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A Study of Lactate Dehydrogenase (LDH) Isoenzyme is a Biochemical Tumour Marker in Cervical Carcinoma Patients

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ABSTRACT To establish Lactate dehydrogenase (LDH) isoenzyme is a biochemical tumor marker in cervical carcinoma patients for diagnosis and treatment monitoring of the disease. The Serum and cervical tissue LDH isoenzyme was analyzed qualitatively by Poly Acrylamide Disc Gel Electrophoresis method. The total LDH activity was estimated quantitatively by the method of UV spectrophotometry. 50 untreated cervical carcinoma patients were taken up and compared with age matched healthy females (controls). Out of 50 patients, 5 patients were histologically classified as well differentiated squamous cell carcinoma (Grade-I), 23 were moderately differentiated squamous cell carcinoma (Grade-II) and 22 were poorly differentiated squamous cell carcinoma (Grade-III). Grade-I patients did not show any change, in the serum and cervical tissue LDH isoenzyme fractions compared to the controls but variation in electrophoretic mobility have been observed. Grade-II patients showed two new additional isoenzyme fractions in LDH_2 and LDH_3 locations, where as Grade-III patients showed only three LDH isoenzyme fractions. The total LDH activity in both serum and cervix tissue of normal individuals are 0.458±0.0796 units/ml; and 0.680±0.0218 units/mg. Grade-I patients did not show any change in total LDH activity (serum: 0.384±0.0404 units/ml; cervical tissue: 0.589±0.0292 units/mg; $P<0.01$). Grade-III patients showed much lower values (serum: 0.284±0.0109 units/ml; cervical tissue: 0.327±0.043 units/mg; $P<0.001$), where as Grade-II patients showed significant increase in total LDH activity (serum: 0.878±0.0531 units/ml; cervical tissue: 1.296±0.0813 units/mg; $P<0.001$) respectively. The results suggest that LDH isoenzyme is useful biochemical tumor marker for diagnosis and to assess the grade of malignancy.

1. INTRODUCTION

Cervical region is a common site of female malignancies in India with 85% of neoplasia having a mortality rate of 5% (Nair and Pillai 1992). Various types of markers are being in use for the early diagnosis and to observe patients response to therapy while there are many diagnostic marker for early diagnosis of malignancy. Lactate dehydrogenase (LDH) isoenzymes is of considerable interest to the biochemical oncologists. An increased levels of serum LDH in gynecological malignancies has been reported by several authors (Wright et al. 1966; Sherwin et al. 1968; Zondag and Klein 1968). Activity of lactate dehydrogenase LDH isoenzyme in blood serum was higher in case of precancerous lesion of cervix uteri than in healthy controls. This index was further increased 1.6 fold in the cases of malignancy of cervix uteri than precancerous lesions (Nugamanova et al. 1981). Kumar et al. (1988) studied serum LDH isoenzyme activity in carcinoma cervix patients. They found out significant increase of LDH_2 and LDH_3 fractions in cervical carcinoma patients compared with control values ($P<0.001$). After radio therapy the patients showed significant lowering of LDH_2 and LDH_3 ($P<0.001$) in the serum. So they concluded that LDH isoenzyme determination in carcinoma cervix is very helpful in assessing, treatment response following radiotherapy and thus may be an important prognostic parameter. Chow et al. (1991) found that the total serum LDH in malignant ovarian tumor was 876.3 (±450.4) IU/L which was significantly higher than in benign ovarian cancer 364.8 (±87.9). Xi et al. (1994) suggested that LDH isoenzyme may be considered as a tumor marker. Von Eyben (2001) concluded that serum LD_1 is useful tumor marker of testicular germ cell tumors. For patients with ovarian germ cell tumors, serum LD_1 was raised more often than for patients with testicular germ cell tumors. Oladipo et al. (2002) reported that five isoenzyme of LD (LD_1-LD_5) were present in both control and

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patient sera. The serum LD_1 and LD_2 isoenzymes were predominant in the diseases groups (malignant and non malignant). The purpose of this study is to analyze LDH of serum and cervix tissues qualitatively and to estimate its activity quantitatively both in normal person and in patients suffering from cancer cervix so as to establish a bio-chemical relationship between LDH and cervical carcinoma in Indian population.

