

Evaluating Alterations in Breast Cancer Patients after Recovery Via A PET/CT-Assisted Metabolomics Approach

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Objectives: Breast cancer is a mortal disease that causes many deaths, especially in women. Improved therapies could contribute positively to survival rates. Metabolomics is an important tool for monitoring the alterations of several metabolites in clinical cases. This study aimed to develop a metabolomics model to observe (via mass spectroscopy) metabolic alterations in patients who suffered from breast cancer (BC), both before and after their recovery.

Materials and Methods: Grades 1 and 2 invasive ductal carcinoma patients were evaluated based on their positron emission tomography/computed tomography results. Fourteen patients who had fully recovered from BC were subjected to metabolomics analysis. Plasma samples were extracted and analyzed via quadrupole time-of-flight mass tandem spectroscopy. A chemometrics analysis was performed in order to determine the statistically significant metabolites. All the metabolites were annotated via the mummichog algorithm.

Results and Discussion: According to the data analysis, glucose, ornithine, phenylalanine, some vitamins, and metabolites in the fatty acid metabolism were statistically altered after recovery of each patient.

Conclusion: Untargeted metabolomics studies can be used to understand the etiopathogenesis of breast cancer, finding new biomarkers and alterations of metabolic pathways. After the tumor burden was removed, homeostasis was restored and the concentration of several metabolites began to normalize. This study elucidated the effects of breast cancer at the molecular level. [*P R Health Sci J* 2023;42(4):276-282]

Key words: Metabolomics, Breast cancer, Mass spectroscopy, Chemometrics, PET/CT

Breast cancer (BC) is known as the most diagnosed cancer and also as having the highest mortality rate for women, worldwide. Approximately 2 million new BC cases were reported in 2018, with nearly 23% of all who suffered from this disease living in and almost 60% of deaths occurring in developing countries. Cancer survival rates vary from 80% to 40%, those in developed countries are higher than those in developing countries (Turkey, Latvia, Czech republic have %80 survival rate while Eastern Africa have %40 survival rate) (1,2). According to epidemiological studies, 1 out of every 12 females is diagnosed with BC (3). In Belgium and Poland, diagnosed BC cases were reported to number 145.2 and 66.3 of 100,000, respectively (4). As can be seen, BC is a very important health problem for both developed and developing countries.

The breast is a complex tubuloalveolar gland that is embedded within asymmetrical connective tissue that differentiates from childhood to maturity (5). During the menstrual cycle and pregnancy, mature tissues of precursor cells initiate the formation of duct lobular units (6). The typical breast is composed of a stratified epithelium covered by a basement membrane and embedded in a mesh of blood vessels,

lymphatics, and stromal cells (7). In a normal, healthy breast, the stratified epithelium contains 2 different cell populations, the myoepithelial and the epithelial, which can be altered by immunohistochemical staining. Mostly, primary development of normal breast is strongly related with the cellular heterogeneity in any breast based disease. Heterogeneity of breast cells may occur because of neoplastic changes in either myoepithelial or epithelial cells or, possibly, in stem cells (8). These neoplastic cells behave differently than do normal cells and proliferate in an uncontrolled way (9).

Breast cancer is simply defined as a malignant disease that originates in the breast cells (10). As is the case with other malignant tumors, several factors can, when present, increase the

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possibility of a woman's developing BC. Damage to the DNA and genetic disposition can lead to BC, both of which have been associated with estrogen exposure. Some women inherit faulty DNA, genetic mutations, or both (e.g., mutations in the p53, BRCA1, and BRCA2 genes). Women with a family history of breast or ovarian cancer are understood to be at significant risk of BC (11). Different diagnostic techniques are used to determine the presence BC, ranging from self-examination to combined positron emission tomography and computed tomography (PET/CT) (12-14). None of these methods, however, is sufficient to make a comprehensive evaluation of a BC patient. Consequently, several tumor markers have been suggested for use in BC diagnosis to attain numerical and precise results. These markers are the Ca 15-3, Ca 27.29, and HER2 antigens and are, for the most part, estrogen and progesterone receptors (15). However, these markers do not provide sufficient information about patients. Therefore, high-throughput -omics studies are performed in order to measure the effects of BC on the human body as a whole (16-20).

Metabolomics is a technique that statistically evaluates all the metabolites in any biological system and reveals alterations in the various entities (e.g., (genes, transcripts, proteins, metabolites) (21). In this study, we aimed to perform a PET/CT-monitored metabolomics study in order to significantly demonstrate the alterations in metabolites between BC patients before and after treatment. By doing so, we hoped to be able to identify the metabolic changes that occurred during successful BC treatment.

