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Original Research

The role of GSTM1 gene polymorphism in pathophysiology, evaluation, and management of constipation of anorectal outlet obstruction

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Abstract: Constipation of anorectal outlet obstruction may be caused by mechanical or functional causes. This complication is a debilitating disease that needs proper and timely treatment. Many studies have shown that there is a direct link between constipation and intestinal cancer. One of the most effective ways to prevent or diagnose intestinal cancer is through genetic studies. Evaluation of people's polymorphism shows how much they are at risk for cancer. Therefore, in this study, the GSTM1 gene polymorphism was evaluated in patients with constipation of anorectal outlet obstruction to assess better and manage this disease and investigate the possibility of anorectal cancer in these people. In this regard, 40 people with constipation of anorectal outlet obstruction were compared with 40 healthy people. In the case group (patients), in addition to demographic and clinical evaluations, the anorectal manometric test was used to diagnose the pathology of the disease. Results showed that out of 40 patients with constipation of anorectal outlet obstruction, 5 cases (12.5%) had megarectum, 7 cases (17.5%) had anismus, 10 cases (25%) had Hirschsprung's disease, 5 cases (12.5%) had descending perineum syndrome, 6 cases (15%) had rectal prolapse, 4 cases (10%) had enterocele, and 3 cases (7.5%) were with rectocele. Also, the results of GSTM1 gene deletion polymorphism showed that patients with constipation of anorectal outlet obstruction were almost two times more exposed to the null genotype than the control group ($P < 0.04$). Therefore, in people with both constipation of anorectal outlet obstruction and null genotype (i.e., deletion in the GSTM1 gene), because they do not have glutathione-S transferase, they appear to be at higher risk for anorectal cancer than healthy people with the same genotype.

Key words: Anorectal cancer; Constipation; GSTM1 gene; Obstructive defecation; Polymorphism.

Introduction

Problems and delays in defecation for seven days or more that lead to obvious discomfort to the patient and the inability to empty the rectum completely is one of the definitions of constipation (1). Constipation can be a complication associated with systemic or neurological disorders or can be a side effect of many common medications (2). Constipation could cause by extraintestinal problems (e.g., hypocalcemia, hypothyroidism, diabetes, hypercalcemia, multiple sclerosis, autonomic neuropathy, Parkinson's disease, spinal cord lesions, muscular dystrophies, systemic sclerosis, eating disorders, depression, or some medications), or it could cause by Intestinal problems (3).

Intestinal problems that cause constipation are divided into two groups. The first group is colon problems. This problem could be functional (e.g., irritable bowel syndrome and slow transit) or organic (e.g., aganglionosis, polyps, strictures, neoplasms, diverticulum disease) (3, 4). The second group is anorectum and pelvic floor problems (e.g., Megarectum, Rectocele, Complete rectal prolapse, Anal stenosis [after surgery, radiation or Crohn's disease], Internal rectal prolapse, Congenital or acquired internal anal sphincter myopathy, Mucosal rectal prolapse, External compression, Descending perineum syndrome, Enterocele, Aganglionosis, Solitary rectal ulcer, Neoplasms, polyps, Anismus) (5).

About 50% of patients referred to medical centers for chronic constipation have obstructive defecation, called anismus, anorectal dysmotility, or pelvic floor dyssynergia. In patients with anorectal outlet obstruction, obstructed defecation happens in the rectosigmoid segment, and the passage of stool through the colon is usually typical (6). Some of these patients have a large rectocele, distended rectum (megarectum), rectal prolapse, descending perineum, or enterocele, whereas others develop pelvic floor muscle spasms resulting in defecation resistance (anismos) (7). Many researchers claimed that constipation could cause intestinal cancers such as colorectal cancer, adenocarcinoma, anorectal cancer, etc (8-10).

Anorectal cancer (anal cancer) is an uncommon type of cancer that occurs in the anal canal at the end of the rectum (11). This cancer results from the accumulation of mutations in genes that play an essential role in regulating cell growth and differentiation. Cancer-related genes include oncogenes, tumor-inhibiting genes, genes involved in xenobiotic metabolism, and growth factors (12).

Xenobiotic is a compound that is foreign to the body, such as drugs, chemical carcinogens, and various compounds that have somehow entered our living environment (13). Chemical carcinogens can directly da-

mage DNA or undergo changes, activate, and become carcinogenic after being exposed to enzymes such as cytochrome 450. The final carcinogenic compounds are usually electrophiles and are easily transported to nucleophilic groups in DNA (14). Glutathione-S-transferase is an enzyme that catalyzes the binding of glutathione to electrophilic xenobiotics. As a result of this reaction, a carcinogenic electrophilic compound becomes an inactive compound and is excreted from the body. GSTs are cytosolic enzymes and are divided into four groups: α, μ, π, and θ based on the similarity of the amino acid sequence and the reaction with the antibody (15). The GST cluster has five genes called GSTM5, GSTM4, GSTM3, and GSTM1. The GSTM1 gene has been mapped to the human chromosome 1P13 (16).

