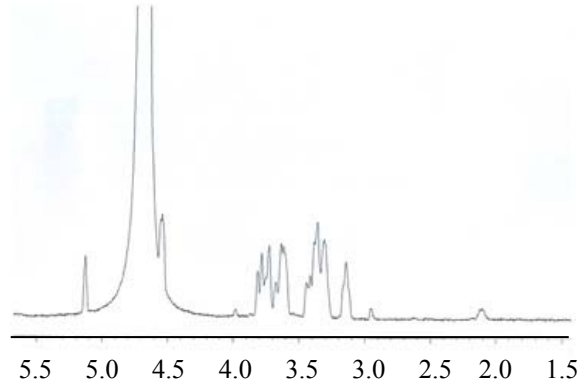


Fig. 3. The 400-MHz NMR spectrum of a 0.05-mL urine sample in 0.95-mL D₂O.

As mentioned earlier, the line shape and linewidth of the NMR spectrum have been used in recent years to study different NMR processes such as the derivation of T_2 , protein dynamics and nuclear spin diffusion, as well as in methodological studies and other analyses [6–18]. Our proposed approach is consistent with these studies and provides a quick way to compare various pathological situations. It may also be used to study other NMR and MRS processes.

It has already been shown that the NMR linewidth increases with concentration [5]. Hence, the concentration dependence of the linewidth shown in Fig. 1b is consistent with the result of previous studies. However, radiation damping distorts the shape, symmetry, and peak linewidth of the spectrum [22]. Therefore, the deviation from linearity shown in Fig. 1a is directly related to radiation damping. In some high-field NMR studies, a RF application angle smaller than 90° was used to avoid radiation damping and obtain a suitable NMR peak [30]. Accordingly, the component of M_0 magnetization was measured instead of M_0 to reduce the peak height. In this case, the linewidth increases to maintain a constant peak area. The dependence of the peak linewidth of a mixture on the RF angles (Fig. 2) shows that the narrowest linewidth is obtained for an RF angle of 90° . However, angles less than 90° can be used to eliminate the radiation-damping effects.

In the H₂O and D₂O mixtures, H protons are replaced by D, and HOD molecules are formed [19–21]. Currently, cysts and abscesses contain H₂O solutions. In the mixture of cysts and abscesses, the chemical exchange of H protons occurs between the solvent (HOD) and sample [20, 21]. The linear relationship shown in Fig. 1b corresponds to rapid chemical exchange. Indeed, in the case of rapid proton exchange between free solvent and solute (e.g., cysts, abscesses), the mean linewidth, $\Delta\nu_{1/2}$, can be expressed as follows:

$$\Delta\nu_{1/2} = P_f(\Delta\nu_{1/2})_f + P_b(\Delta\nu_{1/2})_b, \quad (2)$$

where P_f and P_b are the probabilities of free and bound phases, respectively [23]. For the mixture presented in Fig. 1b, $P_f = 1$ and $P_b = kC$; then Eq. (2) can be simplified to the equation

$$\Delta\nu_{1/2} = m + nC, \quad (3)$$

where m and n are equal to $P_f(\Delta\nu_{1/2})_f$ and $k(\Delta\nu_{1/2})_b$, respectively; C is the amount of cyst or abscess in the sample. The relationship shown in Fig. 1b is as follows:

$$\Delta\nu_{1/2} = 8.2904 + 0.0406C. \quad (4)$$

The experimentally derived Eq. (4) is completely equivalent to Eq. (3), which was theoretically obtained for rapid chemical exchange. Thus, Eq. (4) expresses the contribution of the rapid chemical exchange of H protons between the free and bound HOD to the dipolar broadening of the linewidth. The contribution of rapid chemical exchange to linewidth broadening has also been reported in many previous studies [26].

Conclusions. The mean linewidths of jaw cysts are significantly different from those of jaw abscesses. This suggests that linewidth measurements have strong validity for distinguishing jaw cysts from jaw abscesses. This conclusion may be extended to other body fluids. The linewidths of the mixtures increase with increasing cyst or abscess content; however, they decrease with increasing angles up to 90°. The similarity of Eq. (3) derived for the rapid chemical exchange to the linear relationship obtained in Fig. 1b shows that the rapid chemical exchange of H protons between free HOD and the sample (cystic fluid or abscess) contributes to the linewidths.

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