Original Research Article

Diagnostic efficacy of serum Lactate dehydrogenase, Gamma Glutamyl Transferase, and Alkaline Phosphatase as markers of breast cancer

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Abstract

Introduction: Carcinoma breast is one of the common cancers diagnosed in females and Research is in progress to identify the carcinoma breast in early stages and one of the methods is to detect certain biomarkers for the malignancy. The current study aimed to determine the efficacy of biomarkers like lactate dehydrogenase, Gamma Glutamyl Transferase, and Alkaline Phosphatase.**Methods:** A 5ml of blood sample was obtained from selected cases.The collected blood was allowed to clot following which it was centrifuged at 3000 rpm for 15 minutes. It was stored at minus 20 degrees centigrade until analysis was done. The samples were analyzed by Beckman Coulter AU480 Chemistry Analyzers (Beckman Coulter India Private Limited) Kurla (W), Mumbai. The Histopathological examination and staging of breast cancer were done**. Results:** The LDH, GGT, and ALP levels comparison between group I and group II was not significant. The comparison of group I with group IV found all the parameters were significantly lower in group I than in group IV. Similarly, in the comparison of group II versus group III only LDH and ALP were significantly lower in group II. The intergroup comparison between Group III versus group IV found only ALP significantly lower in group III. **Conclusion:** LDH, GGT, and ALP were found to increase in patients with carcinoma breasts. They may be used as biomarkers for diagnosis and prognosis following treatment. These makers may act as useful adjuncts with more sensitive procedures such as imaging, clinical and histopathological findings.

Keywords: Lactate dehydrogenase (LDH), Gamma Glutamyl Transferase (GGT), Alkaline Phosphatase (ALP), Carcinoma Breast

Introduction

Carcinoma breast is one of the commonly diagnosed in women of middle ages in developed countries. About 22% of all cancers detected in women are breast cancers. [1]Across the world, there are approximately 2.1 million new cases of breast cancer in 2018 and it accounted for 24.2% of all cancers in women. [2]However, recently the incidence of breast cancers is increasing in developing countries like India. The estimated incidence of breast cancer in India is 25.8 per one lakh women and the mortality is 12.7% per one lakh cases in women.[3]The etiology of breast cancer is complex with the interplay of several factors. The genetic factors are estimated to cause 5 – 10% of cases of breast cancers. It appears to occur commonly in women with a family history of breast cancers and with mutation of BRCA1 and BRCA2.[4, 5]BRCA1 is located on chromosome 17 (17q21) it is a tumor supper gene and accounts for 40% of familial cases of breast cancers. Whereas the BRAC2 which is located on chromosome 13(13q12-13) accounts for 30% of familial cases of breast cancers. [6] The other factors related to exposure of women to estrogens include early menarche, nulliparity, short lactation period, late conception, obesity, and Hormone replacement therapy [HRT].[7] The environmental factors include exposure to radiation, alcohol consumption, and the westernization of diets. Enzymes are the products of the cells released into circulation when cells are destroyed. Under normal circumstances, there is a steady-state of enzymatic pattern which is increased in cases of malignancy because of higher rates of cell replication in malignant cells.

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The enzymatic changes are also due to genetic reprogramming of malignant for their survival.[8]Tumor markers are used to screening the population at risk and they also help in diagnosis staging and prognosis of cases. Tumor markers such as CA 15-3 CA 549, TPS, TPA, and CEA are used however the method required for the detection of these requires sophisticated and well-equipped centers and are expensive. Therefore, for population screening simple biochemical investigations are required. Lactate dehydrogenase (LDH) functions for the conversion of pyruvate to lactate during glycolysis.[9]It is expressed in all tissues and it has two subunits A and B coded by two different genes combining to form 5 isomers LDH1 to LDH5 distributed among tissues and in serum. Increased LDH is seen in cancers such as germ cell tumors, lymphoma, melanoma, and renal cell carcinoma.[10, 11] Gamma-glutamyltransferase (GGT) is responsible for glutathione metabolism (GSH) and degradation of extracellular products of GSH metabolism.[12]Because intracellular GSH is an antioxidant, therefore, GGT and GSH can be increased in states of oxidative stress such as carcinogenesis.[13]Alkaline phosphatase [ALP] is a group of enzymes causing hydrolysis of phosphate esters in an alkaline environment leading to the generation of an organic radical and inorganic phosphate.[14]ALP is increased in diseases such as hepatitis, biliary and extra-biliary obstruction, and cancers. [15]Although studies have tried to evaluate the role of LDH, GGT, and ALP in the diagnosis of breast cancers no consensus exists on their utility. We in the current study tried to evaluate the efficacy of the serum enzymes as the diagnostic markers of breast cancers.

Material and methods

This cross-sectional study was conducted in the Department of Biochemistry, Rajiv Gandhi Institute of Medical Sciences, [RIMS] Adilabad. Institutional Ethical committee permission was obtained for the study. Written consent was obtained for the study from all the participants.

