RESEARCH ARTICLE

Association of *GSTM1* and *GSTT1* genes insertion/deletion polymorphism with colorectal cancer risk: A case-control study of Khyber Pakhtunkhwa population, Pakistan

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Abstract

Objective: To evaluate the genetic association of glutathione S transferase M1 and glutathione S transferase T1 genes insertion/deletion polymorphism with the risk of colorectal cancer.

Method: This case-control study was conducted March 2018 and November 2019 at the University of Peshawar, Peshawar, Pakistan, and comprised blood samples from colorectal cancer patients and age- and gender-matched controls. Deoxyribonucleic acid was extracted from blood samples, and glutathione S transferase M1 and glutathione S transferase T1 genotyping was performed using polymerase chain reaction at the Institute of Radiation and Nuclear Medicine, Peshawar. Data regarding age, gender, location, smoking status, cancer stage and node involvement was collected on a predesigned proforma. Data was analysed using Minitab 17.

Results: The frequency of glutathione S transferase M1 was was significantly associated with colorectal cancer risk ($p<0.01$), while glutathione S transferase T1 null genotype showed non-significant association ($p<0.43$). The association between the combined deletion of glutathione S transferase M1 and glutathione S transferase T1 polymorphism and the colorectal risk was significant ($p=0.011$). Glutathione S transferase M1 and glutathione S transferase T1 deletions had non-significant association with age, smoking status, dwelling and tumour location ($p>0.05$) when compared with the wild genotypes in colorectal cancer cases.

Conclusion: Glutathione S transferase M1 gene deletion was found to be associated with the risk of colorectal cancer development.

Keywords: Colorectal cancer, Detoxification, *GSTM1, GSTT1*, Polymorphism. (JPMA 72: 457; 2022)

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Introduction

Cancer has become one of the most serious problems in public health and is one of the leading causes of death globally.[1] Colorectal cancer (CRC) is the second most frequent type of cancer among female and the third most common cancer in men in terms of incidence, and second in terms of mortality.[2] CRC accounted for approximately 0.881 million deaths in 2018 and more than 1.8 million new CRC cases were reported. CRC accounts for approximately 1 in 10 cancer cases and deaths.[3] The incidence rate of CRC in the developed world is about 3 times higher compared to the developing countries.[1]

CRC is a multifactorial disease and its life-time risk in the general population increases 5% with age. This may be due to the ingestion of carcinogenic compounds through food.[4] Susceptibility to CRC varies among individuals which may be due to differences in their metabolism/ detoxification potential of carcinogens in the gastrointestinal tract (GIT). Importantly, these differences are caused by both environmental and genetic factors as they play major roles in CRC development. Gene sequence variation in enzymes involved in detoxification leads to differences in detoxification potentials, which consequently affects the accumulation of toxic (carcinogenic) compounds in the body that may in turn increase the risk of CRC development.[5]

The human glutathione S transferases (GSTs) is a superfamily of ubiquitous multifunctional metabolic enzymes that are present in most epithelial tissues of GIT.5 They possess both enzymatic and non-enzymatic activities and play important biological functions, including cellular phase II metabolism, response to stress, cell proliferation, cell death, drug resistance and tumour progression.[6] They have the ability to enzymatically facilitate the coupling reaction of reduced glutathione with toxic electrophilic endogenous and exogenous molecules, like environmental pollutants, reactive oxygen species, carcinogens and a large spectrum of xenobiotics, and facilitate their elimination from the cell by converting them into more water-soluble products, thus protecting macromolecules and cells from damage.[5,7]

