LACK OF ASSOCIATION BETWEEN GASTRIC CANCER AND HOPQ ALLELES IN Helicobacter pylori

Elham KAZEMI¹ and Danial KAHRIZI²,³*

1-Department of Sexual Medicine, The Rhazes Center for Research in Family Health and Sexual Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran
2. Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
3- Department of Agronomy and Plant Breeding, Faculty of Agriculture, Razi University, Kermanshah, Iran


Helicobacter pylori 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 (such as cagA). The HopQ 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 Helicobacter pylori. This gene has been shown in two types. The purpose of the current study was to investigate the association between different Helicobacter pylori virulence HopQ alleles (types I and II) and patients with gastroduodenal disorders. For this purpose 58 stomach biopsies the 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 Helicobacter pylori infections were diagnosed by PCR for GlmM gene. Then frequencies of hopQI+, hopQII+ and hopQI+ hopQII+ genotypes were determined in Helicobacter pylori infected cases. Statistical analysis showed that there were not significant differences between healthy and diseased ones for genotypes hopQI-, hopQII- and hopQI hopQII.

Keywords: gastric cancer, HopQ genotyping, Helicobacter pylori
INTRODUCTION

Gastric cancer is the most universal lethal cancer with around 738,000 deaths per year (JEMAL et al., 2011). Different frequency of gastric cancer in worldwide can be due to diversity in the genetic conditions, nutritional behaviors and living conditions (HUMANS, 1994).

_Helicobacter pylori_ is a gram negative and successful gastric pathogen which colonizes more than 50% of the world population (COVER and BLASER, 2009).

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 (GRAHAM AND FISCHBACH, 2010). Available information indicates a slight association between gastroduodenal diseases and _H. pylori_ virulence factors (ZHOU et al., 2004).

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 a 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 (MCCOLL and EL-OMAR, 2002).

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 (AUGUSTO et al., 2007). Oxidative stress that caused by ROS is involved in human carcinogenesis (CERUTTI, 1985). 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 (EGAN et al., 2003).

Several putative virulence factors for _H. pylori_ have been identified including vacA, babA, and iceA. _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 (CAO _et al._, 2005). The _H. pylori_ genomes include about 30 different _hop_ genes, which encode outer membrane proteins (CAO and COVER, 2002).

LOH _et al._, (2008) showed that, in certain _H. pylori_, adherence to the gastric epithelial cells are faintly facilitated in strains expressing _hopQ_ (LOH _et al._, 2008), 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 and II) can affect disease outcome.

The purpose of the current study was to investigate the association between different _H. pylori_ virulence _hopQ_ alleles (types I and II) and patients with gastroduodenal 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 Cinnamon, Iran. All chemicals and reagents were prepared from Zagros Bioidea Co, Kermanshah, Iran.

Participants

The population consisted of gastric cancer patients and cancer-free 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 purified from stomach biopsies of the patients with gastric cancer (according to MORADI et al., 2014 method) and saliva samples from buccal epithelial cells of the healthy individuals (according to AIDAR, 2007 method).

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 hopQI+, hopQII+ and hopQI+ 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 hopQ alleles 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

<table>
<thead>
<tr>
<th>Table 1. Primer sequences and amplified fragment length for H. pylori genes</th>
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</thead>
<tbody>
<tr>
<td>Gene</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>glmM</td>
</tr>
<tr>
<td>hopQ1</td>
</tr>
<tr>
<td>hopQ2</td>
</tr>
</tbody>
</table>
Table 2. Materials amount for all PCR reaction in current experiment

<table>
<thead>
<tr>
<th>Materials</th>
<th>Amounts</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgCl₂</td>
<td>1.5 mM</td>
</tr>
<tr>
<td>dNTP</td>
<td>200 mM</td>
</tr>
<tr>
<td>PCR Buffer</td>
<td>50 mM</td>
</tr>
<tr>
<td>F-Primer</td>
<td>50 pmol</td>
</tr>
<tr>
<td>R-Primer</td>
<td>50 pmol</td>
</tr>
<tr>
<td>Template DNA</td>
<td>2 µl</td>
</tr>
<tr>
<td>Taq DNA Polymerase</td>
<td>1 unit</td>
</tr>
<tr>
<td>Double distilled water</td>
<td>16.25 µl</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td>25 µl</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Thermal cycles for PCR reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>glmM</td>
<td>94 ºC (5 min)</td>
</tr>
<tr>
<td>hopQ1, hopQ2</td>
<td>94 ºC (5 min)</td>
</tr>
</tbody>
</table>

RESULTS

Genomic DNA purification

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

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

Figure 2. Diagnosis of *H. pylori* from biopsy specimens and normal 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 *glmM* gene in cancer-free. Lane 3: Size marker. Lane 4: Negative control.

