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ORIGINAL ARTICLE

Does Val/Val genotype of GSTP1 enzyme affects susceptibility to colorectal cancer in Saudi Arabia?

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Abstract

OBJECTIVES: Glutathione S-transferase pi (GSTP1) is a candidate enzyme that may be involved in colorectal cancer susceptibility. Polymorphism of GSTP1 gene may cause changes in expression or structure which lead to alteration in the efficiency of catalytic function of the enzyme variants, i.e., deficient detoxification of carcinogens and consequently influences coloreActal cancer development. The present report examined the possible impact of GSTP1 (Ile105Val) polymorphism and the risk of colorectal cancer.

METHODS: Samples of paraffin embedded tissues from 83 patients with colorectal cancer as well as thirty five non-cancerous colon tissues were collected from the archive of the pathology department at King Abdulaziz University in Saudi Arabia. All cancer and control samples were subjects to DNA extraction then amplification. DNA genetic analyzer from Applied Biosystems was used to sequence the product of amplification for genotypes determination.

RESULTS: None of the genotypes of GSTP1 was associated with the risk of colorectal cancer development. There were no statistical differences in the frequencies of GSTP1 genotypes between colorectal cancer cases and controls.

CONCLUSION: The incidence of (Val/Val) genotype in colorectal cancer cases was three folds higher than controls. This finding is not statistically significant, but it could be of clinical consequence that it may increase the risk of colorectal cancer in Saudi Arabia.

INTRODUCTION

Colorectal cancer (CRC) is ranked first as the type of cancer affecting the Saudi male population and third among females. It accounted for (10.4%) of all recently diagnosed malignant tumors in the Kingdom of Saudi Arabia in 2010 (Al-Eid & Quindo 2014). Mass evidence shows susceptibility to cancer is intermediated by variations in the detoxifying capacity of genetically determined factors which play a role in cellular defense mechanism versus carcinogenic endogenous and exogenous substances (Deakin *et al.* 1996; Inoue *et al.* 2001). Similarly, data exists that connects exposure to reactive oxygen species to the possibility of colorectal cancer development (Yoshida *et al.* 2007). Furthermore, there is indication that around 80% of malignant tumors emerge as a result of exposure to environmental elements (Ates *et al.* 2005; Dang *et al.* 2005). Enzymes engaged in detoxifying carcinogenic substances along with DNA repair may have an impact on susceptibility to many forms of cancer including colorectal tumors (Vlaykova *et al.* 2007; Zhang *et al.* 2007). The efficiency of detoxifying functions of these enzymes is determined genetically (Deakin *et al.* 1996; Inoue *et al.* 2001; Ye & Parry 2002). Polymorphic genes of such enzymes may hence be engaged in colorectal cancer susceptibility.

GSTs are phase 2 enzymes which detoxify and clear the mutagenic and cytotoxic properties of many carcinogens, electrophiles and DNA reactive metabolic products, mainly by glutathione conjugation. Consequently, this process eliminates carcinogens, and protects DNA from injury or adducts formation (Mannervik & Danielson 1988; Zhang *et al.* 1992; Beckett & Hayes 1993; Zhong *et al.* 1994; Ryberg *et al.* 1997). Many studies documented that normal and tumor tissues of the colon expressed GSTP1 (Singhal *et al.* 1992; Hoensch *et al.* 2006; Vlaykova *et al.* 2007; Zhao *et al.* 2012; Zhang *et al.* 2014), in which GSTP1 has key role in detoxifying several carcinogenic and toxic substances such as electrophiles and heterocyclic amines which have been linked with colorectal cancer development (Boone *et al.* 1990; Lin *et al.* 1994; Hengstler *et al.* 1998).

The gene of GSTP1 is located on chromosome 11q. Two polymorphisms (Ile105Val and Ala114Val) of the GSTP1 gene were reported, the first was mapped to exon 5 and the second to exon 6 (Zimniak *et al.* 1994). Both codons rest nearby the GSTP1 binding site. Furthermore, it is known that Ile105Val polymorphism changes enzyme properties (Ryberg *et al.* 1997). Several studies have reported that depending on the substrate, 105Val variant showed low or high activity and affinity in comparison with 105Ile. On the other hand, no influence was found so far for the Ala114Val polymorphism in this regard (Ali-Osman *et al.* 1997; Sundberg *et al.* 1998; Li *et al.* 2013).

