

## Prevalence of hopQ Alleles and Relationship between cagA and vacA s1 with hopQ I Gene in *Helicobacter pylori* Strains Isolated from Patients with Peptic Ulcer Referred to Towhid Hospital in Sanandaj (2014)

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<http://dx.doi.org/10.13005/bbra/2008>

(Received: 10 January 2016; accepted: 20 February 2016)

Infection with *H. pylori* leads to digestive diseases including gastritis, peptic ulcer, and gastric adenocarcinoma. The virulence factors of *H. pylori* outer membrane proteins or Hop (*Helicobacter pylori* outer membrane protein) as an auto transporter and has widely adhesion properties, phase changes and recombination. *H. pylori* genome is widely encoded HopQ (*Helicobacter pylori* outer membrane protein Q), which affect the strains of *H. pylori* binding to human epithelial cells. The aim of this study determine the prevalence of hopQ alleles and associated Between cagA and vacA s1 with hopQ I gene in *H. pylori* strains isolated from patients with peptic ulcer referred to Towhid Hospital in Sanandaj (2014). Biopsy specimens from 100 patients with peptic ulcer *H. pylori* positive were collected and separated, different allele hopQ, vacA, cagA by PCR (Polymerase Chain reaction) was determined. The relationship between genes, cagA and vacA s1 hopQ I with Chi-square test SPSS version 20 (version 19, SPSS Inc., Chicago, IL) was used. P value <0.05 was considered as significant. The frequency of genes hopQ I, hopQ II, cagA, vacA s1, vacA s2, respectively, 54 (54%), 46 (46%), 51 (51%), 83 (83%), 17 (17 %). The relationship between hopQ I and cagA (P<0.01), hopQ I and vacA s1 (P<0.026), respectively. In this study, the presence of the CagA and hopQ I, HopQ I and VacA S1 gene in patients with gastric ulcer statistically significant relationship was found.

**Keywords:** *Helicobacter pylori*, HopQ, Peptic ulcer, outer membrane proteins, Sanandaj.

*Helicobacter pylori* are gram-negative bacteria that persistently colonize the human gastric mucosa. Gastric colonization by *H. pylori* is a risk factor for the development of peptic ulcer disease and distal gastric adenocarcinoma. When *H. pylori* isolates from unrelated humans are compared, a high level of genetic diversity is consistently detected Genetic diversity among *H.*

*pylori* strains helps to account for varying clinical outcomes among persons colonized with *H. pylori* Candidate markers for distinguishing disease-associated *H. pylori* strains (i.e., those associated with peptic ulceration or gastric adenocarcinoma) from less virulent strains include presence of the cag pathogenicity island and s1/m1 polymorphisms in vacA alleles. High rates of *Helicobacter pylori* infection in Iran and increase the number of gastrointestinal complaints led to study whether the presence of the alleles can result in disease affects HopQ. The main objective of this study

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was to evaluate the association between the presence of CagA and VacA genes of *H. pylori* hopQ two major alleles with clinical samples isolated from patients with peptic ulcer using the PCR method.

### METHOD AND MATERIALS

Horoscopes have on patients over 18 years old with sadness in the area of the upper GI endoscopy Towhid Hospital affiliated to Kurdistan University of medical science were presented. Information on factors such as age, sex and drugs obtained by questionnaire and were prepared for all forms of morality with peptic ulcer patients. Pathological diagnosis or procedure was performed by a specialist. At least two biopsies from each patient to test RUT (Rapid urea test) and another sample to study the molecular and pathological study. Extracted DNA (Deoxyribonucleic acid) and PCR (Polymerase Chain reaction). To investigate the molecular sample taken after the transition to 1.5 microliter tubes containing thioglycollate medium in microbiology research lab data transfer Kurdistan University of Medical Sciences and the extraction time were stored in a freezer at -80 ° C. To extract DNA, biopsy samples on the slide was

transferred in sterile conditions and carefully slide the other was crushed sterile. Then DNA extraction kit to help QIAamp DNA Mini Kit (QIAGEN) was performed. Specific primers for genes HopQ I / II, VacA and CagA in Table 1. The final volume of 25 ml and all the PCR using Master kit (Cinnagen, Iran). Thermal cycling for PCR amplification were optimized as shown in Table 2. Green PCR products by 2% agarose gel and stained with Syber green (Cinnagen, Iran) and review by UV (Ultraviolet) was observed. To search for an allele I (Table 3) CagA gene-gene and gene HopQ VacA s1 in patients with peptic ulcer associated and whether this difference was statistically significant? (Table 4), the chi-square test software SPSS-20 (version 20, SPSS Inc., Chicago, IL) was used. 0.05p < was considered as significant.

