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# The association between *iceA* and *vacA* allelic gene variations of *Helicobacter pylori* strains and gastric disorders

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ABSTRACT

*Helicobacter pylori* is a causative agent of gastric disorders and associated with gastric adenocarcinoma. Different kind of gene products such as induced by contact with epithelium (*iceA*), vacuolating cytotoxin (*vacA*), and cytotoxin-associated gene A (*cagA*) confers the ability to this bacterium to destroy gastric cells and can lead to gastric cancer. The aim of the present study was to evaluate the frequency and association of *iceA* and *vacA* allelic genes variations of *H. pylori* strains and gastric disorders. This cross-sectional study included 90 non-duplicated *H. pylori* isolates from gastric biopsy of 300 patients in the north of Iran. DNA was extracted from all isolated strains and polymerase chain reaction (PCR) was used to determine the prevalence of *iceA* and *vacA* genes allelic variants. In overall, from 90 samples of *H. pylori iceA, vacA s1/s2, vacA m1* and *vacA m2* genes were detected in 68.9%, 33.3%, 53.3%, and 37.8% of patients, respectively. Regard to the distribution of the virulence genes, the co-occurrence of *iceA/vacA m1* (20%) was the most prevalent genotype followed by *iceA* (14.4%) and *iceA/vacA m2* (10%). In summary, as a first reports from Guilan province in the north of Iran we showed the association of *iceA* and *vacA* allelic variations in different types of gastric disorders. In this regard, we found a significant association between the presence of *iceA/vacA m1* genotype and gastric diseases.

## 1. Introduction

*Helicobacter pylori* as a successful pathogen that selectively colonizes in the gastric epithelium of human stomach and afflicts half of the world population is the first accepted Gram-negative bacterial carcinogen (Eghbali et al., 2016; Khashei et al., 2016). In addition, it plays as the most significant etiological agent of chronic gastritis and has been linked to several important diseases including gastric cancer, duodenal and gastric ulcer (Mojtahedi et al., 2007; Dara et al., 2017; Sohrabi et al., 2017). *H. pylori* utilize urease to metabolize urea producing ammonia to create a neutral environment in which the bacterium resides. The ammonia production has a negative impact on gastric mucosa. In other words, it seriously damages gastric mucosa through the interruption of tight junctions and the change of gastric epithelium. Furthermore, urease induces activation of phagocytes and production of inflammatory cytokines (Mojtahedi et al., 2007; Zanotti and Cendron, 2010). Thus, both innate and adaptive immune response induced by bacterial infection. The innate response is an initial non-specific process that reacts with different bacterial molecules with the purpose of killing the bacteria. In contrast, the antigen-specific adaptive responses cause to activation of B, T, and memory cells (Portal-Celhay and Perez-Perez, 2006).

The distribution of *H. pylori* infection is varies among different regions even in the same country (Khashei et al., 2016). Also, the rate of bacteria isolation in the developing countries is usually higher than in developed countries (Hosseini et al., 2012). Based on the recent report the pooled prevalence of *H. pylori* infection among Iranian patients was estimated 54%, ranging from 13% to 82% (Moosazadeh et al., 2016).

*Abbreviations:* cagA, Cytotoxin-associated gene A; babA, Blood group antigen-binding adhesin A; iceA, induced by contact with epithelium; cagE, cytotoxin-associated gene E; vacA, vacuolating cytotoxin; GU, gastric ulcer; DU, duodenal ulcer; G, gastritis; NUD, Non-ulcer dyspepsia; GC, gastric adenocarcinoma; FCS, Fetal calf serum

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**Table 1**
Primers sequences for amplification of *glmM*, *iceA* and *vacA* genes.

| Genes | Primers | Sequences (5′ → 3′) | Product size (bp) | Reference |
|---|---|---|---|---|
| *glmM* | glmM-F | AAGCTTTTAGGGGTGTTAGGGGTTT | 294 | (Lu et al., 1999) |
| | glmM-R | AAGCTTACTTTCTAACACTAACGC | | |
| *iceA* | iceA-F | GTGTTTTTAACCAAAGTATC | 247 | (van Doorn et al., 1998) |
| | iceA-R | CTATAGCCATTATCTTTGCA | | |
| *vacA (s1/s2)* | vacA (s1/s2)- F | ATGGAAATACAACAAACACAC | 259 and 286 | (Atherton et al., 1995; Qiao et al., 2003) |
| | vacA (s1/s2) - R | CTGCTGGAATGCGCCAAAC | | |
| *vacA (m1)* | vacA (m1) - F | GGAGCCCCAGGAAACATTG | 290 | (Tummuru et al., 1993) |
| | vacA (m1) - R | CATAACTAGCGCCTTGCAC | | |
| *vacA (m2)* | vacA (m2) - F | GGAGCCCCAGGAAACATTG | 352 | (Tummuru et al., 1993) |
| | vacA (m2) - R | CATAACTAGCGCCTTGCAC | | |

