



Serum levels of LDH and Gamma GT in Libyan Breast Cancer patients

KEYWORDS

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ABSTRACT Background: Carcinoma breast is a major health problem in Libya particularly among the younger women. It is necessary to ascertain the prognostic and diagnostic markers of breast cancer and alteration of enzyme markers in breast cancer can be a supporting guide for its prognosis and treatment outcomes. Studies have shown a positive correlation of serum LDH (Lactate dehydrogenase) and GGT (gamma glutamyl transpeptidase) levels with carcinoma breast. The present study is undertaken to observe any alterations in the levels of LDH and GGT in patients suffering from carcinoma breast in a small population in Libya.

Methods: 40 patients in the age group ranging from 16 to 65 years in various stages of breast cancer have been selected from the department of surgery, 7th October hospital, Benghazi, Libya during the years 2009 and 2010. 38 healthy controls in the age group of 20 to 55 years with mean age of 35 years were selected at random at the similar time period. Out of the 40 cases of carcinoma of breast, 25 cases were premenopausal and 15 post-menopausal. The cut off age was taken as 47 to demarcate between both the groups, selecting the group less than 47 years as premenopausal and 47 and above as postmenopausal group. Serum LDH and GGT estimation was performed on these patients as well as on the healthy controls after an overnight fast.

Results: Serum LDH is elevated significantly in carcinoma of breast when compared with the control group among both premenopausal and postmenopausal women ($p = 0.0001$). Between the two groups, postmenopausal women had significantly higher levels of LDH than premenopausal ($p = 0.0234$). Serum GGT was significantly higher in cases when compared to the controls ($p = 0.006$) and statistically more significant in Premenopausal women ($p = 0.0059$) than that of the postmenopausal women.

Conclusion: An elevation of LDH and GGT levels in cases of carcinoma breast in our study signifies its importance as a marker of the disease. A serial measurement of these enzymes will have a prognostic significance and help treatment decisions.

Introduction

Carcinoma breast is one of the major surgical problems in Libya particularly among the younger women. It is necessary to ascertain the prognostic and diagnostic indicators of breast cancer and alteration of enzyme markers in breast cancer can be a supportive guide for its prognosis and treatment outcome. Studies have shown a positive correlation between the various biomarkers of breast cancer and among these, LDH (Lactate dehydrogenase) and GGT (Gamma glutamyl transpeptidase) levels are considered as important enzyme markers of prognostic and diagnostic importance. Though they lack specificity as diagnostic markers, their levels in blood have shown greater prognostic significance.

Research in cancer studies still remains incomplete in spite of the extensive resources being spent on the studies and thus remains an on-going process. Early detection of carcinoma is an important step towards treatment and a number of biochemical markers are being studied to evaluate malignancy and its impact on human survival rate. However, there is no ideal marker that has been proved to be a sensitive and specific indicator of early breast cancers.[1]

During normal conditions, every tissue maintains a steady and consistent enzymatic pattern which may significantly change in diseased states. In carcinomas, where the cells replicate rapidly, membrane constituents are shed into the surrounding milieu at increased rate. Hence the enzymes

and proteins present in nucleus, cytoplasm and mitochondria are released into circulation when cells are destroyed. These enzymatic changes in malignant tissue may also result from genetic reprogramming to malignant behaviour, a likely strategy for survival of tumour cells [2]. LDH and GGT are some of the enzymes which may rise in malignancies and since being economic and relatively easier to determine, remain as useful and practical markers in terms of prognosis and also early diagnosis. In combination with other tumour markers, these two enzymes show higher specificity and sensitivity when compared with other liver enzymes especially when there is liver metastasis [3]. LDH is a cytosolic enzyme that regulates the inter-conversion of pyruvate to lactate, using NAD as cofactor. The high glycolytic rate is important for rapidly proliferating cancers not only as a major energy source but also to provide such cells with precursors for nucleotide and lipid biosynthesis. Malignant tumours are known to have high rates of glycolytic activity leading to high production of lactic acid.[4]. As a result of this high glycolytic rate, there is an elevation of LDH levels during cancerous conditions [5][6]. Tumour cells have an increased glucose transport and this glucose is metabolized via the anaerobic glycolytic pathway to produce lactic acid and hence this enzyme has significance in the recognition of neoplastic disease.

Gamma-glutamyl transferase (GGT) is a membrane-bound enzyme catabolising reduced glutathione to cysteine and glycine in Meister's γ -glutamyl cycle (Orlowski and Meister,

1970). This delivers cysteine for intracellular synthesis of glutathione, the major thiol anti-oxidant. Elevated serum levels of GGT are markers of oxidative stress, resulting from factors including alcohol, heavy metals, cardiovascular disease and diabetes [7] and has shown similar results in carcinomas.

Materials and Methods

40 patients in the age group ranging from 16 to 65 years in various stages of breast cancer have been selected from the department of surgery, 7th October hospital, Benghazi, Libya during the years 2009 and 2010. 38 healthy controls in the age group of 20 to 55 years with similar characteristics were recruited at random for the study after their consent. Out of the 40 cases of carcinoma of breast, 25 were premenopausal and 15 were post-menopausal. These groups were demarcated taking 47 years of age as the cut-off point with the group less than 47 years as premenopausal and 47 and above as the postmenopausal group. Serum LDH and GGT estimation was performed on the patients as well as on the healthy controls after an overnight fast. Serum LDH was estimated using autoanalyzer Cobas Integra 400 with DeutscheGesellschaft für Klinische Chemie (DGKC) method[8,9]. Serum GGT was estimated using modified Szasz method [10][11].

Statistical evaluation was done by using Graphpad software. The method used was calculation of p-values by using student 't' test.

