STUDIES OF BIOCHEMICAL PARAMETERS IN BREAST CANCER WITH AND WITHOUT METASTASIS

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ABSTRACT

The study includes 102 confirmed cases of carcinoma breast with and without metastasis and 25 healthy non-pregnant females. They were evaluated for blood levels of Ferritin, GSH, LDH, ALP, GGT and Hb before and 21 days after mastectomy. A significant increase ($p < 0.001$) was observed in ferritin, LDH and GSH levels in cancer patients without metastasis in comparison to normal control subjects. Patients with metastasis had further elevated ($p < 0.001$) levels of Ferritin, ALP and GGT as compared to non-metastatic patients. Mastectomy in both the cases i.e. with and without metastasis resulted in non-significant decrease in all the biochemical parameters suggesting that longer follow up could confirm post surgery decrease in the biochemical parameters. The results of the study suggest cost effective, usefulness of Ferritin, ALP, GGT and GSH / Hb ratio in differentiating breast cancer patients with and without metastasis which can be assayed in smaller laboratories.

KEY WORDS

Carcinoma breast, Metastasis, Glutathione, Ferritin, Lactate dehydrogenase, Alkaline phosphatase, Gamma glutamyl transpeptidase, Diagnostic tools.

INTRODUCTION

While cancer account for high morbidity and high mortality rate throughout the world, cancer of breast is common in women in developed countries and more than 40% of all breast cancer cases are found in developing countries (1). Cancer that is detected early can potentially be cured when the tumor is small enough to be completely removed surgically. Unfortunately, most cancers do not produce any symptoms until the tumors are either too large to be removed surgically or cancerous cells have already spread to other tissues i.e. metastasis has taken place. Hence there is need to detect cancer at an early stage. Tumor markers are used for the detection of risk, population screening, diagnosis, staging and prognosis. It can also predict the response to therapy, monitor treatment, detect the presence of occult metastatic disease and monitor the course of the disease (2). Several tumor markers are in vogue now a days, which include alpha-feto protein, Carcino embryonic antigen, Human chorionic gonadotrophin, Prostate specific antigen and relatively less specific makers like Lactate dehydrogenase, Alkaline phosphatase, ferritin and Gamma glutamyl

transpeptidase etc. (3). However, the analytical method of many of these are unapproachable for general population as the facilities for these are available only at sophisticated and well-equipped centers with latest technology and are expensive. There is, therefore, need of simple biochemical investigations, which can be easily assayed, are less expensive and can detect metastasis.

In view of this present study was undertaken to assess the clinical utility of certain biochemical investigations-lactate dehydrogenase (LDH), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), ferritin and reduced glutathione (GSH) in diagnosis of carcinoma and also in metastasis. We have restricted our study to carcinoma breast in young premenopausal women as the frequency of breast cancer has shifted to premenopausal age group and accounts for high morbidity high mortality (4).

MATERIALS AND METHODS

The present study was started with 120 clinically established and histopathologically confirmed breast cancer premenopausal female patients ranging in age from 35 to 48 years with mean age of 42.6 ± 9.1 admitted in S.M.S. Hospital, Jaipur. Out of these 18 patients could not be followed hence the study is based on 102 breast cancer patients and 25 age matched controls. These subjects are categorized as under (Table 1).

Fasting blood samples were collected from healthy controls and cancer patients just before mastectomy (pre level) and 21 days after surgery (post level), and

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Table 1: Categorization of subjects

Subject	Lymph node	Stage of cancer	Lumpsize (cm)
Control (n=25)	Nil	Nil	Nil
Breast Cancer without metastasis (n=58)	Nil	Stage 1	2.0 to 7.0
Breast Cancer with metastasis (n=44)	Regional lymphnode	Stage 2 & 3	2.0 to 7.0

analyzed for, GSH (5), Ferritin (6), Hb (7) and LDH, ALP, GGT on Merck Selectra random access autoanalyzer using standard kits. GSH: Hb and LDH: Ferritin ratios were also calculated to assess their relevance in these patients.

Statistical comparison was done between breast cancer with or without metastasis and control by using students 't' test.

RESULTS AND DISCUSSION

As breast cancer is one of the leading cause of death in women in developed and developing countries, a need is felt for some simple biochemical investigations for the early detection of cancer and can be assayed in smaller laboratories located at remote areas. In view of this, certain biochemical investigations, which are though considered nonspecific, carried out in breast cancer patients to establish their diagnostic value in cancer with and without metastasis.

Blood samples of 58 breast cancer cases without metastasis and 44 cases with metastasis were analyzed before and after mastectomy. The results are presented in table 2. The results of the study show a significant increase in LDH, GSH and ferritin and non-significant increase in ALP and GGT levels in cancer patients without metastasis in comparison to normal control subjects. Individual patient's data revealed that 70% of patients without metastasis had LDH above normal while 65% and 62% patients had ferritin and GSH above normal level respectively which further increased in metastasis.

It is evident from table 2 that when these biochemical parameters were again analyzed 21 days after surgery showed a decreasing trend in most of cases however, this decrease was not found to be statistically significant. 21 days (3 weeks) of surgery seems to be too short a period for any significant change to occur in these parameters and a longer follow up is needed to establish prognostic importance of these investigations. Several workers (8-10) have reported

elevated levels of ferritin and GSH in breast cancer patients in their studies. Kher *et al* (11) correlated post treatment decreased in Ferritin and LDH levels with response to therapy, however persistent rise observed in few cases was attributed to recurrence of metastasis. Higher level of ferritin in cancer patients with metastases has also been reported (12-14). Ferritin has been considered as a useful diagnostic tool for early detection of breast cancer patients and Kokacinaska *et al* (15) compared its usefulness with CA 15-3 in patients of high risk. Rise in serum ferritin has been attributed to the iron requirement for cell growth and malignant cells requiring more iron to modulate transferrin receptor. Transferrin receptors on proliferating and malignant cells are well documented (16).

