Gastric Cancer and Helicobacter pylori: Impact of hopQII Gene

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Abstract: The Helicobacter pylori (H. pylori) is a Gram-negative, microaerophilic bacterium found usually in the stomach and use a number of mechanisms to survive in the stomach lumen. The presence of these bacteria in the stomach can lead to gastritis and reduction in stomach acid production. Acute inflammation can directly damage to the peripheral cells that are responsible for the secretion of acid. The risk of developing gastric carcinoma is associated to heterogeneity of Helicobacter pylori virulence factors. The hopQII is one of the outer membrane proteins involved in bacterial adherence to gastric mucosa and has been suggested to also play a role in the virulence of H. pylori. The purpose of the current study was to investigate the association between different H. pylori virulence hopQII allele and patients with gastrroduodenal disorders. For this purpose 58 stomach biopsies of patients with gastric cancer and 100 saliva samples from healthy individuals were collected. Then genomic DNA was purified and PCR for was done for desired genes via specific primers. The H. pylori infections were diagnosed by PCR for GlmM gene. Then frequencies of hopQII+ and hopQII− genotypes was determined in H. pylori infected cases. Statistical analysis showed that there were not significant differences between healthy and diseased ones for genotype hopQII+.

Key words: Gastric cancer, HopQII genotyping, Helicobacter pylori.

Introduction

The gastric cancer phenomenon is the most universal lethal cancer with around 738,000 deaths per year (1). Different occurrence of gastric cancer in worldwide can be due to diversity in the genetic conditions, nutritional behaviors and living conditions (2).

The Helicobacter pylori is a gram negative and successful gastric pathogen which colonizes more than 50% of the world population (3). The H. pylori infection is the key cause of gastric and duodenal ulcers, as well as a potential risk factor for gastric cancer and mucosa-associated tissue lymphoma (4). Available information indicates a slight association between gastroduodenal diseases and H. pylori virulence factors (5).

The H. pylori is now recognized to be a significant co-factor in the etiology of non-cardia gastric cancer of both the diffuse and intestinal histological type. The latter type develops via a complex multitage and multifactorial process. The first stage involves progression from superficial gastritis to atrophic pangastritis with intestinal metaplasia and correlated hypochlorhydria. This gastric phenotype may then progress to dysplasia and gastric cancer. Many co-factors are concerned in this progression as well as the strain of H. pylori, host genetic factors, host gender and environmental factors. Intestinal colonization with helminthic infection may retard the progression by changing the immune and inflammatory response to H. pylori and colonization of the aichlorhydric stomach with nitrosating bacteria may promote progression to cancer. H. pylori appears to be an necessary co-factor in the etiology of most gastric cancers. Therefore, avoidance of the infection or its eradication in early life should reduce the rate of this widespread and usually fatal tumor (6).

If H. pylori infects the gastric epithelium cells, the interleukin-8 should be induced and production of too much amounts of toxic reactive oxygen species (ROS) may be occurred. It may induce the interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α) and some other interleukins (7). Oxidative stress that caused by ROS is involved in human carcinogenesis (8). ROS generated in normal respiration of cells and during xenobiotics metabolism. It is known as a candidate agent in the growth of cancer and damage to cell membranes, mitochondria and DNA molecule (9).

Several putative virulence factors for H. pylori have been identified including vacA, babA, and iceA. The HopQ is one of the outer membrane proteins involved in bacterial adherence to gastric mucosa and has been found as a virulence factor of H. pylori. In 2005, Cao et al., reported that H. pylori hopQ genotypes are associated with an increased risk for peptic ulcer disease (10). The H. pylori genomes include about 30 different hop genes, which encode outer membrane proteins (11).

LOH et al., (2008) showed that, in certain H. pylori, adherence to the gastric epithelial cells are faintly facilitated in strains expressing hopQ (12), though they did not present further data about disease specific virulence factor of hopQ.

The high rate of H. pylori infection in Iran and the increasing number of digestive complaints lead to the current study on whether the presence of hopQ (typeII)
can affect disease outcome.

The purpose of the current study was to investigate the association between different H. pylori virulence hopQII allele (types II) and patients with gastro-duodenal disorders among a sample of the Iranian population.

Materials and Methods

Materials, chemicals and reagents

Agarose and polymerase chain reaction (PCR) materials were prepared from Fermentas. Specific primers were synthesized by Cinnaclo, Iran. All chemicals and reagents were prepared from Zagros Bioidea Co, Kermanshah, Iran.

