A Quantitative study of various Metabolites in Breast tumors by In-vitro Proton MR Spectroscopy and its Statistical evaluation

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SYNOPSIS

PROTON MR quantitative analysis in breast tumors, fibroadenomas (n=10) and ductal carcinomas (n=13) along with their respective controls (n=23) were carried out on twelve metabolites viz. formate, myo-inositol, glycine, α-GPC, choline, Cr/PCr, glutamine, succinate, glutamate, acetate, alanine and lactate. Multivariate discriminant function analysis (DFA) was performed to find out important descriptors for the discrimination of fibroadenomas and ductal carcinomas (CA), along with their control tissues. The best discrimination between fibroadenomas and ductal carcinomas was obtained with the combined effect of Glycine and Choline as marker metabolites ($p< 0.01$). The proposed model could classify 95.65% cases correctly between the fibroadenoma and ductal carcinoma, with the sensitivity of 92.3% and the specificity of 100%.

INTRODUCTION

The risk of getting breast cancer is increasing worldwide as it is the commonest cancer among women and is a significant cause of morbidity and mortality. For a woman who lives to the age of 90 the odds of getting breast cancer in her entire lifetime is about 12.5% or 1 in 8. Men can also develop breast cancer, although their risk is less than 1 in 1000. Cancer is a disease that can metastasize to other body parts at any age group, however the probability of breast cancer rises with age. In-vitro NMR spectroscopic examinations of tissue extracts can be combined with appropriate pattern-recognition and visualization techniques in order to monitor the characteristic metabolic differences between different types of breast tumors. Substantial work has been done in the area of breast cancer using ^{1}H NMR analysis[1] of breast carcinomas and non-involved breast tissues including the analysis of their water to fat ratios upto the metastatic approach of carcinomas to the lymph nodes. To predict accurately still some more research needs to be addressed regarding its specificity and sensitivity. This has prompted us to explore quantitative ^{1}H NMR information and Multivariate Discriminant Function Analysis (DFA) in order to address these issues.

MATERIALS AND METHODS

Breast tissues comprising fibroadenomas (n=10) and ductal carcinomas (n=13) along with their controls from the same subject were obtained during breast surgery from our local hospital (KGMU, Lucknow) and a small portion of the control from periphery and tumor tissues were immediately snapped frozen in liquid nitrogen in order to stall the metabolic activity while rest of the portion was sent for excisional biopsy studies. Breast tissue samples were taken after measuring their wet weight and processed with the 14% Perchloric acid extract, centrifuged and the pH was maintained between 7.2-7.4 by adding 4M KOH, again centrifuged, lyophilized and samples were prepared in 500 µl D_2O for the purpose of internal locking and immediately subjected to NMR analysis. The NMR experiments were performed on Avance DRX 300 MHz FT-NMR spectrometer equipped with a 5 mm multinuclear inverse probehead with a Z-shielded gradient. The ^{1}H NMR spectra were acquired with water suppression at 298K with 32768 data points, spectral width (SW) of 3172.589 Hz; with 10s relaxation delay and 128 transients were acquired. The quantification of the metabolites in all the samples were carried out using Bruker's NMRQUANT with respect to a known concentration of TSP (49 mg/dl). The data were subsequently subjected to DFA with the step-wise forward variable selection procedure to define important descriptors for the differentiation between fibroadenoma and ductal-CA.

RESULTS

There was a significant mean difference ($p< 0.05$) in almost all the twelve metabolites viz. formate, myo-inositol, glycine, α-GPC, choline, Cr/PCr, glutamine, succinate, glutamate, acetate, alanine and lactate. The comparative values of mean differences between control and pathological tissues for fibroadenoma and ductal carcinoma have been shown in the figure. The DFA was performed to discriminate between the fibroadenomas with the ductal-CA. The DFA performed on the pathological tissues of fibroadenoma and ductal-CA was found highly significant ($p< 0.01$) in discriminating the two groups, in which Glycine and Choline gave the joint importance to discriminate the benign vs. malignant groups. The DFA score can be calculated by the equation as-

$$\text{DFA score} = 16.712 * \text{Glycine} - 6.841 * \text{Choline} \quad (F_{2,\,20=36.32}, p< 0.001).$$

This model can discriminate upto 95.65% cases correctly. A cancerous case will be the one having DFA score less than 10.72. The sensitivity of the model is 92.31% while the specificity is 100% with the false negative rate of 7.7%. The control tissues of both the groups could also be discriminated well after performing DFA. Metabolites showing significant discrimination ($p<0.05$) between the control tissues of both the groups were-myo-inositol (74%), glycine (78%), choline (74%), glutamine (78%), succinate (82%), glutamate (83%), alanine (87%) and lactate (78%). (Values in the parentheses are percent correct classification by the respective metabolites). All the metabolite levels present in the pathological tissues of ductal-CA were very close to the metabolite levels of their control tissues ($p >0.05$), however in the fibroadenomas the level of glycine, choline, glutamine, glutamate and alanine were significantly higher ($p<0.05$) in their respective pathological tissues, except a-GPC and Cr/PCr, which were showing non-significant results.

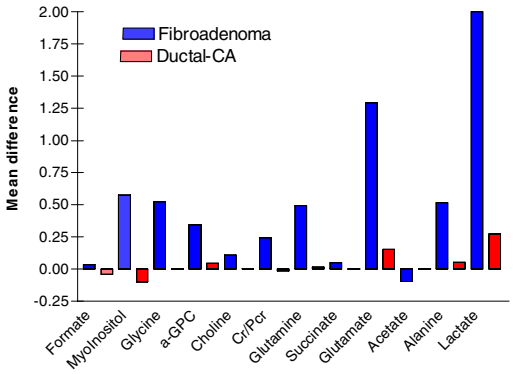

Fig. Comparative mean differences in tissue metabolites of fibroadenoma and ductal carcinoma.

DISCUSSION

Several models were tried, but the combined effect of glycine and choline is able to discriminate a case between fibroadenoma with ductal carcinoma (CA) correctly by 95.65% with high sensitivity, specificity and a low false negative rate. This suggests Glycine and Choline as a marker in the multivariate discriminant function. A significant similarity between the metabolite levels of the control and pathological tissues ($p> 0.05$) in all the ductal carcinoma cases is an indicative of the high proliferation rates of the cancerous cells and rapid cancer manifestation to the normal tissue areas. Whereas, the metabolites were high in the pathological tissues than in the controls of fibroadenomas with glycine, α-GPC, choline, glutamine, glutamate and alanine as significant ($p< 0.05$) descriptors. Though the current reporting has a limitation of small sample size, yet the consistency in results are encouraging because of high discriminant power of the proposed model. Since fine needle aspiration cytology (FNAC) and NMR analysis of different breast areas may not be advised to patients during pre-operative surgery, for better planning due to ethical limitations, nevertheless NMR analysis may provide as an additional diagnostic tool along with incisional biopsy for better predictive diagnosis and chemotherapy management.

REFERNCES

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