Value of glutathion-S transferase pi as a prognostic factor in epithelial ovarian carcinoma

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Summary

The association between glutathione S-transferase pi (GSTπ) and other clinicopathological parameters, response to chemotherapy and clinical outcome were investigated in chemotherapy naive epithelial ovarian cancer patients. Paraffin-embedded material from 55 patients were used for immunohistochemical analysis. All patients had received six cycles of cisplatinum-based chemotherapy and 41 of them were revalued by laparotomy. Pre- and post-chemotherapy GSTπ staining were detected in the cancer tissues of 18/55 (32.7%) and 5/14 (35.7%) patients, respectively. GSTπ expression was not associated with other clinicopathologic parameters. Of 17 patients with postoperative measurable residual disease clinical response was observed in 4/7 of GSTπ positive and in 9/10 GSTπ negative patients (p = 0.25). Pathologic complete response (pCR) was achieved in 5/8 of GSTπ positive and 11/22 of GSTπ negative cases (p = 0.69). There was no significant difference in overall survival and progression-free survival (PFS) according to initial GSTπ status. However the PFS of the five patients (median 22 ± 5.9 months) who had postchemotherapy positive GSTπ was significantly shorter than the nine patients (10.0 ± 2.19 months) who had negative GSTπ (p = 0.006). This difference was not observed in overall survival. These results suggest that initial immunohistochemical staining of GSTπ does not aid in the prediction of pCR and clinical outcome in patients with epithelial ovarian cancer. Nonetheless investigation of GSTπ expression after chemotherapy needs further evaluation.

Key words: Glutathion-S transferase PI; Epithelial ovarian cancer.

Introduction

The development of resistance to standard platinum based chemotherapy is a major obstacle in the clinical treatment of epithelial ovarian cancers. Several resistance mechanisms such as increased rates of drug efflux from the cell [1], decreased drug sensivitiy [2], increased DNA repair mechanisms [3] and altered expression of metabolic and detoxification processes which may be mediated by the glutathione/glutathione-S transferase (GST) detoxification system have been described [4]. The latter mechanism is known to play an important role in the metabolism of platinum compounds, cyclophosphamide and doxurubicin [5]. Enhanced GST content has been described in tumor cell lines resistant to these agents also [6].

GSTs are a phase II metabolism multigene family consisting of the cytosolic isoenzyme classes designed α, π, μ, θ which are responsible for the conjugation of reduced GSH to a broad range of electrophilic compounds [7]. It has been shown that GSTπ is the predominant transferase in malignant ovarian epithelial tissue [8, 9]. Although increased activity of GST in tumor cells has been associated with increased drug resistance, there are conflicting reports about overexpression of GSTπ as an indicator of clinical and pathologic chemotherapy response and as a prognostic factor in ovarian cancer patients [8, 10-14].

The aim of this study was to investigate the association between the expression of GSTπ and clinicopathological parameters, response to chemotherapy and clinical outcome in epithelial ovarian cancer patients.

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Materials and Methods

A computerized search was performed to locate all patients diagnosed with primary ovarian cancer treated at the Istanbul University Oncology Institute between January 1990 and December 1996. Patients with borderline tumors, non epithelial ovarian carcinomas and secondary ovarian tumors were excluded. Fifty-five patients with primary epithelial ovarian carcinoma for whom archival pathologic material obtained at the time of initial diagnosis which was available for the GSTπ immunohistochemical (IHC) analysis were included in the study.