Inclusion Criteria

1. Patients with clinically and histopathologically confirmed carcinoma breast.
2. Aged 25 years and above
3. Those willing to participate in the study voluntarily.

Exclusion criteria

1. History of liver diseases, MI, and Tuberculosis
2. Recurrent breast carcinoma cases
3. History of Renal diseases or Pancreatic disorders

Blood sample collection: Under aseptic precautions, 5 ml of venous blood was collected from the antecubital vein in vacutainer care was taken to prevent hemolysis of blood. The collected blood was allowed to clot following which it was centrifuged at 3000 rpm for 15 minutes. It was stored at minus 20 degrees centigrade until analysis was done. The samples were analyzed by Beckman Coulter AU480 Chemistry Analyzers (Beckman Coulter India Private Limited) Kurla (W), Mumbai. The Histopathological examination and staging of breast cancer were done by the Department of Pathology which was then noted for each case. All the available data was uploaded on an MS Excel spreadsheet and analyzed by SPSS version 21 for descriptive statistics.

Results

Based on the inclusion and exclusion criteria n=50 cases were identified and selected for the study. The mean age was 42.5 ± 5.0 years. The details of the age-wise distribution of cases are depicted in graph 1. it was found that in the current study 82% of cases were between age groups 31 – 55 years. N= 31(62%) were premenopausal age group. N=4(8%) of the cases in the study were the perimenopausal group and N=15(30%) were the postmenopausal group. In this study the measurement of BMI was done which revealed n=30(60%) were with BMI < 25 and n=20(40%) were with BMI > 25.

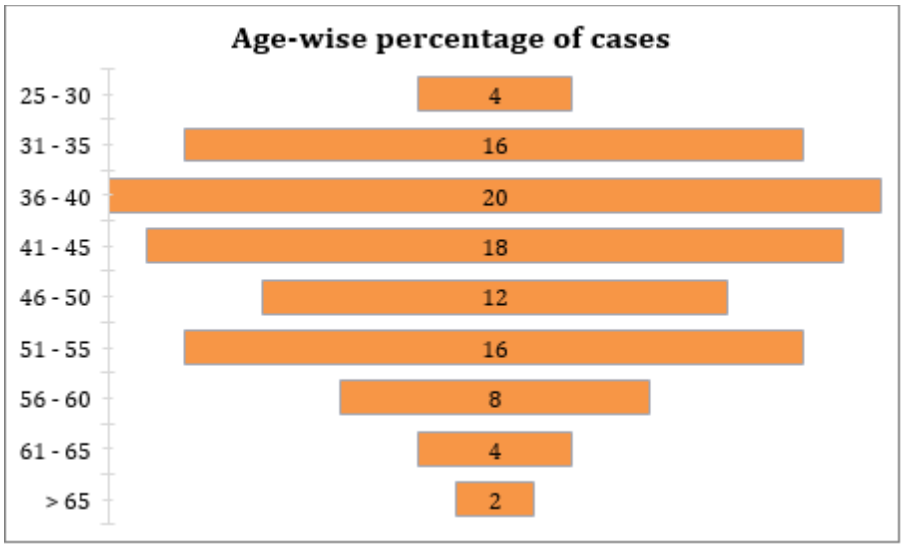

Fig 1: **Age-wise distribution (percentage) of cases included in the study**

The comparison of LDH, GGT, and ALP in pre-menopause, perimenopause, and post-menopause Revealed a significant change in the GGT and ALP levels between pre-menopause and post-menopause cases. The values were highest in post-menopause females whereas the values were in between the two in perimenopause females. Although the values of LDH were also found to be higher in post-menopausal females the p values were >0.05 hence considered as non-significant as shown in table 2.

Table 2: **Comparison of LDH, GGT, and ALP based on different groups**

Group	Frequency	LDH(U/L)	GGT(U/L)	ALP(U/L)
Premenopause	31	390.13±12.55	39.41 ± 3.32	110.33 ± 5.30
Perimenopause	04	402.76±22.36	48.94 ± 3.75	119.52± 7.44
Post Menopause	15	505.89±53.78	59.28 ± 4.62	175.36 ± 23.15
ANOVA		0.0669	0.0426*	0.036*

* significant

In the current study out of the n=10 cases of Stage, I n=6(60%) cases were Ductal carcinoma in situ (DCIS) n=4(40%) cases were Lobular carcinoma in situ (LCIS). In grade II out of n=23 cases, Invasive duct cell carcinoma was found in n=20 cases and Invasive lobular carcinoma (ILC) was found in n=3 cases. In stage III out of the n=12 cases, n=10 cases were Invasive duct cell carcinoma and n=1 case each of Medullary carcinoma and Invasive Lobular Carcinoma (ILC). In stage IV out of the n=5 cases, n=4 cases of invasive duct cell carcinoma and n=1 case of Invasive lobular carcinoma was recorded.

