The relationship between the simultaneity present of cagA and hopQI genes in Helicobacter pylori and the risk of gastric cancer

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Abstract: Helicobacter pylori is a bacterium that causes infections in the gastrointestinal tract. This type of bacterium is very common and contagious at the same time. H. pylori enters the mouth and continues its course along the gastrointestinal tract. H. pylori infection induces an inflammatory response that leads to the activity of neutrophils, lymphocytes, plasma cells, and macrophages. In addition to the bacterial role in gastric mucosa, the host's inflammatory response may also play a role in disease outcome. In inflammation, the risk of carcinogenesis increases due to DNA damage increased proliferation and the creation of an environment rich in cytokines and growth factors. Genetic methods and diagnosis of H. pylori genes are used to identify healthy and healthy gastric cancer patients infected with H. pylori. In relation to the genes associated with H. pylori pathogenesis, the presence of genes such as cagA, hopQI, hopQII and so on is used, and PCR of a part of these genes amplified fragments of different lengths. One of the less-studied cases is the association of two or more pathogenic genes simultaneously with H. pylori. In this research, the frequency of disease and healthy individuals who are infected with H. pylori and have two genotypes cagA and hopQI at the same time, was examined. In order to diagnose H. pylori-infected individuals in healthy and gastric cancer patients, after PCR of glmM gene, PCR product electrophoresis on agarose gel was used. For this purpose, gastric tissue biopsy was used in patients and saliva was used in healthy individuals. For this purpose, 100 gastric biopsy samples were collected from patients with gastric cancer and 100 saliva samples from healthy individuals. According to the data, there is a significant relationship between the simultaneous presence of two genes cagA and hopQI and gastric cancer. In patients, 45.3% showed both genotypes, while in healthy individuals only 10.5% have this genotype and other healthy but infected with H. pylori (90.8%) do not have this genotype. To be. No report was observed on the simultaneous study of cagA and hopQI genes. No report was observed regarding the simultaneous study of cagA and hopQI genes.

Key words: cagA; Genotyping; Gastric cancer; Helicobacter pylori; hopQI.

Introduction

Gastric cancer is a condition in which cancer cells have formed on the lining of the stomach. This type of cancer can spread from the stomach to other parts of the abdomen, especially the liver, lungs, bones, abdominal wall, and lymph nodes. The disease has no clinical signs in the early stages (1-5).

Symptoms of gastric cancer include (6-11): Pain in the upper abdomen and a feeling of heaviness in the upper abdomen, difficulty swallowing (dysphagia), especially in tumors of the primary gastric region, feeling full stomach with pain before eating, nausea, anorexia, weight loss and jaundice, the presence of blood in the stool and black stools, bloody vomiting, especially when the tumor is in the lower part of the stomach, the discovery of a palpable abdominal mass, which in most cases indicates long-term growth and local spread of the tumor (the most common site for the spread of gastric tumors is the liver) and anemia, iron deficiency in men, and occult blood in the stool can be possible symptoms.

To prevent the disease, the following can be mentioned (12-18): 1) Lifestyle modification and avoidance of the mentioned risk factors in the diet are effective in the incidence of gastric cancer. 2) Consistent consumption of fresh fruits and vegetables in multiple meals throughout the day. 3) The effect of taking one 80 mg aspirin tablet daily with a doctor’s prescription, for men over 40 and women over 50. Important global research has shown that taking this pill is very effective not only in preventing stomach cancer but also in preventing other cancers, and in the United States, as a country where the incidence of gastric cancer is greatly reduced, continuous use of this pill is recommended. People who tolerate a daily dose of 80 mg aspirin tablets, use it from the age of 50, permanently and for at least 10 years. 4) Periodic endoscopy and eradication of Helicobacter pylori in people whose first-degree relatives have died of gastric cancer.

The causes of stomach cancer are: severe hypertrophy (overgrowth of cells) in the gastric folds, gastric ulcers and some polyps (polyps that tend to be malignant), atrophic gastritis (a disease in which the stomach wall is degenerated), past gastric surgery (removal of the end of the stomach), decreased gastric acidity, prolonged consumption of high concentrations of nitrates in smoked, dry and salty foods, use salty and salty diets, smoking, hookah, opium, high consumption of red meat, especially grilled meat, high consumption of high-fat cream, consumption of hot drinks, including hot tea, which is very common in the northern regions of the country, family history and a microbial infection called...
H. pylori. The role of this microbe in stomach cancer is very important, but getting this germ is not enough to cause stomach cancer alone, and there must be other risk factors; however, it can be said that 90% of people with gastric cancer are infected with H. pylori (19-22).