The polymorphism of GSTP1 produce replacement of an amino acid isoleucine (Ile) with a valine (Val), which introduces conformational changes, due to the bulky properties of the valine side chain (Mannervik & Danielson 1988; Beckett & Hayes 1993; Zimniak *et al.* 1994). Therefore, it is possible to hypothesize that carcinogen metabolic enzymes with lower activity may be linked to an elevated risk of cancer development. However, to date, the relation between GSTP1 polymorphism and colorectal carcinoma remains somewhat debatable and may vary from population to population (Ates *et al.* 2005; Vlaykova *et al.* 2007; Matakova *et al.* 2009; Epplein *et al.* 2009; Hlavata *et al.* 2010; Khabaz 2012; Sameer *et al.* 2012; Song *et al.* 2014; Kassab *et al.* 2014).

Recognition of susceptibility influences which increase chances of developing colorectal cancer if individuals are subject to certain environmental substances may enlighten our knowledge regarding the etiology of colorectal cancer. One way of examining the protective role of GSTP1 is to research the influences of GSTP1 polymorphisms on susceptibility to malignant colorectal tumors. Hence, this paper investigated the effects of the resultant genotypes of GSTP1 polymorphism and the status of susceptibility to colorectal cancer.

MATERIALS AND METHODS

Paraffin embedded tissue samples of eighty three cases of previously diagnosed colorectal cancer were recruited in this study, in addition to 35 samples of non-cancerous colon tissue as a control group. The patients of this study have undergone colorectal tumor resections with regional lymph node dissection between January 2010 and April 2012 at King Abdulaziz University Hospital. Clinical data (gender, age, type of carcinoma, size and grade of carcinoma) and tissue samples were gathered from 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. Control group was selected from patients who were biopsied for noncancerous conditions (including adenoma, polyps), as well as nearby normal mucosa and distant surgical margins. The control population comprised of 15 (43%) females and 20 (57%) males. The mean age is 56.7 years, ranging from 28 to 87 years. All blocks of noncancerous 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. QIAamp DNA FFPE Kit (Qiagen) was used in accordance to manufacturer's instruction. Purified DNA was eluted in 50 µl elution buffer and saved at –40 °C. Purity and concentration of isolated DNA was analyzed by nanodrop-2000 (Thermo Scientific).

GSTP1 genotyping

Genotyping for the recognition of GSTP1 polymorphism in Saudi colorectal cancer patients was per-

formed using commercial kit (Qiagen; Cyber green PCR kit) along with the manufacturer's recommendations. Approximately 200 ng DNA was magnified in an overall volume of 25 µl/ reaction. We used GSTP1 oligonucleotide primers (F: 5'-ACCCCAGGGCTCTATGGGAA-3', R: 5'-TGAGGGCACAAGAAGCCCCT-3') from MWG-Biotech to amplify GSTP1 fragment. The PCR amplification reaction was done using a thermocycler 480 (Applied Biosystems) starting with 15 min at 94 °C for initial denaturation step, followed by thirty five cycles for denaturation over 1 min at 94 °C, then annealing at 57 °C for 1 min, and extension for 1 min at 74 °C. A final extension for ten min at 72 °C was followed. The products of PCR were examined using 1% agarose gel electrophoresis and visualized using UV-transluminator (SYNGENE).

DNA Sequencing

Applied Biosystems Genetic analyzer 3500 and sequencing kit (big dye terminator v3.1) were used to sequence the amplified PCR products in line with the manufacturer's instruction. The resulting sequence data were studied using Applied Biosystems sequence analysis software (v 5.4). Genotypes were determined as wild type Ile/Ile, heterozygous type Ile/Val or homozygous variant type Val/Val.

Statistical analyses

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

Tab. 1. Clinicopathological characteristics of colorectal cancer patients.

Characteristics		All patients		Female		Male		*p*-value
		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	NA
	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	NA
	Rectosigmoid	12	14.45	7	15.5	5	13.15	
	Sigmoid	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	Well with / or without mucinous	32	38.55	17	37.8	15	39.4	
	Moderate with / or without mucinous	40	48.2	20	44.4	20	52.6	NA
	Poor with / or without mucinous or signet ring cells	11	13.25	8	17.8	3	7.9	
Duke's 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	NA
	C2	29	34.9	11	24.4	18	47.4	
	D	5	6	4	8.9	1	2.6	

RESULTS

Eighty three colorectal cancer cases were revised. The mean age of these cases was 57.8±12.8 years (ranging 22–94 yrs.), with slight preponderance of females 45 (54.2%). More than one third of the tumors (38.55%) were well differentiated, (48.2%) moderately differentiated, while only (13.25%) were poorly differentiated. Moderately differentiated tumors were more frequent among males (52.6%) compared to females (44.4%), and poorly differentiated tumors especially with mucinous or with signet ring cell differentiation were recorded mostly among females (Table 1). Using modified Dukes grading system, the grades of cancer cases were A, B1, B2, C2, and D accounting for 1.2%, 2.4%, 55.5%, 34.9%, and 6% respectively. Almost two thirds (66.7%) of females tumors were graded B2 compared to (42.1%) among males, whereas, grad C2 accounted for (47.4%) and (24.4%) among males and females tumors respectively (Table 1). The commonest anatomic sites of the tumors were, in descending order, the ascending colon (21.69%), sigmoid colon (20.48%), rectum (18.08%), descending colon (15.66%), recto-sigmoid (14.45%) and the least were (4.82%) for each of the transverse colon and cecum. Colorectal cancer was almost equally distributed in both genders regarding sites except for rectum where the tumor occurred in males more than females by 10%. 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 which ranged between 0.6 and 12 centimeters (Table 1).

