Research Paper

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# Clinical Utility of Glutathione-S-Transferase, Alkaline Phosphatase and Lactate Dehydrogenase Activity in Stomach Cancer Patients Before and After Chemotherapy for Diagnosis and Prognosis

Medical Science

**KEYWORDS :** GST, ALP, LDH, Stomach cancer, Chemotherapy

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## ABSTRACT

***Purpose:*** *- To analyze the level of serum glutathione-s-transferase (GSTs), Alkaline Phosphatase (ALP) and Lactate Dehydrogenase (LDH) activity in patients of stomach cancer before and after chemotherapy.*

***Methods:*** *- For the study total 42 cases of carcinoma of stomach of stage II and stage III (after and before chemotherapy) were selected. All patients were clinically and histological diagnosed. 40 age and sex matched healthy normal subjects selected as control. GSTs, ALP, and LDH activity was measured in the serum of control group (n=40) and in patients with stomach cancer (n=50).*

*Results: -Mean GSTs, ALP, and LDH activity in serum was significantly higher in patients with stomach cancer as compared to control group (p<0.001). The patients of stomach cancer after chemotherapy had significantly elevated activity of serum GSTs, ALP, and LDH than before chemotherapy.*

***Conclusion:*** *- In this study, we concluded that Serum GSTs measurement in plasma maybe useful tumor marker in stomach cancer and serum GSTs activity might be helpful to predict the response of chemotherapy in advance stages of cancer. Increased level of LDH and ALP can be use as a prognostic factor and serum LHD and ALP good indicator of stages and bulk of tumor, LDH is also a good prognostic factor in advanced GIT cancer treated with chemotherapy, ALP level greater than 500U/L over a period of 6 month may be indicative of advanced disease progression, which wants a more aggressive treatment. These findings of serum enzyme prompt us to adjust treatment strategies ahead of time, considering for diagnosis and prognosis in both the stages.*

## INTRODUCTION

The digestive tract is major site of cancer in humans. However there are great differences in incidence among the components sites from esophagus to the anus. Furthermore, a number of the histologic types of tumors at these sites differ in their incidence and prognosis [**1**].

Several modifiable environmental, dietary and habitual risk factors have been associated with development of gastrointestinal cancers, causal relationship between tobacco usage and gastrointestinal malignancies have been demonstrated for several decades. A large proportion of human cancers are claimed to be caused by lifestyle or dietary factors [**2**]. Our diet contains many toxic or potentially carcinogenic compounds which are absorbed and metabolized in the gastrointestinal tract. The etiology of gastric cancer is multifactorial. It is multistep pathway. Smoking, drinking alcohol, high consumption of rice with chilli and the consumption of high temperature diet are risk factor for stomach cancer [**3,4**].

Studies comparing different food have noted that western style breakfast; diet high in antioxidants and diet low in salt are show to be inversely proportional to the incidence of stomach cancer. Deity factor that have been closely associated with stomach cancer are the better smoking alcohol consumption, hot food and beverages. Currently smoking and Alcohol consumption are known risk factor for stomach cancer. The incidence of stomach cancer is 5.7 per 100,000 men and 2.8 per 100,000 in women [**5**].

In the recent years glutathione-S-transferase (GSTs) have attracted interest in the field of cancer because their activity is readily increased in chemically induced tumors **[6,7]**. They have a considerably important role in detoxification of carcinogens. GSTs are present in many species and tissues of the human gastrointestinal tract. Likewise, the human GSTs were found

to be over expressed in most of the tumors**[7,8]** and it catalyze the nucleophilic addition of glutathione at electrophilic centre of wide variety of compounds. They also serve as intracellular binding and transport proteins by virtue of their capacity to bind a broad range of lipophilic compounds like bilirubin, bile acids steroid hormones drugs and other xenobiotics **[9, 10, 11**]. GSTs expression in response to tumor formation is probably a resistance mechanism by which cells can survive, and the source of plasma enzyme is mainly transformed cells with over expression of GSTs. Indeed GSTs are one of the enzyme systems induces by anticarcinogens and thus can prevent tumor formation .GSTs have also been suggested to play an important role in multiple drug resistance in cancer chemotherapy **[12]**. In this study, serum GSTs activity has been measured in different stages of stomach cancer patients.

Many enzymes have been used earlier to aid in diagnosis of various malignancies. The increased level of lactate dehydrogenase (LDH) was found patient of neoplastic disease **[13].** The increase activity of LDH is fairly sensitive marker for solid neoplasm serum alkaline phosphate (ALP) was found useful in detecting bone metastasis.

