Spectroscopy 19 (2005) 181–190
IOS Press

Clinical application of *in vivo* proton (^{1}H) MR spectroscopy in musculoskeletal tumors

Chien-Kuo Wang [a,b], Tsyh-Jyi Hsieh [a], Twei-Shiun Jaw [a,b], Jau-Nan Lin [b], Gin-Chung Liu [a,b] and Chun-Wei Li [c,*]

[a] *Department of Medical Imaging, Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung Medical University, No. 100, Tzyou 1st Road, Kaohsiung 807, Taiwan*
[b] *Faculty of Medicine, Kaohsiung Medical University, No. 100, Tzyou 1st Road, Kaohsiung 807, Taiwan*
[c] *Faculty of Medical Radiation Technology, Kaohsiung Medical University, No. 100, Tzyou 1st Road, Kaohsiung 807, Taiwan*

Abstract. A technique called *in vivo* magnetic resonance spectroscopy (MRS) can be performed along with magnetic resonance imaging (MRI) to obtain information about the chemical content of musculoskeletal lesions. This information can be used for several clinical applications, such as improving the accuracy of lesion diagnosis and monitoring the response to cancer therapies. Initial MRS studies of musculoskeletal tumors show promising results, and the technique has been incorporating into the MRI routine protocols. This article introduces ^{1}H MRS of the musculoskeletal tumors, reviews the literature, discusses current methods and technical issues, and describes applications for treatment monitoring and lesion diagnosis.

Keywords: *In vivo* proton MRS, MRI, musculoskeletal tumors

1. Introduction

The first *in vivo* magnetic resonance spectroscopy (MRS) studies of tumors measured resonances from phosphorus atoms (^{31}P). These studies showed that measurable variations in phospholipid metabolism could be detected and used for diagnosing cancer and monitoring the response to treatment [1]. More recently, there has been growing interest in cancer research using hydrogen (^{1}H) MRS [2], because of its higher sensitivity than ^{31}P MRS. The information regarding cellular chemistry obtainable from *ex vivo* or *in vitro* proton (^{1}H) MRS may be helpful.

Choline and its derivatives are thought to represent important constituents in the phospholipid metabolism of cell membranes [3]. A resonance from choline-containing compounds (tCho) is present at a chemical shift of 3.2 ppm with *in vivo* ^{1}H MRS. Elevation of tCho peak is thought to represent increased membrane phospholipid biosynthesis and also to be an indicator of increased cellular proliferation [4,5]. Thus, the increased tCho concentration in neoplastic tissues may be a reflection of increased membrane turnover by replicating cells. *Ex vivo* studies have been performed to identify the different choline compounds giving rise to the tCho resonance at a chemical shift of 3.2 ppm. High-resolution ^{1}H spectra acquired from biopsy tissues have shown that the tCho resonance is actually a superposition

*Corresponding author: Chun-Wei Li. Tel.: +886 73208235; Fax: +886 73154208; E-mail: wangck@kmu.edu.tw.

of several resonances [6]. The primary constituents are those with a trimethylamine moiety, $R–(CH_2)_2–N^+–(CH_3)_3$, including free choline, phosphocholine, phosphatidylcholine, and glycerophosphocholine. Millis et al. [7] reported that *ex vivo* NMR-visible phosphatidylcholine level as an estimate of total tissue cell membrane phospholipid mass in pleomorphic liposarcoma was three times higher than that in dedifferentiated liposarcoma. The pleomorphic liposarcoma is the most aggressive subtype of liposarcoma. Mukherji et al. [8] also reported that the choline/creatine ratio obtained by *in vitro* proton MRS was capable of helping to distinguish malignant tumors of the extracranial head and neck from uninvolved muscle. The choline/creatine ratio is significantly higher in squamous cell carcinoma than in muscle.

