GSTM1 gene polymorphism and the risk of colorectal cancer in a Saudi Arabian population

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ABSTRACT. The enzyme glutathione S-transferase Mu 1 (GSTM1) is encoded by the *GSTM1* gene. Polymorphisms in *GSTM1* affect the detoxifying function of the enzyme variants. This forms the basis of the debate about the impact of the *GSTM1* null/present genotype on colorectal carcinoma risk. We tested the potential influence of *GSTM1* polymorphisms on the development of colorectal cancer. DNA extracted from 83 samples

taken from patients that were previously diagnosed as having colorectal carcinoma and from 35 control subjects who did not have colorectal carcinoma were amplified. *GSTM1* genotypes were determined by DNA sequencing. The current study revealed that the majority (69/83, 83%) of colorectal cancer cases harbored the null genotype (*GSTM1**0/*0), and the remaining 14 (17%) cases harbored either the *GSTM1*wt/wt or the *GSTM1*wt/*0 genotype. In contrast, among the control cases, 23 (65%) had the null genotype (*GSTM1**0/*0) and 12 (35%) had either the *GSTM1*wt/wt or the *GSTM1*wt/*0 genotype. The current report emphasizes the impact of the *GSTM1* null genotype on the increased risk of colorectal carcinoma in Saudi Arabia.

Key words: Polymorphism; *GSTM1*; Colorectal carcinoma

INTRODUCTION

The 2010 annual report on non-communicable diseases released by the Saudi Arabian Ministry of Health placed colorectal cancer (CRC) first among cancers affecting Saudi males and third among those affecting females. Of all recently diagnosed malignant tumors, CRCs accounted for 10.4% in the Kingdom of Saudi Arabia in 2010 (Al-Eid and Quindo, 2014). CRC development is a complicated multifactorial process, and colorectal carcinogenesis is still not well-understood (Fearon, 2011). Genetic influences play a crucial role in the carcinogenesis of many cancers including CRC (Fearon, 2011). Accumulative evidence in the field of epidemiology suggests that numerous geographical differences mirror variations in lifestyle or environmental exposure, possibly interacting with distinctions in genetic factors (Parkin et al., 1993). Polycyclic aromatic hydrocarbons (PAHs), which are present in tobacco and some dietary components, have been associated with colorectal malignancies. As glutathione S-transferases (GSTs) are engaged in the detoxification of PAHs, it has been assumed that GST genotypes may alter the risk of colorectal cancer in individuals exposed to PAHs (Hirvonen, 1995). Therefore, it has been proposed that genetic alterations in GST genes may influence susceptibility to cancer.

GST enzymes are engaged in phase II metabolic reactions and play an essential protective anticancer role via glutathione conjugation with a range of potentially cytotoxic/genotoxic exogenous or endogenous compounds. These compounds include chemotherapeutic drugs, carcinogens (PAHs), N-nitroso compounds, benzo[a]pyrene diol epoxide-DNA adducts, environmental pollutants, products of oxidative stress such as 5-hydroxymethyluracil and DNA hydroperoxides, in addition to a wide range of xenobiotics. Consequently, the latent hazards presented by these compounds are eliminated, leaving DNA and other vital molecules unharmed and safe from adduct formation (Beckett and Hayes, 1993; Hayes and Pulford, 1995).

The major GST enzymes are GSTM1 (mu), GSTT1 (theta), and GSTP1 (pi); genes encoding these enzymes are polymorphic and are broadly expressed in gastrointestinal tissue (de Bruin et al., 2000). In humans, five genes encode the GST mu class of enzymes, and are located at chromosomal locus 1p13.3 (Pearson et al., 1993). Mu class GST genes encode cytosolic enzymes, which form a dimeric protein and show some tissue specificity in their expression (Campbell et al., 1990).

Mu class GST gene polymorphisms have been widely investigated and formerly reviewed in humans (Hayes and Pulford, 1995; Hayes and Strange, 2000). Geographic and ethnic differences affect the incidence of *GSTM1* genotypes. The *GSTM1**0 allele refers to a loss of the entire gene

(the null genotype), which is the most common polymorphism of the *GSTM1* gene. Individuals with the homozygous deleted allele show absence of GSTM1 enzyme activity (Tetlow et al., 2004). The incidence of the *GSTM1* null genotype is 20-100%, and is influenced by ethnicity (Garte et al., 2001). Individuals harboring the null genotypes have decreased carcinogen detoxification capability and are supposedly at an elevated risk of developing cancer (Hayes and Strange, 2000).

