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RESEARCH ARTICLE

mRNA Expressions of Gamma-Glutamyl Transferase Genes in Different Types of Cancer

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SUMMARY

Measurement of gamma-glutamyl transferase (GGT) activity in serum has been found useful in showing tissue damages. In addition to GGT1, different related genes or sequences in the human genome have been reported in pre-genome studies. Two of them, GGT5 and GGT6, are active genes and have similar nucleotide or amino acid sequence to GGT1. The aim of this study was to evaluate the gene expression of GGTs in different types of cancer. In the current study, GGT1, GGT5, and GGT6 mRNA expressions were measured in normal and tumor tissues of 26 patients with breast, gastric and colorectal cancers, using quantitative RT-PCR method. Serum GGT levels were measured by a spectrophotometric method. While GGT1 and GGT5 were expressed in almost all the normal and tumor tissues of the patients, GGT6 expressions were observed in half of both the normal and tumor tissues. In all patients, no significant differences were found in mean mRNA expression levels of GGT1, GGT5 and GGT6 ($p>0.05$), although GGT1 were higher in tumor tissues than those in the normal tissues. GGT1 was overexpressed in half of the patients (13/26) and these over-expressions were positively correlated with gender ($p<0.05$) and age ($p<0.05$). In female patients, serum GGT levels were positively correlated with tumor tissue GGT1 ($p<0.01$) and GGT6 ($p<0.05$). Mostly increased mRNA expressions for GGT1 and GGT5 in tumor tissues, overexpressions in some, show their roles in carcinogenesis. The results given here may suggest that the tumor GGT1 is mainly responsible for the increase in serum GGT levels in female patients.

Key Words: *Gamma-glutamyl transferase, GGT1, GGT5, GGT6, mRNA expression, cancer*

Değişik Kanser Türlerinde Gama-Glutamil Transferaz Genlerinin mRNA Ekspresyonları

ÖZET

Serumda gama-glutamil transferaz (GGT) aktivitesinin ölçümünün doku hasarlarının gösterilmesinde faydalı olduğu bulunmuştur. GGT1'e ek olarak, insan ön-genom çalışmalarında farklı ilgili genler veya sekanslar bildirilmiştir. Bunlardan ikisi, GGT5 ve GGT6, aktif genlerdir ve GGT1'e benzer nükleotid veya amino asit dizisine sahiptir. Bu çalışmanın amacı farklı kanser türlerinde GGT'lerin gen ekspresyonunu değerlendirmektir. Bu çalışmada meme, gastrik ve kolorektal kanserli 26 hastanın normal ve tümör dokularında kantitatif RT-PCR yöntemi ile GGT1, GGT5 ve GGT6 mRNA ekspresyonları ölçüldü. Serum GGT düzeyleri spektrofotometrik bir yöntemle ölçüldü. GGT1 ve GGT5, hastaların hemen hemen tüm normal ve tümör dokularında eksprese edilirken, GGT6 ekspresyonu hem normal doku hem de tümör dokularının yarısında gözlendi. Tüm hastalarda, GGT1 tümör dokularında normal dokulardan daha yüksek olmasına rağmen, GGT1, GGT5 ve GGT6' nın ortalama mRNA ekspresyon seviyelerinde anlamlı farklılıklar bulunamadı ($p>0.05$). GGT1 hastaların yarısında aşırı eksprese edildi (13/26) ve bu aşırı ekspresyonlar cinsiyet ($p<0.05$) ve yaş ($p<0.05$) ile pozitif olarak korele idi. Kadın hastalarda, serum GGT düzeyleri tümör dokusu GGT1 ($p<0.01$) ve GGT6 ($p<0.05$) ile pozitif korelasyon göstermiştir. Çoğunlukla, tümör dokularında GGT1 ve GGT5 için artmış mRNA ekspresyonları, bazılarında aşırı ekspresyonlar, karsinogenezideki rollerini göstermektedir. Burada verilen sonuçlar, tümör GGT1'in esas olarak kadın hastalardaki serum GGT düzeylerindeki artıştan sorumlu olduğuna işaret edebilir.

