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The relationship between gastric cancer and *Helicobacter pylori* cytotoxin-related gene A genotypes

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Abstract: Gastric cancer has been known as the third leading cause of cancer-related death in the world. It is when cancer cells form on the lining of the stomach. Early symptoms include heartburn, upper abdominal pain, nausea, and loss of appetite. *Helicobacter pylori* is the most common microscopic creature that has infected humans worldwide. More than half of the world's population is infected with the bacterium. It is the main cause of diseases such as stomach ulcers and stomach and intestinal disorders. *H. pylori* infection is related to gastric adenocarcinoma and *cag*A genotype is believed to be related to cancer development. cytotoxin-associated gene A (*Cag*A) is a 120–145kDa protein encoded on the 40kb *cag* pathogenicity island (PAI). This study investigates the association between *cag*A *H. pylori* genotypes and gastric cancer. For this purpose, 65 stomach biopsies of the gastric cancer patients and 100 saliva samples were collected from healthy and *H. pylori*-infected individuals. Then genomic DNA was purified and Polymerase Chain Reaction (PCR) was performed for the studied gene using specific primers. The presence of *H. pylori* was investigated by PCR and a pair of specific primers for a protected region in the bacterium glmM gene. Then *cag*A+ and *cag*A- genotypes frequencies were determined in *H. pylori*-infected cases. Statistical analysis showed that there were significant differences between healthy and diseased ones for genotypes *cag*A+ and *cag*A-. Then the *cag*A+ can be a risk factor genotype for gastric cancer.

Key words: *cag*A genotyping; Gastric cancer; *Helicobacter pylori*.

Introduction

Gastric cancer is when cancer cells form on the lining of the stomach. (1). Early symptoms include heartburn, upper abdominal pain, nausea, and loss of appetite. Other symptoms include weight loss, jaundice, vomiting, difficulty swallowing, and blood in the stool, among other symptoms (2). This type of cancer may spread from the stomach to other parts of the body, especially the liver. Infect the lungs, bones, abdominal wall and lymph nodes (3).

The most common cause is an infection by the bacterium *Helicobacter pylori*, which accounts for more than 60% of cases. Certain types of *H. pylori* have greater risks than others (4).

Certain types of *H. pylori* are more dangerous than others. Other common causes of this disease are eating pickled vegetables and smoking. About 10 percent of the disease occurs in families, and between one and three percent of cases are due to genetic syndromes inherited from one's parents, such as gastric cancer spread from heredity. Many cases of gastric cancer include gastric carcinoma. This type of disease is divided into several sub-categories. Lymphomas and mesenchymal tumors may also develop in the stomach. In many cases, gastric cancer progresses through several stages over several years. The diagnosis of the disease is usually made by histology during endoscopy. Medical imaging is usually done next to see if the disease has spread to other parts of the body (3, 4).

H. pylori is the most common microscopic creature that has infected humans worldwide. More than half of the world's population is infected with the bacterium. It is a major cause of diseases such as stomach ulcers and stomach and intestinal disorders. The incidence of this bacterium in European countries and North America is 10 times higher than in other countries (about 74% of the US population). This bacterium is basically a spiral bacterium, but can also be deformed into a spherical shape, which is also habitable and pathogenic, but non-culturable (in vitro) and attaches to the gastric mucosa (generally in both forms. Biological and pathogenic) (5-7).

H. pylori virulence factor *cag*A (cytotoxin-associated gene A) has been known as a protein encoded on the 40kb cag pathogenicity island (PAI) (8). *H. pylori* strains can be separated into *cag*A positive or negative strains. About 60% of *H. pylori* isolates in Western countries are positive, while most of the East Asian isolates are positive (8).

The *cag* PAI also encodes a type 4 secretion system used to "inject" *cag*A into target cells after *H. pylori* has attached. After translocation, *cag*A is located on the inner surface of the cell membrane and undergoes tyrosine phosphorylation by Src family kinases (8).

CagA is also a highly antigenic protein, which is

Table 1. Primer sequences and amplified fragment length for *H. pylori* genes.

Gene	Accession No.	Primer sequence	Amplified fragment length
glmM	900169	5'-AAGCTTTTAGGGGTGTTAGGGGTTT-3' 5'-AAGCTTACTTTCTAACACTAACGC -3'	294 bp
*cag*A	889201	5'-TTGACCAACAACCACAAACCGAAG -3' 5'-CTTCCCTTAATTGCGAGATTCC -3'	183 bp

associated with obvious inflammation by causing the production of interleukin-8 (10).

