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Association of Glutathione S-Transferase A1 Gene Polymorphism-69 Promoter with Colorectal Cancer

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Abstract

Colorectal cancer is the third most common cancer and the second leading cause of cancer deaths in the world. Risk factors such as obesity, low-fiber diet, sedentary, and alcohol have been implicated in the development of this cancer. Cells have many mechanisms in place to fight malignancy and cancer, one of which is the detoxification system that protect the cell against DNA damage. One of the essential components of this system is the glutathione s-transferase. In this study, the genotype of region -69 promoter of this gene in 150 colorectal cancer patients and 150 healthy people was investigated by the RFLP method. T/T genotype is significantly associated with colorectal cancer (P = 0.0001), C/T genotype did not show a significant association with colorectal cancer and its frequency was not significantly different in both healthy and patient groups (P = 0.074), Most genotypes in the two groups were homozygous C / C and did not show a significant association with colorectal cancer.

INTRODUCTION

Colorectal cancer is the third most common cancer and the second leading cause of cancer deaths in the world (1). Currently, one of the most common gastrointestinal cancers in Iran is colorectal cancer, which has the third-highest incidence in men and fourth in Iranian women (2). Colorectal cancer is divided into two groups: hereditary (familial) and sporadic, of which 80% are occasional and 20% genetic. In terms of age, 92% of these cancers occur over 50 years. Risk factors such as obesity, low-fiber diet, sedentary, and alcohol have been implicated in the development of this cancer (3). There are various protective systems in the cells that protect against cancer (4). most notably toxicogenomics, the most well-known members of the order being cytochrome oxidase p450 and glutathione s-transferases (5). The system protects the cell against cancer by disabling carcinogens and converting them into low-risk substances. Glutathione s-transferases are a large family of enzymatically functioning proteins involved in toxicogenomics mechanisms (6). This enzyme catalyzes the glutathione conjugation to a variety of toxic metabolites and can inactivate metabolites that cause DNA damage. Glutathione contains cysteine amino acid, which, through the thiol and sulfur group, triggers the thiol factor of non-nucleophilic electrons to react with electrophiles, which is catalyzed by the glutathione s-transferase enzyme (7). GST oxidizes two glutathione molecules in the presence of substrates such as nitrates and peroxides and converts them to GSSG and a reduced substrate. Substances that can be subjected to glutathione s-transferase include xenobiotics, organophosphorus-like insecticides, carcinogens, as well as endogenous cell-derived materials such as A4 leukotrienes, A1 prostaglandins, bilirubin, hydrocarbons, and hydrocarbons (8). Specific forms of glutathione S-transferases have been identified in precancerous cells that can be used as tumor markers. Different types of GSTs have been identified in different cells and species, the cytoplasm is the primary site of this enzyme. One member of the GST family is GSH 1A, which, like other members of this family, is involved in cell toxicity, and studies have shown that exposure to mice with carcinogens increases the expression of this family in liver, kidney, brain, and eyes Which represents the beginning of the process of protection against toxins (9). According to recent studies, it is essential to regulate the expression of this enzyme when exposed to toxins. The single nucleotide polymorphism in the promoter region (-69) of this gene, which converts cytosine nucleotide to thymine, affects the activity of this enzyme (10). Studies have shown that the person with the CC genotype has the highest enzyme activity at

the time of exposure to the toxins, while the person with the TT genotype has the lowest business in the first case (11). Given its role in neutralizing carcinogens, the cell is expected to become more sensitive to environmental carcinogens by reducing its activity. We studied the -69 gene region genotype in blood samples of 150 colorectal cancer patients and 150 healthy individuals and examined its relationship with age, weight, and family cancer parameters.

MATERIALS AND METHODS

The study population consisted of 150 blood samples from colorectal cancer patients and 150 blood samples from healthy individuals obtained from the Tumor Bank of Imam Khomeini Hospital. The patient population included 94 men and 56 women with a mean age of 62.74. The control population included 80 healthy men and 70 healthy women with an average age of 45.81. Blood samples were obtained from patients with colorectal cancer and patient information, including gender, age, weight, family history of colorectal cancer. Salting out method was used for DNA extraction, and the samples were electrophoresed on 1.5% agarose gel to evaluate the extraction quality. RFLP method was used to study the genotype of the region. Genomic DNA was first amplified with specific primers in Table 1 and amplified by PCR. Following PCR product for Genotype-69 region treated with restriction enzyme Eam11041 according to product protocol. The product was run on 2% agarose gel. The CC genotype is reported if there is only one 480bp band, and the CT genotype is observed at both 380bp and 100bp bands and the CT genotype if the three groups are 480-380-100bp. All statistical calculations were performed using SPSS V16.0 software, and $P \leq 0.05$ was considered significant.

Table 1. Primer Sequence

Primer Name	Primer Sequence	Annealing Tm
GST-F	GTTAAACGCTGTCACCGTCC	56c
GST-R	TGTTGATTGTTTGCCTGAAAT	

RESULTS

The study population included 94 men with a mean age of 58.41 and 56 women with a mean age of 64.14. The results of RFLP-PCR showed that the T/T genotype is very low in the patient and healthy population; however, it is more frequent in the patient population and is significantly associated with colorectal cancer ($P = 0.0001$). The heterozygous C/T genotype did not show a significant association with colorectal cancer, and its frequency was not significantly different in both healthy and patient groups ($P = 0.074$) Most genotypes in the two groups were homozygous C/C, accounting for 48.8% of total cases and more frequency in the healthy group. No significant relationship was found between gender and genotype -69. It was found that the individuals with T/T genotype had a mean weight above 70, and there was a significant relationship between pressure and genotype of the target area ($P = 0.012$). None of the population in their classy relatives had a person with similar cancer.