In humans, GSTM1 is genetically multifaceted and three of its alleles are known as GSTM1*A, GSTM1*B, and GSTM1*O (17). The difference between GSTM1*A and GSTM1*B is in the nucleotide of number 534. Two alleles appear to be quite similar in function. The O allele is obtained due to deletion in the GSTM1 gene (18). If a person has a homozygous deletion for the allele, his/her genotype is null-type. People with this genotype do not have the enzymatic activity of GSTμ in their cells (18, 19).

Given that deletion in the GSTM1 gene causes anorectal cancer (18), the aim of this study was to determine the relationship between deletion of homozygosity in the GSTM1 gene and the increased risk of anorectal cancer in patients with constipation of anorectal outlet obstruction.

Materials and Methods

Patient's evaluation

The research was conducted by the case-control method. Blood samples were taken from 40 patients with constipation of anorectal outlet obstruction (case group) and 40 healthy individuals. Case and control groups were the same in terms of age, gender, economic status, and social status, and there was no kinship relationship between either. 1.5 ml of blood was taken from each person and transferred to tubes containing anticoagulant EDTA. Samples were stored at -20°C until DNA extraction.

In addition to clinical evaluations such as patient history (duration of symptoms, frequency of defecation, abdominal distension, bloating, and abdominal pain and discomfort), anorectal manometric test was performed.

Anorectal manometric test

All 40 patients underwent anorectal manometry to ensure that all patients had constipation of anorectal outlet obstruction. Arnolderfer (low compliance) manometer was used with Gastro sof TM pneumohydraulic perforating system, polygram lower GITM, version 6.4, which has four channels with holes at a distance of 5mm connected to a catheter, the end of which is connected to a balloon. For anorectal manometry, to ensure that the rectum was empty, the patients were treated with paraffin, the night before the procedure. In some patients whose rectum was full of hard stools, it was recommended to evacuate with laxatives 2-3 days before manometry. The balloon attached to the catheter entered the rectum and by injecting air into the balloon, the rectum dilated. The amount of air entering the balloon was 1000ml. By injecting air into the balloon in the rectum, the superficial receptors of the rectal mucosa are stimulated and reduce the pressure of the internal anal sphincter and relax it, which was plotted as a downward curve on the computer. The answer was accepted when the pressure drop was equal to or greater than 20% of the base pressure at rest, and three consecutive steps were repeated. In this case, the anorectal inhibitory reflex was considered positive. In patients whose rectal mucosal nerve cells did not respond to the dilation of the balloon (despite adequate air injection), no reduction in pressure was established in the internal anal sphincter and no curved descent occurred on the computer screen. That is, the reflex was negative and raised the possibility of Hirschsprung's disease.

DNA extraction and PCR

To extract the DNA, about 1.5 ml of blood was poured into 15 ml Falcons and 10 ml of RIPA Lysis Buffer System was used. The microtubes containing DNA were also stored at -20°C until the PCR reaction was performed. A pair of primers with the following sequence was needed to perform PCR and detect deletion in the GSTM1 gene:

Forward: 5'-AGACAGAAGAGGAGAAGATTC-3'
Reverse: 5'-TCCAAGTACTTTTGGTTCAGT-3'

These primers can amplify the region between exons 5 and 6, including intron 5, from the GSTM1 gene. PCR reaction in 0.5 ml tubes containing the following materials with a final concentration of 1μL primer, 200μL DNTP, 0.1μL Taq DNA polymerase, 1.5 μM $MgCl_2$, and 1μL of template DNA was performed for 35 cycles. In each cycle, the samples were placed at a temperature of 72°C for the annealing stage. In the first cycle, the samples were placed at 95°C for 5 minutes. At the end of 35 cycles, the samples were placed at 72°C for 10 minutes. After PCR, the products were electrophoresed on 1.2% agarose gel for 3 hours. After staining with ethidium bromide, the gel was analyzed under ultraviolet light.

Statistical analysis

The results were analyzed using T-test and chi-square tests and the results of anorectal manometry were evaluated based on the presence of anorectal inhibitory reflex with its absence to confirm the diagnosis of constipation of anorectal outlet obstruction.

Results

Demographic and clinical evaluations

The results showed that out of forty patients participating in this study, 18 were men and 22 were women. Among healthy individuals in this study, there were 16 men and 24 women, with no significant difference between the two groups in terms of gender ($P = 0.91$). Also, the mean age in the case group was 35.29 ± 4.21 and the mean age in the control group was 36.10 ± 5.13 that there was no significant difference between the two groups in terms of age ($P = 0.82$) (Table. 1). In terms of mean frequency of defecation, in the case group 1.35 ± 0.92 times a week and in the control group 5.71 ±

Table1. Demographic and clinical evaluations of case study group and control group.

