ORIGINAL ARTICLE

Diagnostic and Prognostic Application of Glutathione-S-Transferase, Lactate Dehydrogenase, Alkaline Phosphatase and Carcinoembryonic antigen pre and post treatment Of chemotherapy in stomach cancer patients.

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ABSTRACT

BACKGROUND: To analyze the level of serum Glutathione-S-Transferase (GST), Lactate Dehydrogenase (LDH), Alkaline Phosphatase (ALP) and Carcinoembryonic antigen (CEA) before and after Cycles Of chemotherapy in patients suffering from stomach cancer. **METHODS**: For the study comprising total 112 cases suffering from stomach cancer stage I, stage II stage III and Stage IV (before and after different cycle of chemotherapy) were selected. All patients were clinically and histologically diagnosed. A total of 42 age and sex matched healthy subjects taken as control. The circulating levels of GST, LDH, ALP and CEA activity were assayed in the in the serum of control group and in patients with stomach cancer. **RESULTS:** Mean GST, LDH, ALP and CEA activity in serum were significantly higher in stomach cancer patients as compared to control ($p<0.001$). After chemotherapy (stage II) the activity of GST, LDH, ALP and CEA were significantly higher than before chemotherapy (stage I). In stage III (after second cycle of chemotherapy) activity was significantly decreased than that of stage II and the activity of GST, LDH, ALP and CEA was significantly decreased in stage IV (after third cycle of chemotherapy) than stage III (after second cycle of chemotherapy). **CONCLUSION:** The study highlights serum GST and CEA measurement are useful marker in stomach cancer, its activity helpful to predict the response of chemotherapy in advanced stage of cancer. An initial increased level of GST and CEA before and first cycle of chemotherapy may not indicate tumor progression, but may represent a transient tumor marker surge phenomenon after second and third cycles of chemotherapy in patients under to treatment. The administration of chemotheraptic drug in stomach cancer patients cause increase in oxidative stress, it is indicated by decreased level of glutathione. Decreased level of GST might be associated with deceased level of GST after different cycle's chemotherapy. Increased levels of serum LDH and ALP indicates infection or blockage or liver damage by treatment. LDH and ALP are good prognostic marker in stomach cancer treated with chemotherapy. Increased levels of ALP over a period of 3 month are an indicative of advanced disease progression which requires more prompt treatment. Increased level of LDH and ALP indicates liver damage during or after treatment. Statistically significant changes in GST, LDH, ALP and CEA levels during the observed treatment of stomach cancer with positive response and no established disease progression during study period near about 18 months after the treatment, which indicate that GST and CEA are important predictive factor.

Keywords: Cisplastin, capecitabine, stomach cancer, tumor marker, chemotherapy, Glutathione-s-transferase.

INTRODUCTION

Cancer is a group of disease characterized by uncontrolled growth and spread of abnormal cell. If the spread is not controlled, it can result in death. Cancer is caused by both external (Tobacco, chemicals, radiations and infectious organisms) and internal factors (inherited mutation, hormones, immune condition and mutations that occur from metabolism)[1]. Its reported Stomach cancer was the fourth most common malignancy in the world with estimated new cases. Approximately 72% new cases occurred in developing countries. Generally stomach cancer rates are about twice as high in men as in women in both low risk and high risk areas. Annual incidence rate of stomach cancer in India is 10.6 per 100000 populations[2]. Stomach cancer is the third leading cause of cancer death in men and fifth leading cause in women. Environmental factors including dietary habits are important in its development, consumption of salted, smoked, pickled and preserved food rich in salt, nitrite and N-nitro compounds have been reported to