Materials & Methods

Subjects and samples

Patients who received a diagnosis of invasive ductal breast carcinoma (IDC) via biopsy and who agreed to a PET/CT scan were asked to participate in this study. Patients who had other chronic diseases or who took prescribed drugs were excluded from the study. A body mass index (BMI) was calculated for each patient and those with a BMI lower than 30 were accepted into the study. The grade of each patient's IDC was determined based on the results of her PET/CT scan. Only patients with grade 1 or 2 disease were included in the study. Only those IDC patients who met our inclusion/exclusion criteria (BMI, comorbid chronic disease, drug use) and who had experienced a total recovery from their IDC (accomplished via surgery), as determined by their PET/CT results, were accepted into our study.

Blood samples (2 cc) were collected from the patients (n = 20) who met our inclusion/exclusion criteria. After that second blood samples (2 cc) were collected for patients who totally recovered from the IDC regarding to PET/CT results (n = 14). The blood samples were gently received and centrifuged (10,000 rpm). The samples were stored at -80 °C until the time of analysis. The mean age of the patients was 51.2 (\pm 10.6) years. A volunteer application form was obtained from each patient, and all the volunteers received information about the study. The study's workflow was suitable for Helsinki declaration and has

an local ethical approval. All the data gathered about the patients are elucidated in Table 1.

Table 1. Ages and SUVmax of IDC patients (grades 1 & 2), including metastatic lymph node SUVmax.

Patients	Age (years)	BMI	SUVmax	Index node*
Patient 1	51	27.21	8.51	NaN
Patient 2	36	24.61	3.46	NaN
Patient 3	42	29.15	27.72	10.16
Patient 4	37	26.41	4.17	NaN
Patient 5	65	28.13	5.99	NaN
Patient 6	70	27.71	13.11	3.21
Patient 7	37	28.04	7.67	NaN
Patient 8	36	29.14	6.60	5.40
Patient 9	47	25.53	9.30	2.77
Patient 10	41	24.34	4.04	2.45
Patient 11	57	26.84	4.93	3.72
Patient 12	64	27.89	15.68	9.72
Patient 13	66	29.00	4.33	NaN
Patient 14	68	28.16	4.47	NaN

*Index node defined as the highest SUVmax of lymph node. Abbreviations: BMI, body mass index; IDC, Invasive Ductal Carcinoma; SUVmax, maximum standardized uptake values

Imaging

The patients were not permitted to eat for 6 hours before their PET/CT scan. The fasting blood glucose levels of patients were measured before the PET/CT study. A PET/CT scan was not performed on patients with fasting blood glucose levels higher than 140 mg/dl.

The patients were injected with 370 to 740 MBq of ¹⁸F-Fluorodeoxyglucose (FDG), iv; 1 hour after the injection, non-diagnostic CT imaging was performed for anatomic localization and attenuation correction. The CT imaging was performed using the 70 mAs and 120 keV values from cranium to thigh with the patient in a supine position. Following CT imaging, PET imaging was performed (with the patient in the same position) from the proximal thigh to the base of the skull, with 7 to 8 bed positions and with 2-minute periods for each one. The Biograph 16 TruePoint PET/CT device (Siemens, Germany) was used on all the patients for FDG-PET/CT.

Sample, quality control, and internal standard preparation

The study was approved by the Atatürk University Human Studies Ethical Committee (IRB ID: B.30.2.ATA.0.01.00/81). Twenty-eight thawed plasma samples (14 recovered and 14 IDC patients) were homogenized on a vortex mixer: 300 μ L of plasma were mixed with 900 μ L of methanol for the extraction of metabolites. The sample was vortexed and then stored in a refrigerator for 15 minutes for cell lysis to enhance extraction efficiency. After that, the samples were centrifuged for 5 min at 16,900 \times g. Finally, 1000 μ L of supernatant was transferred into a clean microtube. In addition to this, quality control (QC) samples were prepared by loading 5 μ L of each sample into a clean microtube. The QC samples were injected in order to remove false positives from the system and correct for within- and between-batch drift.

Q-TOF MS/MS analysis

The quadrupole time-of flight (Q-TOF) tandem mass spectroscopy (MS/MS) system used was the Agilent 1290 Infinity LC coupled with the Agilent 6530 Accurate-Mass Q-TOF liquid chromatography (LC)/MS (Agilent, USA) and the SVEA C18 (95Å, 2.1 x 100 mm, 1.8 µm).

The LC mobile phase consisted of 0.1% formic acid and acetonitrile. A mobile phase flow rate of 0.4 mL/min was employed for gradient elution, with a total run time of 12 min. The column temperature was set at 55 °C during the analysis. The positive ion scan mode (with a capillary voltage of 3.5 kV) was applied during the study. The mass scanning range was from 50 to 1500 m/z. Ionization was performed via electrospray ionization (ESI). The MS absorbance threshold was set at 200. The instrument acquired data using the following optimized conditions: drying gas temperature, 350 °C; drying gas flow rate, 11 L/min; nebulizer, 40 psi; sheath gas temperature, 350 °C; and sheath gas flow, 11 L/min.

