Association of H. pylori Virulence Genes CagA, VacA and Ure AB with Ulcer and Nonulcer Diseases in Iranian Population

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Abstract: To evaluate the association of virulence genes CagA, VacA and UreAB of H. pylori with the development of different gastric disorders, polymerase chain reaction was performed on H. pylori organisms isolated from biopsy samples of stomach of patients with ulcerative disease and nonulcerative disease. The difference between the groups was statistically significant (p<0.05) only for VacA gene. We detected 8 phenotypes, characterized as CagA-VacA-UreAB (phe 1), CagA-VacA-UreAB (phe 2), CagA-VacA-UreAB (phe 3), CagA-VacA-UreAB (phe 4), CagA-VacA-UreAB (phe 5), CagA-VacA-UreAB (phe 6), CagA-VacA-UreAB (phe 7), CagA-VacA-UreAB (phe 8). The prevalence of phenotype 1 was significantly higher in the patients with UD than that in the patients with NUD (p<0.05). These results suggest that in the population under our study, being infected by a H. pylori strain with the genotype CagA-VacA-UreAB may be associated with an increased risk of acquiring an ulcer disease.

Key words: Helicobacter pylori, VacA, CagA, UreAB, ulcer disease, nonulcer disease

INTRODUCTION

Helicobacter pylori organisms colonize approximately half of the world’s human population (The EUROGAST Study Group, 1993). It is considered the etiological agent of Chronic Gastritis (CG) and peptic ulcer and their complications (Marshall, 1986; Cover and Blaser, 1995; Sipponen, 1997; Maaroos et al., 1999). Histological gastritis is essentially universal among H. pylori infected individuals, but only a minority develops a clinically significant outcome, such as peptic ulcer disease or gastric cancer.

The process by which different disease patterns develop has not been fully elucidated. However two putative virulence determinants of H. pylori have been identified as markers which may influence the pathogenicity of different H. pylori isolates, the cytotoxin associated gene A (CagA) and the vacuolating cytotoxin gene A (VacA) (Xiang et al., 1995; Censini et al., 1996). The CagA gene encodes a 120-140 kDa protein of unknown function in about 60-70% of H. pylori strains. Overall, the data support the notion that infection with a CagA positive isolate increases the risk but does not predict the presence of a clinically significant outcome (Konnethisson et al., 2003; Ali et al., 2005; Shibata et al., 2006). VacA codes for another important virulence factor that induced vacuolization on eukaryotic cells in vitro. Differences in VacA gene (the mosaic combination of signal[sg] regions and middle[m] region allelic types) have been identified and attempts have been made to associate specific VacA genotypes with different outcomes, especially with Ulcer Disease (UD) (Atherton et al., 1997; He et al., 2000; Rzovics et al., 2001; Correa, 2005).

The aim of this study was to evaluate the distribution of CagA and VacA genes in H. pylori strains isolated from Iranian patients. Besides that, we studied the prevalence of another pathogenic factor, UreAB gene, as it has been cited that intense urease activity is a key pathogenicity factor (Shen et al., 1998).

MATERIALS AND METHODS

Patients and samples: We performed this study in Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, during November 2004 to October 2005. In this study 116 patients undergoing endoscopy, at endoscopy ward of Nemaze Hospital of Shiraz University of Medical Sciences were enrolled (average age 41.3±1.4, range 16-80, 60 males and 56 females). Histologically, antral biopsy specimens were embedded in paraffin, stained and examined by a central study pathologist for diagnosis of H. pylori infection and

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confirmation of gastric disease. Another piece of antrum biopsy from each patient was obtained and transferred to the lab for isolation of *H. pylori* by previously described culture methods (Farshad et al., 2004a, b). Briefly, the homogenates of the biopsy specimens were cultured on Brucella agar base (Merk, Germany) containing 10% lysed horse blood and appropriate antibiotics. The cultures were kept in a microaerophilic atmosphere (6% O₂, 7.1% CO₂, 7.1% H₂, 79.8% N₂) (ANOXOMAT Mark II, Mart Microbiology BV, Netherlands) at 37°C for 5-10 days, the organisms were identified as *H. pylori* by gram staining, colony morphology and positive oxidase, catalase and urease reactions.

