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Towards Early Diagnosis and Assessment of Cancer: Role of MRI and in-vivo MR Spectroscopy (MRS)

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SUMMARY

Cancer is a major disease that affects men and women, worldwide, while breast cancer is the leading cause of cancer related deaths in women. Early diagnosis is essential for timely initiation of treatment which would improve the quality and overall survival of patients. Last two decades has seen development of non-invasive MRI methods like contrast MRI, diffusion and perfusion MRI for breast cancer diagnosis. MRI is useful for preoperative staging; follow response to therapy, and to detect local recurrences; however it has poor specificity in differentiating benign from malignant lesions, even with the use of contrast agents. Both diffusion MRI and *in vivo* MR spectroscopy (MRS) have shown great potential to increase the specificity of MRI. This article presents a review of the results obtained from our Institute on the potential of various MRI and MRS methods in the early diagnosis and assessment of tumor response of breast cancer patients.

Key Words **:** Magnetic resonance imaging (MRI); *in vivo* proton (^{1}H) magnetic resonance spectroscopy (MRS); breast cancer; diagnosis; assessment; tumor response.

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INTRODUCTION

Cancer affects men and women of all ages, race and class. There is an alarming raise in the incidences of various cancers and considering its progressive nature, early diagnosis and treatment is essential for improving the survival and the quality of life of patients. Despite the availability of large number of investigational methods like biochemical and imaging modalities, the diagnosis of cancer is challenging. Various diagnostic methods are used routinely like X-ray, mammography, ultrasound, computed tomography, magnetic resonance imaging (MRI) and positron emission tomography for diagnosis. However, the 'gold standard' is still the histopathological evaluation of biopsied tissues. Further prognosis and survival rates vary widely depending on the cancer type, staging and treatment regimens given to the patient. Thus, accurate diagnosis of cancer at an early stage would be of immense use to clinicians for timely intervention and for initiation of appropriate treatment that would improve the quality and the overall survival rate of patients.

During the past two decades, extensive research has been directed towards the development of non-invasive imaging methods that are highly sensitive, specific and cost effective to improve the diagnosis. In this direction various MR imaging methods like dynamic contrast enhanced MRI (DCEMRI), diffusion MRI (DWI) and perfusion MRI have been evaluated at various centers. The MR images that are produced using a powerful MRI scanner are the spatial display of the distribution of hydrogen nuclei (protons) present in body tissues. The advantages of MRI include high-resolution anatomical images in multiple planes with high soft-tissue contrast resolution and the use of non-ionizing radiation. In addition to structural characterization, real time brain functions, blood flow etc. can also be measured using MRI. The sensitivity of detection of cancer with MRI is high; however it has poor specificity in differentiating benign from malignant lesions even with the use of contrast agents. In view of this, the addition of *in vivo* MR spectroscopy (MRS) has been shown to improve the specificity (1). *In vivo* MRS allows non-invasive detection of the biochemical composition of the tissues and provides information on both the biochemical and the physiological processes of malignant transformation. Further, the method facilitates obtaining biochemical or metabolic information from a well-defined region of interest (ROI) or volume element (voxel). Since both MRI and MRS are non-invasive methods, they are also useful for repeated monitoring or guiding treatment of cancer and the tumor response to treatments (2-5). MRS can be performed with a large number of nuclei; however most *in vivo* studies are performed using the nuclei hydrogen (^{1}H) and phosphorus (^{31}P) due to their high natural abundance in tissues. However, the focus of this oration article is to review the application of various MRI and *in vivo* proton (^{1}H) MRS methods in the study of breast cancer in Indian population carried out in our Institute over the last two decades for

early diagnosis as well as their role in monitoring the tumor response of patients undergoing therapy.