All the samples and QC aliquots were analyzed via Q-TOF MS/MS. Complex chromatograms usually include thousands of peaks having huge intensity alterations across the spectrum. For that reason, several algorithms were improved for peak detection. In this study, the XCMS centWave algorithm was selected for peak detection. All the samples were imported into the XCMS online software, R package, and peak detection was maintained. The Obiwrap algorithm of the previously mentioned package was used for peak alignment. The retention time of each chromatogram was optimized with this approach. Then, a peak grouping step was performed to obtain a unique retention time and m/z value, which was called a “feature,” for the analysis. After this, an Excel spreadsheet was generated for all the samples and included the retention time, unique m/z value, and intensity for each feature. This Excel spreadsheet was imported into MATLAB for chemometric analysis. The features that had low reproducibility in the QC samples were removed. Quality control support vector regression (QC-SVR) correction was also maintained (22). After cleaning the data, classification was performed by importing the data into PLS Toolbox 8.9. Orthogonal partial least squares discriminant analysis (OPLS-DA) was implemented for each group.

All the receiver operating characteristic (ROC) curve studies and a permutation test have shown that the model was not over-fitted and can perfectly classify temperature

alterations. Recovery groups showed differences with respect to BC patients. These differences (significantly altered metabolites) were identified by Q-TOF MS/MS measurements. In order to annotate these metabolites; a *mummichog* (default *P* value cutoff settings) algorithm were followed in accordance with the Kyoto Encyclopedia of Genes and Genomes (KEGG) database for human research. MetaboAnalyst 5.0 software was used for pathway analysis. According to the data, different pathways were significantly affected between the women with a diagnosis of BC and those who had recovered from it. The variations between groups in the major pathways can be seen in Figure 1.

All the features having a *P* value over .01 were filtered and discarded from the pathway analysis. MetaboAnalyst 5.0 software was used for pathway analysis. According to the data (Figure 1), different pathways were significantly affected upon recovery from BC.

Results

In FDG-PET/CT images, all the areas of focal hypermetabolic activity that were greater than the background activity, except the maximum standardized uptake value (SUVmax) areas of physiological involvement, were accepted as indicating pathological involvement. Patients who had at least 1 lesion

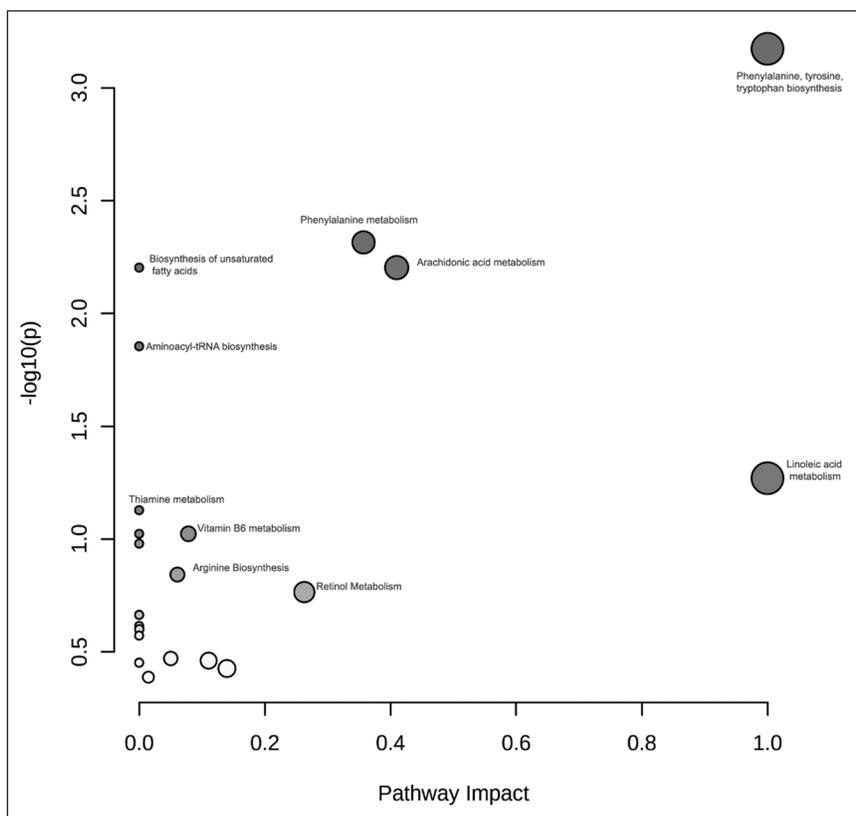


Figure 1. Pathway impact diagram for annotated metabolites that have significant alterations between the groups.

in the targeted region were categorized as being PET/CT+. Each patient's response to therapy was evaluated using the PERCIST (PET response criteria in solid tumors) 1.0 criteria. The average values of SUVmax were found to be $8.57 (\pm 6.58)$ for diagnosed BC patients, and SUV values were determined for recovered patients (Table 1).

