

## Study of Serum Lactate Dehydrogenase and Gamma-Glutamyl Transpeptidase in Breast Cancer Patients Receiving Chemotherapy

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### ABSTRACT

Breast cancer (BC) is the most common type of cancer worldwide, being one of the leading causes of morbidity in the female. In Nepal, it is the second most common type of cancer among women of perimenopausal age group. More than one-quarter of the BC is diagnosed in young Nepalese female, with a familial history of breast cancer, early pregnancy, longer lactation and estrogen exposure, often tumors showing aggressive biological behaviors. Anthracyclines (Doxorubicin) based treatment regime were reported to cause cardiotoxicity by increasing intramyocardial free radical production, lipid alterations and decreasing antioxidant level. Oxidative stress involving cellular reactive oxygen species (ROS) production is widely accepted mechanism, but the molecular basis of chemotherapy-induced organ toxicity remains highly controversial. An increased rate of metabolism and oxidative stress results in rapid turnover of cancer cells that modulates the enzyme level in blood circulation. Serum LDH and GGT level correlate with tumor burden, the metastatic character of BC and intensity of organotoxicity. The aim of our study is to evaluate the serum level of LDH and GGT in BC patients receiving chemotherapy and correlate these enzyme levels with different stages of BC. A total number of 150 subjects were included in the study, comprising 90 histopathologically confirmed 24 to 76 years aged patients of different breast cancer stages, receiving at least 3 cycles of 5-Fluorouracil, Adriamycin, and Cyclophosphamide (FAC) chemotherapy. Sixty age-matched healthy women were enrolled as controls. Blood samples from each were collected after informed consent and analyzed for serum LDH and GGT levels using standard biochemical methods. Data were analyzed using student's paired-T test, Pearson correlation test and ANOVA. Serum LDH and GGT levels were significantly ( $p < 0.001$ ) increased in BC patients as compared to control group. When all 4 stages of BC were compared to control group, LDH and GGT activities showed a progressive increase from stage I to IV. The study concludes that serum LDH and GGT may be sensitive, specific and profitable biomarkers in early diagnosis of breast cancer, assessing cancer prognosis and response to treatment.

**Keywords:** Breast cancer, stages, chemotherapy, Lactate dehydrogenase, Gamma glutamyl transpeptidase

### INTRODUCTION

Breast cancer (BC) is the common type of cancers among women and has become the major public health problem worldwide [1, 2]. It was estimated that over five hundred thousand women died in 2011 due to breast cancer [3]. Among Nepalese women, BC is the second most common type of cancer that accounts for 6% of total cancers in Nepal [4]. Familial history of BC, mutations on tumor suppressor genes like BRCA1,

BRCA2 and p53 and reproductive factors associated with longer exposure to estrogen such as early menarche, late menopause, late age at first childbirth, oral contraceptives use and hormone replacement therapy increases BC risk [1]. Nepalese young women represent more than one-quarter of the BC diagnosed in Nepal, often diagnosed at an advanced stage with tumors showing aggressive biological behaviors [5]. Nearly 50% of BC patients will develop distant metastasis, and at some

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point, more than half patients with metastatic BC will have liver involvement [6]. An increased rate of metabolic changes like anaerobic glycolysis and oxidative stress results in a rapid turnover of cancer cells that modulates the enzyme level in blood circulation [7].

Lactate dehydrogenase (LDH) is an enzyme of anaerobic glycolysis released into surrounding environment at increased rate when cells replicate rapidly [8]. LDH activity correlates with tumor burden, and the metastatic BC exhibits elevated LDH level than normal breast tissue [7]. Gamma Glutamyl transpeptidase (GGT) is an enzyme of glutathione metabolism, involved in cell's detoxification pathway and apoptotic balance including tumor development, progression and chemotherapy resistance [9, 10].

