*Original Article*

# USE OF ENZYMATIC PARAMETERS IN DIAGNOSING DUCTAL AND LOBULAR BREAST CARCINOMA

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## Summary

Ductal and lobular breast carcinomas are one of the leading causes of death among perimenopausal and menopausal women. The aims of study are: determination of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) activities in tumorous tissues of patients with ductal and lobular breast carcinoma, as well as in healthy breast tissue; electrophoretic profiling of MDH isoforms; correlation of these parameters with histological type of breast carcinoma. Tumorous and healthy breast tissue of each woman was analyzed. LDH and MDH activities were quantified spectrophotometrically. MDH isoforms distribution was obtained with electrophoretic zymography. Significant difference was found between levels of MDH activity in tumorous *vs.* healthy tissue of both histological types. A dominant MDH isoform in tumorous and healthy tissue was cytosolic MDH (c-MDH). Correlation coefficients were high, regarding LDH levels in lobular carcinoma and MDH levels in ductal carcinoma. MDH activity was highly sensitive in diagnosing breast carcinoma. LDH activity can be used as a parameter for biochemical estimation of lobular breast carcinoma, as well as MDH levels in ductal breast carcinoma. LDH and MDH levels highly correlate in lobular breast carcinoma and their ratio can be used to detect lobular and ductal breast carcinoma.

**Key words:** ductal breast carcinoma, lactate dehydrogenase, lobular breast carcinoma, malate dehydrogenase

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## Introduction

Breast cancer is the most common cancer in women in developed countries. Survival in breast cancer patients has improved substantially over the years as a result of multimodal treatment, comprising of local treatment by surgery and radiotherapy; systemic treatment by chemotherapy and hormonal therapy [1]. Defining molecular abnormalities in breast cancer is an important strategy for early detection, prognosis, and treatment selection [2]. Ductal breast carcinoma (DBC) is a heterogeneous disease defined as a neoplastic proliferation within the ductal structures of the breast [3]. DBC accounts for a significant percentage of breast cancers diagnosed

nowadays and the greatest increase in incidence rates occurs among women aged 50 or older [4]. Although not as common as DBC, lobular breast carcinoma (LBC) incidence rates also tend to increase. LBC is generally considered a risk factor rather than a precursor of invasive lobular and ductal carcinomas. However, some data suggest that gene mutation in LBC *in situ* and invasive LBC are similar, and that LBC *in situ* might be a precursor of an invasive form of LBC [5, 6]. Carcinoma cells are frequently under persistent oxidative stress. Human tumor cell lines *in vitro* produce reactive oxygen species (ROS) at a far greater rate than do non-transformed cell lines [7]. Unlike nontransformed cells, tumor cells stimulate growth through increased glycolysis despite low pH and hypoxic conditions. Hypoxia precipitates overexpression of many genes of glycolysis, ultimately stimulating key metabolites that may be used as tumor markers [8]. Markers of constitutive oxidative stress have been detected in samples from *in vivo* breast carcinomas [9, 10]. Intensity of oxidative damage in breast carcinoma patients can be determined by numerous parameters including enzymatic. Some of them are activities of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH), which can be used as indicators of anaerobic and aerobic respirations intensity respectively, and biomarkers of cellular turnover [11, 12]. Activity of serum LDH can be elevated in numerous pathological conditions, including neoplastic diseases [13]. Increased MDH activity in sera and tumorous tissue of breast cancer patients can be used as an additional parameter for diagnosis and severity estimation [14]. Also, it has been shown that electrophoretic profiling of MDH isoforms can be a useful tool for diagnosis of various carcinomas, including DBC and LBC [15, 16]. There are two isoforms of MDH in human cells: cytosolic (c-MDH) and mitochondrial (m-MDH) [17, 18]. Comparative metabolic profiling of neoplastic and normal cells improves our understanding of the fundamental mechanisms of tumorigenesis and opens opportunities in target and drug discovery [19].