All the sample members and QCs were analyzed by Q-TOF MS/MS. Complex chromatograms generally contain thousands of m/z values that have intensity alterations across the spectrum. For that reason, several bioinformatic algorithms were developed for peak detection. For this study, the XCMS centWave algorithm was chosen for peak detection. Data from the analysis were transferred into the R package, and the peak detection process was performed using the centWave algorithm. Peak alignment was carried out using the Obiwrap algorithm. The retention time of each spectrum was corrected by this method. Then a peak grouping step was initiated to determine a unique retention time and an m/z value for each feature. Subsequently, an Excel data table containing the retention time, m/z values, and intensity for each detected feature was created. The data were cleaned to correct for batch drift and to remove features that had low reproducibility. The following procedure was applied for data filtering: Acceptable missing values and %Relative Standard Deviation (%RSD) for the features were set at 20% and 30%, respectively. The data were normalized with the k-nearest neighbors (KNN) algorithm. A local polynomial regression model was selected for QC correction.

After cleaning the data, classification was performed by importing the cleaned data into PLS Toolbox 8.0. The OPLS-DA model was used for each group. Two latent variables (LVs) were selected to explain the model. Latent variables 1 and 2 scores plots can be seen in Figure 3, which demonstrates the predictive power of the proposed method. As can be seen in the figure, the groups were precisely classified. A ROC curve was plotted to assess the selectivity and sensitivity of the model. Graphs of specificity and sensitivity show that the model was not over-fitted and that the probability of chance correlation is insignificant

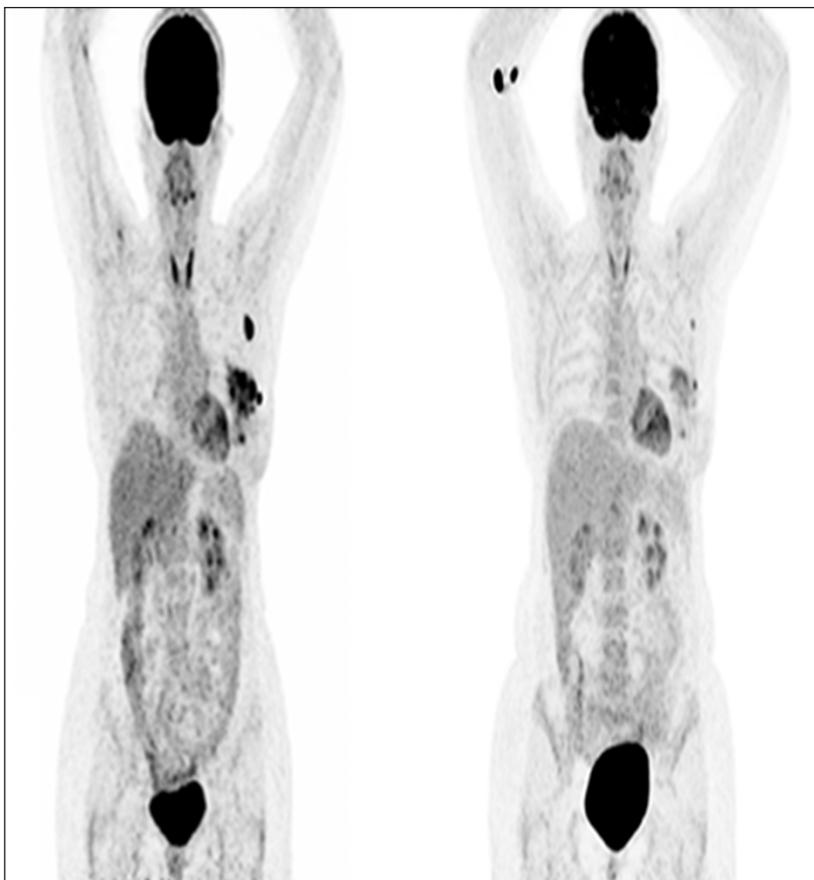


Figure 2. A) PET/CT Image of Breast Cancer patient with lesion on Left Breast. B) Positron Emission Tomography/Computed Tomography (PET/CT) Image of recovered Breast Cancer patient after treatment.

(Figure 4). The main dataset and cross-validation dataset each also has an acceptable area under the curve (1.0000), as can be seen in the figure (ROC curve). The cross-validation dataset was selected using the Venetian blinds method with 10 splits. Root mean square error of calibration (RMSEC)

