GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Determination of erythrocyte Glutathione S-Transferase activity in individuals with gastric and colon cancer

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GSC Biological and Pharmaceutical Sciences, 2022, 20(03), 192–197

Publication history: Received on 04 August 2022; revised on 13 September 2022; accepted on 15 September 2022

Article DOI: https://doi.org/10.30574/gscbps.2022.20.3.0357

Abstract

Glutathione-S-Transferase (GST) in human cells has great importance in the detoxification mechanism of carcinogenic chemicals. Therefore, (GST) may be a useful tumor marker. This study examines whether GST activity in Gastric cancer (GC) and Colon Cancer (CC) is a helpful marker in diagnosing and monitoring the disease course. GST activity was investigated in patients with CC and GC and healthy individuals.

Erythrocyte isolation was performed in 3 ml blood samples from volunteers aged 18-75 years. Hemoglobin amounts were determined from the standard graph drawn by monitoring the conversion of methemoglobin to cyanmethemoglobin in the presence of cyanide at 540 nm. Glutathione S-transferase activity was determined by measuring the amount of enzyme that catalyzes 1 µmol of S-(2,4-dinitrophenyl) glutathione formed per minute using 1-Chloro-2,4-Dinitrobenzene. The mean values of GST activities of patients with CC and GC, respectively; (1.28 ± 0.23 U/gHb; 1.20 ± 0.30 U/gHb), were significantly higher when compared to the mean values of healthy individuals with GST activity (0.59 ± 0.13 U/gHb) ($p < 0.05$). The GST activity of patients with colon cancer, measured as (1.56 ± 0.13 U/gHb) after chemotherapy, was significantly higher than before (1.09 ± 0.12 U/gHb) ($p < 0.05$). GST activity measured as (1.53± 0.24 U/gHb) after chemotherapy in gastric cancer patients was significantly higher when compared to the value measured before chemotherapy (0.97± 0.12 U/gHb) ($p < 0.05$). Our results show that the change in GST activity in CC and GC can be used as a biomarker to monitor the disease course and response to chemotherapy.

Keywords: Colon cancer; Gastric cancer; Glutathione S-Transferase; Biomarker

1. Introduction

Glutathione-S-Transferases (GSTs) (E.C:2.5.1.18) are Phase-II detoxification enzymes widely found in the animal kingdom. Human cytosolic GSTs have been grouped into eight gene independent classes termed Alpha, Mu, Pi, Theta, Sigma, Omega, Kappa, and Zeta. GSTs are a family of inducible enzymes that conjugate reactive oxygen species (ROS), hydrophobic, and multiple electrophile-reduced compounds with glutathione and catalyze their conversion to less toxic metabolites generally more readily excreted [1–3].

Exposure to environmental chemicals and other toxic substances plays a vital role in developing colon and gastric cancer and many other pathological processes. Studies for carcinogenesis emphasize that these chemicals must be metabolized using the body's enzyme systems to exert their effects. Chemicals can transform into electrophilic metabolites in the body and react by covalent binding with DNA, and as a result, they cause mutagenic lesions (oxidative damage) in DNA. Permanent modification of genetic material resulting from these “oxidative damage” events constitutes the first step in mutagenesis, carcinogenesis, and aging [4,5].

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In order to prevent such processes from occurring, GSTs participate in antioxidant defense through various mechanisms, including conjugation of xenobiotic compounds with glutathione. Such conjugation reactions result in the synthesis of mercapturic acid [6,7]. GSTs participate in the neutralization process and play critical roles in protecting hosts against cancer [8,9].

Recently, it has been shown that GST is overexpressed in different tumors in humans and this suggests that the measurement of GSTs levels can be a reliable and sensitive marker in the colon and gastric cancers [9]. And increased GST levels are thought to accelerate the metabolism of chemotherapy drugs, leading to the inability to achieve the targeted effect with the drug, in other words, the development of acquired resistance to the drug [10,11].

This study investigated whether the change in GST activity in GC and CC could be used as a biomarker in monitoring the disease course and response to chemotherapy. In addition, it was aimed to determine the relationship between exposure to toxic chemicals and the amount of GST in both healthy and cancerous individuals.

