BRITISH JOURNAL OF BIOMEDICAL SCIENCE
2018, VOL. 75, NO. 4, 169–174
https://doi.org/10.1080/09674845.2018.1482734

Impact of GSTM1, GSTT1 and GSTP1 genes polymorphisms on clinical toxicities and response to concomitant chemoradiotherapy in cervical cancer

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ABSTRACT

Background: Certain forms of chemoradiotherapy generate toxic reactive oxygen species, which may be ameliorated by antioxidant enzymes such as glutathione S-transferase (GST). Genetic polymorphisms of *GST* may predict treatment outcomes and can be used as genetic marker to screen patients before treatment. We hypothesised an effect of *GST* polymorphisms on the response and toxicities produced by chemoradiation therapy.

Materials and methods: GST polymorphisms were determined by multiplex polymerase chain reaction and PCR-restriction fragment length polymorphism (PCR-RFLP) in 227 women with cervical cancer receiving cisplatin based chemoradiotherapy. Treatment response and toxicities were evaluated by standard internationally recognised criteria (RECIST and RTOG).

Results: Severe (grade 3–4) gastrointestinal and haematological toxicities were present in 22 (9.4%) and 16 (7.0%) patients, respectively. *GSTM1* null, *GSTT1* null and *GSTP1* AG genotypes brought marginally better non-significant associations. In single locus analysis *GSTP1* AG and GG was linked to greatest risk of severe (grade 3–4) gastrointestinal toxicity (OR = 3.12, $P = 0.035$ and OR = 6.99, $P = 0.01$, respectively). In gene–gene interaction analysis, *GSTM1* null-*GSTP1* GG showed 4.2-fold higher risk of severe gastrointestinal toxicity ($P = 0.014$). *GSTT1* null-*GSTP1* AG reached statistical significance with a 3.9-fold higher risk of high grade gastrointestinal toxicity ($P = 0.038$).

Conclusions: Although no significant links were found between *GST* polymorphism and treatment response, null genotypes of *GSTM1, GSTT1* and 'G' allele of *GSTP1* bring a higher risk of severe gastrointestinal toxicity due to chemoradiation therapy in cervical cancer.

ARTICLE HISTORY
Received 4 May 2018
Accepted 28 May 2018

KEYWORDS
Cervical cancer; concomitant chemoradiation; *GST* gene polymorphism; response; toxicity

Introduction

Cervical cancer is the second most common cancer among women and remains a major health problem worldwide [1]. Concomitant cisplatin based chemoradiation, the standard treatment for cervical cancer, is effective in improvement in tumour control and overall survival of patients [2]. Although effective, this treatment is linked to early and late widespread toxicities to various organs [3–5]. These side effects have an impact on treatment outcome, exhibiting a decrease in patient compliance and overall quality of life during treatment. Anticancer treatment has a narrow therapeutic index and administration of maximum dose to achieve the best response may lead to increased risk. The wide variability in toxicity and efficacy of treatment is a major challenge in current clinical practice, and are often due to differences in genetic constitution [6]. Accordingly, knowledge of a patient's response to a particular drug and its dose would be valuable in identifying predictors of toxicity so that cancer treatment regimens can be decided on a personalised basis with maximum efficacy [7,8].

Resistance and toxicity due to specific agents are largely determined by multifaceted enzymatic systems which are cytotoxic targets or members of metabolic pathways of administered drugs. Studies have suggested that genetic polymorphisms in genes encoding such metabolic enzymes and those involved in DNA-repair, signalling and cellular response pathways contribute to inter-patient variability in drug response and toxicity [9].

The glutathione S-transferases (GSTs) superfamily belongs to dimeric phase II metabolic enzymes, acting on various xenobiotics or metabolic by-products, and so play an important role in cell protection. They protect against cellular damage by detoxifying toxic and carcinogenic electrophilic molecules via conjugation with glutathione, and also scavenge free radicals produced by radiation and cytotoxic drugs [10,11]. Most common members of the GST family are coded for by *GSTM1*, *GSTT1* and *GSTP1* that have functional polymorphisms. The genetic polymorphism of *GSTP1* A313G and null polymorphisms of *GSTM1* and *GSTT1* that reduce the enzyme activity have been associated with increased

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drug response in cancer patients [12]. Anti-cancer drugs such as cisplatin, carboplatin, chlorambucil, melphalan, cyclophosphamide and adriamycin are substrates for GST which determines their cytotoxicity [13–17]. Therefore, we hypothesised that *GSTM1*, *GSTT1* and *GSTP1* are linked to acute toxicity in cervical cancer patients undergoing concurrent chemoradiotherapy.