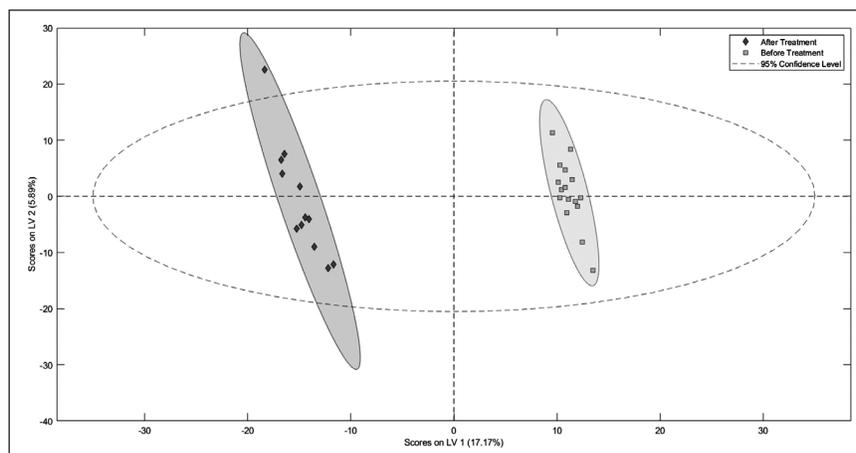


Figure 3. Score plot of latent variables obtained from Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) chemometrics classification model to show alteration of Breast Cancer patients after treatment.

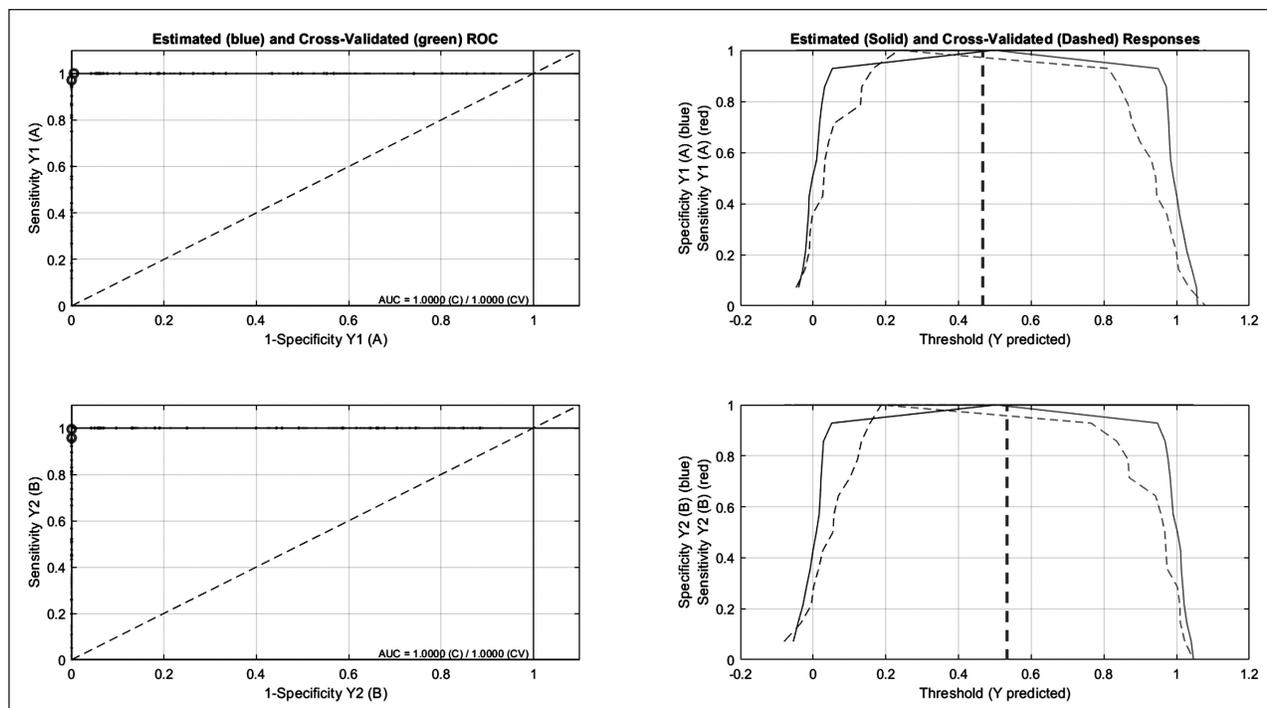


Figure 4. Receiver Operating Characteristic (ROC) Analysis and Area Under the Curve (AUC) Results for Test and Cross-validation Datasets of Each BC Patient Group in Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) Model.

and cross validation (RMSECV) were found to be 0.2304 and 0.3293, respectively. Fifty iterations were performed for the permutation test. All the permuted data were lower than the raw data, which also proved the significance of the OPLS-DA model (Figure 4). The iteration scores of the permutation test can be seen in Figure 5. A significance test (Wilcoxon test) and a random t test were applied to the iterations of both the main dataset and the cross-validation dataset. All the statistics showed that the model was significant ($P \leq .05$). The regression coefficients for the model and the cross-validation dataset were 0.9959 and 0.9714, respectively. In addition, the sensitivity value was calculated to be 0.76 (according to the OPLS-DA model), including 2 LVs.

