CHAPTER FOUR
Lactate Dehydrogenase in Cancer.
INTRODUCTION

A widely distributed intracellular enzyme lactate dehydrogenase (LDH) acts in the glycolytic cycle to catalyze the conversion of lactic acid to pyruvic acid, is known to occur in all glycolyzing cells (1,2). LDH is made up of five isoenzymes, which are composed of two subunits or monomers. The five LDH isoenzymes differ in their mobility in an electrophoretic field. Elevations in the LDH serum level occurs when there is cellular death and leakage of enzyme from the cell.

Total serum LDH was elevated about ten times the normal in myocardial infarction. Elevated levels of total LDH were also noted in pernicious anaemia, megaloblastic anaemia, liver diseases, renal diseases and malignancies. LDH has been very extensively studied to establish a relationship to cancer since well known to be normal components of tissue and all body fluids. Abnormal levels of LDH in body fluids have been reported in relation to leukemia by a number of workers. (3-6). Schenker
demonstrated abnormal levels of LDH in gastric cancer (7). Abnormal levels of serum LDH were also noted in lymphomas.

Several studies of human tumors have appeared in which LDH isoenzyme have been examined in normal and malignant samples from prostate (10) breast (8,9,15,16), lung (11), brain (12) and colon (13). All of these studies suggested that the tumors contain a greater proportion of the isoenzymes than the healthy subjects.

Kolaric et al. (14) reported in histologically verified malignant liver processes that serum total LDH was significantly increased in cancer of the liver than scintigraphy of the liver could register. Skillen et al. (16) measured serum LDH, γ-glutamyl transferase and alkaline phosphatase (ALP) in 105 patients with ovarian cancer for a period of four years. Chemotherapy was started 1 week after surgery at which time 25% of the patients had elevated LDH, elevated γ-GT in 29% and 21% elevated ALP.
Urinary LDH activity was measured in normal subjects and 20 patients suffering from urinary cancer by Rubatto et al. (17). The activity noted by them in normal was 1.24 units compared to 14.39 for patients with renal diseases. They also measured LDH activity in 3 patients after surgery and found LDH activity to be normal after 20-30 days. This method was recommended for the early diagnosis of renal tumors.

Fottrell et al. (19) reported total LDH levels and M-type units of the enzyme to be elevated in hyperplastic endometrium. Hurtado et al. (20) determined relative activity of 5 isoenzymes of LDH in serums of 240 patients with neoplasia. In most of the cases the LDH 4 and 5 activity was found to elevated. In thyroid cancer LDH 3 was also elevated. In mammary cancer LDH 5 was increased and LDH 1 was decreased. Similarly in state III of cervical cancer, LDH 4 was increased and LDH 1 was decreased. In stomach cancer LDH 4 and 5 was increased and LDH 1 decreased. In myeloid leukemia LDH 1 was increased and LDH 5 was decreased.
Saravanan et al. (21) reported elevated levels of serum phosphohexose isomerase (PHI) and LDH in breast tumors. Hofmann et al. (22) measured LDH activity in tissue homogenates of 34 normal human ovaries and 34 ovarian carcinomas. They could not find any correlation in the LDH activity of the tumor and its stage. Robertson et al. (23) presented that serum LDH may be of value in identifying patients with advanced seminoma of the testis. Elevated levels of serum LDH were noted before treatment but after the treatment the levels retained to normal which is a useful indicator of treatment response. Total LDH activity and LDH 5 isoenzyme activities were reported to be higher in ovarian, endometrial and cervical carcinoma tissues than in control tissues as reported by Jandova et al. (24). Golobrod et al. (25) reported that the activity of total LDH in larynx cancer tissue was 2.6 fold higher than the normal tissue. Similarly Ishida et al. (26) presented that the level of serum LDH was higher in almost all types of leukemias when compared with the normal serum. Gar Kavji et al. (27) noted in 85 patients with advanced
carcinoma of ovary, breast and lung, isoenzymes LDH 4 and LDH 5 to be massively elevated than the normals. LDH has been found elevated in the serum of patients suffering from malignant tumors (26-32). Although it has been reported that in cases of leukemia the degree of elevation of serum LDH closely reflects the clinical status of the patients (33), Zondag and Klen (34) reported that of 22 patients with malignant lymphoma 16 (73%) had abnormal LDH isoenzymes pattern and LDH 1 and LDH 2 were elevated. The elevation is due to rapid turnover of tumor cells. Reviewing the observations of most investigators since 1956 that several types of neoplastic disease are associated with high LDH levels, we tried to compare the LDH levels in different cancers depending upon their rural or urban habitat, with different age groups in cervical, ovarian, breast, colon, 41 and stomach cancer.