**Preparation of genomic DNA:** Pure isolates were collected from the surface of the plates, washed with sterile PBS buffer and pelleted in 1.5 mL tubes. The pellets were resuspended in 383 mL of TE Buffer [10 mM Tris-HCl (PH, 8.0), 1 mM EDTA, 10% SDS] and 2 mL of 20 mg mL⁻¹ solution of proteinase K and incubated at 56°C for 2 h in a hot block. DNA was then extracted using phenol-chloroform method (Wilson, 1994).

**Detection of CagA, VacA and UreAB by PCR:** The primers sequences were previously reported and obtained from TIB MOLBIOL Syntheselabor GmbH H (Berlin, Germany) (Han et al., 1998). Description and sequences of the PCR primers used in this study are given in Table 1. Amplifications were carried out in a gradient thermal cycler (Eppendorf, Germany) as described earlier by Han et al. (1998). Individual PCR products were electrophoresed on agarose gels, stained with ethidium bromide and photographed.

**Data analysis:** Fisher’s exact test was used for analysis of data for different groups and diseases. An amount of <0.05 was accepted for p-value as statistically significant.

**RESULTS**

**Patient groups and prevalence of *H. pylori* infection:** According to endoscopic and pathologic findings the patients were categorized to 2 groups: Ulcerative (37) and nonulcerative (77). Totally from antrum of ulcerative and nonulcerative patients 30 (81.08%) and 35 (45.45%) *H. pylori* strains were isolated, respectively.

**Prevalence of CagA, VacA and UreAB among *H. pylori* positive patients:** In polymerase chain reaction analysis totally from 65 *H. pylori* isolates 31 strains (47.69%) were CagA⁺, 37 strains (56.92%) were VacA⁺ and 42 (64.61%) strains were UreAB⁺.

**Relation between CagA, VacA, UreAB status and ulcerative or nonulcerative disease:** Thirteen of 35 (37.14%) *H. pylori* strains isolated from patients with Non Ulcerative Disease (NUD) and 24 of 30 (80%) *H. pylori* strains isolated from patients with Ulcerative Disease (UD) were VacA⁺. The presence of VacA in the patients with UD was significantly higher than that in the patients with NUD (p=0.05). 13 of 35 (37.14%) *H. pylori* strains isolated from patients with NUD and 18 of 30 (60%) *H. pylori* strains isolated from patients with UD were CagA⁺. CagA positivity was higher in the patients with UD than that in the patients with NUD, but this difference between the groups was not statistically significant (p=0.05). Twenty of 35 (57.14%) *H. pylori* strains isolated from patients with UD and 22 of 30 (73.3%) *H. pylori* strains isolated from patients with UD were UreAB⁺. According to the Table 2 CagA, VacA and UreAB positivity was higher in the patients with UD (60, 80 and 73.3%, respectively) than that in the patients with NUD (37.14, 37.14 and 57.14%, respectively), but the difference between the groups was statistically significant (p<0.05) only for VacA gene. We detected 8 phenotypes, characterized as CagA⁺-VacA⁺-UreAB⁺ (phe 1), CagA⁺-VacA⁻-UreAB⁺ (phe 2), CagA⁻-VacA⁺-UreAB⁻ (phe 3), CagA⁻-VacA⁻-UreAB⁻ (phe 4), CagA⁺-VacA⁻-UreAB⁻ (phe 5) CagA⁻-VacA⁻-UreAB⁻ (phe 6), CagA⁺-VacA⁻-UreAB⁻ (phe 7) and CagA⁺-VacA⁻-UreAB⁻ (phe 8) (Table 2). Phenotype 1 was found in 26.15% of the patients (40% UD, 14.28% NUD). The prevalence of this phenotype was significantly higher in the patients with UD than in the patients with NUD (p<0.05). However, phenotype 8 was only seen in the patients with NUD. There was not any significant difference between the groups according to the other phenotypes (p>0.05).
Table 2: Distribution of CagA, VacA and UreAB genes in H. pylori strains isolated from patients with non ulcerative and ulcerative diseases

