



Glutathione-S-Transferase and GST- π in Gastric Carcinoma

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Abstract

Aims: To analyze the level of serum Glutathione-S-Transferase (GST) and Glutathione-S-Transferase pi (GST π) before and after Cycles Of chemotherapy in patients suffering from gastric carcinoma.

Methods: For the study comprising total 30 cases suffering from gastric carcinoma before and after chemotherapy were selected. All patients were clinically and histologically diagnosed. A total of 50 age and sex matched healthy subjects taken as control. The circulating levels of GST and GST π activity were assayed in the in the serum of control group and in patients with gastric carcinoma.

Results: Mean GST and GST π activity in serum were significantly higher in gastric carcinoma patients as compared to control ($p < 0.001$). After chemotherapy the activity of GST and GST π were significantly higher than before chemotherapy. It indicated that plasma GST and GST- π also may be useful in diagnosing and monitoring gastric carcinoma. The generation of free radicals as reflected by increased GST and GST- π activity in carcinoma cases.

Conclusion: In the gastric carcinoma, increased activity of GST and GST Pi isoenzyme was significant as that of the tissue content, the fact that higher serum values of patients with some cancers often reverted to the normal range after treatment of the gastric carcinoma suggested the direct derivation of these enzymes from tumor tissues. Thus, follow up of elevated serum GST and GST Pi levels may be useful for monitoring gastric carcinoma patients during the course of treatment. Our results indicated that plasma GST- π also may be useful in diagnosing gastrointestinal cancer.

Keywords: Cisplatin, capecitabine, stomach cancer, tumor marker, chemotherapy, Glutathione-s-transferase, GST π , ROS, GIT and radiotherapy.

INTRODUCTION

“Carcinoma is a type of malignant neoplasm that develops in epithelial cells. Specifically, a carcinoma begins in a tissue that lines the inner or outer surfaces of the body, and that generally arises from cells originating in the endodermal or

ectodermal germ layer during embryogenesis”. Cancer or malignant neoplasm is referred as a group of diseases characterized with uncontrolled growth with increase of abnormal cells. If the growth is uncontrolled, this can result in death. The development of malignancy is promoted by

internal as well as external factors. Furthermore these external and internal factors may act upon to initiate carcinogenesis. The promotion of most malignancies requires multiple steps that occur over many years. Certain types of malignancies can be prevented by abstinence of tobacco and other factors that promote the development of malignancy.

A large proportion of human malignancies are claimed to be caused by lifestyle or dietary factors. Our diet contains many toxic or potentially carcinogenic compounds, which are absorbed and metabolized in the GIT. Upper GIT is a common site for malignant neoplasm, especially malignant tumors. However there are variations in incidence among the component site from esophagus to anus; furthermore number of histologically confirmed types of tumors at these sites differs in their incidence and prognosis ^[1]. The GIT malignancy includes malignant condition of the GIT and all accessory organs of digestion inclusive ileum, colon and rectum. The disease symptoms pertaining to the tissue affected may include abnormality in functioning or other disease conditions. The conditions for prognosis often require endoscopy followed by biopsy of suspected organ ^[2,3].

Malignancy of stomach is also referred as gastric carcinoma. Worldwide gastric carcinoma was the fourth most common malignancy till 2002, but recently reported that gastric adenocarcinoma is the second most common malignancy upto 2015 and is leading cause of death ^[4]. In India according to the National Registry programme, esophagus and gastric malignancies are the most common malignancies found in men, while esophagus malignancy ranks third among women there after carcinoma of breast and cervix. Gastric carcinoma is the fourth leading malignancy in the world and the second most common cause of death due to malignancy. Nearly 1 million new cases of gastric carcinoma and 0.7 million gastric carcinoma deaths are reported every year. Age standardized incidence rates are approximately twice as high in men as in women, ranging from 3.9 million in

Northern Africa to 42.4 million in Eastern Asia for men and from 2.2 million in southern Africa to 18.3 million in Eastern Asia for women ^[5].

Currently gastric carcinoma is more common in Asia than in United States of America and Europe.

