Helicobacter pylori cagA-positive strains: gastric cancer susceptibility

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Abstract. Despite of decreasing in gastric cancer incidence in western countries, gastric cancer is known as a deadly disease in many of countries. Based on WHO categorize, north of Iran is known as a region with high gastric cancer incidence in the world. Helicobacter pylori (H. pylori) is identified as a risk factor in gastric cancer induction. cagA gene is one of the most important virulence genes of H. pylori. The aim of this research is to study the relation between H. pylori and gastric cancer and also the detection of cag-positive strains in Northern Iran. Participants in this study were 52 gastric cancer cases. For detection of H. pylori infection and presence of cagA gene we used PCR method, with specified primers. About 69% of gastric cancer patients were infected to H. pylori and 67% of H. pylori infected patients were cag-positive. Our results suggest that the H. pylori cagA positive strains increase the gastric cancer susceptibility.

Key words: Gastric cancer, H. pylori, cagA, glmM, PCR, Iran.

Introduction

Although gastric adenocarcinoma death rate has been decreased in most parts of the world (Bertuccio et al. 2009), Northern Iran is still one of the leading causes of cancer-related deaths (Malekzadeh et al. 2009). Gastric cancer (GC) is a multifactorial disease and exact etiologic mechanisms are highly controversial. Environmental factors such as consumption of red meat and dairy products, high salt intake, consuming hot tea, and also H. pylori infection, have been identified as important risk factors for GC in some parts of Iran (Pourfarzii et al. 2009).

Helicobacter pylori was recognized as type I carcinogen in 1994 and now it is recognized as the most common etiologic agent of infection-related cancers (Parkin et al. 2005).

Helicobacter pylori is a gram-negative, spiral shaped bacterial pathogen that penetrate the stomach's protective mucous lining and selectively colonizes the gastric epithelium. In addition, the majority of H. pylori strains express virulence factors that have evolved to affect host cells and molecular signaling pathways (Weeks et al. 2000). Its genome is comprised of about 1.6 mega bases, encoding approximately 1500 predicted open reading frames (ORF). About 20-30% of the genome sequence varies between different strains. This is a relatively high percentage among bacterial species and is thought to be resulted from a high spontaneous mutation rate and a relatively high recombination frequency (Israel et al. 2001).

Some scientific evidence indicates cagA, vacA and novel genes including iceA, BabA, SabA as virulence factors in H. pylori are associated with gastric cancer. (Boren et al. 1993, Peek Jr et al. 1995, Basso et al. 1998, van Doorn et al. 1998) H. pylori's vacA gene encodes a multi-meric vacuolating exotoxin (VacA), a 88 KDa secretory protein with the potential of forming intracellular vacuoles in gastric and other epithelial cells (Cover & Blaser 1992). Helicobacter pylori genome also encodes several adhesions that are important for ensuring tight binding between H. pylori and gastric epithelial cells. These include blood group antigen binding adhesion (BabA) and the sialic acid binding adhesion, SabA (Boren et al. 1993, Gerhard et al. 1999, Yamaoka et al. 2006).

One of the main important virulence factors of H. pylori is the cag pathogenicity island (cag-PAI). It is consisted of 31 genes, also encodes six proteins of a putative type IV secretion system. A variety of protein complexes are transferred across the bacterial membrane to the extracellular space or into the host cells by this system (Censini et al. 1996, Akopyants et al. 1998).

Among cag-PAI genes, cagA is the most studied one. This gene is located on the right half of cag-PAI. It encodes a protein (CagA) that is injected into the cytoplasm of the host cell through the type IV secretion system. CagA interacts with several proteins and interferes with signaling pathways leading to increased expression of pro-inflammatory cytokines, such as IL-8 which appears to be directly related to more severe inflammatory responses, also actin cytoskeleton rearrangements, alterations in cell polarity and invasiveness (Segal et al. 1999, Churin et al. 2003, Hatakayama 2009).

The aim of the present pilot study was to find the relation between gastric cancer and H. pylori cag-positive stains in Northern Iran.

Patients and Methods

Participants

In the present study patients consisted of 52 subjects with pathologically confirmed primary gastric cancer. Informed written consent was obtained from all participants and ethical principles of the Helsinki Declaration were followed. This study (grant number: 91265) was approved by the Ethics Committee of Kermanshah University of Medical Sciences (Iran).

Genomic DNA extraction

Genomic DNA was extracted from endoscopic biopsy samples. then, tissue specimens were suspended in 500 µl of lyses buffer containing 10 mM Tris-HCl (pH 8.0), 2 mM EDTA and 400 mM NaCl. The solution was incubated at room temperature for 20 min, following addition of Proteinase K and sodium dodecyl sulfate (400 ng and 0.6%, respectively), the mixture was incubated at 55°C overnight and extracted with GPP kit (Gen Pajoohan, Iran). DNA was evaluated by gel electrophoresis and Nano Drop (Thermo Scientifíc2000, USA). Ex-
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Molecular detection of\(H.\) pylori
The presence of \(H.\) pylori DNA was detected using PCR for amplification of the phosphoglucomutase gene, \(\text{glmM}\). The oligonucleotide primers used to amplify \(\text{glmM}\) gene were GLMF (5'-AAGCTTTAGGGGTGTTAGGGGTTT-3') and GLMR (5'-AAGCTTACTTTCTACACTAAGC-3') that generate 294-bp products. PCR reaction was put into practice as described in previous articles (Ayazi et al. 2013, Moradi et al. 2013).