GSTs are dimeric and mainly cytosolic proteins. The well-

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characterised human cytosolic GSTs are divided into several classes, including alpha (a), mu (m), pi (p), sigma (s), theta (t), zeta (z), and omega (W). GSTs are different in their amino acid sequences and location on chromosomes.[8] Glutathione S transferase M1 (*GSTM1*) maps to the short arm of the chromosome one (1p13.3), while glutathione S transferase T1 (*GSTT1*) lies on 22q11.2.[6] GSTs' deletion or their specific sequence variation causes a decrease in their enzymatic activity compared to the wild-type (WT) homozygotes. Functional polymorphisms of *GSTT1* and *GSTM1* are evident by the loss of these genes. The *GSTM1* and *GSTT1* variant alleles are represented by the complete deletion of these genes. Individuals who have homozygous deletion (null alleles) of the GST allele have completely absent enzyme activity called the null genotype.[9] *GSTM1* deletion enhances the risk of the digestive tract, bladder, lung and skin cancers. Among *GSTM1*-null individuals the association between the amounts of polycyclic aromatic hydrocarbon (PAH)-deoxyribonucleic acid (DNA) adducts and dietary antioxidants is important.[10] The genetic variants' frequency of these genes depend on the geographic location in general population. Khyber Pakhtunkhwa (KP) is one of the provinces of Pakistan occupied mainly by Pashtun tribes. Genetically, their closest relatives are Persians and Tajiks. The prevalence of *GSTM1* and *GSTT1* deletion polymorphism and their possible effect on CRC development has not been studied in KP population. The current study was planned to fill the gap by ascertaining how *GSTM1* and *GSTT1* null genotypes are linked with CRC development in the area.

Patients and Methods

This case-control study was conducted March 2018 and November 2019 at the University of Peshawar, Peshawar, Pakistan. After approval from the institutional ethics review committee, the sample size was calculated using the World Health Organisation (WHO) formula with 95% confidence level, 5% alpha error, assumed odds ratio (OR) of 3 and 80% power level.[11] The sample was raised using non-probability convenience sampling technique. Those included were individuals of either gender aged 15-60 years belonging to Pashtun tribes of KP. Those who were not willing to provide relevant data and patients who had developed CRC aged >60 years at diagnosis were excluded. Patients from mixed ethnic groups were also excluded. Among the cases, documentary evidence of pathologically confirmed CRC adenocarcinoma was diagnosed with computerised tomography (CT) scan and biopsy. Determination of tumour stages and types was done by experienced pathologists at the Institute of Radiation and Nuclear Medicine (IRNM), Peshawar. For the controls, healthy individuals with no sign of present or previous malignancy and no indication of CRC or nor any family history of cancer were included who had no blood relation with the patients. The controls were selected on the basis of gender, age, smoking history and habit, occupation and food intake.

After taking informed consent from all the participants, or their guardians, data was collected from the cases about age, gender, ethnicity, medical record, pathology reports, drug history, family history, tumour size, tumour location and lymph node status etc. on a pre-designed proforma. Information about colorectal cancer risk factors, such as consumption of red meat, vegetables, fibres, fruits and cooking choices and smoking history, was also obtained. Demographic data was noted for the controls.

Blood samples 3mL were collected through a sterile syringe from both the cases and the controls visiting IRNM. They were stored at -20°C in Thomas scientific sterile vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) till further analysis at the Biochemistry Section of the Institute of Chemical Sciences, University of Peshawar. DNA was extracted from the blood samples using DNA extraction kit (GeneJET Genomic DNA Purification kit, Thermo Scientific, United States) and was quantified using ultraviolet (UV)-visible spectrophotometer (752 PC, China). *GSTM1* and *GSTT1* insertion/deletion polymorphism was determined using polymerase chain reaction (PCR) (Multigene Optimax, Labnet International, USA). PCR was performed in a 20µL reaction mixture containing template DNA (100ng), deoxynucleoside triphosphates (dNTPs) (200 µM of each dNTP), magnesium chloride (MgCl2) (1mM), primers (0.5 µM of each specific primer), Taq polymerase (2.5U) and Taq buffer (1X) (Thermo Scientific, USA).[12] The sequences of primers and PCR conditions for *GSTM1* and *GSTT1* amplification have been described previously[12] (Table 1). The amplified fragments were electrophorosed on agarose gel (2%), followed by staining with ethidium bromide. The fragments were visualised using UV transilluminator (Wealtec, USA). The absence of 480 bp PCR product corresponded to *GSTT1* deletion (null homozygous), while the absence of 215 bp product showed *GSTM1* deletion (null homozygous). All items of the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) Statement-Checklist were followed (Annexure)[13].