The first *in vivo* ^{1}H MRS report of bone and soft tissue tumors did not focus on the level of tCho at 3.2 ppm [9], but subsequent studies performed with ^{1}H MRS noted that a resonance from tCho at a chemical shift of 3.2 ppm was commonly present in malignant lesions of breast [10,11], prostate [12], and cervix [13]. In our initial study about *in vivo* ^{1}H MRS of musculoskeletal tumors, tCho can be used as an indicator of malignancy with clinical 1.5 T scanners [14]. Some groups have also shown that the tCho peak decreases or disappears in response to chemotherapy treatment. The results of these studies are encouraging, and with continued technical development it seems likely that MRS will become a useful tool in detecting and managing bone and soft-tissue malignant tumors.

2. Technical issues

Historically, ^{1}H MRS research has been focused mainly on the brain, in part because use of this technique on the brain poses fewer technical challenges than on other organ sites. Most of the research and development in the field of MR has been focused on brain applications; as a result, commercial MR systems are generally better optimized for brain rather than musculoskeletal studies. As a result of increased interest by clinicians and researchers in the application of MRS, many technical advances are now taking place that are improving the quality and reliability of MRS.

The feasibility of clinical *in vivo* MRS depends heavily on the ability to investigate a well-defined volume of tissue within the organ of interest. Generally, spectra can be acquired using single voxel spectroscopy (SVS) or multiple voxels methods. Single voxel method can define a voxel by the combination of three orthogonal planes, such as stimulated echo acquisition mode (STEAM) [15] and point-resolved spectroscopy (PRESS) [16] in ^{1}H MRS. The advantage of this method is that the position and size of the VOI are controlled easily by changing transmitter frequency and gradient pulse strength. Moreover, the magnetic field homogeneity can be adjusted specifically over the VOI. The primary disadvantage of these sequences is that only data from a single voxel data are collected. The other class of localization techniques which uses pulsed magnetic field gradients is the phase encoding method [17,18]. It maps the spatial distribution of MRS signals arising throughout an excited volume. By employing phase-encoding gradients used in normal imaging in two or three directions, an array of columns (2D-CSI) or rectangular voxels (3D-CSI) can be defined. Local spectroscopic information can be obtained by analyzing each volume independently. The CSI technique has several distinct advantages. First, spectroscopic data is obtained from multiple voxels simultaneously. Second, the spatial location of each voxel can be related easily to the proton image of the same object. Third, the voxels can be translated to center on the VOI after the data collection. Fourth, a metabolic image can also be derived which reflects the local variation of specific metabolite. Fifth, spectra from different voxels can be combined retrospectively to yield better S/N and more precisely define the VOI for irregularly shaped lesions. The disadvantages

of CSI include baseline distortions introduced by the pre-acquisition delay for eddy currents to decay, poorer homogeneity than the single voxel method with localized shimming, and data post-processing is time-consuming.

Most musculoskeletal MRS studies so far have used SVS to localize the chemical signals to a single, cuboid volume centered on the lesion of interest. The acquisition of ^{1}H MRS requires two additions to the sequence to remove the signal from water and fat. One way to suppress these signals is to use the chemical shift selective pulses and gradient dephasing method [19,20]. Another approach for fat suppression includes by carefully selecting the VOI to prevent the contamination of the fat, using a longer TE [21] or by spatially selective pulses [22].

2.1. Position of VOI

A typical MRS study of musculoskeletal tumors is performed immediately after acquiring dynamic contrast-enhanced (DCE) MR images. Decisions about the placement of the MRS voxel are usually based on a review of the lesion morphology and the kinetics of contrast agent uptake while the patient is still in the magnet. With SVS, the correct positioning of the VOI for suspicious lesions during ^{1}H MRS is very important. In DCE gradient-echo MR studies, this technique provides clinically useful information by depicting tissue vascularization and perfusion, capillary permeability, and composition of interstitial space [23]. The identification of well-vascularized, viable areas within a tumor can help to position the VOI and boost the confidence in ^{1}H MRS. The voxel should be placed so that it contains as much of the hypervascular region within the lesion as possible. In studies using MRS to monitor response to treatment, the voxel size and position can be adjusted to cover the same anatomical region of the tumor, decreasing the voxel size as the tumor shrinks.

