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Polymorphisms in *GSTM1* and *GSTT1* influence the response and treatment outcome in lung cancer patients treated with platinum-based chemotherapy

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Lung cancer, the leading cause of cancer-related deaths, is amongst the most frequent cancer types reported, and is mainly classified as small cell lung cancer and non-small cell lung cancer (NSCLC). One of the standard treatments of NSCLC involves the use of platinum-based compounds, such as cisplatin or carboplatin [3], but as with all chemotherapy, these are linked to undesired side effects [4].

Reduced ability to metabolise chemotherapy by a detoxification mechanism renders the host susceptible to lung cancer and also influences their treatment outcome and survival. This detoxification system consists of enzymes such as glutathione S-transferase (GST). Enzymes encoded by the *GST* super-family detoxify carcinogens, therapeutic drugs and environmental toxins, thereby inhibiting their interaction with cellular proteins and nucleic acids [5,6]. Genetic deletions in two family members, *GSTM1* and *GSTT1* result in loss of catalytic activity [5]. *GSTM1* has two active alleles and a non-functional null allele which results from a deletion mutation. *GSTT1* codes for biotransforming enzymes which act on various drugs and industrial chemicals [7]. Platinum-based compounds (cisplatin or carboplatin) are detoxified by the catalytic activity of GST enzymes [8].

Thus, by anticipating an individual's glutathione system activity, responses to platinum drugs could be quantified and could potentially provide clinicians with useful prognostic information. We therefore hypothesised a role for polymorphisms of *GSTM1* and *GSTT1* with overall survival in lung cancer patients and their treatment response related to platinum-based chemotherapy.

We tested our hypothesis in a cohort of 323 subjects, approved by the Institute Ethics committee of the Post Graduate Institute of Medical Education and Research, Chandigarh, India. Clinic-pathological details such as TNM staging (Tumour size, lymph Node involvement, Metastasis) and clinical response towards chemotherapy were obtained from the hospital records. Every 2 months, the patients were followed up until death or till the end of the study. The survival time was from the date of diagnosis till the last follow-up or the date of death.

Genomic DNA was isolated from peripheral blood [9] with slight modifications. A multiplex PCR was used for the genotypic analysis [10]. The presence or absence of *GSTM1* was detected using specific primers: F 5′-GAACTCCCTGAAAAGCTAAAGC-3′ and R 5′-GTTGGGCTCAAATATACGGTGG-3′ to generate a 480 bp product. The presence or absence of *GSTT1* were detected using primers F 5′-TTCCTTACTGGTCCTCACATCTC-3′ and R 5′-TCACCGGATCATGGCCAGCA-3′ to generate a fragment of 215 bp. A fragment of 312 bp of the albumin gene was amplified as an internal standard with F 5′-GCCCTCTGCTAACAAGTCCTAC-3′ and R 5′-GCCCTAAAAAGA AAATCGCCAATC-3′. Twenty-five microlitres of PCR mixture contains: 1X PCR buffer, 1.5 mM $MgCl_2$, 0.5 µM each primer, 200 µM of each dNTP's, 100 µg/mL BSA, 2U Taq polymerase and 400 ng of DNA. The PCR conditions were: initial denaturation at 95°C for 5 min followed by 30 cycles of 94°C for 1 min, 59°C for 1 min and 72°C for 1 min followed by a final extension at 72°C for 5 min. The results were inferred on 1.5% agarose gel electrophoresis by the absence or presence of a band for the respective genes. The association between survival and genetic polymorphism was evaluated using Kaplan–Meier and log-rank test for comparison of survival curves. The hazard rate and effect of genetic polymorphism on survival after adjusting for covariates was evaluated using the Cox proportional model providing hazard ratios (HRs) and 95% CI. After adjusting for gender, age, stage, histology, smoking, chemotherapy regimen and performance status the relation between response and genetic polymorphisms was evaluated using a logistic regression model. All the tests were two-sided, statistical significance was set at $p < 0.05$. MedCalc version 15.11.4 (MedCalc Software, Ostend, Belgium) was used for statistical analysis.

