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INFLUENCE OF GSTP1 Ile105Val POLYMORPHISM AND PROTEIN EXPRESSION ON CLINICAL OUTCOME IN PATIENTS WITH COLORECTAL CANCER

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ABSTRACT

Background: GSTP1 plays a central role in the inactivation of toxic and carcinogenic electrophiles, including platinum compounds. In colorectal cancer (CRC) patients treated with oxaliplatin-based therapy, GSTP1 polymorphism and protein expression found to play significant correlation with prognosis but showed conflicting results. Present study assessed the association of GSTP1 Ile105Val polymorphism and protein expression with survival as well as clinicopathological parameters in CRC patients. **Materials and Methods:** GSTP1 polymorphism was examined by PCR-RFLP and protein expression was studied by immunohistochemistry in 143 untreated CRC patients. **Results:** GSTP1 Ile105Val polymorphism showed 51% A/A wild type, 42% A/G heterozygous and 7% G/G variant genotypes. Significant association of GSTP1 polymorphism was noted with family history (P=0.020) and tumor site (P=0.036). Variant G/G genotype was associated with unfavorable prognosis in total CRC, advanced stage and rectal cancer patients. The subgroup of patients having variant genotypes treated with combined 5-FU/oxaliplatin drug had a significant higher incidence of disease relapse and death as compared to those treated with single 5-FU drug. In relation to protein expression, GSTP1 cytoplasmic and/or nuclear immunoreactivity was noted in 95% CRC patients. Cytoplasmic GSTP1 expression was significantly associated with age (P=0.042) and histologic type (P=0.017). Low cytoplasmic as well as low nuclear GSTP1 expression correlated with worse survival in early stage, whereas showed better prognosis in advanced stage patients. **Conclusion:** GSTP1 Ile105Val polymorphism as well as its protein expression could be useful prognostic biomarkers in CRC patients.

KEYWORDS: GSTP1, Ile105Val polymorphism, Immunohistochemistry, 5-FU/oxaliplatin, Prognosis, Colorectal cancer.

INTRODUCTION

The current scenario of treating colorectal cancer (CRC) has shown enhanced effectiveness of standard regimen 5-fluorouracil (5-FU) in combination with a platinum drug-oxaliplatin. In advanced CRC patients, FOLFOX has improved the response rate of 53% as well as progression free survival (PFS) of 9 months as compared to 5-FU alone having 22% response rate and PFS of 6 months,[1] However, tumor cell drug resistance is the major obstacle for oxaliplatin based therapies. Evidence has suggested that resistant mechanisms of platinum may be via the increase of the DNA repair capacity and of the tolerance to DNA damage; and via the inactivation of platinum compounds through the glutathione metabolic pathway.[2] Hence, detoxification of platinum drugs via the action of glutathione-s-transferases (GSTs) is an important resistant factor in patients treated with oxaliplatin based therapy. GSTs are multi-gene family of enzymes which are crucial for the cell defence.[3] Some of the major genes of GST superfamily are GST mi (GSTM1), theta (GSTT1), and pi (GSTP1)[4] and amongst them, Glutathione S-transferase P1 (GSTP1), located on chromosome 11q13.2, is an important host-defense molecule against a range of toxins, and it participates directly in the detoxification of platinum compounds.[5]

The functional polymorphism of the GSTP1 gene involves an A-G substitution in exon 5 and the conversion of isoleucine to valine at position 105 of the amino acid chain (Ile105Val).[6] This genetic Ile105Val polymorphism has been linked to inter-individual difference in altering its expression levels and enzymatic activity, which in turn affects the clinical outcome and

results in variations in oxaliplatin efficacy and prognosis in CRC patients. Moreover, it has been suggested that patients with variant Val allele may be associated with decreased enzymatic activity and hence may have a better response to platinum-based therapy. On the other hand, overexpression of GSTP1 was observed in several types of cancers such as blood, head and neck, lung, esophagus and breast cancers as well as CRC; and may lead to resistance to anticancer drugs and thus influence the clinical outcome.[7] Several studies showed the effect of GSTP1 high expression with unfavorable prognosis in various malignancies.[7-9] GSTP1 is highly overexpressed in colon cancer, with increased levels in drug resistant tumors.[10]

However, controversial reports were observed for association of GSTP1 Ile105Val polymorphism and GSTP1 immunohistochemical localization with prognosis as well as response to therapy in CRC patients.[11-15] Hence, it necessitates the need for better evaluation of their clinical significance both at genetic and protein levels. Therefore, present study aimed to evaluate the prognostic as well as therapeutic efficacy of GSTP1 Ile105Val polymorphism and protein expression in CRC patients. Additionally, its correlation with clinicopathological parameters was examined.

MATERIALS AND METHODS

Patients

Present study included a total of 143 untreated patients with histologically confirmed CRC at 'The Gujarat Cancer & Research Institute' between 2007 and 2014. Written consent of the patients who underwent surgery at the Department of Surgical Oncology was obtained, prior to sample collection. Clinicopathological details are depicted in Table 1. Out of 143, 113 patients were treated with chemotherapeutic regimen. The main chemotherapeutic treatment included were 5-FU and leucovorin, oral Capecitabine, or in combination with Oxaliplatin (OX). The patients were followed for a minimum period of 36 months or until death within that period. Complete follow-up details were obtained in 114 CRC patients and were included for overall survival (OS) analysis. Out of 114 patients, 13 patients who died due to persistent disease were not included for relapse-free survival (RFS) analysis. Survival analysis was also performed in the subgroups of patients with early stage and advanced stage, as well as in the subgroups of colon cancer and rectal cancer. Further, to evaluate the predictive efficacy of GSTP1 on survival according to adjuvant treatment, patients were subgrouped into those treated with 5-FU alone (single drug group) and with combined 5-FU+OX (combined drug group), irrespective of RT. In relation to adjuvant treatment, out of 101, 83 patients were included for RFS; and out of 114, 94 patients were included for OS analysis.

Sample collection

To detect GSTP1 Ile105Val polymorphism, primary tumor tissue samples were collected on ice directly from the operation theatre. Tumor tissues were selected by a pathologist and divided into two portions. One portion was submitted for the routine histopathological evaluation and the other portion was immediately snap frozen in liquid nitrogen and preserved at -80°C till DNA isolation and consequently proceed for polymorphism study. To study GSTP1 immunohistochemical localization, paraffin embedded tumor tissue blocks of CRC patients were collected from the Pathology Department of the institute.