Our aim was to determine activities of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) in tumorous and healthy tissues of patients with DBC and LBC. Also, we determined electrophoretic profile of MDH isoforms of both histological types of breast cancer. Finally, we conducted correlative analyses of LDH and MDH activities, correlating activities between those values in tumorous and healthy tissues of each patient for both histological types of breast carcinoma. Furthermore, the rate of biochemical polymorphism and metabolic characteristics of DBC and LBC were examined, and we tried to propose another biochemical parameter for diagnosis of breast carcinoma and for differential diagnosis between those two histological types of breast carcinoma based on their biological characteristics.

## Material and Methods

### *Patients and bioptic material*

The study included two groups of patients with breast carcinoma. The first one included 18 women diagnosed with ductal breast carcinoma, aged 57.5 ± 9.6 years. The second group included 18 women diagnosed with lobular breast carcinoma, aged 55.6 ± 8.7 years. Bioptic materials of tumorous and surrounding healthy tissues were provided after excision biopsy of each patient. Healthy tissue was used as control for each woman separately. Samples of lysated bioptic material of each woman tumorous and healthy tissue were provided by National Cancer Research Center. Samples were stored at -20°C and were incubated for 30 min at 37°C before each biochemical analysis. The study was carried out according to all ethical standards. The study protocol was approved by written consent of each patient and the local ethical committee.

### *Biochemical estimation*

Activities of lactate dehydrogenase were determined according to the method of Buhl et al. [20]. The activities were assayed spectrophotometrically, measuring the absorbance downfalls during oxidation of NADH at 340 nm. Test conditions provided that one unit of enzyme activity [U] catalysed transformation of 1 μmol of reduced nicotinamide adenine dinucleotide (NADH) per minute.

The method of Frieden et al. was used to determine activities of malate dehydrogenase [21]. Absorbance downfalls were measured during oxidation of NADH at 340 nm. Test conditions provided that one unit of enzyme activity [U] catalysed transformation of 1 μmol of reduced nicotinamide adenine dinucleotide (NADH) per minute.

Protein concentration of each tumorous and

healthy tissue sample was determined according to the Bradford method [22].

Malate dehydrogenase isoforms were determined with direct electrophoretic zymography, using the method of Yoshimura et al. [23]. For each sample, equal lysate protein concentration was loaded, according to results provided after using the Bradford method. Isoforms were visualized as dark blue bands of formazane, which formed as a product of reduction of nitroblue tetrazolium (NBT) in the presence of phenazine metosulphate (PMS) as an electrone transfer mediator and oxidized coenzyme nicotinamide adenine dinucleotide ($NAD^+$). Zymography was followed by densitometric analysis of MDH isoforms, performed using ImageJ Q.42 software package [24].

### *Statistical analyses and data presentation*

Data were expressed as mean ± SEM. Statistical comparison between values obtained from tumorous and healthy tissue was performed by one-way analysis of variance (ANOVA), followed by Bonferroni-Holm Post hoc test. Pearson`s correlation test was used to establish associations between distributed variables. SPSS 12.0 software package was used for statistical analyses. Differences were considered significant at $p<0.05$.

## Results

### *Activities of lactate dehydrogenase and malate dehydrogenase*

Values of LDH and MDH activities are represented as units per gram of tissue [U/g]. Specific values of LDH and MDH activities in both histological types of breast carcinoma and their controls are presented in Table 1. There was no statistically significant difference ($p>0.05$) between tumorous and healthy (control) tissue regarding LDH activities in both histological types of breast carcinoma. Highly significant difference ($p<0.001$) was found between tumorous and healthy (control) tissue, regarding MDH activities in the group with ductal breast carcinoma, as well as in the group with lobular breast carcinoma.

