Analysis of *Helicobacter pylori* Genotypes in Afghani and Iranian Isolates

HOSSEIN DABIRI1, 2*, MEHDI BOLFION3, AKBAR MIRSALEHIAN2, MARYAM REZADEHBASHI1, FERESHTEH JAFARI1, LEILA SHOKRZADEH1, NAVID SAHEBEKHTIARI1, HOMAYON ZOJAJI1, YOSHIO YAMAOKA1, DARIOUSH MIRSATTARI1, MOHAMMAD REZA ZALI1

1 Research Center for Gastroenterology and Liver Diseases in Shaheed Beheshti University, M.C., Tehran, Iran
2 Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
3 Department of Medicine-Gastroenterology, Michael E. DeBakey Veterans Affairs Medical Center and Baylor College of Medicine, Houston, Texas, USA

Received 2 February 2010, revised 20 January 2010, accepted 21 January 2010

**Abstract**

The geographical variation in *Helicobacter pylori* genotypes is an observed phenomenon. Cytotoxin associated genes A (*cagA*) and E (*cagE*), and vacuolating cytotoxin (*vacA*) genotypes of *H. pylori* are associated with peptic ulcer disease (PUD). This study compared the distribution of these genotypes in Iranian and Afghani isolates and their association with clinical outcomes. *H. pylori* infected patients, as proven by positive culture, were recruited prospectively. A total of 70 patients, 55 Iranian (26 men and 29 women, mean age 48±18 years) and 15 Afghani immigrants (13 men and 2 women, mean age 34.8±11 years) living in Tehran, Iran were enrolled in this study. DNA was extracted from isolated *H. pylori* and polymerase chain reaction was carried out to determine the *cagA* and *cagE* status and *vacA* alleles. The number of gastric cancer, peptic ulcer and gastritis cases was 11, 23 and 36, respectively. The *cagA* positive isolates were more common in Iranian (67%) than Afghani isolates (60%). *cagE* was positive in 53% of Afghani compared to 51% of Iranian isolates. The most common *vacA* s-region genotype was s1; 80% in Afghani and 67% in Iranian. The s1m1 was a frequently observed genotype in Afghani strains (53%) while s1m2 (47%) was more common in strains isolated from Iranian patients. There is a difference in the *H. pylori* strains between Iranian and Afghani groups, for instance Iranian isolates were similar to European isolates while Afghani isolates were similar to isolates from India. However, there was no significant association between *cagA*, *cagE* and *vacA* genotypes and clinical outcomes in Iranian and Afghani patients.

**Key words:** Afghani and Irani isolates of *Helicobacter pylori*, *H. pylori* genotypes, virulence factors, clinical outcome

**Introduction**

*Helicobacter pylori* genotypes and their geographic distribution are linked to the severity of peptic ulcer disease (PUD) (Van Doorn et al., 1998; Covacci et al., 1999). The *H. pylori* genome is genetically diverse, as it can be seen in the *cag* pathogenicity island (PAI) and allelic variation within the *vacA* gene (Van Doorn et al., 1998a; Covacci et al., 1999; Yamaoka et al., 1999). *H. pylori* infects the majority of the global population and more than 90% of the Iranian population is reported to be infected with *H. pylori* (Alborzi et al., 2006). *H. pylori* infection causes chronic gastritis, PUD, gastric carcinoma (GC) and MALT lymphoma (Marshall and Warren, 1984; Wotherspoon et al., 1991; Parsonnet et al., 1991). Several *H. pylori* virulent genes contribute to the risk and severity of the disease outcome. These include the *cag* PAI that encodes a type IV secretion system (Kuipers et al., 1995; Censini et al., 1996). The cytotoxin associated gene A (*cagA*) has been proposed as a marker for the *cag* PAI and is associated with more severe clinical outcomes (Van Doorn et al., 1998a; Atherton, 1997). The *cag* PAI genes contain a *cagE* gene that encodes a secretory protein that is required for the induction of interleukin IL-8 (Tummur et al., 1995) and for translocation and phosphorylation of CagA protein (Odenbreit et al., 2000). The presence of the *cagE* gene has been associated with a severe clinical outcome, especially in developed countries (Fallone et al., 1998).