2.2. Quantification

The measured tumor spectrum can be either presented by the ratio which respect to a stable metabolite, such as creatine or water, or presented by the absolute concentration. The possible large variation of the creatine or water prevents the usage of the ratio in the tumors study, especially in the tumor follow-up study. Absolute quantification of metabolite concentrations offers a number of advantages when evaluating *in vivo* MR spectroscopic data. For example, it improves the ability to characterize pathological and developmental changes in metabolite levels and it further enhances the power of MRS in exploring disease processes through basic biochemical studies. In addition, this process is beneficial when conducting comparative studies for clinical trials based on data obtained from different sites.

Numerous absolute quantification techniques have been proposed for MRS [24–26]. These techniques involve the calibration of *in vivo* signals from a VOI through comparison with either an internal or an external reference. For an internal reference, the signal used is from a known endogenous compound that has a defined concentration, such as creatine [27] or water [28,29]. The disadvantage of this method is the possibility of big variation of the water or creatine concentration inside the tissue on different subjects or disease states. For external reference, a voxel of identical size and identical acquisition parameters is placed in a phantom containing the reference compound at a known concentration. Variations in coil geometry, RF homogeneity, and flip angle over the areas occupied by both the tissue of interest and the calibration phantom must be taken into account and re-calibrated if necessary. Differences in T1 and T2 between the phantom and the tissue should also be considered. A more reliable quantification result can be obtained by this method with the trade-off of more examination time which is an important factor that

needed to be considered in clinical applications. Both internal and external referencing methods need correction for differences in relaxation rates, which are difficult to measure in individual subjects.

3. Applications

3.1. Diagnosis

Improvement in the treatment and outcome of patients with bone and soft tissue tumors requires the development of diagnostic tools that can help to accurately characterize musculoskeletal tumors in a noninvasive and reliable manner. The most important application for musculoskeletal MRS is to distinguish benign from malignant lesions before biopsy. tCho can be used as a marker of malignancy.

In patients with malignant tumors, a resonance at 3.2 ppm attributed to a choline-containing compound was detected (Fig. 1). The position of the VOI, including the early enhanced regions inside the lesions, is determined by DEC MR images. The early enhanced regions represent areas of high biologic activities, such as cellularity, cell turnover time, and neovascularity. In malignant tumors, these areas may contain more choline-containing compounds. Choline might not be detected in the densely ossifying malignant tumor [14] or in the tumors with severe necrosis or hemorrhage. The susceptibility effects due to the mineralization or hemosiderin and less solid components may account for the false-negative choline uptake in these cases.

Signal contribution arising from fat in the 0.9–2.1 and 5.3 ppm regions decreased with increasing TE, resulting in an improved spectral resolution. However, with increasing TE, a decreasing spectral signal-to-noise ratio was observed due to relaxation losses. In our experience, the intermediate echo time (135 ms) seemed to be the most reliable in the multiecho acquisition approach. The scan time to complete the MR spectroscopy was approximately 10 minutes if only using one echo time. Further studies should be performed in a larger group of patients.

In most patients with benign lesions, no choline signal was detected (Fig. 2). Previous reports have demonstrated that benign tumors which are hypercellular may show elevated choline in brain lesions as well as head and neck tumors [30–32]. In addition, a large number of inflammation-related cells produced by inflammatory processes may also result in a high choline peak [33]. Therefore, choline could be detected in the benign lesions with cellular proliferation and/or increased cell density (Fig. 3). Three of 17 benign lesions in our patients were false-positive. These lesions included one perineurioma, one giant cell tumor (GCT), and one abscess. The histology of perineurioma as well as GCT showed hypercellularity. The histopathology of the patient with abscess demonstrated an abundance of inflammatory cells in the wall of abscess.

According to our results, using *in vivo* ^{1}H MRS to evaluate the patients of large bone and soft tissue tumors based on the presence of choline, we found a sensitivity of 95% (18/19), specificity of 82% (14/17), and an accuracy of 89% (32/36) for diagnosis. These results are very encouraging, especially considering that the determination of malignancy was done without considering any other diagnostic or historical information that would normally be available clinically.