According to the pathway analysis, the most affected pathways were phenylalanine, tyrosine, tryptophan biosynthesis (logP: 3.3), the phenylalanine metabolism (logP: 2.4, the arachidonic acid metabolism (logP: 2.3), and the biosynthesis of the fatty acid metabolism (logP: 2.3). These metabolic pathways were strongly associated with alterations in phenylalanine, tyrosine, ornithine, arachidonic acid, lysine, hexadecanoic acid, malic acid, and linoleic acid. For that reason, a pathway impact diagram was generated. The metabolites that were significantly differentiated in the plasma profiles of fully recovered patients compared to those of diagnosed IDC patients are shown in Table 2.

Eighteen different metabolites were found to be significantly altered, according to Welch's *t* test. Vitamins (thiamine, pyridoxine, and retinol), amino acids (ornithine, phenylalanine, tyrosine, lysine) fatty acids (hexadecanoic acid, linoleic acid), carboxylic acids (arachidonic acid, malic acid), sugars (ribose

and glucose), and leukotriene were found to be significantly altered after recovery from BC. The levels of most of the metabolites tended to be significantly reduced, an occurrence that may have been caused by the lower metabolic reactions that were seen after BC recovery.

Discussion

Several metabolites with a strict mass tolerance level (5 ppm) and *P* value (.01) were observed. These metabolites suggest that there are some important signs that indicate that a partial or full recovery has occurred after BC treatment. In particular, the fatty acid metabolism was strongly affected by BC recovery. After that, the alpha linoleic acid metabolism, the retinol metabolism, the linoleic acid metabolism, purine, and the arachidonic acid metabolism were affected by the successful treatment.

Haritwal et al. suggested that the lipid metabolism is strongly associated with BC (23). Lipid alterations are a very common in BC patients, and the metabolisms of *our* patients were strongly affected after recovery (24).

Tyrosine is another important metabolite that is significantly decreased in BC patients after recovery; this is caused by the tyrosine kinase inhibitors which regulate apoptosis and cell proliferation (25). Low level of tyrosine is accepted as a sign for patients who respond to therapy. As revealed herein, post-recovery ornithine levels are upregulated. High ornithine levels may reduce the concentration of ammonia and protect the human body from oxidative stress

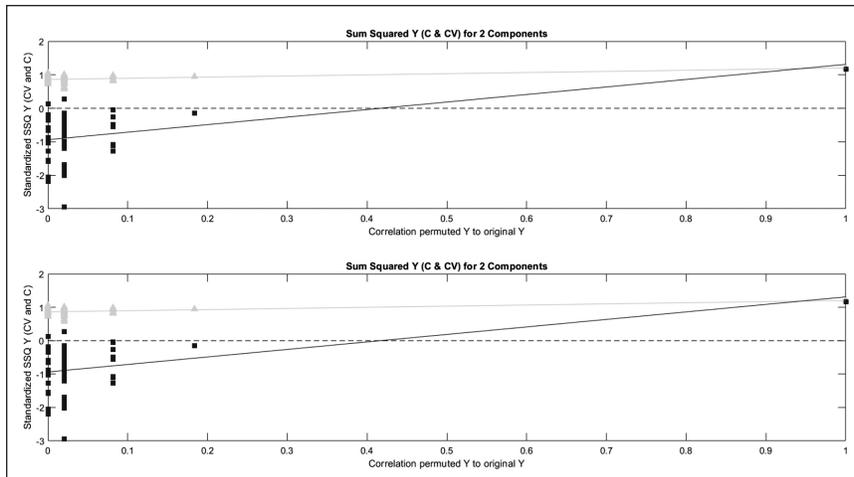


Figure 5. Diagram of correlation between permuted data vs original value after 50 iterations (for each group).

via the urea cycle (26). Furthermore, some studies have shown that ornithine levels are higher in controls than in BC patients (27). The phenylalanine levels were also lower in recovered patients, while phenylalanine, N-acetyl glycoprotein, and glucose levels were all found to be higher in metastatic BC patients (28).

There are several studies claiming that high phenylalanine levels are associated with malignancy (29,30). In BC patients who recover, however, it seems probable that those levels would be lower. Thiamine and pyridoxine levels were found to be higher in BC patients. Their metabolization to active compounds (thiamine diphosphate and pyridoxamine phosphate) may be increased and might be correlated with this process. Leukotriene A4 and B4 were also higher in the BC group. These 2 molecules are known to be important

This was a pilot study of recovered patients, and it has some limitations with regard to sample size. This study could be expanded by increasing the sample size for altered metabolites.

Conclusion

Breast cancer is a very common and usually malignant disease. Survival rates increase with improvements in therapy. In this study, we observed the metabolic alterations that were present in patients who experienced a full recovery of their BC.

Significantly altered after recovery were the arachidonic acid metabolism, phenylalanine, the leukotriene metabolism, and essential amino acids. These pathways are mostly associated with the immune response of the human body and lipid mediators.

Our findings demonstrate that BC directly damages the lipid mechanism and that the immune system works toward mediating the lipid metabolism. Leukotrienes, the arachidonic acid metabolism, and fatty acid levels were significantly altered. We can confidently state that the leukotriene metabolism, fatty acids, and the arachidonic acid levels were decreased after recovery. Furthermore, the amino acid levels were also approaching normal after successful surgery.

