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High frequency of *hopQ* genotypes among Iranian *Helicobacter pylori* clinical isolates

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SUMMARY

Different virulence factors are involved in *Helicobacter pylori* pathogenesis. *H. pylori* outer membrane proteins are a family of virulence factors that have diverse members. HopQ (*H. pylori* outer membrane protein) is the largest of them that contains types I and II alleles. The role of *hopQ* is not exactly known, but it has been considered in *H. pylori* adhesion and colonization. The aim of this study was to determine the frequency of *hopQ* genotypes among *H. pylori* isolates obtained from patients with gastroduodenal disorders and their association with the clinical outcome. The DNA of 100 *H. pylori* clinical isolates was investigated by polymerase chain reaction (PCR) method using specific primers for determining the *hopQI* and *hopQII* genotypes. *hopQI* was present in 35%, while *hopQII* was positive in 55% of the isolates. Amongst the gastritis subjects, the rate of *hopQII* compared to *hopQI* was higher, and a statistically significant difference was found between *hopQ*II genotype and the clinical outcome. With respect to the significant difference between the *hopQ* genotype and clinical outcome in our clinical isolates, it seems that this genotype is a useful marker for evaluating its association with *H. pylori*-related diseases.

Keywords: *Helicobacter pylori*, *hopQ* gene, virulence factor, gastroduodenal disorders, Iran.

INTRODUCTION

It is widely accepted that *Helicobacter pylori* as a Gram-negative and microaerophilic spiral bacterium is characterized by its potency to establish an intimate contact with gastric epithelia, thereby persistently colonizing in the stomach of most people [1]. In most of the infected subjects, it remains as an asymptomatic infection for decades, and only a minority of colonized persons develops gastroduodenal diseases such as chronic active gastritis, peptic ulcer disease (PUD), and gastric malignancies [2-5].

In addition to VacA (vacuolating cytotoxin A) and CagA (cytotoxin-associated gene A) as the putative virulence factors, in recent years using the sequencing technology and whole genome analysis, novel virulence factors such as Hop (*H. pylori* outer membrane protein) proteins has been recognized [1, 2, 6, 7]. The outer membrane proteins of *H. pylori* have been categorized into five families and Hop family is the most important one which includes some adhesins such as HopQ, the newly discovered member of this family [6, 8].

Although the physiological role of *hopQ* gene is not well known, it has been mentioned that HopQ is involved in *H. pylori* colonization, adhesion and progress of peptic ulceration [2, 8, 9].

Two allelic forms of *hopQ* (outer membrane protein=*omp27*) gene are recognized, as type I and type II, and the nucleotide similarity of these alleles is determined as 75-80% [6, 8]. The studies have shown that *hopQI* alleles are commonly present in the isolates containing *cag*-pathogenicity island (PAI) from patients with PUD.

By contrast, strains harboring *hopQII* were found significantly among *cag*-PAI negative isolates and have been reported from patients without peptic ulcer [2, 9].

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Regarding the high prevalence of gastroduodenal diseases due to *H. pylori* infection in Iran, and lack of any report about the evaluation of possible association between *hopQ* gene and respective clinical outcomes in our geographic region, the current study was designed in order to determine the frequency of *hopQ* genotypes among *H. pylori* isolates from patients with dyspeptic diseases in Shiraz, Iran.

MATERIALS AND METHODS

Subjects and specimens

In the current survey, 100 *H. pylori* DNA samples obtained from a previous study which had been conducted on antral biopsies of patients with dyspepsia attending the gastroenterology service at Faghihi Hospital in Shiraz, Southwest of Iran (from January to May 2014) were examined [10]. According to endoscopic diagnosis, the participants were classified into gastritis (Ga, n=63), gastric ulcer (GU, n=15), duodenal ulcer (DU, n=13), and non-ulcer dyspepsia (NUD, n=9) groups. Based on the informed consent, within the previous one month none of patients had ever undergone endoscopy and *H. pylori* eradication therapy. The study protocol was approved by the Shiraz University of Medical Sciences ethics committee (EC-9379-7059).