GSTM1 null genotypes are coupled with a risk of some malignancies such as lung (Seidegård et al., 1986), nasopharyngeal (Wei et al., 2013), hepatocellular (Wang et al., 2010), breast (Maugard et al., 1998), and prostate (Murata et al., 1998) cancers. In contrast, several researchers could not detect any significant relationship between *GSTM1* null genotypes and cancers (Lin et al., 1998; Liu et al., 2000). It has been suggested that homozygotic deletion of the functional alleles of *GSTM1* increases the risk of colorectal cancer, although many studies conducted in several populations have produced debatable outcomes. Several research groups have reported a remarkable incidence of the *GSTM1* null genotype in patients with colorectal cancer (Saeed et al., 2013; Cai et al., 2014; Cong et al., 2014; Ma et al., 2014; Teng et al., 2014; Djansugurova et al., 2015), while others failed to validate this result (Hezova et al., 2012; Kassab et al., 2014; Vogtmann et al., 2014).

Recognition of predisposition factors that increase the risk of CRC if individuals are subjected to certain environmental substances may improve our knowledge of the etiology of colorectal malignancies. One way of examining the role of GSTM1 is to investigate the influence of *GSTM1* polymorphisms on susceptibility to malignant colorectal tumors. Hence, this report studied the influence of the resultant genotypes of *GSTM1* polymorphism and the status of colorectal cancer risk.

MATERIAL AND METHODS

Paraffin-embedded tissue samples from 83 cases of previously diagnosed colorectal cancer were analyzed in this study, in addition to 35 samples of non-cancerous colon tissue as a control group. The patients who participated in this study underwent colorectal tumor resection with regional lymph node dissection at King Abdulaziz University Hospital. Clinical data (gender, age, type of carcinoma, size, and grade of carcinoma) and tissue samples were collected by the Pathology Department of King Abdulaziz University. All cases with familial history of colorectal cancer or those who had received radiation therapy or chemotherapy were excluded from this study. All samples were stored at room temperature. The control group of tissues was obtained from patients who were biopsied for non-cancerous conditions (including adenoma and polyps), as well as nearby normal mucosa and distant surgical margins. The control population comprised 15 (43%) females and 20 (57%) males. The mean age was 56.7 years, and the age range was 28-87 years. All blocks of non-cancerous control and tumor tissues were serially sectioned and used in the present study.

DNA isolation

Paraffin-embedded tissue samples were used to extract genomic DNA. A QIAamp DNA FFPE Kit (Qiagen, Germany) was used in accordance with manufacturer instructions. Purified DNA was eluted in 50 µL elution buffer and stored at -40°C. The purity and concentration of the isolated DNA were analyzed using a NanoDrop 2000 UV-VIS spectrophotometer (Thermo Scientific, USA).

GSTM1 genotyping

Genotyping for the recognition of *GSTM1* polymorphisms in colorectal cancer was performed using a SYBR Green commercial polymerase chain reaction (PCR) kit (Qiagen) in accordance with the manufacturer instructions. Approximately 200 ng DNA was amplified in an overall volume of 25 µL/reaction. We used *GSTM1* oligonucleotide primers (forward: 5'-CTGCCCTACTTGATTGATGGG-3'; reverse: 5'-CTGGATTGTAGCAGATCATGC-3') from MWG-Biotech (Ebersberg, Germany) to amplify the *GSTM1* fragments. The PCR was performed using a Thermal Cycler 480 (Applied Biosystems, USA) apparatus. The PCR regimen comprised: an initial denaturation at 94°C for 15 min; followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 1 min, and extension at 74°C for 1 min; and a final extension at 72°C for 10 min. The PCR products were examined by 1% agarose gel electrophoresis and visualized using a Syngene UV transilluminator.

DNA sequencing

A 3500 Genetic Analyzer and a BigDye Terminator v3.1 sequencing kit (Applied Biosystems) were used to sequence the amplified PCR products by following the manufacturer protocol. The resulting sequence data were investigated using the Applied Biosystems sequence analysis software (v. 5.4).

Statistical analyses

Analyses of the results were completed using SPSS version 20 and the chi-square test to establish any significant differences in polymorphism incidence between the colorectal cancer cases and the control group. Calculation of statistics was performed based on 95% confidence intervals.