Table 3: Comparison of LDH, GGT, and ALP based on the different clinical stages of breast cancer patients

Stage	Frequency	LDH(U/L)	GGT(U/L)	ALP(U/L)
I	10	395.64 ± 9.65	34.69 ± 2.81	101.34 ± 4.12
II	23	449.36 ± 11.25	44.18 ± 3.43	111.87 ± 6.87
III	12	560.98 ± 61.89	59.37 ± 7.23	169.24 ± 21.69
IV	05	601.41 ± 75.51	68.53 ± 8.21	210.36 ± 30.74
ANOVA		0.036*	0.012*	0.023*

* significant

The intergroup comparison of various parameters was done as depicted in table 4. The LDH, GGT, and ALP levels comparison between group I and group II found all the p-values >0.05 hence it was not significant. A comparison of the same parameters in group I versus group III revealed LDH and ALP levels were significantly lower in group I p-values less than 0.05. The comparison of group I with group IV found all the parameters were significantly lower in group I than group IV p-values (<0.05). Similarly, in the comparison of group II versus group III only LDH and ALP were significantly lower in group II. The comparison of group II versus group IV revealed all the parameters significantly lower in group II. The intergroup comparison between Group III versus group IV found only ALP significantly lower in group III.

Table 4: Intergroup comparison of parameters (p-values)

Inter-group	LDH(U/L)	GGT(U/L)	ALP(U/L)
I vsII	0.226	0.159	0.256
I vs III	0.045*	0.178	0.021*
I vs IV	0.011*	0.045*	0.001*
II vs III	0.0491*	0.065	0.036*
II vs IV	0.021*	0.049*	0.015*
III vsIV	0.692	0.569	0.05*

* Significant

Discussion

Carcinoma breast is the second common malignancy detected in females after carcinoma cervix. The prognosis largely depends on its early detection and treatment. Therefore, there is a particular interest in biomarkers for the detection, prognosis, staging, and management of tumors. This study is a scientific quest to carry forward the importance of biomarkers in the detection of breast cancers. Some of the promising biomarkers are enzymes such as Lactate dehydrogenase,gamma-glutamyl transferase,and Alkaline phosphatase. They are easy to identify inexpensively and can be used for diagnostic and prognostic significance. In this study we found a mean LDH value of 395.64 ± 9.65 (U/L) at stage I compared to 601.41 ± 75.51 (U/L) at stage IV. Basnyat AS et al., [16] found the mean values of LDH significantly increased in patients with breast cancers as compared to controls. M Saheb SK et al., [17] found the values of LDH were 403.33±10.0(U/L) at stage I and 744.43±140.57 (U/L) at stage IV. G Rajeshwar et al., [18]in a similar study in Andhra Pradesh found LDH levels significantly increased in carcinoma breast patients (mean 543.22± 127.37) compared to controls (mean 304.85± 47.07). They also observed that the mean values were higher in carcinoma breast cases of the post-menopausal age group. We in the current study also observed that the mean values were 505.89±53.78 (U/L) higher compared to the other age groups (Table 2). The growing tumor causes the induction of LDH synthesis in the normal tissue of the host. Therefore, more aggressive growth of the tumor is correlated with greater LDH levels which is an important diagnostic and prognostic marker. Khurana P et al., studying the total serum LDH levels also found that its isoenzymes levels were increased in carcinoma breast. [19] Yea-TsuN et al.,[20] Maity CR et al.,[21]and Rao YN et al., [22]concluded that serum LDH levels raised only in the laterstages of carcinoma breast but no rise in the initial stages and the measurement ofserum LDH level would be of very little help in the diagnosis and prognosis of carcinoma breastexcept in cases of distant metastasis. Serum GGT levels in this study were highest in the post-menopausal group with mean values 59.28 ± 4.62 compared to other age groups (Table 2). The intergroup comparison of the GGT values (table 4) revealed GGT significantly elevated in group IV as compared to group I and group II. This study found a steady increase in GGT activity in groups I to IV. Seth LR et al., and Mishra S et al., in similar studies observed that there is a tremendous increase in mean serum GGT activity from stage I to stage IV in agreement with observations of the current study. In contrast some studies.[23 - 25] have reported that there is a non-significant increase in serum GGT in cases of breast cancers and the values tend to rise significantly in cases of liver metastasis therefore GGT estimation may be used as a biochemical marker for distant metastasis. Chandrakanth KH et al., [26] and Swathi S et al., [27] have also reported a significant increase in serum ALP levels in breast cancer cases as compared to controls. The cause of elevation of ALP in cancer breast occurs due to neoplastic cells infiltrating the liver and the adjacent tissues which may lead to localized pressure and necrosis of the liver cells and increased enzyme formations in the proliferating cells are responsible for elevated ALP levels.

Conclusion

The present study concludes within its limitations that serum markers LDH, GGT, and ALP were found to increase in patients with carcinoma breast. They may be used as biomarkers for diagnosis and prognosis following treatment. Although, these biomarkers are less accurate, specific, and sensitive,yet they are cost-effective and can be easily assayed with limited resources. These makers may act as useful adjuncts with more sensitive procedures such as imaging, clinical and histopathological findings.

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