Anahtar Kelimeler: *Gama-glutamil transferaz, GGT1, GGT5, GGT6, mRNA ekspresyonu, kanser.*

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INTRODUCTION

Gamma-glutamyltransferase (GGT; EC 2.3.2.2) is also known as 5-L-glutamyl-peptide: amino acid 5-glutamyl transferase and being found in bacteria, plants and in the animal kingdom, including humans (Whitfield, 2001). GGT is located on the outer surface of the plasma membrane of cells, and its active site is fixed to the plasma membrane having ecto-activity (Horiuchi, 1978; Terzyan, 2015). The enzyme belongs to the N-terminal nucleophile hydrolase superfamily and is able to cleave the gamma-glutamyl peptide bond in glutathione (GSH) and other proteins, and transfer the gamma-glutamyl moiety to acceptors, such as other amino acids, water, or even GSH itself (Lieberman, 1995; Corti, 2010). GGT also cleaves and removes the gamma-glutamyl moiety from leukotriene (LT) C4 to give leukotriene (LT) D4. GGT has also been shown to have activities on induction of bone resorption in transgenic mice (Hiramatsu, 2007). GGT deficiency is very rare in humans. In these people, glutathione is secreted in the urine of humans with deficiency, and they cannot metabolize LTC4 (Heisterkamp, 2008).

Presence of the enzyme was shown in many places, such as kidney, liver, pancreas, prostate, testis, small intestine, brain, heart, lung, spleen, central nervous system, platelets, leukocytes, fibroblasts, bone marrow cells and serum, but enzymatic activity is relatively higher in the kidney, pancreas, and intestine (Meister, 1981; Castellano, 2013). Increased levels of GGT has been observed in various cancers, such as lung, ovary, breast, colon, liver, pancreas, prostate, brain, skin, astrocytic glioma, soft tissue sarcoma, melanoma and leukemia (Hanigan, 1999; Pompella, 2006). Though the evidence shows that GGT predicted cancer mortality even at physiologic high levels (Long, 2014) and might be involved in tumor biology (Kunutsor, 2015), the underlying pathways remain unclear.

Genome research indicated that besides GGT1, the human genome, contains additional related genes or sequences (Heisterkamp, 2008). All human sequences related to GGT1 using genomic and cDNA database searches were evaluated and 13 genes have been identified to belong to the GGT family. At least six of these genes appeared to be active. From the perspective amino acid sequences, genes with substantial (GGT5 and GGT7) or limited (GGT6) similarity to GGT1 have been identified, and these have been included into separate families (Heisterkamp, 2008).

GGT genes show widespread expressions in both human normal and tumor tissues. According to a database study, while GGT1 mRNA expressions are normally observed in blood and tissues of mammary gland, lymph and prostate and were also detected in tissues of the breast, uterus and lung cancers, and leukemia (Heisterkamp, 2008). There are studies which demonstrated that a protein is made from GGT1 with enzymatic activity (Visvikis, 1991).

Cysteinyl leukotrienes, including leukotriene C4 (LTC4), D4 (LTD4) and E4 (LTE4), are the members of eicosanoid-GSH conjugates well known as pro-inflammatory mediators in allergic diseases. Recent studies underline the role of these inflammatory mediators in various diseases, such as the central nervous system (CNS) disorders, atherosclerosis, and cancer (Ghosh, 2016). While GGT1 has functions in the metabolism of glutathione and LTC4, GGT5 (formerly Gamma-Glutamyl Leukotrienase (GGL), GGTLA1/GGT-rel) has a role in inflammation by converting LTC4 to LTD4 (Carter, 1997). GGT5 gene shows expression in normal tissues of adrenal gland, spleen, blood vessels, thymus, and bladder and is also detected in human tumor specimen in the cancers of primitive neuroectodermal tumor (PNET), kidney, glioma and esophageal. Its expression may be dynamically regulated in some conditions. GGT5 also has a protein with enzymatic activity (Heisterkamp, 2008). Previous studies using GGT1 and GGT5 null mutants showed that GGT1 was the main enzyme involved in glutathione metabolism (Carter, 1997; 1998), whereas GGT5 was mainly involved in LTC4 metabolism (Han, 2002). In a recent study carried out in cell lines, it has been shown that GGT1 rapidly catalyzed the LTD4 formation in the epithelial lung cancer cells, GGT5 catalyzed slower in primary bronchial epithelial cells (Lukic , 2016).