In view of this present study was under taken to assess, the clinical utility of GST, LDH and ALP enzymes in stomach cancer.

## MATERIALANDMETHODS

For the study total 21 cases of carcinoma of stomach of stage II and 21 cases of stage III were selected. All patients were clinically and histological diagnosed. All patients with stage-III received chemotherapy including cisplastin, cyclophosphamide and doxorubicin. There are 28 males & 14 female of stomach cancer. For control total 40 normal healthy age and sex matched persons were selected. Subjects with stomach cancer and those without any evidence of any type of cancer participated in this study as listed in tab.

**TABLE1: Distribution for control and patients**

| | Number of subjects (male/ female) | Age-range (years) |
|---|---|---|
| Control | 40 (24/16) | 40-55 |
| Stomach cancer | 42 (28/14) | 40-60 |
| Stage II | 21 (14/07) | 40-60 |
| Stage III | 21 (14/07) | 45-60 |

**TABLE 2: Comparison of serum GST, ALP and LDH activity in control with stomach cancer**

| | No. Of cases | Mean ± SD | No. of cases (Value> normal) | " P" Value |
|---|---|---|---|---|
| GST | 42 | 10.30±2.35 | 39 (93%) | <0.001 |
| GST Control | 40 | 5.36±0.59 | 40 (100%) | - |
| ALP | 42 | 276.28 ± 164.89 | 22 (52.38%) | <0.05 |
| ALP Control | 40 | 166.62 ± 25.80 | 40 (100%) | - |
| LDH | 42 | 750.52 ± 344.22 | 34 (81%) | <0.001 |
| LDH Control | 40 | 274.10 ± 35.38 | 40 (100%) | - |

**All Values are expressed in IU/L**

**Collection of samples**

5ml blood sample were collected in plain bulb. Serum was separated and used to estimation of glutathione-s-transferase, Alkaline Phosphatase, and Lactate Dehydrogenase. Serum GSTs activity measured by, using 1-chloro-2, 4 dinitrobenzene (purchased from Sigma company) as substrate, was measured according to the procedure described by Habig et al **[12]**.

For quantitative estimation of ALP in serum kinetic method (pNPP) is used **[14]** and Estimation of serum lactate dehydrogenase was done by using commercial kits from AGAPPE diagnosis on semi auto analyzer (Transasia ERBA CHEM -5 plus) by kinetic method based on SCE recommended method**[15].**

Data were expressed as mean ±SD. Mean values were assessed for significance by unpaired student –t test. Probability values p < 0.05were considered statistically significant.

**RESULTS**

AS shown in**TABLE2** mean serum GSTs activity (mean±SD) in control using CDNB as substrate was 5.36±0.59 IU/L. Serum GSTs activity of esophageal cancerous patients was 10.30±2.35IU/L. GSTs activity was significantly higher in stomach cancer patients than control (p<0.001).The 39 of 42 patients of stomach cancer had elevated activity of serum GSTs.

ALP activity (mean±SD) in control using pNPP method was 166.62±25.80. Serum ALP activity of stomach cancer patients was 276.28 ± 164.89. ALP activity was significantly higher in stomach cancer patients than control (p<0.05). The 22 of 42 patients of stomach cancer had elevated activity of serum ALP.

LDH activity (mean±SD) in control using semi auto analyzer by kinetic method was 274.10±35.38. Serum LDH activity of oesophagus cancer patients was 750.52 ± 344.22. LDH activity was significantly higher in stomach cancer patients than control (p<0.001). The 34 of 42 patients of stomach cancer had elevated activity of serum LDH.

**Table 3: Serum GST activity in stomach cancer patients before and after chemotherapy.**

| | No. Of Cases | Mean ±SD | p-value |
|---|---|---|---|
| Control | 40 | 5.36 + 0.59 | - |
| Before Chemotherapy | 21 | 8.43 + 1.95 | < 0.001 |
| After Chemotherapy | 21 | 12.02 + 1.09 | < 0.001* |

**Table 3: Serum ALP activity in stomach cancer patients before and after chemotherap**

| | No. Of Cases | Mean ±SD | p-value |
|---|---|---|---|
| Control | 40 | 166.62 + 25.80 | - |
| Before Chemotherapy | 21 | 199.05+ 28.26 | < 0.05** |
| After Chemotherapy | 21 | 396.23 + 162.17 | < 0.001* |