2. Material and methods

2.1. Patient and control groups

This study was conducted from patients who diagnosed with colon cancer and the control group was selected from healthy subjects according to age and sex group of patients (Table 1). The approval of Human Ethics Committee of Cumhuriyet University (2010-04/10 numbered) and informed consent was obtained from all the participants. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Table 1 Control group, stomach and colon carcinoma patient's data

	Control	**Gastric**	**Colon**
No of Cases	n =30	n =30	n =30
Age ± SD	41.46 ± 2.60	58.30 ± 1.83	56.06 ± 1.75
Male	12	21	16
Female	18	9	14
Smoke	13	15	11
Non-smoke	17	15	19
Stage I (Before Chemotherapy)	-	15	15
Stage II (After Chemotherapy)	-	15	15

Stage I- Without any treatment (Surgery, chemotherapy, Radiotherapy); Stage II- After First Cycle of Chemotherapy

2.2. Blood samples

3 mL of venous blood samples were taken from the patient and control group of the subjects by using a tube containing K3-EDTA and transferred to the laboratory at the tube 4 °C. Laboratory erythrocyte isolation process was started without loss of time. Blood samples were centrifuged at 2500 x g for 10 minutes at 4 °C (Selecta Centrofriger BL II). After receiving the supernatant, 3 times the volume of isotonic NaCl (Carlo Erba, Italy) solution was added to the shaped components remaining in the tube. The erythrocytes were slowly washed down and centrifuged again at 2500 x g for 10 minutes at 4 °C. This process was repeated 3 times. Afterwards, erythrocytes were hemolyzed by freeze-thaw method by adding distilled water at a ratio of 1: 5 v / v. After this process, hemolysate was centrifuged at 22.000 x g for 60 minutes at + 4 ° C to allow separation of cell membranes. The resulting erythrocyte hemolysates were stored at -20 ° C in 1.5 ml eppendorf tubes for use in all analyzes [12].

2.3. Determination of glutathione S-transferase activity

GST activity was studied according to the method described by Habig et al. [13]. It was determined by measuring the amount of enzyme that catalyzes one μmoll of S-(2,4-dinitrophenyl) glutathione formed per minute using GSH (Sigma, ABD) and 1-Chloro-2, 4-dinitrobenzene (CDNB) (Sigma-Aldrich) at 37˚C at 340 nm. Results are given as U/gHb. Total hemoglobin amount was determined in g/dl by cyanmethemoglobin determination [14].

2.4. Statistical analysis

The findings were analyzed by using variance analysis, Tukey test, Kuruskal-Wallis test, Man Whidney U test and correlation analysis. A *p*-value of<0.05 was considered statistically significant.

3. Results

Individuals with colon cancer (n=30), stomach cancer (n=30), and a healthy control group (n=30) were included in the study. The characteristic features of the patient and control groups are shown in Table 1.

GST activity was significantly increased in gastric cancer patients compared to the control group (Table 2). The difference between the GST value of the control group and the GST activity of the patients with gastric cancer was statistically significant ($p<0.05$).

Compared with the control group, GST activity was significantly increased in colon cancer patients (Table 2). The difference between the GST value of the control group and the GST activity of the patients with colon cancer was statistically significant ($p<0.05$).

When the GST activities of patients with gastric and colon cancer were compared, no statistically significant difference was found, as shown in Table 2 ($p>0.05$).

Table 2 Mean GST activity of control and patient groups (U/gHb)

Parameters	No of Subjects (n)	Mean ± SD	p- value
Control group	30	0.59 ± 0.13	0.001
Gastric cancer	30	1.20 ±0.30	
Control group	30	0.59 ± 0.13	0.001
Colon cancer	30	1.28 ±0.23	
Gastric cancer	30	1.20 ±0.30	0.787
Colon cancer	30	1.28 ±0.23	

*Statistically significant compared to the control group ($p<0.05$)

The change in GST activity according to gender in each group was statistically insignificant, as shown in Table 3 ($p>0.05$).

The change in GST activity according to smoking in each group was statistically insignificant, as shown in Table 3 ($p>0.05$).

Table 3 Control group, gastric and colon carcinoma patient's GST activities (U/gHb)

Parameters	Gender			Smoke		*p*-Value
	Male	Female	*p*-Value	Yes	No	
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ±SD	
Control	0.55 ± 0.10	0.61 ± 0.14	0.390	0.59 ± 0.16	0.58 ± 0.10	0.691
Gastric Cancer	1.15 ± 0.24	1.32 ± 0.40	0.331	1.15 ± 0.26	1.25 ± 0.34	0.443
Colon Cancer	1.35 ±0.17	1.19 ±0.25	0.390	1.23 ± 0.21	1.30 ± 0.23	0.426

*Statistically significant compared to the control group ($p<0.05$)

The GST activity of individuals with gastric cancer who received chemotherapy treatment was higher than the GST activity of individuals with gastric cancer who did not receive chemotherapy (Table 4). When these two GST values are compared statistically, the difference is significant ($p<0.05$).

The GST activity of individuals with colon cancer who received chemotherapy treatment was higher than the GST activity of individuals with colon cancer who did not receive chemotherapy (Table 4). When these two GST values are compared statistically, the difference is significant (p<0.05).

