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VALUE OF GLUTATHION S-TRANSFERASE IN DIAGNOSIS OF MALIGNANT PLEURAL EFFUSIONS

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ÖZET

Bu çalışmada plörezili 89 hastanın plevra sıvısı ve serumları ile kontrol grubu olarak seçilen 15 sağlıklı bireyin serumlarında glutathion S-transferase (GST) düzeyleri araştırıldı. Plörezinin nedeni 27 olguda malignite iken 62 olguda malignite dışı hastalıklardı. Maligniteli hasta grubunda elde edilen ortalama serum GST düzeyi 18.39±1.18 U/L olup gerek malignite dışı plörezi grubu (p<0.01) ve gerekse kontrol grubundakilerden (p<0.001) anlamlı derecede yüksekti. Malignite ve malignite dışı plörezi gruplarında saptanan ortalama plevra sıvısı GST düzeyleri sırasıyla 28.46±1.48 U/L ve 10.59±0.87 U/L olup aralarındaki fark istatistiksel olarak anlamlı düzeydeydi (p<0.001). Maligniteli hasta grubunun ortalama plevra sıvısı/serum GST oranı da diğer hasta grubuna göre anlamlı derecede yüksek bulundu. Malignite kaynaklı plörezilerin malignite dışı nedenlere bağlı olanlardan ayırdedilmesinde 20 U/L'yi aşan değerler için plevra sıvısı GST düzeyi tayininin spesifitesi % 97 ve sensitivitesi %85 olarak bulundu. Plevra sıvısı/ serum GST oranının spesifite ve sensitivitesi ise 1.4 sınır değer ile sırasıyla % 87 ve %78 idi. Bulgularımız plevra sıvısı GST düzeyi tayininin malignite kaynaklı plörezilerin tanısında değerli bir inceleme olduğunu göstermektedir.

Anahtar kelimeler: Glutathion-S transferase, plevra sıvısı

SUMMARY

Value Of GST in Diagnosis of Malignant Pleural Effusions

We meauserd pleural fluid and serum glutathion S-tranferase (GST) activity in 89 patients with pleural effusion and serum GST activity in 15 healthy person choosen as control group. The cause of 27 pleural effusion was malignancy, and nonmalignant diseases were determined as the cause of 62 cases. Mean serum GST activity was 18.39±1.18 U/L in patients with malignant effusions, which was significantly higher than those in both nonmalignant cases (p<0.01) and control group (p<0.001). Pleural fluid GST level of malignant and nonmalignant effusions were 28.46±1.48 U/L and 10.59±0.87 U/L, respectively. The difference between the pleural fluid GST values of malignant and nonmalignant effusions was statistically significant (p<0.001). The mean pleural fluid to serum GST ratio of patients with malignant effusion was also significantly higher than that of nonmalignant group. The specifity and sensitivity of the determination of pleural fluid GST level in excess of 20 U/L in distinguishing malignant effusions were 97 percent and 85 percent, respectively. These values for pleural fluid to serum GST ratio with the cutoff level of 1.4 were 87 percent and 78 percent. Our findings indicate that determination of GST activity in pleural fluid has a diagnostic value in differential diagnosis of malignant effusions.

Key words: Glutathion S-transferases, pleural effusion.

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Pleural effusion is an important and common finding, and often represent a diagnostic task to the physicians (1,2). Although, it is evolve in the course of a variety of diseases, malignancies are most common causes of exudative effusions (3). A correct diagnosis of underlying disease is essential to rational management of effusions. In patients with pleural effusions, accurate diagnosis can be made if cytologic, histologic, biochemical, or bacteriologic results of pleural fluid and biopsy are positive. Despite a careful and detailed investigations, however, the cause often presents a difficult diagnostic challenge and remains obscure in 10 to 20 percent of all cases (2,4,5).

Accurate diagnosis of malignant effusions is often difficult and presence of malignancy frequently could not be proved. Cytologic analysis of pleural fluid is reported to be diagnostic of malignant disease in 9 % to 80 % of cases, but in most series the success rate is about 60 percent. The reported yield from pleural biopsy in patients with documented malignant effusions ranges from 11 % to 70 % and pleural biopsy offers an overall diagnosis of 60 percent (6).

The most critical question about a patient with an undiagnosed pleural effusion is whether the patient has a benign or a malignant effusion (7). Therefore, various tumor markers and biochemical parameters in pleural fluid have been investigated that may be helpful for diagnosis (5,8). Although a plenty of substances have been extensively studied for their import on diagnosis, the use of most of them appears to be of limited value.

