Evaluation of Glutathione-S-Transferase, Lactate Dehydrogenase, Alkaline Phosphatase and Carcinoembryonic Antigen in Monitoring of Response to Chemotherapy in Stomach Cancer Patients

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Abstract

A large proportion of human cancers are claimed to be caused by lifestyle or dietary factors [1]. Our diet contains many toxic or potentially carcinogenic compounds which are absorbed and metabolized in the digestive tract. Several biochemical markers have been used for diagnosis and prognosis in various cancers. Serum tumor markers such as Carcinoembryonic antigen and enzymes such as alkaline phosphatase, lactate dehydrogenase and glutathione-s-transferase are assessed in 50 patients of stomach carcinoma. Serum levels are measured before initiation of chemotherapy as well as during and after chemotherapy. The patients received chemotherapy in three cycles at 6 months intervals under standard protocol, which included the cisplatin and/or 5-Fluorouracil. The results show significantly higher baseline mean values of carcinoembryonic antigen, glutathione-s-transferase, lactate dehydrogenase and alkaline phosphatase in the patients of stomach cancer compared to the controls. The mean levels of glutathione-s-transferase, lactate dehydrogenase and alkaline phosphatase rise after first cycle of chemotherapy thereafter the mean levels were found to decline. The rate of decrease in glutathione-s-transferase was parallel to carcinoembryonic antigen. It is concluded that serum glutathione-s-transferase is the suitable surrogate biochemical marker for assessing the prognosis in the chemotherapy of gastric carcinoma. In comparison to alkaline phosphatase and lactate dehydrogenase, glutathione-s-transferase is sensitive and preferable biochemical marker in monitoring chemotherapy in gastric carcinoma.

Keywords: Alkaline phosphatase; Lactate dehydrogenase; Glutathione-s-transferase; Carcinoembryonic antigen; Cisplatin chemotherapy; 5-Fluorouracil

Introduction

A large proportion of human cancers are claimed to be caused by lifestyle or dietary factors [1]. Our diet contains many toxic or potentially carcinogenic compounds which are absorbed and metabolized in...
the gastrointestinal tract. Important metabolizing or biotransformation enzyme such a glutathione-S-transferase (GST) is present in the epithelial cells along the human gastrointestinal tract [2]. The digestive tract is a major site of cancer in humans. A number of reports [3-5] particularly from Japan have suggested that serum Pi-class GST levels may be elevated in a wide range of gastrointestinal and haematology malignancies and thus the measurement of serum GST level might provide a useful tumor marker [6]. Niitsu et al. reported that elevated serum Pi-class GST concentrations were found in 28 patients with gastric cancer [4]. Recently, a significant negative correlation was demonstrated between GST enzyme activity in the mucosa along the gastrointestinal tract and the tumor incidence [7]. Deficiencies of GST-μ and/or GST-θ have been claimed to have increased the risk of developing gastric or colonic cancer [8,9], although other studies deny such genetic predisposition [10]. Low or reduce levels of GSH and GSTs may result in a lower capacity to detoxify carcinogens, which may result in more cytogenetic damage and account for an increased tumor risk [11-13].

The Carcinoembryonic antigen (CEA) is a high molecular weight glycoprotein present in foetal gut and colonic adeno-carcinoma [14]. It is well known tumor marker. Increased values of CEA have been observed in cancers of colon, rectum, lung, breast, liver, pancreas, prostate, stomach and ovary [14-16]. Many enzymes have been used earlier to aid in diagnosis of various malignancies [17-19]. The elevated level of serum lactate dehydrogenase (LDH) was found patient of neoplastic disease [19]. The increased activity of LDH is a fairly sensitive marker for solid neoplasm. Serum alkaline phosphatase (ALP) was found useful in detecting liver cancer and bone metastasis and also in monitoring cancer therapy [20]. In view of this, present study was undertaken to assess, the clinical utility of GST, LDH and ALP enzymes in gastrointestinal cancers and to assess the status of tumor markers such as CEA along with the serum enzymes GST, LDH, ALP in stomach cancer patients. The study was also undertaken to monitor the response to chemotherapy with the help of serum enzyme levels.