However, all differences regarding clinical data between tumors in males and females population are not statistically significant $p<0.05$ (Table 1).

PCR and sequencer genetic assays were completed to test the effect of GSTP1 polymorphism on susceptibility to colorectal malignancy. Our results (Table 2) showed the majority of colorectal cancer cases have either Ile/Ile or Ile/Val genotypes of glutathione S-transferase-pi, 40.95% for each, and the remaining cases were of Val/Val genotype accounting for 18.1%. On the other hand, 48.6%, 45.7% and 5.7% of control cases were of Ile/Ile, Ile/Val and Val/Val respectively. GSP1 genotype Ile/Ile was recorded more among males (50%) compared to (33.3%) among females. The comparison of GSTP1 genotypes between tumor cases and controls showed that the Val/Val genotype is more common among colorectal cases, (as much as three folds) than controls. Statistically this difference is not significant $p>0.05$; however, it might be of clinical importance. Overall, the outcomes of the present study suggest that these GSTP1 genotypes do not influence colorectal carcinoma susceptibility in the tested Saudi population.

The alleles recorded percentage were 71.43% for Ile and 28.57% for Val in the control population. In 83 colorectal tumor cases, the matching incidences of GSTP1 alleles were 61.45% and 38.55% respectively (Table 3). No remarkable differences were found regarding the alleles incidence of the GSTP1 (Ile105Val) polymorphism between colorectal cancer cases and control group. Furthermore, no associations were found between the GSTP1 genotypes incidences and clinical and histopathological features (gender, age, site, grade, type, and lymph node metastases) of the tumors.

DISCUSSION

Several factors such as low penetrance susceptibility genes, environmental exposures to air pollution, carcinogens in diet, cigarette smoke, in addition to the interaction between the environment and the genotypes, may have a complex role in the causation of colorectal tumors. GSTs are detoxification enzymes of phase II which are engaged in detoxifying a wide verity of potential carcinogens. One of the possible candidates of colorectal cancer susceptibility genes is GSTP1. GSTP1 gene encrypts an acidic enzyme engaged in the metabolism of a broad panel of possible carcinogens and free radicals, leading to their detoxification, however in a few occasions, to their activation (Bostrom *et al.* 2002; Anders 2004; Anders *et al.* 2004; Hayes *et al.* 2005).

A polymorphic site in the fifth exon at the codon 105 of GSTP1 is well established, where an A to G change triggers substitution of Ile to Val (Ile105Val). The result of this transition is low enzyme activity towards several electrophilic molecules (Hayes *et al.* 2005; McIlwain *et al.* 2006). Millar and colleagues showed that the mutated enzyme (Valine genotype) has lowered activity towards its substrate (Millar *et al.* 1999). Furthermore, different types of tumors (pancreas, kidney, lung, brain, esophagus, breast and bladder) displayed variant genotypes

Tab. 2. GSTP1 genotypes of colorectal cancer cases and controls.

GSTP1 genotypes	Cancer cases						Controls		*p*-value
	Total	%	Female	%	Male	%	Total	%	
Ile/Ile	34	40.95	15	33.3	19	50	17	48.6	0.304
Ile/Val	34	40.95	21	46.7	13	34.2	16	45.7	
Val/Val	15	18.1	9	20	6	15.8	2	5.7	
Total	83	100	45	100	38	100	35	100	

of GSTP1 (Harries *et al.* 1997; To-Figueras *et al.* 1999; Simic *et al.* 2009; Vrana *et al.* 2009; Ou *et al.* 2014; Xie *et al.* 2014; Tan & Chen 2015; Khabaz *et al.* 2015). Similarly, colon tissues express GSTP1 at high levels, therefore, any alteration in the expression pattern may have serious impact on susceptibility to colorectal cancer, and especially GSTP1 has been shown to participate in the deactivation of mutagenic and carcinogenic heterocyclic amines (Lin *et al.* 1994; Nijhoff *et al.* 1995).