GSTP1 Ile105Val polymorphism by PCR-RFLP

DNA isolation was performed using the frozen tumor tissues by phenol-chloroform extraction method. The quantification of extracted DNA samples was performed by agarose gel electrophoresis using Lambda Hind III digest. Also, the purity of the DNA samples was checked spectrophotometrically at 260 and 280 nm. For GSTP1 Ile105Val polymorphism study, polymerase chain reaction (PCR) analysis was carried out in a total volume of 50 µl using PCR core kit (Qiagen, USA). Primers used for GSTP1 amplification were 5' ACC CCA GGG CTC TAT GGG AA 3' (forward) and 5' TGA GGG CAC AAG AAG CCC CT 3'(reverse). 0.1 µg of genomic DNA was added per reaction. PCR was performed in a ProFlex PCR system (Applied Biosystems, Life Technologies Corporation, USA) using the following conditions: initial denaturation at 95°C for 5 minutes followed by 35 cycles of amplification (denaturation at 94°C for 45 seconds, annealing at 61°C for 45 seconds, extension at 72°C for 1 minute) and final extension at 72°C for 10 minutes. Then digestion of these PCR products was performed with BsmAI restriction enzyme (NEB, USA) at 55°C overnight and separated on 2.5% ethidium bromide stained agarose gel.

GSTP1 protein expression by immunohistochemistry

Immunohistochemical localization of GSTP1 was performed from formalin fixed paraffin embedded tumor tissue blocks of CRC patients. The immunohistochemical staining was carried out using primary mouse monoclonal GSTP1 antibody (Clone: 3F2C2, Santa Cruz, Biotechnology, Inc.; dilution- 1:100) and Mouse and Rabbit specific HRP/DAB (ABC) Detection IHC kit from Abcam, as per manufacturer's protocol recommendations. Prior to application of the primary antibody, antigenicity was retrieved by heating the tissue sections in 10mM tri-sodium citrate buffer solution (pH-6.0) for 20 minutes in a pressure cooker. All sections were scored independently by two independent researchers in a blinded manner. The staining intensities and the percentage of positive cells were assessed independently for all primary tumor tissues (N=143). Previously used modified histoscore (H-score) method was performed for scoring of GSTP1 immunostaining.[16]

Statistical analysis

The data was statistically analyzed using the Statistical Package for Social Sciences (SPSS) software version 17 (SPSS Inc., USA). The distribution of genotypes in patients and healthy individuals was first tested for the Hardy-Weinberg equilibrium (HWE) by a goodness-of-fit Chi-square (χ^2) test to compare the observed genotype frequencies to the expected ones. Two-tailed χ^2 test was used to assess the associations of GSTP1 polymorphism and protein expression with clinicopathological parameters. Correlation between two parameters was calculated using Spearman's correlation coefficient (r) method. RFS and OS were calculated using Kaplan-Meier estimates and the difference in survival curve was calculated using Log rank test. P value ≤0.05 was considered significant.

RESULTS

Incidence of GSTP1 Ile105Val polymorphism and association with clinicopathological parameters

In CRC patients, GSTP1 Ile105Val polymorphism showed 51% (72/143) A/A homozygous wild type genotype, 42% (60/143) A/G heterozygous variant genotype, whereas only 7% (11/143) G/G homozygous variant genotype. The genotype distribution of this polymorphism was consistent with HWE among CRC patients (χ^2=0.095, P=0.757). The representative gel image is shown in Figure 1. With regard to clinicopathological parameters, a significant higher incidence of variant genotypes (A/G+G/G) was observed in patients with family history (P=0.020) and rectal cancer (P=0.036) as compared to those without family history and colon cancer, respectively (Table 1).

Table 1: Correlation of GSTP1 polymorphism and protein expression with clinicopathological parameters.

Characteristics	N	GSTP1 Ile105Val polymorphism		P	Cytoplasmic GSTP1 protein expression		P	Nuclear GSTP1 protein expression		P
		Wild type A/A N (%)	Variant type A/G+G/G N (%)		Low N (%)	High N (%)		Low N (%)	High N (%)	
Age (years) Median: 52 years										
<52	68	38 (56)	30 (44)	0.210	36 (53)	32 (47)	**0.042**	39 (57)	29 (43)	**0.080**
≥52	75	34 (45)	41 (55)		27 (36)	48 (64)		32 (43)	43 (57)	
Gender										
Female	58	31 (53)	27 (47)	0.544	26 (45)	32 (55)	0.879	29 (50)	29 (50)	0.945
Male	85	41 (48)	44 (52)		37 (43)	48 (57)		42 (49)	43 (51)	
Habit*										
No	77	39 (51)	38 (49)	0.939	34 (44)	43 (56)	0.979	38 (49)	39 (51)	0.939
Yes	66	33 (50)	33 (50)		29 (44)	37 (56)		33 (50)	33 (50)	
Family history										
No	133	71 (53)	62 (47)	**0.020**	58 (44)	75 (56)	0.950	67 (51)	66 (49)	0.760
Yes	10	01 (10)	09 (90)		05 (50)	05 (50)		04 (40)	06 (60)	
Diet										
Vegetarian	95	48 (51)	47 (49)	0.953	42 (44)	53 (56)	0.959	48 (51)	47 (49)	0.770
Veg+Non-veg	48	24 (50)	24 (50)		21 (44)	27 (56)		23 (48)	25 (52)	
Tumor site										
Colon	69	41 (59)	28 (41)	**0.036**	35 (51)	34 (49)	0.123	36 (52)	33 (48)	0.563
Rectum	74	31 (42)	43 (58)		28 (38)	46 (62)		35 (47)	39 (53)	
Tumor size										
T2	36	20 (56)	16 (44)		16 (44)	20 (56)	0.905	21 (58)	15 (42)	0.644
T3	95	48 (51)	47 (49)	0.261	42 (44)	53 (56)		42 (44)	53 (56)	
T4	12	04 (33)	08 (67)		05 (42)	07 (58)		08 (67)	04 (33)	
Nodal status										
Negative	90	47 (52)	43 (48)	0.563	41 (46)	49 (54)	0.641	47 (52)	43 (48)	0.426
Positive	53	25 (47)	28 (53)		22 (42)	31 (58)		24 (45)	29 (55)	
TNM stage										
I	24	13 (54)	11 (46)		10 (42)	14 (58)		14 (58)	10 (42)	
II	64	33 (52)	31 (48)	0.473	31 (48)	33 (52)	0.508	32 (50)	32 (50)	0.331
III	51	25 (49)	26 (51)		22 (43)	29 (57)		23 (45)	28 (55)	
IV	04	01 (25)	03 (75)		00 (00)	04 (100)		02 (50)	02 (50)	
Tumor differentiation										
Well	29	16 (55)	13 (45)	0.564	11 (38)	18 (62)	0.460	13 (45)	16 (55)	0.564