As far as we know no previous study has determined the pleural fluid contcentration of GST, which is a family of widely distributed intracellular enzymes thet exhibit a variety of biological function. Changes in the level of GST activity were measured in the cytosols of different human tumor tissues (9-13) and an increasing body of evidence suggest that GST is overexpressed in tumor cells (10). Significant increases in serum GST activity were also reported in various gastrointestinal malignancies and lung cancer (11).

In this study, therefore, we investigated the level of GST in pleural fluids from patients with pleural effusions caused by various etiology to assess its potential role as tumor marker in distinguishing malignant effusions from benign.

MATERIALS AND METHODS

Patients

We studied 89 consecutive patients (28 females and 61 males) with pleural effusion together with 15 healthy controls. Patients ranged in age from 16 to 79; the mean age was 51 years and the controls had a mean age of 29 (range 18 to 45). In all patients, a standard clinical and radiologic evaluation, and routine hematologial and biochemical tests were followed up and the final diagnosis was recorded. The causes of the effusions are listed in table 1.

A sample of pleural fluid was aspirated for its protein, LDH, sugar content, cytologic examination, and culture of acid-fast bacilli. Pleural biopsy with a Cope needle was performed in all patients except when a transudate established. A sample of blood was also obtained by venous arm puncture from the patients and controls. Pleural fluid and blood samples were taken at the same time from fasting patients at the admission to the hospital and before beginning any treatment. A proportion of both samples were centrifugated for 10 minutes at 3000 rpm, and supernants were then divided into multipl small aliquots and stored at -20 C until assyed.

According to the final diagnosis, the pleural effusions were classified as malignant or non malignant;

1) Malignant effusions: This group included 27 patients with maligant effusions. In 16 cases lung cancer was established. In the other patients, tumour was localized in breast (2 patients), gastrointestinal tract (1 patients). Six patients had a pleural mesothelioma and in 2 patients the initial tumour site was uknown.

2) Nonmalignant effusions: This group consisted of 62 patients with tuberculous effusions (n=24), exudates caused by various etiologies other than malignancy or tuberculous (n=28), and transudates (n=10).

Assay

Total glutathion S-transferase activity was determined by the method of Habig et al., using 1 mM 1-chloro-2,4-dinitro benzene (CDNB) as substrate (12).

Data Analysis

The results are assessed in mean ± SE and the statistial signifiance of differences between the means was estimated using "t test "with $p<0.05$ as the minimum level of significance. The diagnostic value of each parameters was determined on the-basis of commonly given definitions for a screening test: Sensitivity was determined on the basis of commonly given definitions for a screening test: Sensitivity was defined as TP/(TP+FN), specifity TP/(TN+FP), and positive predicdive value (PPV) TP/(TP+FP), where TP indicates the number of true positive diagnosis (malignant effusions); FN, false negatives; FP false positives; TN, true negatives (nonmalignant effusions).

RESULTS

Serum GST activity in patients with malignant effusions and nonmalignant effusions averaged 18.39±1.28 U/L, and 13.22±0.73 U/L, respectively. The mean GST level of control group was 10.42±1.03 U/L. These values show that serum GST level of each patient group was higher than that of control group, and differences between patient and control groups were statistically significant ($p<0.001$, $p<0.05$). Serum GTS concentration in the patients with malignancies was also higher than that in the patients with nonmalignant diseases. These values were also statistically significantly different ($p<0.01$).

The mean pleural fluid GST level of malignant effusions was 28.46±1.48 U/L, which was significantly different ($p<0.001$), higher than that of nonmalignant effusions (10.59±0.87 U/L). Distribution of GST levels in serum samples and pleural fluids were shown in figure 1. Table 2 represents all the mean values of GST in serum samples and pleural effusions.

Tablo 1. Etiology of the pleural effusions

	No. of cases
Tuberculous	24
Neoplastic	
Lung	16
Breast	2
Stomach	1
Mesothelioma	6
Unknown origine	2
Pneumonic	22
Pulmonary thromboembolism	1
Pancreatitis	1
Congestive heart failure	10
Undetermined etiology	4

Another results in our study was difference, which was statistically significant ($p<0.01$), between pleural fluid to serum GST ratios of each patients group. The mean ratios in malignancies and nomalignant cases were 1.67+0.11 and 1.04+0.17, respectively. Table 3 describes the sensitivity, specifity, and positive predictive value of serum GST above 15 U/L, of pleural fluid GST exceeding 20U/L, and of fluid to serum GST ratio with the cutoff level of 1.4.