**Table 1.** Lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) activites in tumorous and in healthy (control) tissue among patients with ductal and lobular breast carcinoma

| Parameter | Ductal breast carcinoma | | Lobular breast carcinoma | |
|---|---|---|---|---|
| | Tumor | Control | Tumor | Control |
| LDH [U/g] | 2665.2 ± 653.4 | 1123.4 ± 598.2$^{\dagger}$ | 2241.8 ± 421.8 | 2012.5 ± 389.2$^{\dagger\dagger}$ |
| MDH [U/g] | 43256.2 ± 4235.1 | 17254.3 ± 2278.1$^{\ddagger}$ | 38245.7 ± 3845.1 | 16789.6 ± 2163$^{\ddagger\ddagger}$ |

*$^{\dagger}p>0.05$ Tumorous vs. healthy tissue; $^{\dagger\dagger}p>0.05$ Tumorous vs. healthy tissue; $^{\ddagger}p<0.001$ Tumorous vs. healthy tissue; $^{\ddagger\ddagger}p<0.001$ Tumorous vs. healthy tissue*

### *Electrophoretic profile of malate dehydrogenase isoforms and relative activities*

After electrophoretic zymography, two bands appeared on electrophoregrams. Cytosolic (c-MDH) isoform appeared on gel as a band of higher electrophoretic mobility, and the mitochondrial (m-MDH) isoform – as a band of lower electrophoretic mobility. Representative electrophoregram with samples of tumorous and healthy tissue of both histological types is shown on Figure 1. Cytosolic MDH isoform appeared as a dominant isoform in tumorous, as well as in healthy tissue of both histological types of breast carcinoma. Both ductal and lobular breast carcinoma patient groups showed highly significant difference ($p<0.001$) between c-MDH *vs.* m-MDH isoforms. Distributions of MDH isoforms are represented on Figure 2 as relative activities (% of total).

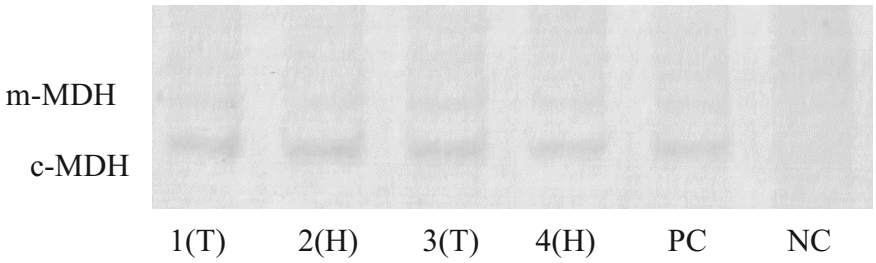


**Figure 1.** Representative electrophoretic zymogram of malate dehydrogenase isoforms. T-tumorous tissue; H-healthy tissue; PC-positive control; NC-negative control. Positions 1 and 2 represent MDH isoforms distribution in group of ductal breast carcinoma patients. Positions 3 and 4 represent MDH isoforms distribution in group of lobular breast carcinoma patients

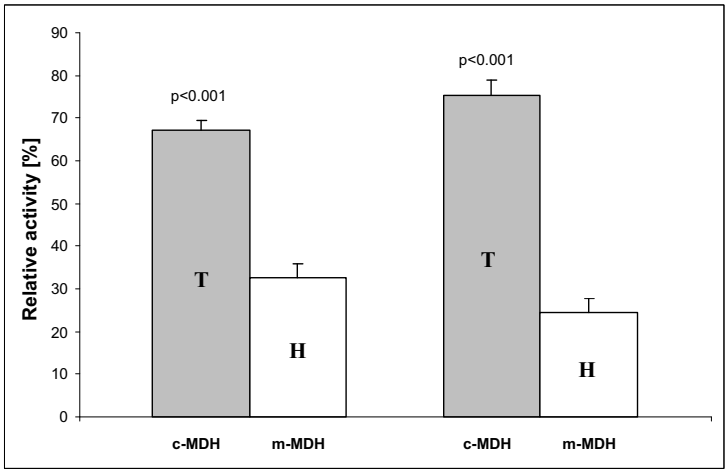


**Figure 2.** Activities of c-MDH and m-MDH are represented as relative activites in tumorous (T) and healthy (H) tissue

### *Correlation analyses*

Figure 3 shows a correlation histogram of MDH activities in the group with ductal breast carcinoma. There is a medium correlation (R=0.46) between tumorous and healthy tissue. Correlation analysis of MDH activities in the group with lobular breast carcinoma is shown in Figure 4. There is a high correlation (R=0.6) between tumorous and healthy tissue. A correlation histogram of LDH activities in ductal breast carcinoma patients is shown in Figure 5, demonstrating a medium correlation (R=0.32) between tumorous and healthy tissue. Regarding LDH activities, there is a high correlation (R=0.61) between tumorous and healthy tissue in the group with lobular breast carcinoma (Figure 6).