Materials and methods

The study was performed in cervical cancer patients assigned to receive chemoradiotherapy, enrolled from the Department of Radiotherapy, King George's Medical University, Lucknow, India, from which Institutional Ethics Committee approval was obtained. Inclusion criteria were histopathologically proven cervical cancer, age between 30 and 70 years with similar ethnicity, FIGO stage II-II, Karnofsky Performance Status (KPS) ≥70, and normal haematological, renal and hepatic functions. Exclusion criteria were age >70 years, history of other cancers and any co-morbid conditions such as diabetes, cardiovascular disease, allergy, infection and inflammatory response, prior chemotherapy, radiotherapy or surgery. Clinical diagnosis and staging was performed as per guidelines of International Federation of Gynecology and Obstetrics. Under these criteria, 227 women were recruited.

All patients received external beam radiation therapy (EBRT) 50 Gy/25 fractions for 5 weeks by AP-PA/4 Field box techniques to whole pelvis along with weekly concurrent cisplatin (40 mg/m^2). This was followed by three vaginal insertions of high dose rate intracavitary brachytherapy of 7Gy/fraction at one-week intervals. Patients were assessed every week during treatment for acute toxicities. Weekly haematology and renal function tests were done in all patients. Several protection measures were undertaken during irradiation in order to prevent gastrointestinal and urinary toxicities. The patients who did not followed protocol of chemoradiation treatment were excluded from the study.

During treatment, adequate bladder filling protocol was followed in EBRT, appropriate and meticulous insertion of applicator with adequate packing of vagina in brachytherapy was practised in order to protect the bladder and rectum. Patients were assessed every week during the treatment for acute toxicity. The most common toxicities observed were gastrointestinal, haematological, skin and genitourinary. Patients were evaluated and classified according to the Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer (RTOG/EORTC) criteria for grading the toxicities which follows a scale of 0–4 [18]. Grade 0 represented no toxicity, grades 1–2 were considered low grade while grade >2 was considered high grade/severe toxicity. Gastrointestinal toxicities were nausea, vomiting, diarrhoea and proctitis. Haematological toxicities were anaemia, leucopenia, neutropenia and thrombocytopenia, while genitourinary toxicity was cystitis. Skin toxicity was from development of dull erythema to ulceration [18]. Nutritional support, counselling and supportive care were provided before initiating, during and after completion of treatment until all acute toxicities were resolved. Patients who experienced severe toxicities were managed with intravenous infusion, blood transfusion, low fibre diet, administration of antibiotics, anti-diarrheals, anti-spasmodics, colony stimulating factors and treatment breaks as and when required, depending on the type of toxicity. Treatment response to CRT was assessed according to Response Evaluation Criteria in Solid Tumours (RECIST) as complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD) [19].

Five millilitre (ml) of blood was collected from cases in EDTA vials after informed consent and ethical approval. Genomic DNA was extracted from blood samples of all patients by standard salting out method with slight modifications [20,21]. The DNA quantity and quality was checked by a biophotometer (Eppendorf, Germany) and 1% agarose gel. The genotypes of *GSTM1* and *T1* polymorphisms were detected by using multiplex polymerase chain reaction using specific primers: forward 5′GAACTC CCTGAAAAGCTAAAGC-3′ and reverse 5′GTTGGGCTCAA ATATACGGTGG-3′; forward 5′TCCTTACTGGTCCTCACA TCTC-3′ and reverse 5′TCACCGGATCATGGCCAGCAC-3′, respectively. PCR amplification was performed in a 25 µl reaction mixture of genomic DNA (100–150 ng), 5 pmol of each primer, 200 µM of each dNTPs, and 0.5 U of Taq DNA polymerase (MBI-Fermentas, USA) per tube using a gradient Master Cycler (Eppendorf, Germany). The PCR products were visualised on ethidium bromide stained 2% agarose gels in a Gel Documentation System (Vilber Lourmat, France). The null genotypes of *GSTM1* and *T1* were determined by absence of gene products. *TCF7L2* was co-amplified and used as positive control for *GSTM1* and *GSTT1*. The *GSTP1*A313G (Ile105Val) polymorphism was analysed using PCR-Restriction Fragment Length Polymorphism (PCR-RFLP). The PCR reaction mixture of 25 µl was prepared as described above by using primers: forward 5′ACCCCAGGGCTCTATGGGAA-3′ and reverse 5′TGAGGGCACAAGAAGCCCCT-3′. The PCR products were digested with two units of restriction enzyme *Alw26*I at 37 °C for 16 h. The digested products were electrophoresed on 12% polyacrylamide gel (PAGE) and visualised with ethidium bromide.

