# Glutathione-S transferase-π expression in non small cell lung cancer in the assessment of response to chemotherapy

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## ABSTRACT

**Objective:** To determine the relation of glutathione-S transferase-π (GST-π) expression and cisplatin resistance in non small cell lung cancer (NSCLC).

**Methods:** This study was carried out on 61 patients who were admitted to Chest Diseases Clinic, Ondokuz Mayis University, Samsun, Turkey, from 1997 to 1999. Twenty-seven NSCLC patients out of 61 lung cancer cases whose biopsy specimens were evaluated for GST-π, received multiagent chemotherapy including cisplatin. The correlations between GST-π expression and age, sex, performance score, histology, stage of the disease and response to chemotherapy were investigated.

**Results:** There was a significant correlation between GST-π expression and the histological type of the disease ($p<0.05$). However, no significant relation was found with age, sex, performance score or stage of the disease ($p>0.05$). Glutathione-S transferase-π staining characteristics of the 27 patients receiving chemotherapy were: less than 10% in 3 patients (11.1%), 10-50% in 9 patients (33.3%) and more than 50% in 15 patients (55.5%). One of the 3 patients (33.3%) with GST-π staining percentage of less than 10%, 3 of 9 patients (33.3%) with staining percentage of 10-50% and 4 out of 15 patients (26.6%) with staining percentage of more than 50% had an objective response to chemotherapy. No significant correlation was found between GST-π expression and response to chemotherapy in the 3 groups ($p>0.05$).

**Conclusion:** Glutathione-S transferase-π expression might not always predict the response to combination chemotherapy regimens containing cisplatin. Several other mechanisms may play a role in cisplatin-resistance.

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Non-small cell lung cancers (NSCLC) correspond to 75%-80% of all lung cancers. They are usually diagnosed at late stages and for that reason, are candidates for chemotherapy. However, treatment results are satisfactory in only 30-50%.[1,2] On the other hand chemotherapeutic agents may have toxic effects and worsen the life quality of the patients. Therefore, prediction of the chemotherapeutic response is an important issue. By this prediction, patients may be protected from the toxic effects of chemotherapy and excessive and expensive drug use. Glutathione-S transferases are a group of enzymes which catalyse and conjugate glutathione and a large group of electrophilic components and result in detoxification.[3,4] Glutathione (GSH) system is responsible for the metabolism of some chemotherapeutic agents such as cyclophosphamide, doxorubicin and cisplatin. Glutatione-S transferases induces the conjugation of glutathione and electrolytes in these drugs. By this way, drugs are inactivated and resistance to chemotherapy is established.[5,6] In many studies it is stated that increase in GST-π activity

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indicates resistance to cisplatin in various cancer types such as nasopharyngeal carcinoma, ovarian carcinoma, and non-Hodgkin's lymphomas.[7-9]

Glutathione-S transferases are categorized into 4 groups as α, μ, π and μ according to their enzymatic and structural properties.[10] Especially GST-π is found in NSCLC tumor tissue[11] and it is important in assessing cisplatin resistance.[12] Thus, GST may be used as a tumor marker.[13,14] Besides, the response to the chemotherapy in NSCLC patients may be analyzed by examining the expression of GST in the tissues.[15,16]

In our study, GST-π expression in lung cancer patients was evaluated immunohistochemically, and the relationship between GST-π expression and response to cisplatin-based chemotherapy regimens in NSCLC patients were determined.

**Methods.** ***Cases.*** The study was performed on 61 patients with lung cancer who were admitted to Chest Diseases Clinic, Ondokuz Mayıs University Hospital, Turkey, 1997 to 1999. Thirty-eight NSCLC and 23 small cell lung cancer (SCLC) patients were diagnosed by bronchoscopic biopsies. The patients have not received chemotherapy and radiotherapy previously. They were staged according to the International Staging System[17] according to the results of the chest x-ray, computerized tomography of the lung, upper abdomen and brain, whole body bone scanning and the fiberoptic bronchoscopic examination. Their performances were assessed according to the Eastern Cooperative Oncology Group criteria. Complete blood count and renal and liver function tests were evaluated before initiating the chemotherapy. Median age of all lung cancer patients, including 4 female and 57 male was 61.1. There were 38 NSCLC cases; 8 at stages I-II, 7 at stage III and 23 at stage IV. Among all lung cancer patients, performance scores were 0-1 in 52 and 2 in 9 patients **(Table 1)**.