3.2. Monitoring response to treatment

A second and perhaps more promising application is the use of MRS for predicting response to treatment. Current clinically available methods such as palpation and imaging rely on changes in tumor size, which take several weeks before any changes are detectable. *In vivo* MRS, in contrast, detects changes in

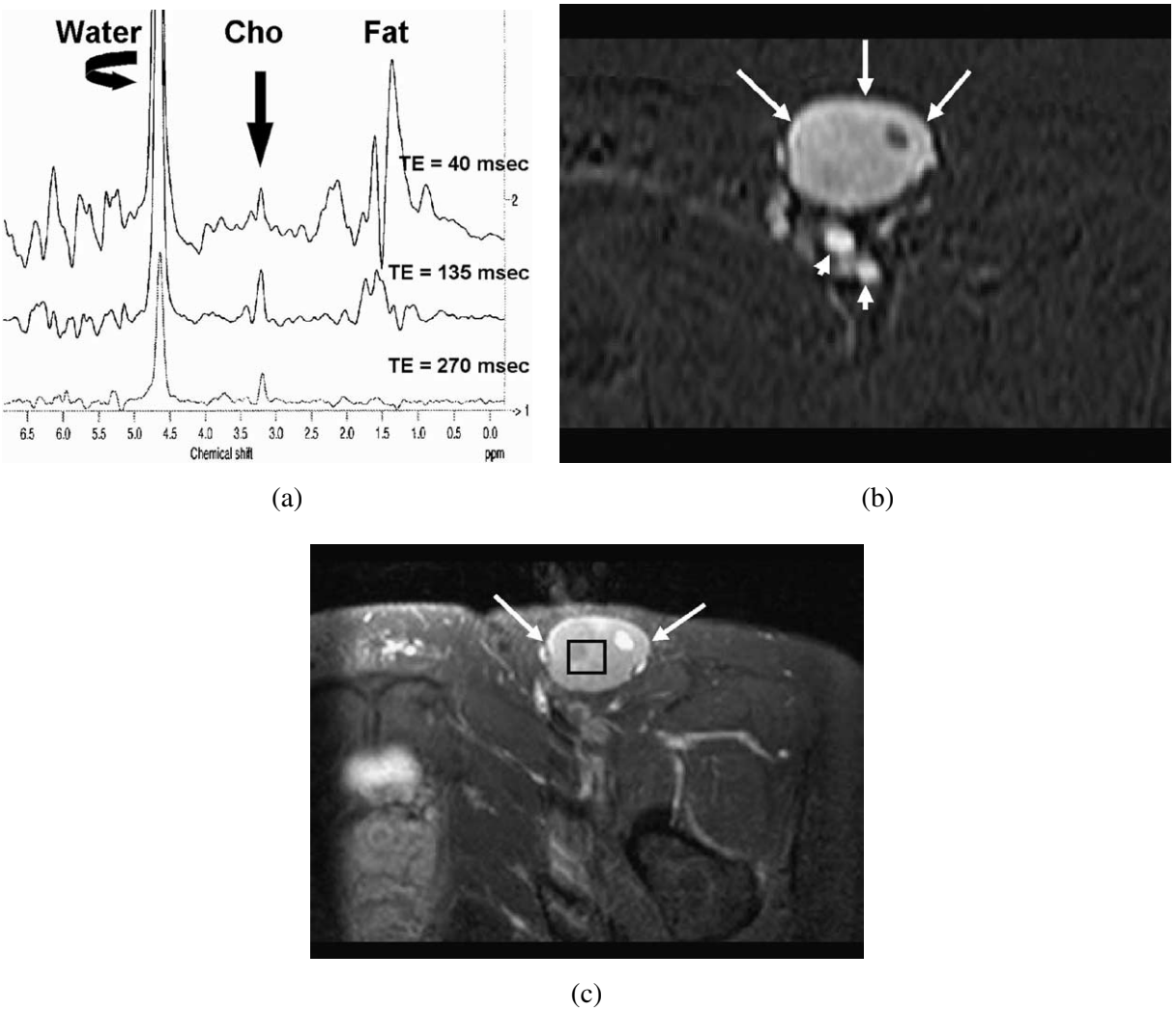


Fig. 1. (a) Spectra were acquired at different TEs in a patient with lymphoma. Choline (3.2 ppm) was found in all three spectra. This was a true-positive MR spectroscopy for malignancy. Resonances derived from fat and water were present. The peaks of water were the result of water suppression. Cho = choline. (b) An early enhancing tumor (arrows) at left inguinal area was demonstrated in the arterial phase of transverse dynamic contrast-enhanced subtraction MR image (15/4.1). The femoral arteries (small arrows) were also evident. (c) This tumor (arrows) was homogeneously enhanced on the corresponding delayed contrast-enhanced MR image with fat suppression (500/14). The position of volume of interest was showed.

