

# Diagnosis of Breast Cancer by Some Remarkable Enzymes

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## Abstract

**Objectives:** The aim of our research was to evaluate the activity of several remarkable enzymes in the serum of breast cancer and estimate their potential value in the diagnosis of breast cancer.

**Methods:** The study comprised of 70 patients with breast cancer and 50 age and sex-matched healthy subjects as controls. Levels of (5–Nucleotidase), Acid phosphatase activity (ACP), Glutamate dehydrogenase (GDH), Serum  $\beta$ -glucuronidase, Aldehyde dehydrogenase (ALDH), Alcohol Dehydrogenase (ADH), Superoxide dismutase (SOD) and Adenosine deaminase ADA were measured using enzymatic methods.

**Results:** There was a significant increase in the catalytic activities of 5–Nucleotidase ( $P < 0.001$ ), ACP ( $P < 0.05$ ), GDH ( $P \leq 0.001$ ), Serum  $\beta$ -glucuronidase ( $P < 0.0001$ ), ADH ( $P < 0.001$ ), SOD ( $P < 0.001$ ) and ADA ( $P < 0.001$ ), while there was no significant increase in the level of Aldehyde dehydrogenase (ALDH) ( $P = 0.231$ ) in sera of breast cancer patients compared to the control.

**Conclusion:** The present study strongly indicates the potential role of SOD, GDH, ADH, GDH, 5–Nucleotidase, GLU and ACP as important biomarkers in breast cancer.

**Keywords:** Breast cancer, remarkable enzymes

## Introduction

Carcinoma of Breast is a distractive disease that dependent on a number of factors, especially genetic or environmental factors. It is characterized by its uncurbed growth and metastasis of irregular breast cells.<sup>1,2</sup> Breast cancer is one of the most afraid diseases, which caused women and recognized as fatal world-wide diseases. The increased prevalence may unfortunately be due to the fact that most cancers are symptomless until the tumours are too large, and should be removed surgically or cancerous cells have already reach to other tissues.<sup>3</sup> In the normal conditions, every tissue maintains a steady consistent enzymatic level which may significantly change in diseases. In carcinomas, where the cells rapidly replicate, membrane constituents are surrounding milieu at elevated rate.<sup>4</sup> When the cells are destroyed The enzymes and proteins present in cells, nucleus, cytoplasm and mitochondria, released in the circulation. The enzymatic alterations in cancer tissue may attribute to the reprogramming of genetic materials to cancer feature for preserving of malignant cells.<sup>5</sup> The hepatic enzymes are increased in some conditions, the most common elevations are liver diseases (liver cirrhosis), infections, inflammations, and secondaries from malignant tumours. In spite of the considerable study for many years, the etiopathogenesis of cancer still remains unresolved. Variety of biochemical markers have been examined to assess the malignancy for the untimely investigation the cancer of different origins. However, no sole marker has confirmed to be a unique marker as well as sensitive of untimely malignancy.<sup>6</sup> Moreover, the cell protection from toxic actions of free oxygen reactive species is performed by superoxide dismutase (SOD), it is an enzyme seen to shield DNA molecule, components of cell membrane and proteins from oxidative damages. Besides, many quantitative alterations in serum proteins in cancers of various origins have been elucidated. Hence the present study is aimed at presuming some of the biomarkers, directly linked with breast cancer which are inexpensive, accurate, identified by easy

methods of detection and validated, that may be of some diagnostic significance.<sup>7</sup> Since enzymes are the products of genes, the quantitative changes in (total) enzymatic activity represent an indicator for reactivities of the corresponding genes.<sup>8</sup> Many enzymes and its isoenzymes are often used as markers in identification cancer diseases. The enzymes activity is changed in cancer diseases. Although these enzymes represent results of malignant cells, they are still not too specific for malignity because they can sometimes appear in some other diseases.<sup>9</sup>

In this study we have examined catalytic activity of 8 enzymes (5–Nucleotidase, lactate dehydrogenase, Glutamate dehydrogenase, Serum  $\beta$ -glucuronidase, Aldehyde dehydrogenase, Alcohol Dehydrogenase and SOD) the serum of breast cancer patients with either potentially curable disease or disseminated metasases. The objective of this investigation was to determine whether the levels of these enzymes in the serum can be correlated with the extent of the disease in these patients and to identify the most sensitive marker(s) for subsequent follow-up study in cancer patients.