DNA extraction and PCR-based amplification

Genomic DNA extraction was performed from *H. pylori* pure cultures using the Cinna-pure kit (Cinnagen Co., Iran) according to manufacturer's instructions. Confirmation of *H. pylori* DNA was conducted by presence of *ureC* (*glmM*) gene in PCR assay as previously described [11]. Then, the DNAs were subjected to PCR for determining of *hopQ* genotypes (type I and type II alleles) with OP and BA primers, respectively, as described by Cao et al. [6]. The amplified products were separated by electrophoresis on a 1.5% (w/v) agarose gel containing TAE (Tris-Acetic acid-EDTA) buffer and stained with ethidium bromide (0.5 µg/ml), and visualized under Ultraviolet Light. *H. pylori* ATCC 26695 was used as a positive control for *ureC* gene.

Gene sequencing

To confirm the *hopQ* genotypes, the resulting PCR products of both *hopQI* and *hopQII* alleles (two positive samples from each allele) were submitted for sequencing (Macrogen, South Korea), and the sequences were edited, aligned, and analyzed using the CLC Sequence Viewer (ver 6.4; CLC Bio Co., Aarhus, Denmark).

Statistical analysis

Categorical data were coded using Microsoft office Excel program (Microsoft, Mountain View, Calif.), and statistical tests were performed using the statistical package SPSS for Windows 21.0 (IBM Co., Armonk, NY, USA). To identify possible associations between *hopQ* genotypes and clinical outcomes, χ^2 test was used and a p-value of <0.05 was accepted as statistically significant.

RESULTS

The study population consisted of 50 men (mean age 42.9, SD=15.32) and 50 women (mean age 40.3, SD=13.40), ranging from 18 to 75 years old. A significant association was observed between genders with the four investigated disease groups (P<0.014) and strains distribution with disease status (P<0.001) [10].

All the DNAs were positive for *glmM* (*ureC*) with 294 bp size. The PCR using the OP and BA prim-

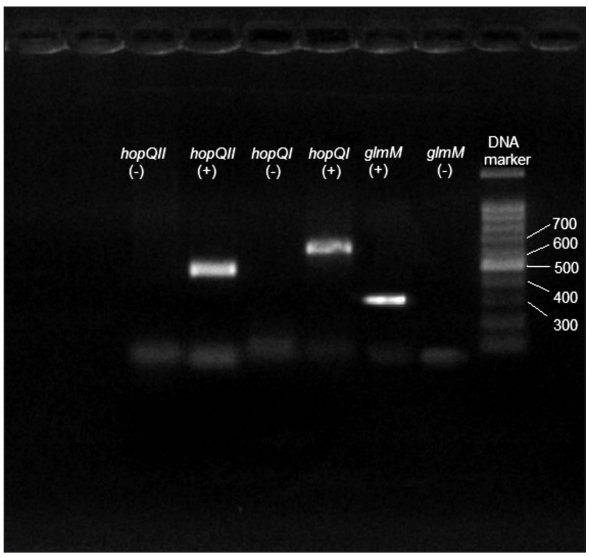

Figure 1 - Gel electrophoresis of *hopQI*, *hopQII*, and *glmM* PCR products. PCR product size of *hopQI* is 640 bp, *hopQII* 430 bp, and *glmM* 294 bp.

Table 1 - Distribution of hopQ alleles within disease groups.

Clinical disease	hopQ*I* *No. (%)*	hopQ*II* *No. (%)*	*Significant level (P value)*
Ga	20 (31.7)	34 (54)	0.012
GU	7 (46.7)	9 (60)	0.46
DU	4 (30.8)	6 (46.1)	0.42
NUD	4 (44.4)	6 (66.7)	0.34
Total	35 (35)	55 (55)	0.004

Ga: gastritis
GU: gastric ulcer
DU: duodenal ulcer
NUD: non-ulcer dyspepsia

er sets corresponding to the *hopQ*I and *hopQ*II genes yielded amplicons (630 bp and 430 bp) for 35 (35%) and 55 (55%) *H. pylori* isolates tested, respectively (Figure 1). In 28 specimens, there were both *hopQ* type I and type II alleles in the same *H. pylori* isolate, and their distribution is as follows: Ga=16, GU=7, DU=3 and NUD=2 subjects. Overall, the frequency of *hopQ* in our samples was found 90%.
Distribution of *hopQ* type I and type II alleles within disease groups is shown in Table 1. The *hopQ*II genotype was significantly found to be predominant among patients with gastritis (P=0.012). In total, a statistically significance association was shown between *hopQ*II genotype and clinical manifestations (P=0.004).