Tablo 2. Serum and Pleural Fluid GST Levels and Pleural Fluid to Serum GST Ratios (Mean+SE)

	Serum (U/L)	Pleural fluid (U/L)	Pleural fluid /serum ratio
Malignant effusions	18.39±1.18	28.46±1.48	1.67±0.11
Nonmalignant effusions	13.22±0.73	10.59±0.87	1.04±0.17
Control	10.42±1.03		

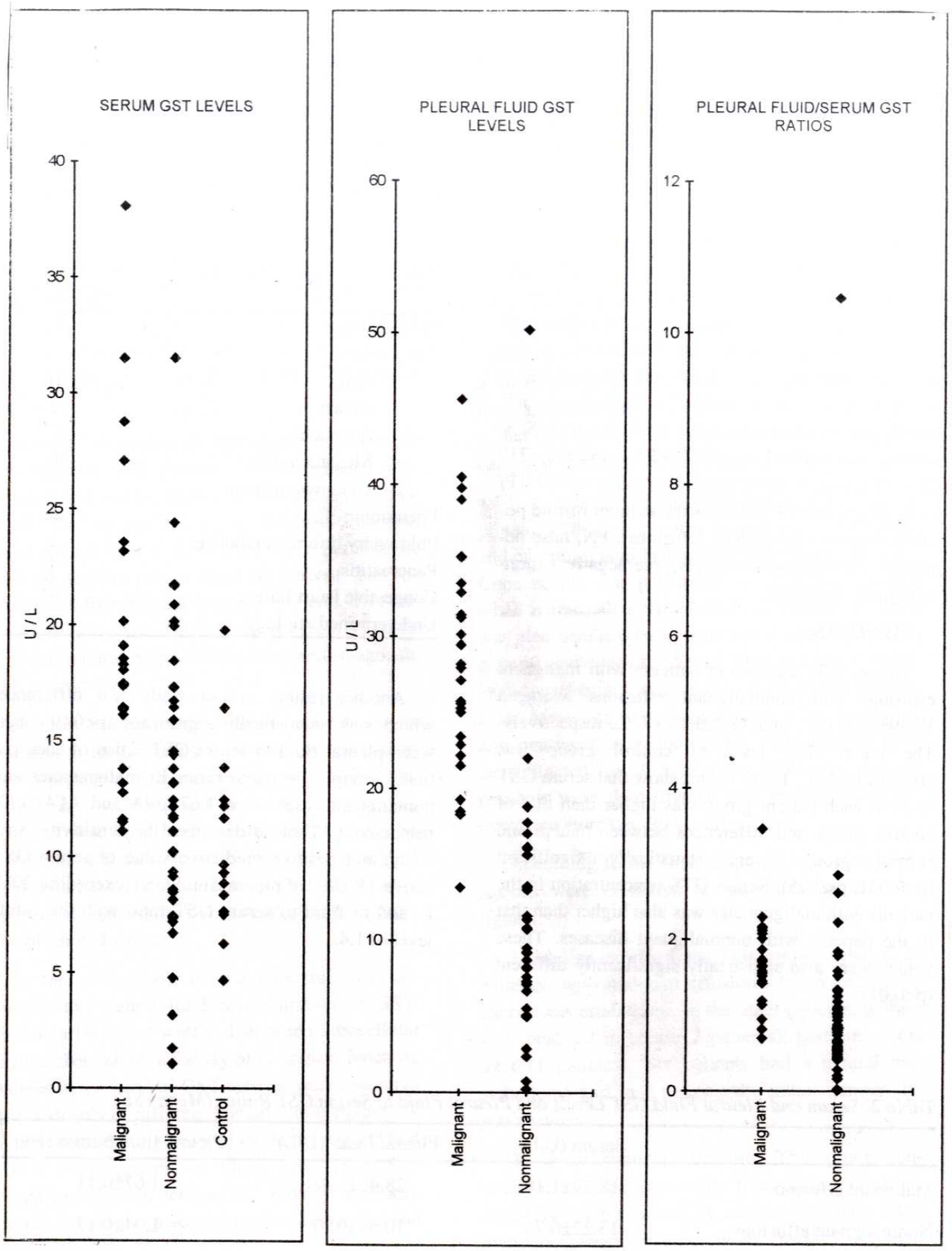

Figure 1. The overall distribution of GST levels of serum and pleural fluid samples and pleural fluid to serum ratios.

Tablo 3. Discriminative Values of the GST in malignant and Nonmalignant Effusions

	Sensitivity (%)	Specifity (%)	PPV* (%)
Serum GST >15U/L	70	63	45
Pleural fluid GST>20 U/L	85	97	92
Pleural fluid /serum GST>1.4	78	87	72

* PPV: Predictive value of positive test.

DISCUSSION

The activation of potentially toxic or carcinogenic substances occurs via the phase I mixed-function oxygenases and the reactive intermediates formed by this oxidative metabolism may then be detoxified by phase II enymes (13). Phase II enzymes catalyse the conversion of reacting intermediates coming from the cytochrome p-450 system to less reactive conjugates (14,15). Glutathion S-transferases (GST, EC 2.5.1.18) are a family of multigene cytosolic phase II detoxification enzymes (16) and have been studied intensively since 1961 that were first identified (17).