Variables		Case Study Group (n=40)	Control Group (n=40)	*P*-Value
Age (years)		35.09±4.21	36.10±5.13	0.82
Mean frequency of defecation		1.35±0.92 times a week	5.71±1.26 times a week	0.001
Duration of symptoms (months)		2.21±0.71	0	0.001
Abdominal distension	Positive	38	5	0.001
	Negative	2	35	0.001

Table 2. Distribution of individuals according to GSTM1 genotype in study groups.

Genotype	Case Study Group	Control Group	*P*-Value
Non- Null	21 (52.5%)	31 (77.5%)	0.012
Null	19 (47.5%)	9 (22.5%)	0.043
Total	40 (100%)	40 (100%)	-

1.26 times a week (P <0.001). Also, the duration of the symptoms in patients averaged 2.21 ± 0.71 months. Abdominal distension was positive in 38 patients and negative in only 2 cases (Table 1).

Anorectal manometric test results

The results of this section showed that out of 40 patients with constipation of anorectal outlet obstruction, 5 cases (12.5%) had megarectum, 7 cases (17.5%) had anismus, 10 cases (25%) had Hirschsprung's disease, 5 cases (12.5%) had descending perineum syndrome, 6 cases (15%) had rectal prolapse, 4 cases (10%) had enterocele, and 3 cases (7.5%) were with rectocele.

Gene polymorphism evaluations

The amplified region between exons 5 and 6, which includes introns 5, is observed in gel electrophoresis as a single band of about 1050bp. People with two alleles of GSTM1*O (null genotype) do not have this band. The results of GSTM1 gene deletion polymorphism showed that patients with constipation of anorectal outlet obstruction were almost 2 times more exposed to the null genotype than the control group (P <0.04) (Table 2).

Discussion

Many large-scale and small-scale clinical studies have been performed to evaluate whether chronic constipation increases the risk of intestinal cancer (20). The results of these studies are conflicting and some studies show that constipation raises the risk of cancer and other studies conclude that there is no such risk, and some studies have even shown that chronic constipation can reduce the risk (21-24). Many studies in this area are on people with cancer in comparison with those who do not have cancer. The problem with this type of study is that these people with cancer suffer from constipation due to cancer problems and even due to the use of chemotherapy medicines. Therefore, the result obtained from these experiments cannot be one-hundred percent confirmed (25).

Therefore, one of the best ways to evaluate the effect of constipation on cancer can be genetic evaluating of people with constipation for cancer-causing genes, so that the risk of cancer in people with constipation can be assessed and the risk will be reduced through prevention (26). Numerous studies have shown that the GSTM1 gene polymorphism plays an important role in the development of various cancers, including anorectal cancer (27, 28). The results of the present study showed that 47.5% of the case group had null genotype, which was 22.5% in the control group. Therefore, people in the case group have almost twice as many genotypes as controls, which could increase the risk of cancer in these people.

In individuals with the null genotype, homozygous deletions have occurred in the GSTM1 gene that produces the GSTM1*O alleles. In these individuals, the activity of the enzyme glutathione-S transferase is lost due to this deletion in the gene. The glutathione S-transferase family is a metabolic enzyme that plays an important role in the metabolism and detoxification of mutagens and carcinogens (16). GST genes encode a family of stage 2 enzymes (with a molecular mass of 23 to 30 kDa) that catalyze the binding of glutathione to a wide variety of hydrophobic and electrophobic substrates and carcinogens such as benzopyrene and ROS radicals. These enzymes can make up to 10% of the intracellular matrix proteins of various mammalian organs (29). This gene family includes at least 6 classes, of which the two classes GSTT1 and GSTM1 are the most important, with the highest probability of the possible consequences of GST polymorphisms on polymorphisms at the locus of these genes. They are on chromosome 22 and chromosome 1, respectively (30). GSTM1 isozymes detoxify a wide range of toxic reactions and mutagenic compounds, including metabolic phase 1-induced epoxides in aromatic polysilicon hydrocarbons such as benzopyrene, as well as oxidative stress products such as DNA hydroperoxidase and 5-Hydroxymethyl. Therefore, homozygous individuals with the GSTM1*O genotype are thought to be more susceptible to cancer and inflammatory lesions (31,32). A genome-wide association study is necessary to identify candidate genes for constipation of anorectal outlet obstruction (33,34).

In general, as mentioned earlier, glutathione-S transferase is an important metabolic enzyme in the detoxification of mutagens and the elimination of carcinogens (16). On the other hand, constipation of anorectal outlet obstruction causes the accumulation of toxins and carcinogens in the intestinal tract (35). Therefore, in people with both constipation of anorectal outlet obstruction and null genotype (i.e. deletion in the GSTM1 gene), because they do not have this enzyme, they are much more likely to get anorectal cancer than healthy people

with a null genotype.

The results of this study showed that the evaluation of GSTM1 gene polymorphism can help to evaluate and manage people with constipation of anorectal outlet obstruction. People with the GSTM1*O genotype are at higher risk for anorectal cancer due to the inactivity of the enzyme glutathione-S transferase. Therefore, for better management of this disease, and prevention of cancer in these people, the necessary treatments can be started immediately after determining the genotypes.

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