DISCUSSION

Colorectal cancer is one of the seven deadly cancers in developing countries. Genetic and environmental factors, like other cancers, are also involved in this type of cancer. However, the nutrition factor is more important in this cancer. Studies have shown that a high-fat diet, frequent use of red meat, and a lack of fiber in the diet can increase the chance of developing cancer (12). Cells have many mechanisms in place to fight malignancy and cancer, one of which is the detoxification system, which neutralizes toxins and dangerous metabolites that protect the cell against DNA damage. One of the essential components of this system is the glutathione s-transferase. The enzyme oxidizes two glutathione molecules in the presence of substrates such as nitrates and peroxides and converts them to GSSG and a reduced substrate. In this study, the genotype of region -69 promoter of this gene was investigated. Studies have shown that the genotype of this region has an effect on the activity of the enzyme so that the protein with CC genotype has the highest business and the TT genotype has the least movement. Therefore, people with the TT genotype are expected to have a higher risk of developing cancers. In this study, despite the low number of TT genotypes in the target population, there was a significant association with colorectal cancer. Similar results were also found in other studies, such as the study by Coles et al. Who studied this polymorphism (13). But in the study of Sweeney et al., Which examined genotype on breast cancer, no significant relationship was reported (11). Studies on the GST promoter region -69 gene and the risk of various cancers have been few studies. Given the role of this enzyme in different cancers, especially gastrointestinal cancers, further studies with larger population sizes, are recommended.

REFERENCE

1. Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RGS, Barzi A, et al. Colorectal cancer statistics, 2017. *CA Cancer J Clin*. 2017;**67**(3):177-193. **doi:** 10.3322/caac.21395 **pmid:** 28248415
2. Ansari R, Mahdavinia M, Sadjadi A, Nouraie M, Kamangar F, Bishehsari F, et al. Incidence and age distribution of colorectal cancer in Iran: results of a population-based cancer registry. *Cancer Lett*. 2006;**240**(1):143-147. **doi:** 10.1016/j.canlet.2005.09.004 **pmid:** 16288832
3. Haggar FA, Boushey RP. Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors. *Clin Colon Rectal Surg*. 2009;**22**(4):191-197. **doi:** 10.1055/s-0029-1242458 **pmid:** 21037809
4. Lee C, Raffaghello L, Longo VD. Starvation, detoxification, and multidrug resistance in cancer therapy. *Drug Resist Updat*.

2012;**15**(1-2):114-122. **doi:** 10.1016/j.drup.2012.01.004 **pmid:** 22391012
5. Yao SJ, Wolfson SK. Blood and tissue detoxification apparatus. Google Patents; 1976.
6. Salinas AE, Wong MG. Glutathione S-transferases-a review. *Curr Med Chem*. 1999;**6**(4):279-310.
7. Dusinska M, Staruchova M, Horska A, Smolkova B, Collins A, Bonassi S, et al. Are glutathione S transferases involved in DNA damage signalling? Interactions with DNA damage and repair revealed from molecular epidemiology studies. *Mutat Res*. 2012;**736**(1-2):130-137. **doi:** 10.1016/j.mrfmmm.2012.03.003 **pmid:** 22450146
8. Keen JH, Habig WH, Jakoby WB. Mechanism for the several activities of the glutathione S-transferases. *J Biol Chem*. 1976;**251**(20):6183-6188. **pmid:** 977564
9. Townsend DM, Tew KD. The role of glutathione-S-transferase in anti-cancer drug resistance. *Oncogene*. 2003;**22**(47):7369-7375. **doi:** 10.1038/sj.onc.1206940 **pmid:** 14576844
10. Kusama M, Kubota T, Matsukura Y, Matsuno K, Ogawa S, Kanda Y, et al. Influence of glutathione S-transferase A1 polymorphism on the pharmacokinetics of busulfan. *Clin Chim Acta*. 2006;**368**(1-2):93-98. **doi:** 10.1016/j.cca.2005.12.011 **pmid:** 16448639
11. Sweeney C, Ambrosone CB, Joseph L, Stone A, Hutchins LF, Kadlubar FF, et al. Association between a glutathione S-transferase A1 promoter polymorphism and survival after breast cancer treatment. *Int J Cancer*. 2003;**103**(6):810-814. **doi:** 10.1002/ijc.10896 **pmid:** 12516103
12. Benito E, Stiggelbout A, Bosch FX, Obrador A, Kaldor J, Mulet M, et al. Nutritional factors in colorectal cancer risk: a case-control study in Majorca. *Int J Cancer*. 1991;**49**(2):161-167. **doi:** 10.1002/ijc.2910490202 **pmid:** 1652565
13. Coles BF, Morel F, Rauch C, Huber WW, Yang M, Teitel CH, et al. Effect of polymorphism in the human glutathione S-transferase A1 promoter on hepatic GSTA1 and GSTA2 expression. *Pharmacogenetics*. 2001;**11**(8):663-669. **doi:** 10.1097/00008571-200111000-00004 **pmid:** 11692074