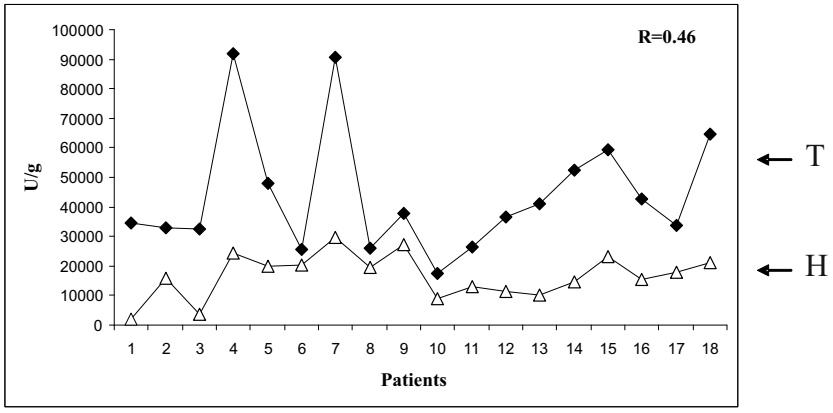


**Figure 3.** Correlation histogram of MDH activities in tumorous (T) and healthy (H) tissue among patients with ductal breast carcinoma

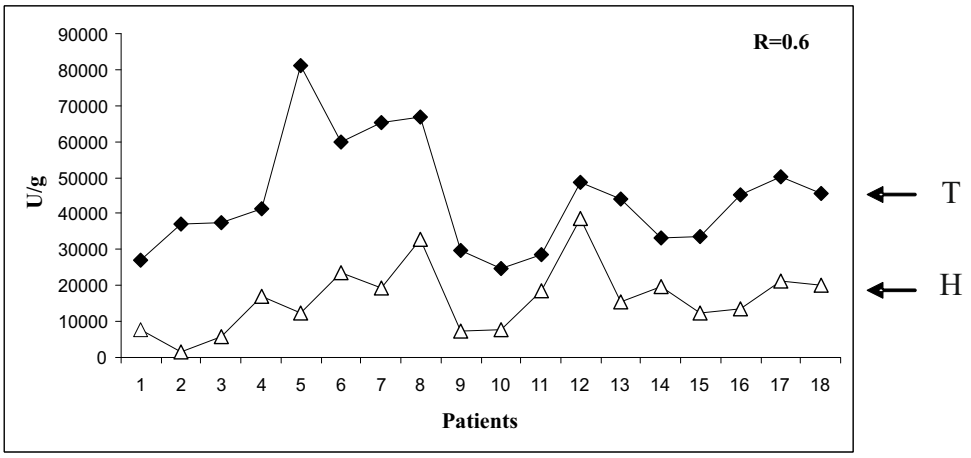


**Figure 4.** Correlation histogram of MDH activities in tumorous (T) and healthy (H) tissue among patients with lobular breast carcinoma