The medical records of these 55 patients were reviewed. Age, histopathological subtype, grade, stage, ability to perform optimal cytoreductive surgery (< 2 cm largest residual tumor), clinical and pathological response to chemotherapy and survival data were obtained from these records. Follow-up information on surviving patients was updated through June 2002. Histopathologic diagnosis was based on World Health Organisation (WHO) criteria [15]. Histologic types included 33 serous, five mucinous, nine endometrioid, two clear cell, and six undifferentiated. Thirteen tumors were grade 1, 17 were grade 2 and 13 were grade 3. Stage was determined by surgical staging with use of criteria recommended by International Federation of Gynecology and Obstetrics (FIGO) [16]. Of the 55 patients nine had Stage IC, six had Stage II, 38 had Stage III, and two had Stage IV disease. Patients underwent initial surgical evaluation and tumor debulking followed by six cycles of cisplatinum-based combination chemotherapy as initial therapy. The residual tumor size was ≥ 2 cm in 17 patients and < 2 cm in 38 patients. After the chemotherapy cycles clinical responses were evaluated by computed tomography (CT) or magnetic resonance imaging (MRI) according to the WHO response criteria. Among the 48 patients who were in clinical complete response (CR) (n:35) or partial response (PR) (n:13), second-look laparo-

tomy (SLL) or interval debulking was done in 41 of them. Pathologic complete response (pCR) was observed in 16, while the remaining 25 had residual tumor. GSTπ staining could be performed to the 14 suitable blocks of these residual tumors. Pathologic complete responders received consolidative intraperitoneal chemotherapy which consisted of three cycles of cisplatin and others were treated according to ongoing phase II studies.

All pathological materials were obtained by exploratory laparotomy or by primary cytoreductive procedures at the beginning and by second-ook laparotomy or interval debulking procedures after six cycles of chemotherapy. Immunohistochemical staining for GSTπ was performed at the immunotyping laboratory of the Istanbul University Pathology Department under the direct supervision of one of the co-authors. The pathologist reviewed microscopic slides of the cases, confirmed the diagnosis and selected sections for immunostaining. A median of four slides was examined for each patient. Sections from the formaldehyde solution-fixed paraffin-embedded blocks judged to be most representative of the tumor were used for IHC. Five-micron specimens were dewaxed in xylene and hydrated in descending concentrations of alcohol. Antigen retrieval with pressure cooking using 1000 ml 0.01 mol/l sodium citrate buffer (pH 6) was applied. After using 3% H_2O_2 treatment for 20 minutes to block endogenous peroxidase activity, nonspecific blockage with Ultrablock nonspecific blocking agent (Labvision Co.) was applied to all sections for 15 minutes. Then the slides were incubated with primary antibodies (Novocastra, NCL-GST-pi, in 1/100 dilution) at 4°C overnight. After washing the sections, they were exposed to a biotynlated secondary antibody (Ultravision- Labvision Co.) for 25 min and to peroxidase labelled streptavidin (Ultravision Streptavidin/HRP, Labvision Co.). AEC was used as the chromogen and Meyer hematoxylin was used as the counterstain. Percentage of nuclear and cytoplasmic immunoreactivity with both antibodies was assessed with an Olympus BX 50 microscope. Nuclear or cytoplasmic staining more than 10% was considered as GSTπ positive.

Chi-square tests for proportion were used to analyze the distribution of GST staining in relation to clinicopathologic characteristics. Progression-free survival (PFS) and overall survival (OS) were calculated from the date of first surgery to the date of clinical progression or death. The Kaplan-Meier method was used to estimate survival rates. The log-rank test was used to compare survival distribution among subgroups. Information obtained from univariate analysis was applied to a survival analysis with covariates by using the Cox model of proportional hazards to determine which variables were independently predictive of outcome. The Wilcoxon signed rank test was used to evaluate the effect of chemotherapy on GST staining.

Results

Fifty-five patients with primary ovarian invasive epithelial cancer were studied. The median age of the patients was 51 years (range 25-72). Nuclear and/or cytoplasmic staining of GSTπ was found in 18 of 55 (32.7%) specimens obtained from the initial surgery. In 13 specimens GSTπ was cytoplasmic, in two specimens nuclear and in three specimens both cytoplasmic and nuclear. All specimens with ≥ 10% positive GSTπ staining were regarded as one group. Table 1 demonstrates the association between GSTπ overexpression and the clinicopathologic variables. Expression of GSTπ was not associated with age, menopausal status, tumor histology, grade of differentiation, stage of disease, initial serum CA125 levels, or residual tumor after initial surgery. None of the undifferentiated tumors (0/6) showed positive staining.

