Original Article

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Preliminary Study on Serum Lactate Dehydrogenase (LDH)-Prognostic Biomarker in Carcinoma Breast

Biochemistry Section

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ABSTRACT

Introduction: Serum Lactate Dehydrogenase (LDH) is one of the biochemical markers for breast cancer. Serum LDH is enzyme required for anaerobic glycolysis. One of its isoenzyme is increased in breast cancer due to up-regulation in its gene. It leads to increase in serum LDH level in breast cancer patients. Serum LDH is economical, easily available and easy to estimate.

Aim: In the present study, we evaluated the LDH levels in circulation of newly diagnosed patients of breast cancer and tried to correlate it with different TNM staging of carcinoma breast before interventions and after adjuvant therapy of these patients.

Materials and Methods: This prospective study was done on 83 diagnosed patients of breast cancer was conducted among poor patients in rural area. This study was conducted in the Department of Surgery between October 2008 to October 2010, at MGIMS, Sevagram, Maharashtra, a rural medical college located in Central India. Out of total 83 participants, 10 participants were having adverse events following surgery and remaining 73 participants were without adverse events following surgery. The significant difference in serum LDH levels between two groups, with and without adverse surgical outcome was calculated by Mann-Whitney U test.

Results: Patients with higher clinical TNM staging were having higher serum LDH levels. The serum LDH levels at sixth months following surgery showed a trend of statistically significant difference between patients with and without adverse events. As increased serum LDH levels in breast cancer patients shows poor prognosis, surgical outcome or advanced metastases.

Conclusion: Serum LDH monitoring can be used as a prognostic biomarker in patients of breast cancer. For confirmation of this finding, we require further more studies on larger sample size and long-term follow-up in patients specifically with higher serum LDH levels.

Keywords: Breast cancer, Metastasis, Postoperative, Preoperative

INTRODUCTION

With over one million new cases in the world each year, breast cancer is the commonest malignancy in women (23% of all the cancers), ranking second overall when both sexes are considered together [1]. For the early detection of carcinoma of various origins, a number of biochemical markers have been studied to evaluate the malignancy [2]. For breast malignancy, serum biochemical markers namely ferritin, Gamma- Glutamyl transpeptidase (GGT), Carcinoembryonic Antigen(CEA), superoxide dismutase(SOD), alkaline phosphatase (ALP), gluthathione, phosphohexose isomerase, leucine aminopeptidase and cholinesterase have been evaluated but none of them is ideal [3]. Repeating these tests during the follow up makes the cost of health care much more expensive; amongst all these markers, LDH is the simplest and cheapest to measure. Serum LDH enzyme is required for anaerobic glycolysis. A rapid increase in number of malignant cells modulates the LDH level in cytoplasmic compartment of cells, due to upregulation in its gene [4]. It leads to increase in serum LDH level in breast cancer patients. This increased LDH level is helpful for fulfillment of metabolic requirements and anaerobic glycolysis of these malignant cells. The value of serum LDH was found to be specific in patients with breast malignancy and also corresponds with clinical TNM staging, while patients with benign breast disease such as fibroadenomas have LDH value close to carcinoma (308±60 IU/l) [5].

AIM

In the present study, we evaluated LDH levels in circulation of newly diagnosed patients of breast cancer. In this preliminary study, we tried to analyse status of serum LDH with different TNM staging of carcinoma breast, before interventions and after adjuvant therapy of these patients.