MATERIAL AND METHODS

483 patients with different cancers from Surgical Department proved by histopathological studies were obtained from Government Medical College
and Hospital, Aurangabad. These comprised of 165 cervical carcinomas, 60 ovarian carcinomas, and 135 breast carcinomas staged according to the International Federation of ObGy, 60% of cancer colon and 36% of stomach cancer. Rural patients were those who came from villages having population less than 50,000 without any educational background. These patients normally were of stage III categories. The patients were divided into three age groups 20 to 40, 41 to 60 and 61 to 80 years. Control subjects consisted of students and employees from Government Medical College and Hospital, Aurangabad. The enzyme determinations were carried out within 5 to 8 hrs. of collection of the blood samples, as described in earlier chapter.

Total LDH was determined by the method of Wooton (35). The reaction mixture contained 1 ml of buffered substrate pH 7.4 (0.75 mM) and 0.1 ml. serum. The mixture was incubated at 25° for 5 minutes. To it 0.1 ml of NADH (1 x 10⁻⁴ M) was added and the tubes were incubated for 15 minutes. Then 1 ml of 2.4 DNP (2 mM) was added. The tubes were allowed to stand for 20 minutes at room
temperature, 10 ml. of NaOH (0.4 N) was added and the coloured complex was measured at 530 nm. Sodium pyruvate (0.75 mM) was utilized as a standard.

\[
\frac{T-C}{S-B} \times 0.75 \times \frac{1}{15} \times \frac{1000}{0.1} = \frac{T-C}{S-B} \times 500
\]

IU/L.

RESULTS

Table - 1 indicates the values of total serum LDH in healthy controls and cancer patients. Serum LDH was found to be increased in cervical, ovarian and breast cancer. The magnitude of increase in LDH of cervical cancer was found to be highest in age group of 21 - 40 in rural subjects, and 41 - 60 in urban subjects. However, ovarian tumor subjects registered total LDH to be significantly elevated in urban patients in the age group 41 - 60 years when compared to normals.

A statistically significant increase in patients with breast tumors was noted both in rural and urban
### Table 1
Serum total lactate dehydrogenase in Genital cancers

<table>
<thead>
<tr>
<th>Type</th>
<th>Age</th>
<th>Rural</th>
<th>n</th>
<th>Urban</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>21 - 40</td>
<td>112.50 ± 1.2</td>
<td>(15)</td>
<td>114.4 ± 1.5</td>
<td>(25)</td>
</tr>
<tr>
<td></td>
<td>41 - 60</td>
<td>116.60 ± 1.8</td>
<td>(20)</td>
<td>118.6 ± 2.0</td>
<td>(40)</td>
</tr>
<tr>
<td></td>
<td>61 - 80</td>
<td>125.00 ± 2.0</td>
<td>(16)</td>
<td>120.0 ± 1.2</td>
<td>(25)</td>
</tr>
<tr>
<td>Cervical</td>
<td>21 - 40</td>
<td>125.00 ± 2.5</td>
<td>(25)</td>
<td>118.75 ± 1.2</td>
<td>(15)</td>
</tr>
<tr>
<td></td>
<td>41 - 60</td>
<td>118.75 ± 1.9</td>
<td>(30)</td>
<td>150.00 ± 2.6</td>
<td>(40)</td>
</tr>
<tr>
<td></td>
<td>61 - 80</td>
<td>137.50 ± 3.2</td>
<td>(30)</td>
<td>125.00 ± 1.9</td>
<td>(25)</td>
</tr>
<tr>
<td>Ovarian</td>
<td>21 - 40</td>
<td>142.50 ± 3.0</td>
<td>(10)</td>
<td>148.75 ± 2.8</td>
<td>(14)</td>
</tr>
<tr>
<td></td>
<td>41 - 60</td>
<td>175.00 ± 2.8</td>
<td>(10)</td>
<td>200.00 ± 3.6</td>
<td>(18)</td>
</tr>
<tr>
<td>Breast</td>
<td>21 - 40</td>
<td>B 275.00 ± 4.6</td>
<td>(15)</td>
<td>B 260.00 ± 4.8</td>
<td>(10)</td>
</tr>
<tr>
<td></td>
<td>A 200.50 ± 3.1</td>
<td>(15)</td>
<td>A 180.50 ± 6.0</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>41 - 60</td>
<td>B 400.00 ± 5.0</td>
<td>(40)</td>
<td>B 425.00 ± 5.2</td>
<td>(25)</td>
</tr>
<tr>
<td></td>
<td>A 310.00 ± 4.0</td>
<td>(40)</td>
<td>A 325.00 ± 7.0</td>
<td>(25)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>61 - 80</td>
<td>B 350.00 ± 3.6</td>
<td>(25)</td>
<td>B 360.00 ± 4.2</td>
<td>(20)</td>
</tr>
<tr>
<td></td>
<td>A 288.00 ± 2.8</td>
<td>(25)</td>
<td>A 310.40 ± 5.0</td>
<td>(20)</td>
<td></td>
</tr>
</tbody>
</table>