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be associated with an increased risk of stomach cancer[3]. Smoking and alcohol consumption have been proposed as risk factor for stomach cancer in some epidemiological studies but their role has been inconsistent[4,5]. Similarly dietary factor have been studied in some epidemiological studies from India, but their role has not been consistently proven[6]. In recent years GSTs have attracted interest in the field of cancer because their activity is readily increased in chemically induced tumors[7,8]. These enzymes catalyze the conjugation of GSH to a variety of electrophilic compounds, reactive compounds indeed GSTs are one of the enzymes by ant carcinogens and thus can prevent tumor formation. GSTs have also been suggested to play an important role in multiple drug resistance in cancer chemotherapy. They have considerably important role in detoxification of carcinogens; GSTs are present in many species and tissues of the human gastrointestinal tract[9]. Number of reports have been suggested that serum GSTs level may be increased in stomach cancer and serum pi-GST level may be increased in a wide range of gastrointestinal and hematology malignancies and thus the measurement of serum GST concentration might provide a useful tumor marker[10, 11]. GSTs expression in response to tumor formation is probably a resistance mechanism by which cell can survive and the source of plasma enzyme is mainly transformed cells with over expression of GSTs. Indeed GSTs are one of the enzyme system 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]. Lactate Dehydrogenase (LDH) has been used earlier to aid in the diagnosis of various malignancies. The increased level of LDH is response to tissue injury, necrosis, hypoxia, hemolysis, multiple cancers and myocardial infection. LDH play a key role in regulating glycolysis by catalyzing the final step of anaerobic glycolysis, therefore its regulation facilitates the efficiency of anaerobic glycolysis in tumor cell and reduce their dependency on oxygen[13]. Increased level of serum LDH are prognostic biomarker for poor survival in multiple cancers. The increased activity of LDH is fairly sensitive marker for solid neoplasm. Though serum LDH level is frequently elevated in the patients with advanced stomach cancer, its clinical significance is still elusive. Moreover, the patients with advanced stomach cancer, high serum LDH level were related to better response to chemotherapy. The normalization of elevated serum LDH level after chemotherapy was related to good response to treatment[14]. LDH play an important role in tumor maintance and invasion. Alkaline Phosphatase (ALP) comprises a group of enzymes that catalyze the hydrolysis of phosphate esters in an alkaline environment, generating an organic radical and inorganic phosphate. Like other enzymes, this enzyme has many isoenzymes. Serum ALP levels are frequently elevated in patients with multiple cancers. These are anecdotal reports and small studies suggesting that the elevated ALP can aid in detecting metastatic liver disease[15]. This is an important issue as biological detection of liver metastases represents an important factor in the prognosis of patients with stomach cancer and it is useful in detecting bone metastasis. Carcinoembryonic antigen (CEA) is a glycoprotein. It was first identified in 1965 by Gold and Freedman in human colon cancer tissue extracts[16]. In the recent years CEA has been widely used as a tumor marker in the diagnosis and monitoring of some malignancies[17]. Science the tumor marker including CEA and other have been widely used to monitor stomach cancer progression and even to assess the prognosis of stomach cancer patients although their specificities have not been satisfactory[18,19,20]. Baseline level of CEA is commonly observed to increase before the initiation of chemotherapy in recurrent gastric cancer. CEA is normally found in the gastrointestinal tract of embryos and in smaller concentration in normal adult tissue, CEA function as an intercellular

adhesion molecule promoting the aggregation of human gastrointestinal cancer cells[21]. During chemotherapy, the response to treatment is estimated by radiographic assessment, but if radiologic assessment is difficult, the change in tumor marker level may be used as adjuvant in treatment monitoring[22]. Clinicians predict the effect of chemotherapy by obtaining serial level of tumor markers during chemotherapy. In general a rising tumor marker level means tumor progression in patients who are receiving chemotherapy. In present study, serum GST, LDH, ALP and CEA activities have been measured before and after chemotherapy in stomach cancer patient.

MATERIAL AND METHODS

Selection of Patients: Present study comprising total 28 cases of carcinoma of stomach stage I, Stage II, Stage III and stage IV. All patients were clinically and histologically diagnosed. All patients with stage-II, stage-III and stage-IV received chemotherapy including cisplastin, capecitabine, cyclophosphamide, Transtuzumab and doxorubicin. There are 15 males & 13 female of stomach cancer. For control total 42 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 table.

Collection of samples: Overnight fasting venous 5ml blood samples were collected before and after different cycle of chemotherapy in plain bulb. Serum was separated and used to estimate glutathione-s-transferase, Lactate Dehydrogenase Alkaline Phosphatase and Carcinoembryonic antigen. 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 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[23]**.** For quantitative estimation of ALP in serum kinetic method (pNPP) is used[24]**,** and Estimation of serum CEA was carried out by using commercial available kits from accu-bind USA, using ELISA micro plate Immunoenzymometric assay[25].