Table 1. — *Patient characteristics according to GSTπ staining status.*

	N (X ± SD)	GSTπ (+) (Min.-Max.)	GSTπ (–)	P
All cases	55	18	37	
Age				NS
< 60	40	14	26	
> 60	15	4	11	
Menopause				NS
Pre	21	9	12	
Post	34	9	25	
Tumor histology				NS
Serous papillary	33	10	23	
Mucinous	5	2	3	
Endometroid	9	5	4	
Clear cell	2	1	1	
Undifferentiated	6	0	6	
Tumor grade*				NS
1	13	7	6	
2	17	6	11	
3	10	1	9	
FIGO stage				NS
I+II	15	7	8	
III + IV	40	11	29	
Initial CA125				NS
Normal	8	3	5	
Elevated	35	11	24	
Initial surgery				NS
Optimal	38	11	27	
Suboptimal	17	7	10	
Clinical response				NS
Complete	7	3	4	
Partial	10	1	9	
Pathologic response				NS
Second-look negative	16	5	11	
Second-look positive	25	6	19	

NS: Not significant, * for the statistical analysis 1 vs 2+3.

Seventeen patients with measurable residual disease after initial surgery were investigated for clinical response. The clinical overall and complete response rates of these patients were 76% and 23.5%, respectively. Clinical response was observed in four of seven (57%) GSTπ positive and in nine of ten (90%) GSTπ negative patients (Fisher's 2-sided exact test p = 0.25).

The pathologic response rate was evaluated by SLL or secondary debulking after six cycles of chemotherapy in 41 patients. Pathologic complete response was observed in 16 (39%) patients. In 11 of the 30 (33%) cases with negative staining at the initial surgery pathologic complete response was achieved whereas in five of the 11 (45%) cases with positive staining at the beginning showed pathologic complete response (Fisher's 2-sided exact test p = 0.72).

Since platinum therapy may induce expression of GSTπ, this marker of drug resistance was also evaluated in 14 of 25 patients with positive SLL or interval debulking after six cycles of cisplatinum-based chemotherapy.

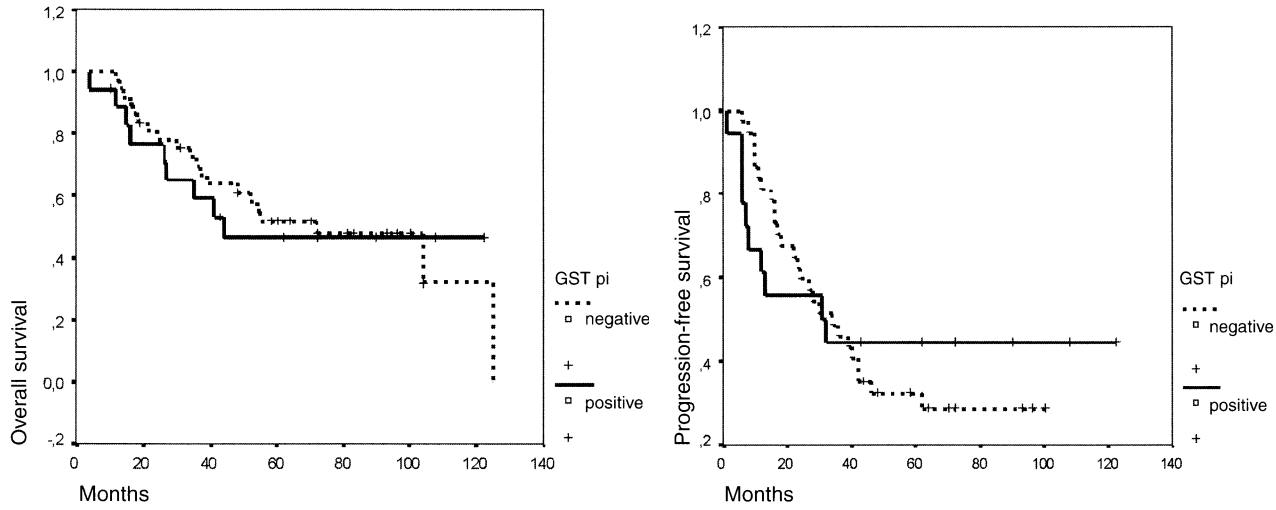

Figure 1. — Survival cures according to initial GST pi status.