RESULTS

Eighty-three colorectal cancer cases were revised. The mean age of the cases was 57.8 ± 12.8 years (range 22-94 years), with a slight preponderance of females (45; 54.2%). More than one-third of the tumors (38.55%) were well differentiated, 48.2% were moderately differentiated, and only 13.25% were poorly differentiated. Moderately differentiated tumors were more frequent among males (52.6%) compared with females (44.4%), and poorly differentiated tumors, especially with mucinous or with signet ring cell differentiation, were mostly recorded among females (Table 1). Using a modified Dukes' grading system, 1.2, 2.4, 55.5, 34.9, and 6% of the cancer cases were categorized as grades A, B1, B2, C2, and D, respectively. Almost two-thirds (66.7%) of female tumors were graded B2 as opposed to 42.1% among males, whereas grade C2 accounted for 47.4 and 24.4% among male and female tumors, respectively (Table 1). The most common anatomic sites of the tumors were, in descending order, the ascending colon (21.69%), the sigmoid colon (20.48%), the rectum (18.08%), the descending colon (15.66%), and the rectosigmoid colon (14.45%); and the least common sites (4.82%) were the transverse colon and cecum. Colorectal cancer was almost equally distributed in both genders regarding sites except for the rectum where the tumors occurred 10% more frequently in males than in females. At the time of surgical removal of colorectal tumors, lymph nodes were found to be involved in more than one-third of the tumor cases (38.6%). The average size of the tumor was 5.0 ± 2.6 cm, and the size range was 0.6-12 cm

(Table 1). However, there were no statistically significant differences between the clinical data for the tumors in the male and female populations (P < 0.05) (Table 1).

Table 1. Clinicopathological characteristics of patients with colorectal cancer.

Characteristics		All patients		Female		Male	
		No.	%	No.	%	No.	%
Total cases		83		45	54.2	38	45.8
Age							
	< 40	6	7.23	5	11.11	1	2.6
	40-49	12	14.45	6	13.3	6	15.8
	50-59	28	33.74	13	28.8	15	39.5
	60-69	23	27.72	12	26.6	11	28.9
	> 70	14	15.66	9	20	5	13.2
Average age	57.8 (22-94)						
Tumor location							
	Ascending colon	18	21.69	11	24.4	7	18.4
	Transverse colon	4	4.82	3	6..6	1	2.6
	Descending colon	13	15.66	7	15.5	6	15.78
	Rectum	15	18.08	6	13.3	9	23.68
	Rectosigmoid colon	12	14.45	7	15.5	5	13.15
	Sigmoid colon	17	20.48	9	20	8	21.05
	Cecum	4	4.82	2	4.4	2	5.26
Average size of tumor	5 cm (0.6-12)						
Lymph node involvement							
	Yes	32	38.6	15	33.3	17	44.7
	No	51	61.4	30	66.7	21	55.3
Tumor differentiation							
	Good with/without mucin	32	38.55	17	37.8	15	39.4
	Moderate with/without mucin	40	48.2	20	44.4	20	52.6
	Poor with/without mucin or signet ring cells	11	13.25	8	17.8	3	7.9
Dukes' grading system							
	A	1	1.2	0	0	1	2.6
	B1	2	2.4	0	0	2	5.3
	B2	46	55.5	30	66.7	16	42.1
	C2	29	34.9	11	24.4	18	47.4
	D	5	6	4	8.9	1	2.6

PCR and gene sequencing assays were performed to test the effect of the *GSTM1* polymorphisms on susceptibility to colorectal malignancy. Our results (Table 2) showed that the majority of colorectal cancer cases (69, 83%) were of the null genotype (*GSTM1**0/*0), and the remaining cases (14, 17%) were either of the *GSTM1*wt/wt or *GSTM1*wt/*0 genotypes (wt = wild-type).

The incidence of the null genotype in the current study was higher than that in similar studies that reported significant results (Table 3). However, 23 (65%) and 12 (35%) of the control cases were of the null genotype and the *GSTM1*wt/wt or *GSTM1*wt/*0 genotype, respectively. The results showed a significant association between deletion of the *GSTM1* gene and colorectal malignancies [P value (two sided) = 0.03706; odds ratio = 2.571 with 95%CI = 1.041-6.35] (Table 2). However, no associations were found between the incidence of *GSTM1* genotypes and the clinical and histopathological features (gender, age, site, grade, type, and lymph node metastases) of the tumors (Table 1).

Table 2. *GSTM1* genotypes observed in colorectal cancer cases and controls.

GSTP1 genotypes	Cancer cases		Controls		P
	Total	%	Total	%	
Present	14	17	12	35	0.03706
Null	69	83	23	65	
Total	83	100	35	100	

P = Comparison between the GSTM1 deletion and non-deletion groups.

DISCUSSION

Colon tissues express GSTM1 at significant levels. Therefore, any modification in the phenotype may have a considerable influence on susceptibility to colorectal cancer, and GSTM1 in particular has been reported to contribute to the deactivation of mutagenic and carcinogenic heterocyclic amines (Beckett and Hayes, 1993; Hayes and Pulford, 1995).