Limited expressions of GGT6 (formerly rat ggt6 homologue) were observed in normal tissues of the esophagus, trachea, kidney, bladder, adrenal gland and the intestine and tumor tissues of the adrenal gland, colorectal and the breast. The function of GGT6 as an enzyme has not yet been described and there are no studies showing its translation into protein (Heisterkamp, 2008).

In this study, the mRNA expressions of GGT1, GGT5 and GGT6 were measured in normal and tumor tissues in breast, gastric and colorectal cancers by qRT-PCR. It was aimed to see whether the expressions of GGT1, GGT5 and GGT6 were tumor specific.

It is believed that this is the first study investigating GGT1, GGT5 and GGT6 expressions in normal and tumor tissues of the same patients with colon, breast and gastric cancers. The aim was to measure and compare mRNA expressions of GGT1, GGT5 and GGT6 in normal and tumor tissues of cancer patients. It was also aimed to investigate overexpressions of the genes and their association of patients' clinicopathological features.

MATERIALS AND METHODS

Patients

Thirteen patients with colon cancer, 7 patients with breast cancer and 6 patients with gastric cancer, who were followed up by Ankara Oncology Training and Research Hospital, were included in this study. Seventeen out of the 26 patients were female and 9 were male. All the patients have recently been diagnosed by biopsy, and have not received any clinical or radiological anti-cancer treatment at the time of the study. Tumor and non-tumor tissue samples were obtained from patients, who were decided to have resected tissues for treatment. The patients were classified according to the TNM system developed by American Joint Committee on Cancer (Greene, 2006). Informed consents were obtained according to the Helsinki Declaration from all the patients before the study. This study was approved by the Clinical Research Ethics Committee of Mersin University (22.11.2012; decision no: 2012/355). Baseline characteristics of the patients are given in Table1.

Table 1: The clinicopathologic characteristics and laboratory findings of the patients

	Patients (n=26)
Age (SD)	55.48 (10.41)
Cancer type	
Colorectal Cancer (CRC), n (%)	13 (50)
Gastric Cancer (GC), n (%)	6 (23)
Breast Cancer (BC), n (%)	7 (22)
Stage of Disease (Stage I, II,III,IV) *, n	7,7,7,5
Gender	
Female: CRC, GC, BC (n)	8, 2, 7
Male: CRC, GC, BC (n)	4, 5, 0
Menopausal status	
Premenopausal, n (%)	5 (29.4)
Postmenopausal, n (%)	12 (70.6)
Laboratory Findings, mean(SD)	
Glukoz (mg/dl)	132.3 (48.3)
Uric acid (mg/dl)	3.3 (1.1)
Blood urea nitrogen (BUN, mg/dl)	13.7 (5.6)
Total Protein (g/dl)	5.5 (0.8)
Albumin (g/dl)	3.0 (0.6)
Aspartate transaminase (AST, U/L)	23.5 (14.2)
Alanine transaminase (ALT, U/L)	16.7 (14.9)
Gamma glutamyl transferase (GGT, U/L)	
Female	31.9 (21.1)
Male	20.5 (9.4)

*Only one missing data in gastric cancer group

Sample collection and storage

All tumor tissue samples and distant normal tissue samples were collected and stored in RNA SaferTM RNA Stabilization Reagent (SA Biosciences, USA) at 4°C for 12-24 hours, then kept at -80°C until used. Sufficiently distant non-tumor tissues of the colon, breast or gastric were obtained from the same patients as control tissues.

Tissue processing, RNA extraction and cDNA synthesis

Frozen tissue samples (approx.100 mg) were defrosted and homogenized in Tripure Isolation Reagent in a Roche MagNALyser instrument. Samples were separated into three phases. RNA phase was transferred to a nuclease-free polypropylene tube and the method for RNA isolation was applied according to the manufacturers' instructions. Obtained RNA samples were kept frozen at -80°C, to be used later. cDNA was synthesized by RT-PCR using a Roche Transcriptor High Fidelity cDNA Synthesis Kit.