All human organs contain GST and although most abundatly present in the liver, differing amounts of GST are present in each tissue. Apart from one monomeric microsomal form, all GSTs are dimeric cytosolic proteins (18,19). All cytosolic forms of GST are composed of identical or nonidentical subunits (20). On the basis of physicochemical, immunological, enzymatic, and structural properties, GST's are classified into three groups; designed as the Alpha, Mu, and Pi classes (17,21). These enzymes exhibit both catalytic and binding activities that promote conjugation of glutathion (GSH) with electrophilic center of a large number of hydrophobic substances, thereby protecting cells against their potential toxicity (20,22). Numerous toxic chemicals including compounds considered to be carcinogens and mutagens, were found to be substrates of GST (9).

It is hypothesized that the oxidised metabolites have been responsible for tissue damage. In this case the local levels of GST would be crucial importance in protecting the tissues from these toxic metabolites (14). There is growing body of evidence which indicates that GST play an important role in both carcinogenesis and the acquisition of drug resistance in tumor which are initially responsive to therapy (9,23,24).

It is widely known that malignant tumors are known to secrete a large variety of substances, many of them extensively studied (25) and the expression of many enzymes is altered in preneoplastic foci (26). Expression of GST has been investigated biochemically or immunologically in human cancer tissues and cell lines. It has been reported that there was relationship between the expression of GST (pi) and malignancy in tissues (11). It has been found that the level of GST in solid tumors was higher than that observed in the cell lines (24).

Di ilio et al. (9) have shown that GST activity in the cytosolic fractions of human lung tumor tissues was significantly increased compared with surrounding non-tumor tissue. They have also found that more than 90 % of the activity was GST-Pi in both tumor and non-tumor cytosols and elevated GST activity measured in lung tumor cytosols was mainly due to an increased quantity of this isoenzyme. In another study, plasma GST-pi concentration in patients with lung cancer was also found significantly higher than that measured in control group consisted of patients with respiratory disorders other than malignancy (16). Elevated serum GST-pi levels were also observed in patients with various gastrointestinal maligancies including, gastric esophageal, colonic, pancreatic, hepatocellular, and biliary tract (11).

In the present study, serum GST content in the malignancies was significanly higher than that of

control group and of nonmalignant diseases. This result was consistent with previous reports. We found that nonmalignant diseases have had an elevated serum GST compared to that of controls, but this elevation was not as high as in that of malignant group.

Our study also showed that GST value in the malignant pleural effusions, which was 28.49 U/L, was significantly higher than that in nonmalignant effusions (10.59 U/L). The increase in the pleural fluids from patients with malignancies was more vigorous compared to that in serum of the same group of patients. This is reflected in high specifity and sensitivity of measurement of pleural fluid GST contents exceeding 20 U/L, which were 97% and 85%, respectively. Whereas, serum GST with the cutoff of 15 U/L had a specifity of 63% and a sensitivity of 70%, in distinguishing malignancies from nonmalignant disease.

We found that mean pleural fluid to serum GST ratio was 1.67 in malignant and 1.04 in the benign group. Difference between them was also significant. This study showed that the GST ratio has also a diagnostic value in the differential diagnosis of malignant effusions, with the specifity of 87% and sensitivity of 78% in excess of 1.4.

Elevated serum GST levels in patients with malignancies may originates from the tumor cells themelves, because increased GST level in cytosols of transformed cells from many tumor samples was well documented (11). Tumor cells probably secrete GST into the circulation. We suggest that implants of tumor cells in the pleural space, in addition to the diffusion from serum, could produce effusion with elevated concentration of GST. The higher increase in the fluid GST activity compared to that in serum of the patients with malignant diseases supports this suggestion. Pleural fluid to serum GST ratio was, therefore, important in determining contribution of GST in pleural fluid originated from malignant cells implanted on pleura.

Many GST subunit may contribute to the serum and effusion GST level. We suppose, howewer, that the increased GST levels in this study was probably due to pi-isoenzymes. Because, it was stated that the pi class GST was the major isoenzyme expressed in most of the human tumors (24) and more than 90% of activity was GST-pi in both tumor and montumor lung tissues (9). Our cases in the malignannt group were also mostly patients with lung cancer.

In conclusion, our study revealed that GST may be a useful addition to tests currently available in the diagnosis of malignancies, although cytologic examination is the most sensitive method. Measurement of pleural fluid GST activity, especially appears to be of more value compared to serum GST in the differential diagnosis of pleural effusions of unknown etiology.

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