2. MATERIALS AND METHODS

A total of 50 cervical cancer patients who attended Out Patient Department (OPD) of the Government Aringnar Anna Memorial Cancer Institute and Hospital, Kanchipuram were taken up for this present study and compared with same age matched healthy women as control. All patients were examined clinically and histo-pathologically. The final diagnosis was made after histopathological examination. The pap smear results were not obtained when collecting the samples however, the stages of the tumor where conformed by the histopathological observation by the qualified experts. In all the 50 patients serum and cervical tissue LDH isoenzymes have been analyzed.

$$\text{Percentage activity of each fraction} = \frac{\text{Activity in fraction comprising a peak}}{\text{Activity in all the fractions}} = \text{x } 100$$

2.1 Qualitative Analysis

Before the onset of treatment, blood samples from the patients were collected and serum was separated. Serum was maintained at 20ºC in freezer until the analysis were carried out. Tissue samples were collected from the patients at the time of biopsy and the samples were homogenized with 0.1 M phosphate buffer at pH 7.4 and centrifuged at 10,000 Xg for 20 minutes. Samples were taken separately in each tube of PAGE (Poly Acrylamide Disc Gel Electrophoresis) for analysis of LDH isoenzyme. The results were conformed by running duplicates.

The percentage activity of each fractions of isoenzymes were calculated by the following formula (using densitometric scanning).

2.2. Quantitative Analysis

The quantitative analysis of lactate dehydrogenase was assayed according to the method described by King (1965). One ml of buffered substrate and 0.1 ml of enzyme extract was added and the tubes were incubated at 37ºC for 5 minutes. After the incubation period, 0.2 ml of NAD^+ solution was added to the test and 0.2 ml of distilled water to the control and the incubation was continued for another 15 minutes. The reaction was then arrested by the addition of 1 ml of DNPH reagent and the tubes were incubated for a further period of 15 minutes at 37ºC. After the last incubation period, 7 ml of 0.4 N NaOH solution was added and the colour development was measured at 420 nm in a UV spectrophotometer. Suitable aliquotes of standard and blank were also analysed by the same procedure in normal women serum and cervix tissues and all the cervical cancer patients serum and cervix tissues. For serum analysis 0.1 ml of sample was taken in test tube and the same procedure was followed for estimating total serum LDH activity.

2.3. Statistical analysis

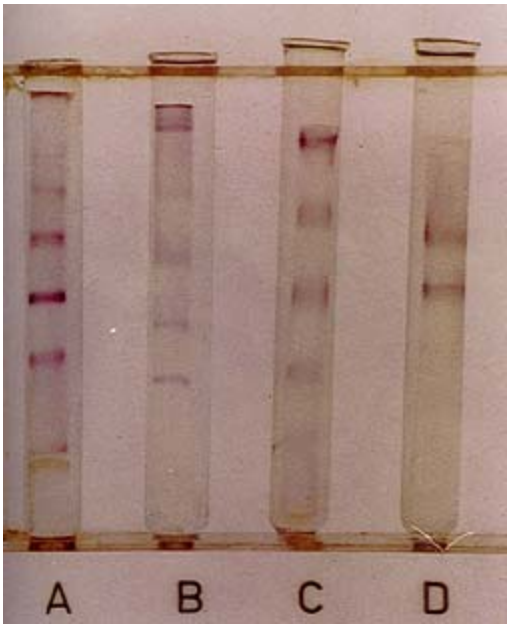
Mean ($\bar{x}$), standard error (SE) were calculated for each patient. To understand the level of significance between normal and cancer cervix patients, Student t-test was applied (Zar 1984).

3. RESULTS

Among 50 patients studied, 5 patients were histologically classified as well differentiated squamous cell carcinoma (Grade-I), 23 were moderately differentiated squamous cell carcinoma (Grade-II) and 22 were poorly differentiated squamous cell carcinoma as (Grade-III). The densitometric scanning was done in each samples but the picture was not incorporated.