The sample size for *GST* polymorphisms was calculated by QUANTO software [22] using minor allele frequency and prevalence. Odds ratio (OR) and 95% confidence intervals (95% CI) were calculated by multivariate logistic regression analysis. The association of different combinations of *GST* genotypes with response and different toxicities were analysed. All *P* values were two-sided and

Table 1. Clinical characteristics of the participants.

Characteristics	Patients (*n*)	Frequency (%)
Tumour stage		
Stage IIB	117	51.5
Stage IIIA/IIIB	110	48.5
Histopathology		
SCC	216	95.2
AD	11	4.8
Response		
CR	162	71.4
PR	32	14.1
SD	22	9.7
PD	11	4.8
Toxicities		
Gastrointestinal toxicity		
Grade 0–2	205	90.6
Grade 3–4	22	9.4
Haematological toxicity		
Grade 0–2	211	93.0
Grade 3–4	16	7.0
Skin toxicity		
Grade 0	210	92.5
Grade 1	17	7.5
Genitourinary toxicity		
Grade 0	187	82.4
Grade 1	40	17.6

SCC, squamous cell carcinoma; AD, adenocarcinoma; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Table 2. Clinical responses to cisplatin based concomitant chemoradiation according to genotypes.

	Clinical response			
Genotypes	CR + PR (%)	SD + PD (%)	OR (95% CI)	*P* value
	N = 194 (85.5)	*N* = 33 (14.5)		
GSTM1				
Present (*n* = 147)	121 (62.4)	26 (78.8)	1.0 (Ref.)	
Null (*n* = 80)	73 (37.6)	7 (21.2)	0.45 (0.18–1.11)	0.074
GSTT1				
Present (*n* = 176)	148 (76.3)	28 (84.8)	1.0 (Ref.)	
Null (*n* = 51)	46 (23.4)	5 (15.2)	0.60 (0.21–1.68)	0.333
GSTP1 A313G				
AA (*n* = 128)	108 (55.7)	20 (60.6)	1.0 (Ref.)	
AG (*n* = 81)	74 (38.1)	7 (21.2)	0.54 (0.21–1.37)	0.194
GG (*n* = 28)	12 (6.2)	6 (18.2)	2.02 (0.63–6.45)	0.236
Alleles				
A# (*n* = 337)	290 (74.7)	47 (71.2)	1.0 (Ref.)	
G# (*n* = 117)	98 (25.3)	19 (28.8)	1.20 (0.67–2.14)	0.545

CI, confidence interval; OR, odds ratio; 1.0 (Reference), adjusted for age, stage and histopathology; Alleles#, total number of chromosomes (unadjusted). See Table 1 for other abbreviations.

differences were considered statistically significant at $P < 0.05$. The statistical analyses were performed on SPSS (Version 21.0).

Results

Clinical characteristics distribution of 227 women (age mean [SD] 48.5 [8.3 years]) are summarised in Table 1. Of these, 85.5% were responders and 14.5% were non-responders. After comparing treatment response with GST polymorphisms, the frequency of non-responders was higher in *GSTM1* present, *GSTT1* present and *GSTP1* AA genotypes but did not reach significance (Table 2). Severe gastrointestinal toxicity (grade 3–4) was more frequent among women with *GSTM1* null, *GSTT1* null and *GSTP1* AG genotypes (Table 3). *GSTM1*, *T1* and *P1* polymorphisms were associated with gastrointestinal, but not with haematological toxicity. Patients with the *GSTP1* AG and GG genotypes showed significantly higher risk of developing severe gastrointestinal toxicity (Table 3). In combined genotype analysis, patients with *GSTM1* null/*GSTP1* GG and *GSTT1* null/*GSTP1* GG showed higher risk of severe (grade 3–4) gastrointestinal toxicity. However, the model for clinical response did not reach statistical significance (Table 4). Genitourinary and skin toxicities were low grade and there was no significant link with *GST* genotypes.