Material and Methods

The study was conducted in tertiary care centre with specialty facility in collaboration with department of medicine and biochemistry during the period of June 2015 to Dec 2017. The study was initiated on approval of institutional ethical committee. The study involved total 50 patients of stomach carcinoma comprised of 29 males and 21 females. The patients were clinically and histologically diagnosed. All the patients received chemotherapy including cisplatin and 5-Fluorouracil (5FU) in standard protocol of four cycles. 10 ml blood was collected in a clean and dry plain bulb from patients in the morning. The blood samples were processed by standard protocol in clinical biochemistry laboratory for separation of serum. Serum enzyme assay was carried out on the same day of sample collection without any delay. A total of 40 normal healthy persons, age and sex matched with the study group were selected as controls. The values were compared between the phases. Data were expressed as mean ± SD. The statistical significance of changes in the mean values was assessed by paired student ‘t’ test. Probability level p<0.05 (min) and p<0.001 (max) was considered as statistically significant. All the statistical analysis was performed using SPSS (version-26).

Treatment protocol

According to the protocol, 63.82% (27 patients out of 50) of the patients completed four cycles of chemotherapy which included the cisplatin and/or 5FU. All the chemotherapy regimens were used under standard protocol. The combination of cisplatin (60-100 mg/day) 80 mg/m2 IV on day 1 in combination with and 5FU 12 mg/kg body weight (750-1000 mg/day) 800 mg/m2/day continuous IV infusion given by continuous intravenous infusion for 4-5 days.

Follow up

Overall 27 patients were followed up after starting the treatment. Seven patients expired during the follow up period. The follow up program included clinical examination, hematological analysis, tumor marker and ALP, LDH, GST enzyme assays at the next day of completion of each cycle. All the patients followed up for at least 27 months after the initiation of chemotherapy. Total ALP in serum was estimated by kinetic method [21]. The reagent kit of Strachem diagnostic was used in the assay with p-Nitro-phenyl phosphate as the substrate. Serum total LDH was measured by kinetic method using commercial kits from Agappe Diagnostics Ltd., on semi-auto analyzer Transasia ERBA CHEM-5 Plus [22]. Serum level of GST was measured by the method as described by Haiilig et al. [23]. Enzyme activity was monitored at 340 nm by measuring the conjugation of 1-Chloro 2, 4-dinitrobenzene with glutathione. The chemicals and reagents were procured from Sigma Chemical Company [24]. All other reagents used were of analytical and reagent
grade. Serum CEA level was measured by using commercial kit from Accu-bind on ELISAC micro plate Immuno-enzymometric assay [14].

**Results**

The results are shown in Table 1 and are also expressed graphically by Figure 1. The results on CEA show higher mean value before starting the chemotherapy and subsequently there is a drastic decline after first cycle. Thereafter the decline rate is reduced. The results on GST shows higher mean values in the patients as compared to the normal controls. The mean GST level was found to be increased after the first cycle of chemotherapy and thereafter in second cycle of chemotherapy, it shows a steady decline which was parallel to CEA levels. The results on LDH shows higher mean value of LDH in the patients compared to the normal controls. The mean value of LDH was found to be higher after first cycle of chemotherapy. It remains higher after subsequent cycles, thereafter it declines to slightly lower level as compared to the pre-treatment phase. Mean value of ALP in the patients was not much different from normal controls. Mean value was found to show a rise after first cycle of chemotherapy. Thereafter, it shows decline after second phase. Subsequently, after third phase, it does not change much when compared with previous phase/stage.

<table>
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<tr>
<th></th>
<th>GST (IU/L)</th>
<th>CEA (ng/dl)</th>
<th>LDH (IU/L)</th>
<th>ALP (IU/L)</th>
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<tbody>
<tr>
<td>Control (n=40)</td>
<td>5.36 ± 0.59</td>
<td>1.52 ± 0.28</td>
<td>274.10 ± 35.38</td>
<td>166.52 ± 25.80</td>
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<td>Phase I (n = 50)</td>
<td><em>9.80 ± 2.45</em></td>
<td><em>10.02 ± 2.45</em></td>
<td><em>512.03 ± 42.39</em></td>
<td><em>199.72 ± 39.58</em></td>
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<td>(Before Chemotherapy)</td>
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<tr>
<td>Phase I (n = 27)</td>
<td>12.43 ± 1.00</td>
<td>4.68 ± 1.20</td>
<td>722.33 ± 64.53</td>
<td>399.05 ± 28.26</td>
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<tr>
<td>(After First Cycle)</td>
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<td>Phase II (n = 27)</td>
<td>7.90 ± 1.09</td>
<td>2.33 ± 0.40</td>
<td>676.52 ± 361.84</td>
<td>200.02 ± 162.17</td>
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<tr>
<td>(After Second Cycle)</td>
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<tr>
<td>Phase III (n = 27)</td>
<td>5.10 ± 0.49</td>
<td>1.65 ± 0.42</td>
<td>401.08 ± 29.32</td>
<td>178.88 ± 31.50</td>
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<td>(After Third Cycle)</td>
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Table 1: Serum Enzymes and Tumor Marker activity in various stages of stomach cancer patients on chemotherapy.

