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Original Research

Association between *Helicobacter pylori hopQ*I genotypes and human gastric cancer risk

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Abstract: The *Helicobacter pylori* use a number of mechanisms to survive in the stomach lumen and can lead to gastritis and reduction in stomach acid secretion. It has been found that the risk of developing gastric carcinoma is associated to heterogeneity of *H. pylori* virulence factors such as *HopQ*. The *HopQ* is one of the outer membrane proteins involved in bacterial adherence to gastric mucosa and has been suggested to also main role in the virulence of *H. pylori*. The purpose of the current study was to investigate the association between different *H. pylori* virulence *hopQ*I (types I) genotyping and patients with gastroduodenal disorders. For this purpose 58 stomach biopsies of the patients with gastric cancer and 100 saliva samples from healthy and *H. pylori* infected individuals were collected and studied. Then genomic DNA was purified and PCR was done for desired gene via specific primers. The *H. pylori* infections were diagnosed using PCR for *Glm*M gene. Then frequencies of *hopQ*I+ and *hopQ*I- genotypes were determined in *H. pylori* infected cases. Statistical analysis showed that there were not significant differences between healthy and diseased ones for genotypes *hopQ*I+ and *hopQ*I-. Then the *hopQ*I+ cannot be as a risk factor genotype for gastric cancer.

Key words: Gastric cancer, *HopQ*I genotyping, *Helicobacter pylori*.

Introduction

Gastric cancer is the most universal lethal cancer with around 738,000 deaths per year (1). Different frequency 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 aetiology of non-cardia gastric cancer of both the diffuse and intestinal histological type. The latter type develops via a complex multistage 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 achlorhydric stomach with nitrosating bacteria may promote progression to cancer. *H. pylori* appears to be an necessary co-factor in the aetiology of most gastric cancers. Therefore, avoidance of the infection or its eradication in early life should reduce the occurrence 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* (typeI)

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

Gene	Accession No.	Primer sequence	Amplified fragment length
*glm*M	900169	5'-AAGCTTTTAGGGGTGTTAGGGGTTT-3' 5'-AAGCTTACTTTCTAACACTAACGC -3'	294 bp
*hopQ*1	7010294	5'- ACGAACGCGCAAAAACTTTA-3' 5'-TTGCCATTCTCATCGGTGTA-3'	187 bp

Table 2. Materials amount for all PCR reaction in current experiment.

Materials	Amounts
$MgCl_2$	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

can affect disease outcome.

The purpose of the current study was to investigate the association between different *H. pylori* virulence *hopQ*I allele (types I) and patients with gastroduodenal disorders among a sample of the Iranian population.

Materials and Methods

Materials, chemicals and reagents

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.

Participants

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 58 gastric cancer patients and 100 cancer-free that 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 extracted and 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 *hopQ*I+ and *hopQ*I- genotypes were determined in *H. pylori* infected cases. All materials amount and optimized conditions for PCR reactions are shown in Tables 2 and 3.

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

Statistical analysis

The χ^2 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

The genomic DNA was extracted and purified from stomach biopsies of the patients with gastric cancer and saliva samples from buccal epithelial cells of the healthy individuals.

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

A quantitative spectrophotometric test of DNA was performed using a UV-visible spectrophotometer (Zagros Bioidea Co.). The absorbance was measured at wavelengths of 260 and 280 (A260 and A280, respectively) nm. The absorbance quotient (OD260/OD280) was about 1.9 that showed high DNA purity.

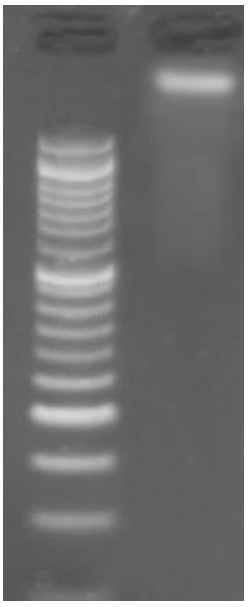

Figure 1. Purified genomic DNA from *H. pylori* infected samples. Lane 1: Size marker. Lane 2: genomic DNA.

Table 3. Thermal cycles for PCR reaction for different *H. pylori* genes.

Gene	Thermal cycles for PCR reaction				
	1	2	3	4	5
glmM	94 ºC (5 min)	94 ºC (30 sec)	58 ºC (30 sec)	72 ºC (30 sec)	72 ºC (5 min)
*hopQ*I	94 ºC (5min)	94 ºC (30 sec)	54 ºC (30sec)	72 ºC (30 sec)	72 ºC (5 min)

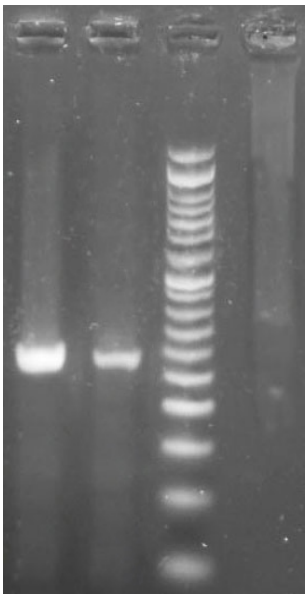

Figure 2. Diagnosis of *H.* pylori from biopsy specimens and normal samples. PCR products and agarose gel electrophoresis for *glm*M gene detection from *H. pylori* infected samples. Lane 1: *glm*M gene amplification in gastric cancer patients. Lane 2: PCR product for *glm*M gene in cancer-free. Lane 3: Size marker. Lane 4: Negative control.