MATERIALS AND METHODS

This prospective study was carried out in the Department of Surgery between October 2008 and October 2010 at MGIMS, Sevagram, Maharashtra. All newly diagnosed patients of breast cancer attending Surgery OPD were selected as participants in this study. New patients with complaint of breast lump, breast ulcer, or axillary lymph nodes were also included after proper clinical examination and TNM staging. Patients already treated by mastectomy in other hospitals and those who had distant metastasis (stage IIIc and IV) were excluded from this study. Patients suffering from myocardial infarction, jaundice or liver disease, polycythaemia, megaloblastic anaemia, haemolytic anaemia, diabetes mellitus, pancreatic disease, rheumatic fever and tuberculosis were also excluded. Detailed clinical history and examination findings of the breast and axilla were noted. Routine investigations and fine needle aspiration cytology (FNAC) of breast and axillary lymph nodes and Ultra-Sonography (USG) of abdomen and pelvis, x-ray chest were performed in all cases. X-ray of the limbs, spine and CT scan of abdomen were performed as indicated. All patients were classified according to TNM staging.

Serum LDH level in all patients was assessed preoperatively, postoperatively and on day seven, one month, three months and six months. Fine me 5ml blood was collected from antecubital vein in sterile test-tube. Then sample was centrifuged at 2000 rpm for 10 minutes and serum was separated. This serum sample was used for LDH estimation. LDH was estimated by DGKC method in Hitachi 912 Autoanalyser [6]. This method is based on the use of pyruvate substrate as per Henry et al., [7]. Normal value of serum LDH is 100-270IU/L [8].

Depending upon the histopathology reports, patients were subjected to chemotherapy (Cisplatin, Epirubicin and 5-FU

or Paclitaxel and 5-FU), radiotherapy and hormone therapy (Tamoxifen 20mg once a day for 5 years). All data were entered in excel sheet.

As data did not show normal distribution, Mann-Whitney U test was used to assess the significant difference in serum LDH levels among two groups of patients (with and without adverse outcome of surgery).Mann-Whitney U test was used to assess significant difference in LDH levels between preoperative and postoperative values and a p-value of less than 0.05 was taken as statistically significant. All statistical calculation was done by using SPSS 16.0 statistical software.

RESULTS

During the study period, 77966 patients attended the Surgery OPD, of which 11532 patients were treated as in-patients in the Department of Surgery. Incidence of breast cancer was 9.28% of all the surgical malignancies and 5.07% of all the malignancies when taken together in our hospital. All patients with breast cancer were females. Majority (75.89%) of the patients were in the age group 31-50 years [Table/Fig-1]. Majority of the participated patients were in TNM Stage IIIa and Stage IIIb. Four patients (4.81%) were in Stage IIa [Table/Fig-2]. Fine needle aspiration cytology (FNAC) report of all patients depicted ductal cell carcinoma and histopathology report showed that 82 (98.80%) were invasive ductal cell carcinoma NST (nonspecific type) and one (1.20%) was medullary carcinoma.

LDH measurement was done on preoperative day, postoperative day seven, one month, three months and six months period. It was observed that, patients with higher TNM stage had higher LDH levels compared to patients with lower TNM Stage. It shows difference of LDH levels among all participated patients (83), with (10) and without (73) adverse events over six month period [Table/Fig-3]. It was found that the LDH levels were initially increased on Postoperative day seven and then LDH level decreased to normal levels. However, LDH level remained elevated in 10 participated patients with adverse events [Table/Fig-4]. These ten patients developed adverse events in the form of pleural effusion (two patients), satellite nodules (two patients), and liver metastasis (two patients) and four with recurrence of breast cancer.

LDH levels failed to distinguish in patients with and without adverse effects in the early period. But in later period (at six month interval), there was a statistically significant difference in serum LDH levels among breast carcinoma patients, with and without adverse events postoperatively.

Age Group	No of patients (%)
31 – 40 yrs	26(31.32)
41 – 50 yrs	37(44.57)
51 – 60 yrs	18(21.68)
>60 yrs	2 (2.40)
Total	83(100)

[Table/Fig-1]: Age-wise distribution of cases with carcinoma breast.