Values are given as mean ± SD. Control to Phase I – *P < 0.05 #P<0.001; Phase I to Phase II – P < 0.001; Phase II to Phase III – P < 0.001; Phase III to Phase IV – P < 0.001

GST = Glutathione-s-transferase; CEA = Carcinoembryonic antigen; LDH = Lactate dehydrogenase; ALP = Alkaline phosphatase; IU/L = International units per litre; ng/dl = nanogram per decilitre.

**Discussion**

A number of enzymes are present on the cell membrane. Changes in tissue enzyme activities are often reflected in blood. Rapid turnover of malignant cell results in release of ecto and endogenous enzymes into the blood stream. Membrane constituents are shed into surrounding milieu at increasing rate when cell replicated more rapidly. Enzymes present in the nucleus, cytoplasm and mitochondria are also released when cells are destroyed. Also enzymatic changes may reflect the overall changes in metabolism that occur in the cells. ALP is an enzyme that is mainly involved in bone growth. It is processed in the liver and excreted into digestive tract through the bile. A higher value of ALP indicates bone or liver disorders. In cancer patients, elevated ALP may indicate that cancer has spread to bones or that liver damage is possible due to chemotherapy drugs that affected the bile excretion [18]. In Cancer metastasis of bone, the activity of ALP can be six times greater than upper limit of normal [20,24,25]. The results on the ALP level in the present study show a significant increase in the stomach cancer patients in comparison to the normal control subjects.

Individual patient's data revealed that 27 of 50 patients of stomach cancer had ALP level above...
normal limits. Several workers [18,25,26] have reported elevated level of ALP in esophagus, stomach and ovarian cancers in their studies. It is assumed that the increased total ALP activity could be due to placental ALP isoenzyme, which probably originates from cancer itself [24,25]. Isoenzyme studies could reveal more exact reasons for the rise of ALP level. The elevated value of total ALP was observed after first cycle of chemotherapy (phase II) of stomach cancer which was four times greater than normal limits. It is suggested that high serum ALP activities in stomach cancer patient may result from the higher tumor activity in the patients.

A significant rise in serum total LDH activity was observed in stomach cancer patients than the normal control group. In present study, it was observed that 81% of stomach cancer patients had LDH activity greater than 500 IU/L in phase II and 13 of 27 stomach cancer patients had LDH activity greater than 500 IU/L. In phase III, all patients of had value of LDH greater than 500 IU/L. Thus serum LDH activity increases with progression of disease. Neoplastic tissue show increased glycolytic activity and it has been shown that LDH content of tumor is greater than the normal homologous tissue [19]. Several reports [19,26,27] found that serum LDH activity rise in gastrointestinal cancer and stated that LDH has positive correlation with stages of malignancy. Our present observations agree with these reports. LDH was termed as an old enzyme which is reborn as cancer marker [28]. The high levels seen in malignancies are due to high rate of glycolysis by tumor cells. The increase in LDH could be due to overproduction by tumor cell, or due to changes in the permeability of cell membrane allowing leakage of soluble enzymes in circulation or it could be because of blockage of duct system by tumor through which the enzyme passes. Release of LDH from dying tumor cells and induction of synthesis in normal tissue of host due to tumor could be other possible mechanisms which may explain the raised LDH levels. High value of LDH may reflect the mass of rapidly growing tissue, which results from extension of carcinoma form primary site. This dissemination usually, but not invariable, includes liver. LDH may also rise without clinical evidence of metastasis. So increase in LDH reflects the mass of tumor tissue rather than presence or absence of hepatic metastasis [27]. The patients who are treated with cisplatin based combination therapy had lower level of LDH and considered as independent prognostic factor for survival [29].