Table-1: Primer sequences and amplification conditions for glutathione S transferase M1 (GSTM1) and glutathione S transferase T1 (GSTT1) polymosrphisms.[12]

Gene	Primer sequences	Amplification conditions	Cycles
GSTM1	Forward:5/-GAACTCCCTGAAAAGCTAAAGC-3/ Reverse:5/-GTTGGGCTCAAATATACGGTGG-3/	95°C for 5 min, 95°C for 1 min,	37
GSTT1	Forward:5/-CCTTACTGGTCCTCACATCTC-3/ Reverse:5/-TCACCGGATCATGGCCAGCA-3/	64°C for 1 min, 72°C for 1 min and 72°C for 10 min	35

Annexure: STROBE Statement: Checklist of items that should be included in reports of *case-control studies*.

	Item No	Recommendation	Section	Paragraph No
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	Title	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	Abstract	1
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Introduction	1-3
Objectives	3	State specific objectives, including any prespecified hypotheses	Introduction	4
Methods				
Study design	4	Present key elements of study design early in the paper	Methods	1
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Methods	1
Participants	6	(a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	Methods	1
		(b) For matched studies, give matching criteria and the number of controls per case	Methods	1
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Methods	1 and 2
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Methods	1and 2
Bias	9	Describe any efforts to address potential sources of bias		Not applicable
Study size	10	Explain how the study size was arrived at	Methods	1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why		4
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	Methods	3
		(b) Describe any methods used to examine subgroups and interactions	Methods	3
		(c) Explain how missing data were addressed		Not applicable
		(d) If applicable, explain how matching of cases and controls was addressed	Methods	3
		(e) Describe any sensitivity analyses	Methods	3
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Results	Table 3
		(b) Give reasons for non-participation at each stage		Not applicable
		(c) Consider use of a flow diagram		Not applicable
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Results	Table 2
		(b) Indicate number of participants with missing data for each variable of interest		Not applicable
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	Results	Table 2 and Table 3
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Results	Table 2-5
		(b) Report category boundaries when continuous variables were categorized		Not applicable
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period		Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Results	Table 4-5
Discussion				
Key results	18	Summarise key results with reference to study objectives	Discussion	1-3
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Discussion	4
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Discussion	1-4
Generalisability	21	Discuss the generalisability (external validity) of the study results	Discussion	4
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Funding disclosure	1

*Give information separately for cases and controls; STROBE: Strengthening the Reporting of Observational studies in Epidemiology.

Data was analysed using Minitab 17 and was presented as mean±standard deviation (SD). ORs were calculated to find out the strength of correlation between *GSTM1* and *GSTT1* deletion with CRC while 95% confidence interval (CI) was used to find out the precision of OR. The non-random association between GST polymorphism and CRC was calculated using relative risk and Fisher's exact text, while chi-square test was used to compare characteristics and allele-genotype between the cases and the controls. P≤0.05 was considered statically significant.[14]

Results

Of the 340 subjects, 170(50%) were cases; 66(38.8%) females and 104(61.2%) males. The remaining 170(50%) were controls; 58(34.1%) females and 112(65.9%) males. The Inter-group differences related to age, gender, dwelling and food consumption patterns were non-significant (p>0.05), while smoking status was a significant factor (Table 2). The tumour location among the CRC case was non-significant (p>0.05) as 83(48.8%) patients were diagnosed with rectal carcinoma and 87(51.2%) had colon

Table-2: Demographics and risk factors in colorectal CRC cases and healthy controls.

Variable	Cases (n=170) n (%)	Control (n=170) n (%)
***Age** (years)		
≥40	112 (65.8)	103 (60.6)
<40	58 (34.1)	67 (39.4)
***Gender**		
Male	104 (61.2)	112 (65.9)
Female	66 (38.8)	58 (34.1)
Smoking status		
**Ever	30 (17.6)	15 (8.8)
*Never	140 (82.4)	155 (91.2)
***Dwelling**		
Rural	67 (39.4)	57 (33.5)
Urban	103 (60.6)	113 (66.5)
***Food consumption**		
Mainly vegetables	90 (53.0)	82 (48.2)
Mixed Food	80 (47.0)	88 (51.8)

*$p>0.05$ Cases vs Control, **$p=0.02$ Cases vs Control

carcinoma.