H. pylori is a gram-negative spiral curved bacterium that was identified by Marshall and Warren in 1983 by microscopic examination of the gastric epithelium of patients with chronic active gastritis (23). H. pylori is the most common cause of chronic bacterial infection and the main cause of the peptic ulcer, chronic active gastritis - is known in the world that it is accumulated in the stomach of more than 50% of the world's population (24). The World Health Organization (WHO) classifies H. pylori as a carcinogen from group 1. The method of transmission of this infection is person to person through fecal-oral or oral-oral contact (25).

Epidemiologically, H. pylori is one of the most common infectious diseases in the world. This bacterium causes 95% of chronic gastritis, 70-80% of gastroduodenal and also the development of gastric cancer. Chronic H. pylori infection may be associated with chronic gastritis, peptic ulcer disease, adenocarcinoma gastritis. Approximately 20% of people infected with H. pylori develop gastroduodenal disorders during their lifetime. Infection with this bacterium can play a significant role in gastric cancer. The annual incidence of H. pylori infection is 4 to 15% in developing countries but 0.5% in industrialized countries (26).

The doctors prescribe special antibiotics to kill H. pylori. Most common wound healing medications are also prescribed. Most of the time, killing this bacterium will prevent the wound from coming back. Sometimes not all bacteria are killed or come back. If this happens, another wound will form (27).

In general, H. pylori is associated with about 70% of all gastric cancers. Seroepidemiological studies in different parts of Iran have shown nearly 90% infection with this bacterium in people over 35 years of age. H. pylori in the stomach leads to general gastritis and decreased production of gastric acid. Acute inflammation can directly damage the peripheral cells that are responsible for secreting acid. In addition, inflammatory responses lead to the secretion of IL-1β (a pro-inflammatory cytokine with the property of inhibiting acid secretion). Acute inflammation is progressively replaced by chronic inflammation and acid secretion resumes. Gastritis is usually associated with acid secretion. Excess acid secretion reduces inflammation of the corpus and induces inflammation of the antrum, anthrax stimulates acid secretion, so antral gastritis is associated with high acid secretion and corpus gastritis is associated with decreased acid secretion. Only a small percentage of people infected with H. pylori develop gastric carcinoma. Scientists believe that the mismatch between the number of infected people and the number of people with cancer is due to environmental factors, genetic predisposition to inflammation in the host, and bacterial species diversity (13,20,28).

H. pylori regulate apoptosis of gastric epithelial cells through several mechanisms. Following the attachment of bacteria to the surface of epithelial cells, signaling through the cag secretory system activates an unknown factor called nuclear factor B (NF-B). are activated. NF-B is transported into the nucleus to activate the transcription of pre-apoptotic genes. H. pylori can also induce apoptosis by stimulating the expression of Fas. H. pylori urease protein can induce apoptosis by binding to MHC class II tissue compatibility complex molecules (29-32).

The risk of developing gastric carcinoma is associated with a heterogeneity of H. pylori virulence factors, especially cagPAI (Pathogenesis Island). cagPAI is a cluster of 45 kb (or 45,000 bp) and 31 genes, containing the cagA terminal gene, which typically acts as a marker for the whole island. Following binding to H. pylori to host epithelial cells, the CagA protein enters the cytoplasm of host cells through the secretory cagPAI coding secretory system (T4SS) (28,33,34).

In host cells, CagA interacts with several proteins and through signaling leads to increased expression of proinflammatory cytokines, skeletal actin markets, changes in cell polarity, and increased invasive power. The presence of T4SS, a CagA protein, is highly associated with gastrointestinal ulcer inflammation and an increased risk of gastric carcinoma (14,28,30).