Moderate/Poor	114	56 (49)	58 (51)		52 (46)	62 (54)		58 (51)	56 (49)	
Histologic type										
Adenocarcinoma	103	48 (47)	55 (53)	0.152	39 (38)	64 (62)	**0.017**	53 (52)	50 (48)	0.492
Mucinous/Signet ring cell	40	24 (60)	16 (40)		24 (60)	16 (40)		18 (45)	22 (55)	
Pre-op circulating CEA levels (ng/ml) (N=131)										
< 5.0	68	37 (54)	31 (46)	0.555	31 (46)	37 (54)	0.499	34 (50)	34 (50)	0.528
≥ 5.0	63	31 (49)	32 (51)		25 (40)	38 (60)		28 (44)	35 (56)	

*Tobacco chewing, smoking, alcohol, snuff (anyone or in combination)

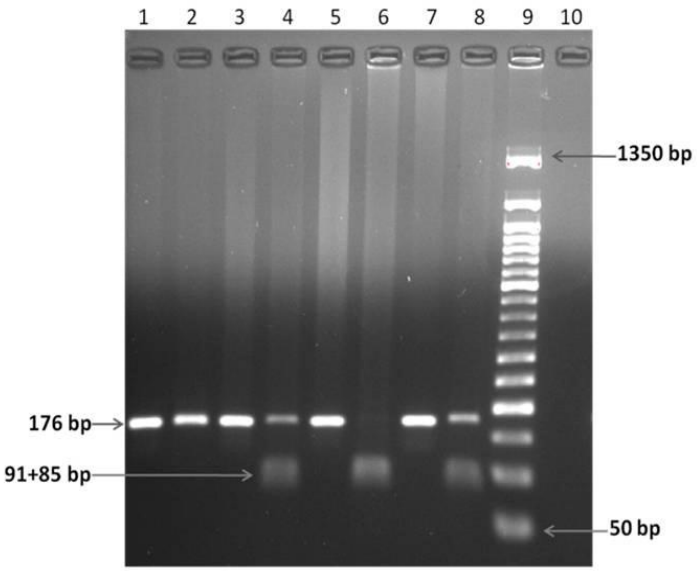

Figure 1: Representative image of GSTP1 Ile105Val polymorphism on 2.5% agarose gel in CRC patients.
Lanes 1, 3, 5, 7: undigested PCR products at 176 bp
Lane 2: presence of A/A homozygous wild type genotype at 176 bp
Lanes 4, 8: presence of A/G heterozygous variant genotypes at 176, 91 and 85 bp
Lane 6: presence of G/G homozygous variant genotype at 91 and 85 bp
Lane 10: negative control
Lane 9: 50 bp ladder

Incidence of GSTP1 protein expression and its association with clinicopathological parameters

GSTP1 exhibited cytoplasmic and/or nuclear expression with heterogenous immunoreactivity in 95% of CRC patients (Figure 2). For statistical evaluation, cytoplasmic and nuclear expressions were scored independently and compared separately. Cytoplasmic GSTP1 expression was observed in 94% of tumors and nuclear GSTP1 expression was present in 75% of tumors. The median H-score values of cytoplasmic and nuclear GSTP1 expressions were used as cut-off value to divide the patients into low (H-score <median) and high (H-score ≥median) expression groups. Cytoplasmic GSTP1 expression was found to be significantly higher in patients with older age as compared to younger age group (P=0.042); and adenocarcinoma as compared to mucinous/signet ring cell adenocarcinoma (P=0.017). Also, a trend of higher nuclear GSTP1 expression was observed in older age group patients as compared to younger age group patients (P=0.080) (Table 1).

Survival analysis of GSTP1 Ile105Val polymorphism

Survival analysis was performed amongst individual three genotypes as well as wild vs combined variant genotypes. In total patients, Kaplan-Meier univariate survival analysis demonstrated that patients with G/G genotype showed a trend of reduced RFS (P=0.051; Figure 3a) and a significant reduced OS (P=0.030; Figure 3b) as compared to those with A/A or A/G genotypes. Further, in early stage disease, A/A genotype was associated with a trend of reduced RFS (P=0.053; Figure 3c), while G/G genotype was associated with a trend of reduced OS (P=0.074; Figure3d). In advanced stage patients, a trend of reduced OS was observed with combined variant genotypes (A/G+G/G) as compared to A/A genotype (P=0.088; Figure 3e). G/G variant type also showed a significant reduced OS in the subgroup of rectal cancer patients (P=0.015; Figure 3f).

In relation to adjuvant treatment, a significant higher incidence of disease relapse in the subgroup of patients

having variant G/G genotype treated with combined drug (67%, 2/3) was observed as compared to those treated with single drug (50%, 1/2; Log rank=6.226, df=2, P=0.044). Also, a trend of higher incidence of death with variant G/G genotype (75%, 3/4) was noted in the subgroup of patients treated with combined drug as compared to those treated with single drug (50%, 1/2; Log rank=5.505, df=2, P=0.064).