Quantitative real-time PCR analysis

cDNA samples were amplified with the real-time PCR with a Roche LightCycler480 Probes Master Kit using TaqDNA Polymerase. The method was applied according to the manufacturer's instructions and specific forward and reverse primers and probes were used for each gene amplification designed by the kit manufacturer.

Measurements of GGT1, GGT5 and GGT6 mRNA expression levels in normal and tumor tissues were carried out in parallel, and were repeated 3 times. Target gene mRNA expressions were quantified and standardized according to the β-actin reference gene signal. This method does not require precise quantification of starting material. Relative quantification (RQ) values were calculated by formula of the Pfaffl method shown below (Pfaffl, 2001).

$$RQ = 2^{[C_T\,\text{(tumor, ref. gene)} - C_T\,\text{(tumor, targ. gene)}]} / 2^{[C_T\,\text{(calibrator, ref. gene)} - C_T\,\text{(calibrator, targ. gene)}]}$$

Target mRNA expressions were calculated in tumor and normal tissues (calibrator sample), compared to reference mRNA expressions on the basis of the difference between C_T values of the target and reference genes (ΔC_T) (as proportional).

Measurement of serum GGT

GGT activity was measured at 0.05 mM 2-amino-2-methyl-1,3-propanediol buffer pH 8.6 in the presence of $MgCl_2.6H_2O$, Gly-Gly and L-gamma-glutamyl-p-nitroanilide as GGT substrate. The reaction was monitored by following the increase in absorbance at 405 nm linked to the release of p-nitroanilide (Burtis, 1994).

Statistical analysis

Statistical analyses were performed using SPSS 22.0 Software (SPSS Inc., Chicago, IL, USA). The data were presented as mean (standard deviation, SD), and statistical analyses were performed by Mann-Whitney U test. χ^2 tests were performed to evaluate whether mRNA expressions were correlated with clinicopathologic parameters. Spearman's correlation coefficients were calculated for the relationship between the parameters. For all analyses, a P-value <0.05 was regarded as statistically significant.

RESULTS

The GGT expression profiles were determined normalized against β-actin in order to correct for varying amounts of tissue between samples. According to relative quantification (RQ) values of GGT1, GGT5 and GGT6, tumor mRNA expression greater than twofold relative to the corresponding gene expression in normal tissues was considered to be an overexpression.

mRNA expressions in all the patients

In this study, mRNA expressions of GGT1 were found higher, but not statistically significant in tumor tissues, than those in the normal tissues for all of the patients (p>0.05) (Table 2, Figure 1a). GGT5 and GGT6 mRNA expressions were similar in both the normal and tumor tissues for all of the patients (p<0.05) (Table 2, Figure 1b, c). The highest relative expression ratio was found in GGT5, followed by GGT1 and GGT6.

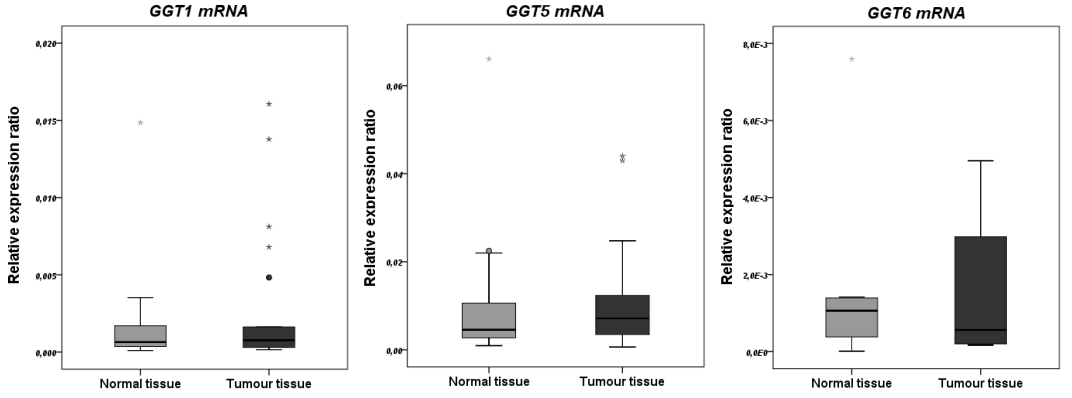
For all the patients, 57.7% (15/26) of samples for GGT1, 61.5% (16/26) of samples for GGT5, 11.5% (3/26) of samples for GGT6 showed higher mRNA expression ratios (RQ>1) in tumor tissues than those in the normal tissues (Table 2).