Of 323 subjects, 281 (87%) were males and 42 (13%) females: their mean [SD] age was 55.2 [10.6] yrs. There were 270 (83.6%) smokers, 115 (35.6%) had a squamous

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Table 1. Relationship of genotype with response to chemotherapy.

Genotype	Response to chemotherapy CR+PR (n=105) n (%)	SD+PD (n=82) n (%)	AHR[a] (95% CI)
GSTT1 +ve	87 (82.9)	67 (81.7)	1.00 (Reference)
GSTT1 −ve	18 (17.1)	15 (18.3)[a]	1.00 (0.44–2.28)
GSTM1 +ve	72 (68.6)	41 (50.0)	1.00 (Reference)
GSTM1 −ve	33 (31.4)	41 (50.0)[b]	2.00 (1.04–3.84)

Adjusted HR (95%cl) [a]1.00 (0.44–2.28)(p=0.11) (X^2 p=0.838) versus GSTT 1 −ve, [b]2.00 (1.04–3.84)(p=0.018) (X^2 p=0.01) versus GSTM 1 −ve. CR= complete remission, PR= partial remission, SD= stable disease, PD= progressive disease.

carcinoma, 101 (31.3%) had an adenocarcinoma, 151 (46.7%) were at TNM stage III, 134 (41.5%) were at TNM stage IV. Of 187 subjects, 105 (32.5%) showed complete or partial response to chemotherapy whereas 82 (26.4%) exhibited stable or progressive disease (i.e. non-response). The T4 type of tumour was present in 162 (50.2%) patients, T3 in 72 (22.3%), T2 in 35 (10.8%) and T1 in 22 (6.8%). Of these, 40.9% had distant metastasis (M1), 11.8% had no lymph node involvement, 9.3% N1 involvement, i.e. 42.4% had N2 and 25.7% had N3 involvement.

Variation in *GSTT1* had no effect on median survival period (6.3 vs. 9.0 months, HR 1.06 95% CI = 0.77–1.45; log rank p = 0.69), and no significant association was observed between *GSTM1* null genotype and outcome survival. There was no association between *GST* genes and overall survival. A positive relationship was found between *GSTM1* genotype and treatment response, in that the null *GSTM1* genotype was linked to poor response (stable disease + progressive disease) towards chemotherapy. However, there was no such link with *GSTT1* (Table 1). When stratified on the basis of histological subtypes, no significant association was observed between *GSTM1* and *GSTT1* polymorphisms and outcome survival. In other studies, the relationships of *GSTM1* and *GSTT1* genotypes and the survival rates in lung cancer are conflicting: some have not found significant links [11,12], while others have found significant associations [8,13].

No significant link was seen between *GSTM1* or *GSTT1* genotypes and survival rates in smokers or non-smokers, although the median survival time of non-smokers having the null *GSTT1* genotype was almost half as compared to that of patients carrying the wild type *GSTT1* genotype (5.0 vs. 9.5 months, HR 1.67 95% CI = 0.62-4.44; log rank p = 0.20), suggesting a possible false negative as only 14% of the cohort were non-smokers. Subjects with null *GSTT1* genotype had an increased risk of death as compared to the subjects having the wild *GSTT1* genotype (HR 2.72 95% CI = 1.09-6.79; p = 0.03). When the association was analyzed between the effects of genetic polymorphism of *GSTT1* and *GSTM1* with Karnofsky Performance Status (KPS), the *GSTT1* null genotype was linked with the performance status of patients under the scale of 70–80, showing increased risk of death compared to the patients with wild *GSTT1* genotype (HR 2.34 95% CI = 1.44-3.82; p = 0.0006) but no such statistically significant association was found with *GSTM1*. Goto et al. [11] reported that *GSTM1* polymorphism was significantly associated with the KPS. Patients with null *GSTT1* genotype had a higher risk of adverse clinical stage and an increased risk of metastases. Patients with the *GSTM1* null genotype were less susceptible to lymph node invasion compared to those carrying the wild *GSTM1* genotype (Table 2), in accordance with Goto et al. [11].

