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Glutathione S-transferase activity in patients with cancer of the digestive tract

Giancarlo Severini

Laboratorio di Biochimica Clinica, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Roma, Italia

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Abstract. Glutathione *S*-transferase (GST) and carcinoembryonic antigen were measured in the plasma of 95 patients with neoplasm of digestive tract, in 40 patients suffering from non-neoplastic diseases and in 40 healthy subjects. The mean value of the GST activity was significantly ($P<0.001$) elevated in patients with gastric, liver and colorectal cancer (10.4 U/l, 14.1 U/l and 12.3 U/l respectively) as compared with the reference population (3.2 U/l). GST elevations above normal were observed in 26 (90%) patients with gastric cancer, in 18 (100%) with liver cancer and in 25 (89%) with colorectal cancer. Carcinoembryonic antigen appeared less sensitive. In 15 patients the postoperative levels of serum GST were increased after surgery then gradually declined, and after 1 month showed a normalization in 10 patients. Our data suggest that GST measurement may be useful as a tumour marker in gastric, liver and colorectal cancer. Moreover the combined determination of GST and other markers increase the sensitivity for cancer detection.

Key words: Glutathione *S*-transferase – Digestive tract cancer – Tumour marker

Introduction

The glutathione transferases are a group of enzymes that are found in the cytosol of most cells (Jackoby 1978). Although their physiological significance has not yet been defined exactly, they are thought to play a role in the detoxification of both exogenous and endogenous compounds by catalysing the conjugation of reduced glutathione with a wide range of electrophils to form a thioether (Jacoby and Keen 1977). Studies on biochemical markers involving the use of cell culture in human tumour cell lines have indicated that glutathione *S*-transferase (GST; EC 2.5.1.18) and its itoenzymes may be a useful addition to the tests currently available (Whelan et al. 1992; Volm et al. 1991; Chao et al. 1992). The main purpose of this study was to evaluate the diagnostic significance of serum GST as a tumour marker and to examine the possibilities of using GST for screening. In the present investigation we measured the preoperative serum level and postoperative change of GST in patients with malignant tumours of the digestive tract. A correlation with the carcinoembryonic antigen (CEA) was also examined.

Abbreviations: GST, glutathione *S*-tranferase; CEA, carcinoembryonic antigen

Patients and methods

The determination of GST was carried out in 40 normal healthy subjects (controls), in 95 patients with oesophageal cancer (9), gastric cancer (29), liver cancer (18), pancreas cancer (11) and colorectal cancer (28) and in 40 patients with various non-neoplastic diseases (10 epatic, 16 gastric, 5 pancreas and 9 colorectal). In all tumour patients the diagnosis was histologically confirmed. Before carrying out the enzyme estimation, it was ascertained that the patients had not received any prior treatment. Sera were promptly separated, stored at –20° C and examined within 1 week. The method of assaying GST was a modified version of that described by Habig et al. (1974). It consisted of 0.60 m*M* 1-chloro-2,4-dinitrobenzene (CDNB) and serum (75 µl) in 100 m*M* potassium phosphate buffer, pH 6.25, to which 100 µl 50 m*M* glutathione was added to initiate the reaction; addition of glutathione was carried out within 3 min after the addition of CNDB. The final volume was 1 ml. Formation of the *S*-conjugate was followed by measuring absorbance (*A*) at 340 nm in a Beckman DU 50 spectrophotometer. The assay temperature was 25° C. Blanks, i.e. A_{340}/min, obtained without the serum, were subtracted from each assay value. One unit of enzyme activity is defined as the amount of enzyme that catalyses the formation of 1 µmol *S*-conjugate/min under the assay conditions. Calculations used a molar absorption coefficient of 9.6 mM^{-1} cm^{-1} for CDNB. All samples were run in duplicate; the mean coefficient of variation between assays was 5%. CEA was quantified by using a commercial kit. Means and standard deviations were calculated and student's *t*-test was used to evaluate the results.

Results

The mean value for GST concentration in the control subjects was 3.2 U/l (range 0–5) with a standard deviation of 0.8 with the upper limit of the normal range set at 4.80±2 U/l (x±SD), two of the control GST contents (5%) exceeded the normal range. In the case of CEA the cut-off level of 5 ng/ml

Table 1. Comparison of positive rates of glutathione transferase (GST) with carcinoembryonic antigen (CEA)