The less studied hopQ gene encodes the extracellular protein HopQ, which can regulate the binding of some strains of H. pylori to gastric epithelial cells, so it may play an important role in early colonization and long-term resistance of bacteria to the gastric stroma. The hopQ gene exists in two forms, type I and type II. It should be noted that the prevalence of I&II HopQ type alleles in Iran has not been studied so far. Recently, a degree of covariance between hopQ and cagPAI has been shown, and it has been reported that the hopQI allele is commonly present in cagPAI-containing strains of Helicobacter, although some strains have both alleles (35,36).

The hopQ gene is one of the newly identified genes involved in CagPAI protein translocation. Then, this experiment aimed to determine the relationship between cagA+ strains of hopQI genotypes and its relationship with gastric cancer.

Materials and Methods

Statistical population

In this study, in order to investigate the hopQ type genotypes of Helicobacter pylori and its relationship with cagA virulence factor, 100 gastric biopsy specimens were collected from patients with gastric cancer and 100 saliva samples from healthy individuals, from Affiliated Yueqing Hospital, Wenzhou Medical University, Wenzhou, China.

All samples were collected endoscopically by a physician with the informed consent of the subjects. For all participants in the questionnaire, information including age, sex, history of previous illness or family members, eating habits and lifestyle were considered. The rest of the work was done in a molecular research laboratory.

DNA extraction, PCR reaction and primers used

After DNA extraction from biopsy specimens (from patients with gastric cancer) and saliva from healthy individuals (without any history or symptoms of gastric upset), the presence of H. pylori was investigated by polymerase chain reaction (PCR) and the use of a pair
of specific primers for one conserved area in the glmM gene of this bacterium. Genotyping of cagA and hopQ type I genes was performed by PCR using a specific primer pair. Pairs of specific primers are shown in Table 1.

**DNA extraction from healthy individuals infected with *H. pylori***

In order to find healthy people infected with *H. pylori* and extract DNA from them, gastric juice, feces or saliva can be used to extract DNA. Due to the low risk for volunteers, hygiene and simplicity of sampling, the DNA extraction method was used from saliva. For this purpose, after identifying the target individuals, cleaning the mouth and holding 5 ml of 3% sucrose solution for one minute in the mouths of volunteers, DNA was extracted from saliva according to the following instructions.

At first lysis Buffer was prepared. Then the following operation was performed. Centrifuge sample tubes at 3000 rpm for 10 minutes. Removal of supernatant. Adding 1 ml of TNE buffer and 20 μl of lysozyme and keep at 37 °C for one hour. Centrifuging the samples at 2000 rpm for 5 minutes. Removing the supernatant and the sample vortex for 5 seconds. Adding 1.3 ml of lysis buffer and 10 μl protease k and 0.8% agarose gel (horizontal electrophoresis).

**DNA extraction from biopsy specimens of patients with gastric cancer infected with *H. pylori***

At first lysis Buffer was prepared. Then the following operation was performed. Placing the gastric biopsy tissue sample in a 2 ml tube and add 1 ml of lysis buffer, 100 μl of 10% SDS and 20 μl of proteinase K (40 mg / ml). After vortexing, incubation was performed for one hour at 58 °C. After cooling the samples on ice, 350 μl chloroform and 350 μl NaCl (5 M) were added to them and mixed. The samples were centrifuged at 6000 rpm for 10 min, in which 3 phases were formed. Separating the supernatant and transfer to a new 2 ml microtube. Adding 1 ml of cold absolute ethanol to the samples and centrifuge for 15 min at 12000 rpm and 4 °C. The supernatant was discarded and 1 ml of 70% ethanol was added to the precipitate and centrifuged for 15 min at 12000 rpm and 4 °C. Discarding the supernatant and keep the tubes at room temperature until the pellets are dry. 50 ml of distilled water was added to each sample twice.

Evaluation of DNA quality was performed using 0.8% agarose gel (horizontal electrophoresis).

**Polymerase chain reaction (PCR)**

In all polymerase chain reactions, the reaction materials were mixed with the concentrations listed in Table 2 and the final volume of the mixture reached 25 μl. It was then placed in a thermocycler to perform a PCR reaction.

The PCR reaction program was defined for the thermocycler for each locus according to Table 3. In DNA samples obtained from biopsy 30 cycles and in the case of DNA obtained from saliva 40 cycles of repetition from steps 2 to 4 were defined.