## Materials and Methods

The study included 120 participants aged 36–77 years who were divided into two groups: breast cancer and control. The first group included: 70 breast cancer patients who had been diagnosed to have breast cancer by colonoscopy and biopsy. The second group included: 50 healthy people who had been diagnosed as free from breast cancer by colonoscopy. Exclusion criteria included: treatment by chemotherapy, radiotherapy, Blood, after coagulation, was centrifuged for 10 minutes at 4000 x g at 4°C. The supernatant (serum) was transferred to Eppendorf (safe-lock) tubes and frozen at –80°C. for the measurement of some remarkable enzymes parameters such as: Serum Lactate Dehydrogenase (LDH) was determined by an enzymatic method using kit manufactured by biocon (Germany). Serum Adenosine Deaminase (ADA) was determined by an enzymatic method using a kit manufactured by

BioMerieux (France). Serum gamma glutamyl transpeptidase (GGT): was determined by an enzymatic method using kit manufactured by biocon (Germany). Serum Superoxide dismutase (SOD) was determined by an enzymatic method using a kit manufactured by BioMerieux (France). ADH and ALDH were determined by an enzymatic method using a kit manufactured by Beta-glucuronidase (GLU) was determined by an enzymatic method using a kit manufactured by BioMerieux (France). Glutamate dehydrogenase (GDH) & lactate dehydrogenase (LDH), were determined by an enzymatic method using a kit manufactured by BioMerieux (France).

## Statistical Analysis

For the statistical analysis, SPSS 8.0 for Windows PL (SPSS, Chicago, IL, USA) was used. The differences between groups were evaluated using U test. Statistically significant differences were defined as comparisons resulting in  $P < 0.05$ .

## Results

The results in Table 1 is for (5-Nucleotidase, lactate dehydrogenase (LDH), Glutamate dehydrogenase (GDH), Serum  $\beta$ -glucuronidase, SOD and Adenosine deaminase (ADA) activity showed that there were significant increase ( $P < 0.001$ ,  $P = 0.009$ ,  $\leq 0.001$ ,  $P < 0.0001$ ,  $P < 0.001$ ,  $P < 0.001$  respectively) were significant in breast cancer women compared with control group.

The activities of ADH and ALDH in the sera are presented in Table 1. The serum level of total ADH activity was significantly higher in the cancer patients group compared with the control group. The analysis of ALDH activity did not show a significant difference between the total tested group and healthy persons.

## Discussion

Tumours were associated markers considered as a changed feature from tissue to blood, that responsible in the alteration in the concentration of enzymes, proteins and hormones in malignancies tissue and blood due to uncurbed multiplication of cells.<sup>10</sup> It was recorded that the concentration of serum ADA was remarkably higher in breast cancer patients when compared to marched number of healthy women. The activity of serum ADA is delicate to stimulation by chemicals such as cytokines and some growth factors through fast tissue

multiplication.<sup>11</sup> The high concentrations in the serum ADA may attribute to the lymphoid multiplication in the spread lymph nodes or passing of the enzyme from the chief tumour cells. High concentrations of ADA could be helpful biochemical marker for the identification carcinoma of breast for surveillance the development and cancellation after surgical treatment or chemotherapy.<sup>12</sup>

Current finding demonstrated elevated concentration of serum 5'-nucleotidase in carcinoma of breast cancer indicate a paperback data and are in concert with the theory that the untypical concentrations of serum glycosyltransferases may derived from the tumor. Since 5'-nucleotidase has been generally confirmed as a marker of plasma membrane, elevation in this enzyme in the serum would probability specify accelerated degradation and secretion of cell surface material. Determination the serum concentrations of 5'-nucleotidase as markers will be of value in observing the appearance of metastasis or cancellation.

The current study showed, remarkably higher enzymatic levels of of glutamate dehydrogenase and (GDH), lactate dehydrogenase (LDH) in serum samples of patients with breast cancer when compared to serum of healthy control group. The potential cause for elevate catalytic activities of GDH in cancer cells may be due to the fact that either it is important for redox homeostasis in cancer cells<sup>13</sup> or overexpression of GDH promoted cell proliferation, migration, and invasion in vitro, whereas loss of function of GDH had the opposite effect.<sup>14</sup>