intracellular metabolism that would occur before any gross morphological change [34]. The first report using tCho measurements to detect treatment response in extracranial lymphoma and germ cell tumors was by Schwarz and colleagues, who observed that the changes in the tCho : water ratio following treatment were found to predict subsequent patient response.

Expanding on this observation, our group performed a study designed to determine whether changes in tCho could provide a biomarker of clinical response for malignant musculoskeletal tumors after each course of chemotherapy. Two cases of lymphomas showed significant decrease of tCho and tCho : water ratio and it was compatible with the response to the chemotherapy in the changes of tumor size (Fig. 4). One case of alveolar soft part sarcoma with no prominent decrease of tCho or tCho : water ratio had

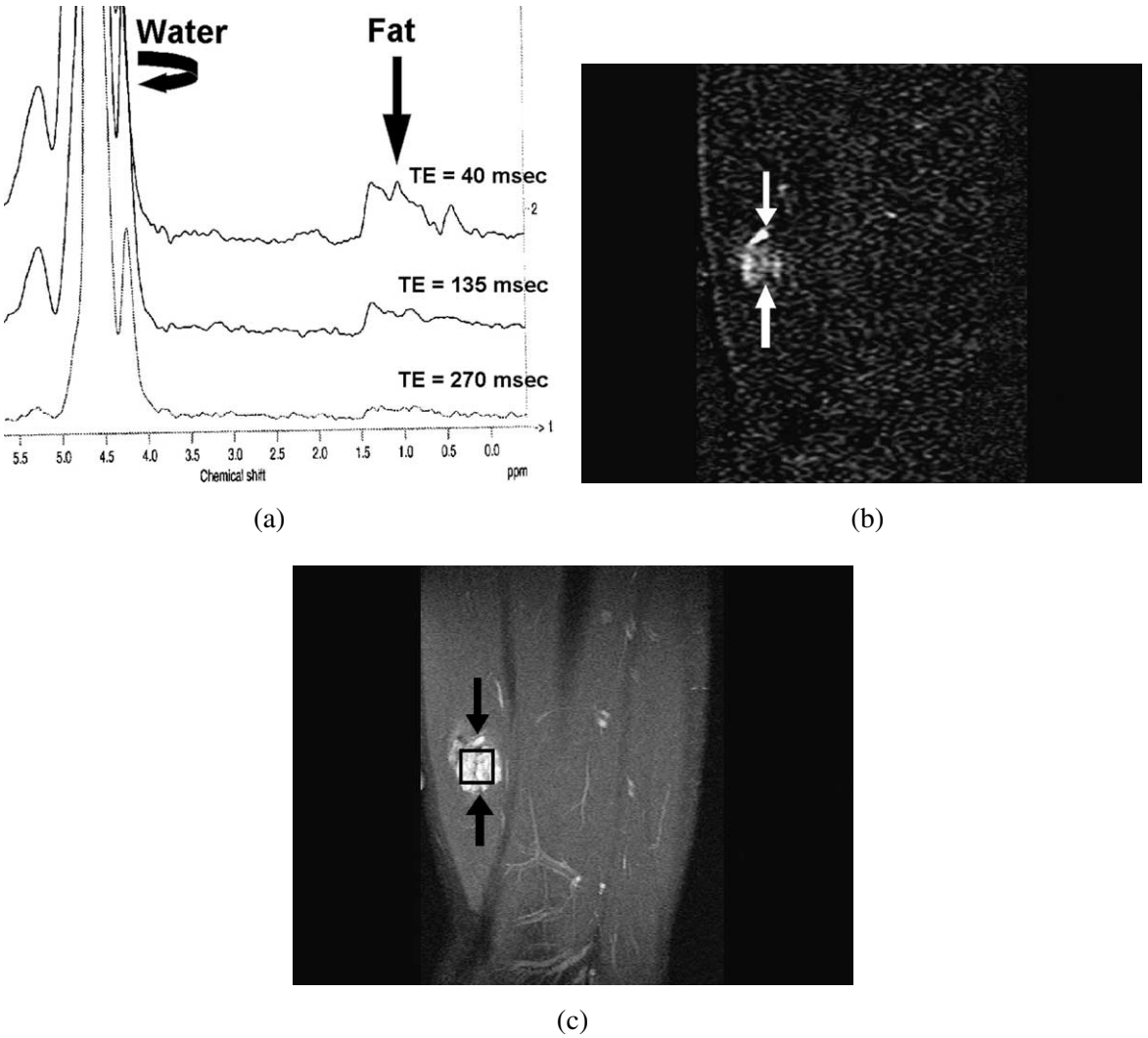
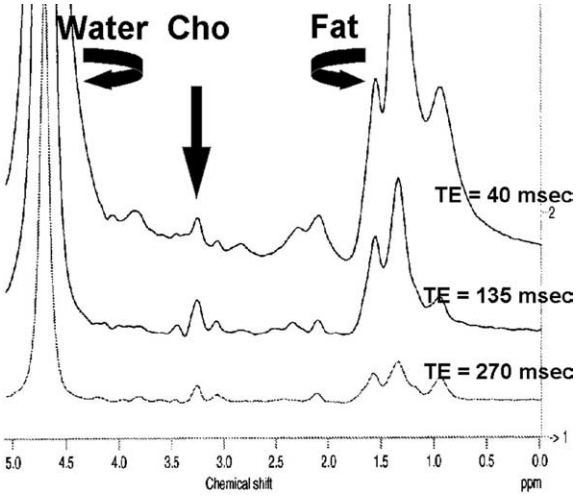