The *GSTM1* gene is known to be highly polymorphic. *GSTM1* gene polymorphisms may exert an effect on the functioning of enzymes encoded by this gene through the change in both gene expression level and the activity of the protein itself. Thus, *GSTM1* has an influence on the detoxification of carcinogens, and consequently, the level of DNA damage; in this way, an individual's susceptibility to toxins and carcinogens can vary owing to genetic variations that also affect the toxicity and effectiveness of particular drugs (Gong et al., 2012). The polymorphism of the *GSTM1* gene observed in the human population comprises a hereditary homozygous deletion (null/null), a deletion of a gene fragment, resulting in a deficiency in the protein product, which is related to the total loss of enzymatic activity (Gong et al., 2012). The *GSTM1* null genotype seems to be related to a low capacity for detoxification of selected xenobiotics and a reduced capability for controlling oxidative stress, which is equivalent to the damage to the cell caused by free radical activity (Wei et al., 2012). Null genotypes of this mu class gene have been associated with larger numbers of malignant tumors, probably due to an augmented susceptibility to carcinogens and environmental toxins (Procopciuc and Osian, 2014).

Many studies have assessed the possibility of colorectal cancer in individuals with the null

genotype of *GSTM1*; nevertheless, the outcomes are quite debatable (Hezova et al., 2012; Cai et al., 2014; Kassab et al., 2014; Procopciuc and Osian, 2014; Teng et al., 2014; Djansugurova et al., 2015). The current paper describes *GSTM1* genotypes and the impact of *GSTM1* polymorphism on susceptibility to colorectal malignancies in the tested Saudi population. Despite the small sample size in the current study, our results regarding the incidence of the genotypes of *GSTM1* polymorphism in the 35 controls were almost in agreement with the results of other studies from different parts of the world (van der Logt et al., 2004; Kassab et al., 2014). The slight differences observed were shown to be insignificant.

In respect of the risk of CRC, there are inconsistent reports regarding the impact of *GSTM1* polymorphism on CRC susceptibility. Many studies (Houlston and Tomlinson, 2001; van der Logt et al., 2004; Hezova et al., 2012; Kassab et al., 2014) have shown that a relationship between *GSTM1* polymorphism and colorectal tumors does not exist. In contrast, the current study, which recruited a comparatively small number of controls and CRC cases, supports the idea that homozygous and heterozygous wild-type allele genotypes of *GSTM1* are likely to protect against colorectal malignant tumors. Furthermore, *GSTM1* null genotype carriers are at a greater risk of developing colorectal malignancies. The results of this study are in agreement with those of numerous studies that have reported a significant effect of the *GSTM1* null genotype on susceptibility to colorectal cancer (Sachse et al., 2002; Huang et al., 2006; Csejtei et al., 2008; Hlavata et al., 2010; Cai et al., 2014; Teng et al., 2014). The incidence of the null genotype in the current study was higher than that in similar studies that reported significant results (Table 3).

Table 3. Percentage of colorectal cancer cases harboring the *GSTM1* null genotype that have been reported in the literature.

Authors	Djansugurova et al. (2015)	Cong et al. (2014)	Sachse et al. (2002)	Hlavata et al. (2010)	Csejtei et al. (2008)	Huang et al. (2006)	Aghajany-Nasab et al. (2011)	Darazy et al. (2011)	Current study
GSTM1 null genotype percentage	50.20	53.8	58	53.90	58.80	57.10	71.80	43.90	83

Furthermore, in India, a study that included more than 300 patients with gastrointestinal cancer confirmed the association between the *GSTM1* null genotype and elevated risk of rectal carcinoma. In addition, it has been suggested that the concomitance of polymorphisms in three genes, *GSTM1*, *GSTT1*, and *GSTP1*, may be crucial in the development of colorectal malignancies in the Hindu population (Wang et al., 2011). A further study conducted in Iran indicated that individuals aged over 60 years, harboring the *GSTM1* null genotype, were predisposed to development of CRC (Aghajany-Nasab et al., 2011). In a Lebanese population, an increased risk of gastric and colorectal malignancies was found in individuals with the null genotype. The researchers stated that these findings confirm the outcomes of similar studies conducted on a Caucasian population (Darazy et al., 2011).

However, bearing in mind the heterogeneity of mutagenic and carcinogenic substances, as well as the complexity of xenobiotic metabolic reactions, larger comprehensive research projects are essential for evaluating genetic predisposition to colorectal cancer. Additionally, future studies should investigate the interaction between different environmental exposures and a wide range of high- or low-penetrance genes in addition to *GSTM1*. In general, this study confirms the suggestions of previous studies regarding the consequences of *GSTM1* polymorphisms and susceptibility to colorectal cancer. Furthermore, this research has demonstrated a convincing association between *GSTM1* polymorphism and colorectal cancer risk.

Conflicts of interest

The authors declare no conflict of interest.

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