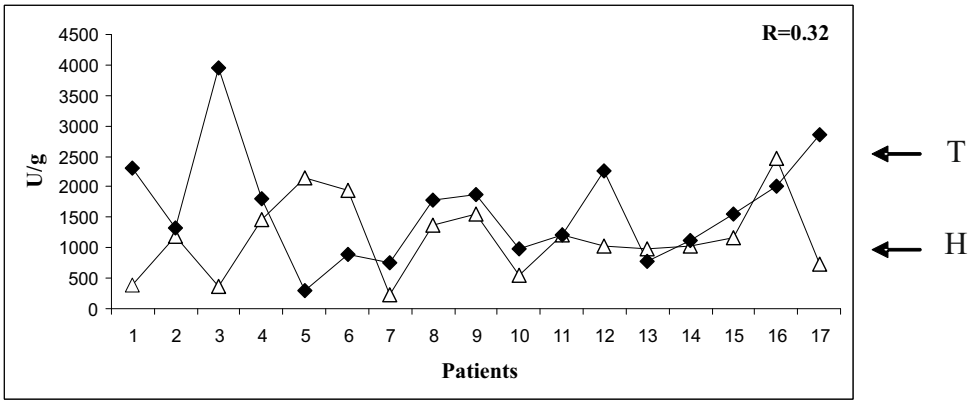


**Figure 5.** Correlation histogram of LDH activities in tumorous (T) and healthy (H) tissue among patients with ductal breast carcinoma

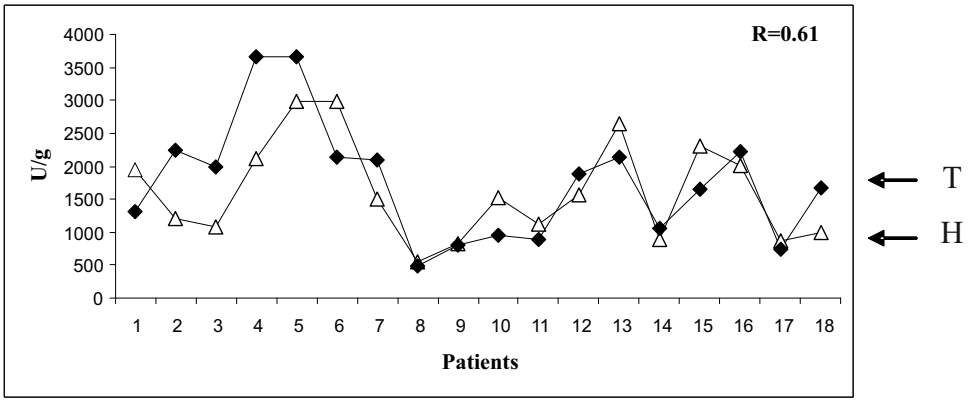


**Figure 6.** Correlation histogram of LDH activities in tumorous (T) and healthy (H) tissue among patients with lobular breast carcinoma

## Discussion

Only a limited number of tumor markers for breast cancer are currently available. Much of the effort in the past was focused on the discovery and characterization of single tumor-associated antigens as cancer markers [25]. Enzymes alone or in combination with tumor markers or other factors may be used. Lactic dehydrogenase (LDH) is perhaps the most common clinical enzyme used in cancer patients for prognostic purposes. Patients can be stratified into treatment protocols based on LDH activity [26]. There exists documented evidence that MDH activity can be elevated in numerous carcinomas and that this activity is in a direct correlation with tumor grade [27]. Our study considered a pathobiochemical aspect of DBC and LBC tissue, investigating activities of LDH and MDH, and profiling MDH isoforms. We also determined the biological and metabolic behavior of DBC and LBC, examining correlation coefficients between LDH and MDH activities in tumorous and healthy tissue of each patient. We established statistically highly significant ($p<0.001$) increase of LDH activity in both DBC and LBC. However, DBC showed higher values. Increased LDH activity in tumorous tissue points to a higher rate of utilization of anaerobic sources of energy [28]. Correlation analyses displayed high correlation ($R=0.61$), regarding LDH activity, in women with LBC. On the contrary, the correlation was medium ($R=0.32$) in the group of DBC patients. A high correlation coefficient, concerning LDH activities in the LBC group lead us to the conclusion that LBC shows a better expressed biochemical and metabolic homogeneity than DBC does. MDH activities were increased significantly ($p<0.001$) in tumorous tissues of both histological types, but DBC showed higher values than LBC, which points to its higher aerobic respiration potential as compared to than LBC. Unlike LDH, MDH activities increase was absolutely sensitive for tumorous tissue of both DBC and LBC. The ratio between values of MDH activity in tumorous and healthy tissue was higher than 1 in each of the patients. Correlation between MDH activities in tumorous and healthy tissues was high ($R=0.6$) in the LBC group. On the other hand, a medium correlation ($R=0.46$) between MDH activities in tumorous and healthy tissue was found in DBC patients. This finding, as well as LDH correlation pattern, also confirmed that DBC possesses a lower metabolic homogeneity than that of LBC. A dominant MDH isoform in tumorous and healthy tissues of both histological types was cytosolic (c-MDH). The relative activity of c-MDH *vs.* m-MDH isoform differed significantly ($p<0.001$) in both DBC and LBC groups. On the other hand, increased LDH and MDH activities in tumorous tissues of both histological types are indicators of increased cellular turnover [11].

