

Study of serum enzymes Lactate dehydrogenase (LDH) and Gamma-glutamyltransferase (GGT) in patients of cervical cancer

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Abstract

In patients of cervical cancer, a simple and easy blood based tumor marker system might be an additional tool to derive tumor status and to correlate with the response to anticancer treatment and disease recurrence. Being simple, rapid, inexpensive, easily assayable, serum levels of LDH and GGT were measured in 50 histopathologically diagnosed cases of cervical cancer and compared with age matched healthy controls in the age group of 35-75 years and analysed by unpaired Student's 't' test (2-tailed) for parametric analysis. The cases of cervical cancer were further divided into study subgroups i.e. stage I, II, III. The comparison was also done among study subgroups. The biochemical parameters were compared among three study subgroups using one way analysis of variance (ANOVA). Results of the study showed significant higher levels of serum Lactate dehydrogenase, Gamma-glutamyltransferase in cases of cervical cancer as compared to their age matched controls. Interstage comparison between study subgroups was also significant. Thus it is concluded that Serum LDH and GGT may be better indicators of cervical cancer and may help in early detection of the disease and assist in assessing the extent and therefore prognosis of this malignancy along with clinical findings.

Keywords: Cervical cancer, Gamma-glutamyltransferase (GGT), Lactate Dehydrogenase (LDH)

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INTRODUCTION

Cervical cancer is a malignant neoplasm arising from cells originating in the cervix uteri. It is the second most common cancer among women worldwide, with an estimated 5,30,000 new cases and 2,75,000 deaths annually, about 88% of which occur in developing

countries.¹⁻³ Cervical cancer ranks as the 1st most frequent cancer among women in India, and the 1st most frequent cancer among women between 15 and 44 years of age.^{4,5} In India as there is no population based screening programme, 70 to 80% of cervical cancer patients are diagnosed at advanced stages (stage III and IV), with very poor long term survival.⁶ For better long term survival in cervical cancer early diagnosis is important which is generally missed in many patients. The cause for missing early diagnosis may be lack of awareness, inadequate access to healthcare facility, lack of effective and organized population based screening programmes.⁷ In patients of cervical cancer, a simple and easy blood based tumor marker system might be an useful tool to know extent of tumor progression. Subsequently, such markers can be useful to correlate with the response to anticancer treatment and disease recurrence.⁸ Lactate dehydrogenase (LDH) is the important enzyme in glycolytic pathway.

Increased rate of glucose utilization producing more lactate by tumor cells has been reported by Warburg. Lactate dehydrogenase (LDH) activity has been studied abundantly and found to be increased in various malignancies. It has been documented to correlate positively with the clinical stage of disease.⁹ In clinical practice, serum gamma-glutamyltransferase (GGT) evaluation is a commonly used diagnostic test, mainly as a indicator for hepatobiliary disease and a marker of alcohol intake.^{10,11} It has been reported in various studies that GGT was related to higher incidence, metastasis, recurrence and prognosis in patients with malignant neoplasm of digestive organs, the hepatobiliary tract, the respiratory system, female genital organs, the genitourinary tract, breast cancer, lymphoid and hematopoietic cancers.¹²⁻¹⁵ Gamma-glutamyltransferase (GGT) is a key enzyme of glutathione (GSH) metabolism, transferring gamma-glutamyl functional groups. The glutathione (GSH) metabolism plays an important role in protecting cells against oxidants, which are produced during normal metabolism.¹⁰ Glutathione (GSH) and GGT modulate the cellular proliferative and apoptotic balance, and play a role in tumor progression, invasion and anticancer-drug resistance. High gamma-glutamyltransferase (GGT) has been found to be associated with advanced tumor stages in patients with cervical cancer.¹⁵ The role of gamma-glutamyltransferase (GGT) is being extensively studied in different types of cancer. There are very less studies documenting the alteration of this parameter in cervical cancer. Hence, this study was carried out to evaluate the levels of serum GGT in cervical cancer patients and compare them with their age matched healthy controls.

MATERIALS AND METHODS

The present study entitled "Study of serum enzymes lactate dehydrogenase (LDH) and gamma-glutamyltransferase (GGT) in patients of cervical cancer" has been carried out in department of Biochemistry, Government Medical College and Hospital, Nagpur during the period of January 2012 to April 2013. All the study subjects were examined and investigated according to proforma that was predesigned. The study protocol was approved by the ethical committee of the Institute. Informed written consent was obtained from all the study subjects enrolled in the study.