Incidence of breast cancer :

In India, breast cancer it is the second leading cause of death among females and the age-adjusted incidence is 28.6/100,000 with more cases detected in Delhi and Mumbai (6). A recent report by the Indian Council of Medical Research predict the number of breast cancer cases in India would rise to 106,124 in 2015 and to about 123,634 in 2020 (2). It has become a disease of major socio-economic importance due to high morbidity and mortality and hence diagnosis and treatment are recognized as priorities in research. The survival rates in developed countries are high while in the developing countries the survival rates are much lower. Thus, early detection using appropriate techniques along with better treatment options is necessary to improve the clinical outcome and to reduce the mortality.

Diagnosis of breast cancer :

Physical examination, mammography, ultrasound and fine needle aspiration cytology or core biopsy are routine methods used for the diagnosis of breast lesions. The primary screening technique for detection of breast cancer however, is mammography but it has limitations in identifying lesions in dense breast or micro-calcification. Ultrasonography is used for diagnosis of cyst, mammographically occult lesions and in screening young women with dense breast. Both mammography and ultrasonography have low specificity leading to unnecessary biopsies with associated complications such as hemorrhage, pain and complications related to anesthesia.

Magnetic resonance imaging (MRI) :

Recently, considerable interest is focused on the evaluation of various MRI methods in the characterization of breast lesions in view of the limitations of mammography, ultrasonography and other techniques (7-13). MRI has been used as complimentary modality for preoperative evaluation of lesion size, staging of cancer, to monitor the response to therapy, and to detect local recurrences. In addition, it plays an important role in studying the integrity of breast implants and delineates breast cancer around or behind the implant.

Several studies showed that the sensitivity and specificity of MRI for detection of cancer can be significantly increased with the use of paramagnetic contrast agents through DCEMRI (7-13). In DCEMRI, tumor angiogenesis is the basis of contrast enhancement and rapid imaging is used to detect the differential enhancement between malignant tumor and normal breast parenchyma. Imaging at high spatial resolution enables tumor characterization based on size, shape, margins and the internal features observed. In DCEMRI, two sets of T1-weighted images are acquired; one before and one after contrast administration with

identical parameters, and the differences in contrast enhancement is calculated. However, most DCEMRI studies report poor specificity (ranging from 20% to 100%) (14-16). DCEMRI is very useful for the detection of multi-focal, multi-centric disease, preoperative evaluation and for accurate staging. Additionally, MRI is used in the screening of high-risk women (9, 17-20) and has the sensitivity in the range of 95-100% for the early detection of breast cancer (21).

In recent times various researchers have also exploited the differences in various biophysical, biochemical and physiological characteristics of various breast tissue types using DWI (to study water diffusion), perfusion weighted imaging (to study vascularity) and MRS (to identifying biochemical markers). Studies using DWI have shown potential in differentiating malignant, benign and normal breast tissues (22, 23) as well as in monitoring the treatment response (24-27). Recently we studied about 203 subjects and among them 141 were infiltrating ductal carcinoma (IDC) patients, 34 were benign breast pathology and 28 were normal volunteers who did not have any breast abnormalities (28). Our data showed that the mean ADC of malignant lesion was significantly lower (1.03 ± 0.18) compared to benign (1.63 ± 0.28) and normal (1.80 ± 0.12) breast tissues. We used ROC analysis to determine the cut-off values of mean ADC among malignant, benign and normal breast tissues. Accordingly, a cut-off value of 1.18 ($x10^{-3}$ mm^2/s) was obtained to differentiate malignant from benign diseases. Similarly, a cut-off value of 1.42 ($x10^{-3}$ mm^2/s) was obtained for the differentiation of malignant and normal breast tissues. Similarly, a cut-off value of 1.62 ($x10^{-3}$ mm^2/s) was obtained to differentiate benign from normal breast tissues. These data indicated that ADC of breast cancer patients was significantly lower compared to benign patients and controls. The lower ADC values seen in malignant breast tissues reflects the underlying histological pattern of densely packed randomly organized tumor cells that inhibit effective motion of water molecules, thus restricting the diffusion and hence a lower ADC value. These results clearly indicated the diagnostic potential of DWI in characterizing the breast lesions.