According to the cancer groups, high RQ values (>1) were observed 85.7% (6/7) for GGT1, 42.9% (3/7) for GGT5, and 14.3% (1/7) for GGT6 in breast cancer group; 66.7% (4/6) for GGT1 and 50% (3/6) for GGT5 in gastric cancer group; 46.2% (6/13) for GGT1, 76.9% (10/13) for GGT5, and 15.4% (2/13) for GGT6 in colon cancer group (Table 2).

Table 2. Relative quantitative (RQ) values for GGT1, GGT5 and GGT6 genes in the patients

No	Type	Sex	Age	Stage	GGT1 RQ	GGT5 RQ	GGT6 RQ
1	BC	Female	44	1	4,97	,76	--*
2	BC	Female	57	2	--*	--*	--*
3	BC	Female	39	2	5,03	,15	--*
4	BC	Female	31	2	2,60	,74	405,33
5	BC	Female	74	2	2,60	,79	--***
6	BC	Female	53	2	3,86	1,74	--*
7	BC	Female	67	3	,56	2,43	,56
8	GC	Female	68	1	12,87	,26	--*
9	GC	Male	61	1	,13	,38	--***
10	GC	Male	62	3	--*	1,85	--*
11	GC	Male	66	3	1,49	4,36	--***
12	GC	Male	46	4	,075	,72	--**
13	GC	Female	51	4	2,63	6,07	,19
14	CRC	Male	55	1	,21	1,19	--**
15	CRC	Male	50	1	,96	1,67	--**
16	CRC	Male	63	1	1,09	1,71	--**
17	CRC	Female	48	1	--*	2,30	--**
18	CRC	Female	59	1	,99	12,61	,38
19	CRC	Male	63	2	,29	1,46	--**
20	CRC	Female	53	2	13,73	2,60	,33
21	CRC	Female	57	3	--*	,19	--***
22	CRC	Female	48	3	,22	,68	--**
23	CRC	Female	53	3	11,05	,70	--*
24	CRC	Female	51	4	,09	1,24	3,11
25	CRC	Male	68	4	1,20	5,33	,02
26	CRC	Female	70	4	,97	6,13	9,91

* No Expression in normal tissues
** No Expression in tumour tissues
*** No Expression in both normal and tumour tissues



(a) (b) (c)

Figure 1: Expressions of GGT1 (a), GGT5 (b) and GGT6 (c) in normal and tumor tissues (p>0.05). The GGT1, GGT5 and GGT6 mRNA expressions in the tumor tissues and corresponding normal tissues of same patients were evaluated by RT-PCR.

mRNAs of GGT1, GGT5 and GGT6 were overexpressed in samples from 13 (50 %), 9 (34.6%) and 3 (11.5%) patients, respectively. According to the cancer groups, overexpressions were observed 85.7% (6/7) for GGT1, 28.6% (2/7) for GGT5 and 14.3% (1/7) for GGT6 in breast cancer group. In gastric cancer patients, overexpressions were found as 50% (3/6) for GGT1 and 33.3% (2/6) for GGT5. In colorectal cancer group, 30.8% (4/13) of the patients had overexpressions for GGT1 and 38.5% (5/13) for GGT5 and 15.4% (2/13) for GGT6 and 15.4% (2/13) for GGT6 (Table 2).

Association of patients' clinicopathological features with mRNA overexpressions

The correlation of mRNA expressions with patients' clinicopathological features, such as gender, age, clinical stage, tumor size, and lymph node metastasis were examined (Table 3). A significant association between GGT1 overexpression with gender and age was found. Of 13 patients with tumors that overexpressed GGT1, 11 (84.6%) were female (p=0.039). Furthermore, 11 of the 13 (84.6%) tumor tissues with overexpressed GGT1 were of patients' under the age of 60 (p=0.016). Similarly, although 9 (69.2%) of 13 tumors that overexpressed GGT1 were in clinical stage I-II, only a weak association between the stage and GGT1 expression (p=0.073) was found. Seven (77.8%) of 9 patients had GGT5 overexpressions in tumor tissues had lymph node metastasis (p=0.044) (Table 3). Due to some undetectable expressions, GGT6 overexpressions could not have been examined.