B = Before treatment;  
A = After treatment  
\( +p < 0.05 \)  
\( n \) in parentheses indicate number of subjects.
subjects. Levels of total LDH in urban patients were higher than the rural patients. The magnitude of induction paralleled with increase in age of both rural and urban subjects. It was observed in follow up cases in breast cancer that the LDH activities were lowered on treatment of patients with stage II cancers whereas stage III patients did not turn up for the study. The post therapeutic level of serum LDH was followed in few breast cancer patients though systematic follow up was limited. There was no consistent decrease in the post therapeutic level of LDH. One 5-6 cases who were subjected to bilateral oophorectomy and chemotherapy showed significant decrease in the levels of LDH. These changes may be due to the alterations in the membrane permeability due to chemotherapeutic drugs.

Table-II indicates the decrease in LDH activity with colon and stomach cancers in both male and female subjects. The magnitude of decrease in LDH activity was more in male as compared to female in both the cases of rural subjects, whereas in the urbans female showed 25% decrease in colon cancers as
| Stage/type | Rural | | | Urban | | | 
|---|---|---|---|---|---|---|---|
| | Male | n | Female | n | Male | n | Female | n | 
| Normal | 118.70 ± 2.9* (25) | 119.00 ± 2.3 (20) | 118.00 ± 1.5 (20) | 118.00 ± 2.4 (20) | 
| Colon | A 75.00 ± 5.0* (15) | 100.00 ± 1.6 (20) | 106.25 ± 2.0 (10) | 87.50 ± 3.1* (15) | 
| | A 95.00 ± 4.2* | 115.00 ± 2.0 | 110.00 ± 1.0 | 100.50 ± 2.5* | 
| Stomach | B 105.00 ± 2.6* (16) | 115.00 ± 2.0 (15) | 105.00 ± 2.2 (14) | 104.00 ± 2.8* (10) | 
| | A 110.00 ± 1.8 | 120.00 ± 1.6 | 115.00 ± 1.6 | 110.50 ± 1.6 | 

B - Before treatment;  
A - After treatment;  
n - in parentheses indicate number of subjects  
* \( p < 0.05 \)
compared to 10% of the male. However, in urban stomach cancers, marginal decrease in LDH was observed in both male and female subjects. Alteration in serum LDH activity was noted by treatment with chemotherapeutic agents.

DISCUSSION

The total serum LDH levels were found to be elevated in most of the malignancies studied, except in the colon cancer. Serum LDH is known to be elevated in conditions associated with muscular and hepatic necrosis. Breast cancer patients having high level of LDH are in the late stage of cancer. Bardwill and Chang (36) reported marked elevation in the serum LDH in patients with carcinoma of the pancreas. They suggested that elevation in serum LDH may be due to malignancy per se. Thus increased serum LDH activity would be a useful adjunct to the diagnosis of malignancy involving hepatic system. Similar results were reported by Rao et al. (37) in cancers of breast, GIT, bone, genitourinary tract, skin and oral cancers. Murakami et al. (38) reported elevated levels of LDH in patients with germ cell
neoplasms, to be derived directly from neoplastic tissue and explained the relationship between serum LDH levels and tumor burden.

Analysis of total serum LDH and their isoenzymes may be an effective criterion for diagnosis of malignant or nonmalignant type of tumor since corelation of serum total LDH is significantly higher than the normal. Analysis of the LDH isoenzyme spectra proves to be a very informative method giving an answer to many question. The very important feature of LDH is isoenzyme that it is specific of all types of human neoplasms, both benign and malignant tumors of the most diverse localizations. This features consists in a sharp decrease in activity of LDH isoenzyme I, and an increase in LDH isoenzyme is very sharp. On the basis of such analysis it is possible to distinguish reliably a malignant neoplasm from a benign neoplasm specially by using the LDH \(-V/LDH\) I coefficient.

The observed high levels of total LDH in cervical, ovarian and mammary cancers are supported by a number of workers (40-49). These elevated levels may be partly and/or wholly due to distant metastasis.
The decrease in total LDH in patients with stomach and colon cancer agrees with the results obtained by other author (40). Thus it appears that there are several different mechanisms to explain the alterations in LDH activity in serum with various carcinomas. The decrease serum LDH in stomach and colon cancers could be explained on the basis of nutritional status of the patients. The present studies indicate that the understanding of known mechanisms contributing to LDH activity alterations in body fluids becomes necessary in order to correlate the quantitative and serial changes in LDH activity with experimental and/or clinical factors.

The increase in serum LDH in the subjects studied could be due to the changes in the cell membrane of malignant tumors which release LDH into the circulation before accumulation of the enzyme in the tumor tissue can be demonstrated. The release of large quantities of cytoplasmic enzymes into the interstitial fluids of solid tumor transplants has been shown (51,52). These findings suggest that changes of LDH in serum are an expression of fundamental metabolic in the total organism or due to tumor progression.
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