### RESULTS

The study of 100 samples from patients with upper gastrointestinal disorders and in patients with *H. pylori*-positive peptic ulcer disease 94-93 years were collected. The frequency of genes HopQ I, HopQ II, CagA, VacA S1, VacA S2, respectively, 54 (54%), 46 (46%), 51 (51%), 83 (83%), 17 (17 percent). A significant correlation between the

**Table 1.** Primer Designer

Gene or locus	Primer designation	Primer 5'→3'	Amplimer size(bp)	reference
HP1177	hopQ I F R	ACGAACGCGCAAAAACCTTTA TTGCCATTCTCATCGGTGTA	187	[1]
HP1177	hopQII F R	ACAGCCACTCCAATCCAGAA AACCCACCGTGGATTTAG	160	[1]
cagA	cagA F R	TTGACCAACAACCACAAACCGAAG CTTCCCTTAATTGCGAGATTCC	183	[1]
vacA	VA1 F V136 R	ATGGAAATACAACAAACACAC CAACAATGGCTGGAATGAT	136	[1]

**Table 2.** Program thermocycler instruments

Steps of PCR technique 35 cycles of:	Temperature and time
First denaturation	95° (15 min)
Denaturation	95° (1 min)
Annealing	56° (1 min)
Extention	72° (65 sec)
Final extention	72° (5 min)

presence of the gene HopQ I CagA (0.01P <) and gene hopQ I VacA S1 (0.026P <) and VacA S2 II HopQ with CagA-negative and statistically shown.

### DISCUSSION

Progress by the interaction of several factors in the pathogenesis of *H. pylori* infection as well as infection by Helicobacter pylori host of

**Table 3.** Frequency of hopQ, vacA and cagA Genotypes in Peptic ulcer patient

Allele	Genotypes				
	<i>hopQ I</i>	<i>hopQ II</i>	<i>vacA s1</i>	<i>vacA s2</i>	<i>cagA</i>
Positive	n*=54 (54%)	n=46(46)	n=83(83%)	n=17(17%)	n=51(51%)
Negative	n=46(46%)	n=54(54%)	n=17(17%)	n=83(83%)	n=49(49%)

\*(n=100)

**Table 4.** Relationship between *hopQ* Alleles and *cagA* and *vacA*

Gene or Allele	<i>hopQ</i>		Two-sided P* value Pearson Chi-Square	
	<i>hopQ I</i>	<i>hopQ II</i>		
<i>cagA</i>	Positive	34(66.7%)	17(33.3%)	<0.010
	Negative	20(40.8%)	29(59.2%)	
<i>vacA</i>	s1	49(59.0%)	34(41.0%)	<0.026
	s2	5(29.4%)	12(70.6%)	

\*p value of &lt;0.05 was considered as statistically significant

superficial inflammation of the stomach and inevitable role in the etiology of peptic ulcer disease show<sup>2,3</sup>. Access to the successful colonization of the biological concepts and long for a bacterial adhesion mechanism is complex. The bacterial produced the largest share among all products should be made to account for bacterial colonization<sup>4</sup>. HopQ gene in the bacterium *Helicobacter pylori* is a major protein of the outer membrane of *H. pylori* DNA is the largest family of proteins. To determine the relationship between HopQ and gastrointestinal diseases may be a point to answer questions about the adhesion of the bacteria to the stomach cells to provide. The study to determine the frequency of genotypes associated HopQ and HopQ I CagA gene and hopQ I VacA S1 gene isolated from biopsy samples was designed. It is believed that the analysis based on the genotyping of *Helicobacter pylori* isolated from clinical specimens can be useful in disorders infection<sup>5</sup>. According to the results of recent studies HopQ I allele correlated significantly with an increased risk of developing ulcers stomach in Western countries is also HopQ II alleles in this population are checked frequently<sup>6</sup>. Sicinski *et al.*,<sup>1</sup> on 86 children Colombian asymptomatic patients with *H. pylori* by urea breath test labeled PCR and examined, the frequency of allele HopQ, VacA and CagA in samples alleles HopQ and VacA detected was 91.7% VacA s1 and 73.7% HopQ

showed type 1. HopQ type 1 allele and genotype samples with positive CagA and VacA s1 (P <0.0001), respectively. Belogolova *et al.*,<sup>7</sup> presented a study on protein secretion system HopQ as a virulence factor associated with the type 4 strain P12 protein HopQ outer membrane of *H. pylori* cagA and answers related to displacement, including host cell cagA determination of cell morphology and cell dispersion was essential. According to the results, the researchers HopQ as a cofactor Code Type 4 plays an important role in the secretory system. In our study of 100 gastric ulcers between the presence of the gene HopQ CagA (0.01p <) and also the presence of VacA s1 Gene I HopQ (0.026p <) was a statistically significant relationship. It can be HopQ impact on virulence genes and CagA and VacA relationship between gene is developing a stomach ulcer argue. Further studies in this direction would be to identify strategies for physicians and the anti-peptic ulcer disease, gastritis and gastric cancer to improve.

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