Treatment: According to the protocol, 71.79% of the patients completed one cycle of preoperative and three cycle of postoperative chemotherapy included the cisplastin, capecitabine, cyclophosphamide, transtuzumab and doxorubicin. All the chemotherapy regimens were used under standard protocol.

a. Preoperative Treatment

1. Cisplastin + capecitabine Day 1- Cisplastin 30 mg/ m^2 (IV), Day 2- Capecitabine 800 mg/ m^2 (IV) orally twice daily. Repeat cycle for 5 week.

b. Postoperative Treatment

1. Cisplastin + capecitabine Day 1- Cisplastin 80 mg/ m^2 (IV), Day 2 to day 14- capecitabine 1000 mg/ m^2 orally twice daily. Repeat cycle after every 3 weeks.

Follow Up: Overall 39 patients were followed up admitted in hospital and after hospital discharge. Out of 7 patients follow up were lost during the follow up period. The follow up system consisted of measurement of serum GST, LDH, ALP and CEA level after chemotherapy countinely 3 months intervals for first 6 month and at 6 month intervals thereafter. The follow up program included, clinical examination, hematological analysis, tumor marker and enzyme assess at every check up, abdominal ultrasound were scheduled came for treatment. Criteria for the establishment of recurrent disease included histological conformation or disease evident radiological findings with subsequent clinical progression and supportive biochemical data. The follow up ends in 4^{th} December 2015. All survived patients followed up for at least 30 months. Four patients expired during the follow up period. Data were expressed as mean ±SD. Mean values were assessed for significance by unpaired student –t test.

A statistical analysis was performed using the Stastical Package for the Social Science program (SPSS, 21.0). Frequencies and percentages were used for the categorical measures. Probability values $p < 0.001$ were considered statistically significant.

Table1: Distribution for control and patients

	Number of subjects (male/female)	Age-range (years)
Control	42(25/17)	25-60
Stomach cancer patients	112 (60/52)	25-60
Stage I	28(15/13)	25-60
Stage II	28(15/13)	25-60
Stage III	28(15/13)	25-60
Stage IV	28(15/13)	25-60

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

Tumor Markers	No. Of cases	Mean ± SD	" P" Value
GST IU/L	28	8.34 ± 1.02	<0.001
GST Control	42	5.05 ± 0.51	-
ALP IU/L	28	274.23 ± 52.94	<0.001
ALP Control	42	184.69 ± 28.96	-
LDH IU/ L	28	604.06 ±130.46	<0.001
LDH Control	42	293.47 ± 39.83	-
CEA ng/ml	28	4.63 ± 0.76	<0.001
CEA Control	42	1.55 ± 0.30	-

RESULTS

As shown in table 2 mean serum GSTs activity (mean±SD) in control using CDNB as substrate was 5.05±0.51 IU/L. Serum GSTs activity of stomach cancerous patients was 8.34 ± 1.02 IU/L. GSTs activity was significantly higher in stomach cancer patients than control ($p<0.001$). ALP activity (mean±SD) in control using pNPP method was 184.69±28.96. Serum ALP activity of stomach cancer patients was 274.23 ± 52.94. ALP activity was significantly higher in stomach cancer patients than control ($p<0.001$). LDH activity (mean±SD) in control using semi auto analyzer by kinetic method was 293.47±39.83. Serum LDH activity of stomach cancer patients was 604.06 ± 130.46. LDH activity was significantly higher in stomach cancer patients than control ($p<0.001$). CEA activity (mean±SD) in control using commercial kits from accu-bind on ELISA micro plate Immunoenzymometric assay was 1.55±0.30. Serum CEA activity of stomach cancer patients was 4.63 ± 0.76. LDH activity was significantly higher in stomach cancer patients than control ($p<0.001$).

Table 3: Serum GST (IU/L) levels before and after I, II, III, IV comprised with control counterpart.

(Values are expressed in IU/L) * Control vs Stage-I, **Stage-I vs Stage-II, $ Stage II vs Stage III and $$ Stage III vs Stage IV.