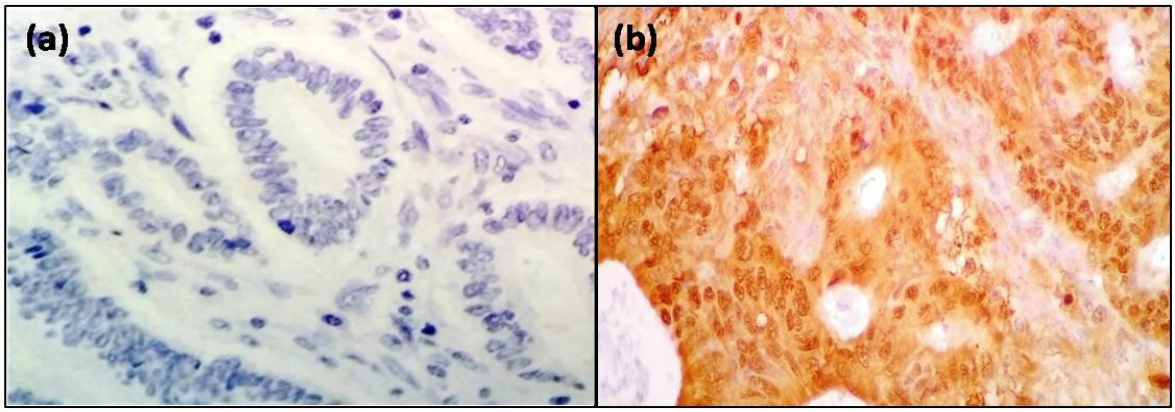

Figure 2: Representative photomicrographs showing (a) Negative (b) cytoplasmic/nuclear staining of GSTP1 (40x).

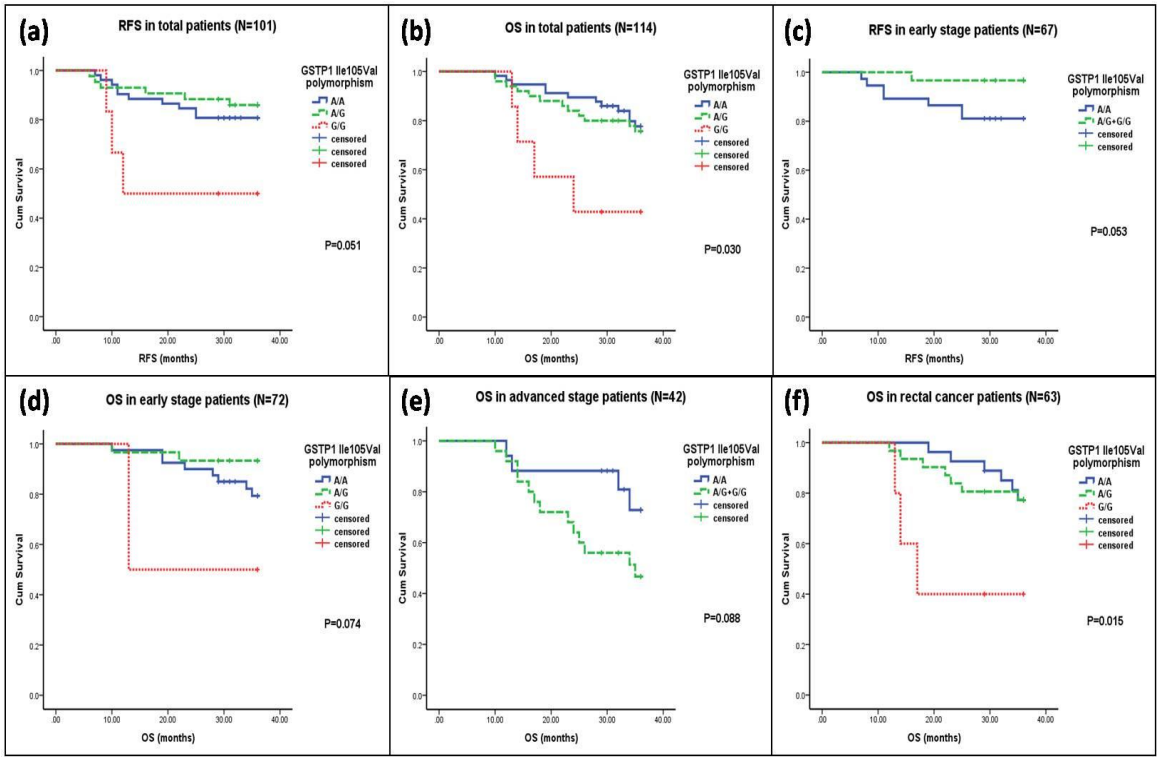

Figure 3: Kaplan-Meier survival curves in relation to GSTP1 Ile105Val polymorphism (a) RFS (b) in total patients; (c) RFS and (d) OS in early stage patients; (e) OS in advanced stage patients (f) OS in rectal cancer patients.

Survival analysis of GSTP1 protein expression

In early stage patients, low cytoplasmic GSTP1 expression was associated with a significant reduced OS (P=0.026; Figure 4a). While, in advanced stage disease, patients with high cytoplasmic GSTP1 expression showed a trend of reduced OS (P=0.086; Figure 4b). Similarly, low nuclear GSTP1 expression showed a significant reduced RFS (P=0.029; Figure 4c) and OS (P=0.005; Figure 4d) in early stage patients; whereas in advanced stage patients, a significant reduced OS was observed with high nuclear GSTP1 expression (P=0.012; Figure 4e). Further, no significant correlation of cytoplasmic or nuclear GSTP1 expression was observed with survival in relation to adjuvant treatment.