Discussion

Radiotherapy with concurrent platinum-based chemotherapy is a standard treatment for locally advanced cervical cancer [23]. Severe toxicities resulting from this treatment cause reduced efficacy and contribute to patient morbidity. Therefore, predictive factors such as particular genotypes and haplotypes may help to screen patients at risk of increased toxicity. Cisplatin is one of the most cytotoxic platinum agents used in carcinoma of uterine cervix [24]. The platinating agents have the ability to generate DNA cross-links and intra-strand N-7 adducts which are the major causes of cytotoxicity. When cisplatin is used concomitantly with radiation, it acts as a radio-sensitizer causing significant increase in cell death. During radiation, there are two mechanisms of interaction by which a platinum compound acts: forming free radicals with altered binding to DNA and inhibiting repair of sublethal damage [25].

The cisplatin adducts become aquated in tissue and interact with thiol containing molecules like glutathione and metallothioneins. GST detoxifies cisplatin by conjugation with glutathione and increases its excretion from the body [26]. Acquired resistance to cisplatin involves increased inactivation by glutathione and related enzymes [27]. Furthermore, the cytotoxic effects of radiation result principally from damage to DNA, either directly or indirectly by formation of hydroxyl radicals and reactive oxygen species, which can be detoxified by GSTs [14]. Polymorphisms in many genes contribute to significant treatment-related toxicities in patients by attenuating pathways such as DNA repair, drug metabolism and cell cycle progression, impairing the survival of normal cells under stress during radiotherapy or chemotherapy. Genetic polymorphisms can affect protein expression and alter biological pathways that are integral in response to chemoradiation therapy in tumour cells [28]. Many studies showed that polymorphisms in *GST* are linked to variation in cytotoxic effects of many chemotherapeutic drugs, and are linked to survival and toxicity in many types of cancers

Table 3. Association of *GSTM1, T1* and *P1*A313G gene polymorphisms with risk of gastrointestinal toxicity.

Genotypes	Gastrointestinal toxicity Grade 0–2 (%)	Grade 3–4 (%)	OR (95% CI)	*P* value
	N = 205 (90.4)	*N* = 22 (9.6)		
GSTM1				
Present (*n* = 147)	137 (66.8)	10 (45.5)	1.0 (Ref.)	0.077
Null (*n* = 80)	68 (33.2)	12 (54.5)	2.2 (0.91–5.50)	
GSTT1				
Present (*n* = 176)	161 (78.5)	15 (68.2)	1.0 (Ref.)	0.384
Null (*n* = 51)	44 (21.5)	7 (31.8)	1.55 (0.58–4.13)	
GSTP1 A105G				
AA (*n* = 128)	122 (59.5)	6 (27.3)	1.0 (Ref.)	
AG (*n* = 81)	69 (33.7)	12 (54.5)	3.12 (1.08–8.98)	0.035
GG (*n* = 18)	14 (6.8)	4 (18.2)	6.99 (1.58–30.9)	0.01
Alleles				
A# (*n* = 337)	313 (76.3)	24 (54.5)	1.0 (Ref.)	
G# (*n* = 117)	97 (23.7)	20 (45.5)	2.70 (1.42–5.08)	0.002

CI, confidence interval; OR, odds ratio; 1.0 (Reference), adjusted for age, stage and histopathology; Alleles#, total number of chromosomes (unadjusted).

viz. leukaemia, lymphoma, glioma, breast, lung, ovarian, gastric, colorectal and germ cell tumours [29,30].

The detoxification of various exogenous and endogenous reactive species was affected by genetic polymorphisms in *GST*s [31]. The gene deletion polymorphisms of *GSTM1* and *T1* have been described as null genotypes resulting in the absence of functional enzyme [32]. A single nucleotide substitution (A > G) at position 313 leads to amino acid substitution of isoleucine to valine at codon 105 (Ile105Val) of *GSTP1* which results in reduced enzymatic activity [33]. Genotypes resulting in lower GST activity may be advantageous for individuals undergoing chemoradiation treatment because a reduced detoxification may enhance the effectiveness of the treatment [34]. Decreased enzyme activity due to deletion polymorphisms of *GSTM1* and *T1* genotypes increases treatment response as well as toxicity in patients receiving platinum-based drugs like cisplatin and oxaliplatin [35].