	No. Of Cases	Mean ±SD	p-value
Control	42	5.05 ± 0.51	-
Before Chemotherapy (Stage I)	28	8.79 ± 2.15	< 0.001*
First Cycle of Chemotherapy (Stage II)	28	12.28 ± 1.01	< 0.001**
Second Cycle of Chemotherapy (Stage III)	28	7.05 ± 1.11	< 0.001$
Third Cycle of Chemotherapy (Stage IV)	28	5.22 ± 0.59	< 0.001$$

Table 4: Serum ALP (IU/L) levels before and after I, II, III, IV comprised with control counterpart.

(Values are expressed in IU/L) * Control vs Stage-I, **Stage-I vs Stage-II, $ Stage II vs Stage III and $$ Stage III vs Stage IV.

	No. Of Cases	Mean ±SD	p-value
Control	42	184.69± 28.96	-
Before Chemotherapy (Stage I)	28	206.79± 36.58	< 0.001*
First Cycle of Chemotherapy(Stage II)	28	375.39±109.68	<0.001**
Second Cycle of Chemotherapy(Stage III)	28	277.43 ± 62.07	< 0.001$
Third Cycle of hemotherapy (Stage IV)	28	237.32 ± 44.93	< 0.001$$

Table 5: Serum LDH (IU/L) levels before and after I, II, III, IV comprised with control counterpart.

	No. Of Cases	Mean ±SD	p-value
Control	42	293.47 ± 39.83	-
Before Chemotherapy (Stage I)	28	526.50 ± 63.70	< 0.001*
First Cycle of Chemotherapy (Stage II)	28	811.43 ± 313.48	< 0.001**
Second Cycle of Chemotherapy (Stage III)	28	669.18 ± 168.87	< 0.001$
Third Cycle of Chemotherapy (Stage IV)	28	409.14 ± 40.09	< 0.001$$

(Values are expressed in IU/L) * Control vs Stage-I, **Stage-I vs Stage-II, $ Stage II vs Stage III and $$ Stage III vs Stage IV.

Table 6: Serum CEA (ng/ml) levels before and after I, II, III, IV comprised with control counterpart.

	No. Of Cases	Mean ±SD	p-value
Control	42	1.55 ± 0.30	-
Before Chemotherapy (Stage I)	28	9.90 ± 2.34	< 0.001*
First Cycle of Chemotherapy (Stage II)	28	4.60 ± 0.97	< 0.001**
Second Cycle of Chemotherapy (Stage III)	28	2.35 ± 0.41	< 0.001$
Third Cycle of Chemotherapy (Stage IV)	28	1.66 ± 0.45	< 0.001$$

(Values are expressed in ng/ml) * Control vs Stage-I, **Stage-I vs Stage-II, $ Stage II vs Stage III and $$ Stage III vs Stage IV.

DISCUSSION

Knowledge of diagnostic and prognostic factors are essential for the management of individual patients and these factors should be taken into account in the design of randomised trials and in interpreting the result of such trials. Serum tumor markers have been used in aiding the diagnosis of gastrointestinal cancers for a long time. Previous studies reported that the elevated serum values reflect the increased secretion of tumor antigens by tumor itself[26]. However mild elevation of serum tumor marker levels in number of early-stages of cancer has been always difficult to justify as many benign pathologies may frequently cause such changes. The clinical use of tumor markers is much more beneficial in determination of prognosis assessing response to treatment and detection of early recurrence[27, 28]. The present study demonstrates that elevated level of GST, LDH, ALP and CEA occur in stomach cancer patients as composed to those obtained from normal healthy control group (Table 2). Similar findings reported by G.S. Mahammadzadeh et. al (7,9). Table 3, Table 4, Table 5 and Table 6 shows that the activity of serum GST, LDH, ALP and CEA significantly increased ($p<0.001$) in stage II after first cycle of chemotherapy than stage I (before chemotherapy) similar findings reported by N. R. Hazari[28] and in my previous study[29]. But after 3 weeks after second cycle of chemotherapy means in stage III level of GST, LDH, ALP and CEA significantly decreased ($p<0.001$)observed in present study than stage II (after first cycle). This result indicates that patients were responded to the treatment and may in the direction of recovery. Similarly in stage IV after third cycle of chemotherapy the activity of GST, LDH, ALP and GST significantly decreased ($p<0.001$)than stage III (after second cycle), and activity become in normal range. This shows that patients were responding and totally recovered by cisplastin based treatment. Studies reported progressive increase of GSTs with advancing cancer and has been associated with poor prognosis and development of drug resistance[30-32].K.Johansson et al[33] reported GSTs protect the cells from lipid peroxidation and H_2O_2which is increased by cisplastin based chemotherapeutic drug .Our results showed at association of serum GST and chemotherapy in stomach cancer. Charushila Y. Kadam, Subodhini A. Abhang[34] observed that serum GST level was significantly higher in post-operative stage II in breast cancer patients before chemotherapy as compared to healthy controls. After 3 weeks of receiving 1st adjuvant chemotherapy cycle, GST level was significant decreased as compared to levels before chemotherapy in these patients. Increased activity of serum GST in stomach cancer is probably a resistance mechanism by which cell can survive and source of plasma enzyme is mainly transformed to cell with over expression of GST. The findings of the present study showed a significant increase ($p< 0.001$)in ALP level in stomach cancer patients in comparison to normal control subjects, Nishio H. et. al. observed, that rise in ALP level in 59% in stomach cancer concluded that the total ALP activity increased due to placental alkaline phosphatase isoenzymes which is probably originates from cancer itself. A significant rise in serum LDH activity was observed in stomach cancer than control group. In present study it was observed that 92 % of stomach cancer patients had LDH activity greater than 500 lU /Liter. In before chemotherapy 18 of 28 of stomach cancer had LDH activity greater than 500 lU/Liter and after chemotherapy 26 of 28 patients of stomach cancer had value of LDH greater than 500