TNM Stage	No of patients (%)
Stage I (Ta N0 M0)	0
Stage IIa (T0-2 N0-1 M0)	4 (4.81%)
Stage IIb (T2-3 N0-1 M0)	13(15.66%)
Stage IIIa (T1-2 N1-2 M0)	38(45.78%)
Stage IIIb (T4 N0-2 M0)	28(33.73%)
Stage IIIc (Tany N3 M0)	0
Stage IV (Tany Nany M1)	0
Total	83(100)

[Table/Fig-2]: Distribution of cases and TNM Staging

Interval at which serum LDH is estimated	Mean rank	Z score	p-value
Preoperative Serum LDH level	69.7	- 3.69**	p<0.01
At 7th Day after treatment	97.28**		
Preoperative Serum LDH level	98.5	- 4.021**	p<0.01
At 1st month after treatment	68.5**		
Preoperative Serum LDH level	107.5	- 6.44**	p<0.01
At 3rd month after treatment	59.45**		
Preoperative Serum LDH level	107.19	-6.35**	p<0.01
At 6th month after treatment	59.81**		

[Table/Fig-3]: Comparison of LDH mean rank at postoperative interval with preoperative, in all patients using Mann-Whitney U test.
*p-value<0.05- significant, ** p<0.01- highly significant

	Pre-operative level	Post-operative day 7	Post-operative at 1 month	Post-operative at 3rd month	Post-operative at 6th month
Mann-Whitney U test	319.500	283.000	336.000	320.000	7.500
Wilcoxon W test	3020.500	2984.000	3037.000	3021.000	2708.500
Z score	-.637	-1.147	-0.406	-0.630	-5.002
Asymp. Sig. (2-tailed)	0.524	0.251	0.685	0.529	0.000**

[Table/Fig-4]: Serum LDH mean rank in patients with adverse events versus without adverse events at various interval of pre and postoperative
*p-value<0.05- significant, ** p<0.01- highly significant

DISCUSSION

Serum LDH enzyme is required for anaerobic glycolysis. A rapid increase in number of malignant cells modulates the LDH level in cytoplasmic compartment of cells, due to up regulation in its gene. It leads to increased in serum LDH level in breast cancer patients. These increased LDH level is helpful for fulfillment metabolic requirements and anaerobic glycolysis of these malignant cells [9].

For the early detection of carcinoma of various origins, a number of biochemical markers have been studied. However, no marker specific for breast cancer has been discovered and those currently available lack the sensitivity and specificity for early detection of the disease or for determining the tumour burden. Other biochemical markers available in the market for screening, confirming diagnosis, staging, monitoring treatment and prognosis are expensive and increases the overall cost of the treatment. Serum LDH is economical and easy to estimate. It does not require sophisticated centre or any latest technology and can be performed even at our rural centers.

In this study, 79.53% of patients were in stage III which is in accordance with the study of Bhattacharya and Adhikary, who found 88% patients in stage III and IV [10]. [Table/Fig-5] shows stage-wise distribution of patients among different studies. In our study, the maximum numbers of patients were among TNM stage III. Outcome of adjuvant therapy as well as surgery depends on the TNM staging. Late presentation of breast cancer in our country is mainly because of illiteracy, ignorance, shyness and poor economic conditions as well as less number of well equipped hospitals in periphery.

Age of occurrence for breast cancer rises from 30 years and reaches a peak at 45-54 years and a second peak at 65 years [11]. Higher proportions of breast cancer patients in developing Asian countries are younger than patients in developed Asian and western countries [12]. In our study, the maximum numbers of patients were in the age group 31-50 years (75.89%), which is in accordance with the report of Sandhu et al., who similarly found 65.8% patients to belong to the same age group [13].

In our study, the mean rank of serum LDH was higher than certain previous studies. This may be attributed to the higher number of cases in stage III in our group of patients. In our study, serum LDH

levels were found to be higher in patients with breast malignancy and also corresponded with clinical stage and bulk of the tumour [14]. Similar results were depicted by one of the previous studies [15].