The use of molecular components from both normal and neoplastic breast tissue as markers for breast tumors has long been recognized as of major potential for breast cancer diagnosis, and has been an area of active research [29]. The potential use of patient tumor patterns of LDH and other glycolytic enzyme activities in conjunction with estrogen receptor analysis for selecting breast cancer patients for hormone and/or chemotherapy has also been postulated [29]. Various extracellular and intracellular dehydrogenases have been the focus of many investigations that explain metabolism in tumor cells and they will certainly remain a sphere of interest, considering possible diagnostic, prognostic and therapeutic application in neoplastic diseases.

## Conclusions

Ductal and lobular breast carcinomas have increased catabolism intensity, both anaerobic and aerobic, than normal breast tissue. An increase in MDH activity is absolutely sensitive for tumorous tissue, so if relevant range intervals could be established, MDH activity might possibly be used to distinguish tumorous from healthy breast tissue. Further analyses of MDH activity in other breast tumors, including benign tumors are needed to achieve insight in gradation intervals of MDH activities of each tumorous alteration. Dominant MDH isoform is cytosolic due to increased cell turnover but future and more precise quantitative investigations are needed. Based on LDH and MDH activities, correlative analyses showed that DBC had higher metabolic polymorphism than LBC, and according to that, its biological behavior was more heterogenous with consequently higher rates of anaplasticity and severe clinical presentation. LDH and MDH levels highly correlated in lobular breast carcinoma and their ratio could be used for a distinction of DBC and LBC. LDH and MDH examinations showed their important role in accessing data that describe biological and metabolic behavior of ductal and lobular breast carcinoma.