***Chemotherapy.*** Twenty-seven NSCLC patients received cisplatin-based multiagent chemotherapy **(Table 2)**. All of these patients received at least 3 chemotherapy cures. Each chemotherapy cycle was repeated every 3 weeks. Response to the therapy was controlled at least at the end of the third cure.

***Response criteria.*** Response to chemotherapy was evaluated according to World Health Organization criteria.[18] A complete response (CR) was defined as the complete disappearance of all evidence of tumor for at least 4 weeks. A partial response (PR) was defined as a greater than 50% decrease in the sum of the products of the longest perpendicular diameters of all measurable lesions, or a partial resolution of evaluable lesions for a minimum of 4 weeks without the development of new lesions. Progressive disease was defined as an increase of 25% in the sum of the products of the perpendicular diameters of any available lesion, or the development of a new lesion. All remaining patients were classified as stable diseases.[18] Either a complete response or a partial response was accepted as an objective response to chemotherapy in our study.

***Immunohistochemical staining.*** Slides were prepared from the tissues that were fixated in 10% phosphate added neutral formaline and were placed in paraffin. Immunohistochemical staining was carried out by Biotin-Streptavidin Amplified (BioGenax, United States of America) method and by using GST-π (polyclonal, rabbit, Biogenex) primary antibody. The case was accepted to be positive even if one cell was stained. A hundred cells were counted from different areas of the tumor. Tumors were categorized in 3 groups: less than 10%, 10-50% and more than 50% positive.

**Table 1 -** Comparison of the clinicopathological characteristics of lung cancer patients according to glutathione-S transferase-π expression.

| Characteristics | GST-π <10% | Expression 10-50% | Levels >50% | p-value* |
|---|---|---|---|---|
| ***Age*** | | | | |
| Medium (Range) | 65 (50-73) | 60.5 (35-82) | 63.5 (41-76) | >0.05 |
| ***Sex*** | | | | |
| Male | 7 | 24 | 26 | |
| Female | 2 | 2 | - | >0.05 |
| ***Histology*** | | | | |
| Small cell carcinoma | 6 | 13 | 4 | |
| Non small cell carcinoma | 3 | 13 | 22 | <0.05 |
| ***Performance status*** | | | | |
| 0,1 | 9 | 24 | 19 | |
| 2 | | 2 | 7 | >0.05 |
| ***Stage*** | | | | |
| Non small cell carcinoma | | | | |
| I+IIIA+IIB | 3 | 4 | 4 | |
| IIIa | | 1 | | |
| IIIb | 1 | 2 | 3 | |
| IV | 2 | 6 | 12 | |
| ***Small cell carcinoma*** | | | | |
| Limited | 5 | 7 | 2 | |
| Extensive | 1 | 6 | 2 | |
| | | | | >0.05 |

* according to Kruskal-Wallis and chi-square tests
GST-π – glutathione-S transferase π

**Table 2 -** Chemotherapy regimens administered to the patients.

| Chemotherapeutic regimens | Cases | Dosage | |
|---|---|---|---|
| ***MIC*** | | | |
| Mitomycin C (M) | 23 | 6 mg/m$^2$ iv | 1 day |
| Ifosfamide (I) | | 3 g/m$^2$ iv | 1 day |
| Cisplatin (C) | | 50 mg/m$^2$ iv | 1 day |
| ***NC*** | | | |
| Navelbin (N) | 3 | 30 mg/m$^2$ iv | 1-8 days |
| Cisplatin (C) | | 80 mg/m$^2$ iv | 1 day |
| ***GS*** | | | |
| Gemcitabin (G) | 1 | 1250 mg/m$^2$ iv | 1-8 days |
| Cisplatin (C) | | 80 mg/m$^2$ iv | 1 day |