On postoperative day seven, serum LDH levels were significantly found to increase. But, subsequently there was decline in LDH levels postoperatively at one month, three months and six months, proportional to the therapeutic response. This elevation in serum LDH levels in first week following surgery suggests radical destruction of cancer cells resulting in the release of enzyme into the circulating system and could be initiated by tumour itself in a bid to survive hypoxia induced by destruction of tumour vasculature and tissues, the underlying principle behind all anti-cancer treatment [16].

One of the previous studies reported no significant difference in pre and post-treatment serum LDH levels at 7 days, whereas they correlated post treatment decrease in serum LDH levels at one month with response to treatment which is similar to our study [17]. Persistent rise in serum LDH levels is because of poor response to treatment or metastasis or recurrence. In our study, all patients had serum LDH levels nearly normal at six months follow up except for ten patients who showed persistently elevated levels. Six patients of these had developed metastasis (two had pleural effusion, two had satellite nodules and two had liver metastasis) and four were having recurrence of disease. One of the previous studies evaluated serum LDH values between first and second weeks post surgery and found the values to be in normal range but few patients who showed high value was found to show relapse of primary disease with massive bone metastasis and lethal outcome [18].

We found that serum LDH mean rank at one month, three and six months (postoperatively) was significantly low ($p<0.01$), as shown in [Table/Fig-6]. However, all studies including our study had a short follow up and were unable to draw a significant conclusion. Long term studies with large patient group are required to conclude the usefulness of serum LDH as a prognostic marker.

Study[references]	Year	Stage I	Stage II	Stage III	Stage IV
Kanhere et al., [19]	1988	22.2%	50%	22.2%	5.5%
Seth RK et al., [20]	2003	20%	64%		16%
Mishra S et al., [21]	2004	56.87%	43.13%		0%
Jha AK et al., [22]	2010	10.78%	24.62%	40.84%	23.75%
Present study	2010	0%	20.47%	79.53%	0%

[Table/Fig-5]: Distribution of patients as per TNM staging in present versus previous studies.

Interval of LDH estimations / Occurrence of Adverse events	Pre-operative Level	Post-operative day 7	Post-operative at 1 month	Post-operative at 3rd month	Post-operative at 6th month
Mean rank of Serum LDH for patients with adverse effects (10 patients)	46.55	50.20	44.9	46.5	77.75
Mean rank of LDH for patients without adverse effects (73 patients)	41.38	40.88	41.6	41.38	37.10

[Table/Fig-6]: Serum LDH (U/L) mean rank in patients with adverse events versus without adverse events at various interval (pre and postoperative days).

LIMITATION

As our study period was short, we were not able to monitor LDH level beyond six month interval. So, we need further studies and long term follow-up in patients who have high levels of serum LDH without metastasis.

CONCLUSION

As increased serum LDH levels in breast cancer patients shows poor prognosis, surgical outcome or advanced metastasis. Monitoring of serum LDH levels can be used as a prognostic indicator in patients with breast cancer who are undergoing treatment. Persistently high levels of serum LDH or sudden increase in serum LDH levels postoperatively at six months or for long term may indicate poor outcome or metastasis. Higher levels of serum LDH in these patients may be an alarming pre-warning sign of recurrence or metastasis