Diagnosis	No. cases	No. positive for	
		GST	CEA
Esophageal	9	4 (44) [a]	2 (22)
Gastric	29	26 (90)	15 (52)
Early stage	5	3 (60)	0 (0)
Locally advanced	15	14 (93)	8 (53)
Metastatic	9	9 (100)	7 (78)
Liver	18	18 (100)	8 (44)
Pancreas	11	6 (54)	8 (73)
Early stage	6	2 (33)	4 (67)
Metastatic	5	4 (80)	4 (80)
Colorectal	28	25 (89)	12 (43)
Stage I	1	0 (0)	0 (0)
Stage II	9	8 (89)	1 (11)
Stage III	10	9 (90)	5 (50)
Stage IV	8	8 (100)	6 (75)
Total	95	79 (83)	45 (47)
Benign disorders			
Epatic	10	2 (20)	4 (40)
Gastric	16	3 (20)	6 (37)
Pancreas	5	0 (0)	1 (20)
Colorectal	9	1 (11)	2 (22)
Total	40	6 (15)	13 (32)

[a] Percentage positive in parentheses

Table 2. Postoperative change in the plasma levels of glutathione transferase (mean±SD)

Patients	Preoperative level (U/l)	Level (U/l) on postoperative day:			
		1	4	10	1 month
With high preoperative plasma GST (n=15)	20±0.6	22±0.8	26±1.1	21±0.6	6.2±0.9
With normal preoperative plasma GST (n=15)	3±0.4	4.6±0.9	6.4±1	3.6±0.8	3.4±0.6

was used. GST activity was frequently increased in all the cancer patients considered together and ranged from 0 to 24 U/l with a mean value of 12.3 U/l (SD 3.6). In total, 79 of 95 patients (83%) showed increased levels of serum GST (Table 1). The average GST contents in patients with gastric, liver and colorectal cancer (10.4, 14.1 and 12.3 U/l respectively) were significantly (P<0.001) higher than those of healthy controls. Increased GST activities were found in 4/9 (44%) patients with oesophageal cancer, in 26/29 (90%) with gastric cancer, in 18/18 (100%) with liver cancer, in 6/11 (54%) with pancreas cancer and in 25/28 (89%) with colorectal cancer. We measured the postoperative level of serum GST in 15 patients with a high preoperative serum GST. As shown in Table 2 serum GST in patients with a high level was increased after surgery and reached a maximum by the 4th postoperative day. Then it gradually declined. Observation of patients over a longer period of time showed a normalization of GST in 10 patients. In patients with a normal GST level, the postoperative change was similar. Determination of CEA showed no correlation with GST. Serum CEA was elevated in 45 of 95 patients (47%) and in 36 of them (76%) this was accompanied by an elevation in serum GST. If we consider GST or CEA separately in detecting cancer, the sensitivity of the two markers is 83% and 47% respectively. On the other hand, if we consider all patients with altered values of GST or CEA, the sensitivity of detection of cancer is significantly increased (93%). It is known that GST, like other ubiquitous enzymes, increases in many non-neoplastic diseases and we therefore investigated the specificity of the test by measuring the serum levels of GST and CEA in patients with benign diseases. Specificity of tumour markers is expressed as a percentage of the correct normal serum levels. For GST a specificity of 85% can be calculated in all patients considered together while CEA shows correct normal serum values in 62.5%. Finally we evaluated the diagnostic value of the GST assay by the criteria of Galen and Gambino (1975); if all the patients are considered together, GST reveals a sensitivity of 84%, a specificity of 85%, a predictive value of positive and negative tests of 93% and 69%, and an efficency of 84%. If we consider patients with gastric, liver and colorectal cancer separately, GST reveals a sensitivity of 90% and a specificity of 87% in gastric cancer, a sensitivity of 100% and a specificity of 90% in liver cancer and a sensitivity of 89% and a specificity of 77% in colorectal cancer.

Discussion

During the last decade several groups have studied enzymes or isoenzymes in patients with malignant conditions (Hirata et al. 1992; Hayes et al. 1991; Ranganathan and Tew 1991; Clapper et al. 1991). However, the identification of a tumour marker that is highly sensitive as well as specific for the early detection of cancer and can be assayed by simple, reproducible and cheap techniques remains elusive. This is mainly due to the lack of sensitivity and/or specificity of the markers. The present study confirms these findings with regard to CEA. By comparison with CEA the results obtained with GST are more promising in this respect. Our data suggest that the GST assay has sufficient value as a serodiagnostic test in gastric, liver and colorectal cancer and only limited value in pancreas and oesophageal cancer. The mechanism underlying the elevation of serum GST is unclear. In general, the increase of certain subtances in the sera of cancer patients could be attributed to either their production by cancer cells or an acute-phase response to host defence against cancer proliferation. The observation that the higher serum GST in advanced cancer patients remained for more than 4 days and then gradually decreased suggests that the elevation could be due to an acute-phase response. Thus it is possible that as elevated GST level in the sera of patients could be a useful biological parameter for the detection of malignancy, although further evaluations are needed before the actual prognostic potential of this enzyme is known.

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