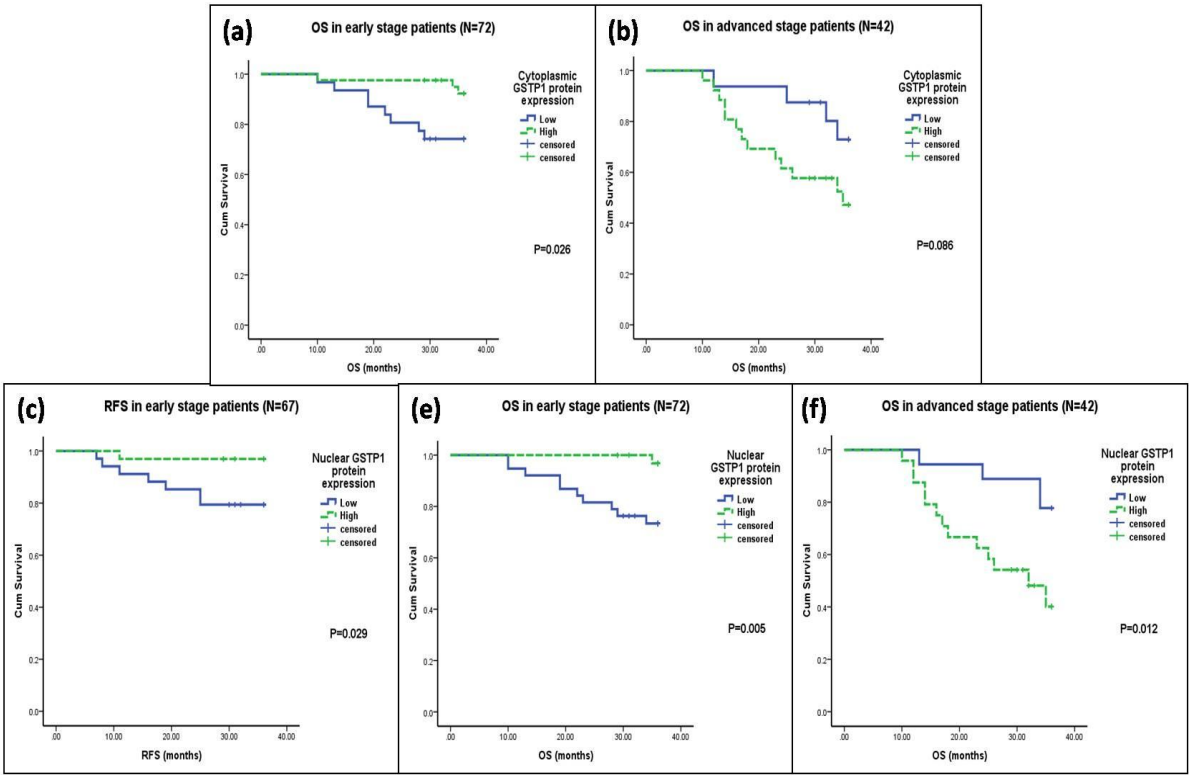

Figure 4: Kaplan-Meier survival curves in relation to cytoplasmic GSTP1 protein expression (a) OS in early stage patients (b) OS in advanced stage patients; Kaplan-Meier survival curves in relation to nuclear GSTP1 protein expression (c) RFS and (d) OS in early stage patients; (e) OS in advanced stage patients.

Intercorrelation

When GSTP1 polymorphism and protein expressions were intercorrelated, a trend of positive correlation was observed between GSTP1 polymorphism and cytoplasmic GSTP1 expression (r=+0.149, P=0.076). Moreover, a significant positive correlation was observed between cytoplasmic GSTP1 and nuclear GSTP1 protein expressions (r=+0.387, P<0.001).

DISCUSSION

In present study, GSTP1 Ile105Val polymorphism showed only 7% G/G (Val/Val) homozygous variant genotypes as compared to 51% of A/A (Ile/Ile) wild type and 42% of A/G (Ile/Val) heterozygous variant genotypes in CRC patients. In concordance with present results, the frequencies of GSTP1-105 were 55% (A/A), 37% (A/G) and 8% (G/G) in advanced CRC patients.[17] Likewise, in mCRC, the frequencies of Ile/Ile, Ile/Val and Val/Val were observed to be 46%, 44% and 9%, respectively;[18] and 40.3%, 48.1% and 11.6%, respectively.[19] Analogous frequencies were also observed in advanced CRC patients[20,21] and colon cancer patients.[22] Present study was conducted in Western India (Gujarat) and in discordance with present results, in geographically different region of Kashmir, prevalence of Ile/Ile (75.6%) was observed as compared to Ile/Val (16.3%) and Val/Val (8.1%) in CRC cases.[11] Another study in same region by Pandith et al also observed similar frequencies for GSTP1 Ile105Val genotypes in bladder cancer patients.[23] While, in small number of CRC patients (N=16) amongst South Indian population, Ramalakshmi et al reported Ile/Ile (37.5%), Ile/Val (37.5%) and Val/Val (25%) frequencies.[24] These variable results attributed that the differences in genotypic frequencies are mostly based on ethnicity and the Indian population comprised multiple ethnic groups [25] differing in their lifestyle and environmental factors.

Current study suggests the association of variant genotypes with aggressiveness of tumor as it showed a high incidence of variant genotypes (A/G+G/G) in patients with family history and biologically aggressive tumor site rectum. In contrast, Holley et al in CRC showed a trend for increasing frequency of Ile/Ile compared with combined Ile/Val and Val/Val, with more advanced Dukes' stage and that this genotype was more common in patients presenting with metastases.[26] However, no significant correlation between clinicopathological characteristics and GSTP1-105 polymorphism was observed in colon cancer patients,[22] CRC patients [21,27] and in epithelial ovarian carcinoma.[28]

When the prognostic importance of GSTP1 polymorphism was explored, the present results revealed that G/G variant genotype was associated with unfavorable survival as compared to A/A and A/G genotypes in total and rectal cancer patients. Contradictorily, other studies described the association of variant genotypes with longer survival and higher response rate to oxaliplatin based therapy in CRC

patients.[13,21,29] In accordance with present results, in other malignancies there exist reports on the association of Val/Val genotype with worse disease outcome. In ovarian cancer, Khrunin et al found that patients with Ile/Ile genotype had an increased PFS compared with that of patients with one or two Val alleles.[30] Moreover, association of Val/Val genotype was noted with worse outcome in breast cancer[31], basal cell carcinoma[32] and epithelial ovarian cancer.[28] Nevertheless, GSTP1 105 polymorphism was reported to be not associated with survival in CRC patients,[17,26] colon cancer,[22] ovarian cancer,[33] and breast cancer.[34,35]

Interestingly, present study reported that in early stage disease, patients with wild type A/A genotype had poor RFS and those with variant G/G genotype had poor OS. While, in advanced stage patients, carriers of variant genotypes (A/G+G/G) were associated with poor OS. It probably suggests that GSTP1 genotypes functioned differently in both early and advanced disease stage.

Further, the current finding of association of variant G allele with worse clinical outcome in the subgroup of patients treated with combined drug as compared to single drug would be explained as follows. The direct involvement of GSTP1 in detoxification of platinum compound has experimentally been well established.[36] Also, both in-vitro and in-vivo studies are indicative of involvement of GSTP1 in the resistance to platinum compounds.[37,38] Hence, the worse outcome in patients with variant G allele could be due to decrease effectiveness of 5-FU+OX combination therapy as compared to 5-FU single agent therapy. Thus, it could be inferred that, CRC patients with presence of G allele had survival benefit when treated with single agent 5-FU as probably they were sensitive and responded better to 5-FU therapy.

