Activity of lactate dehydrogenase and superoxide dismutase in the circulation of patients with breast carcinoma

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SUMMARY

Background: *A rapid increase in the turnover of malignant cells modulates the enzymes level in circulation and may be a prognostic sign of disease progression. We evaluated the activity of serum lactate dehydrogenase and superoxide dismutase in erythrocytes of breast cancer patients immediately after surgery and before adjuvant therapy.*

Methods: *Fourteen newly operated breast cancer patients were included in the study. Lactate dehydrogenase and superoxide dismutase activity were estimated using standard tests for evaluation of enzyme activity.*

Results: *Activity of lactate dehydrogenase was in normal range in all but one sample obtained from patient at clinical status IIIB. Superoxide dismutase activity was elevated in 11 out of 14 patients, and was 2 to 8 fold higher compare to control values. The highest activity of superoxide dismutase was found in samples of two patients at clinical status I.*

Conclusion: *Determination of lactate dehydrogenase and superoxide dismutase activity might be useful in clinical follow up of breast cancer patients.*

Key words: *Breast Neoplasms; Oxidative Stress; Lactate Dehydrogenases; Superoxide Dismutase; Postoperative Period*

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Abbreviations:
Ca – Carcinoma, Cu, ZnSOD – Superoxide dismutase which contains Cu and Zn, EDTA – Ethylene-diammin-tetraascorbic acid, FAC – Therapeutic protocol for breast cancer patients, H_2O_2 – Hydrogene peroxide, LDH – Lactate-dehydrogenase, MnSOD - Superoxide dismutase which contains Mn, O_2^- – Superoxide anion radical, ROS – Reactive oxygene species, SOD – Superoxide dismutase

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INTRODUCTION

Breast cancer is the third most common cancer in women worldwide and accounts for the highest morbidity and mortality (1). Annually 910,000 new patients are diagnosed of breast cancer and 376,000 women die from the disease (2-4). According to the Registry for Malignant Neoplasms of Vojvodina, about 9000 women were diagnosed with breast cancer and 4800 died from the disease from 1995 to 2004 (5). Based on epidemiological studies, it has been established that risk factors for breast cancer include age, reproductive events (menarche, menopause, pregnancy), exogenous hormones (oral contraceptive and hormone replacement therapy), lifestyle risk factors (alcohol, diet, obesity) as well as genetic factors (high- and low-penetrance breast cancer susceptibility genes) among others (3,4). The increasing global incidence of breast cancer emphasizes the need to understand the various mechanisms involved in breast tumor genesis.

A rapid increase in the turnover of malignant cells modulates the enzymes level in the blood circulation (6). In addition, enzymatic changes reflect the overall changes in metabolism that occur in malignancy (7). Increased anaerobic glycolysis and activity of antioxidant enzymes were shown to be characteristics of cancer cell metabolism (6,8)

Lactate dehydrogenase (LDH) is released into the surrounding medium at an increased rate when cells replicate more rapidly (7,9). It was shown that LDH levels were higher in malignant than in normal tissue (10). LDH activity correlates with tumor mass or prognosis in human breast cancer. Metastatic breast cancer frequently exhibits elevated level of LDH as compared to normal breast tissue, but this observation is not consistent and still remains controversial (7,9).

Reactive oxygen species (ROS)-induced cell damage has been implicated in malignant transformation (11). The formation of free radicals is physiologically prevented or scavenged by host antioxidative defense mechanisms. Low levels of essential antioxidants in the circulation have been associated with an increased risk of cancer (12). Previously, impairment in antioxidant defense mechanisms has been demonstrated in a wide variety of malignancies including breast cancer (13-16). Basic and clinical studies showed that over-expression of manganese containing superoxide dismutase (MnSOD) may lead to changes of superoxide anion radical/ hydrogen peroxide (O_2^- / H_2O_2) balance causing changes in the cell redox state. Changes in cell redox state affects signal transduction pathways that modulate cell proliferation (17-19). Over-expression of Cu,ZnSOD also significantly decreases growth and survival of breast cancer cells (14). Therefore, it is suggested that both MnSOD and Cu,ZnSOD can act as a tumor suppressors (14,20). On the other hand, some studies have reported correlation between high MnSOD level and invasiveness of breast cancer (20-23).

In the present study, we evaluated LDH and superoxide dismutase (SOD) activity in the circulation of patients with breast cancer. This is a preliminary report of results from the study on 14 newly diagnosed breast cancer patients obtained immediately after surgical intervention and before any adjuvant therapy.

