# Diffusion MRI and in-vivo proton MR Spectroscopy Study of the Differentiation of Malignant Breast Tissue of Breast Cancer Patients and the Normal Breast tissue of Healthy Lactating Women Volunteers

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**Introduction**: Various magnetic resonance (MR) methods have been explored for the differentiation of malignant from benign breast lesions. Breast cancer is also seen during pregnancy and lactation. Pregnancy associated breast cancer is one of the most common pregnancy associated malignancies, second only to cervical cancer[1]. Although such incidences are low, it is associated with poor prognosis[2]. tCho detected through in-vivo proton MRS and ADC measurements from diffusion imaging (DWI) have emerged as strong MR biomarkers for the differentiation of malignant, benign and normal breast tissues. tCho is not specific to malignant breast lesions but can be seen in benign lesions, normal breast tissues of healthy volunteers and in healthy lactating women, thereby decreasing the specificity of in-vivo proton MRS in breast cancer diagnosis[3]. The observation of tCho has been reported in normal lactating breast and cancer affected lactating breast tissues[4] but no attempt was made to quantify the tCho concentration. This emphasizes the need for quantitative assessment of tCho levels during lactation to develop an unambiguous approach for the differentiation of breast malignancy from normal physiological changes. Recently we reported[5] the use of DWI and MRS in such patients and in this study the results of the MR characteristics (tCho concentration and ADC values) in more number of malignant breast tissue of locally advanced breast cancer (LABC) patients and the normal breast tissue of lactating women volunteers are reported.

**Material and Methods**: For this study 16 LABC patients (n=16; age: 43.5 ± 7.2 yrs; range 36-55) with histologically proven infiltrating ductal carcinoma (IDC) and thirteen normal healthy lactating women volunteers (n=13; age: 28.8 ± 4.8 yrs; range 22-35 yrs) were recruited. Written informed consent was obtained and Institute ethics committee approved the study. MR investigations were carried out using a phased array breast matrix coil at 1.5 T (Siemens, Avanto). T2-weighted coronal images were obtained using standard SE sequence and fat suppressed MR images in the transverse, sagittal and coronal planes. The in-vivo proton MRS using a single voxel PRESS sequence was carried out. Typical water peak line-width ranged from 8 to 22 Hz. A frequency-selective pre-saturation pulse for water suppression was used with a bandwidth of 50 Hz. Spectral lipid suppression was achieved using a bandwidth of 1.8 ppm with the start and end frequencies for the fat region set at 2.2 and 0.4 ppm, respectively. The experimental parameters used were: TR=1500 ms, TE=100 ms, averages=128, total acquisition time was 3.18 minutes. An additional spectrum of the same voxel without water and lipid suppression was obtained for the concentration calculation using water signal as an internal reference. tCho concentration was calculated using water as an internal reference as reported earlier by us (4). DWI was carried out in malignant and lactating women, using a single-shot EPI sequence with TR=5000 ms, TE= 87 ms, EPI factor =128, acquisition matrix = 128 x 128, and slice thickness = 4 to 5 mm without any inter slice gap, and b=0, 500 and 1000. ADC was calculated drawing small circular ROIs of 5 pixels on the tumor region in malignant and whole breast in lactating women.

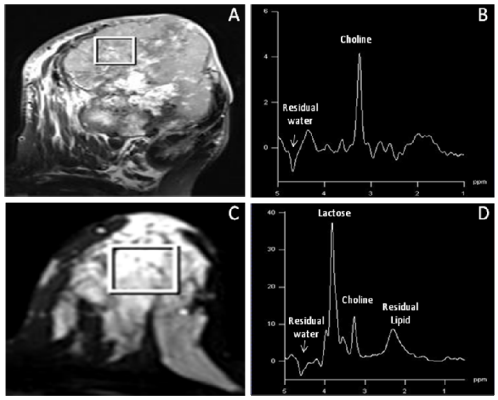


Figure 1: (A) T2-weighted fat suppressed axial image of a LABC patient showing the location of the voxel from which the proton MR spectrum (B) was obtained with water and lipid suppression. (C) T2-weighted fat suppressed axial image from the normal breast tissue of a lactating women volunteer showing the voxel from which the proton MR spectrum (D) was obtained with water and lipid suppression showing the residual water and lipid peaks along with the tCho and the lactose peaks