CLINICAL FEATURES OF GASTRIC CARCINOMA

- Usually asymptomatic until late.
- Symptoms: weight loss, abdominal pain, nausea, vomiting, altered bowel habits
- Metastases to Supraclavicular nodes (Virchow's node) may be first clinical manifestation.
- Sites: pyloric antrum and cardia; lesser and greater curvature.
- Japan: mass endoscopy programs led to 35% early gastric cancers vs. 10% in US.
- Well differentiated tumors may grow very slowly rarely occurs in gastric stump after partial gastrectomy for ulcer.
- Minute (< 5 mm) poorly differentiated tumors may show no gross features, however, chromo endoscopy increases their detectability

INCIDENCE RATE OF GASTRIC CARCINOMA

Through in India, the incidence of gastric carcinoma reported is very low as compared to that of western countries, the number of new gastric carcinoma cases reported is approximately 34,000; with male's predominance, (male to female ratio is 2:1). It was estimated that by the year 2020, approximately 50,000 new cases of gastric carcinoma will be reported annually in India. National survey of malignancy mortality in India reported gastric carcinoma as the second most common cause of malignancy related deaths amongst men and women ^[6]. Annual incidence rate of gastric carcinoma in India reported 10.6 per 100,000 populations, whereas the incidence rate in male 5.7 per 100,000 men and in female 2.8 per 100,000 women ^[7]. It is documented that GIT malignancy has high prevalence in southern

part of India, however recent data highlights that the incidence rates are higher in the north-eastern part of India also [8]. As per latest reports available from National Cancer Registry Programmed the

incidence rate of gastric carcinoma documented as below [9,10]

TABLE 1: Incidence of Gastric carcinoma in India as per National Cancer Registry Programme (NCRP) of India.

Registry	Mumbai	Bangalore	Chennai	Thiruvan-anthapuram	Delhi	Auran Gabad	Barshi	Bhopal
Men	4.2 %	9.1%	12.2%	4.8%	3.4%	1.7%	1.6%	1.6%
Women	2.4%	5.5%	5.2%	1.9%	1.6%	0.8%	1.0%	1.3%

CLASSIFICATION OF GASTRIC CARCINOMA

The World Health Organization (WHO) and the Japanese classifications are describe elaborately several histopathological types of gastric carcinoma and are useful for the prognosis based on the grade of the histological differentiation of early lesion.

- Adenocarcinoma Adenosquamous carcinoma
- Squamous cell carcinoma
- Small cell carcinoma
- Undifferentiated carcinoma
- Other carcinoma

Gastric carcinoma is defined as adenocarcinoma limited to the mucosa and submucosa of the stomach, regardless of lymph node status. The morphology of gastric carcinoma can be sub classified as protruded or elevated, and flat, depressed or excavated a relevant classification, in consideration with endoscopic section. In Japan when aggressive screening programmes were employed, close to 50% of treated cases represent early gastric carcinoma. This presentation is usually well differentiated (70%) lymph node metastasis and is present in about 10% of the cases. The overall cure rate of these malignant neoplasms is 95% when resection and lymphadenectomy is performed [11].

RISK FACTORS OF MALIGNANCY IN INDIA

The malignancy causes in India are almost same as in other parts of the world. The chemical,

biological and other environmental identities are responsible for uncontrolled and unorganized proliferation of cells i.e. carcinogens. Basically under special circumstances carcinogens interact with DNA of the normal cell resulting into series of complex multistep processes responsible for uncontrolled cell proliferation or tumor. The causes for malignancy can be both either internal factors like inherited mutations, hormones, immune conditions or external factors like environmental factors such as tobacco, diet, alcohol, radiation and infectious agents. There are significant variations in incidence of malignancy due to life style and food habits [12,13]. It is interesting to mention here that the rates of these malignancy incidences increase substantially when Asians migrates to the Western Countries; indicating a clear relationship of carcinogenesis with food habits and life styles.

Glutathione-S-Transferase (GSTs E.C.2.5.1.18) is a family of dimeric enzymes that are widely distributed in animal kingdom, divided into the main classes' α , μ , π and θ respectively [48] and it detoxify of foreign compounds. They participate in antioxidant defenses through several mechanisms including reactive oxygen species (ROS) and conjugation of reduced glutathione with large number of electrophiles [14]. Such conjugation reactions results in the synthesis of mercapturic acid and represent an important excretory route for xenobiotics including carcinogens toxins and drugs [15].

GSTs catalyze the binding of large variety of electrophils to the sulphhydryl group of GSH

yielding less harmful and more water soluble molecules, which can be excreted via bile or urine. Since most reactive, ultimate carcinogenic forms of chemicals are generally electrophils, GSTs takes considerable importance as a mechanism for carcinogen detoxification^[16]. Our diet contains many toxic or potentially carcinogenic compounds which are absorbed and metabolized in the gastrointestinal tract (GIT). Metabolization or biotransformation of enzymes such GSTs also occur in the epithelial cells all over the human GIT^[17].