Proton (^{1}H) MR spectroscopy :

The ^{1}H MR spectrum of breast tissue is not rich with many metabolites in comparison to the spectrum from brain (29, 30). The normal breast tissue without water suppression is dominated by lipid (at 1.33 ppm due to methylene [-(CH_2)n-] protons) and water (at 4.7 ppm) resonances (see **Fig. 1**) and showed

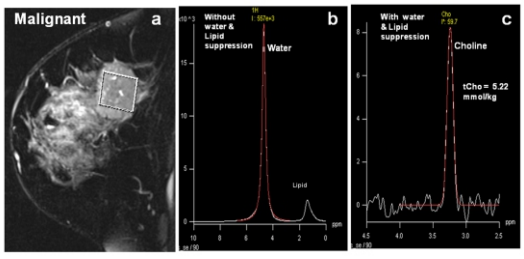

Figure 1.(a) T2-weigted sagittal image of a normal volunteer showing the voxel from which the ^{1}H MR spectrum shown in (b) was obtained without water and lipid suppression..

variation due to physiology, heterogeneity and hormonal variation during various phases of menstrual cycle (31). A change in the lipid composition of the normal breast parenchyma throughout the menstrual cycle has also been reported (32). We recently showed that within the normal breast and depending on the location of the VOI, due to the amount and distribution of adipose and fibro-glandular tissues, the ^{1}H MR spectral characteristics and the water-to-fat (W-F) ratio value vary considerably (31). Also, the W-F value of the para-areolar region is strongly influenced during the menstrual cycle with increase in the water content during menstruation and a gradual decrease, thereafter. The malignant breast tissues showed high water content with low contribution from lipids (see Fig. 2a & b) and thus a high W-F ratio compared to the normal breast tissues (33-35). Many studies have shown that W-F ratio can be used as a biomarker for diagnosis as well as to monitor the progression of cancer (34, 36). However, there are some limitations of using W-F ratio in diagnosis since substantial overlap of W-F values between benign and malignant breast tissues are also reported (33, 34-36).

Figure 2c is the water suppressed ^{1}H MR spectrum from a malignant breast tissue of a patient suffering from infiltrating ductal carcinoma showing clearly a peak at 3.2 ppm that correspond to several choline containing compounds (tCho) like phosphocholine, glycerophosphocholine, and free choline (1). The high level of tCho in tumor cells is attributed to the proliferative activity and

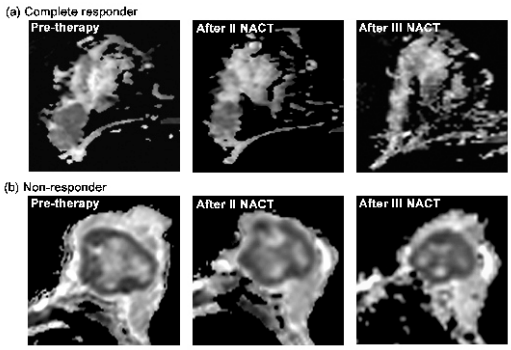

Figure 2.(a) T2-weigted sagittal image of a locally advanced breast cancer patient showing the voxel from which the ^{1}H MR spectrum shown in (b) was obtained without water and lipid suppression, while (c) shows the ^{1}H MR spectrum obtained with both water and lipid suppression.

increased membrane synthesis (37). Further, both increased synthesis by choline kinase and catabolic activity by specific phospholipase may also be responsible for high level of tCho in tumors (38).

A sensitivity of 83% and a specificity of 85%, respectively was reported when combined analysis of the MRS data available on breast cancer patients were carried out by Katz Brull *et al*. (39). With the addition of more data, Bartella *et al* showed an increase in both the sensitivity and the specificity of MRS as 87% (40). The potential application of using multi-voxel MRS to assess multiple lesions in a single study in breast cancer patients have also been reported (41-44). The advantages include distinguishing lesion borders and infiltration into the surrounding tissues.