To conclude, BC tumor burden strongly affects essential amino acids, the lipid metabolism, and the immune system. These findings may contribute to those of future studies that aim to increase our understanding of the pathogenesis of BC and find new therapeutics.

Table 2. Annotated metabolites including KEGG ID and regulation properties.

Compound	KEGG ID	Exact mass	Fold change	P value*	Regulation
Glucose	C00031	181.0701	0.42	.039	down
Lysine	C00047	169.0951	0.32	.035	down
Ornithine	C00077	155.0796	1.84	.041	up
Phenylalanine	C00079	188.0686	0.02	.012	down
Tyrosine	C00082	204.0638	0.04	.019	down
Ribose	C00121	151.0594	0.54	.048	down
N-acetylglucosamine	C00140	222.0980	0.51	.046	down
Malic Acid	C00149	157.0114	0.30	.035	down
Arachidonic Acid	C00219	327.2280	0.18	.021	down
N-acetylphosphate	C00227	140.9944	0.24	.030	down
Hexadecanoic Acid	C00249	279.2297	0.20	.028	down
Pyridoxine	C00314	192.0634	0.40	.044	down
Thiamine	C00378	266.1191	0.59	.050	down
Retinol	C00889	269.2276	0.25	.029	down
Leukotriene A4	C00909	341.2082	0.36	.041	down
7-Dehydrocholesterol	C01164	385.3474	0.57	.05	down
Linoleic Acid	C01595	303.2286	0.21	.025	down
Leukotriene B4	C02165	359.2185	0.29	.033	down

*P values were calculated by Welch's test. Outliers were filtered. Abbreviations: KEGG ID, Kyoto Encyclopedia of Genes and Genomes Identifier

Resumen

Objetivos: El cáncer de seno es una enfermedad mortal que causa muchas muertes especialmente en mujeres. Las terapias mejoradas podrían tener una contribución positiva en las tasas de supervivencia. La metabolómica es una herramienta importante para monitorear la alteración de varios metabolitos en cualquier caso clínico. En este estudio se pretende desarrollar un modelo metabolómico para observar las alteraciones metabólicas antes/ después de la recuperación de pacientes que padecen cáncer de mama mediante el método de espectroscopia de masas. **Material y Método:** Los pacientes con carcinoma ductal invasivo de grado 1-2 fueron evaluados de acuerdo con los resultados de tomografía por emisión de positrones/tomografía computarizada. Se tomaron en cuenta 14 pacientes que se recuperaron por completo para el análisis metabolómico. Las muestras de plasma se extrajeron y analizaron mediante espectroscopia Q-TOF MS/MS. Se realizó un análisis quimiométrico para determinar los metabolitos estadísticamente significativos. Todos los metabolitos se anotaron mediante el algoritmo mummichog. **Resultados y Discusión:** De acuerdo con el análisis de datos, la glucosa, ornitina, fenilalanina, algunas vitaminas y metabolitos en el metabolismo de los ácidos grasos se alteraron estadísticamente después de la recuperación de los pacientes. **Conclusión:** Los estudios de metabolómica no dirigidos se pueden utilizar para comprender la etiopatogenia del cáncer de seno, encontrando nuevos biomarcadores y alteraciones de las vías metabólicas. Después de que desaparece la carga tumoral, se proporciona homeostasis y la concentración de varios metabolitos se normaliza. Este estudio dilucida el efecto del cáncer de seno a nivel molecular.