**Polymerase chain reaction for hopQ1 gene detection:**

The agarose gel electrophoresis for hopQ1 gene detection in the *H. pylori* infections via PCR has been shown in Fig. 3. The PCR reaction for this gene in hopQ1+ samples amplified a fragment in 187 bp.
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.

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 $\chi^2$ analysis showed that there was not a significant difference between gastric cancer and healthy individuals for presence of allele in their strains ($P<0.05$).

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

<table>
<thead>
<tr>
<th></th>
<th>$hopQ$I$^+$ (%)</th>
<th>$hopQ$I$^-$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>53.8</td>
<td>46.2</td>
</tr>
<tr>
<td>Normal</td>
<td>46.4</td>
<td>53.6</td>
</tr>
</tbody>
</table>

* P value = 0.308

Polymerase chain reaction for $hopQ$II gene detection

The agarose gel electrophoresis for $hopQ$II gene detection in the $H.\ pylori$ infections via PCR has been shown in Fig. 4. The PCR reaction for this gene in $hopQ$II$^+$ samples amplified a fragment in 160 bp.
Figure 4. The agarose gel electrophoresis for hopQII gene amplification in the H. pylori infections via PCR. Lanes 1: DNA size marker. Lane 2 and 3: hopQII- strains. Lane 4: Negative control. Lane 5: hopQII+ strains.

The hopQII gene frequency in the H. pylori infections

The frequencies for the hopQII gene frequency in the H. pylori infections has been shown in table 5. The $\chi^2$ analysis showed that there was not a significant difference between gastric cancer and healthy individuals for presence of allele in their strains ($P<0.05$).

Table 5. The hopQII gene frequency in the H. pylori infections

<table>
<thead>
<tr>
<th></th>
<th>hopQII+ (%)</th>
<th>hopQII- (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>43.6</td>
<td>56.4</td>
</tr>
<tr>
<td>Normal</td>
<td>42.9</td>
<td>57.1</td>
</tr>
</tbody>
</table>

P value =0.555

The hopQI hopQII genes simultaneously frequency in the H. pylori infections

The frequencies for the hopQII hopQII genes frequency as simultaneously in the H. pylori infections has been shown in table 6. The $\chi^2$ analysis showed that there was not a significant difference between gastric cancer and healthy individuals for presence of allele in their strains ($P<0.05$).
Table 6. The hopQI hopQII gene simultaneously frequency in the H. pylori infections

<table>
<thead>
<tr>
<th></th>
<th>hopQ1⁺</th>
<th>Others (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>23.1</td>
<td>76.9</td>
</tr>
<tr>
<td>Normal</td>
<td>10.3</td>
<td>89.7</td>
</tr>
</tbody>
</table>

P value =0.067

**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 (MOGES et al., 2006).

*Helicobacter pylori* plays a key role in the pathogenesis of chronic gastritis, peptic ulceration, and noncardia gastric cancer. As it has been shown in Fig. 2, the PCR product from gastric cancer patients biopsies (lane 1) was more efficient rather than saliva samples from healthy individuals (lane 2).

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 (GATTI et al., 2005).

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* 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 I and II genotype among Iranian patients with gastric cancer and healthy individuals that are infected to *H. pylori*. TALEBI BEZMIN ABADI and 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.

It has been suggested that specific genotyping-based analysis of *H. pylori* isolates can be helpful for predicting post infection disorders (LU et al., 2005).

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 (OHNO et al., 2009).

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 hopQ type I and II alleles and other virulence factors such as cagA and vacA in terms of clinical outcomes. 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. In contrast to a study from United States (CAO and COVER, 2002) 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). It has been reported that hopQ type II strains have low frequency (less than 1%) among the Far East countries. Data from western countries indicate that the prevalence of hopQ type II strains (36%) is more common than countries from eastern Asia (1%). Our data about hopQ type II frequency is related to H. pylori infected persons not in a random population.

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

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REFERENCES