**Table 3: Serum LDH activity in stomach cancer patients before and after chemotherapy**

| | No. Of Cases | Mean ±SD | p-value |
|---|---|---|---|
| Control | 40 | 274.10 + 35.38 | - |
| Before Chemotherapy | 21 | 522.33 + 64.59 | < 0.001 |
| After Chemotherapy | 21 | 976.52 + 361.84 | < 0.001* |

(All Values are expressed in IU/L) ** Control vs Stage-II and * Stage-II vs Stage-III

**DISCUSSION**

The ability of the GSTs to provide cellular protection against a wide variety of xenobiotics makes this enzyme family an attractive candidate biomarker of both cancer susceptibility and chemopreventive activity **[3,6]**.

In the present study serum GST, ALP, LDH was significantly higher in patients with stomach cancer as compared to those obtained from normal healthy control group (TABLE 2). Similar findings reported by G.S.Mohammadzadeh et al**[7]** Tsuchicla et.al **[16]**..The increased activity of total GSTs in serum can be due to over expression of isoenzymes of GST in tumor tissues .GST-π class was found to be over expressed in most of tumor **[17,18]**.However, there are doubts over the use of total GSTs activity as a marker for all types of tissues . The GSTs activity of plasma represents a non invasive biomarker of the cellular protection .The strong correlation between the GST- π activities of plasma and gastric tumor tissues has been reported**[5]**.

Our result showed a significant increased(p<0.001) activity of GSTs in stage-III (after chemotherapy) than stage-II patients (TABLE 3).Many studies also showed progressive increase of GSTs with advancing cancer and has been associated with poor prognosis and development of drug resistance **[17-19].**K.Johansson et al**[20]** reported GSTs protect the cells from lipid peroxidation and $H_2O_2$which is increased by cisplastin, a chemotherapeutic drug .Our results show the association of serum GST and chemotherapy in stomach cancer.

Alkaline Phosphatase an enzyme that involved in bone growth. It is processed in the liver and excreted into digestive tract in the bile. A higher value of ALP indicates bone or liver problems. In cancer patients elevated ALP may indicate that cancer has spread to the bones or that liver damages possible due to some chemotherapy drugs (in treatment) has caused problems with bile excretion.

Cancer metastatic to bone the activity of ALP can be six times greater than upper limit of normal **[21]**

The result of the present study shows a significant increase in ALP level in stomach cancer before and after chemotherapy (Table 4).Several workers **[22,23]** have reported elevated level of ALP in stomach cancer in their studies Gou Li. et. Al**[ 24]** observed, that rise in ALP level in 59% of stomach cancer concluded that the total ALP activity increased due to placental alkaline phosphatase isoenzymes which is probably originates from cancer itself. The elevated value of ALP was observed in stage III of stomach cancer but only three times greater than normal limits. It is suggested that high serum ALP activities in stomach cancer patient may result from the tumor production in the patients.

A significant rise in serum LDH activity was observed in stomach cancer than control group. In present study it was observed that 81% of stomach cancer patients had LDH activity greater than 500 lU/Liter. In before chemotherapy 13 of 21 of esophagus cancer had LDH activity greater than 500 lU/Liter and after chemotherapy all patients of esophagus cancer had value of LDH greater than 500 lU/Liter.LDH was termed as an old enzyme which reborn as cancer marker **[25]**Also increase in LDH due to overproduction by tumor cell, change in permeability of cell al-

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lowing leakage of soluble enzymes in circulation and because of tumor blockage of duct system through which the enzyme passes **[26].R. Domiguer et.al.[27]** reported that LDH **4** and LDH5 *activity* and the LDH5/ LDH1 ratio increased in neoplasm's of gastrointestinal cancer an alteration associated with prolific of "M" type monomers of LDH by neoplastic Cells.

**CONCLUSION**

In this study, we concluded that Serum GSTs measurement in plasma maybe useful tumor marker in stomach cancer and serum GSTs activity might be helpful to predict the response of chemotherapy in advance stages of cancer. Increased level of LDH and ALP can be use as a prognostic factor and serum LHD and ALP good indicator of stages and bulk of tumor, LDH is also a good prognostic factor in advanced GIT cancer treated with chemotherapy, ALP level greater than 500U/L over a period of 6 month may be indicative of advanced disease progression, which wants a more aggressive treatment. These findings of serum enzyme prompt us to adjust treatment strategies ahead of time, considering for diagnosis and prognosis in both the stages.

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