In CRC, considerably less work has been carried out on GSTP1 (GST π) protein expression. In present study, GSTP1 protein expression was observed to be located in cytoplasm and/or nucleus of tumor cells. Cytoplasmic positive staining was noted in 94% tumors, while nuclear staining was present in 75% tumors. Kim et al showed positive GST π nuclei or cytoplasmic immunoreactivity in 71.4% of cases in advanced CRC.[39] This finding is in accord with the reports in nasopharyngeal cancer[40] and NSCLC.[41,42] In breast cancer too, GSTP1 immunoreactivity showed cytoplasmic or nuclear staining in more than 50% of patients.[8,43,44] In epithelial ovarian cancer, GSTP1 cytoplasmic expression was observed in 65% of patients, whereas 71% of patients showed nuclear staining.[28]

In current study, a significant higher incidence of cytoplasmic GSTP1 expression was found in patients with older age and adenocarcinoma as compared to their respective counterparts. Moreover, a trend of high nuclear GSTP1 expression was observed in patients with older age as compared to those with younger age. Ali-Osman et al also found a strong positive correlation between the presence of GST-π expression in cell nuclei and patient age in human gliomas.[45] However, several others have shown no significant association of GSTP1 positive expression with clinicopathological parameters in CRC,[46] NSCLC,[41] breast cancer,[8] and gastric cancer.[36]

In relation to prognostic role of GSTP1 protein, Allen et al found that positive GSTP1 nuclear and cytoplasmic staining was associated with decreased survival in stage I and II squamous cell lung carcinomas.[9] Gilbert et al suggested that increased GST-pi expression could be an important predictor of early recurrence and death in node negative breast cancer patients.[47] However, experimental evidence shows that breast epithelial cells with lack of GSTP1 expression would suffer from DNA damage more easily upon exposure to carcinogen pointing towards GSTP1 expression might play a protective tool from cancer initiation. It indicates potential role of GSTP1 in an early event in breast carcinogenesis.[44,48] Hence, it might probably the reason of significant association between low nuclear as well as cytoplasmic GSTP1 expressions and worse disease outcome in subgroup of early stage patients in current study. Although modulation of other signaling pathways mediated by GSTP1 can also be responsible, since present study observed contradictory findings in advanced stage patients showing association of both high cytoplasmic and nuclear GSTP1 expressions with worse OS. In accordance, Yamamoto et al in esophageal squamous-cell carcinoma (ESCC) demonstrated that high GSTP1 protein expression level was associated with worse prognosis than low GSTP1 expression level in the patients treated by adjuvant chemotherapy.[7] Similarly, patients with GSTPi positive tumors had worse DFS as compared to those with GSTPi negative tumors in breast cancer.[8] High GST-pi expression in tumor cells and the presence of the GST-pi protein in tumor cell nuclei are associated with clinically more aggressive gliomas and are strong predictors of poor patient survival.[45] Thus, current study observed dual function of GSTP1 protein expression in early and advanced stage CRC. The exact reason for the same however needs to be elucidated.

The positive intercorrelation between GSTP1 Ile105Val polymorphism and protein expression in present study suggests that GSTP1 wild type Ile/Ile genotype associated with low GSTP1 expression and variant type associated with high GSTP1 expression. Thus, wild or variant type of polymorphism may affect the levels of protein expression. However, in epithelial ovarian cancer, Howells et al (2004) reported a significant association between the GSTP1 Ile/Ile genotypes and increased overall GSTP1 expression (P=0.049), and the GSTP1 Ile/Val genotypes and reduced overall GSTP1 expression (P=0.046).[28] Hence, further studies are needed to examine the correlation between GSTP1 Ile105Val polymorphism and GSTP1 protein expression in CRC.

CONCLUSION

Variant G allele of GSTP1 Ile105Val polymorphism could predict reduced survival and poor response to oxaliplatin based treatment in CRC patients. On the other side, GSTP1 protein expression had differential role in early and advanced stage patients. Hence, GSTP1 may be an effective tool to predict clinical outcome and a useful biomarker for the identification of high risk group of CRC patients with unfavorable prognosis.

