

Original Research Article

A Study on Blood Biochemical Markers in Carcinoma BreastGopalakrishnan Bhuvanewari¹, K. Ramadevi², M. Vijayalakshmi³¹Department of Biochemistry, Government Vellore Medical College, Vellore, India 632011²Professor of Biochemistry, Madras Medical College, Chennai- 600003, India³Associate Professor of Biochemistry, Stanley Medical College, Chennai-600001, India***Corresponding author**

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Abstract: Carcinoma breast is the most common malignancy and the most common cause of cancer deaths in Indian women. Tumor markers are biochemical indicators of the presence of tumor. Various tumor markers have been studied singly or in combination, in breast malignancies. Most of these are high on running costs, in terms of laboratory infrastructure. In this case-control study, we analyzed the levels of three biochemical parameters- namely superoxide dismutase (SOD), lactate dehydrogenase (LDH) and gamma-glutamyl transferase (GGT). The primary objectives were to determine the reference ranges for SOD, LDH and GGT in women aged between 35 and 75, and in women with carcinoma breast, to determine whether the levels of these biochemical parameters differ significantly in individuals with carcinoma breast when compared to apparently healthy individuals and the secondary objectives was determine if any of the risk factors have an association with breast cancer. The study was done during the period February 2007 to June 2007. It was carried out in two groups- 27 apparently healthy female as controls and 50 females with newly diagnosed carcinoma breast as cases. LDH levels were significantly elevated in the women with breast cancer. The levels of serum GGT were elevated in women with breast cancer. The levels of blood SOD were found to be significantly decreased in women with breast cancer. The mean and standard deviations of the biochemical parameters for the cases were: SOD 151.7 ±10.7 U/mL. LDH 423±198 IU/L, GGT 20 ±6 IU/L. The mean and standard deviations of the biochemical parameters for the controls were: SOD 189.8 ±14.8 U/mL, LDH 213±112 IU/L, GGT 20 ±6 IU/L. The levels of biochemical markers LDH and GGT were significantly elevated, and the levels of antioxidant enzyme SOD significantly lowered in women with breast carcinoma.

Keywords: Biochemical markers carcinoma breast

INTRODUCTION

Carcinoma breast is the most common malignancy and the most common cause of cancer deaths in Indian women [1]. Various factors have been attributed as risk for breast cancer- family history of breast cancer in a first-degree relative [2], socioeconomic factors [3], reproductive behaviors such as parity, age at first child birth, early onset menarche, late menopause [4], exogenous hormone use including oral contraceptive pills and post-menopausal hormone replacement therapy [5], obesity [6], consumption of alcohol [7], ionizing radiation exposure [8]. While these factors have helped at primary prevention strategies, focus has also been on understanding the pathogenesis of breast cancers. Tumor markers are biochemical indicators of the presence of tumor. Various tumor

markers have been studied singly or in combination, in breast malignancies [9]. Most of these are high on running costs, in terms of laboratory infrastructure. In this case-control study, we analyzed the levels of three biochemical parameters- namely superoxide dismutase (SOD), lactate dehydrogenase (LDH) and gamma-glutamyl transferase (GGT), that are potentially feasible to measure in resource constrained settings, and analyzed for possible correlation of their values to breast cancer.

Superoxide dismutase (SOD) is a key antioxidant in nearly all cells exposed to oxygen, and has been studied for potential prognostic and predictive role [10]. LDH activity is present in all cells of the body and is invariably found only in the cytoplasm of

the cell. Patients with breast malignancies have been shown to have increased serum LDH that has a potential to be used as a prognostic marker [11]. GGT activity in serum comes primarily from liver. GGT has also been shown to have a potential predictive role in prognosticating breast cancer [12].

AIM OF THE STUDY

Primary objectives

- To determine the reference ranges for SOD, LDH and GGT in women aged between 35 and 75, and in women with carcinoma breast
- To determine whether the levels of these biochemical parameters differ significantly in individuals with carcinoma breast when compared to apparently healthy individuals.

Secondary objectives

- To determine if any of the risk factors have an association with breast cancer

MATERIALS AND METHODS

The study was done during the period February 2007 to June 2007. It was carried out in two groups- apparently healthy female controls and females with newly diagnosed carcinoma breast.