Table 4 Comparison of GST activity in cases before and after chemotherapy

Parameters	No of Subjects (n)	Mean ± SD	p- Value
Gastric Cancer before Chemotheraphy	15	0.97 ± 0.12[a]	0.001
Gastric Cancer after Chemotheraphy	15	1.53 ± 0.24[b]	
Colon Cancer before Chemotheraphy	15	1.09 ± 0.12[a]	0.001
Colon Cancer after Chemotheraphy	15	1.56 ± 0.13[b]	

*When the patients are compared according to the treatment type, the same letters are statistically insignificant compared to each other. Different letters are important (p<0.05)

4. Discussion

GSTs are the most important class of phase II detoxification enzymes involved in the metabolism of carcinogens, environmental pollutants, and therapeutic drugs. Any factor that disrupts the detoxification process can lead to the accumulation of carcinogens and thus an increased risk of cancer. Therefore, GSTs can be considered biomarkers of cancer susceptibility and chemo-preventive activity [2,3,15,16].

In many studies conducted with patients with different cancers, it has been shown that the amount of GST in the tissues and erythrocytes of individuals with cancer is higher than in healthy individuals [2,3,9,17]. Patel et al. [18], in their study with patients with oral cancer and Gromadzinska et al. [19] with patients with lung cancer, found erythrocyte GST activity to be higher in individuals with cancer than in healthy control groups. Again, in the studies conducted by Upadhya et al. [20] and Saygılı et al.[21], in individuals with colon cancer, it was reported that the GST activity in individuals with colon cancer was significantly higher than the GST activity in healthy individuals. Likewise, in their study of patients with gastrointestinal system tumors, Scibior et al. [22]. Mandal et al., [23], stated that GST activity in cancerous gastric and colon tissues was statistically significantly higher than in the control group. In our study, the erythrocyte GST activity of both individuals with colon cancer and gastric cancer was significantly higher than the GST values of control individuals (p<0.05) (Table 2). These results show that our study is parallel with the studies mentioned above.

The lack of antioxidant substances such as reduced glutathione in GST activity or the presence of any defect in the genetic expression of GST may cause a decrease in the antioxidant capacity of the organism. Consequently, low levels of GST in tissues and blood reduce the capacity to detoxify carcinogens and carry a higher risk of tumors. It has been observed that the tumor risk will be less in tissues with sufficient GST activity [24–26].

However, as mentioned above, many studies have shown that GST in cancerous tissues and erythrocytes is considerably higher than in healthy tissues. The increase in the GST level accelerates the metabolism of many drugs metabolized by this system in chemotherapy and other drug treatments, thereby eliminating the harmful side effects. Therefore, GST activity is increased in cancer patients receiving chemotherapy treatment. GSTs protect cells from lipid peroxidation and H_2O_2 as the cisplatin-based chemotherapy drug increases them. In addition, in various studies, as cancer progresses, the increase in GST levels has been associated with poor prognosis and drug resistance [3,11,27]. In this study, the GST activity of individuals with gastric and colon cancer who received chemotherapy treatment was significantly higher than the GST values of individuals who did not receive chemotherapy (p<0.05) (Table 4). It was thought that the use of GST inhibitors could be beneficial in regulating the effectiveness of traditional electrophilic cancer drugs in chemotherapy, and various compounds targeting this system were developed. Because the increased GST activity in the erythrocytes of individuals with cancer reduces the effects of chemotherapy drugs and strengthens the idea that resistance in tumor cells may develop to these drugs [28]. In all the groups we studied, the effect of gender difference on GST activity was statistically insignificant (p>0.05) (Table 3). Similar to the studies of Patıl et al. [2], the differences in GST activities of smokers and non-smokers in our study were statistically insignificant in all groups studied (p>0.05) (Table 3).

5. Conclusion

Progressive increase in level of GST with progression of colon and gastric and colon cancer may be assosiaced with drug resistance as well as poor prognosis. Measuring GST activity may be helpful in the evaluation of preventive treatment in studies considering antioxidant strategies. Determination of changes in erythrocyte GST activity is a promising indicator for monitoring oxidative stress conditions occurring in CC and GC. We think that the measurement of GST activity will be helpful in the evaluation of prophylactic treatment in antioxidant strategy trials.

Compliance with ethical standards

Acknowledgments

This study was supported by Republican University Scientific Research Projects Commission [F-330].

Disclosure of conflict of interest

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Statement of ethical approval

Cumhuriyet University, Faculty of Medicine, Scientific Research Assessment Board has been working with the permission of decision 20120-04 / 10.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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