2.1 Study design Case control study

2.2 Study population

50 clinically and histopathologically confirmed cases of cervical cancer, attending Radiation Therapy and Oncology OPD / ward of this institute and who were willing to participate in the study were selected for the present study. Cases were grouped into subgroups i.e.

stage I, II and III by FIGO classification. No case of stage IV was found in our study.

50 age-matched healthy and apparently normal females as controls were also selected for study. The cases and controls were females in the age group of 35-75 years.

All the cases and controls were divided into two groups, viz.

1. **Group A :** 50 cases of cervical cancer
2. **Group B:** 50 age matched healthy controls

2.3 Selection criteria

A. Inclusion criteria

1) Criteria for cases (cervical cancer patients)

Clinically and histopathologically confirmed cases of cervical cancer, between 35-75 years of age.

2) Criteria for controls

Age matched healthy females without family history of cervical cancer.

B. Exclusion criteria for cases and controls

Patients suffering from Myocardial infarction, Hypothyroidism, Hepatocellular damage, Pancreatic disease, Renal failure, Diabetes mellitus, Hypertension, Other malignancies, Alcoholics and smokers, Pulmonary diseases, Hemolytic anemia, Sickle cell anemia, Muscular dystrophy, Patients on drug treatment like [16] Phenytoin, Phenobarbitone, Oral contraceptives, Estrogen, Corticosteroids.

2.4 Collection of blood sample

5 ml of fasting venous blood sample was withdrawn from the anti-cubital vein of each participant after taking all aseptic precautionary measures using sterile, disposable syringe and needle. The blood samples were then immediately transferred to a clean dry sterile plain bulb. The blood was allowed to clot and serum was separated by centrifugation. The estimation of serum parameters was carried out immediately.

2.5 Equipments and facility for analysis

Serum parameters were estimated by following methods on Semiautoanalyser - Biochemical Analyzer WP 213 Ver 0.1

| No. | Parameter | Method |
|-----|-----------|------------------------------|
| 1 | Serum LDH | Modified IFCC Method[17] |
| 2 | Serum GGT | Carboxy substrate method[16] |

2.6 Statistical analysis

Statistical data was expressed as mean \pm SEM (Standard error of mean). The level of significance was calculated by applying unpaired Student's 't' test (2-tailed) for parametric analysis. $p < 0.05$ was taken as significant, $p < 0.001$ was taken as highly significant.

$p > 0.05$ was taken as non-significant (NS)

The biochemical parameters were compared among three study subgroups using one way analysis of variance (ANOVA), F^* value and p value were calculated. All statistical analyses were performed with GRAPH PAD PRISM version 6.00 software[18].

* F Value

Estimation of $(\sigma)^2$ based on the variation between the groups

$$= \frac{\text{Estimation of } (\sigma)^2 \text{ based on the variation within the groups}}{\text{Estimation of } (\sigma)^2 \text{ based on the variation within the groups}}$$

 $(\sigma)^2 = \text{Variance}$, $F = \text{Variance ratio}$

have been carried out in serum levels of lactate dehydrogenase (LDH), gamma-glutamyltransferase (GGT) in cases of cervical cancer and controls. Stage-wise comparison of cases divided in stage I, II and III has also been carried out with their respective age matched controls.

Staging of cases was done by FIGO staging

| Clinical stage | No. of cases |
|----------------|--------------|
| Stage I | 5 |
| Stage II | 15 |
| Stage III | 30 |

3. OBSERVATIONS AND RESULT

The present study comprises of 50 diagnosed cases of cervical cancer (Group A: $n = 50$) and 50 age matched healthy and apparently normal females (Group B: $n = 50$). Cases were further divided into subgroups i.e. stage I, II and III. Respective group-wise comparisons

On comparing age groups of cases and controls by unpaired student's 't' test p value was statistically non significant. Hence, cases and controls were comparable.