Control Group:

The group comprised of 27 apparently healthy female subjects with no significant medical illness, and they were selected from the patients attending the general outpatient department of Madras Medical College, Chennai.

Test Group: This group comprised of 50 females recently diagnosed with carcinoma breast through tissue diagnosis, being managed under the general surgery wards of Madras Medical College.

Inclusion criteria:

Females with confirmed diagnosis of carcinoma breast through tissue diagnosis, any stage of carcinoma, of any age-group.

Exclusion criteria:

1. Individuals who have undergone any surgical intervention for the carcinoma breast other than tissue biopsy
2. Individuals who have received chemotherapy or radiotherapy for the carcinoma breast.
3. Individuals diagnosed to have diabetes mellitus, or liver disease, or pancreatic diseases
4. Individuals with a history of recent acute myocardial infarction.

Sample collection

5ml of peripheral venous blood was withdrawn under sterile conditions with disposable syringes from all the 77 subjects of the study. 1.5 ml of blood was transferred into a test tube containing EDTA, for superoxide dismutase estimation. After thorough mixing, the contents were transferred into a 2 ml Eppendorff tube and labeled properly. These Eppendorff tubes were then stored at a temperature of -20° C till the samples were analyzed.

The remaining blood of 3.5 ml from each subject was transferred to another test tube without any anti-coagulant. Serum separated from this tube was pipetted into a centrifuge tube and was centrifuged at 2000 rpm for 5 minutes to obtain clear fluid without cells. The clear serum was then analyzed for the estimation of serum Lactate Dehydrogenase, serum Gamma glutamyl transpeptidase, serum total proteins, and serum albumin. The biochemical parameters undertaken for the study were determined by semiauto analyzer ERBA CHEM- 5 plus V2.

RESULTS

The mean age of the controls was 51.88±8.59 years and the mean age of the cases was 52.28±10.93 years.

The mean and standard deviations of the biochemical parameters for the controls were: SOD 189.8 ±14.8 U/mL, LDH 213±112 IU/L, GGT 20 ±6 IU/L. The mean and standard deviations of the biochemical parameters for the cases were: SOD 151.7 ±10.7 U/mL. LDH 423±198 IU/L, GGT 20 ±6 IU/L, Table-1 shows the comparison of biochemical parameters among the cases and controls. Figure-1 to Figure-3 illustrates the distribution of these values. The statistical significance of the difference in the mean values of the biochemical parameters between the cases and the controls was calculated using Mann-Whitney U test for LDH and Student's t-test for the other parameters. The *p-values* for serum total protein, albumin, LDH, GGT and blood SOD were less than 0.01. Table-2 shows the comparison of biological risk factors among the cases and controls. The statistical significance was determined by Chi-square test. The *p-value* for positive family history was 0.54, for nulliparity 0.15, for age more than 30 at first child birth 0.29, for duration of exposure to estrogen more than 30 years 1.00. Table-3 shows comparison of biochemical parameters in cases with stage-II and stage-III breast cancer. Statistical significance of the difference between the parameters was calculated using Mann-Whitney U test for LDH and Student's t-test for the other

parameters. The *p-values* for serum SOD, LDH and GGT were 0.01, 0.18, and 0.28 respectively.

Table 1: Comparison of biochemical parameters in cases and controls

SI No	Study group	LDH	GGT	SOD
		Mean ± SD IU/L	Mean ± SD IU/L	Mean ± SD U/mL
1	Controls (n=27)	213±112	20±6	189.8±14.8
2	Cases (n=50)	423±198	29±6	151.7±10.7
p-value		0.00	0.00	0.00
Level of significance*		S	S	S

*S = SIGNIFICANT

Table 2: Distribution of biological risk factors among cases and controls

SI No	Study group	Family history of CA Breast	Nulliparity	First child birth at > 30 yrs	Exposure to Estrogen > 30 yr
		%	%	%	%
1	Control (N=27)	0	10	8	64
2	Cases (N=50)	4	3.7	0	33.3
p-value		0.54	0.15	0.29	1.00
Level of significance*		NS	NS	NS	NS