5-Fluorouracil, doxorubicin, and cyclophosphamide (FAC) is the most common combination of chemotherapy drugs used in breast cancer treatment that is often associated with oxidative stress, hepatotoxicity and cardiotoxicity [11]. Elevated levels of cardiac enzymes LDH and creatine kinase (CK) after FAC treatment is evidence of the cardiotoxic effect of chemotherapy [12]. Similarly, elevated serum GGT level is the markers for oxidative stress resulting from carcinomas with liver involvement and liver toxicity [13].

Although oxidative stress involving cellular reactive oxygen species (ROS) production is widely accepted, the molecular mechanism of chemotherapy-induced organ toxicity remains highly controversial. Therefore the present study was carried out to evaluate the serum levels of LDH and GGT in BC patients, of Nepal, receiving chemotherapy and correlate these organ toxicity markers with different stages of BC.

## MATERIALS AND METHODS

### *Study design*

The present hospital based cross-sectional study was conducted at Department of Pathology, Bhaktapur Cancer Hospital (BCH), Bhaktapur, Nepal from September 2013 to February 2015 and the study was approved by Ethical Review Board of Nepal Health Research Council (NHRC). The study subjects were divided into two groups; BC patients receiving chemotherapy and controls group.

### *Study subjects*

The study subjects comprised 90 clinically and histopathologically confirmed breast cancer patients of age 24 to 76 years, receiving at least 3 cycles of 5-fluorouracil, doxorubicin, and cyclophosphamide (FAC) chemo-

therapy whereas control group consisted 60 normal healthy females of same age group. Breast cancer patients with the previous history of tuberculosis, rheumatic fever, diabetes, hypertension, hepatobiliary disease or myocardial infarctions were excluded. Informed consent from each individuals was taken before blood sample, and other demographic information were collected.

### *Histopathological grading*

The patients selected for the study were divided into 4 different groups based on their tumor stages, stage I (n = 9), stage II (n = 31), stage III (n = 27) and stage IV (n = 23). Based on the Nottingham modification of the Scarff Bloom and Richardson's (SBR) grading system and TNM staging, tumor grades and stages were determined (where T describes the size of a tumor, N describes number of nodes involved and M describes metastasis [14].

### *Sample analysis*

About 3 mL of blood sample was collected from the antecubital vein, allowed to clot and centrifuged at 3000 rpm for 10 minutes to obtain the serum. Serum samples were then stored at -20°C until analyzed.

Serum level of LDH was determined by catalyzing reaction between pyruvate and NADH to produce NAD and lactate measuring absorbance at 340 nm. Using semi-automated analyzer (Stat Fax 3300, USA). Similarly, serum GGT level was estimated by measuring the amount of 5-amino-2-nitrobenzoate released during the catalytic reaction of L- $\gamma$ -Glutamyl-3-carboxy-4-nitroanilide with glycylglycine at 405 nm. Enzyme levels were determined using Analyticon reagent kits (Analyticon Biotechnologies AG Muhlenberg, Germany) on the semi-automated analyzer (Stat Fax 3300, USA).

### *Statistical analyses*

The data obtained from biochemical analyses was tabulated and analyzed in *Statistical Package for the Social Sciences version 17 (SPSS, Chicago, IL)*. Means and standard deviations of LDH and GGT were calculated for pre and postmenopausal patients with different cancer stages. One way ANOVA was used for multiple group comparison. Pearson correlation (p) was used to measure the relationship between parameters and p-value of 0.05 or less was considered statistically significant.

## RESULTS AND DISCUSSION

In this study, serum LDH and GGT level was sig-

nificantly higher in pre and postmenopausal breast cancer patients when compared with controls ( $p < 0.05$ ). Postmenopausal BC women showed higher LDH level than premenopausal women but in the case of serum GGT premenopausal women showed significantly increased level as compared to postmenopausal as shown in [Table 1].