Genotyping of the deletion polymorphism of *GSTM1* and *GSTT1* genes was performed for both the case and the controls, and representative images of WT and deleted mutant for both the groups were preserved (Figure). Among the 340 subjects, *GSTM1* deletion was found in 155(45.59%) subjects, while *GSTT1* null genotype was present in 124(36.47%). Inter-group differences were also noted (Table 3).

Subjects carrying combined deletion of *GSTM1* and *GSTT1* (*GSTM1-/GSTT1-*) had higher risk of CRC development over individuals with *GSTM1-/GSTT1+* genotypes ($p=0.01$). *GSTM1*-null/*GSTT1*-present were more prone to CRC development ($p=0.02$) than *GSTM1*-present/*GSTT1*-null for CRC development ($p=0.23$). Moreover, no significant difference was observed between the *GSTM1*-present and *GSTT1*-null genotypes and CRC risk ($p>.05$). Individuals with double-null *GSTM1-* and *GSTT1-* genotypes showed an increased risk for the development of CRC ($p=0.01$) (Table 4). The effect of *GSTT1* and *GSTM1* deletion on clinicopathological variables was analysed in the patient group by comparing individuals with null and present genotypes (Table 5).

The association of *GSTM1* and *GSTT1* deletion on clinicopathological variables was also analysed in patients by comparing individuals with null and present genotypes.