Many different kinds of effector proteins and toxins have been implicated in the pathogenesis of *H. pylori* infection. In addition, a high level of genetic variation in bacterium genome contributes to the persistence and adaption of *H. pylori* in gastric environments and subsequently outcome of infection (Roesler et al., 2014). The most considerable genes associated with virulence in *H. pylori* are the cytotoxin-associated gene A (*cagA*), Blood group antigen-binding adhesin A (*babA*), induced by contact with epithelium (*iceA*), cytotoxin-associated *gene* E (*cagE*) and vacuolating cytotoxin (*vacA*) (Ramis et al., 2013; Sedaghat et al., 2014; Haddadi et al., 2015; Sohrabi et al., 2017). VacA protein has been introduced as an important virulence factor and it plays a crucial role in the pathogenicity of the bacterium (Jones et al., 2010). VacA stimulates cytoplasmic vacuolation in eukaryotic cells and carried by a majority of *H. pylori* strains (Jones et al., 2010); however, the functional VacA protein is produced by half of *H. pylori* strains (Aguilar et al., 2001). The biological activity of VacA protein is significantly intensified by exposure to acidic pH and the acid activated VacA is considered to be the most remarkable cause of increased mucosal damage in *H. pylori* infection (Jones et al., 2010). Allelic variants of *vacA* gene have been identified in three main region [s (signal), i (intermediate), and m (middle)]. Different combinations between two main alleles of each region (s1, s2, i1, i2, m1, m2) have a considerable effect on VacA toxins with the distinct capability of stimulating vacuolation in epithelial cells (Ramis et al., 2013).

Recently, the *iceA* gene was introduced as a potential virulence factor that significantly associated with peptic ulcer. The *iceA* gene has two main allelic variants, *iceA1* and *iceA2*. The *iceA1* allele is considerably up-regulated by the contact of the bacterium with gastric epithelium and has been associated with peptic ulcer, while *iceA2* allele has been generally associated with asymptomatic gastritis and non-ulcer dyspepsia (da Costa et al., 2015).

Regard to the importance of *vacA* and *iceA* genes and their association with *H. pylori* pathogenicity and the prevalence of *H. pylori* infection in the north of Iran, the present study aimed to investigate the frequency and association of *iceA* and *vacA* allelic genes variations of *H. pylori* isolates in patients with gastric diseases.

## 2. Materials and methods

### 2.1. Patients and H. pylori isolation

In this cross-sectional study, 90 *H. pylori* isolates were included from biopsy of 300 patients referred for endoscopy at the teaching hospitals of Rasht city in the north of Iran during two years, from March 2013 to February 2015. To avoid any bias in results, the equal number of patients in both gender and number of samples in each group of disease was considered. Hence, gastric biopsy were taken from patients with gastric ulcer (GU), duodenal ulcer (DU), gastritis (G), Non-ulcer dyspepsia (NUD) and gastric adenocarcinoma (GC). An informed consent was signed by all patients after getting a detailed explanation from a physician about the study.

Two biopsy specimens were taken from each patient. One sample was used for histopathological analysis and the other for culture purpose. The biopsy specimens were transported within a short time to the laboratory in microtubes containing Brucella broth for further experiments. The biopsy specimens after cutting and homogenized with a sterile scalpel were cultured on the surface of Columbia agar plates supplemented with 5% sheep blood, 10% FCS (Fetal calf serum) and 6 g/mL cefsulodin, 10 g/mL trimethoprim and 5 g/mL vancomycin and incubated at 37 °C for 5–7 days under microaerophilic condition (Eghbali et al., 2016). Gram-negative bacilli with catalase, oxidase, and urease positive reaction were considered as *H. pylori*. All isolated strains were stored at −80 °C.

### 2.2. DNA extraction and identification of H. pylori by PCR

DNA samples from all isolated strains were extracted using DNA extraction kit (Roche, Germany) according to the manufacturer's instruction. Then, DNA density was evaluated by Nanodrop. A fragment of 294 bp from the *ureC* (*glmM*) gene was amplified using specific primers for molecular confirmation (Table 1) by automatic thermocycler (Eppendorf Personal 5332, Germany) (Lu et al., 1999). This gene is considered a “housekeeping” gene specific to *H. pylori*, and it participates directly in cell wall synthesis (Espinoza et al., 2011). PCR reaction was carried out in the final volume of 25 μL containing 2.5 μL 10× buffer, 0.75 μL $MgCl_2$ (50 mM), 2.5 unit Taq DNA polymerase (Cinnagen, Iran), 0.5 μL dNTP (10 mM) (Cinnagen, Iran), 20 Picomole from each primers and 1 μL from genomic DNA as a template. Amplification was performed of pre-incubation (94° for 3 min), followed by 35 cycles of denaturation (94° for 1 min), annealing (55 °C for1 min), extension (72 °C for1 min) and a final extension (72 °C for 5 min). PCR products were electrophoresed in 1.5% agarose gel (Roche, Germany) containing Sybr safe. DNA ladder (Roche, Germany) was used to detect the molecular weights of observed bands under UV lamp.

### 2.3. Amplification of iceA and vacA genes

PCR amplification of *iceA* and *vacA* genes was performed according to an above-mentioned method which used to amplifying the *ureC* gene and by using synthetic primers listed in Table 1. (Tummuru et al., 1993; Atherton et al., 1995; van Doorn et al., 1998; Qiao et al., 2003). All PCR products were analyzed using 1.5% gel electrophoresis. The sybr safe stain was used for visualizing of DNA bands under UV lamp.

## 3. Statistics

The analysis was performed by using SPSS™ software, version 22.0 (IBM Corp., USA). The results are presented as descriptive statistics in terms of relative frequency. Values were expressed as the mean ± standard deviation (continuous variables) or percentage of the group (categorical variables). Chi-square and ANOVA test were used to determine the significance of differences. $P$ values $< 0.05$ was considered