Pearson correlation coefficient ( $r = 0.622$ ) showed the positive correlation between LDH and GGT in BC patients receiving chemotherapy. Serum LDH and GGT levels when compared in different stages of BC, the significant rise was observed with increased disease severity (stage). One way ANOVA did not show significant differences between the various stages of BC as shown in [Table 3].

Breast cancer that is detected in them early stage can be cured when the tumor is small enough which can be surgically removed completely [15]. Unfortunately, most cancers do not show any symptoms until tumors are either too large to remove surgically or cancerous cells have already spread to other organs [16].

In present study, serum LDH level was significantly ( $p < 0.001$ ) increased in BC patients (mean  $531.66 \pm 138.86$  U/L) as compared to controls (mean  $375.27 \pm 60.34$  U/L) but further increased level was observed in postmenopausal women ( $543.22 \pm 15.53$  U/L). A markedly increased LDH level was seen in postmenopausal BC patients than in premenopausal as similar to our finding [13, 17]. A significant rise in LDH level was observed in BC patients than fibroadenoma patients [16]. Guddanti et al. [17] reported due to aggressive tumor growth; serum LDH level was observed significantly elevated in BC cases than in controls. Serum LDH levels when compared in different stages of BC, the significant rise was observed with increased disease severity (stage) similar to the study by Chandrakanth et al. [16]. A study by Bogdavic et al. [7] observed LDH activity in stage

IIIB patient was approximately 2 fold higher as compared to lower stage samples.

Serum LDH was significantly increased in preoperative BC patients with and without lymph node metastasis in comparison to controls [8]. Similarly, Cao et al. [6] reported LDH levels were significantly higher in BC patients with liver metastasis than those without liver metastasis. An elevated serum LDH enzyme activity is the consequence of increased anaerobic glycolysis in rapidly dividing malignant cells and subsequent cell destruction [18]. Increased level of serum LDH suggests the increase rate of enzyme leakage from mitochondria as a result of doxorubicin-induced toxicity [11]. A study by Basnyat et al. [19] reported that elevated serum lipids level increased the risk of cardiovascular disease during BC management by FAC combination.

GGT is a membrane-bound enzyme involved in the metabolism of glutathione, a non-protein thiol molecule that protects cells against oxidative stress [15]. GGT is crucially involved in cell's detoxification pathway and apoptotic balance- including tumor development, progression and chemotherapy resistance [9, 10]. An elevated GGT level significantly increased the risk of developing neoplasms of breast, female genital organs, digestive organs, lymphoid and hematopoietic cancers [20]. Similarly, Grimm et al. [9] reported elevated serum GGT level is associated with increased cancer risk and worse prognosis of gynecologic cancers.

In this study, we found significantly ( $p < 0.001$ ) increased serum GGT level in BC patients (mean  $64.71 \pm 26.16$  U/L) as compared to controls ( $27.00 \pm 13.12$  U/L), but the further increased level was observed in premenopausal BC women ( $64.82 \pm 26.10$  U/L). A study by Jarari et al. [13] reported significantly higher level of serum GGT in breast cancer cases as compared to controls, but the significant hike was observed in the premenopausal group when compared with that of post

Table 1. Distribution of serum LDH and GGT level in controls, premenopausal and postmenopausal BC patients

| Parameters | Menstrual status | Controls ( $n = 60$ ) | Patients ( $n = 90$ ) | $p$ value |
|------------|------------------|-----------------------|-----------------------|-----------|
| LDH (U/L)  | Premenopausal    | $370.88 \pm 64.92$    | $532.99 \pm 138.58$   | 0.013     |
|            | Postmenopausal   | $384.05 \pm 50.39$    | $543.22 \pm 15.53$    | 0.007     |
| GGT (U/L)  | Premenopausal    | $26.08 \pm 12.76$     | $64.82 \pm 26.10$     | 0.030     |
|            | Postmenopausal   | $28.85 \pm 13.99$     | $64.44 \pm 23.92$     | 0.093     |