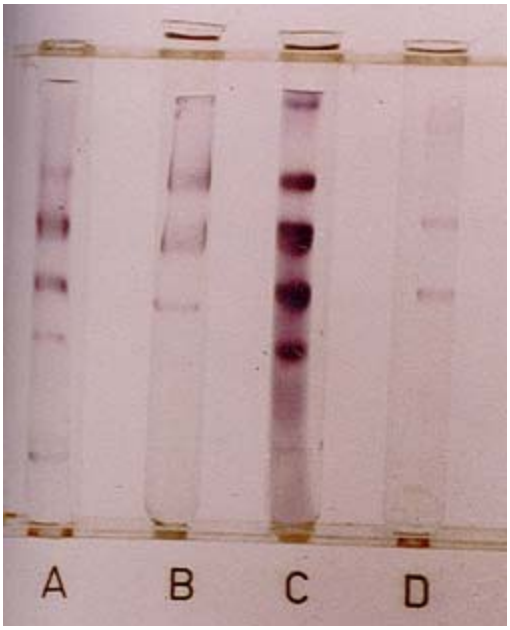
3.1. Serum and Cervical Tissue LDH Activity in Normal Women

Five LDH isoenzyme fractions namely LDH_1, LDH_2, LDH_3, LDH_4 and LDH_5 have been observed in both serum and cervical tissue of normal women (Fig. 1 and 2). Serum LDH showed that LDH_1 fraction has the highest activity (38.7%) and LDH_5 has the lowest activity (14.5%). cervical tissue LDH showed that the LDH_5 fraction has the highest activity 80.1% while the LDH_4 has the slowest activity (27.8%) (Table 1 and 2; Fig. 3 and 4).



A-Normal, B-Grade I, C-Grade II, D-Grade III

Fig. 1. Electrophoretogram showing the serum LDH isoenzymes of normal woman and patients suffering from cancer cervix (Grade I, Grade II and Grade III)



A-Normal, B-Grade I, C-Grade II, D-Grade III

Fig. 2. Electrophoretogram showing the cervix tissue LDH isoenzymes of normal woman and patients suffering from cancer cervix (Grade I, Grade II and Grade III)

Table 1: LDH isoenzyme activity (percentage of total) in sera of normal woman and patients suffering from cancer cervix (Grade-I, Grade-II and Grade-III)

LDH isoenzyme	*Normal woman*	*Well differentiated squamous cell carcinoma (Grade-I)*	*Moderately differentiated squamous cell carcinoma (Grade-II)*	*Poorly differentiated squamous cell carcinoma (Grade-III)*
LDH_1	38.7±1.347	17.6±0.632	83.5±9.015 60.2±5.253	
LDH_2	23.5±0.636	22.6±0.769	30.3±2.100 52.6±4.125	55.80±5.520
LDH_3	20.3±0.413	20.0±0.736	26.9±1.315	18.78±1.627
LDH_4	26.3±0.777	9.20±0.182	43.7±3.660	24.40±1.982
LDH_5	14.5±0.197	35.7±0.046	80.1±7.990	

Table 2: LDH isoenzyme activity (percentage of total) in cervix tissues of normal woman and patients suffering from cancer cervix (Grade-I, Grade-II and Grade-III)

LDH isoenzyme	*Normal woman*	*Well differentiated squamous cell carcinoma (Grade-I)*	*Moderately differentiated squamous cell carcinoma (Grade-II)*	*Poorly differentiated squamous cell carcinoma (Grade-III)*
LDH_1	54.8±1.675	44.6±2.625	50.3±3.898 70.5±3.253	
LDH_2	60.8±1.708	35.8±2.860	65.6±3.206 90.7±6.425	63.50±5.180
LDH_3	39.4±0.941	33.7±1.885	72.8±3.672	44.38±3.280
LDH_4	27.8±0.686	18.5±0.713	52.6±5.448	35.90±2.875
LDH_5	80.1±6.120	90.7±0.450	32.5±5.629	

3.2. Serum and Cervical Tissue LDH Activity in Grade I Patients

LDH isoenzyme fractions of the serum of Grade-I patients showed no abnormality. However a reduction in the electrophoretic mobility of LDH isoenzyme fractions and LDH_4 fraction was sharply declined in its activity from 26.3% to 9.2% (Table 1 and Fig. 3).