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REFERENCES

1. Neugut AI, Lin A, Raab GT, Hillyer GC, Keller D, O'Neil DS, Accordino MK, Kiran RP, Wright J, Hershman DL. FOLFOX and FOLFIRI Use in Stage IV Colon Cancer: Analysis of SEER-Medicare Data. Clin Colorectal Cancer, 2019; 18(2): 133-40.
2. Ye H, Shao M, Shi X, Wu L, Xu B, Qu Q, Qu J. Predictive assessment in pharmacogenetics of Glutathione S-transferases genes on efficacy of platinum-based chemotherapy in non-small cell lung cancer patients. Sci Rep, 2017; 2670. https://doi.org/10.1038/s41598-017-02833-7.
3. Goekkurt E, Hoehn S, Wolschke C, Wittmer C, Stueber C, Hossfeld DK, Stoehlmacher J. Polymorphisms of glutathione S-transferases (GST) and thymidylate synthase (TS)–novel predictors for response and survival in gastric cancer patients. Br J Cancer, 2006; 94(2): 281-86.
4. Rodrigues-Fleming GH, de Mendonça Fernandes GM, Russo A, Biselli-Chicote PM, Netinho JG, Pavarino ÉC, Goloni-Bertollo EM. Molecular evaluation of glutathione S transferase family genes in patients with sporadic colorectal cancer. World J Gastroenterol, 2018; 24(39): 4462-71.
5. Jiang WQ, Fu FF, Li YX, Wang WB, Wang HH, Jiang HP, Teng LS. Molecular biomarkers of colorectal cancer: prognostic and predictive tools for clinical practice. Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology), 2012; 13: 663-75.
6. Klusek J, Nasierowska-Guttmejer A, Kowalik A, Wawrzycka I, Lewitowicz P, Chrapek M, Głuszek S. GSTM1, GSTT1, and GSTP1 polymorphisms and colorectal cancer risk in Polish nonsmokers. Oncotarget, 2018; 9(30): 21224-30.
7. Yamamoto Y, Konishi H, Ichikawa D, Arita T, Shoda K, Komatsu S, Shiozaki A, Ikoma H, Fujiwara H, Okamoto K, Ochiai T. Significance of GSTP1 for predicting the prognosis and chemotherapeutic efficacy in esophageal squamous cell carcinoma. Oncol Rep, 2013; 30(4): 1687-94.
8. Huang J, Tan PH, Thiyagarajan J, Bay BH. Prognostic significance of glutathione S-transferase-pi in invasive breast cancer. Mod Pathol, 2003; 16(6): 558-65.
9. Allen TC, Granville LA, Cagle PT, Haque A, Zander DS, Barrios R. Expression of glutathione S-transferase π and glutathione synthase correlates with survival in early stage non–small cell carcinomas of the lung. Hum Pathol, 2007; 38(2): 220-27.
10. Stoehlmacher J, Park DJ, Zhang W, Groshen S, Tsao-Wei DD, Mimi CY, Lenz HJ. Association between glutathione S-transferase P1, T1, and M1 genetic polymorphism and survival of patients with metastatic colorectal cancer. J Natl Cancer Inst, 2002; 94(12): 936-42.
11. Sameer AS, Qadri Q, Siddiqi MA. GSTP1 I105V polymorphism and susceptibility to colorectal cancer in Kashmiri population. DNA and Cell Biol, 2012; 31(1): 74-9.
12. Jones BA, Christensen AR, Wise JP, Yu H. Glutathione S-transferase polymorphisms and survival in African-American and white colorectal cancer patients. Cancer Epidemiol, 2009; 33(3-4): 249-56.
13. Zarate R, Rodríguez J, Bandres E, Patino-Garcia A, Ponz-Sarvise M, Viudez A, Ramirez N, Bitarte N, Chopitea A, Gacia-Foncillas J. Oxaliplatin, irinotecan and capecitabine as first-line therapy in metastatic colorectal cancer (mCRC): a dose-finding study and pharmacogenomic analysis. Br J Cancer, 2010; 102(6): 987-94.
14. Jankova L, Robertson G, Chan C, Tan KL, Kohonen-Corish M, Fung CL, Clarke C, Lin BP, Molloy M, Chapuis PH, Bokey L. Glutathione S-transferase Pi expression predicts response to adjuvant chemotherapy for stage C colon cancer: a matched historical control study. BMC Cancer, 2012; 12: 196. doi: 10.1186/1471-2407-12-196.
15. Glasgow SC, Yu J, Carvalho LP, Shannon WD, Fleshman JW, McLeod HL. Unfavourable expression of pharmacologic markers in mucinous colorectal cancer. Br J Cancer, 2005; 92(2): 259-64.
16. Gajjar KK, Vora HH, Kobawala TP, Trivedi TI, Ghosh NR. Deciphering the potential value of 5-fluorouracil metabolic enzymes in predicting prognosis and treatment response of colorectal cancer patients. Int J Biol Markers, 2018; 33(2): 180-88.
17. Ruzzo A, Graziano F, Loupakis F, Rulli E, Canestrari E, Santini D, Catalano V, Ficarelli R, Maltese P, Bisonni R, Masi G. Pharmacogenetic profiling in patients with advanced colorectal cancer treated with first-line FOLFOX-4 chemotherapy. J Clin Oncol, 2007; 25(10): 1247-54.
18. Stoehlmacher J, Park DJ, Zhang W, Yang D, Groshen S, Zahedy S, Lenz HJ. A multivariate analysis of genomic polymorphisms: prediction of clinical outcome to 5-FU/oxaliplatin combination chemotherapy in refractory colorectal cancer. Br J Cancer, 2004; 91(2): 344-54.