Acknowledgements

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References

- Coleman, M.P., et al. Cancer survival in five continents: a worldwide population-based study (CONCORD). *The lancet oncology* 9, 730-756 (2008).
- Zaidi, Z. & Dib, H.A. The worldwide female breast cancer incidence and survival, 2018. (AACR, 2019).
- Han, S.-J., et al. Prognostic significance of interactions between ER alpha and ER beta and lymph node status in breast cancer cases. *Asian Pacific Journal of Cancer Prevention* 14, 6081-6084 (2013).
- Bien, A.M., Korzynska-Pietas, M. & Iwanowicz-Palus, G.J. Assessment of midwifery student preparation for performing the role of breast cancer educator. *Asian Pacific Journal of Cancer Prevention* 15, S633-S638 (2014).
- Stingl, J., Raouf, A., Emerman, J.T. & Eaves, C.J. Epithelial progenitors in the normal human mammary gland. *Journal of mammary gland biology and neoplasia* 10, 49-59 (2005).
- Villadsen, R., et al. Evidence for a stem cell hierarchy in the adult human breast. *The Journal of cell biology* 177, 87-101 (2007).
- Stingl, J., Raouf, A., Eirew, P. & Eaves, C.J. Deciphering the mammary epithelial cell hierarchy. *Cell cycle* 5, 1519-1522 (2006).
- Gusterson, B.A., et al. Distribution of myoepithelial cells and basement membrane proteins in the normal breast and in benign and malignant breast diseases. *Cancer research* 42, 4763-4770 (1982).
- Evan, G.I. & Vousden, K.H. Proliferation, cell cycle and apoptosis in cancer. *Nature* 411, 342-348 (2001).
- Curtis, C., et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature* 486, 346-352 (2012).
- Hartwell, L.H. & Kastan, M.B. Cell cycle control and cancer. *Science* 266, 1821-1828 (1994).
- Husarik, D.B. & Steinert, H.C. Single-photon emission computed tomography/computed tomography for sentinel node mapping in breast cancer. in *Seminars in nuclear medicine*, Vol. 37 29-33 (Elsevier, 2007).
- Gursoy, A.A., et al. Comparison of three educational interventions on breast self-examination knowledge and health beliefs. *Asian Pac J Cancer Prev* 10, 765-772 (2009).
- Al-Naggar, R.A., Al-Naggar, D.H., Bobryshev, Y.V., Chen, R. & Assabri, A. Practice and barriers toward breast self-examination among young Malaysian women. *Asian Pac J Cancer Prev* 12, 1173-1178 (2011).
- Akram, M., Iqbal, M., Daniyal, M. & Khan, A.U. Awareness and current knowledge of breast cancer. *Biological Research* 50, 33 (2017).
- Yanovich, G., et al. Clinical proteomics of breast cancer reveals a novel layer of breast cancer classification. *Cancer research* 78, 6001-6010 (2018).
- Gámez-Pozo, A., et al. Functional proteomics outlines the complexity of breast cancer molecular subtypes. *Scientific Reports* 7, 1-13 (2017).
- Moore, S.C., et al. A metabolomics analysis of body mass index and postmenopausal breast cancer risk. *JNCI: Journal of the National Cancer Institute* 110, 588-597 (2018).
- Denkert, C., et al. Metabolomics of human breast cancer: new approaches for tumor typing and biomarker discovery. *Genome medicine* 4, 1-9 (2012).
- Yang, C., Richardson, A.D., Smith, J.W. & Osterman, A. Comparative metabolomics of breast cancer. in *Biocomputing 2007* 181-192 (World Scientific, 2007).
- Monteiro, M., Carvalho, M., Bastos, M. & Guedes de Pinho, P. Metabolomics analysis for biomarker discovery: advances and challenges. *Current medicinal chemistry* 20, 257-271 (2013).
- Kuligowski, J., Sánchez-Illana, Á., Sanjuán-Herráez, D., Vento, M. & Quintás, G. Intra-batch effect correction in liquid chromatography-mass spectrometry using quality control samples and support vector regression (QC-SVRC). *Analyst* 140, 7810-7817 (2015).
- Haritwal, A.K., Chourasia, R.K. & Ojha, S. A comparative study of serum lipid profile and glucose level between breast cancer patients and controls at tertiary care hospital in India. *International Journal of Medical Science Research and Practice* 2, 16-19 (2015).
- Sharma, M., et al. Chemotherapy agents alter plasma lipids in breast cancer patients and show differential effects on lipid metabolism genes in liver cells. *PLoS one* 11, e0148049 (2016).
- Hynes, N.E. Tyrosine kinase signalling in breast cancer. *Breast cancer research* 2, 1-4 (2000).
- Vong, L.B., et al. Poly(ornithine)-based self-assembling drug for recovery of hyperammonemia and damage in acute liver injury. *Journal of Controlled Release* 310, 74-81 (2019).
- Manni, A., Mauger, D., Gimotty, P. & Badger, B. Prognostic influence on survival of increased ornithine decarboxylase activity in human breast cancer. *Clinical cancer research* 2, 1901-1906 (1996).
- Oakman, C., et al. Identification of a serum-detectable metabolomic fingerprint potentially correlated with the presence of micrometastatic disease in early breast cancer patients at varying risks of disease relapse by traditional prognostic methods. *Annals of Oncology* 22, 1295-1301 (2011).
- Neurauter, G., et al. Serum phenylalanine concentrations in patients with ovarian carcinoma correlate with concentrations of immune activation markers and of isoprostane-8. *Cancer Letters* 272, 141-147 (2008).
- Miyagi, Y., et al. Plasma free amino acid profiling of five types of cancer patients and its application for early detection. *PLoS one* 6, e24143 (2011).
- Haeggstrom, J.Z. Structure, function, and regulation of leukotriene A4 hydrolase. *American journal of respiratory and critical care medicine* 161, S25-S31 (2000).
- Chen, X., Wang, S., Wu, N. & Yang, C. Leukotriene A4 hydrolase as a target for cancer prevention and therapy. *Current cancer drug targets* 4, 267-283 (2004).
- Wang, D. & DuBois, R.N. Eicosanoids and cancer. *Nature Reviews Cancer* 10, 181-193 (2010).