Many studies assessed the possibility of colorectal cancer development in persons having variant Val allele of GSTP1; nevertheless, the outcomes are quite debatable (Kiyohara 2000; Grubben *et al.* 2001; Loktionov *et al.* 2001; Seow *et al.* 2002; Sun *et al.* 2005; Ateset al. 2005; Khabaz 2012; Sameer *et al.* 2012; Kassab *et al.* 2014). The current study describes the results of GSTP1 genotypes and the effect of GSTP1 polymorphism (Ile105Val) on susceptibility to colorectal malignancy in the Saudi population. Despite the small sample size in the current study, the incidences of the resultant genotypes of GSTP1 Ile105Val polymorphism in 35 controls (48.6% Ile/Ile, 45.7% Ile/Val, and 5.7% Val/Val) are almost in harmony with the results of other studies from different parts of the World such as East Anglia region (Loktionov *et al.* 2001), Newcastle in England (Welfare *et al.* 1999), Swedish (Sun *et al.* 2005), Edinburgh city (Harries *et al.* 1997) and Jordan (Khabaz 2012). A slight difference was shown not to be significant.

In respect of the risk of colorectal cancer, there are inconsistent reports regarding the impact of GSTP1 polymorphisms on colorectal cancer susceptibility. It was reported that wild allele type enzymes may have protective effects against colorectal tumors (Harries *et al.* 1997). Moreover, Vlaykova and colleagues proposed that Ile105Val polymorphism of GSTP1 affects the susceptibility to colorectal cancer depending on the hypothesis that Ile/Val genotype of GSTP1 has protective effects (Vlaykova *et al.* 2007). However, the present study, which recruited a comparatively small number of controls and colorectal cancer cases, contradicts the idea that wild type allele homozygous and heterozygous genotypes of GSTP1 are likely to have protective effects from colorectal cancer. These two GSTP1 genotypes were almost equally distributed among cancer cases and control samples in the present study (Table 2). Our results are in harmony with the findings of many other researchers reporting that there was no effect of the wild type allele homozygous and heterozygous GSTP1 genotypes on susceptibility to colorectal cancer (Loktionov *et al.* 2001; Seow *et al.* 2002; Ates *et al.* 2005; Sun *et al.* 2005).

Our results also showed the Val/Val GSTP1 genotype exists in 18% of cancer patients and in 5.7% of controls; Val/Val genotype is more common among colorectal cases(as much as three folds) than controls. Statistically this difference is not significant; however, it might be of clinical importance. Our results are similar to the findings of many other reports, stating the greater risk of colorectal cancer is allied with Val/Val genotype (Ates *et al.* 2005; Wang *et al.* 2011; Wang *et al.* 2012; Sameer *et al.* 2012; Song *et al.* 2014). In 1999, a research team reported contradicting results of which GSTP1 genotypes have no impact on susceptibility to colorectal cancer (Welfare *et al.* 1999). They also found the incidences of Val-105 allele are almost equal in cases and controls. However, the present study proposes that GSTP1 variant alleles are statistically improbable to increase the risk of cancer, even though the risk of a small effect is possible. Our result analyses regarding the presence of mutant allele revealed no significant association among homozygous mutant allele and/or the heterozygous mutant and elevated risk of colorectal cancer (Table 3). Ile allele was present in 61.4% and 71.4% of the cases and control, respectively. Hence, the wild type allele seems to have no protective effect. The Val allele present in 38.5% of cancer cases, and in 28.5% of controls. These outcomes show, statistically, there is no association between the frequency of mutant allele and colorectal cancer susceptibility. Our results are in harmony with the outcomes of many other studies, which found no effect of the GSTP1 genotype on colorectal tumor susceptibility (Welfare *et al.* 1999; Loktionov *et al.* 2001; Seow *et al.* 2002; Ates *et al.* 2005; Sun *et al.* 2005).

Tab. 3. Allele incidences in colorectal cancer cases and controls.

Allele	Number of alleles in 83 cancer cases	Percentage of alleles in 83 cancer cases	Number of alleles in 48 controls	Percentage of alleles in 48 controls
Ile	102	61.45	50	71.43
Val	64	38.55	20	28.57
Total	166	100	70	100

Bearing in mind the heterogeneity of the mutagenic and carcinogenic substances as well as the complex xenobiotic metabolic reaction, larger comprehensive research projects are essential for evaluating the susceptibility to colorectal cancer. These studies should investigate the interaction between a wide panel of high or low penetrance genes in addition to GSTP1 and environmental exposures.

CONCLUSION

In general, this study did not confirm the suggestions of previous studies about the effects of GSTP1 polymorphisms on colorectal cancer susceptibility. Furthermore, this study has not drawn sharp convincing outcomes for the influence of GSTP1 Ile105Val polymorphism on susceptibility to colorectal cancer.

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