Fig. 2. (a) Spectra acquired at different TEs in a patient with intramuscular hemangioma showed no choline resonance above the baseline noise at 3.2 ppm. This was a case of true-negative MR spectroscopy study. (b) Sagittal dynamic contrast-enhanced subtraction MR image (15/4.1) of thigh in the same patient showed a heterogeneously enhancing tumor (arrows) in the arterial phase. The tumor was gradually enhanced in the late phase (not shown). (c) Sagittal delayed contrast-enhanced MR image with fat suppression revealed a diffusely enhancing tumor (arrows). The volume of interest fitted the size of tumor.

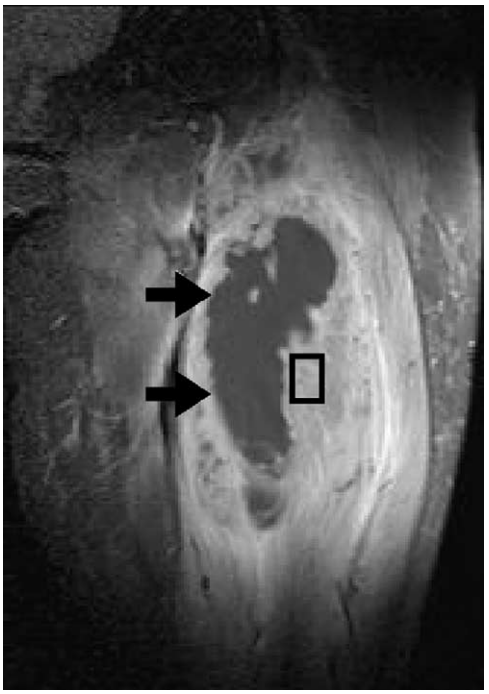
no shrinkage of tumor. Pulmonary metastatic tumors occurred in this patient several months later. In our experience, the variations of water were large during the period of chemotherapy. The cell death might result in the changes of water. Water might not be a good internal reference in the follow-up of chemotherapy. tCho changes were more sensitive than tCho : water ratio under this situation.