Table 1: Age-wise distribution of cases and controls

| Age in (years) | Cases (Group A) (n=50) | Controls (Group B) (n=50) | p value | Significance |
|----------------|------------------------|---------------------------|---------|-------------------------------|
| 35 - 44 | 12 | 12 | 0.88 | Statistically non-significant |
| 45 - 54 | 23 | 23 | | |
| 55 - 64 | 11 | 11 | | |
| 65 and above | 4 | 4 | | |
| Mean age | 49.14 \pm 8.99 | 49.4 \pm 8.51 | | |

Table 2: Serum levels of LDH and GGT in study groups

| Serum Parameter | Cases (Group A) (n=50) | Controls (Group B) (n=50) | p value | Significance |
|-----------------|------------------------|---------------------------|---------|--------------------|
| LDH (U/L) | 487.8 \pm 17.84 | 248.5 \pm 2.88 | <0.0001 | Highly significant |
| GGT (U/L) | 39.76 \pm 0.84 | 17.16 \pm 0.65 | <0.0001 | Highly significant |

Table 3: Serum levels of lactate dehydrogenase (LDH) in study subgroups

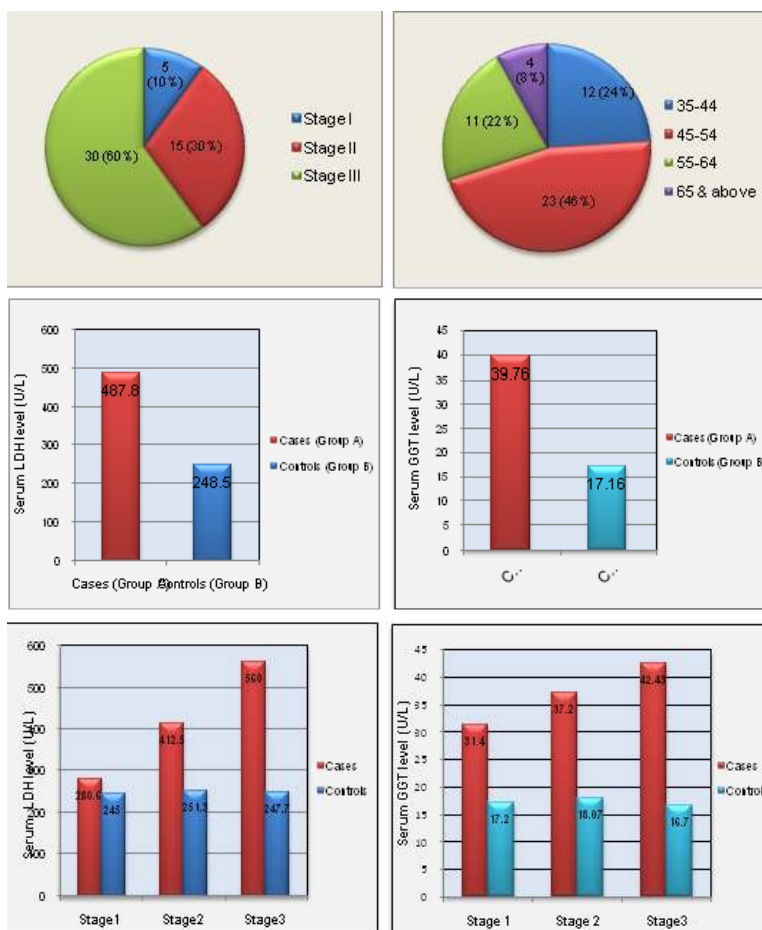
| Serum LDH (U/L) | Stage I (n=5) | Stage II (n=15) | Stage III (n=30) |
|------------------------|-------------------|--------------------|--------------------|
| Cases | 280.6 \pm 9.44 | 412.5 \pm 20.89 | 560.0 \pm 16.26 |
| Controls (age matched) | 245.0 \pm 11.38 | 251.3 \pm 5.54 | 247.7 \pm 3.56 |
| p value | 0.0427 | <0.0001 | <0.0001 |
| Significance | Significant | Highly significant | Highly significant |

F value for LDH= $F(2, 47) = 33.14$, p value <0.0001. (One way ANOVA test)