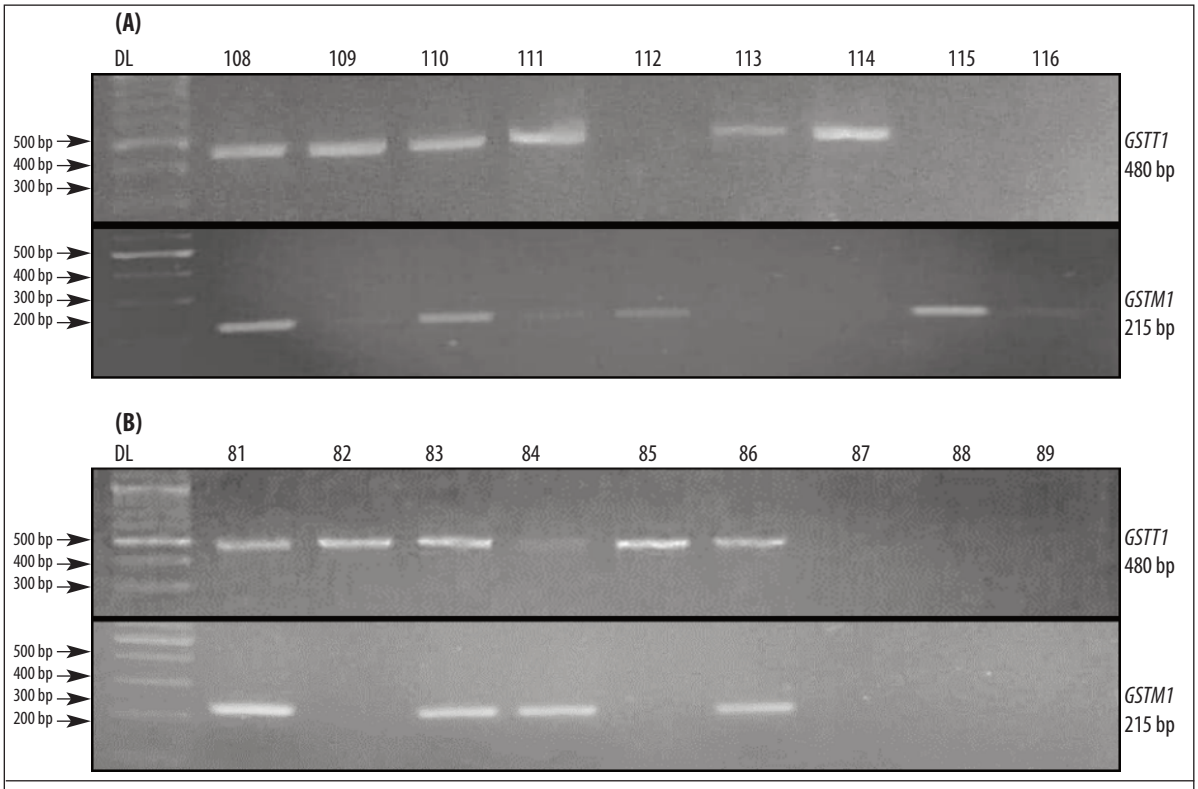

Figure: Glutathione S transferase M1 (GSTM1) and glutathione S transferase T1 (GSTT1) genotyping by polymerase chain reaction (PCR) in healthy Controls (A) and colorectal cancer (CRC) patients (B). The presence of 480bp band for GSTT1 and 215bp band for GSTM1 represents the wild type (insertion) genotypes, while the absence of these bands indicates their deletion (mutant). Representative image has been shown for control (Subjects ID: 108-116) and CRC patients (Subjects ID = 81-89).
DL: Deoxyribonucleic acid (DNA) ladder.

Table-3: Glutathione S transferase M1 (*GSTM1*) and glutathione S transferase M1(*GSTT1*) gene polymorphism frequencies in colorectal cancer (CRC) cases and healthy controls.

Genotype	Cases (n=170) n (%)	Controls (n=170) n (%)	OR (95% CI); Fψ	χ^2; P (overall)	Relative Risk
GSTM1					
Present	80 (47.1)	105 (61.8)	1.82 (1.18-2.78); 0.01	7.41; 0.01	1.4
Null	90 (52.9)	65 (38.2)			
GSTT1					
Present	103 (60.6)	113 (66.5)	1.29 (0.83-2.01); 0.31	1.27; 0.41	1.0
Null	67 (39.4)	57 (33.5)			
GSTM1 **(Overall)**	Cases+ Controls n=340				
Present	185 (54.4)				
Null	155 (45.6)				
GSTT1 **(Overall)**	Cases+ Controls n=340				
Present	216 (63.5)				
Null	124 (36.5)				

OR: Odds ratio; CI: Confidence interval.

Table-4: Distribution of genotype combination between glutathione S transferase M1 (*GSTM1*) and glutathione S transferase T1 (*GSTT1*) gene polymorphism in colorectal cancer (CRC) cases and controls and risk analysis.

Genotype	Cases (n=170) n (%)	Controls (n=170) n (%)	OR (95% CI); Fψ	χ^2; P (overall)	Relative Risk
GSTM1+/GSTT1+	47 (27.6)	70 (41.2)	Reference		0.67
GSTM1−/GSTT1+	56 (32.9)	44 (25.9)	1.90 (1.10-3.26) ;0.02	5.39; 0.02	1.30
GSTM1+/GSTT1−	34 (20.0)	35 (20.6)	1.45 (0.79-2.64) ;0.28	1.46;0.23	0.97
GSTM1−/GSTT1−	33 (1.9)	21 (1.2)	2.34 (1.21-4.53) ;0.01	6.47; 0.01	1.57

OR: Odds ratio; CI: Confidence interval.

Table-5: Association of glutathione S transferase M1 (*GSTM1*) genotypes with various clinicopathological variables in colorectal cancer (CRC) patients.