4. Conclusions

The reliability and quality of MRS data will only improve as further refinements in MR systems and techniques continue to occur. So far, the promising results from multiple institutions suggest that MRS,



(a)



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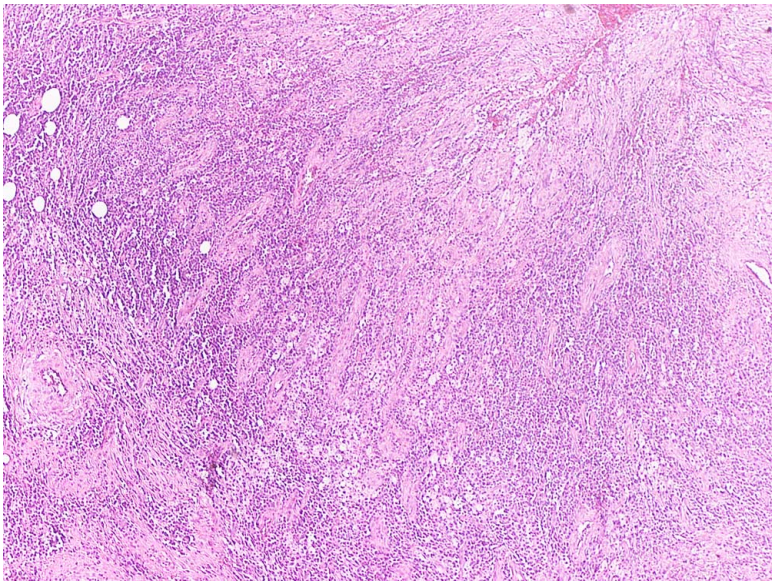

(c)

Fig. 3. (a) False-positive spectra acquired at different TEs in a patient with extensive abscess of thigh showed positive choline findings. (b) Coronal delayed contrast-enhanced MR image with fat suppression (500/14) depicted a central unenhanced area (arrows) compatible with abscess formation. The position of volume of interest was marked. (c) Photomicrograph of a pathologic specimen (H&E stain; original magnification, $\times 8$) from the abscess wall demonstrated dense inflammatory infiltrate in soft tissue with vascular proliferation.

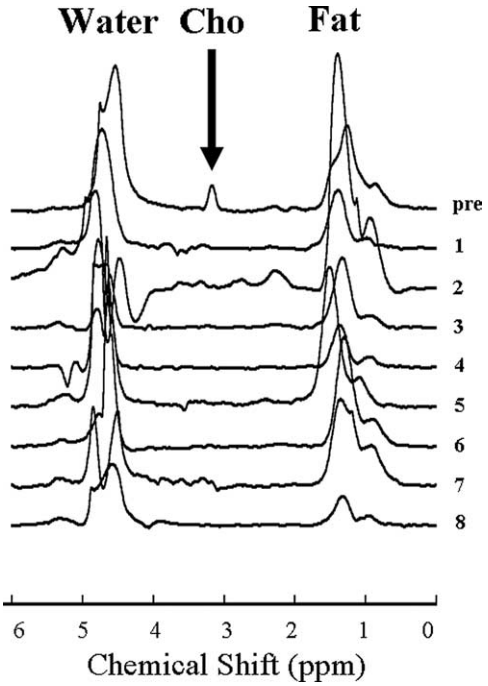

Fig. 4. The proton MRS changes of a patient with lymphoma after 8 courses of chemotherapy were showed.

along with MRI, will have an increased role in the clinical assessment of musculoskeletal tumors in the future. However, large multicenter trials are still needed before the tCho biomarker can be widely used to guide diagnostic decisions and to predict response to therapy.

Acknowledgements

The authors acknowledge support from the grant (NSC 93-2314-B-037-047) of National Science Concil, Taiwan and special thanks to Yuan-Yu Chiau, RT, and Feng-O Shu, RT, for MR technical assistance and Sharon Huang and Faline Lin for preparation of this manuscript.

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