H. pylori infection is associated with MALT lymphoma and gastric adenocarcinoma, while *cag*A is thought to be associated with cancer development (9). Phosphorylated *cag*A is can interact with the SHP-2 tyrosine phosphatase, rendering it functionally active, triggering a host cell morphological change to a more motile phenotype called the "hummingbird phenotype"(8). This phenotype mimics the effect produced by the hepatocyte growth factor which may be involved in all aspects of cancer, including metastasis (9). *Cag*A is also a highly antigenic protein, which is associated with obvious inflammation by causing the production of interleukin-8 (10).

The purpose of this research was to study the association between different *H. pylori* virulence *cag*A allele and patients with gastroduodenal disorders among a sample of the Iranian population.

Materials and Methods

The agarose and required materials for polymerase chain reaction (PCR) were prepared from Fermentas. Specific primers were synthesized by Cinnaclon, Iran. All chemicals and reagents were prepared from Zagros Bioidea Co, Kermanshah, Iran.

The population consisted of gastric cancer patients and cancer-free individuals as controls. All desired population was *H. pylori*-infected. Gastric biopsies were taken from 65 gastric cancer patients and 100 cancer-free that were infected with *H. pylori*. The patients and controls were age and sex-matched. The experiment materials included stomach biopsies of the patients with gastric cancer and saliva samples from healthy individuals.

The genomic DNA was extracted and purified from stomach biopsies of the patients with gastric cancer according to Moradi *et al.,* 2014 method (11) and saliva samples from buccal epithelial cells of the healthy individuals according to Aidar, 2007 method (12).

The PCR was done for desired genes via specific primers (Table 1). The *H. pylori* infections were diagnosed by PCR for the *glmM* gene. Then frequencies of *cag*A+ and *cag*A- genotypes were determined in *H. pylori* infected cases (Tables 2). All materials amount and optimized conditions for PCR reactions are shown in and (Tables 3).

The presence of *H. pylori* and *cag*A allele in gastric biopsy specimens and saliva healthy samples were identified by specific PCR assays.

Statistical analysis

The $\chi 2$ analysis was applied for the study of different frequencies in patients and healthy people. The SPSS V19 was used for statistical analysis.

Table 2. The *H. pylori* containing cagA gene frequency in the gastric cancer patients and Infected healthy individuals.

Situation	*cag* A^+ (%)	*cag* A^- (%)
Patients	66.7	33.3
Healthy	39.3	60.7
P value =0.008		

Table 3. Materials amount for all PCR reaction in current experiment.

Materials	Amounts
MgCl2	1.5 mM
dNTP	200 mM
PCR Buffer	50 mM
F-Primer	50 pmol
R-Primer	50 pmol
Template DNA	2 µl
Taq DNA Polymerase	1 unit
Double distilled water	16.25µl
Total volume	25µ l

Results and discussion

Gastric cancer is one of the most important and the most common diseases diagnosed in the world and Iran, which in Iran, it is the most common lethal cancer. Gastric cancer occurs as a result of many factors such as genetic conditions, environmental, nutritional behaviors, living conditions and the prevalence of *Helicobacter pylori* infection that the most common cause of the pathogenesis of chronic gastritis, peptic ulceration, and non-cardia gastric cancer, which accounts for more than 60% of cases (4, 13).

To detect infected people to *H. pylori* in gastric cancer patients and cancer-free individuals, the *glm*M gene was used. After the Extraction of genomic DNA and doing PCR, it was determined that the PCR product from gastric cancer patient's biopsies (lane 1) was more efficient rather than saliva samples from healthy individuals (lane 2). (Figure 1)

In Figure 1, it is clear that the band of biopsy specimens is sharper than normal ones because the DNA sample in gastric ones was denser rather than gastric free. This result can be obtained due to the use of the different samples for DNA extraction such as biopsies and saliva. DNA extraction from biopsies is more efficient than saliva samples.