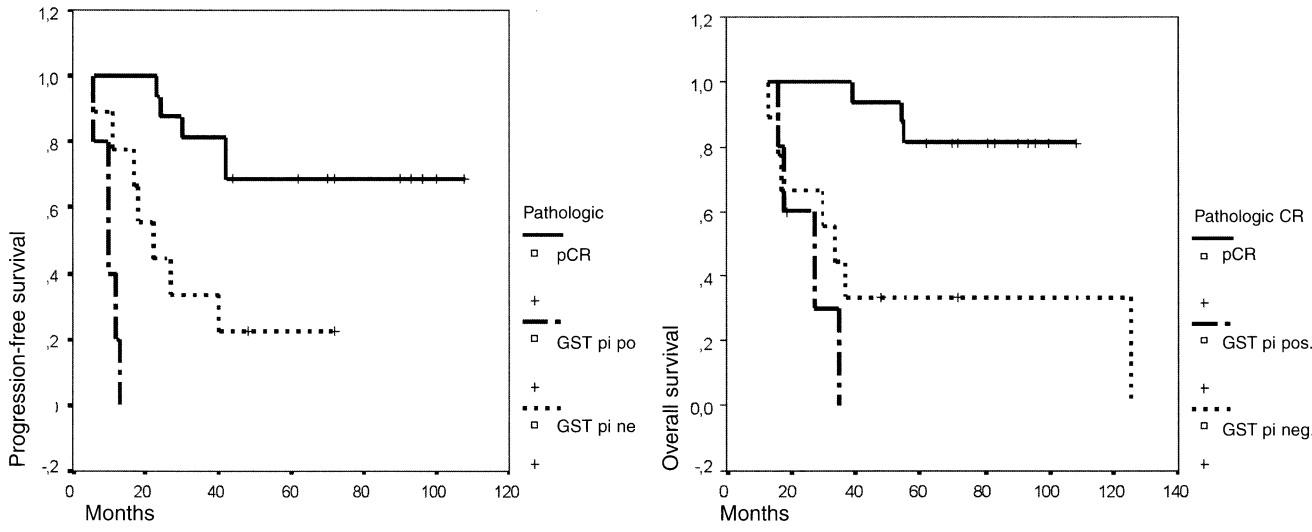

Figure 2. — Survival curves according to postchemotherapy GST pi status and pathologic complete response.

In 11 cases the amount of residual tumor was not suitable for immunostaining. Five of 14 specimens (35.7%) obtained from the SLL or secondary debulking showed positive GSTπ staining. The intensity of GSTπ staining was increased in three, decreased in three and no change was observed in eight cases. Although three of 11 (27%) cases with negative initial staining had secondary positive staining, two of three (67%) cases with initial positive staining had secondary positive staining. However the number of patients was insufficient for statistical analysis. Moreover positive staining after chemotherapy was not associated with the clinicopathologic parameters.

Median follow-up was 48 months (range 4-125). During the follow-up period 36 recurrences or progressions and 29 deaths were observed. Three patients were lost to follow-up after 10, 19 and 31 months. The median PFS and OS of the patients according to their initial, postchemotherapy GSTπ status and pathologic response are shown in Table 2. There was no significant difference in clinical outcome according to initial GSTπ status. However the PFS of the five patients (median 22 months) who had positive GSTπ staining at the SLL or secondary debulking was significantly shorter than the nine patients (10.0 months) who had negative GSTπ staining ($p = 0.006$). The difference was not significant in overall survival. Figure 1 shows PFS and OS curves in our population according to initial GSTπ staining status. Figure 2 shows PFS and OS curves of the patients with pathologic