LDH activity of the cervical tissue of Grade-I patients showed similar number of LDH isoenzyme fractions like the normal. The percentage activity of LDH_5 isoenzyme fractions showed an increase from 80.1% to 90.7%, where as the percentage activity of LDH_4 fraction was decreased from 27.8% to 18.5% (Table 2 and Fig. 4).

3.3. Serum and Cervical Tissue LDH Activity in Grade II Patients

In Grade II patients, two new additional isoenzyme fractions in LDH_2 and LDH_3 locations with 60.2% and 52.6% were observed in the serum. The activity of LDH_1 and LDH_5 significantly increased from 38.7% to 83.5%, 14.5% to 80.1%. The percentage activity of LDH_2 was increased from 23.05% to 30.3% (Table 1 and Fig. 3).

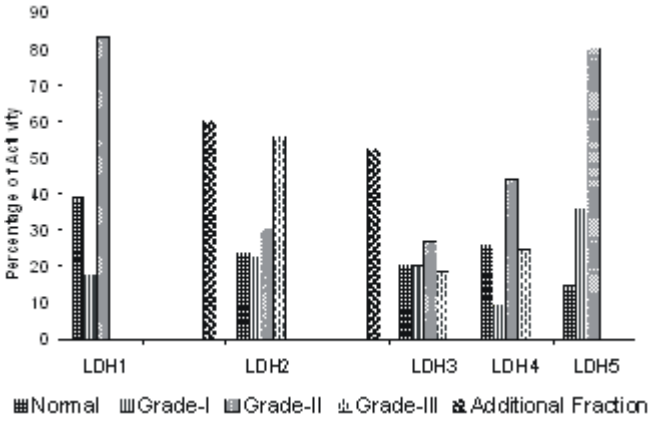


Fig. 3. LDH isoenzyme activity (percentage of total) in sera of normal woman and patients suffering from cancer cervix (Grade-I, Grade-II and Grade-III)

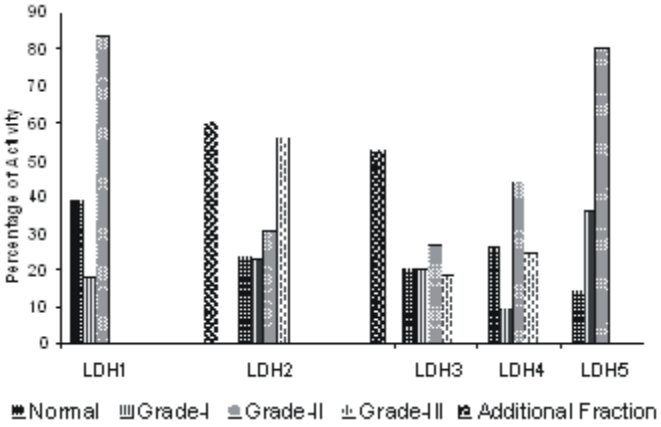


Fig. 4. LDH isoenzyme activity (percentage of total) in cervix tissues of normal woman and patients suffering from cancer cervix (Grade-I, Grade-II and Grade-III)

Similar to serum, cervical tissue LDH activity also showed two new fractions near LDH_2 and LDH_3 locations. The new LDH fractions showed highest activities 70.5% and 90.7%, where as the activity of LDH_1, LDH_2, LDH_3 and LDH_4 fractions were showed a marked increase but LDH_5 fraction showed sharply declined its activity from 90.7% to 32.5% (Table 2 and Fig. 4).

3.4. Serum and Cervical Tissue LDH Activity in Grade III Patients

The serum of Grade III patients showed only three LDH isoenzyme fractions and disappearance of LDH_1 and LDH_5 isoenzyme fractions (Fig. 1 and 2). The activity of LDH_3 and LDH_4 isoenzyme fractions declined from 26.9% to 18.7% and 43.7% to 24.4% respectively, where as the LDH_2 fraction increased from 30.3% to 55.8% (Table 1 and Fig. 3).