Vacuolating cytotoxin (*vacA*) is another important virulence factor of *H. pylori* that is associated with injury to epithelial cells. The *vacA* gene is virtually present in all *H. pylori* and has at least two variable parts, the signal or s-region, and the middle or m-region (Atherton et al., 1995). To date, s region and

* Corresponding author: H. Dabiri, Research Center for Gastroenterology and Liver Diseases in Shaheed Beheshti University, M.C., Tehran, Iran; phone: (0098)9122839824; fax: 0098-21-22432517; e-mail: hdabiri@razi.tums.ac.ir
m region can be differentiated to s1a, s1b, s1c, s2 and m1a, m1b, m1c, m2 subtypes respectively. The different combination of s- and m-region allelic types determines the production of the cytotoxin and the pathogenicity of the organism. *vacA* m1 strains are associated with greater gastric epithelial damage than m2 strains (Atherton et al., 1995). *vacA* s1m1 strains produce a large quantity of toxins, and s2m2 strains produce very little or no toxins. *vacA* s1a strains appear to be more pathogenic than s1b or s2 strains (Covacci et al., 1999; Saribasak et al., 2004; Blaser, 1995), although these are not consistent (Mohammadi et al., 2003; Mukhopadhyay et al., 2000; Tan et al., 2005). Respect to geographic variation in the *vacA* genotypes (Yamaoka et al., 1999; Tan et al., 2005; Van Doorn et al., 1999), it is suggested that such variations may contribute to the different prevalence of gastric diseases in these areas.

Iran and Afghanistan are neighboring countries and there are many Afghani refugees in Iran. Although there are several recent studies examining the relationship between *H. pylori* virulence factors and clinical outcomes in Iran (Mohammadi et al., 2003; Hussein et al., 2008); however, there is no report about relationship between Iranian and Afghani *H. pylori* virulence factors. We therefore compared the distribution of *vacA* alleles, *cagA* and *cagE* status in Iranian and Afghani residing in Tehran, Iran, and their association with clinical outcome.

**Experimental**

**Material and Methods**

**Studied Population.** A total of 70 patients from Iran and Afghanistan living in Tehran, Iran who were subjected to upper endoscopy from February 2007 to May 2008, were enrolled in this study. None of the patients had received non-steroid anti-inflammatory drugs or antibiotics within the previous three months. Informed consent was obtained from all patients, and the protocol was approved by the ethical committee of Research Center for Gastroenterology and Liver Diseases in Shaheed Beheshti University of Medical Science.

**Isolation and identification.** Three biopsy specimens were taken from the greater curve of the antrum; two were used for histological examination and one for *H. pylori* culture. Gastric biopsy specimens for culture were kept in transport medium consisting of thioglycolate with 1.3 g/l agar (Merck Co, Homburg, Germany) with 3% yeast extract (Oxoid Ltd., Basingstoke, UK) and were delivered to the laboratory on the day of endoscopy. In each case, the gastric biopsy specimens were cultured on Brucella Agar with 7% sheep blood and supplements with different antibiotics (Merck, Homburg, Germany) and incubated under microaerophilic conditions at 37°C for 3–10 days. The organisms were identified as *H. pylori* by colony morphology, gram staining results and positive reactions to oxidase, catalase, and urease activities. The identified *H. pylori* were sub-cultured and single colonies were used for DNA extraction.

**Preparation of genomic DNA and Polymerase Chain Reaction (PCR).** DNA from each *H. pylori* isolate was extracted using a commercially available kit (Qiagen, Hilden, Germany). The genotypes of *vacA* s-region (s1 or s2) and m-region (m1 or m2), the presence of the *cagA*, *cagE* and *glmM* (ureC) genes were determined by PCR. The *glmM* (ureC) gene was used as controls for detecting *H. pylori* DNA. Primers sequences used in this study are listed in Table I. All PCR mixtures were prepared in a volume of 25 µL containing 1× PCR buffer, 500 nM of each primer, 1.5 mM MgCl$_2$; 200 µM each dNTP, 1.5 U Taq DNA
polymerase, and 300 ng DNA sample. The products were amplified in an Eppendorf AG 22331 thermocycler (Eppendorf, Hamburg, Germany), and visualized by electrophoresis in 1.5% agarose gel, stained with ethidium bromide, and examined under UV illumination. Mixed infection of different genotypes and vacA negative samples were excluded in the analyses.