We hypothesised that *GSTM1*, *GSTT1* and *GSTP1* are linked to acute toxicity in cervical cancer patients undergoing concurrent chemoradiotherapy. We found that individuals with *GSTT1* null (*T1*-) genotype did not show significant association with toxicity, in agreement with other studies such as chemotherapy-induced toxicity in testicular cancer survivors and response to chemotherapy in head and neck squamous cell carcinoma [36,37]. We also found that individuals having *GSTM1* null (*M1*-) genotype showed a higher risk of high grade gastrointestinal toxicity, but this did not reach statistical significance, and although we believe our study is well-powered, we cannot deny the possibility of a false negative. The enzyme product of *GSTP1* is known to detoxify platinum compounds cisplatin and oxaliplatin, and *GSTP1* polymorphism is linked to differences in chemotherapy response and cancer susceptibility [38]. A study reported that patients with *GSTP1* AA genotype had a higher risk of developing neurological toxicity [10]. In our study, patients having *GSTP1* AG or GG genotypes showed higher risk of high grade gastrointestinal toxicity, whilst the combination of genotypes *GSTM1* null/*GSTP1* GG and *GSTT1* null/*GSTP1* GG was linked to a significant higher risk of high grade gastrointestinal toxicity.

Our results suggest that screening of genetic polymorphisms of *GST* in cervical cancer patients before chemoradiation could act as independent predictors of side effects. We suggest that assessment of *GST* phenotypes may become a routine laboratory method. This may enable clinicians to select optimal doses of chemoradiation with maximum treatment efficacy and reduced side effects leading to a personalised therapy. However, larger sample sizes are required for confirmation of possible interactions between different *GST* polymorphisms and treatment outcome. This study represents an advance in biomedical science as it shows that women with *GSTP1* AG/GG and in combination with *GSTM1* null (M1-) and *GSTT1* null (*T1*-) genotypes are more likely to experience high grade (≥3) gastrointestinal toxicity.

Table 4. Gene–gene interactions among *GSTM1, T1* and *P1*A313G polymorphisms in cervical cancer treatment outcome.

Genotype interactions	Clinical response		Grade 3–4 gastrointestinal toxicity		Grade 3–4 haematological toxicity	
	OR (95% CI)*	*P* value	OR (95% CI)*	*P* value	OR (95% CI)*	*P* value
GSTM1–GSTT1						
Null/null	0.67 (0.16–2.26)	0.455	2.50 (0.71–8.74)	0.154	1.18 (0.22–6.20)	0.846
***GSTM1–GSTP1*A105G**						
Null/AG	2.03 (0.58–7.13)	0.269	1.18 (0.128–10.79)	0.887	0	–
Null/GG	0.80 (0.13–4.72)	0.803	4.20 (1.34–12.91)	0.014	2.00 (0.65–6.15)	0.223
***GSTT1–GSTP1*A105G**						
Null/AG	0.80 (0.16–3.97)	0.786	1.37 (0.148–12.75)	0.78	3.17 (0.54–18.51)	0.201
Null/GG	0.49 (0.13–1.84)	0.293	3.90 (1.08–14.16)	0.038	1.36 (0.25–7.45)	0.727

CI, confidence interval; OR, odds ratio; 1.0 (Reference); *Adjusted for age, stage and histopathology.

Summary table

What is known about this subject?
- Genetic polymorphisms affect drug efficacy of treatment.
- Toxicity due to concomitant chemoradiation therapy increases morbidity and limits therapeutic effectiveness and is attributed to genetic variability.
- Genetic polymorphisms in *GST* may predict treatment outcomes and can be used as genetic marker to screen patients before treatment.

What this paper adds:
- Patients with *GSTP1* AG or GG genotypes have a 3.12–6.99 fold higher risk of high grade gastrointestinal toxicity.
- Patients with *GSTM1* null/*GSTP1* GG and *GSTT1* null/*GSTP1* GG have a 3.9–4.2 fold higher risk of high grade gastrointestinal toxicity.

Acknowledgements

MA is thankful to ICMR for research fellowship.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was supported by Council of Science & Technology-Uttar Pradesh (CST-UP), Lucknow; Centre of Excellence (Higher Education), UP Government; ICMR, New Delhi, India; and DST, New Delhi, India.

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