<table>
<thead>
<tr>
<th>CagA</th>
<th>VacA</th>
<th>UreAB</th>
<th>NUD (%)</th>
<th>UD (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(phenotype 1)</td>
<td>5(14.28)</td>
<td>12(40)</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>(phenotype 2)</td>
<td>7(20)</td>
<td>2(6.66)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>(phenotype 3)</td>
<td>4(11.42)</td>
<td>2(6.66)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>(phenotype 4)</td>
<td>2(5.71)</td>
<td>3(10)</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(phenotype 5)</td>
<td>2(5.71)</td>
<td>7(33.3)</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>(phenotype 6)</td>
<td>2(5.71)</td>
<td>1(3.33)</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>(phenotype 7)</td>
<td>2(5.71)</td>
<td>3(10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(phenotype 8)</td>
<td>11(31.42)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>35</td>
<td>30</td>
</tr>
</tbody>
</table>

NUD - Non-Ulcerative Disease, UD - Ulcerative Disease

DISCUSSION

It has been suggested that H. pylori may induce more or less severe gastroduodenal diseases according to the strain virulence. In this study, we employed VacA, CagA and UreAB genotyping to characterize 65 individual H. pylori isolates derived from two groups of patients. The presence of VacA, was significantly more prevalent in the patients with UD than those in patients with NUD (p<0.05). The positivity of CagA was higher in the patients with UD than that in the patients with NUD, but difference was not statistically significant (p=0.05). However, we found a significantly higher prevalence of VacA positive strains in UD than that in NUD patients (80 and 37.14%, respectively). It was reported from different centers that in patients with ulcer diseases, the positivity rates of CagA and VacA and both CagA, VacA were 71-100, 47.5-92, 37-75%, respectively (Kidd et al., 1999; Lamarque et al., 1999; Brito et al., 2003). In all of these studies, the positivity of CagA and VacA was higher in the patients with UD, however, some was statistically significant (Hennig et al., 1999; Lin et al., 2000; Martin Guerrero et al., 2000; Leite et al., 2005) and some not when it was compared to patients without ulcer (Kodama et al., 1999; Mahachai et al., 1999; Audibert et al., 2000). In patients with NUD, the positivity rates of CagA and VacA were reported to be 37-89.7% (Hennig et al, 1999; Kodama et al., 1999; Lamarque et al., 1999; Tan et al., 2006) and 33.3-73% (Weel et al., 1996; Lamarque et al., 1999; Mahachai et al., 1999), respectively. Nearly in all of these studies, CagA and VacA positivity rate in the patients with NUD was found to be low compared to that in the patients with ulcer, however, this difference was statistically significant in some studies (Ito et al., 1997; Warburton et al., 1998; Martin Guerrero et al., 2000; Chen et al., 2005) but not in some others (Mitchell et al., 1996; Mahachai et al., 1999; Zheng et al., 2000; Buletn et al., 2003). In this research, the VacA positivity in the patients with NUD was significantly lower than that in the ulcer patients (p<0.05). Although CagA positivity was higher in the patients with ulcer than that in the patients with NUD, this was not statistically significant and did not seem to be an important risk factor for the development of ulcer in our patients. However, determination the VacA genotypes and the presence of CagA gene together may contribute to potential clinic determination of patients who have different levels of risk. It has been shown that VacA type s1/m1 strains produce more cytotoxins than type s1/m2 and that type s2/m2 strains do not produce active cytotoxins (Yamacka et al., 1997). Many studies have confirmed these findings (Evans et al., 1998; Stephens et al., 1998; Rudni et al., 1999) but some other reports have found no association between the presence of CagA or s1 allelic variant of VacA and the clinical outcome of an H. pylori infection (Faurdez et al., 2000). In the literature, it has been controversial that CagA and VacA positive isolates cause more serious gastroduodenal lesions (Mitchell et al., 1996; Martin Guerrero et al., 2000). The results for UreAB gene showed that UreAB gene doesn’t have any role in ulceration process without any combination with VacA genes. Positivity of UreAB in NUD patients was significantly higher than that in UD patients, when there was no other virulence gene. On the other hand, in our study significant difference between distribution of CagA-VacA-UreAB phenotype in UD patients and NUD patients (40% vs 14.28%) showed that the most virulent strains of H. pylori harbor these three virulence genes together. Consequently, it was seen that duodenal ulcer incidence increased in the patients with CagA, VacA and UreAB positive.

In conclusion, these results suggest that in the population under our study, being infected by a H. pylori strain with the genotype CagA- VacA- UreAB may be associated to an increased risk of acquiring an ulcer disease. The genetic predisposition of the population and local environmental factors, may also be important factors in the development of diseases caused by H. pylori, which may explain the differences observed with respect to other countries.

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