## References


1. Veronesi U, Cascinelli N, Mariani L, Greco M, Saccozzi R, Luini A et al. Twenty-year follow-up of a randomized study comparing breast-conserving surgery with radical mastectomy for early breast cancer. N Engl J Med. 2002;347:1227-32.
2. Beenken SW, Grizzle WE, Crowe RD, Conner MG, Weiss HL, Sellers MT et al. Molecular biomarkers for breast cancer prognosis: coexpression of c-erbB-2 and p53. Ann Surg. 2001;233(5):30-8.
3. Jones JL. Progression of ductal carcinoma in situ: the pathological perspective. Breast Cancer Res. 2006;8:204-7.
4. Li CI, Daling JR, Malone KE. Age-specific incidence rates of in situ breast carcinomas by histologic types, 1980 to 2001. Cancer Epidemiol Biomarkers Prev. 2005;14:1008-11.
5. Lishman S, Lackhani S. Atypical lobular hyperplasia and lobular carcinoma in situ: surgical and molecular pathology. Histopathology. 1999;35:195-200.
6. Vos CB, Cleton-Jansen AM, Berx G, de Leeuw WJ, ter Haar NT, van Roy F et al. E-cadherin inactivation in lobular carcinoma in situ of the breast: an early event in tumorigenesis. Br J Cancer. 1997;76:1131-3.
7. Szatrowski TP, Nathan CF: Production of large amounts of hydrogen peroxide by human tumor cells. Cancer Res. 1991;51:794-8.
8. Yang C, Richardson AD, Osterman A, Smith JW. Profiling of central metabolism in human cancer cells by two-domensional NMR, GC-MS analysis, and isotopomer modeling. Metabolomics. 2008;4(1):13-29.
9. Toyokuni S, Okamoto K, Yodoi J, Hiai H. Persistent oxidative stress in cancer. FEBS Lett. 1995;358(1):1-3.
10. Portakal O, Ozkaya O, Erden Inal M, Bozan B, Kosan M, Sayek I. Coenzyme Q10 concentrations and antioxidant status in tissues of breast cancer patients. Clin Biochem. 2000;33:279-84.
11. Naik SG, Rathnasabapathy C, Chenthil H, Sangal A, Dumlao T, Kodali S et al. LDH in solid tumors as a surrogate marker for tumor burden and response to treatment. J Clin Oncol. 2008;26(15S):22164.
12. Onda M, Emi M, Nagai H, Nagahata H, Tsumagari H, Fujimoto T et al. Gene expression patterns as marker for 5-year postoperative prognosis of primary breast cancers. J Cancer Res Clin Oncol. 2004:130:537-45.
13. Clement L. Markert Lactate Dehydrogenase Isozymes: Dissociation and Recombination of Subunits. Science. 1963;140:(3573):1329-30.
14. Kogan EA. Histo- and cytochemical enzymatic characteristics of breast cancer. Arkh Patol. 1979;41(11):19-25.
15. Balinsky D, Cayanis E, Geddes EW, Bersohn I. Activities and isoenzyme patterns of some enzymes of glucose metabolism in human primary malignant hepatoma. Cancer Res. 1973;33:249-55.
16. Balinsky D, Platz CE, Lewis JW. Isozyme Patterns of Normal, Benign, and Malignant Human Breast Tissues. Cancer Res. 1983;43:5895-901.
17. Tanaka T, Inazawa J, Nakamura Y. Molecular Cloning and Mapping of a Human cDNA for Cytosolic Malate Dehydrogenase (MDH1). Genomics. 1996;32:128-30.
18. Loeber G, Maurer-Fogy I, Schwendenwein R. Purification, cDNA cloning and heterologous expression of the human mitochondrial NADP(+)-dependent malic enzyme. Biochem J. 1994;304:687-92.
19. Richardson AD, Yang C, Osterman A, Smith JW. Central carbon metabolism in the progression of mammary carcinoma. Breast Cancer Res Treat. 2008;110:(2):297-307.
20. Buhl SN, Jackson KY, Lubinski R, Vanderlinde RE. Optimal conditions for assaying human lactate dehydrogenase by the lactate-to-pyruvate reaction: Arrhenius relationships for lactate dehydrogenase isoenzymes 1 and 5. Gun Chem. 1977;23:1289.
21. Frieden CJ, Fernandez S. Kinetic studies on pig heart cytoplasmic malate dehydrogenase. J Biol Chem. 1975;250:2106-13.
22. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding Anal Biochem. 1976;72:248-54.
23. Yoshimura Y, Kawano T, Kuroi M, Morishita M, Mori M, Kawakatsu K. Zymographic demonstration of lactate and malate dehydrogenases isoenzymes in the rodent salivary glands. Histochem Cell Biol. 1970;22:337-46.
24. ImageJ-RSB [Internet] Image Processing and Analysis in Java. Available from: http://rsb.info.nih.gov/ij
25. Zhong L, Ge K, Zu J, Zhao L, Shen W, Wang J et al. Autoantibodies as potential biomarkers for breast cancer. Breast Cancer Res. 2008;10(3):R40.
26. Schwartz MK. Enzymes as prognostic markers and therapeutic indicators in patients with cancer. Clin Chim Acta. 1992;206:77-82.
27. Ross CD, Gomaa MA, Gillies E, Juengel R, Medina JE. Tumor grade, microvessel density, and activities of malate dehydrogenase, lactate dehydrogenase, and hexokinase in squamous cell carcinoma. Otolaryngol Head Neck Surgery. 2000;122:195-200.
28. Ceriani R, Anderson EP. Tumor markers and their significance in the management of breast cancer. Breast Cancer Res Treat. 1985;6:249-54.
29. Savlov ED, Wittliff JL, Hilt A. Further studies of biochemical predictive tests in breast cancer. Cancer. 1977;39:539-41.