MIC - mitomycin, NC - navelbin
GS - gemcitabin, IV- intravenous

**Table 3 -** Comparison of clinicopathological characteristics and response to chemotherapy with the expression of glutathione-S transferase-π in non small cell lung carcinoma.

| Characteristics | The rate of glutathione-S transferase π expression | | | p-value* |
|---|---|---|---|---|
| | <10% | 10%-50% | >50% | |
| ***Age*** | | | | |
| Medium (Range) | 66.5 (66-67) | 60 (35-71) | 65.5 (54-74) | >0.05* |
| ***Sex*** | | | | |
| Male | 2 | 9 | 15 | >0.05* |
| Female | 1 | | | |
| ***Performance status*** | | | | |
| 0,1 | 3 | 9 | 11 | >0.05* |
| 2 | | | 4 | |
| ***Stage*** | | | | |
| IIIa | | 1 | | |
| IIIb | 1 | 2 | 3 | |
| IV | 2 | 6 | 12 | >0.05* |
| ***Response to chemotherapy*** | | | | |
| CR + PR | 1 | 3 | 4 | |
| PD | 2 | 3 | 6 | |
| SD | | 3 | 5 | >0.05* |
| ***Median survival (months)*** | 2 | 9 | 6 | >0.05** |

CR - complete response, PR - partial response, PD - progressive diseases, SD - stable disease
* according to Kruskal-Wallis and chi-square tests
** according to Kaplan-Meier test

***Statistics.*** Kruskal-Wallis variance analysis, x2 test, Fisher's Exact x2 test and Kaplan-Meier methods were used in statistical analyses. The results were accepted to be significant when p value was <0.05.

**Results.** ***Clinical characteristics and glutathione-S transferase-π expression.*** Glutathione-S transferase-π staining was found to be less than 10% in 6, 10%-50% in 13 and more than 50% in 4 of 23 SCLC patients. These values were 3, 13 and 22 in NSCLC. There were no cases with complete absence of staining. There was a significant difference in GST-π expression between SCLC and NSCLC cases. No relationship was found between Gllutathione-S transferase-π expression and age, sex, performance status and stage of the disease ($p > 0.05$). **(Table 1)**

***Response to chemotherapy and glutathione-S transferase-π expression.*** An objective response was obtained in 8 of 27 NSCLS patients receiving chemotherapy. Glutathione-S transferase-π expression was less than 10% in one, 10-50% in 3 and more than 50% in 4 patients. Glutathione-S transferase-π expression was found to be less than 10% in 2, 10-50% in 3 and more than 50% in 5 patients with stable disease. In patients with progressive disease, 2 had less than 10%, 3 had 10-50% and 6 had more than 50% GST-π expressions. No significant relationship was detected between GST-π expression and response to chemotherapy ($p>0.05$). **(Table 3)**

***Survival and glutathione-S transferase-π expression.*** Median survival periods were 6 months, 9 months and 6 months for GST-π expressions of less than 10%, 10-50% and more than 50%. No significant difference was found between the median survival of the 3 groups (>0.05). **(Table 3)**