Variable	GSTM1+ (n=80) n (%)	GSTM1− (n=90) n (%)	p-value	GSTT1+ (n=103)	GSTT1− (n=67)	p-value
Age (years)						
≥40	51 (63.8)	61(67.8)	0.58	62 (60.2)	50 (74.6)	0.05
<40	29 (36.2)	29 (32.2)		41 (39.8)	17 (25.4)	
Gender						
Male	42 (52.5)	63 (70.0)	0.02	65 (63.1)	39 (58.2)	0.52
Female	38 (47.5)	27 (30.0)		38 (36.9)	28 (41.8)	
Smoking status						
Ever	10 (12.5)	20 (22.2)	0.10	20 (19.4)	10 (14.9)	0.45
Never	70 (87.5)	70 (77.8)		83 (80.6)	57 (85.1)	
Dwelling						
Rural	32 (40.0)	35(38.9)	0.88	40 (38.8)	20 (29.9)	0.23
Urban	48 (60)	55 (61.1)		63 (61.2)	47 (70.1)	
Tumour location						
Colon	39 (48.8)	48 (53.3)				
0.55	56 (54.4)	31 (46.3)	0.30			
Rectum	41 (51.2)	42 (46.7)		47 (45.6)	36 (53.7)	
Node involvement						
Yes	45 (56.2)	60 (66.7)	0.16	65 (63.1)	42 (62.7)	0.96
No	35 (43.8)	30 (33.3)		38 (36.9)	25 (37.3)	
Stages						
I	0 (0)	1 (1.1)		0 (0)	1 (1.5)	
II	15 (18.8)	14 (15.6)		21 (20.4)	8 (11.9)	
III	41 (51.2)	53 (58.9)		60 (58.2)	34 (50.8)	
IV	24 (30.0)	22 (24.4)		22 (21.4)	24 (35.8)	

Both *GSTM1* and *GSTT1* risk genotype (null) were not associated with age (p=0.58 for *GSTM1*− and p=0.05 for *GSTT1*−), smoking status (p = 0.01 for *GSTM1*− and p= 0.45 for *GSTT1*−), dwelling (p=0.88 for *GSTM1*− and p=0.23 for *GSTT1*−), tumour location (p=0.55 for *GSTM1*− and p=0.30 for *GSTT1*−), and node involvement (p=0.16 for *GSTM1*− and p=0.96 for *GSTT1*−) compared to WT (present) genotype in CRC cases. However, *GSTM1*− was significantly associated with gender (p=0.02), while *GSTT1*− did not show significant (p=0.52) association with gender in CRC patients.

Discussion

The current study investigated the possible correlation between *GSTM1* and *GSTT1* genes deletion/null (*GSTM1*- and *GSTT1*-) polymorphism and the risk of CRC development in Pashtun tribes of KP. GSTs, an important class of xenobiotics metabolising enzymes, play a significant role in the defensive mechanism against a wide range of carcinogens.[15] Carcinogens, such as heterocyclic amines (HAAs) and PAHs, are involved in CRC development. PAHs as well as other tobacco-related carcinogens are triggered by detoxification enzymes of phase I, like cytochrome P450 (CYP) 1A1. and are then detoxified by GSTs (phase II detoxifying enzyme).[16] Active metabolites of PAHs which are present in the fossil fuel gases and tobacco products, and HAAs and its metabolites produced in meat, cooked at high temperature, are detoxified by *GSTM*.[17] The impact of GST polymorphism and its effect in cancer susceptibility have been studied in several researches due to their active metabolising role and biological effects on carcinogens. Genetic variants of GSTs are less effective in metabolising carcinogens, contributing to the susceptibility of individual diseases depending on the metabolised substrate.[15] The GST genetic polymorphism and their relation with cancer risk reveals some affirmative or negative relations between these polymorphisms and CRC risk. GST gene polymorphism can affect GST enzymes' function either by changing the level of gene expression or activity of protein itself, or both. In this manner, it can indirectly affect the

development of cancer in the body because of its influence on the detoxification of carcinogens and thus the level of DNA damage.[18]

In the current study, *GSTM1* was observed to be deleted from 45.59% population, while *GSTT1* null genotype was 36.5% prevalent in Pashtun tribes of Pakistan residing in KP province (Table 2). Previous research shows that the frequency of *GSTM1* null genotype is higher in Caucasians (34.0%–58.3%), Asians (47.6%–56.2%), and Arabs (44.0%–56.3%) than in Africans (17.0%–46.7%) and in native Latin-American populations (0.0%–43.0%). *GSTT1*-null genotype is lower in Caucasians, South American natives (0%–38.2%), and increases considerably in Asian populations (64.4%) and shows similar frequencies between Arabian and African descendants.[19,20]