Similarly, only three LDH isoenzyme fractions were observed in cervix tissue of Grade-III patients. The fast moving LDH_1 and slow moving LDH_5 isoenzyme fractions are disappeared. The percentage activity of LDH_2, LDH_3 and LDH_4 fractions decreased from 65.6% to 63.5%, 72.8% to 44.3% and 52.6% to 35.9% respectively (Table 2 and Fig. 4).

3.5.Serum and Cervical Tissue Total LDH Activity (Quantitative Analysis)

The total activity of LDH isoenzymes in both serum and cervical tissue of normal individuals were 0.458±0.796 units /ml and 0.680±0.0218 units / mg respectively. The total LDH activity of Grade-I Patients were 0.384±0.0404 4 units/ml and 0.589±0.0292 units/mg (Table 3 and 4). The percentage changes in both serum and cervical tissues were -16.29 % and -13.86 % respectively and this value was changed from normal individual (Fig. 5 and 6).

The activity of Grade-II patients showed higher level of total LDH in both serum and cervical tissue from 0.458±0.0796 units/ml to 0.8780±.0531 units/ml and from 0.680±0.0218 units/mg to 1.296±0.0813 units/mg respectively. Grade-III patients showed significant reduction of total LDH activity in serum and cervical tissue from 0.458±0.0796 units/ml to 0.284±0.0109 units/ml and 0.680±0.0218 units/mg to 0.327±0.043 units/mg respectively (Table 3 and 4). The percentage of changes in both serum and cervical tissue of Grade-III patients were -37.98 % and -51.83 % respectively (Fig. 5 and 6).

Table 3: Total LDH activity (unit/ml) in sera of normal woman and patients suffering from cancer cervix (Grade-I, Grade-II and Grade-III)

Normal Woman	*Well differentiated squamous cell carcinoma Grade-I*	*Percentage of Change*
0.458±0.0796	0.384±0.0404	-16.29**
Normal Woman	*Moderately differentiated squamous cell carcinoma Grade-II*	*Percentage of Change*
0.458±0.0796	0.878±0.0531	+91.37***
Normal Woman	*Poorly differentiated squamous cell carcinoma Grade-III*	*Percentage of Change*
0.458±0.0796	0.284±0.0109	-37.98***

Table 4: Total LDH activity (unit/mg tissue) in cervix tissues of normal woman and patients suffering from cancer cervix (Grade-I, Grade-II and Grade-III)

Normal Woman	*Well differentiated squamous cell carcinoma Grade-I*	*Percentage of Change*
0.680±0.0218	0.589±0.0292	-13.86**
Normal Woman	*Moderately differentiated squamous cell carcinoma Grade-II*	*Percentage of Change*
0.680±0.0218	1.296±0.0813	+90.58***
Normal Woman	*Poorly differentiated squamous cell carcinoma Grade-III*	*Percentage of Change*
0.680±0.0218	0.327±0.043	-51.83***

Mean±Standard error
* p<0.05
** p<0.01
*** p<0.001

4. DISCUSSION

The study revealed a decrease in LDH in the serum of Grade I cervical cancer patients indicating the onset of malignancy (Goldman et al. 1960). These variations of serum LDH isoenzymes are being used as markers. The electrophoretic mobility of all the fractions of cervix tissue showed a significant increase suggesting the impact of malignant cancer on LDH fractions. (Wright et al. 1966).