Serum GGT Levels

Serum GGT levels were found to be higher, but not statistically significant, in female patients, than those in the male patients (p>0.05) (Table 1). Significant positive correlations between serum GGT with GGT1 and GGT6 mRNA expressions were observed in tumor tissues of female patients (n=17, R=0.597, p<0.01 for GGT1 and n=9, R=0.686, p<0.05 for GGT6).

DISCUSSIONS

GGT genes show widespread expressions in both normal and tumor tissues of humans. The role of GGT in cancer is mainly due to its activity on the metabolism of GSH and leukotrienes (Ortega, 2011; Samuelsson 1983). In addition, GGT might promote the release of free iron from transferrin, thus supplying iron to malignant cells. This effect is probably mediated through redox interactions of GGT-derived reactive thiols and/or pro-oxidant species (Dominici, 2003). Previous genome researches indicated that besides GGT1, the human genome contains additional related genes or sequences. All the human sequences related to GGT1 using genomic and cDNA database searches were evaluated and 13 genes have been identified belonging to the GGT family. At least six of these genes appear to be active. From the perspective amino acid sequences, genes with substantial (GGT5 and GGT7) or limited (GGT6) similarity to GGT1 have been identified, and have been divided into separate families (Heisterkamp, 2008).

GGT1 mRNAs is accepted to encode for enzyme, which catalytic properties are identical to those of GGT from human tissues (Visvikis, 1991). In this study, while four patients did not have any expression of GGT1 in normal tissues, fifteen of the patients had higher (RQ>1) expressions, and 13 had overexpression in tumor tissues. One of breast and gastric cancer patients, and two patients with colorectal cancer did

not show GGT1 expression in normal tissues. In a previous study, Hanigan et al. (1999) showed GGT1 expression in tumor tissues in liver, renal, prostatic, pancreatic, and breast carcinomas. They also found that GGT was normally expressed in all breast ductal epithelium (Durham 1997, Hanigan 1996), but not in some breast tumors (Durham, 1997). Hochwald et al. (1997) showed that GGT activity was higher in high-grade tumors than those in low-grade tumors in soft tissue sarcomas. In this study, GGT1 overexpressions were observed in 9 patients with stage I and II, and six of these were in the breast cancer group.

The human GGT5 gene has 40% amino acid identity to GGT1, but has less than 4% of the activity of GGT1 in hydrolyzing GSH, GSSG and leukotriene C4 (Heisterkamp, 1991; Wickham, 2012). However, previous studies using GGT1 and GGT5 null mutants showed that GGT1 was the main enzyme involved in glutathione metabolism (Carter, 1997; 1998) whereas GGT5 was mainly involved in LTC4 metabolism (Han, 2002). There is no published clinical study showing GGT5 expression in cancer. However, in a database study, it was claimed that GGT5 gene showed expression in normal tissues of adrenal gland, spleen, blood vessels, thymus and bladder, and was also detected in human tumor specimen in the cancers of PNET, kidney, glioma and esophageal (Heisterkamp, 2008). In this study, almost all patients had both normal and tumor tissue GGT5 expressions. Only one patient with breast cancer did not show normal tissue expression. Higher GGT5 expressions were found in tumor tissues of 10 colorectal cancer patients than in their normal tissues. Five of them showed overexpressions. An interesting result of this study was that GGT5 had higher relative expression ratio than GGT1.

Limited expressions of GGT6 (formerly rat ggt6 homologue) observed in normal tissues of the esophagus, trachea, kidney, bladder, adrenal gland and intestine, and the tumor tissues of the adrenal gland, colorectal and breast. The function of GGT6 as an enzyme has not yet been described and there are no studies showing that its translation into protein (Heisterkamp, 2008). There is no clinical study on GGT6 expression in cancer to compare with the results shown in this study. In the breast cancer group, the lack of the expression of GGT6 in most normal tissues of the patients (6/7, 86%), but its presence in tumor tissues in stage I and II, was probably a potentially valuable early diagnostic marker for this type of cancer. Unlike the database study, it has been observed here that GGT6 expression was lost in most tumor tissues of colorectal cancer patients (7/13, 54%). These results stipulate the investigation of the function of this GGT type.