Table 2. — *Progression-free and overall survival.*

Survival	Event/total	Mean ± SE (95% CI) (months)	Median ± SE (95% CI) (months)	p Log-rank
Progression-free survival				
All cases	36/55	55.7 ± 6.6 (42.6 - 68.7)	32 ± 6.4 (19.5 - 44.5)	
Initial GSTπ (+)	10/18	61.0 ± 12.98 (35.6 - 86.5)	31.0 ± 20.2 (0 - 70.5)	
Initial GSTπ (-)	26/37	46.7 ± 5.96 (35.0 - 58.4)	34.0 ± 7.3 (19.7 - 48.3)	NS
Pathologic CR	5/16	84.3 ± 8.9 (66.95-101.7)	Not reached	
No Pathologic CR	21/25	26.95 ± 4.5 (18.1 - 35.9)	16.0 ± 1.7 (12.7 - 19.3)	0.0001
Second GSTπ (+)	5/5	10.2 ± 1.2 (7.9-12.6)	10.0 ± 2.2 (5.7 - 14.3)	
Second GSTπ (-)	7/9	31.7 ±7.8 (16.4 - 46.95)	22.0 ± 5.9 (10.3 - 33.7)	0.006
Overall survival				
All cases	29/55	74.02 ± 6.8 (60.7 - 87.4)	72 ± 23.7 (25.6 - 118.4)	
Initial GSTπ (+)	9/18	70.0 ± 12.1 (46.4 - 93.7)	44.0 ± (..-..)	
Initial GSTπ (-)	20/37	74.3 ± 8.2 (58.4 - 90.2)	72.0 ± 17.5 (37.8 - 106.2)	NS
Pathologic CR	3/16	97.0 ± 5.8 (85.7 - 108.3)	Not reached	
No pathologic CR	19/25	51.2 ± 8.98 (33.6-68.8)	35.0 ± 5.4 (24.4-45.6)	0.0001
Postchemotherapy GSTπ (+)	4/5	25.4 ± 4.1 (17.4-33.4)	27.0 ± 7.2 (12.96-41.0)	
Post chemotherapy GSTπ (-)	7/9	58.0 ± 17.2 (24.1 - 91.9)	34.0 ± 5.96 (22.1- 45.7)	0.30

SE: standard error; CI: confidence interval; CR: complete response; NS: not significant.

complete response, postchemotherapy GSTπ positive and GSTπ negative staining. Patients with pathologic complete responses had a significantly better prognosis than postchemotherapy GSTπ positive and GSTπ negative patients in terms of progression free (p = 0.0000) and overall survival (p = 0.0001).

Univariate analysis for PFS and OS were performed on the following parameters; age (≤ 60 vs > 60), menopause status (pre vs post), stage (I + II) vs (III + IV), grade of differentiation (1 vs 2 + 3), residual tumor after initial surgery (< 2 cm vs ≥ 2 cm), GST pi staining at the primary surgery (+ vs -), initial CA125 levels (normal vs abnormal), clinical response (CR + PR vs others) and pathological response (pCR vs other responses). OS was significantly longer in patients who were optimally operated, with normal CA125 levels, early stage, well differentiated tumors and clinical and pathologic complete response. PFS was significantly longer in patients who were optimally operated, with early stage, well differentiated tumors, and clinical and pathologic complete response.

The influence of residual tumor, stage, GSTπ staining, pathologic complete response on OS and PFS was analyzed with Cox regression analysis (forward stepwise LR method). Optimal surgery and pathologic complete response were the independent factors which had an impact on survival parameters.

Discussion

The primary aim of this study was to investigate the association between the expression of GSTπ and response to chemotherapy (especially pathologic response) and clinical outcome in epithelial ovarian cancer patients. There are conflicting reports about overexpression of GSTπ as an indicator of chemotherapy response and as a prognostic factor in ovarian cancer patients [8, 10-14, 17-20]. This may be due to different patient populations with different response rates and prognosis that were included in these studies.

Our results suggest that immunohistochemical staining of GSTπ at diagnosis does not aid in the prediction of pathologic complete response to chemotherapy and outcome in patients with epithelial ovarian cancer. Pathologic complete response was achieved in 11 of the 30 (33%) cases with initial negative staining and in five of the 11 (45%) cases with initial positive staining. Most of the studies, except Zee *et al.'s* which did not find any correlation like ours, dealt with clinical response in patients with residual tumor but did not evaluate the pathologic response.