19. Kweekel DM, Koopman M, Antonini NF, Van der Straaten T, Nortier JW, Gelderblom H, Punt CJ, Guchelaar HJ. GSTP1 Ile105Val polymorphism correlates with progression-free survival in MCRC patients treated with or without irinotecan: a study of the Dutch Colorectal Cancer Group. Br J Cancer, 2008; 99(8): 1316-21.
20. Kweekel DM, Gelderblom H, Antonini NF, Van der Straaten T, Nortier JW, Punt CJ, Guchelaar HJ. Glutathione-S-transferase pi (GSTP1) codon 105 polymorphism is not associated with oxaliplatin efficacy or toxicity in advanced colorectal cancer patients. Eur J Cancer, 2009; 45(4): 572-8.
21. Jun L, Haiping Z, Beibei Y. Genetic polymorphisms of GSTP1 related to response to 5-FU-oxaliplatin-based chemotherapy and clinical outcome in advanced colorectal cancer patients. Swiss Med Wkly, 2009; 139(49-50): 724-8.
22. Zaanan A, Dalban C, Emile JF, Blons H, Fléjou JF, Goumard C, Istanbullu M, Calmel C, Alhazmi K, Validire P, Louvet C. ERCC1, XRCC1 and GSTP1 single nucleotide polymorphisms and survival of patients with colon cancer receiving oxaliplatin-based adjuvant chemotherapy. J Cancer, 2014; 5(6): 425-32.
23. Pandith AA, Lateef A, Shahnawaz S, Hussain A, Malla TM, Azad N, Shehjar F, Salim M, Shah ZA. GSTP1 gene Ile105Val polymorphism causes an elevated risk for bladder carcinogenesis in smokers. Asian Pac J Cancer Prev, 2013; 14(11): 6375-8.
24. Ramalakshmi S, Kavimani S, Srineevas S, Vettriselvi V, Bhaskar LV. A pharmacogenetic study of capecitabine-oxaliplatin therapy in colorectal cancer patients among South Indian population. European Journal of Pharmaceutical and Medical Research, 2016; 3(9): 520-28.
25. Umamaheswaran, G., Kumar, D. K., & Adithan, C. Distribution of genetic polymorphisms of genes encoding drug metabolizing enzymes & drug transporters - A review with Indian perspective. Indian J Med Res, 2014; 139(1): 27-65.
26. Holley SL, Rajagopal R, Hoban PR, Deakin M, Fawole AS, Elder JB, Elder J, Smith V, Strange RC, Fryer AA. Polymorphisms in the glutathione S-transferase mu cluster are associated with tumour progression and patient outcome in colorectal cancer. Int J Oncol. 2006; 28(1): 231-6.
27. Gorukmez O, Yakut T, Gorukmez O, Sag SO, Topak A, Sahinturk S, Kanat O. Glutathione S-transferase T1, M1 and P1 Genetic Polymorphisms and Susceptibility to Colorectal Cancer in Turkey. Asian Pac J Cancer Prev, 2016; 17(8): 3855-9.
28. Howells RE, Dhar KK, Hoban PR, Jones PW, Fryer AA, Redman CW, Strange RC. Association between glutathione-S-transferase GSTP1 genotypes, GSTP1 over-expression, and outcome in epithelial ovarian cancer. Int J Gynecol Cancer, 2004; 14(2): 242-50.
29. Chen YC, Tzeng CH, Chen PM, Lin JK, Lin TC, Chen WS, Jiang JK, Wang HS, Wang WS. Influence of GSTP1 I105V polymorphism on cumulative neuropathy and outcome of FOLFOX-4 treatment in Asian patients with colorectal carcinoma. Cancer Sci, 2010; 101(2): 530-35.
30. Khrunin AV, Moisseev A, Gorbunova V, Limborska S. Genetic polymorphisms and the efficacy and toxicity of cisplatin-based chemotherapy in ovarian cancer patients. Pharmacogenomics J, 2010; 10(1): 54-61.
31. Zhang BL, Sun T, Zhang BN, Zheng S, Lü N, Xu BH, Wang X, Chen GJ, Yu DK, Lin DX. Polymorphisms of GSTP1 is associated with differences of chemotherapy response and toxicity in breast cancer. Chin Med J (Engl), 2011; 124(2): 199-204.
32. Ramachandran S, Hoban PR, Ichii-Jones F, Pleasants L, Ali-Osman F, Lear JT, Smith AG, Bowers B, Jones PW, Fryer AA, Strange RC. Glutathione S-transferase GSTP1 and cyclin D1 genotypes: association with numbers of basal cell carcinomas in a patient subgroup at high-risk of multiple tumours. Pharmacogenetics, 2000; 10(6): 545-56.
33. Cong LX, Zhai XH, Wu FX, Zhu DY, Wang AC. Single nucleotide polymorphisms in glutathione S-transferase P1 and M1 genes and overall survival of patients with ovarian serous cystadenocarcinoma treated with chemotherapy. Oncol Lett, 2016; 11(4): 2525-31.
34. Wang J, Wang T, Yin GY, Yang L, Wang ZG, Bu XB. Glutathione S-transferase polymorphisms influence chemotherapy response and treatment outcome in breast cancer. Genet Mol Res, 2015; 14(3): 11126-32.
35. Wang X, Zhang R, Zhang X, Wu W, Bai J. Polymorphisms in GSTM1 gene influences the chemotherapy response and treatment outcome in breast cancer patients. Int J Clin Exp Pathol, 2016; 9(10): 10781-87.
36. Kwon HC, Roh MS, Oh SY, Kim SH, Kim MC, Kim JS, Kim HJ. Prognostic value of expression of ERCC1, thymidylate synthase, and glutathione S-transferase P1 for 5-fluorouracil/oxaliplatin chemotherapy in advanced gastric cancer. Ann Oncol, 2007; 18(3): 504-9.
37. Ban N, Takahashi Y, Takayama T, Kura T, Sakamaki S, Niitsu Y. Transfection of glutathione S-transferase (GST)-p1 antisense complementary DNA increases the sensitivity of a colon cancer cell line to adriamycin, cisplatin, melphalan, and etoposide. Cancer Res, 1996; 56(15): 3577-82.
38. Shiga H, Heath EI, Rasmussen AA, Trock B, Johnston PG, Forastiere AA, Langmacher M, Baylor A, Lee M, Cullen KJ. Prognostic value of p53, glutathione S-transferase π, and thymidylate synthase for neoadjuvant cisplatin-based chemotherapy in head and neck cancer. Clin Cancer Res, 1999; 5(12): 4097-4104.
39. Kim SH, Kwon HC, Oh SY, Lee DM, Lee S, Lee JH, Roh MS, Kim DC, Park KJ, Choi HJ, Kim HJ. Prognostic value of ERCC1, thymidylate synthase,

and glutathione S-transferase π for 5-FU/oxaliplatin chemotherapy in advanced colorectal cancer. Am J Clin Oncol, 2009; 32(1): 38-43.
40. Jayasurya A, Yap WM, Tan NG, Tan BK, Bay BH. Glutathione S-transferase π expression in nasopharyngeal cancer. Arch Otolaryngol Head Neck Surg, 2002; 128(12): 1396-9.
41. Bai F, Nakanishi Y, Kawasaki M, Takayama K, Yatsunami J, Pei XH, Tsuruta N, Wakamatsu K, Hara N. Immunohistochemical expression of glutathione S-transferase-π can predict chemotherapy response in patients with nonsmall cell lung carcinoma. Cancer, 1996; 78(3): 416-21.
42. Zhu WY, Hunag YY, Liu XG, He JY, Chen DD, Zeng F, Zhou JH, Zhang YK. Prognostic Evaluation of CapG, Gelsolin, P-gp, GSTP1, and Topo-II Proteins in Non-Small Cell Lung Cancer. Anat Rec (Hoboken), 2012; 295(2): 208-14.
43. Pakdeethai S, Fongchaiya V, Pongtheerat T, Iampenkhae K, Sampatanukul P. Relationship between promoter methylation and protein expression of glutathione S-transferase gene class P1 in breast cancer. Asian Archives of Pathology, 2012; 8(2): 45-53.
44. Vecanova J, Hodorova I, Mihalik J, Benicky M, Kluchova D, Rybarova S. Immunohistochemical evaluation of Pi class glutathione S-transferase expression in invasive breast carcinoma. Bratisl Lek Listy, 2011; 112(2): 67-70.
45. Ali-Osman F, Brunner JM, Kutluk TM, Hess K. Prognostic significance of glutathione S-transferase pi expression and subcellular localization in human gliomas. Clin Cancer Res, 1997; 3: 2253-61.
46. Mulder TP, Verspaget HW, Sier CF, Roelofs HM, Ganesh S, Griffioen G, Peters WH. Glutathione S-transferase π in colorectal tumors is predictive for overall survival. Cancer Res, 1995; 55(12): 2696-702.
47. Gilbert L, Elwood LJ, Merino M, Masood S, Barnes R, Steinberg SM, Lazarous DF, Pierce L, d'Angelo T, Moscow JA. A pilot study of pi-class glutathione S-transferase expression in breast cancer: correlation with estrogen receptor expression and prognosis in node-negative breast cancer. J Clin Oncol, 1993; 11(1): 49-58.
48. Lee JS. GSTP1 promoter hypermethylation is an early event in breast carcinogenesis. Virchows Arch, 2007; 450(6): 637-42.