Glutathione-s-transferase pi (GST π) isoform present in many species and tissues and also in relatively large amounts in the epithelial tissues of the human gastrointestinal tract. This enzyme has been used as a possible marker for gastrointestinal cancer. GST π , the predominant isoform in the normal cell, is present in a wide range of normal human tissues, as well as in various malignant tumors of urinary, digestive, and respiratory tracts^[18]. No consensus has been achieved yet regarding to the association between GST π expression and malignant transformation. Some studies suggest an increased activity of GST pi indicator for premalignant and malignant changes^[19]; whereas, in others, GST pi expression is indicated to be a marker of carcinogen exposure in the upper GIT tract^[20]. Meanwhile, in some studies, loss of GST pi expression is suggested as a phenotype associated with carcinogenesis^[21].

As to the alternation of GST π in development of gastric carcinoma, several studies have been performed on Barrett's metaplasia and adenocarcinoma with results suggesting deficiency of GST pi may contribute to an increased cancer risk^[22]. However, limited knowledge is available in terms of GST π alternation in gastric carcinoma, as well as its connection with clinical parameters. Therefore, in the present study, we report results of an immunohistochemical survey of GST and GST π in 30 gastric carcinoma patients with 3 years follow-up. In this our study, serum GST and GST π activity has been measured before and after different cycle of chemotherapy in patients

suffering from gastric carcinoma compared with normal healthy control group.

MATERIAL AND METHODS

Selection of Patients

For the study total 30 cases of gastric carcinoma before and after chemotherapy were selected. All patients were clinically and histologically diagnosed, whom underwent potentially curative surgery during 2013-Apr 2017 at Chandulal Chandrakar Memorial Medical College and Apollo Hospital Durg, were enrolled in this retrospective study. The median age at diagnosis was 55.42 ± 4.67 years (range 35-75 years). No preoperative chemotherapy and radiotherapy were given. All patients with stage-II received chemotherapy including cisplatin, 5-FU capecitabine, cyclophosphamide, Transtuzumab and doxorubicin. There are 17 males & 13 female of gastric carcinoma. For control total 50 normal healthy (the median age 35.40 ± 5.72) age and sex matched persons were selected.

Collection of samples

Overnight fasting 5ml blood sample were collected before and after different cycle of chemotherapy in plain bulb. Serum was separated and used to estimation of glutathione-s-transferase, 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^[14] and Estimation of serum GST π was carried out by using commercial available kits from accu-bind. On ELISA micro plate Immunoenzymometric assay^[23].

Follow Up

Overall 47 patients were followed up at time of admitted in hospital and after discharge from hospital. Out of 12 patients follow up were lost during the follow up period. The follow up system consisted of measurement of tumor marker GST and GST π level before and after chemotherapy.

The follow up program included, clinical examination, hematological analysis, tumor marker and enzyme assay at each check up. Criteria for the establishment of recurrent disease included histological conformation or disease evident radiological with subsequent clinical progression and supportive biochemical data. Seven patients expired during the follow up period.

Data Analysis

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 < 0.05 were considered statistically significant.

TABLE-2 Control group and gastrointestinal carcinoma patient’s data.

	Control	Stomach
No of Cases	n=50	n=28
Age ± S.D yrs	35.40 ± 5.72	55.42 ± 4.67
Male	31	17
Female	19	13
Stage I (before chemotherapy)	50	30
Stage II (After Chemotherapy)	50	30

OBSERVATIONS AND RESULTS

TABLE 3: Comparison of serum GST and GST pi activity in control with oesophagus cancer

Biochemical Parameters	No. of cases	Mean ± SD	“ P” Value
GST Control	50	5.05 ± 0.51	-
GST in patients IU/L	30	9.13 ± 1.71	<0.001
GST π control	50	8.13 ±3.11	-
GST π in patients IU/L	30	18.95 ±11.71	<0.001

Table 3 shows the activity of GST highly significantly increased in gastric carcinoma patients than control group; level of GST in gastric carcinoma patients 9.13 ± 1.71 and in control group was 5.05 ± 0.51

The level of GST π 18.95 ± 11.71 in gastric carcinoma patients and 8.13 ± 3.11in normal

control group; means the activity of GST π expression was highly significantly increased found in gastric carcinoma patients compared to normal control group.

TABLE 4: Activity of GST and GST pi before and after Chemotherapy

Parameters	Control	Before Chemotherapy	After Chemotherapy	‘p’ Value
No of cases	50	30	30	
GST (Mean ± SD)	5.05 ± 0.51	9.13 ± 1.71	14.06 ± 1.95	<0.001
GST π (Mean ± SD)	8.13 ±3.11	18.95 ±11.71	23.06 ± 12.96	<0.001

All values are given as mean ± S. D.