**Data analysis**

To compare the different genotypes of the genes in

| Gene  | Accession No. | Primer sequence | Amplified fragment length |
|-------|---------------|----------------|--------------------------|
| glmM  | 900169        | 5'-AACCTTTTAGGGTGTTAGGGTTT-3' | 294 bp |
|       |               | 5'-AACCTTACTTCTCTAATACAC -3' |       |
| cagA  | 889201        | 5'-TTGACAAACAACCACACACACGGAAG -3' | 183 bp |
|       |               | 5'-CTTCCCCCTAATGGCCGAGATTCC -3' |       |
| hopQ1 | 7010294       | 5'-ACGAAACCCGCAAAAACCTTTA-3' | 187 bp |
|       |               | 5'-TTGCCATTTCTCATCGGTGA-3' |       |

**Table 3.** Thermal cycle of PCR reaction and names of primers for the studied genes.

| Primer name | 1  | 2  | 3  | 4  | 5  |
|-------------|----|----|----|----|----|
| glmM        | 94°C (5 min) | 94°C (30 sec) | 58°C (30 sec) | 72°C (30 sec) | 72°C (5 min) |
| cagA        | 94°C (5 min) | 94°C (30 sec) | 55°C (30 sec) | 72°C (30 sec) | 72°C (5 min) |
| hopQ1       | 94°C (5 min) | 94°C (30 sec) | 54°C (30 sec) | 72°C (30 sec) | 72°C (5 min) |
different groups, χ² test was used and to match the background variables, χ² and t-test were used. SPSS V26 software was also used for statistical analysis.

Results and discussion

Detection of genes used to identify and pathogenicity of H. pylori

In order to identify healthy gastric cancer patients infected with H. pylori, genetic methods and detection of genes related to H. pylori were used. In this study, after extracting genomic DNA, bacterial glmM gene was used to identify infected individuals (Figure 1). The PCR reaction resulting from primers designed for this gene in this study produced a 294 bp fragment (Figure 2).

In relation to the genes associated with H. pylori pathogenesis, the presence of cagA and hopQI genes was used, and PCR of these genes was amplified into fragments of 183 and 187 bp, respectively (Figure 2).

Identify people infected with H. pylori

In order to diagnose H. pylori-infected individuals in healthy and gastric cancer patients, after PCR of glmM gene, PCR product electrophoresis on an agarose gel and observation of 294 bp fragment was used (Figure 4-2). For this purpose, gastric tissue biopsy was used in patients and saliva was used in healthy individuals (Figure 3).

Figure 3 shows the agarose gel electrophoresis for the glmM gene to detect H. pylori-infected individuals in gastric cancer patients and healthy individuals. Infected samples were used for further analysis and identification of genes associated with pathogenicity.

Relationship between co-presence of cagA and hopQI genes in H. pylori and risk of gastric cancer

In this section, the simultaneous presence of cagA and hopQI genes in H. pylori, the frequencies of these two genes are shown in Table 4.

As shown in Table 4 and Figure 4, there is a significant relationship between the simultaneous presence of cagA and hopQI genes and gastric cancer. In patients' 45.3% show both genotypes, while in healthy individuals only 10.5% have this genotype and the rest of the healthy but infected with H. pylori (89.5%) do not have this genotype.

Previous studies have examined the effect of cagA

Table 4. Association between the presence of H. pylori and the presence of cagA and hopQI genes in gastric cancer patients and healthy individuals.

| Case    | cag A+ , hopQI(%) | Other modes |
|---------|------------------|-------------|
| Patient | 45.3             | 54.7        |
| Healthy | 10.5             | 89.5        |

p-value = 0.000
(28,30), hopQII (36) and hopQII (35) genes. However, in this study, the simultaneous effect of two genes (cagA and hopQI) was studied. For further study, it is necessary to do more studies on more genes as well as to study a wide range of gene networks (37–42). The prevalence of H. pylori infection in developing countries is 4 to 15% and this is a serious warning for more attention and prevention (43–50).

A less researched case is that two or more pathogenic genes are associated with H. pylori at the same time. In this section, the frequency of diseased and healthy individuals infected with H. pylori and both genotypes cagA and hopQI are studied. According to the data, there is a significant relationship between two genes cagA and hopQI and gastric cancer.

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