PATIENTS AND METHODS

Fourteen recently diagnosed women with breast cancer, median age 43 [30-49] from Institute of Oncology of Vojvodina, Serbia, were included in the study. They underwent surgical treatment and none of them was treated by hormonal, chemotherapy or radiotherapy. Patients' characteristics are shown in Table 1. Two out of 14 patients were at clinical stage I, five were classified as stage IIA, six as stage IIA, and one as stage IIIB. Informed consent was obtained from all the patients and local ethical board approved the study.

Blood samples were obtained from patients between the first and the second week after surgical treatment by venepuncture in tubes with EDTA. Plasma was separated by centrifugation at 3500 g for 10 min (Megafuge 1.0 R, Heraeus, Germany). After separation of plasma, the buffy coat was removed and the packed cells washed three times with physiologic saline, and centrifuged at 3500 g for 10 min. A known volume of erythrocytes was

Table 1. Characteristics of patients with breast carcinoma included in the study

No	TNM class.	Clinical stage	Type of surgery	Hystological type
1	$T_1N_0M_0$	I	Mastectomy	Lobular Carcinoma
2	$T_1N_0M_0$	I	Breast conserving surgery	Lobular Carcinoma
3	T_1N1M_0	IIA	Breast conserving surgery	Lobular Carcinoma
4	$T_2N_0M_0$	IIA	Breast conserving surgery	Medullar Carcinoma
5	$T_2N_0M_0$	IIA	Mastectomy	Ductal Carcinoma
6	$T_2N_0M_0$	IIA	Breast conserving surgery	Ductal Carcinoma
7	$T_2N_0M_0$	IIA	Mastectomy	Ductal Carcinoma
8	$T_2N_1M_0$	IIB	Mastectomy	Ductal Carcinoma
9	$T_2N_1M_0$	IIB	Breast conserving surgery	Ductal Carcinoma
10	$T_2N_1M_0$	IIB	Mastectomy	Ductal Carcinoma
11	$T_2N_1M_0$	IIB	Breast conserving surgery	Mucinous Carcinoma
12	$T_2N_1M_0$	IIB	Mastectomy	Ductal Carcinoma
13	$T_2N_1M_0$	IIB	Mastectomy	Ductal Carcinoma
14	$T_4N_1M_0$	IIIB	Mastectomy	Ductal Carcinoma

lysed with distilled water. Biochemical estimation for LDH was carried out immediately on ECTACHEM 250 apparatus according to original prescription by The Orthoclinical Jonhson & Jonhson (USA) producer.

Samples for SOD analysis were stored at -80°C until analysis (less than 1 month).

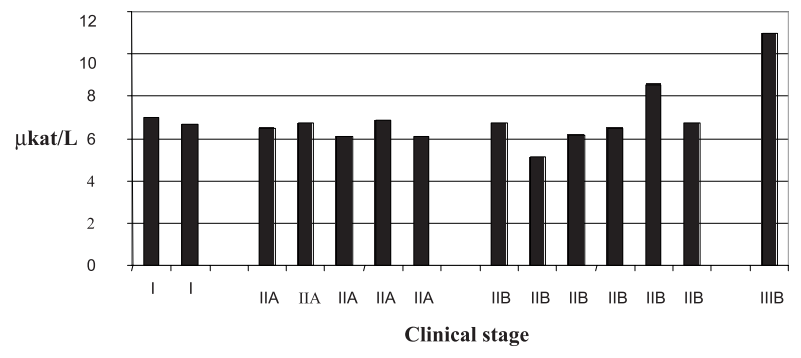

Figure 1. Activities of LDH in the serum of patients with breast cancer

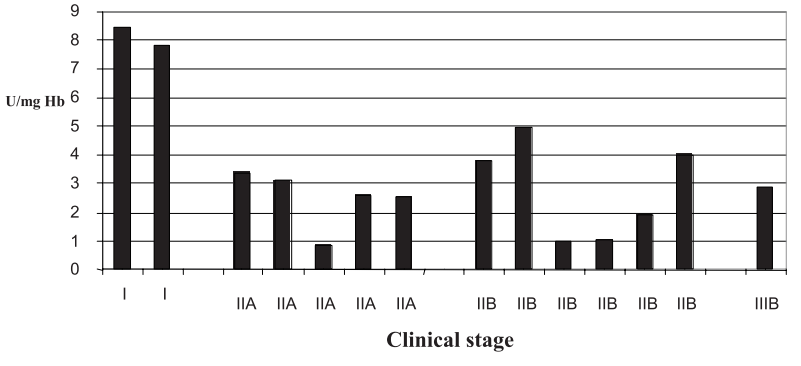

Figure 2. Activities of SOD in erythrocytes of patients with breast cancer

Erythrocyte SOD activity was assayed using the spectrophotometric indirect inhibition technique of Misra and Fridovich, based on the ability of SOD to inhibit auto-oxidation of adrenalin to adrenochrome at alkaline pH (24).