*NS= NOT SIGNIFICANT

Table 3: Comparison of biochemical parameters in stage 2 and stage 3 carcinoma breast

SI No	Study group	LDH	GGT	SOD
		Mean±SD IU/L	Mean±SD IU/L	Mean±SD U/mL
1	Stage 2	410±228	27±7	160.5±7.9
2	Stage 3	439±190	29±6	147.3±6.3
p-value		0.18	0.28	0.00
Level of significance*		NS	NS	S

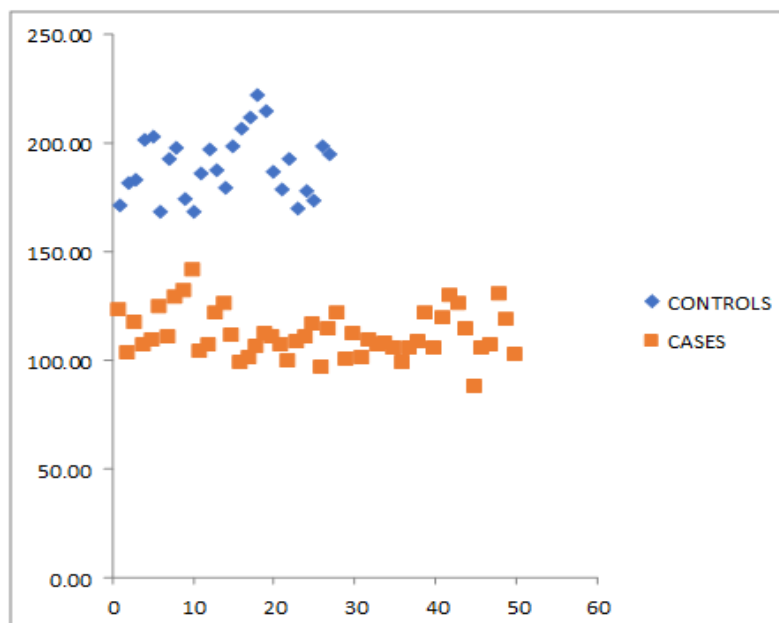


Fig-1: SOD levels in cases and controls

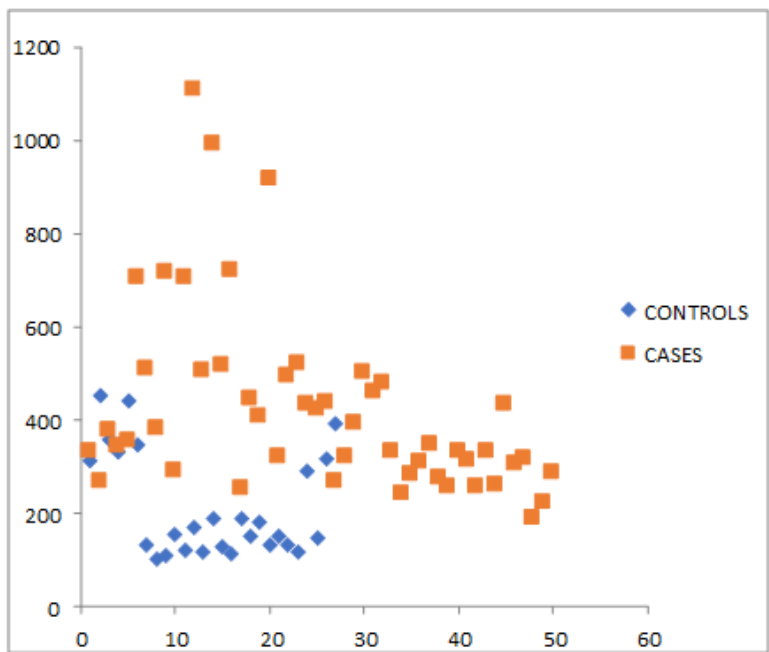


Fig-2: LDH levels in cases and controls

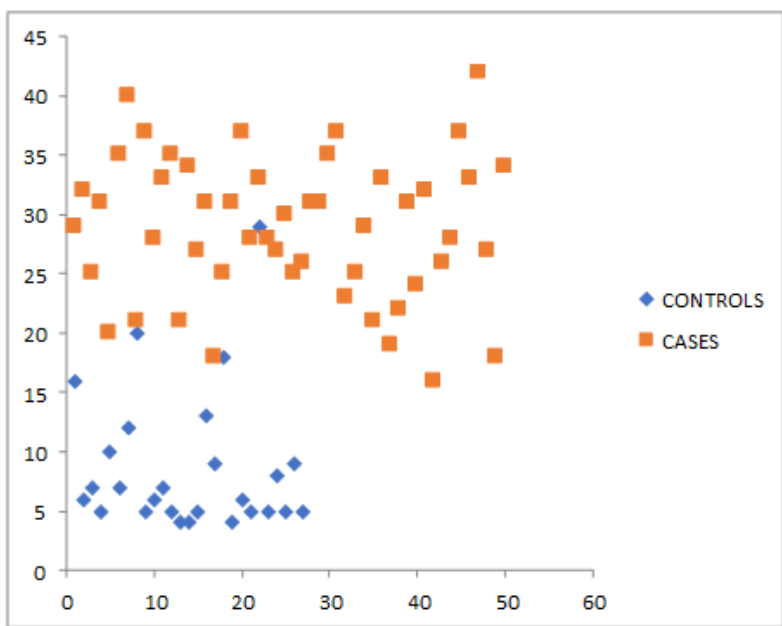


Fig-3: GGT levels in cases and controls

DISCUSSION

The reference ranges for the analyzed biochemical parameters of the apparently healthy female fall within the accepted reference ranges, [13] and therefore could be accepted as valid for this study. LDH levels were significantly elevated in the women with breast cancer. Postulated factors for this include increased production of enzymes by tumor cells, change

in the permeability of the cells, allowing leakage of soluble enzymes into circulation, consequent to the blockade of the duct system through which the enzyme passes [14]. Release of LDH from dying tumor cells and induction of LDH synthesis in the normal tissues of the host by the tumor could also contribute to raised LDH levels [15].

The levels of serum GGT were elevated in women with breast cancer. GGT is a membrane-bound glycoprotein enzyme present in normal human serum. It initiates cleavage of extra cellular glutathione and has been shown to promote oxidative damage to cells. Glutathione level decreases when there is increase in transpeptidation [16]. This is believed to be one of the mechanisms of free radical damage to tissues.

The levels of blood SOD were found to be significantly decreased in women with breast cancer. This could possibly be due to

- 1) Known sensitivity of these enzymes to radical induced inactivation [17].