**Discussion.** There are various histopathological types of lung cancer. The susceptability of the tumor to chemotherapy differs according to these types. It is known that SCLC is sensitive to various chemotherapeutics. However, NSCLC may be resistant to chemotherapy or tumor may relapse after some time. Different responses to chemotherapy may be observed among different histopathological subtypes of NSCLC. The response to chemotherapy is unpredictable especially in late stages of NSCLC. For these reasons, therapy is hard in late stages of NSCLC. However, survival of the patients receiving chemotherapy is better than the others.[19,20] Although new drugs have been used for NSCLC, cisplatin has been the standard drug in the combination chemotherapy protocols.[2] However effectiveness of therapy decreases with the increase in drug resistance. If sensitive and resistant tumors can be detected before the therapy, the patient can be free of excessive drug use by predicting the chemotherapy response. Glutathione-S transferase-π is expressed in lung cancer tissue and it has been now realized that the degree of expression differs according to the histologic subtype. Some investigators have graded GST-π expression as zero when there was complete absence of plasma membrane staining or cytoplasmic staining, while some have accepted the expression to be positive when more than 20% of the cells were stained.[20,21] Eimoto et al[21] have reported that GST-π expression was positive in squamous cell lung cancer, but negative in adenocancer and SCLC. Chung et al,[22] have reported a higher degree of positive staining in squamous cell cancer cases compared to adenocancer cases. Another study revealed 83% and 67% positive staining in squamous cell carcinomas and adenocancer of the lung. In the same study, a significant difference in GST-π expression was observed between SCLC and NSCLC, but not between SCLC and adenocancer.[15] Some investigators have even demonstrated that mesaurement of serum GST-π could be used as a tumor marker in lung cancer.[13,14] Howie et al[13] have observed that serum levels of GST-π would be higher in adenocancers. On the other hand Hida et al,[14] have stated that GST-π serum levels might have limited value as a tumor marker for NSCLC.

In our study GST-π expression has been evaluated in the 2 major histologic subtypes of lung cancer tissue and the tumors have been categorized in 3 groups as having less than 10%, 10-50% and more than 50% positive GST-π expression. To our knowledge, such a classification has not been used in the literature previously, but we think that this type of classification defines GST expression more clearly. Our results indicate significantly higher GST-π expression in NSCLC compared to SCLC in accordance with the literature. Bai et al,[15] have not detected any significant relationship between GST-π expression and age, sex, performance status and stage in NSCLC. Nakanishi et al,[16] have not observed any relationship with age, sex, performance status, weight loss, histopathological type and stage of the disease in NSCLC cases. No significant relationship has been reported between the degree of expression and clinicopathological properties of the patients in several other malignant tumors expressing GST-π, either.[12,23,24] Similar results were obtained in our study.

There are many studies in the literature in which several substances such as p-glycoprotein, GST-π, topoisomerase-II and metallothioneine were investigated as an adjunct to drug resistance in cancer cases.[25-27] High GST-π expression in some cancer tissues was correlated to cisplatin resistance.[8,9,28] Hida et al,[14] claimed that high GST-π expression as well as elevated serum levels of GST-π in NSCLC might indicate resistance to the treatment regimens containing cisplatin. In the study of Bai et al,[15] negative GST-π expression indicated a higher response rate to cisplatin-based multiagent chemotherapy in NSCLC. Glutathione-S transferase-π and p53 expressions were studied simultaneously to evaluate the cisplatin resistance in NSCLC by Nakanishi et al.[16] It was stated that both GST-π and p53 could

predict drug resistance. Arai et al[28] also claimed that response to cisplatin containing chemotherapy is better in NSCLC patients whose GST-π expression is negative, and GST-π can be used to predict the chemotherapy response. We could not demonstrate any significant correlation between GST-π expression and response to chemotherapy in our study. Hsu et al,[29] have studied a relatively small cohort of nasopharyngeal carcinoma cases and declared that GST-π expression was not correlated with chemoresistance to cisplatin in that study group. It was reported that no significant relationship was detected between GST-π expression and survival rates in neuroblastoma.[23] Resistance to cisplatin is claimed to be due to several mechanisms such as increase in cellular detoxification by glutathione-like proteins, decrease in cellular accumulation of the drug, increase in deoxyribonucleic restoration.[30-32]

However, none of these mechanisms can explain why resistance to cisplatin occurs. As previously stated, contrary to the results of several studies in the literature, in our study, resistance to cisplatin was not correlated with GST expression. This might be as our study group was small. Therefore, further studies with larger groups are necessary to clarify a detailed mechanism of chemoresistance in patients with lung cancer.