Data Analysis. Chi Square and Fisher’s exact tests were used for analysis of categorical data and ANOVA for continuing data. Analyses were done using Sigma Stat for Windows V2.03 (SPSS, Chicago, IL). A p value less than 0.05 was accepted as statistically significant.

Results

A total of 70 patients, 55 Iranian (26 men and 29 women, mean age 48±18 years) and 15 Afghani immigrants (13 men and 2 woman, mean age 34.8±11 years) living in Tehran, Iran, were enrolled in this study (Table II). Distribution of gastric cancer, peptic ulcer, and gastritis, was 11, 23 and 36, respectively. The cagA positive isolates were more common in Iranian isolates 37 (67%) than Afghani isolates 9 (60%). The cagA gene was present in 62%, 64% and 74% of H. pylori strains isolated from Iranian patients with, PUD, GC and NUD, respectively, while 50% PUD and 61% NUD of Afghani patients were infected with cagA positive H. pylori isolates. The presence of the cagA gene was not statistically correlated with the presence of PUD or GC in both countries. cagE was positive in 53% of Afghani compare to 51% of Iranian isolates. Regardless of ethnic differences, statistically there was no association between cagE status and clinical outcome.

The most common vacA s-region genotype was s1; the numbers vary from 80% in Afghan to 67% in Iranian isolates. For the vacA m-region, m1 genotype was the most common among Afghan strains (53%), while m2 genotype was the most common (69%) in strains isolated from Iranian patients. The s1m1 (53%) was a genotype frequently observed in Afghan strains while s1m2 (47%) was more common in strains isolated from Iranian patients. In Iranian samples 2 (4%) isolates were s0m2 (with no PCR product for s region) while such genotype was not seen in Afghan isolates. there was no statistically significant association between clinical outcomes and vacA genotypes in each ethnic group, however the allotype distribution of vacA in clinical outcome groups (NUD, PUD and GC) showed a different profile in the two nations (p = 0.08). There was also no significant difference in the prevalence of triple positive (vacA s1, cagA and cagE) strains between PUD and NUD patients.

We also classified the gastritis cases with no acute inflammation (polymorphonuclear cell infiltration), none to severe chronic inflammation (mononuclear cell infiltration) as “mild to severe gastritis”, and analyzed separately; however the prevalence of the cagA gene in strains isolated from patients with mild gastritis was even higher than those with total gastritis as well as those with PUD and GC , indicating that the cagA gene was not involved in the severity of gastritis in either nationality.

Discussion

This study investigated the cagA, cagE and vacA status of H. pylori isolated from patients from Iran and Afghanistan living in Tehran. All Afghani patients were born in Afghanistan and immigrated to Iran. Because the strains were obtained from symptomatic patients, the results reflect the findings in these groups of patients rather than entire populations. The current study confirms the distinctive difference in H. pylori genotypes in Iranian and Afghani patients even they residing within the same city. This is consistent with other studies from the USA, Malaysia and Kuwait (Tan et al., 2005; Yamaoka et al., 2000; Qabandi et al., 2005). The difference in H. pylori strains among different ethnic groups living in the same area suggests that they were brought by immigrants and have remained in that population for many generations (Dabiri et al., 2009; Tan et al., 2005).

Table II

Demographic characterization and clinical outcomes in 70 studied patients

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Number</th>
<th>Sex</th>
<th>Mean age</th>
<th>Gastritis (NUD)</th>
<th>PUD</th>
<th>GC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mild</td>
<td>Moderate</td>
<td>Sev</td>
</tr>
<tr>
<td>Iranian</td>
<td>55</td>
<td>Male</td>
<td>26 (47%)</td>
<td>48±18</td>
<td>12 (22%)</td>
<td>4 (7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>29 (53%)</td>
<td>35</td>
<td>5 (34%)</td>
<td>6 (40%)</td>
</tr>
<tr>
<td>Afghan</td>
<td>15</td>
<td>Male</td>
<td>13 (87%)</td>
<td>31 (44%)</td>
<td>12 (17%)</td>
<td>18 (26%)</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td></td>
<td>39 (56%)</td>
<td>46±18</td>
<td>0.06</td>
<td>0.8</td>
</tr>
</tbody>
</table>

P value is calculated by comparing demographic characterization and clinical outcomes (NUD, PUD and GC) in two nations.