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

The *H. pylori* infections were identified by PCR with specific primersfor *glm*M gene. The PCR reaction for this gene amplified a fragment in 294 bp length in the *H. pylori* infections (Figure 2.). The amplification of this fragment showed the presence of *H. pylori* in samples.

Polymerase chain reaction for *hopQ*I gene detection:

The PCR was done for *hopQ*I gene via specific primers. The agarose gel electrophoresis for *hopQ*I gene detection in the *H. pylori* infections via PCR has been shown in Figure 3. The PCR reaction for this gene in *hopQ*I$^+$ samples amplified a fragment in 187 bp. The amplification of this fragment indicate the *hopQ*I$^+$ allele.

The *hopQ*I gene frequency in the *H. pylori* infections

The frequencies for the *hopQ*I gene frequency in the *H. pylori* infections has been shown in Table 4. The χ^2

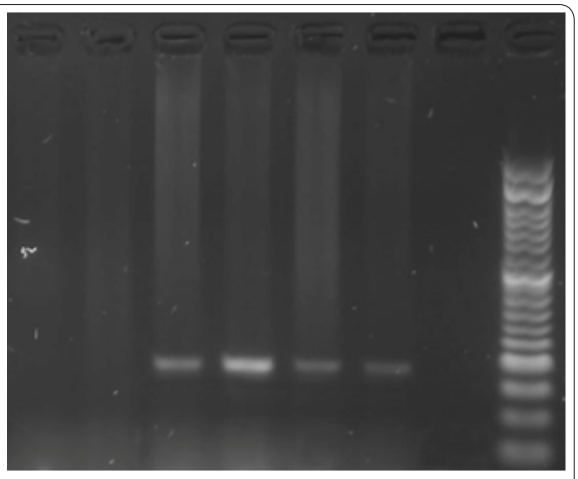

Figure 3. The agarose gel electrophoresis for *hopQ*I gene amplification in the *H. pylori* infections via PCR. Lanes 1 and 2: *hopQ*I$^-$ strains. Lanes 3-6: *hopQ*I$^+$ strains. Lane 7: Negative control. Lane 8: DNA size marker.

Table 4. The *hopQ*I gene frequency in the *H. pylori* infections.

	hopQI$^+$ (%)	hopQI$^-$ (%)
Case	53.8	46.2
Normal	46.4	53.6

P value =0.308

analysis showed that there was not a significant difference between gastric cancer and healthy individuals for presence of allele in their strains (P<0.05). Then the *hopQ*I$^+$ allel cannot be a risk factor for gastric cancer in Iranian population.

Discussion

Gastric cancer 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 environmental, genetic factors, adverse living conditions, dietary habits and the prevalence of *Helicobacter pylori* infection (15).

The *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 1) was more efficient rather than saliva samples from healthy individuals (lane 2).

The band of biopsy specimens is sharper than normal ones. This indicates that the DNA sample in gastric ones was denser rather than gastric free. Then it can be resulted that DNA extraction from biopsies is more efficient than saliva samples. However, this does not impact on our results. Because just presence and absence of bands are important to us not their intensity. The negative control reaction included all PCR materials minas DNA template.

The concentration of DNA in a sample was estimated by running the sample on an agarose gel. Such concentration estimates are semi-quantitative at best and are time consuming and confounded when numerous bands or a 'smear' of DNA are observed. For a more accurate determination of the concentration of DNA in a sample, a UV spectrophotometer was used for DNA solutions.

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 role in the etiology of peptic ulcer disease (16).

According to the biologic concepts, achieving successful and long term colonization requires composite adhesion mechanisms for bacteria. Therefore, all potential bacterial products were under focus for investigating the possible contribution in bacterial colonization. The *H. pylori Hop*Q 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 I genotype among Iranian patients with gastric cancer and healthy indivi-

duals that are infected to *H. pylori* (10, 11, 17 and18).

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

In contrast to results, the recent findings have shown that the *hopQ* type I allele is strongly associated with an increased risk of peptic ulcer diseases (PUD) in western countries and that *H. pylori hopQ* II is frequently detected among investigated population (18).

Furthermore, 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 *hop*Q type I and II alleles and other virulence factors such as *cag*A and vacA in terms of clinical outcomes (18). Their finding is according to our results.

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

In an investigation by OHNO *et al.,* (2009) the prevalence of *hopQ* I among gastritis and gastric cancer patients reported 58% and 68%, respectively. However, our results indicate that the frequency of *hopQ* I was almost similar in both H. pylori infected healthy individuals and gastritis patients (46.4% and 53.8%, resp.). TALEBI BEZMIN ABADI and MOHABBATI MOBAREZ (2014) reported that *hopQ* I is the less prevalent genotype among the *H. pylori* isolates recovered from the Iranian population (19). 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 ($P > 0.05$).

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

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