No relation with clinical response to chemotherapy or prognostic importance with regard to survival was found in other studies as well [11, 13, 17, 20]. In contrast to these there are reports suggesting a strong correlation between GSTπ expression and drug resistance and poor prognosis [8, 10, 12, 18, 19]. In our 17 patients with residual tumor GSTπ expression was also more common in nonresponders than responders (9 of 10 patients vs 4 of 7 patients) but no statistically significant result could be obtained. The reported clinical response rate of Cheng *et al.'s* [10] and Hamada *et al.'s* [12] studies were much lower (50% and 43%) than our population (76%). This difference in response rates between their patient population and ours may explain the discrepancy of the results. The clinical response rate of Green *et al.'s* study [8] was high (72%) but they assessed the intensity of the staining and scored like the studies of Satoh *et al.* and Mayrs *et al.* [19]. In these studies patients showing resistance to cytotoxic chemotherapy were found to have a higher intensity of staining for GSTπ. Due to the low number of patients (n: 55) and low staining rate (32.7%) we could not divide our patients according to their stainability levels.

GSTπ staining was found in 32.7% of our chemotherapy naive cases and 32.7% of treated cases. This amount is lower than other (50-89%) reports which used the IHC method [8, 10-12, 18]. Germain *et al.* also found 37% positive GST staining in their study group [20]. The frequency of GSTπ changed according to the method used for detection [10, 14]. The frequency was 100% in the Western blot analysis but around 50% in the IHC analysis [10]. In the study of Cheng *et al.* the expression of GSTπ remained unchanged in each patient before and after chemotherapy by the IHC method [10]. However they changed when Western blot analysis was used. They found Western blot analysis detected GSTπ expression

more reliably. In contrast we have seen changes in six patients before and after chemotherapy with the IHC method. The intensity of GSTπ staining was increased in three, decreased in three and no change was observed in eight cases. Although three of 11 (27%) cases with negative initial staining had secondary positive staining, two of three (67%) cases with initial positive staining had secondary positive staining. Nonetheless the number of patients was not high enough for statistical analysis. Some cases of ovarian cancer did not express GSTπ but resistance to chemotherapy suggests that other mechanisms are also involved in resistance of ovarian cancer cells to chemotherapeutic agents.

We did not select any subgroup which could effect the prognosis purposely. However a high percentage of optimally operated and early-stage patients puts our population in the good prognosis category. Even in Silvestrini *et al.'s* study, which only included Stage III-IV patients in a randomized prospective protocol, no definitive patterns of predictivity for short-term and long-term clinical outcomes were observed for GSTπ staining [13].

Pathologic complete response is one of the most important prognostic factors in ovarian carcinoma which predicts outcome. Pathologic response was evaluated in 41 patients either by second-look laparotomy or interval debulking after six cycles of chemotherapy. Pathologic complete response and residual tumor after the initial operation were the independent prognostic factors which predicted PFS and survival in this study. The GSTπ staining in the untreated patients had no impact on overall survival and progression-free survival (Figure 1). Although the number of patients was small, a statistically significant increase in PFS was observed in nine patients (median 22 months) who had negative GSTπ staining at SLL or secondary debulking which was significantly longer than for the five patients who had negative GSTπ staining (median 10 months) ($p = 0.006$). This factor was not put in the Cox regression analysis because the number of patients (n: 14) was too small. Nonetheless this finding suggests that acquired resistance after chemotherapy may be a more important factor than the resistance in untreated patients. Patients with pathologic complete response had significantly better prognosis than postchemotherapy GSTπ positive and GSTπ negative patients in terms of progression-free ($p = 0.0000$) and overall survival ($p = 0.0001$) (Figure 2).

These results suggest that initial immunohistochemical staining of GSTπ at diagnosis does not aid in the prediction of pathologic CR and clinical outcome in patients with epithelial ovarian cancer. Investigation of GSTπ expression after chemotherapy however needs further evaluation.

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