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Clinical utility of glutathione-S-transferases, Alkaline Phosphatase and Lactate Dehydrogenase activity in oesophagus cancer patients before and after chemotherapy for Diagnosis and Prognosis

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Abstract
Purpose: - *To analyze the level of serum glutathione-s-transferases (GSTs), Alkaline Phosphatase (ALP) and Lactate Dehydrogenase (LDH) activity in patients of oesophagus cancer before and after chemotherapy.*
Methods: - *For the study total 50 cases of carcinoma of oesophagus 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 oesophagus cancer (n=50).*
Results*: -Mean GSTs, ALP, and LDH activity in serum was significantly higher in patients with oesophagus cancer as compared to control group (p<0.001). The patients of oesophagus cancer after chemotherapy had significantly elevated activity of serum GSTs, ALP, and LDH than before chemotherapy.*
Conclusion*: - Measurement of serum GSTs, ALP and LDH activity may be useful as a tumor marker in oesophagus cancer. Alterations in serum GSTs levels might be helpful to predict the response of chemotherapy. LDH and ALP are good indicator of stages and bulk of tumor LDH is also a good prognostic factor in advanced GIT cancer treated with chemotherapy.*
Keywords: *GST, ALP, LDH, Oesophagus cancer, Chemotherapy*

I. Introduction

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. Dietary factors that have been closely associated with oesophagus cancer are betel nut chewing, hot foods and beverages. The incidence rate of oesophagus cancer is 7.6 per 100,000 men and 5.7 per 100,000 women. Tobacco, which is widely used in India, is major cause of the cancer of the upper digestive and respiratory tract **[1,2]**. Upper gastrointestinal cancers are highly lethal diseases unless diagnosed early. Efforts for early diagnosis of oesophagus cancer have been spread over the past two decades with limited success and tumor markers are appealing tools for this purpose.

In the recent years glutathione-S-transferases (GSTs) have attracted interest in the field of cancer because their activity is readily increased in chemically induced tumors **[3,4]**. 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**[4,5]**.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 **[6]**. In this study, serum GSTs activity has been measured in different stages of esophageal 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 **(7).** 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 gastointestingnal cancer.

II. Materialandmethods

For the study total 25 cases of carcinoma of oesophagus of stage II and 25 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 32 males & 18 female of oesophagus carcinoma. For control total 40 normal healthy age and sex matched persons were selected. Subjects with esophageal 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)	40-70
Oesophagus cancer	50 (32/18)	40-75
Stage II	25 (17/8)	42-69
Stage III	25 (15/10)	40-75

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

	No. Of cases	Mean ± SD	No. of cases (Value> normal)	" P" Value
GST	50	11.80±2.40	46 (92%)	<0.001
GST Control	40	5.36±0.59	40 (100%)	-
ALP	50	313.20 ± 169.15	34 (68%)	<0.001
ALP Control	40	166.62 ± 25.80	40 (100%)	-
LDH	50	797.60 ± 368.09	44 (88%)	<0.001
LDH Control	40	274.10 ± 35.38	40 (100%)	-

All Values are expressed in IU/L

Collection of samples

5ml fasting 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 **[8].**

For quantitative estimation of ALP in serum kinetic method (pNPP) is used **[9]** 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**[10].**

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

III. Results

AS shown inTABLE2 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 11.80±2.40 IU/L. GSTs activity was significantly higher in esophageal cancer patients than control ($p<0.001$).The 46 of 50 patients of esophageal 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 oesophagus cancer patients was 313.20±169.15. ALP activity was significantly higher in oesophagus cancer patients than control ($p<0.001$). The 34 of 50 patients of oesophagus 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 797.60 ± 368.09. LDH activity was significantly higher in oesophagus cancer patients than control ($p<0.001$). The 44 of 50 patients of oesophagus cancer had elevated activity of serum LDH.

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

	No. Of Cases	Mean ±SD	p-value
Control	40	5.36 ± 0.59	-
Before Chemotherapy	25	10.03 ± 1.13	< 0.001
After Chemotherapy	25	13.56 ± 0.85	< 0.001*

Table 3: Serum ALP activity in oesophagus cancer patients before and after chemotherapy

	No. Of Cases	Mean ±SD	p-value
Control	40	166.62 + 25.80	-
Before Chemotherapy	25	218.08 + 48.71	< 0.001
After Chemotherapy	25	409.52 + 192.22	< 0.001*

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

	No. Of Cases	Mean ±SD	p-value
Control	40	274.10 + 35.38	-
Before Chemotherapy	25	522.72 + 72.76	< 0.001
After Chemotherapy	25	1072.76 + 341.82	< 0.001*

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

IV. 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 ($p<0.001$) in patients with oesophagus cancer as compared to those obtained from normal healthy control group (TABLE 2). Similar findings reported by G.S.Mohammadzadeh et al**[4]**.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**[11,12]**.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 esophageal tumor tissues has been reported**[4]**.

Our result showed a significant increased($p<0.001$) activity of GSTs in stage-III (received 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**[11-13]**.K.Johansson et al**[13]** reported GSTs protect the cells from lipid peroxidation and H2O2which is increased by cisplastin, a chemotherapeutic drug .Our results show the association of serum GST and chemotherapy in oesophagus 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 has caused problems with bile excretion.

Cancer metastatic to bone the activity of ALP can be six times greater than upper limit of normal **[14, 15, 16]**

The result of the present study shows a significant increase in ALP level in esophagus cancer before and after chemotherapy Table 4.Several workers **[17, 18, 19, 20]** have reported elevated level of ALP in esophagus cancer in their studies Nishio H. et. al**.[19]** observed, that rise in ALP level in 73% of esophageal 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 esophagus cancer but only three times greater than normal limits. It is suggested that high serum ALP activities in esophagus cancer patient may result from the tumor production in the patients.

A significant rise in serum LDH activity was observed in esophagus cancer than control group. In present study it was observed that 88% of esophagus cancer patients had LDH activity greater than 500 lU/Liter. In before chemotherapy 19 of 25 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**[21]**Also increase in LDH due to overproduction by tumor cell, change in permeability of cell allowing leakage of soluble enzymes in circulation and because of tumor blockage of duct system through which the enzyme passes**[22].**R. Domiguer et.al.**[23]** reported that LDH **4** and LDH5 *activity* and the LDH_5/ LDH! ratio increased in neoplasm's of gastrointestinal cancer an alteration associated with prolific of "M" type monomers of LDH by neoplastic Cells.

V. Conclusion

Serum GSTs measurement in plasma maybe useful tumor marker in esophageal cancer and serum GSTs activity might be helpful to predict the response of chemotherapy in advance stages of cancer.
LDH and ALP are good indicator of stages and bulk of tumor, LDH is also a good prognostic factor in advanced GIT cancer treated with chemotherapy

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