Previous studies show that GSTs deletion can be associated with different types of cancers, varying greatly among the population. In Pakistani population, *GSTM1* and *GSTT1* have been observed to have increased susceptibility to the cancer of head, neck[21] and lungs.[22] *GSTM1* has also been shown to have significant association with squamous cell carcinoma (SCC) of cervix.[23] The current study attempted to find out the association of *GSTM1* and *GSTT1* deletion with CRC risk in KP population. There was a strong association of *GSTM1*-null genotype with CRC development (p=0.01), while *GSTT1*-null was found to be non-significantly associated (p=0.41), indicating that individuals with *GSTM1*-null genotype are more prone to CRC development compared to WT (Table 3). A meta-analysis indicated high risk of CRC among *GSTM1* deleted individuals in Caucasian populations, while among the Chinese subjects, non-significant association was observed. Likewise, Caucasian populations who carried GST-null allele showed higher CRC risk, but in the Chinese population, no significant relationship was observed.[20] Conversely, Nissar et al. found no substantial association of *GSTM1*-null genotype carriers with amplified risk of CRC.[24] Cotterchio et al. determined that *GSTT1* gene polymorphism substantially altered the association between the consumption of red meat and CRC risk, whereas this risk was not altered by *GSTM1* gene polymorphism.[25]

Similarly, individuals who had a double-null genotype (*GSTM1–/GSTT1–*) were found in the current study to have a greater risk for CRC growth and progression (Table 4). There was a strong correlation between polymorphism from *GSTM1* and risk of CRC. Given some literature divergences, the current results are consistent with several other studies that also discovered strong correlation. In Caucasian populations, the combined deletion of *GSTM1* and *GSTT1* genotypes imparted increased risk to CRC.[25] A research showed that the *GSTM1*-null allele frequency was substantially associated with higher rectal cancer, while the *GSTT1*-null genotype allele frequency was related to a higher colon cancer risk. Additionally, it was speculated that the relationship in the three-genes polymorphism -- *GSTM1*, *GSTT1* and *GSTP1* -- could be a significant influencing factor for the production of CRC in the Hindu population.[26]

The association between *GSTT1* deletion carriers and reduced risk of CRC could be attributed to their role in isothiocyanate disposal, glucosinolate breakdown products that are plentiful in cruciferous vegetables and carcinogen clearance. Isothiocyanates, which possess anticarcinogenic activities, are strong inducers of GSTs and other enzymes involved in detoxification.[27] *GSTT1* deletion, causing a decrease in their enzymatic activity, may lead to longer circulating half-life of anticarcinogenic isothiocyanates and possibly greater chemo-preventive effects of cruciferous vegetables, thus opposing the primary hypothesis that individuals carrying the *GSTT1*-null polymorphism are at higher risk of cancer due to low deposition of carcinogenic compounds in vivo.[28] Also, no statistically significant relation was observed between *GSTT1* deletion and risk of CRC development. This lack of association is in line with some other studies.[9] It is worth mentioning that in this study, *GSTM1* and *GSTT1* null genotypes also did not show any correlation (p>0.05) with age, smoking status and geographical location of patients, node involvement and tumour location among CRC patients. *GSTM1* was observed to have significant (p=0.02) association with gender in patients (Table 5). However, this relation seems to be due to the low level of CRC in the female population of KP. These variations in the findings from various studies can be due to the fact that environmental or genetic factors are of utmost importance to the risk of disease and may be affected by ethnic diversity.[20] In addition, it is assumed that certain populations may be more vulnerable to chemical-induced carcinogenesis than others, and the impact of cancer genetic determinants can be modulated by other influencing genes, different environmental factors, other illnesses, and a lifestyle that has a strong or moderate effect.[27]

In terms of limitations, the current study investigated the effect of *GSTM1* and *GSTT1* deletion on the risk of CRC development, but the underlying mechanism, which possibly involves lipid peroxidation and DNA damage, were not determined. Moreover, the effect of *GSTM1* deletion on chemotherapeutic effect of various drugs need to be elucidated as it would uncover important findings related to CRC treatment.

Conclusion

GSTM1 deletion was an important contributor towards CRC development, while *GSTT1* deletion did not confer an additional risk for CRC in the studied population.

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