DISCUSSION

Long-term persistence of *H. pylori* in the gastric mucosa is depending on the host-parasite complex interaction, including adhesion. One of the candidate adhesins is proposed to be HopQ [7, 12]. The *H. pylori hopQ* I and II alleles are mostly associated with *cagA+/vacA* s1 and *cagA-/vacA* s2 strains, respectively. Also, geographical and population differences in the distribution of *hopQ* genotypes have been reported previously [1, 6, 9, 13]. Genotypic analysis of *H. pylori* strains is a useful method to evaluate the gastroduodenal disorders [7]. In the present study, we examined 100 clinical isolates of *H. pylori* from persons with gastrointestinal diseases for the presence of *hopQ* alleles. To the best of our knowledge, this work is the fourth report of *hopQ* genotypes in Iran. In the survey of Talebi et al., the rates of *hopQ*I and *hopQ*II were found 33 and 52%, respectively; this is consistent with our findings as well [7]. In another report from Iran, no correlation between *hopQ*I and gastric cancer was found, while this association had been shown with *hopQ*II allele in a previous study [7, 14].
On the other hand in the third report from Iran, a significant association was shown between both *hopQ* alleles and gastric cancer; this is in contrast with our observations [15]. In contrast to Talebi et al. and our work, the prevalence of *hopQ*I in studies conducted in Colombia (healthy children), Western and Asian (East) countries have been reported very high (72, 55.5, and 89%, respectively) [2, 7, 8]. However, this frequency among Italian population was lower (29%), which is similar to our study [1]. In a study from Pakistan, out of 241 *H. pylori* isolates, *hopQ*I and *hopQ*II were positive in 29% and 25%, respectively [16]. Indeed, both types of I and II were detected in 46% of the isolates. In the mentioned research, NUD was correlated with *hopQ*II, whereas a significant association was found between *hopQ*I and gastric carcinoma. Yakoob and colleague identified *hopQ*I in 47% and 86% of local residents and expatriates of their population [17]. This frequency for *hopQ*II was found in 85% and 59% of local residents and expatriates, respectively. The difference between these results and our study is probably due to geographical and population differences; meanwhile there is a genetic diversity in *H. pylori* clinical isolates worldwide. In the investigation of Cao et al., a significant association between peptic ulcers and *hopQ*I has been found among the United States population, but such relationship was not found in our study and some other studies [3, 7, 8, 13, 14, 18]. On the contrary, compared to other studies, the frequency of *hopQ*II in our work was higher, and there was a significance difference between *hopQ*II genotype and clinical outcome [1, 2, 8].
This finding is in contrast with the reported data from East Asian countries, as their isolates rarely possessed *hopQII* [8]. Therefore, it may be concluded that *hopQ* genotypes' pattern in Iran is similar to those observed in Western *H. pylori* isolates [9, 12]. In each population, availability of reliable data regarding the different virulence factors, including *hopQ* genotypes, could be a useful marker for predicting the clinical outcome of di-

gestive diseases caused by *H. pylori*. In addition, *H. pylori* genotyping is a suitable tool for predicting of infection sequels [1, 7]. Regarding to our results, if further surveys are done, the data could leads to more diagnostic strategies for gastroenterologists.

The limitations of the current study are lack of gastric cancer specimens and evaluation of *cagA* status and *vacA* genotypes.

In conclusion, the current study found a high frequency of *hopQ* among *H. pylori* isolates examined form our region. Therefore, with regard to genetic diversity of *H. pylori* strains and the results obtained from different geographic regions about *hopQ* genotypes, further investigations are required to clarify the exact role of this gene in *H. pylori* pathogenesis and its association with clinical manifestations.

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Conflict of interest. The authors have no conflicts of interest to disclose.

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