Table 2. Overall distribution of LDH and GGT level in BC patients and controls

| Parameters | Controls           | Patients            | $p$ value   |
|------------|--------------------|---------------------|-------------|
| LDH (U/L)  | $375.27 \pm 60.34$ | $531.66 \pm 138.86$ | $p < 0.001$ |
| GGT (U/L)  | $27.00 \pm 13.12$  | $64.71 \pm 26.16$   | $p < 0.001$ |

Table 3. Comparison of LDH, GGT in various clinical stages of BC patients

| Stages     | No. of cases | LDH (U/L)       | GGT (U/L)     |
|------------|--------------|-----------------|---------------|
| I          | 9            | 460.67 ± 58.34  | 57.78 ± 25.46 |
| II         | 31           | 501.39 ± 133.55 | 61.55 ± 4.09  |
| III        | 27           | 522 ± 64.174    | 63.15 ± 22.29 |
| IV         | 23           | 616.78 ± 190.35 | 73.96 ± 33.19 |
| ANOVA      | F            | 4.75            | 1.37          |
|            | P            | 0.04            | 0.256         |
| Ivs. II    |              | 0.084           | 0.510         |
| Ivs. III   |              | 0.260           | 0.189         |
| Ivs. IV    |              | 0.073           | 0.57          |
| II vs. III |              | 0.036           | 0.196         |
| II vs. IV  |              | 0.319           | 0.109         |
| III vs. IV |              | 0.012           | 0.022         |

Note: Significant at  $p < 0.05$

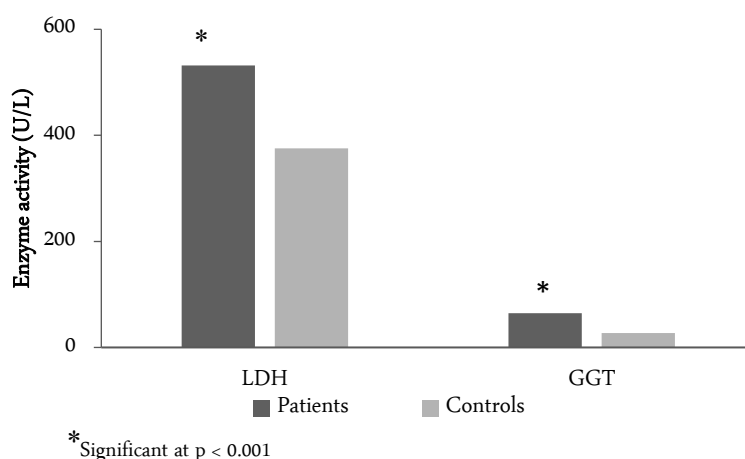


Figure 1. Overall comparison of LDH and GGT in BC patients and controls

menopausal similar to our findings. In contrast to our study, increased GGT level was observed in postmenopausal breast cancer patients as compared to premenopausal age group [17, 21].

Serum GGT level when compared with patients having different stages of BC, we found proportional increased GGT level from stage I to stage IV and the finding was similar to the study by Chandrakanth et al. [16]. Choudhari et al. [15] also observed significantly increased serum GGT level in all four stages of breast cancer patients as compared to controls, and interstage comparison showed a progressive increase from stage I-IV as similar to our study. Serum GGT level was significantly higher in patients with liver metastasis than without liver metastasis [6]. An increased GGT expression is often seen in malignant and large sized tumors as compared to smaller tumors [22]. Furthermore, GGT expression is increased as a response to oxidative stress

which causes induction of GGT mRNAs by multiple signaling pathways [15].

## CONCLUSION

As a conclusion, our study shows significantly increased in the level of serum LDH and GGT in different stages of breast cancer patients receiving chemotherapy with or without metastasis. A rapid increase in malignant cells turn over, oxidative stress and organ toxicity due to chemotherapy modulated the LDH, GGT expression level in blood circulation. These non-specific enzyme markers can be routinely used for diagnosing breast cancer, detecting metastasis and monitoring the cancer progression and treatment.

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