In our study, we observed that the response to cisplatin therapy was poor when LDH levels were considered. The mean levels of LDH remained higher in the patients till 2nd cycle of treatment. This shows that LDH levels do not predict the proper response of the chemotherapy. Recently GSTs have attracted interest in the field of diagnosis and monitoring of malignancy [6]. They have considerable important role in the detoxification of carcinogens [30]. GSTs are present in many species and tissue and also in relatively large amounts in the epithelial tissues of the human gastrointestinal tract [12,30,31]. This enzyme has been used as a possible marker for gastrointestinal cancer [4,7,31,32]. In the present study serum GST was significantly higher (P<0.001) in patients with stomach cancer as compared to those of normal control group [7]. Observed similar results, in which plasma activity was significantly higher in esophagus cancer and gastric cancer patients.

The GST activity in plasma represents a biomarker of the cellular protection. The activity of serum GST was higher in 93% patients of stomach cancer in this study and it is in accordance with the earlier reports [4,5]. The observed increased activity of GSTs can be due to over expression is enzymes of GSTs in response to metabolic changes in tumor cells. The human GST π class was found to be over expressed in most of tumors [5-7]. Our results showed a significant increased activity of GST after 1st cycle of chemotherapy than subsequent cycles, which suggests that initial rise, is an early acute response to the chemotherapy. GST π expression in malignant tissues and plasma GST π levels in human colorectal and gastric cancer are believed to increase depending on the stages of tumor [4,25,31]. GSTs have also been suggested to play important role in multiple drug resistance in cancer chemotherapy [33]. Many studies showed progressive increase of GST with advancing cancer and has been associated with poor prognosis and development of drug resistance [33,34]. The observed elevation of serum GST activity in stomach cancer is most probably appear to be an adaptation mechanism by which cells can survive and source of plasma enzyme is mainly from the transformed cell with over expression of GST.

In the present study, the serum GST level in patients which received 2nd cycle of chemotherapy for cancer was significantly elevated than that after 1st cycle and control group this suggests that enhanced antioxidant defence and support made the tumor tissue less susceptible to the oxidative stress conferring growth advantage. It is reported that GST protect the cells from lipid per oxidation which is increased by cisplatin and also from hydrogen peroxide [33]. Thus it can be said that elevated level of GST may be associated with development of drug.
resistance in stomach cancer. Tumor marker such as CEA is used in prediction and in monitoring patients with advanced cancer [14-16]. Tumor markers alone cannot be used to assess response, but it could be used with other biomarkers to confirm complete response. Serum tumor markers have been used in aiding diagnosis of gastrointestinal cancers for a long time [14,15]. Previous studies reported that the elevated serum values reflect the increased secretion of tumor antigen [15]. However, mild elevation of serum tumor marker level in a number of early stage cancers has always been difficult to justify as many benign pathologies may frequently cause such changes [14].

The clinical use of tumor markers is much more beneficial in determination of prognosis and in assessing response to treatment and detection of early recurrences [16,35]. Various tumor markers such as CEA have been investigated in the serum of gastric adenocarcinomas. Lylas Tuncer et al have reported that the serum CEA level was higher in 70% cases [36]. CEA is one of the most reliable tumor associated markers used for the detection of malignancy. Serum CEA levels are used for cancer detection, determination of cancer stage, recurrence and evolution of cancer therapy, especially in patients with colorectal cancer [15]. It has been reported that the positivity rate of CEA was correlated with the stage of the disease [37]. The present results indicate that CEA levels show a steady decline from phase I to IV. The mean levels of serum CEA show a drastic and acute fall after the chemotherapy. It shows a sensitive response to the chemotherapy and is an ideal tumor marker for monitoring the response of chemotherapy. Similar response is seen with GST but after first cycle of chemotherapy which was parallel to CEA. Therefore, GST can be considered as a useful surrogate serum marker which can help in monitoring the response to chemotherapy, in the absence of serum CEA assays. Validation of serum GST assay as predictor of chemotherapy response in gastric as well as in other carcinoma is warranted [38].

References


22. Agappe. Quantitative determination of Lactate Dehydrogenase in serum or plasma. (Based on SCE recommended method) Assay kit Package insert, version # ADL/V.01/110314, Product # 12005016, Agappe Diagnostic Ltd, Ernakulam, Kerala, India.