NUD, Non Ulcer Dyspepsia; PUD, Peptic Ulcer Disease; GC, Gastric Carcinoma.
Helicobacter pylori vacA s1 was the predominant genotype in both country isolates. The vacA s1m2 genotype was predominant in Iranian strains similar to reports from Western countries (Saribasak et al., 2004). In contrast, s1m1 genotype was predominant in Afghani strains similar to reports from India (Yamaoka et al., 1999; Chattopadhhyay et al., 2002). Different pattern of vacA alleles in association with clinical outcomes in each nation is probably suggesting that Iranian and Afghani H. pylori isolates possess different virulence.

The prevalence of the cagA gene has been reported to vary from 26% in Jordan to 100% in India (Nimri et al., 2006; Datta et al., 2003). Such differences were attributed to the genetic diversity and geographic locations (Covacci et al., 1999). cagA presence in Iranian isolates has been reported to vary from 44% to 91% according to different reports (Dabiri et al., 2009; Siavoshi et al., 2005; Talebkhan et al., 2008). In the present study, 67% of the Iranian patients were infected with cagA positive strains similar to an Iranian report (67%) (Dabiri et al., 2009; Jafarzadeh et al., 2007). This is relatively different from studies in Asian countries such as Japan, Korea, China, Hong Kong, Malaysia, Thailand and India where more than 90% of the strains carry the cagA gene, regardless of clinical outcomes (Yamaoka et al., 1999; Tan et al., 2005; Datta et al., 2003; Pan et al., 1998; Wong et al., 2001; Chomvarin et al., 2008). Our results regarding strains isolated from Iranians are rather consistent with studies from Europe and the US where the prevalence of cagA positive strains is between 60–70% (Van Doorn, L.J., et al., 1998a; Miehlke et al., 2000). The prevalence of cagA positive strains in Afghans (60%) is in agreement with previous reports from neighborhood of Afghanistan; Iran (~70%) and Bangladesh (55%) and despite of high prevalence of cagA positive strains (100%) in India (Datta et al., 2003; Talebkhan et al., 2008; Rahman et al., 2003).

The prevalence of the cagE gene was also reported to vary in different geographic regions and/or ethnic groups (e.g., 64% in the US, 71% in UK, 70% in Malay, 88% in Thailand, 28% in Turkey and 77% [NUD] to 92% [PUD] in India) (Tan et al., 2005; Chomvarin et al., 2008; Podzorski et al., 2003; Kauser et al., 2005; Tiwari et al., 2005; Salih et al., 2007). The prevalence of the cagE gene in studied isolates from both countries was close to each other (51% in Iranian vs. 53% in Afghani isolates) and different with other countries. This finding was different with very limited report on only three Afghani samples (Dabiri et al., 2009).

The present study did not reveal any associations of the vacA and cagA status with PUD and NUD in both ethnics. This finding is in agreement with other reports from Iran (Hussein et al., 2008; Talebkhan et al., 2008) but was different from that in many studies in Western countries where vacA s1 and cagA positive strains are more often isolated from patients with PUD than with NUD (Blaser, 1995). The difference in the cagA status and its association with clinical outcomes might be due to large genomic variations in the H. pylori genomes. There may be several distinct forms of the cagA gene with an uneven geographical distribution, and that only some forms of the cagA gene are associated with severe gastro-duodenal diseases (Miehlke et al., 1996; Pan et al., 1997; Zhou et al., 2004).

Conclusions. The presented study detected differences between H. pylori isolates from Iranian and Afghani patients. Afghani isolates (in the case of vacA allele) are more similar to Indian isolates while in cag alleles are more similar to Iranian isolates. We could not detect any statically associations of the vacA, cagA and cagE status with clinical outcomes in Iranian and Afghani patients. Therefore, we suggest a different marker of H. pylori as a useful virulence marker for more serious disease in Iran and further studies with large number of samples for Afghanistan.

Acknowledgements

The authors are thankful to Dr M. Molaei and K. Baghaei for their technical helping. This study was supported by a grant from Research Center for Gastroenterology and liver Disease of Shaheed Beheshti University Research Fund, project number 407.

Literature