Interestingly, several groups have

also reported the presence of tCho in normal, benign and in normal breast tissue of lactating women (45, 46). Thus, it is essential to accurately quantify the concentration of tCho instead of using the qualitative assessment of its presence or the absence for the differentiation of various breast tissue types. The two widely used approaches are: (a) semi-quantitative method of estimating tCho by calculating the signal-to-noise ratio (SNR), and (b) determination of the absolute concentration of tCho. The absolute concentration of tCho can be determined using both the external and internal water referencing methods (47, 48).

Studies from the literature showed a wide range of tCho concentrations in breast cancer patients and the reported values are in the range of 0 to 21.2 mmol/kg (47-55). These studies used water as an internal reference. Such a wide variation in tCho may be due to the heterogeneous nature or other molecular features of breast cancer. It is difficult to detect tCho in diffusive enhancement type cancers because of the intermingling of tumor cells with the adipose tissues (56).

Recently our group evaluated the potential of quantitative MR imaging and ^{1}H MRS in characterizing malignant, benign and normal breast tissues in a large cohort of women (57). The tCho concentration was found to be significantly higher in early breast cancer patients compared to LABC patients. Further, there was no association of tCho concentration with human epidermal growth factor receptor 2 (HER2), estrogen receptor (ER) and progesterone receptor (PR) status of malignant breast cancer patients. Our results also revealed that tCho concentration was not related to the tumor volume, age and menstrual status of patients. The lack of expression of ER, PR and HER2 are described as triple-negative (TN) breast cancer, while triple-positive (TP) patients have the expression of all the three ER, PR and HER 2. In our study when all three molecular markers were taken into account (i.e, TN, non-TN and TP groups), significant differences in the tCho concentration and the age were observed. Our results indicated that TN patients were younger in age and had lower tCho concentration compared to non-TN and TP patients. In view of the data from large cohort of women were available, we also worked out a cut-off value for tCho concentrations for the differentiation of malignant, benign and normal breast tissues. Larger tumor volumes were seen in LABC patients of various stages compared to early breast cancer patients. ER- patients showed larger tumor volumes than in ER+ patients which are suggestive of aggressive tumor behavior combined with higher angiogenesis in ER- patients. These results demonstrated the molecular heterogeneity of breast lesions and its relation with the tumor volume and tCho concentration (57). Further, our group and several others have shown that addition of MR spectroscopy to MRI increases the specificity of diagnosis with the detection of high levels of choline-containing compounds in malignant breast tissues (58-60).

Therapy monitoring :

As indicated earlier, both MRI and *in vivo* MRS have rapidly evolved not only as sensitive tools for diagnosis but also as a tool for therapy monitoring in cancer research. For patients with advanced stage of the disease or LABC, neoadjuvant chemotherapy (NACT) is the standard treatment option, which is followed by surgery and post-operative therapies (61,62). The advantages of NACT include reduction in the tumor size, option of breast conservation surgery and inhibition of distant metastases (61,63-65) and its drawbacks include toxicity and variation in response of individual patients. Thus, it is necessary to identify the non-responders from responders so that non-responders may be offered alternate line of treatment. Hence, early and accurate assessment of tumor response to treatment is essential for patient management.

Normally, the tumor response to therapy in a clinical setting is assessed by physical examination of the palpable change in the tumor size. Also, techniques like mammography and ultrasonography are used for the evaluation of treatment response but are not accurate in differentiating chemotherapy-induced fibrosis and the residual tumor (61, 66-68). In this regard, MRI was shown to be useful for measuring the residual disease by measurement of the tumor size, both prior to and after the therapy (69, 70). Additionally, the use of DCEMRI has been reported to be more effective for estimation of the residual disease following chemotherapy (71-74). DCEMRI also has limitations like antiangiogenic treatment that may lead to decreased contrast uptake and the residual disease may be missed in diffused tumors due to partial volume averaging. It is reported that in comparison to histology, MRI underestimates the amount of residual tumor, especially in tumors that respond well to chemotherapy (75). The measurement of changes in the tumor size is the basis for assessment of the tumor response in most imaging methods, which is evident only at the late stage of therapy.