Previous works suggest that GDH enzymes are important in cancer cell either for synthesizing Krebs cycle intermediates ( $\alpha$ -ketoglutarate and subsequent metabolite fumarate) or for synthesizing protein and fatty acid from citrate which originate from  $\alpha$ -ketoglutarate. In addition to that, the substrate of GDH, glutamate itself is a substrate for antioxidant (GSH) and nucleotide synthesis in the cancer cell. These metabolic changes support the production of intermediates for cell growth and division and are regulated by both oncogenes and tumor suppressor genes, in a number of key cancer-producing pathways.<sup>15,16</sup> The findings of this research have been reveal that female with carcinoma of breast have elevate levels of ACP when compared with the healthy control. It appears that elevated acid phosphatase activity is a stable character in neoplastic transformation.<sup>17</sup> Acid phosphatase activity has been utilized to investigate that lysosomes involved in the execution of cell death in a variety of tissues<sup>18</sup> and in the regression of mammary carcinomas. Cell death could describe a barrier to the development of invasive cancer.

Table 1. The mean  $\pm$  SD of serum enzymes activities in patients with breast cancer and control

Enzymes	Patients group Mean $\pm$ SD	Control group Mean $\pm$ SD	P-value
5-Nucleotidase Mmol/mg protein/hr	70 $\pm$ 114	20 $\pm$ 29.7	$P < 0.001$
Acid phosphatase (ACP) (mU/l)	14.555 $\pm$ 6.737	9.855 $\pm$ 7.369	$P < 0.05$
Glutamate dehydrogenase (GDH) GDH (mU/l)	5.20 $\pm$ 0.82	3.16 $\pm$ 0.64	$P \leq 0.001$
SOD (U/g Hg)	1121.4 $\pm$ 237	1510 $\pm$ 219	$P < 0.001$
Serum $\beta$ -glucuronidase pKat/mL	287.46 $\pm$ 87.32	149.28 $\pm$ 64.47	$P < 0.0001$
Aldehyde dehydrogenase (ALDH) mU/l	3.09 $\pm$ 1.85	4.24 $\pm$ 1.11	$P = 0.231$
Alcohol dehydrogenase (ADH) mU/L	843 $\pm$ 202	496 $\pm$ 140	$P < 0.001$
ADA(U/L)	88 $\pm$ 107	25 $\pm$ 30.7	$P < 0.001$

In our study also, the SOD activity was significantly ( $P < 0.0001$ ) lower in (Table 1) in patients of breast carcinoma as compared to healthy females. The antioxidant enzyme Superoxide dismutase and catalase are the backbone of the cellular antioxidant defense system.<sup>19</sup> The low activity of these antioxidant enzymes might be due to depletion of antioxidant defense system. The activity of the hydrogen peroxide detoxifying enzymes catalase was significantly decreased in the more metastatic line.<sup>20</sup> Several researches were considered SOD and CAT enzymes acts as anti carcinogens, antitoxins and inhibitors at initiation, promotion and transformation of carcinogenesis.<sup>21-24</sup> In the present study, SOD and CAT activities were significantly decreased in the sera and/or plasma of Breast Cancer patients than healthy controls, thus occurred due to high production of free radicals that led to accumulation of ROMs that it observed the role of antioxidants to lower incidence of various human morbidities or mortalities molecules as ROMs.

The average activity of GLU in the serum of patients with breast carcinoma was significantly greater ( $P < 0.0001$ ) in the serum of patients group with breast cancer in comparison to the healthy individuals. GLU is a lysosomal exoglycosidase whose activity usually increases in different catabolic (e.g. inflammatory) conditions. In mild oxidative stress, some lysosomes fracture and release hydrolytic enzymes into the cytosol,

which is accompanied by apoptosis and further release of the hydrolytic enzymes from the cells.<sup>25</sup>

In our study, the catalytic activities of serum aldehyde dehydrogenase (ALDH) and alcohol dehydrogenase (ADH) were significantly higher in breast cancer (BCA) patients in comparison to serum of control group. The expression of these enzymes in cancer cells is reflected by increased enzyme activity in the sera and thus could be helpful for diagnosing pancreatic cancer. Higher levels of ADH in patients with cancer might result from enzyme release by cancer cells and could be helpful for the diagnosis of pancreatic cancer. The diagnostic criteria for disease markers are sensitivity, specificity and area under the curve (AUC).

## Conclusion

The mean serum SOD, GDH, ADH, GDH, 5-Nucleotidase, GLU and LDH activities in patients with carcinoma breast were tremendously increased as compared to controls. Therefore these enzymes play a critical role in breast cancer progression and can be used as biomarkers for breast cancer.

## Conflicts of Interest

None. ■

## References

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