In 2011 Espinoza and et al., to determine of *H. pylori* used of *glm*M gene and ureA gene. In this study, the

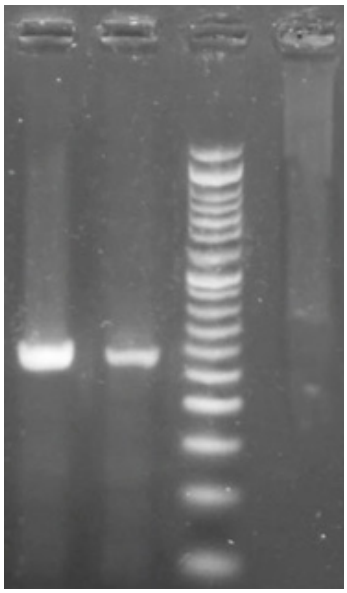

Figure 1. Diagnosis of *H. pylori* from biopsy specimens and nor¬mal samples. PCR products and agarose gel electrophoresis for glmM gene detection from *H. pylori*-infected samples. Lane 1: glmM gene amplification in gastric cancer patients. Lane 2: PCR product for the glmM gene in cancer-free. Lane 3: Size marker. Lane 4: Negative control.

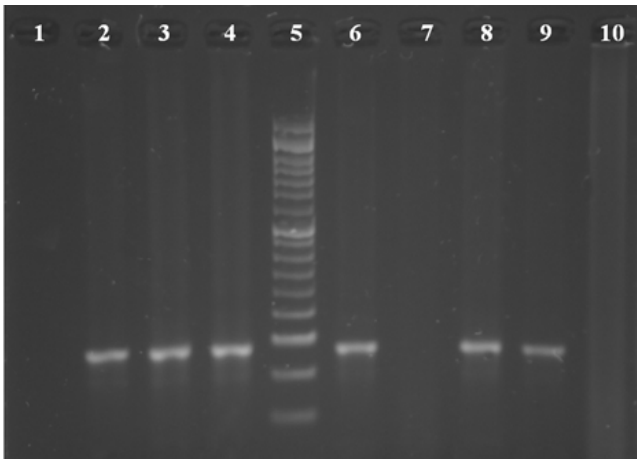

Figure 2. PCR products and agarose gel electrophoresis for the *cag*A gene in cancer patients and healthy individuals. Wells: 2, 3, 4, 6, 8, 9 are *H. pylori*-infected samples and with *cagA* gene, 7 is *H. pylori*-infected sample without *cagA* gene, 5 is a size marker, 10 is the negative control.

performance of the glmM gene was reported 100% but the ureA gene only 80% was able to detect of Infected people to *H. pylori*(14).

*cag*A gene is studied to investigate its relationship to pathogenicity in *H. pylori*. PCR is done and it is obtained fragments with 183 bp length (Figure 2).

frequency Table of the bacterial strain containing *cag*A gene in the Infected people to *H. pylori* and statistical analysis (Table 2) showed that it was a significant relationship between the Infected people to *H. pylori* (patient and healthy individuals) for the presence of *cag*A gene in Iranian population. This result showed that the product of the *cag*A gene is effective in the incidence of gastric cancer.

The risk of developing gastric carcinoma is associated with the heterogeneity of the *cag PAI* (Pathogenesis Island) factor. *Cag PAI* is a cluster of 45 kb and 31 genes, which includes the terminal *cag*A gene that normally is known as a marker for the whole island. After binding of *H. pylori* to epithelial cells of the host, *cag*A protein enters into the cell cytoplasm of the host by *cag PAI* encoding the secretory system of type IV (T4SS) (15)

In the host cells, *cag*A had interaction with several proteins and it causes to increasing in Pro-inflammatory cytokines expression, rearrangement of cytoskeleton actin, changing in cell polarity and Increasing of offensive power by messaging (16).

Saribasak et al., (2004) in Turkey reported that 9 people from 10 Cancer patients (90%) and Infected with *H. pylori* were positive *cag*A (17).

In the study of Miehlke (2000) in Germany, It was observed that 30 people from 34 cancer patients (88.2(were positive *cag*A (18).

In the study in Mexico, 1710 people from 1931(88.5%) Infected sample to *H. pylori* had the *cag*A gene. Thus in this study, Infection by bacterial strain containing the *cag*A gene had a significant relationship with increased risk of mortality due to gastric cancer (19).

Talebkhan et al., (2008) in Iran, reported that *cag*A$^+$ was the dominant *H. pylori* genotype (94% in cancer patients) in Iran (20).

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