Table 4: Serum gamma-glutamyltransferase (GGT) levels in study subgroups

| Serum GGT (U/L) | Stage I (n=5) | Stage II (n=15) | Stage III (n=30) |
|------------------------|--------------------|--------------------|--------------------|
| Cases | 31.40 \pm 2.20 | 37.20 \pm 0.90 | 42.43 \pm 0.97 |
| Controls (age matched) | 17.20 \pm 1.68 | 18.07 \pm 1.36 | 16.70 \pm 0.82 |
| p value | 0.0009 | <0.0001 | <0.0001 |
| Significance | Highly significant | Highly significant | Highly significant |

F value for GGT= $F(2, 47) = 14.21$, p value <0.0001. (One way ANOVA test)



4. DISCUSSION

The present study was conducted to evaluate and compare serum levels of LDH and GGT in cervical cancer patients and their respective age- matched normal healthy controls. The comparison was also done in study subgroups. Significant higher levels of serum Lactate dehydrogenase, Gamma-glutamyltransferase were observed in cases of cervical cancer as compared to their age matched controls ($p < 0.0001$) and also in different stages of cervical cancer cases as compared to their respective age-matched controls. Interstage comparison between study subgroups was also significant. The levels of LDH, GGT were significantly associated with stages of cervical cancer.

4.1 Serum LDH

The levels of LDH are known to be elevated in the blood stream following severe tissue injuries and necrosis. Cancer is a proliferating and invasive disease known to cause severe tissue damage necrosis resulting in the release of intracellular enzymes like lactate dehydrogenase (LDH) into the blood stream by dead cells.¹⁹ The raised levels of LDH in cervical cancer patients obtained in our study are in accordance with the results found in studies done by Marshall MJ *et al*²⁰,

Nugmanov EU *et al*²¹, Das HK *et al*²², Iglesias J *et al*²³, Patel P *et al*⁸, Chougule A *et al*.²⁴ The present study confirms the documentation of various workers that there is significant acceleration of oxidative metabolic processes in cancerous tissues. This being the reason for enhanced glycolysis of cancer cells, hence increased demand of enzymes required for it. Increased tumor mass encroaching the surrounding parenchyma leads to leakage of water soluble, cytosolic enzyme, lactate dehydrogenase (LDH). This may be the possible reason for increased activity of LDH in serum of cervical cancer patients.

4.2 Serum GGT

GGT is critical for maintaining glutathione (GSH) and cysteine homeostasis and its deficiency results in oxidative stress and cellular susceptibility to oxidant injury. Although the relationship between cellular GGT and serum GGT is not known, but serum GGT has been assumed to reflect properties of cellular GGT^{25,26} It has been consistently suggested by series of epidemiological studies that serum GGT within its normal range might be an early and sensitive enzyme related to oxidative stress.^{25,26} It is well known that cervical cancer is a state of oxidative stress showing statistically significant increase of lipid peroxidation (LPO) in cervical cancer

cases while the antioxidant status was depicted by decreased glutathione (GSH) and superoxide dismutase (SOD)²⁷⁻³⁰. Hence, expression of GGT is significantly increased as an adaptive response to protect against oxidative stress by facilitating intracellular recovery of cysteine from extracellular glutathione (GSH) in rapidly dividing neoplastic cells of cervix for synthesis of proteins and intracellular glutathione (GSH). It is considered as a factor influencing tumor growth and conferring survival advantages to rapidly dividing neoplastic cells. The presence of elevated GGT levels seems to reflect a state of persistent oxidative stress as a part of biological pathway related to cancer development. Elevated serum GGT might be produced from the tumor itself as a part of tumor microenvironment. The exact mechanism of GGT elevation in cancer patient is still unclear.¹⁵ Our findings of raised GGT levels in cervical cancer cases were supported by the studies of Malkin *et al*³¹, Desai P, Chetna K³², Polterauer S *et al*.¹⁵

5. CONCLUSION

The evaluation of lactate dehydrogenase (LDH) and gamma-glutamyltransferase (GGT) enzymes in cervical cancer is helpful as these can be easily assayed, less expensive, simple and rapid. Not only these enzymes are able to differentiate malignant growths from normal, but also assist in assessing the extent and therefore prognosis of this malignancy along with clinical findings. Serum LDH and GGT may be better indicators of cervical cancer and may help in early detection of the disease. It is difficult to ascertain their diagnostic importance in cancer patients, yet their prognostic importance by comparing initial activities of these enzymes, cannot be undermined.

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