Stage I- Without any treatment (Surgery, chemotherapy, Radiotherapy)

Stage II- After First Cycle of Chemotherapy

Table 4 shows the activity of GST and GST π highly significantly increased in after chemotherapy (14.06 ± 1.95, 23.06 ± 12.96) than before chemotherapy (9.13 ± 1.71, 18.95 ±11.71) and control group (5.05 ± 0.51, 8.13 ±3.11). It indicated that plasma GST and GST-π also may be useful in diagnosing and monitoring gastric

carcinoma. The generation of free radicals as reflected by increased GST and GST-π activity in carcinoma cases.

DISCUSSION

The present study was carried out in the Dept. of Biochemistry in collaboration with Dept. of Pharmacology, Medicine and Surgery Chandulal Chandrakar Memorial Medical College and Hospital Kachandur, Durg. Serum sample obtained from 30 gastric carcinoma patients

admitted for evaluation & treatment were analyzed for the assay of Glutathione-s-transferase (GST), Glutathione-s-transferase pi (GST π) and routine investigation. Latter on these patients were referred for treatment to specialized cancer hospital.

It is important to know that the some symptoms of carcinoma are malignant tumors, space occupying lesions, nagging cough, disturbances in bowel movement, change in bladder habits, bleeding, stomach upset, fever, fatigue, weight loss, pain, skin changes, white spot on tongue, sores that do not heal and indigestion but a single clinical sign or symptom is not enough to specify diagnosis. Malignancy is the second most common cause of death in developed countries followed by cardiovascular disease.

Oral cavity gastrointestinal tract carcinoma occurs most often in India. The malignancy etiology is a result of combination of the genetic, environmental and behavioral factors ^[24]. Smoking & alcohol ingestion are proven etiologic factor in the development of esophagus carcinoma and cigarette smoking has also been linked with gastric carcinoma ^[25].

The activity of serum GST was higher in 78.57% and GST π in 100% patients of gastric carcinoma in this study supports the finding of Niitsu et al. and Tsuchida et al. ^[26,27] the increased activity of GSTs in tumor tissue can be due to over expression isoenzymes of GSTs in response to metabolic changes in tumor cells.

In the present study serum GST was significantly higher in gastric carcinoma patients (before and after chemotherapy $P < 0.001$) as compared to those obtained from normal healthy control group. G.S.Mahammadzadeh et.al ^[28] and N. R. Hazari et. al ^[29] observed similar result which is stastically insignificant in which plasma activity was significantly higher in esophagus carcinoma and gastric carcinoma patients. The GST activity in plasma represents a non invasive biomarker of the cellular protection.

Previously, GST pi expression in tissue and serum was suggested as a cancer marker in several

studies^[19], with results showing inconsistent GST pi expression patterns in various carcinomas. The human GST π class was found to be over expressed in most of carcinoma patients. GST π expression in response to tumor formation is probably a defence mechanism to aid cells to survive and the source of plasma enzyme is mainly the transformed cells with over expression of GST π . In gastric cancer, an increased serum GST pi was hypothesized to correlate to the advanced stage, and its expression in tissue was found inversely correlated to survival. Palanisamy Pasupathi et. al 2009 ^[28] studied 100 patients of gastric carcinoma and reported significant decrease in the activity of SOD, CAT, GPx, GR and GST in the erythrocytes lysate in gastric carcinoma patients as compared to control. Lee et al. proposed that early loss of GST π expression could lead to increased susceptibility to carcinogens and promoting mutation and cancer development. It is also possible that loss of expression is a bystander effect of some other critical event in prostate cancer development such as methylation of broader chromosomal region or loss of transcriptional factor, which is necessary for maintenance for GST π expression.

CONCLUSION

In the gastric carcinoma, increased activity of GST and GST π isoenzyme was significant as that of the tissue content, the fact that higher serum values of patients with some cancers often reverted to the normal range after treatment of the gastric carcinoma suggested the direct derivation of these enzymes from tumor tissues. Thus, follow up of elevated serum GST and GST π levels may be useful for monitoring gastric carcinoma patients during the course of treatment. Our results indicated that plasma GST- π also may be useful in diagnosing gastrointestinal cancer. However the role of these findings in clinical practice and their utility in early detection, prognosis and treatment planning of gastric carcinoma may need further research in depth

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