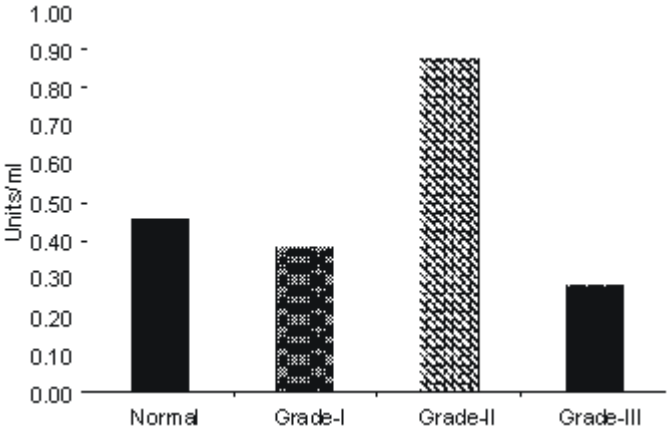


Fig. 5. Total LDH activity (unit/ml) in sera of normal woman and patients suffering from cancer cervix (Grade-I, Grade-II and Grade-III)

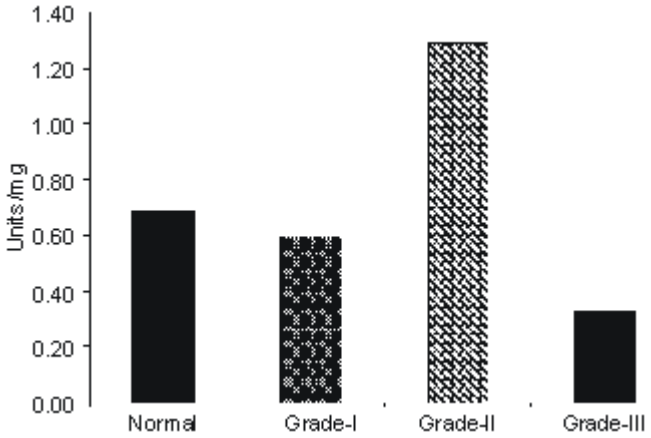


Fig. 6. Total LDH activity (unit/mg tissue) in cervix tissues of normal woman and patients suffering from cancer cervix (Grade-I, Grade-II and Grade-III)

Appearance of two additional new fractions were observed in the serum and cervical tissues of Grade-II patients. It is inferred that the intensity of cervix cancer increased with increased LDH pattern of serum and cervical tissues. Scoetens et al. (1964) found that two additional LDH isoenzymes in brain tumor. Klimove et al. (1989) observed seven LDH isoenzyme fractions in uterus cancer patients. Presence of multi isoenzyme forms of LDH and its variations in brain tumor enhanced the potentialities in neoplastic processes. The occurrence of addi-tional LDH fractions in cervix cancer patients may be the result of malignancy (Cori and Cori 1925). Increase in total serum and cervical tissue LDH activity was mainly due to genomic changes during malignant transformation. Main reason for increased LDH bands was mainly due to increased mitotic index and tumor cells also produce more amount of lactic acid. Increase of LDH level was mainly due to the break down of glycoprotein into lactic acid formation.

Reports of Grade-III showed an appearance of only three LDH fractions and disappearance of two LDH fractions (Pandit et al. 1990). Bhatnagar et al. (1983) reported that LDH_1 and LDH_5 are inhibited by the factors arising out of cervical cancer in patients suffering from breast

and cervical cancer. It is suggested that advance stage of cervical cancer has tremendous influence in preventing LDH activity both qualitatively and quantitatively. This may lead to decreased lactate to pyruvate conversion resulting in anomaly in the regeneration of NAD^+ which may interfere with glycolysis part of carbohydrate metabolism. LDH isoenzyme determination in carcinoma cervix is an important prognostic parameter (Kumar et al. 1988). LDH activity in vaginal secretion is a diagnostic criterion which detects cancer (Horbach et al. 1984). Augoff and Grabowski (2004) were found out LDH isoenzyme and its clinical significance are very much useful in diagnosis and prognosis of neoplastic diseases. Niklasson et al. (1981) were reported that the Lactate dehydrogenase (LD) enzyme techniques proved to be more accurate than cytology with approximately the same number of false positives.

5. CONCLUSION

The variation in electrophoretic mobility and changes in the total LDH activity in cervical carcinoma patients were found in serum and cervical tissue samples. The appearance of two new additional fractions in Grade-II patients and disappearance of two fractions in the Grade-III patients suggest that Lactate dehydrogenase (LDH) isoenzyme is one of the important biochemical tumor marker in cervical carcinoma patients for diagnosis and to assess the grade of malignancy.

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