lU/Liter.LDH was termed as an old enzyme which reborn as cancer marker[35]**.** 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[36]**.R. Domiguer et.al.**[37] 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. Carcinoembryonic antigen (CEA) is used predicting & in monitoring patients with advanced cancer. Tumor markers alone cannot be used to asses response, but could be used to confirm complete response – serum tumor markers have been used in aiding the diagnosis of gastrointestinal cancers for a long time. Previous studies reported that the elevated serum values reflect the increased secretion of tumor antigen of tumor itself. However mild elevation of serum tumor markers level in a number of early stage cancers has always been difficult to justify as many benign pathologies may frequently cause such changes. The clinical use of tumor markers is much more beneficial in determination of prognosis is assessing response to treatment & detection of early recurrences[38]. In the study various tumor markers such as CEA has been investigated in the serum of gastric adenocarcinomas to markers. Llyas Tuncer show the serum CEA level was found to be higher in 70% cases in both cases[39]. CEA is one of the most reliable tumor associated markers used for the detection of malignancy serum CEA level are used for cancer detection determination of cancer stage recurrence ,& evolution of cancer therapy, especially in patients with colorectal cancer. **Gion et. Al[40] ,** reported that CEA was positive in 27% of the patients with oespohagas cancer. In the same study it has been reported that the positivity rate of CEA was correlated with the stage of the disease. In present study the activity of serum GST, LDH, ALP and CEA in stage II after first cycle of chemotherapy was significantly higher than stage I (before chemotherapy) and control but after second and third cycle of chemotherapy activity of serum GST, LDH, ALP and CEA was significantly decreased

CONCLUSION

Based on the data from our study, it can be stated that serum GST and CEA measurement in plasma may be useful tumor marker in stomach cancer, its activity might helpful to predict the response of chemotherapy in advance stage of cancer. An initial increased level of GST and CEA before and first cycle of chemotherapy may not indicate tumor progression, but may be represent a transient tumor marker surge phenomenon after second and third cycle of chemotherapy in patients responding to treatment. The administration of chemotheraptic drug in stomach cancer patients cause increase in oxidative stress, it is indicated by decreased level of glutathione. Decreased level of GST might be associated with deceased level of glutathione after different cycle chemotherapy. Increased level of serum LDH and ALP indicates infection or blockage or liver damage by treatment. Both are good prognostic factor in stomach cancer treated with chemotherapy. Increased level of ALP over a period of 3 month may be indicative of advanced disease progression which wants more aggressive treatment. Increased level of LDH and ALP shows liver damage during or after treatment Statistically significant change in GST, LDH, ALP and CEA concentration level during the process of treatment in stomach cancer patients with a positive response and no established disease progression during study period near about 18 months after the treatment, point to GST and CEA as an important predictive factor.

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