Results:

Serum LDH levels were significantly elevated in carcinoma breast cases when compared with the control group among both premenopausal and postmenopausal ($p= 0.0001$) women. Between the pre- and postmenopausal group, postmenopausal women had significantly higher levels of LDH than premenopausal ($p= 0.0234$) women. Serum GGT was also significantly higher in cases when compared to the controls ($p= 0.006$), however the hike was more significant in the premenopausal group ($p= 0.0059$) when compared with that of the postmenopausal.

Results of serum LDH levels are summarized as in table no.1.

LDH

	Controls (n=38)	Total cases (n=40)	Premeno-pausal (n=25)	Postmeno-pausal (n=15)
Mean values	260.53	329.93	313.35	363.10
STD	53.60	57.99	51.45	58.47
p-Value		0.0001	0.0006	0.0001

The results of GGT are given in table no.2.

GGT

	Controls	Total cases	Premeno-pausal	Postmeno-pausal
Mean values	25.19	35.30	36.40	33.10
STD	11.23	16.16	16.40	16.3
p-Value		0.006	0.0059	-----

Discussion

A number of biomarkers have been studied to evaluate malignancies. However, no specific markers for breast cancer have been discovered and those that are currently available lack the sensitivity and specificity for early detection of cancer and timely treatment [12]. This study involving serum LDH and GGT levels aims to find the correlation of breast cancer with the enzyme levels in determining their prognostic significance if not for their diagnostic role. This may improve the patient survival rate with a timely treatment [13]. Serum levels of LDH and GGT may differentiate benign and malignant lesions and help detection of early disease. These two enzymes are better markers in terms of prognosis [14].

In a study, LDH levels were estimated in 130 patients with a confirmed diagnosis of breast cancer and also in 110 healthy age matched randomly selected controls and Lactate dehydrogenase levels were found to be significantly elevated in breast cancer which suggest an immense potential for LDH as a prognostic marker for breast cancer[15].

Lactate dehydrogenase-A (LDH-A) is one of the main isoforms of LDH expressed in breast tissue, controlling the conversion of pyruvate to lactate of the cellular glycolytic process. It has been shown that LDH-A plays a key role in glycolysis, growth properties and tumour maintenance of breast cancer cells. [16].

A shift toward muscle-type LDH was seen in carcinomas of the breast in a study. In the 130 samples of infiltrating ductal carcinomas, there was a significant increase (approximately three to six-fold) in the proportion of LDH-5 compared to the proportion of LDH-5 in normal tissue[17].

LDH enzyme activity is up regulated in cancer tissue as well as in serum in breast cancers and certain ovarian tumors, mostly the LDH 5 variant. The up regulation of the LDH isoenzyme in blood is due to the up regulation of its gene LDHA to some extent. [18].

In a study on nipple discharge, LDH isozymes in the nipple discharge of patients with benign diseases displayed various patterns. Isozyme levels in breast cancer nipple discharge tended to increase in ascending order from LDH₁ to LDH₅. Breast cancer tissue showed a similar pattern, suggesting that the LDH isoenzymes in breast cancer nipple discharge were derived from breast cancer. Use of LDH isoenzymes assay of nipple discharge may prove a useful technique for providing a supporting diagnosis of breast cancer [19].

One study in India showed a rise of biochemical parameters in increasing order from ferritin, ALP, GGT, LDH and GSH with non-metastatic breast cancer and in metastasis were much higher and they concluded that LDH and GSH are nonspecific parameters for metastasis. They concluded that combination of Ferritin, ALP, GGT along with GSH/HB can become important biomarker for breast cancer with and without metastasis [20].

Significant linear correlations were observed between the expression level of LDHA gene and the glycolytic marker LDH assayed in tumor biopsies indicating the concerted adaptation of the cancer cell to a glycolytic phenotype [21].

Also potentially of interest in human breast cancer is the demonstration that only the fifth isoenzyme of LDH is present in MCF-7 cell (breast cancer cell line). Several investigators have reported an elevation in the proportion of LDH-5 in human breast neoplasia compared to that in normal breast tissue (4, 6, 16), and estradiol increases the rate of LDH-5 synthesis in uteri of immature rabbits and rats (5).LDH itself has the potential to control metabolic functions crucial to growth and may thus prove to be quite useful [22]

The role of GGT in breast cancer has also been highlighted in many studies .In the Guernsey Breast Cancer Cohort Study, GGT was measured in sera from 1803 normal women. Among these women, 251 subsequently developed cancer and from these, 96 developed breast cancer.

These results suggest that premenopausal women with high normal (above median) serum GGT or elevated levels are at increased risk of breast cancer and might benefit from close surveillance, possibly with breast magnetic resonance imaging scans. Serum GGT may mark previous exposure to carcinogens and lead to the identification of DNA adducts involved in mammary carcinogenesis.[23]

A study was conducted to see the association of GGT with

overall and site-specific cancer incidence in a population-based cohort of 92,843 Austrian women with 349,674 in which a serial GGT measurements were prospectively followed-up for a median of 13.5 years. During the follow-up, 4,884 incidence cancers were observed. Compared to normal, cancer risk was associated with higher levels of GGT [24].

CONCLUSIONS:

The present retrospective study has shown a significant association between the serum LDH levels in cases diagnosed with carcinoma breast especially in postmenopausal women. Likewise, a linear correlation was also observed between the serum GGT levels and carcinoma breast in the selected population especially in the premenopausal women. Similar studies can be replicated using a larger population.

Though these enzyme markers have only nonspecific diagnostic significance, their role as prognostic markers seems more significant if a serial measurement of the enzyme levels can be performed as a follow up treatment plan.

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