## References


1. Inde DC. Chemotherapy for lung cancer. ***N Engl J Med*** 1992; 327: 1434-1441.
2. American Society of Clinical Oncology. Clinical practice guidelines for the treatment of unresectable non-small cell lung cancer. ***J Clin Oncol*** 1997; 15: 2996-3018.
3. Moscow JA, Fairchild CR, Madden MJ, Ransom DT, Wieland HS, O'Brien EE et al. Expression of anionic glutathione S-transferase and P-glycoprotein genes in human tissues and tumors. ***Cancer Res*** 1989; 49: 1422-1428.
4. Morrow CS, Cowan K. Drug resistance and cancer. ***Adv Exp Med Biol*** 1993; 330: 287-305.
5. Arrick BA, Nathan CF. Glutathione metabolism as a determinant of therapeutic efficacy. ***Cancer Res*** 1984; 44: 4424-4432.
6. Ciaccio PJ, Tew KD. Adaptive response to glutathione S-transferase inhibitors. ***Br J Cancer Suppl*** 1996; 27: S93-S98.
7. Hsu CH, Chen CL, Hong RL, Chen KL, Lin JF, Cheng AL. Prognostic value of multidrug resistance 1, glutathione-S-transferase-pi and p53 in advanced nasopharyngeal carcinoma treated with systemic chemotherapy. ***Oncology*** 2002; 62: 305-312.
8. Cheng X, Kigawa J, Minagava Y, Kanamori Y, Itamochi H, Okada M et al. Glutathione--S transferaz–pi expression and glutathione concentration in ovarian carcinoma before and after chemotherapy. ***Cancer*** 1997; 79: 521-527.
9. Ribrag V, Massaad L, Janot F, Morizet J, Gouyette A, Chabot GG. Main drug-metabolizing enzyme systems in human non-Hodgkin's lymphomas sensitive or resistant to chemotherapy. ***Leuk Lymphoma*** 1995; 18: 303-310.
10. Hayes JD, Pulford DJ. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. ***Crit Rev Biochem Mol Biol*** 1995; 30: 445-600.
11. Howie AF, Forrester LM, Glancey MJ, Schlager JJ, Powis G, Beckett GJ et al. Glutathione S- transferase and glutathione peroxidase expression in normal and tumor human tissues. ***Carcinogenesis*** 1990; 11: 451-458.
12. Okuyama T, Maehara Y, Endo K, Baba H, Adachi Y, Kuwano K et al. Expression of glutathione S-transferase-pi and sensitivity of human gastric cancer cells to cisplatin. ***Cancer*** 1994; 73: 1377-1382.
13. Howie AF, Douglas JG, Fergusson RF, Beckett GJ. Measurements of glutathione S-transferase Pi isoenzyme in plasma, a possible marker for adenocarcinoma of the lung. ***New York, Clin Chem*** 1990; 36: 453-456.
14. Hida T, Kawasaki M, Ariyoshi Y, Takahashi T, Sugiura T, Hosoda K et al. Serum glutathione S-transferase-pi level as a tumor marker for non-small cell lung cancer. Potential predictive value in chemotherapeutic response. ***Cancer*** 1994; 73: 1377-1382.
15. Bai F, Nakanishi Y, Kawasaki M, Takayama K, Yatsunami J, Pei XH et al. Immunohistochemical expression of glutathione S-transferase-pi can predict chemotherapy response in patients with nonsmall cell lung carcinoma. ***Cancer*** 1996; 78: 416-421.
16. Nakanishi Y, Kawasaki M, Bai F, Takayama K, Pei XH, Takano K et al. Expression of p53 and glutathione S- transferase-p relates to clinical drug resistance in non-small cell lung cancer. ***Oncology*** 1999; 57: 318-323.
17. Mountain CF. Revisions in the Internatıonal System for Staging Lung Cancer. ***Chest*** 1997; 111: 1710-1717.
