

Evaluation of in-vitro NMR spectroscopy in detecting metastasis in axillary nodes in breast cancer in a clinical setting

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INTRODUCTION

Axillary nodal status is currently the most well established prognostic factor predicting the outcome in patients with breast cancer [1]. Serial sectioning and focused evaluation of the axillary and sentinel lymph nodes with immunohistochemistry and reverse transcriptase-polymerase chain reaction (RT-PCR) have shown that the incidence of metastases is more than that detected by conventional histopathology by around 13-30%. MR spectroscopy detects metastases based on specific biochemical changes, which occur consistently with metastases, like elevated water-fat ratio, choline containing compounds and lactate [2-4]. In the present study, we evaluated in a clinical setting the potential of in vitro MR spectroscopy in the detection of axillary metastases in comparison to conventional histopathology.

MATERIAL AND METHODS

Axillary lymph nodes (n = 88) were obtained from thirty patients with breast cancer who underwent either modified radical mastectomy (n = 22) or breast conservation surgery (n = 8). Each lymph node was bisected into two equal halves. One half was immediately frozen and stored in liquid nitrogen for MRS evaluation while the other half was sent for histopathological evaluation. Lymph nodes, with suspicion of metastases on MRS were subjected to re-evaluation with serial sectioning and immunohistochemistry using pancytokeratin staining. The perchloric acid extracts of the lymph nodes were prepared using the standard protocol and lyophilized powder obtained was dissolved in 0.6 ml of D₂O solvent. Sodium trimethyl silyl-(2,2,3,3-H₄) propionate (TSP) was added as a standard for chemical shift and quantification of concentrations of metabolites. 1D proton spectra with water suppression were acquired using DRX-400 (BRUKER, Switzerland) spectrometer with a relaxation delay of 14 seconds. The concentration of metabolites were determined by comparing the integrated intensity of isolated resonances of the compounds of interest with that of the TSP signal. In addition, the intensity ratio for metabolites, GPC and Thr (GPC/Thr) was also determined. The result of MR spectroscopy and histopathology were correlated using Mc Nemar's test and student t test were performed to test the significance of results observed.

RESULTS

Histopathology revealed metastases in 20 lymph nodes from 11 patients and further immunohistochemistry evaluation did not reveal any occult metastases. The metastatic nodes showed significantly higher concentration of the GPC-PC, choline, alanine, uridine-di-phosphate and lactate (Table 1). The GPC-PC/Thr ratio for involved nodes was found to be 1.05 ± 0.51 which is significantly higher ($p < 0.0005$) than that observed for non-involved nodes, the value being, 0.53 ± 0.35 . An ROC curve was plotted (using SPSS 7.0 software) for the GPC-PC/Thr ratio and the histopathological status of the lymph nodes and a cut-off value of 0.80 for the GPC-PC/Thr ratio was chosen to obtain a maximum accuracy of 89% for the classification of the lymph nodes. Using this criterion for differentiation between the non-involved and metastatic nodes, the MRS accurately predicted the nodal status with a sensitivity, specificity and accuracy of 80%, 91% and 88%, respectively. The sensitivity, specificity and accuracy of detecting metastases in the subset of patients who received NACT (n = 9) were 73%, 75% and 75%, respectively which were significantly lower compared to the patients who did not receive NACT (n = 21), the values being 89%, 96% and 96%, respectively.

Table1. Concentration (mM/Kg wet weight, Mean \pm SD) of metabolites in involved and non-involved nodes from breast cancer patients.

Metabolites	Lactate	GPC/PC	Choline	Threonine	Alanine	UDPG
Involved nodes (who received NACT) (n=11)	4.38 ± 3.95	0.52 ± 0.45	0.43 ± 0.37	2.05 ± 1.51	4.24 ± 3.41	0.89 ± 0.4
Involved Nodes (who did not receive NACT) (n=9)	$5.60 \pm 2.31^*$	$0.74 \pm 0.51^*$	$0.67 \pm 0.35^*$	$1.93 \pm 1.32^*$	$5.75 \pm 3.78^*$	$0.93 \pm 0.30^*$
Non-Involved Nodes (n=68)	3.45 ± 2.35	0.35 ± 0.24	0.35 ± 0.22	2.33 ± 1.51	2.90 ± 2.67	0.59 ± 0.22

NOTE: *, $p < 0.05$ between involved and non-involved nodes.

DISCUSSION

To our knowledge, this is the first study profiling the metabolite characteristics of axillary nodes in breast cancer in a clinical setting. The ratio of GPC-PC/Thr and the concentrations of GPC-PC and choline in the nodes harboring metastases were higher than those not involved with metastases. Increase in the concentration of GPC-PC in involved as compared to the non-involved nodes was observed which may be attributed to the increased membrane synthesis in rapidly proliferating tumor cells [2,3]. Recent in vivo MR spectroscopic studies [4] revealed that primary human breast cancers tumors have elevated levels of choline containing compounds. The accuracy of MRS in our study in the subset of patients who received neoadjuvant therapy was only 75% compared to an accuracy of 92% the patients who did not receive NACT. Jagannathan et al [4] reported decreased levels of choline containing compounds after tumors responded to chemotherapy. Thus, in patients who received neoadjuvant chemotherapy, MRS may not be accurate in predicting axillary nodal status. Since NMR examines the whole of the lymph node, it minimizes the probability of missing out micrometastases in between the sections. The single metabolite ratio from NMR spectroscopy used for the detection of micrometastases is an advantage over the long panel of markers to be tested in RT-PCR.

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