Gender differences in serum GGT levels are well known by clinicians, and due to the high GGT activity in prostate tissues in males, serum GGT levels are higher in healthy males than in females. Strasak et al. (2008a; 2008b) suggested that elevated GGT was significantly associated with increased cancer risk in females and males. However in this study group,

Table 3: The associations of GGT1 and GGT5 expressions with clinicopathological parameters in cancer patients. c^2 test, $p<0.05$ indicates a significant relationship among the variables.

		GGT1 mRNA expression			GGT5 mRNA expression		
Characteristics	n	High(%)	Low (%)	P	High(%)	Low (%)	P
Total	26	13 (50.0)	13(50.0)		9 (34.6)	17 (65.4)	
Gender							
Male	9	2(15.4)	7 (53.8)	**0.039**	2 (22.2)	7 (41.2)	0.334
Female	17	11 (84.6)	6 (46.2)		7 (77.8)	10(58.8)	
Age							
£60	10	11 (84.6)	5 (38.5)	**0.016**	5 (55.6)	11(84.6)	0.648
>60	16	2 (15.4)	8 (61.5)		4 (44.4)	6(15.4)	
Clinical stage*							
I-II	14	9 (69.2)	4 (30.8)	0.073	4 (44.4)	9(52.9)	0.571
III-IV	11	4 (30.8)	8 (61.5)		5 (55.6)	7 (41.2)	
Tumor size*							
T1-T2	12	7 (53.8)	5 (38.5)	0.543	4 (25.0)	8 (47.1)	0.790
T3-T4	13	6 (46.2)	7 (53.8)		5 (38.5)	8(47.1)	
Lymph node metastasis*							
N0-N1	23	13 (100)	10 (76.9)	0.125	7 (77.8)	16 (94.1)	**0.044**
N2-N3	2	--	2 (15.4)		2 (22.2)	0 (0)	

*Only one missing data

N0 no regional lymph node metastasis, **N1** metastasis in 1–2 regional lymph nodes, **N2** metastasis in 3–6 regional lymph nodes, **N3** metastasis in seven or more regional lymph nodes

higher serum GGT levels were observed in females than in males. In addition, overexpressions of all three genes were mostly observed in female patients, but there was only a significant correlation between GGT1 and gender. Serum GGT levels positively correlated with tumor tissue GGT1 and GGT6 in females. When these results are combined, it can be suggested that the tumor GGT1 is mainly responsible for the increase in the serum GGT levels in female patients. GGT1 overexpressions are also correlated with age. Overexpressions of GGT1 and GGT6 are mostly observed in age under 60 patients, but there are only significantly correlation between GGT1 and age.

In conclusion, this is the first study examining GGT1, GGT5 and GGT6 mRNA expressions in normal and tumor tissues of the same patients simultaneously, showing GGT5 for the first time in both normal and tumor tissues of the breast, gastric and the colon. Mostly increased mRNA expressions for GGT1 and GGT5 in tumor tissues, overexpressions in some, show their role in carcinogenesis. Overexpressions of all three genes were mostly observed in female patients, but there was only significantly correlation between GGT1 and gender. An interesting result of this study was that GGT5 had higher relative expression ratio than GGT1. This result suggests that more examinations are needed in more detail on the translational level and on the function of GGT5. When the patients are evaluated individually, increased relative expression ratios of GGT1 and GGT5 in tumor tissues and GGT6 in normal tissues suggest that these genes play a role in cancer by different mechanisms. GGT6, due to lack of the expression in most normal tissues, may be a potentially valuable early diagnostic marker for breast cancer and requires the clarification of the function. Additional studies with a larger number of patients with all types of cancer are needed to strengthen the results achieved herein.

Lack of protein expression analysis is the major limitation of this study. Results should further be confirmed with more patients in each group.

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Conflict of interest

The authors declared that this article content has no conflicts of interest.

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