Platinum-based drugs are the standard first-line chemotherapy for NSCLC, especially in advanced disease. Our data shows that patients with the *GSTM1* genotype have a good response to chemotherapy compared to subjects having the null genotype. These results suggest that *GSTM1* plays an important role in influencing the chemotherapy outcome and response. On the contrary, no association was seen in case of the *GSTT1*, in accordance with the earlier study [12] which reported that the null *GSTM1* was associated with a better response to chemotherapy than the non-null *GSTM1* type in NSCLC patients who received platinum-based chemotherapy. Yang and Xian [14] performed a meta-analysis study, finding that *GSTM1* may influence the treatment response of platinum-based chemotherapy in an East-Asian population. The study conducted by Li et al. [15] observed a significant difference in *GSTM1* polymorphism between the response and non-response groups. Thus, *GSTM1* SNP might contribute to the design of individualised cancer treatment for patients with lung cancer [6].

We note limitations in our study. Patients were selected from a single hospital, which might not be representative of the general population. Other variants might influence the treatment outcome of advanced NSCLC in addition to *GSTT1* and *GSTM1*, and the sample size was relatively small, which could limit the power to identify the differences between groups. Further studies with large sample sizes are needed to clarify the association of glutathione S-transferases gene polymorphisms with the prognosis of advanced NSCLC.

Our data represent an advance in biomedical science as it shows that certain *GST* polymorphisms are linked to response to chemotherapy and outcome survival, and so should be adopted as part of the routine management of patients with NSCLC.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Table 2. Relationship of different genotypes with the clinical stage, tumour extension, lymph node invasion and metastasis.

		GSTT1		*GSTM1*	
Genotype		*GSTT1+*	*GSTT1−*	*GSTM1+*	*GSTM1−*
Clinical Stage	III (151) n (%)	132 (87.4)	19 (12.6)	97 (64.2)	54 (35.8)
	IV (134) n (%)	101 (75.4)	33 (24.6)	75 (56)	59 (44)
	AHR (95% CI)	1.00 (Reference)	2.64 (1.38–5.04)	1.00 (Reference)	1.35 (0.82–2.22)
	p-value	-	-	-	-
		-	0.003	-	0.23
Primary tumour extension	Tx+T1+ T2 (57) n (%)	49 (85.96)	8 (14.04)	33 (57.9)	24 (42.1)
	T3+ T4 (234) n (%)	189 (80.8)	45 (19.2)	137 (58.55)	97 (41.45)
	AHR[a] (95% CI)	1.00 (Reference)	1.38 (0.60–3.17)	1.00 (Reference)	1.08 (0.58–2.01)
	p-value	-	0.44	-	0.78
Lymph node invasion	N0 (38) n(%)	33 (86.8)	5 (13.2)	15 (39.47)	23 (60.53)
	N1–N4 (253) n (%)	205 (81.03)	48 (18.97)	155 (61.26)	98 (38.74)
	AHR[a] (95% CI)	1.00 (Reference)	1.45 (0.53–3.96)	1.00 (Reference)	0.40 (0.2–0.84)
	p-value	-	0.46	-	0.014
Metastasis	No (161) n (%)	138 (85.7)	23 (14.3)	97 (60.25)	64 (39.75)
	Yes (132) n (%)	102 (77.3)	30 (22.7)	75 (56.8)	57 (43.2)
	AHR (95% CI)	1.00 (Reference)	2.12 (1.13–3.98)	1.00 (Reference)	1.05 (0.64–1.73)
	p-value	-	0.019	-	0.82

AHR, Adjusted Hazard ratio; CI, confidence interval.

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