Participants

The population consisted of gastric cancer patients and cancer-free subjects as controls. All desired population was H. pylori infected. Gastric biopsies were taken from 58 gastric cancer patients and 100 cancer-free subjectsthat were infected to 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.

DNA purification and gene amplification

The genomic DNA was purified from stomach biopsies of the patients with gastric cancer according to MORADI et al., 2014 method (13) and saliva samples from buccal epithelial cells of the healthy individuals according to AIDAR, 2007 method (14).

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

The presence of H. pylori and hopQII allele in gastric biopsy specimens and in saliva healthy samples was identified by specific PCR assays.

Statistical analysis

The χ² analysis was applied for study of different frequency in patients and healthy people. The SPSS V19 was used for Statistical analysis.

Results

Genomic DNA purification

Genomic DNA from 58 gastric cancer patients and 100 cancer-free subjects was purified successfully (Figure 1). The quality and quantity of purified genomic DNA was studied via spectrophotometry.

Identification of H. pylori infected samples via glmM gene PCR amplification

The H. pylori infections were identified by PCR for glmM gene. The PCR reaction for this gene amplified a fragment in 294 bp length in the H. pylori infections (Figure 2).

Polymerase chain reaction for hopQII gene detection

The agarose gel electrophoresis for hopQII gene detection in the H. pylori infections via PCR has been shown in Figure 3. The PCR reaction for this gene in hopQII+ samples amplified a fragment in 160 bp.

The hopQII gene frequency in the H. pylori infections

The frequencies for the hopQI gene frequency in the
The gastric cancer phenomenon is the most numerous diseases diagnosed in worldwide and it is the most common lethal cancer in Iran. Epidemiologic investigations have reported frequent risk factors for gastric cancer, including genetic factors, environmental, adverse living conditions, dietary habits and the prevalence of *Helicobacter pylori* infection (15).

*Helicobacter pylori* plays a key role in the pathogenesis of chronic gastritis, peptic ulceration, and noncardia gastric cancer. As it has been shown in Figure 2, the PCR product from gastric cancer patients biopsies (lane 4) was more efficient rather than saliva samples from healthy individuals (lane 3).

Clinical development of *H. pylori* infection is affected by the interaction of numerous virulence factors as well as by the host. The *H. pylori* infection is the key causative agent of superficial gastritis and confirms an expected task in the etiology of peptic ulcer disease (16).

Based on the biologic concepts, achieving successful and long term colonization requires composite adhesion mechanisms for bacteria. Consequently, all potential bacterial products were under focus for investigating the possible contribution in bacterial colonization. The *H. pylori* HopQ is one of the main outer membrane proteins on the bacterial surface and is the major outer membrane protein family observed in *H. pylori* genome. Determining a link between *H. pylori* hopQ and convinced digestive diseases may provide a start point for answering questions regarding *H. pylori* adherence to gastric cells. This study was designed to determine the frequency of *H. pylori* hopQ genotypes isolated from biopsy specimens. Our findings demonstrate a moderate prevalence of *H. pylori* hopQ types II genotype among Iranian patients with gastric cancer and healthy individuals that are infected to *H. pylori*.

It has been suggested that specific genotyping-based analysis of *H. pylori* isolates can be helpful for predicting post infection disorders (17).

Talebi Bezmin Abadi & Mohabbati Mobarez (2014) reported a high prevalence of *H. pylori* hopQ type II genotype among Iranian patients with gastric cancer that is not according to our finding (18).

In addition, outer membrane proteins of *H. pylori* have shown a strong potential for increasing the severity of related gastroduodenal disorders. Ohno *et al.* (2009) did not identify any relationship between hopQ type II allele and other virulence factors such as cagA and vacA in terms of clinical outcomes.

However, the exact relationship between virulence factors of *H. pylori* and hopQ alleles needs further investigation especially in genetically different populations.

In contrast to a study from United States (11) which reported a significant association between the carriage of *H. pylori* hopQ type I among the peptic ulcer patients, OHNO *et al.* (2009) did not identify a relationship between both hopQ alleles and clinical outcomes of infection (*P* > 0.05).

Kazemi *et al.* (2016) showed that there is not significant relationship between HopQI and gastric cancer in Iranian population (19).

In conclusion, this study showed that hopQ II is frequently present in *H. pylori* strains isolated from gastric cancer patients and healthy individuals in Iran. Then hopQII can not be a virulence and risk factor in our population.
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References