ABBREVIATIONS

LDH – Lactate Dehydrogenase

REFERENCES

[1] Wang W-J, Lei Y-Y, Mei J-H, Wang C-L. Recent progress in HER2 associated breast cancer. *Asian Pac J Cancer Prev Apjcp*. 2015;16(7):2591–600.
[2] Tashkandi H, Shah N, Patel Y, Chen H. Identification of new miRNA biomarkers associated with HER2-positive breast cancers. *Oncoscience*. 2015;2(11):924–29.
[3] Hadi NI, Jamal Q. "OMIC" tumour markers for breast cancer: A review. *Pak J Med Sci*. 2015;31(5):1256–62.
[4] Duffy MJ, Crown J. A personalized approach to cancer treatment: how biomarkers can help. *Clin Chem*. 2008;54(11):1770–79.
[5] Koukourakis MI, Kontomanolis E, Giatromanolaki A, Sivridis E, Liberis V. Serum and tissue LDH levels in patients with breast/gynaecological cancer and benign diseases. *Gynecol Obstet Invest*. 2009;67(3):162–68.
[6] Committee on Enzymes. Recommended methods for the determination of four enzymes in blood. *Scand J Clin Lab Invest*.1974;33:291-306.
[7] Henry RJ, Chiamor N,Golub OJ,Berkman S. Revised spectrophotometric methods for the determination of glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase and lactic acid dehydrogenase. *Am J Clin Pathol*.1960;34:381-98.
[8] Rao YN, Singh GP, Chakravarthy M, Khanna NN. Serum lactic dehydrogenase, leucine aminopeptidase and alkaline phosphatase in the diagnosis of cancer. *Ind J Cancer*.1978;15:39-44.
[9] Khan N, Tyagi SP, Salahuddin A. Diagnostic and prognostic significance of serum cholinesterase and lactate dehydrogenase in breast cancer. *Ind J Pathol Microbiol*.1991;34(2):126-30.
[10] Bhattacharya S, Adhikary S. Evaluation of risk factors, diagnosis and treatment in carcinoma breast– a retrospective study. *Kathmandu Univ Med J*. 2006;4 (1):54-60.
[11] Gupta A, Shridhar K, Dhillon PK.A review of breast cancer awareness among women in India: Cancer literate or awareness deficit? *Eur J Cancer*. 2015;51(14):2058-66.
[12] Parsa P, Kandiah M, Abdul Rahman H, Zulkefli NM. Barriers for breast cancer screening among Asian women: a mini literature review. *Asian Pac J Cancer Prev*. 2006;7(4):509-14.
[13] Sandhu DS, Sandhu S, Karwasra RK, Marwah S. Profile of breast cancer patients at a tertiary care hospital in north India. *Ind J Cancer*. 2010;47(1): 16-22.
[14] Radenkovic S, Milosevic Z, Konjevic G, Karadzic K, Rovcanin B, Buta M, et al. Lactate dehydrogenase, catalase, and superoxide dismutase in tumour tissue of breast cancer patients in respect to mammographic findings. *Cell Biochem Biophys*. 2013;66(2):287–95.
[15] Khurana P, Tyagi N, Salahuddin A, Tyagi SP. Serum lactate dehydrogenase isoenzymes in breast tumours. *Indian J Pathol Microbiol*. 1990;33(4):355–59.
[16] Brown JE, Cook RJ, Lipton A, Coleman RE. Serum lactate dehydrogenase is prognostic for survival in patients with bone metastases from breast cancer: a retrospective analysis in bisphosphonate-treated patients. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2012;18(22):6348–55.
[17] Kher A, Meghe G, Deshpande A. Significance of serum ferritin and lactate dehydrogenase in benign and malignant disease of breast. *Ind J Patho Micro*.1997;40(3):321-26.
[18] Brown JE, Cook RJ, Lipton A, Costa L, Coleman RE. Prognostic factors for skeletal complications from metastatic bone disease in breast cancer. *Breast Cancer Res Treat*. 2010;123(3):767–79.
[19] Kanhere MH, Sharma YR, Kalra A. Role of bone marrow examination in staging of carcinoma breast. *Ind J Surg*.1988;1:54-58.
[20] Seth LRK, Kharb S, Kharb DP. Serum biochemical markers in carcinoma breast. *Indian J Med Sci*. 2003;57(8):350–54.
[21] Mishra S, Sharma DC, Sharma P. Studies of biochemical parameters in breast cancer with and without metastasis. *Indian J Clin Biochem*. 2004;19(1):71–75.
[22] Jha AK, Hamal PK, Jha J, Banthia P, Thakali K, Basnet BK. Pattern of breast cancer in a tertiary care centre. *J Nepal Med Assoc*. 2010;49(177):1-5.

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