In this context, the utility of DWI and *in vivo* MRS have been explored especially in evaluating the early response of breast cancers to therapy. Recently, we evaluated the role of apparent diffusion coefficient (ADC) of tumors measured using DWI to predict the early response (see Fig. 3) compared with the anatomical

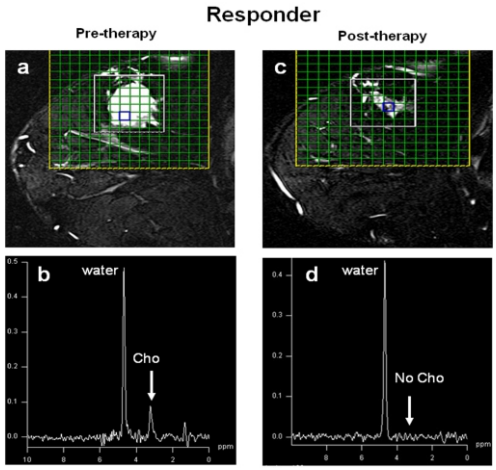

Figure 3. Representative ADC map of a breast cancer patient acquired prior to therapy and after II and III NACT: (a) complete responder, and (b) non-responder. [Reprinted from reference 27 with permission from John Wiley & Sons]

parameters like tumor volume and tumor diameter (27, 76). Our data on 56 patients showed that the specificity of differentiating responders from non-responders after III NACT was found to be 100% for ADC compared to volume and diameter (27). These results suggest the potential of DWI as an important tool in clinical imaging to predict the therapeutic response of cancer patients undergoing chemotherapy. Further, an interesting observation that emerged from our work was that the clinical responders showed significant change in tumor ADC as early as after I NACT. Whereas changes in structural parameters like tumor diameter and volume were evident only after II NACT (27).

A survey of the breast MR literature revealed that during the past decade, there is increasing interest in the use of ^{1}H MRS methods for monitoring the therapeutic response of breast cancer patients (50, 77). As discussed earlier, *in vivo* MRS provides biochemical information of tumor metabolism which is clinically valuable in the diagnosis as well as in the assessment of tumor response to therapy. Our laboratory and other centers have used proton ^{1}H MRS to complement breast MRI studies to improve the specificity of diagnosis and therapy monitoring. The malignant breast tissues have elevated W-F ratio and high levels of tCho and thus any effect of therapy can be expected to manifest as changes in their levels (36, 45). In responders, our sequential ^{1}H MRS data showed significantly reduced W-F ratio and tCho levels compared to that obtained prior to therapy during the course of therapy. These changes occur along with the reduction of the primary tumor size compared to the pre-therapy value. While in non-responders the decrease was insignificant (36, 45). Further study carried out in our laboratory showed that tCho peak was either reduced or absent in responders after III and/or VI NACT (45).

Recently we also evaluated the potential of SNR of tCho resonance and the tumor volume in the assessment of tumor response of patients undergoing NACT by sequential MR spectroscopic imaging (MRSI) and conventional MRI (78, 79). The MR response was compared with the clinical response. In responders, the pre-therapy tCho SNR was high which reduced after III NACT (see **Fig. 4**) with corresponding reduction of tumor

