Objective: To study the metabolic profile of surgically excised involved and non-involved breast tissues by high resolution in-vitro $^1$H NMR.

Introduction: The characterization of different types of tissue based on their biochemical composition can be assessed using in-vitro NMR technique. It provides insight into the tumor metabolism by detecting various metabolites, thus enabling to study the different metabolic pathways involved in breast cancer. Only few metabolites could be observed by in-vivo MRS method and also the spectral quality is compromised by low magnetic field strength. These limitations can be partly overcome by using high-resolution in-vitro NMR spectroscopy. High-resolution NMR has been used for quantitative analysis of tissue extracts to study different pathologies. But there are only few reports available demonstrating the metabolic profile of involved (malignant) and non-involved (normal) breast tissues in a qualitative way. In the present study the absolute concentration of metabolites was determined to investigate the metabolic changes associated with involved and non-involved tissues of the breast by in-vitro $^1$H NMR.

Material and Methods: A total of 23 samples [non-involved (n=13) and involved (n=11)] breast tissues were collected. The high-resolution proton NMR spectroscopy experiments of perchloric acid extracted samples were carried out on a DRX (BRUKER, Switzerland) spectrometer operating at 400.13 MHz. 1-D spectra with water suppression were acquired using a single 90° pulse using a spectral width of 5208 Hz with 128 scans and a relaxation delay of 14s. 8 dummy scans were acquired. For assignment of peaks observed in 1-D and 2-D (DQF-COSY and TOCSY) experiments were performed. The absolute concentration of metabolites was determined using the formula described earlier. Using Student’s t-test a P value of < 0.05 was considered as significant between involved and non-involved breast tissues.

Results: The assignments of various metabolites were carried out using 2-D COSY and TOCSY experiments. In all, 33 metabolites could be assigned unambiguously in the extract of breast tissue samples. The concentration of Lactate (Lac) ($14.0 \pm 11.8 \text{ mmol/kg}$), Creatine (Cr) ($1.9 \pm 1.2 \text{ mmol/kg}$), Choline (Cho) ($3.1 \pm 2.2 \text{ mmol/kg}$), Phosphoethanolamine (PE), Glycero phosphocholine (GPC)/Phosphocholine (PC) ($5.2 \pm 4.1 \text{ mmol/kg}$) and Glutamate (Glu)/Glutamine (Gln) ($8.3 \pm 5.4 \text{ mmol/kg}$) in involved tissues was significantly higher compared to the non-involved breast tissues (see Figure 1). Figure 2 shows the representative example of 1-D in-vitro NMR spectra showing various metabolites observed in the non-involved breast tissue and in the involved breast tissue.

Discussions: Studies have been reported on the breast tissue extracts, axillary lymph nodes and FNAC samples demonstrating the differences between various metabolites. Previous studies from our laboratory demonstrated the role of in-vitro $^1$H NMR in the evaluation of breast tissues in axillary lymph node metastasis. In the present study, a detailed absolute quantitation (concentration) of various metabolites was carried out in the perchloric acid extracts of breast tissues using $^1$H NMR spectroscopy. The results revealed significant changes in the concentration of various metabolites in the involved breast tissues compared to the non-involved tissues. Significant increase was observed in the levels of Lac in involved as compared to non-involved breast tissues. The higher concentration of Lac in involved tissue indicates presence of cancer cells that derive energy via anaerobic glycolysis since energy utilization in these tissues is much higher than that provided by aerobic respiration. Increased Lac concentration is an indicator of metabolic adaptation of tumors and it changes the endothelial phenotype thereby leading to tumor vascular morphogenesis and perfusion. Elevated Lac levels have also been reported in the involved breast tissues as well as in the malignant axillary lymph nodes. The significant increase in the concentration of membrane metabolites like Cho and GPC/PC in involved breast tissues have also been reported as seen in the present study. This increase may be attributed to the increased membrane synthesis that occurs in the proliferating tumor cells which requires rapid degradation and synthesis of membrane phospholipids reflecting changes in the membrane phospholipid metabolic pathway. The data also showed a significant increase in the Cr concentration in involved breast tissues. An increase Cr may be due to the increased production of energy during cell replication. Our data also showed significant increase in the concentration of Glu/Gln in involved breast tissues compared to the non-involved tissues. The increased concentration of Glu/Gln in the involved tissues is attributed to increased protein synthesis, as these amino acids serves as building block for protein synthesis. Studies have also shown that glutamine plays an important role in the number of signaling pathways that contribute to the tumor growth.

Conclusion: A significant increase was observed in the concentration of Lac, Cr, Cho, GPC/PC and Glu/Gln metabolites in involved compared to non-involved breast tissues indicating an increase in the metabolic activity of tumor tissues, providing an insight into the tumor metabolism.