Glutathione as a reductant is very important in maintaining stability of erythrocyte membranes, its sulfhydryl group reduces peroxides formed during O₂ transfer and thus it provides protection against free radical injury (17). The role of GSH in tumors is not clearly understood. Similar to the present study its level was reported to be elevated in malignant lesions (18-19), however contrary report are also available (20-22). Carcinogenesis begins with the interaction of carcinogen with SH groups of an enzyme system directly associated with cell division and this results in high level of free (acid soluble) SH which has role in mitotic initiation and cell division (23). The SH materials involved in carcinogenesis may be found within one of the system in which glutathione is reduced, oxidized or synthesized. If GSH is considered as the principal free SH group compound in cells and tissues, a increase in the level could be due to imbalance between the reducing and oxidizing system of glutathione as observed in this study (table 2). We have further examined GSH levels in terms of per gram Hb (table 3). The rise in ratio in patients without metastasis was almost 85% and in metastasis the ratio has increased to almost three times. It was also interesting to note that at the same time LDH to ferritin ratio decreased significantly in cancer patients, which was further

Table 2 : Serum enzymes, GSH, Ferritin levels in breast cancer patients
(Values are mean $\pm$ SD and range)

Group	ALP U/L	LDH U/L	GGT U/L	GSH mg%	Ferritin ng/ml	Hb. gm%
Normal control (n = 25)	130.0 $\pm$ 15.2 (110-146)	228.8 $\pm$ 54.5 (195-270)	20.1 $\pm$ 7.0 (10-25)	30.2 $\pm$ 3.0 (25-36)	98.0 $\pm$ 35.2 (68-102)	13.1 $\pm$ 2.7 (11-15)
Breast cancer without metastasis (n = 58)						
Pre level	157.0 $\pm$ 32.6 (120-190)	433 $\pm$ 99.6** (350-505)	23.9 $\pm$ 4.9 (17-29)	49.0 $\pm$ 6.5** (41-57)	214.0 $\pm$ 36.2** (180-250)	10.0 $\pm$ 1.9** (7-12)
Post level	139.7 $\pm$ 25.3 (110-160)	360.2 $\pm$ 119.0 (250-460)	26.6 $\pm$ 5.2 (20-35)	38.0 $\pm$ 4.7 (32-45)	180.0 $\pm$ 40.8 (140-225)	9.3 $\pm$ 1.7 (7-11)
Breast cancer with metastasis (n = 44)						
Pre level	750.3 $\pm$ 102.6** (610-850)	730.0 $\pm$ 136.7** (565-950)	92.3 $\pm$ 16.9** (75-126)	67.4 $\pm$ 12.2* (55-82)	647.5 $\pm$ 58.2** (590-705)	9.1 $\pm$ 2.4 (7-12)
Post level	615.9 $\pm$ 96.2 (531-700)	690.0 $\pm$ 128.9 (535-830)	73.9 $\pm$ 27.2 (56-105)	55.7 $\pm$ 13.2 (41-72)	581.2 $\pm$ 32.8 (530-650)	8.6 $\pm$ 2.1 (6-10)

Statistical comparison was done between - Normal and cancer patients without metastasis ; Pre and Post levels of cancer patients without metastasis ; Pre and Post levels of cancer patients with metastasis and Pre levels of cancer patients with and without metastasis.

** p < 0.001; * p < 0.05; rest non significant.

Table 3: GSH / Hb and LDH / Ferritin ratio in breast cancer patients
(values are mean $\pm$ SD)

Group	GSH / Hb	LDH / Ferritin
Normal control	2.6 $\pm$ 0.3	2.3 $\pm$ 0.3
Breast cancer without metastasis		
Pre level	4.8 $\pm$ 0.25**	1.9 $\pm$ 0.13**
Post level	3.9 $\pm$ 0.5	1.9 $\pm$ 0.18
Breast cancer with metastasis		
Pre level	7.4 $\pm$ 0.3 **	1.12 $\pm$ 0.12 **
Post level	6.4 $\pm$ 0.7	1.19 $\pm$ 0.14

Statistical comparison was done between - Normal and cancer patients without metastasis ; Pre and Post levels of cancer patients without metastasis ; Pre and Post levels of cancer patients with metastasis and Pre levels of cancer patients with and without metastasis.

** p < 0.001; rest non significant.

decreased to 50% in metastatic patients. ALP and GGT- which showed a non-significant rise in non-metastatic cases, registered a significant rise of 6 fold and 4 fold in metastatic patients respectively. Similar to the present study Von Hoof *et al* (24) and Steiber (25) also did not find any significant difference in ALP levels in non-metastatic breast cancer while others have revealed a significant rise in ALP and GGT in metastasis (26,27) suggesting involvement of bone and liver.

The extent of rise in the parameters can be one of the criteria to establish its diagnostic value. Patients without metastasis showed highest rise in Ferritin and lowest in GGT (Ferritin> LDH> GSH> ALP> GGT), which correlates well with our previous study indicating ferritin as the most important parameter for diagnosis of breast cancer (28). However, in patients with metastasis the extent of rise is Ferritin >ALP>GGT>LDH>GSH, showing that LDH and GSH are nonspecific for diagnosis of metastasis. Although these are non-specific parameters and it is difficult to ascertain their diagnostic importance in cancer patients, yet their prognostic importance cannot be undermined. A combination of Ferritin, ALP & GGT along with GSH/Hb could be used as important biochemical parameter for differentiation of breast cancer with and without metastasis, which are cost effective, and can be easily assayed in smaller laboratories not yet exposed to any sophisticated technology for more reliable cancer marker.

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