18. Non-small Cell Lung Cancer Collaborative Group. Chemotherapy in non-small cell lung cancer: a meta analysis using uptated data on individual patients. ***BMJ*** 1995; 311: 899-909.
19. Marino P, Pampallona S, Preatoni A, Cantoni A, Inverzinni F. Chemotherapy versus supportive care in advanced non-small cell lung cancer: results of a meta analysis of the literature. ***Chest*** 1994; 106: 861-865.
20. Volm M, Mattern J, Samsel B. Overexpression of P-glycoprotein and glutathione S-transferase-pi in resistant non-small cell lung carcinomas of smokers. ***Br J Cancer*** 1991; 64: 700-704.
21. Eimoto H, Tsutsumi M, Nakajima A, Yamamoto K, Takashima Y, Maruyama H et al. Expression of the glutathione S-transferase placental form in human lung carcinomas. ***Carcinogenesis*** 1988; 9: 2325-2327.
22. Chung H, Chieh HH, Hu H, Tsa C. Immunohistochemical investigation on the expression of glutathione S-tansferases (GSTS) in lung cancer. ***Zhonghua Jie He He Hu Xi Xi Ji Bing Za Zhi*** 1993; 16: 141-143.
23. Kutlik MT, Ayhan A, Gogus S, Yalçın B, Çaglar M, Büyükpamukçu M. Glutathione S-transferase and p-glycoprotein expressions in neuroblastoma. ***Pediatr Hematol Oncol*** 2002; 19: 337-345.
24. Mulder TP, Verspaget HW, Sier CF, Roelofs HM, Ganesh S, Griffioen G et al. Glutathione S-transferase pi in colorectal tumors is predictive for overall survival. ***Cancer Res*** 1995; 15: 55: 2696-2702.
25. Scheper RJ, Broxterman HJ, Scheffer G, Kaaijk P, Dalton WS, Heijningen TH et al. Overexpression of a 110 kD vesicular protein in non-P-glycoprotein mediated multidrug resistance. ***Cancer Res*** 1993; 53: 1475-1479.
26. Kasahara K, Fujiwara Y, Nishio K, Ohmori T, Sugimoto Y, Komiya K et al. Metallothionein content correlates with the sensitivity of human small cell lung cancer cell lines to cisplatin. ***Cancer Res*** 1991; 51: 3237-3242.
27. Volm M, Mattern J. Expression of topoisomerase II catalase, metallothionein and thymidylate synthase in human squamous cell lung carcinomas and their correlation with doxorubicin resistance and with patients smoking habits. ***Carcinogenesis*** 1992; 13: 1947-1950.
28. Arai T, Yasuda Y, Takaya T, Hayakawa K, Toshima S, Shibuya C et al. Immunohistochemical expression of glutathione transferase-pi in untreated primary non-small-cell lung cancer. ***Cancer Detect Prev*** 2000; 24: 252-257.

29. Hsu CH, Chen CL, Hong RL, Chen KL, Lin JF, Cheng AL. Prognostic value of multidrug resistance 1, glutathione-S-transferase-pi and p53 in advanced nasopharyngeal carcinoma treated with systemic chemotherapy. ***Oncology*** 2002; 62: 305-311.
30. Andrews PA, Howell SB. Cellular pharmacology of cisplatin: perspectives on mechanisms of acquired resistance. ***Cancer Cell*** 1990; 2: 35-43.
31. Masuda H, Tanaka T, Matsuda H, Husaba I. Increased removal of DNA-bound platinum in a human ovarian cancer cell line resistant to cis Diamminedichloroplatinium (II). ***Cancer Res*** 1990; 50: 1863-1866.
32. Ishikawa T. The ATP-dependent glutathione-S-conjugate export pump. ***Trends Biochem Sci*** 1992; 5: 164-166.