Molecular detection of\(\text{cagA}\)
PCR amplification of \(\text{cagA}\) gene using specific oligonucleotide primers accomplished by cagF (5'-CTGCCTGCTAGTTTGTCAGCG-3') and cagR (5'-TTCGAAACCTTGTAGTCG-3') that produce 402-bp fragment.

The amplification procedure was carried in a total reaction volume of 25 \(\mu\)l, containing 2.5 \(\mu\)l 10X PCR buffer, 2 \(\mu\)l dNTPs (1.25 mmol/L), 0.5 \(\mu\)l MgCl\(_2\) (25 mmol/L), 1.25 \(\mu\)l of each primer (25 mmol/L), 15.3 \(\mu\)l dH\(_2\)O, 2 \(\mu\)l DNA (100 ng/\(\mu\)l) and 0.2 \(\mu\)l Taq DNA polymerase (5 U/\(\mu\)l) (Biflux, Japan). After an initial denaturation at 94°C for 3 min, the DNA was amplified by 35 cycles of 94°C for 30s, 52°C for 30s, and 72°C for 30s, with a final extension at 72°C for 5 min on the Mini PCR (Bio-Rad), then the products electrophoresed on a 2% agarose gel, to allow detection by ethidium bromide staining.

Results
Present study were included fifty two patients of gastric cancer, 22 female and 30 male, with mean age of 67.8 ± 8.84 years. Gastric cancer patients were recruited between May 2011 and June 2012. All enrolled subjects underwent endoscopy, and endoscopic findings were reviewed by two experienced endoscopists. For \(H.\) pylori detection a conserved fragment of \(\text{glmM}\) gene was selected and specific oligonucleotide primers designed that amplify a 294-bp fragment. Expected PCR product for \(H.\) pylori detection was observed in 39 (69%) patients (Fig. 1). For detection of cagA-positive strains one pair of specific primers was used as described above, that revealed 26 (67%) of \(H.\) pylori positive samples, were cagA positive (Fig. 2). Negative samples were tested twice to confirm the obtained results.

Discussion
In the last decades, gastric cancer has been vastly studied due to high worldwide frequency, poor prognosis and high mortality rate. Different clinical and epidemiological studies as well as basic research have demonstrated that \(H.\) pylori infection is one of the important risk factors for gastric tissue...
carcinogenesis. Also carrying the cag pathogenicity island has been consistently related to more severe gastric inflammation (Segal et al. 1999, Uemura et al. 2001, Charun et al. 2003, Fukase et al. 2008, Hatakeyama 2009).

The aim of this study was better understanding the association of *H. pylori* cag-positive strains infection and the gastric cancer susceptibility among Northern Iranian.

Although there is an agreement about *H. pylori* pathogenicity, the mechanism underlying the bacterial linked to carcinogenesis remains obscure. The carcinogenic gastric inflammatory reaction to *H. pylori* includes oxidative stress. In this process, a higher density of polymorphonuclear cells and large amounts of proinflammatory cytokines in the gastric mucosa produces oxidative stress by the production of reactive nitrogen and oxygen species, which may cause DNA damage in the gastric cells, promoting genetic modifications that are potentially carcinogenic (Baik et al. 1996, Yamaoka et al. 1999, Danese et al. 2001, Ashour et al. 2002, Wang et al. 2002, Thomazini et al. 2006, Farinati et al. 2008)

The present study was driven, to better elucidate if *H. pylori* cagA-positive strains are involved in gastric cancer susceptibility in Northern Iran. *H. pylori* infection was present in 69% of tumor tissues and 67% of them were cagA-positive; so there is a significant correlation between the presence of *H. pylori* cagA-positive strains and gastric cancer susceptibility. This result and high incidence of cagA-positive cases is similar to the study which carried out in Sweden and demonstrated by immunoblotting that 91% of *H. pylori* infected cases are cagA-positive and the study from Japan and Hawaii with 94% of positive cases by serology. In Taiwan, *H. pylori* with positive cagA strains are found in the majority of patients (83%) with gastric cancer or non-cancer patients; also in another study in Brazilian gastrointestinal patients the cagA gene was detected in 73.4% of the strains using the PCR method (Nomura et al. 1991, Evans et al. 1998, Ekström et al. 2001, Lin et al. 2004, Gatti et al. 2006, Thomazini et al. 2006).

Studies from Asian countries have proved that there is a majority of difference in the frequency of cag-positive strains in this area. Results indicate that the frequency of cag-positive strains in the countries close to the Europe, such as those located in West Asia is lower, which is similar to the frequency of European *H. pylori* strains. Instead, the frequency of cag-positive *H. pylori* strains is the most among East Asian countries. Iran, unlike her neighboring countries, can be found among those countries showing the highest prevalence of cagA-positive *H. pylori*, such as China and Japan (Talebkhani et al. 2008).

Most strains of *H. pylori* carry the cag pathogenicity island. Strains lacking this region of the genome, cag-negative strains, are less likely to be associated with gastric cancer. In different studies *H. pylori* infection was associated with an additional risk for infections with cag-positive compared with cag-negative strains. The proportion of strains that are cag-positive varies among geographical regions, but in general, almost all strains are cag-positive where gastric cancer prevalence is high (Huang et al. 2003, Yamaoka 2009, Kim et al. 2011).

In conclusion, accordance with other studies, cagA-positive *H. pylori* strain is an important risk factor for gastric cancer susceptibility in Northern Iran. Therefore based on our study, we suggest that more virulence factors of *H. pylori* related to gastric cancer should be evaluated.

**References**


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