Statistical analysis

Data for biochemical analyses are expressed as individual and median values.

RESULTS

Individual values of LDH and SOD activity for all 14 breast cancer patients are shown in Table 2. Median values of LDH and SOD were 6.73 [5.12 - 11.00] (µkat/L) and 3.00 [0.87 - 8.45] (U/mg Hb) respectively.

LDH activity was in the normal range in all but one breast cancer patient. LDH activity in patient at clinical stage IIIB was approximately 2 fold higher compared to LDH values in lower stage samples (Table 2, Figure 1).

SOD was 2 to 8 fold increased in 11 patients in comparison to median physiological range for erythrocytes (27-29). Both patients at clinical stage I had the highest (~8 fold increased) level of SOD (Table 2, Figure 2). Significant individual differences in SOD activity were present in patients at IIA and IIB stage of disease.

Table 2. Activities of LDH and SOD in circulation of breast cancer patients

No	Age	Clinical stage	LDH* (µkat/L)	SOD* (U/mg Hb)
1	30	I	6.98	8.45
2	47	I	6.70	7.81
3	39	IIA	6.50	3.38
4	43	IIA	6.76	3.12
5	43	IIA	6.12	0.87
6	35	IIA	6.89	2.59
7	45	IIA	6.07	2.54
8	43	IIB	6.76	3.76
9	32	IIB	5.12	4.96
10	43	IIB	6.23	0.98
11	49	IIB	6.49	1.02
12	44	IIB	8.54	1.88
13	39	IIB	6.75	4.00
14	35	IIIB	11.00	2.88

*Ref. values: LDH: 5.22-10.30 µkat/L; SOD: 0.8-1 U/mg Hb (25-29).

DISCUSSION

In this preliminary study, we investigated the activity of serum lactate dehydrogenase and superoxide dismutase in erythrocytes of patients with breast cancer. This investigation is a part of screening study dealing with effects of chemotherapy on antioxidative status and chromosomal damages in breast cancer patients. Results showed that only patient at clinical stage IIIB without distant metastases at the time of investigation had an increased LDH activity. Clinical follow-up of that patient showed relapse of primary disease with massive bone metastases and lethal outcome one year after ending of the chemotherapy. This result suggested that elevated level of LDH might be a prognostic sign of disease progression, which is in correlation with investigations of Seth et al. (7).

Total LDH activity is elevated in the serum of about 65% cancer patients with metastases in the liver and in 20-60% cancer patients without hepatic metastases (25). LDH has also been found to correlate with tumor mass or prognosis in human breast cancer (7,30). Ryberg et al. reported that about 73% of breast cancer tissues had elevated serum LDH levels (30). According to Mehreen, LDH was not increased in patients without metastases (9), and we found normal LDH values in 13 samples in our screening investigation.

We found 2 to 8 fold increase of SOD in 11 patients in comparison to physiological level in erythrocytes (27-29). Both patients at clinical stage I had the highest (~8 fold increased) level of SOD.

The enzymatic antioxidant profile in the circulation of breast cancer patients shows an increase in SOD and GSH-Px activities compared with normal subjects, whereas the CAT activity showed a significant decrease compared with the respective controls (31). Decreased SOD in erythrocytes of patients with breast cancer has also been reported (27). Portakal et al. found that total SOD activity in breast tumor tissues was significantly higher than that in corresponding cancer-free tissues (32). Our findings are in accordance with that of Ray et al who reported that SOD activity was significantly raised in erythrocytes of breast cancer patients (16). Oxidative environment in newly established malignant phenotype may provoke oxidants to activate gene expression through the antioxidant responsive elements (33-35). It is supposed that overexpression of MnSOD, and probably, Cu,ZnSOD are important components of antioxidative defense through the process of malignant transformation (14). These facts might explain strongly enhanced SOD activity at lower stage of malignant disease that we found in two patients at clinical stage I.

In conclusion, lactate dehydrogenase and superoxide dismutase activity might be useful in clinical follow up of breast cancer patients.

Conflict of interest

We declare no conflicts of interest.

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