**Results**: A typical in-vivo proton MR spectrum of a malignant breast tissue of a patient and that from the normal breast tissue of a lactating woman volunteer is shown in Figure 1. Choline peak (comprising of free choline, phosphocholine (PC) and glycerophosphocholine (GPC) and termed as total choline, tCho) at 3.2 ppm was observed in all malignant patients and in 11/13 lactating women. The mean tCho concentration for malignant cases were 3.71 ± 2.31mmol/kg (range 0.31-9.58) and is similar to that obtained from lactating breast i.e., 3.66 ± 1.68 mmol/kg (range 1.50-7.73). In addition to tCho resonance, a lactose peak at 3.8 ppm was observed in 11/13 lactating women which was absent in all malignant breast cancer patients. Mean ADC for malignant breast tissues was $1.01\pm0.10\times10^{-3}$ $mm^2/s$ (range 0.81-1.16) whereas in the lactating breast issue of women volunteers the value was $1.61\pm0.21\times10^{-3}$ $mm^2/s$ (range 1.23-2.11).

**Discussion**: MRS studies showed that tCho can be used as a biomarker of breast malignancy however it has been observed in many benign lesions, normal breast tissues of volunteers and in the normal parenchyma of lactating women, which decreases the specificity of proton MRS in the diagnosis of breast cancer. Thus in the present study, we evaluated quantitatively the concentration of tCho in healthy lactating women and in LABC patients by in-vivo proton MRS and DWI for the differentiation of normal breast tissue of healthy lactating women from that of the malignant lesions of breast cancer patients. The concentration of tCho was similar in both the groups[5]. The high tCho in malignant tissues of breast cancer patients is due to increased cellular replication and increased synthesis of cellular membranes associated with malignancy. While increased tCho concentration seen in lactating breast tissue may be due to the process of ongoing lactogenesis in the breast. However the observation of lactose resonance peak at 3.8 ppm might be unique to the breast tissue of lactating women and can be used to differentiate from malignant breast tissue of LABC patients. The lactose peak was assigned on the basis of previous literature reports[6] and also verified by using in-vitro NMR. Further our data indicated that low ADC in malignant breast tissues which may be due to high cellularity, reflecting the underlying histological pattern of densely packed randomly organized tumor cells. However, the ADC of the normal breast tissue of lactating women volunteers was similar to that seen for normal healthy volunteers ($1.88 \pm 0.20 \times 10^{-3}$ $mm^2/s$). Increased ADC seen in the normal breast tissue of lactating women might indicate increased parenchymal density, more glandular content and expanded extracellular space that contains myxoid matrix with high water content and the presence of milk[7]. In conclusion, our study demonstrated that the observation of a lactose peak at 3.8 ppm may be unique to lactating women and they also show a high ADC value compared to malignant tissue of patients. Thus, the presence of a lactose peak with high ADC value may be useful for the differentiation of the changes that occur in breast tissues due to normal physiological conditions as compared with malignant transformations of tissues.

**References**: (1) Loibl S, von Minckwitz G, Gwyn K, et al. Breast carcinoma during pregnancy. Cancer 2006;106:237-46; (2) DiFronzo LA, O'Connell TX. Breast cancer in pregnancy and lactation, Surg Clin N Am 1996;76:267-278; (3) Sharma U et al., In vivo $^1$H MR spectroscopy in the assessment of the therapeutic response of breast cancer patients, NMR Biomed. 2011; 24, 700-711; (4) Stanwell P, Gluch L, Clark D, et al. Specificity of choline metabolites for in vivo diagnosis of breast cancer using $^1$H MRS at 1.5 T. Eur Radiol 2005; 15:1037-43; (5) Sah RG, Khushbu A, et al. J Magn Reson Imaging (accepted); (6) Jagannathan NR, Kumar M, Seenu V, et al. Evaluation of total choline from in vivo volume localized proton MR spectroscopy and its response to neoadjuvant chemotherapy in locally advanced breast cancer. Br J Cancer 2001;84:1016-1022; (7) Espinosa LA, Daniel BL, Vidarsson L, Zakhour M, Ikeda DM, Herfkens RJ. The lactating breast: contrast-enhanced MR imaging of normal tissue and cancer. Radiology 2005;237:429-36.