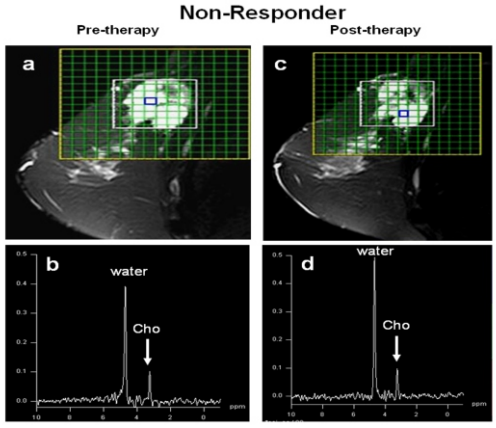

Figure 4. (a) Sagittal fat suppressed T2-weighted image of a LABC patient obtained prior to therapy who is a responder with the MRSI grid. (b) Spectrum obtained from a voxel shown in (a) with tCho resonance peak. (c) T2-weighted MR image of the same patient obtained after III NACT. (d) Spectrum obtained from a voxel highlighted in (c) that showed no tCho resonance peak. (Reprinted from reference 79 with permission from John Wiley & Sons].

volume. Non-responders showed no statistically significant changes in tCho SNR (see **Fig. 5**) and the tumor volume (78, 79). The changes in the tCho concent-

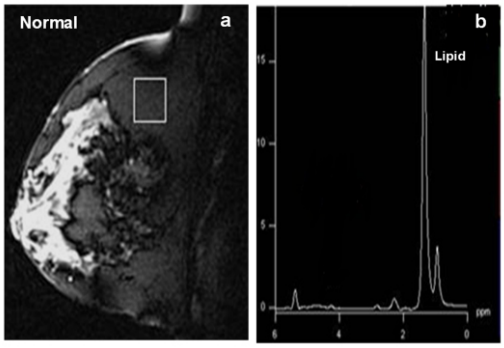

Figure 5. (a) Sagittal fat suppressed T2-weighted image of a LABC patient obtained prior to therapy who is a non-responder with the MRSI grid. (b) Spectrum obtained from a voxel highlighted in (a) showing tCho signal. (c) Post-therapy T2-weighted sagittal fat suppressed image of the same patient after III NACT. (d) Spectrum obtained from a voxel highlighted in (c) showing tCho signal. [Reprinted from reference 79 with permission from John Wiley & Sons].

-ration within 24 hours of administering chemotherapy that correlated positively to lesion size changes has also been reported by Meisamy et al (80). Further, we also reported changes in the absolute concentration tCho in predicting the tumor response of breast cancer patients undergoing NACT (81). The pre-therapy concentration of tCho showed significant reduction as early as after I NACT in responders compared to non-responders. Further reduction was observed after II and III NACT.

Summary :

The last two decades has seen the tremendous growth of various MRI methods as an important imaging tool in cancer management especially in breast cancer with a high sensitivity, high spatial resolution and 3D imaging capability. This is because MR is noninvasive; it avoids ionizing radiation and has the ability to generate high-resolution images. Additionally, through MRS it provides biochemical information at the molecular level. Dynamic contrast MRI shows high sensitivity for breast cancer detection, but with variable specificity. Both routine and DCEMRI are useful adjunct for mammography and ultrasonography. They are useful and have a distinct role in pre-operative staging, assessment of multifocal and multicentric disease, as well as chest wall involvement. Another advantage of various MRI methods is the ability of bilateral breast imaging at the same sitting, which is useful in detecting cancer of the contralateral breast.

Moreover, the advanced methods such as diffusion and perfusion MRI techniques and MR spectroscopy showed great potential for breast lesion characterization and have shown promise to increase the current level of specificity. DWI has the ability for evaluating the cellular changes while the vascular changes using DCEMRI in the same imaging session as MRS. Also, the development of various MRS procedures with water and lipid suppression and editing techniques has enabled obtaining non-invasive biopsy information. The

important feature of *in vivo* MRS is the ability to measure endogenous metabolites noninvasively as well as changes in tissue metabolism. Further, MR spectroscopy is also useful for monitoring therapeutic response of tumors, measuring the distribution, pharmacodynamic and pharmacokinetics of drugs *in vivo*. More studies are required to improve the sensitivity and specificity of *in vivo* MRS for several disease patterns particularly for small lesions before it is incorporated in clinical practice. For example in breast cancer, MRS acts as a complementary tool to histology, mammogram and other accepted techniques.

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