- 2) Exhaustion of free radical scavenging enzymes
- 3) Decreased expression of anti-oxidant enzymes by reactive oxygen species.

In comparing the levels of the biochemical markers among the subjects with stage-II and stage-III breast cancers, only SOD levels were lowered which is significant (p -value <0.01) - mean blood level of 147.3 IU/mL in stage-III compared to the mean blood level of 160.5 IU/mL in stage-II. This again could be indicative of further radical induced inhibition of SOD activity as well as exhaustion of free radical scavenging enzymes in advanced stages of breast cancer.

The levels of these biochemical markers in non-malignant breast tumors need to be studied. Definite association of these markers with carcinoma breast might open-up the scope for use of these biochemical markers as adjuvant in breast cancer screening. This also would warrant further studies of these biochemical markers in patients before and after treatment (surgery or chemotherapy or radiotherapy) which could open up possibilities of these biochemical markers to be used in estimation of these in the diagnosis, management and follow-up of individuals at risk of and those diagnosed to have carcinoma breast which will considerably help in reducing morbidity and mortality associated with cancer breast. Differences in positive family history of breast cancer, parity, age at first childbirth, and duration of exposure to estrogen among the cases and controls were not statistically significant. While these have been determined to be definitive risk factors in literature, contrary observations in our study could be attributed to the sample size and the study design.

CONCLUSION

The levels of biochemical markers LDH and GGT were significantly elevated, and the levels of

antioxidant enzyme SOD significantly lowered in women with breast carcinoma.

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