THE PREVALENCE OF HELICOBACTER PYLORI CAGA AND ICEA GENOTYPES AND POSSIBLE CLINICAL OUTCOMES

KIANOOSH DADASHZADEH1*, MORTEZA MILANI2,3, MOHAMMAD H. SOMI1
1Department of Laboratory Sciences, Marand Branch, Islamic Azad University, Marand, Iran - 2Liver and Gastrointestinal Diseases Research Center, Tabriz, Iran - 3School of Advanced Medical, Tabriz University of Medical Sciences, Tabriz, Iran

ABSTRACT

Objective: There is continuing interest in identifying Helicobacter pylori virulence factors that might predict the risk for symptomatic clinical outcomes. It has been proposed that iceA and cagA genes are such markers and can identify patients with peptic ulcers and gastric cancer.

Methods: To determine the prevalence of specific genotypes of H. pylori, clinical isolate of H. pylori obtained from 102 patients through endoscopic biopsies was cultured. The cagA alleles, iceA genotypes were determined by PCR.

Results: Distribution of cagA and clinical outcome was shown that the frequency of cagA-positive isolates in PUD, NUD and GC patients was 81.25% and 65.5% and 100%, respectively. Also the iceA1 allele was identified in 2 (100%) GC patients but iceA2 allele was not detected in these patients. Overall cagA and iceA1 alleles were detected in GC patients.

Conclusions: The cagA gene and iceA1 genotype was found to predominate in gastric adenocarcinoma patients, and also iceA2 genotype was also associated with PUD.

Key words: H. pylori - cagA - iceA.

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Introduction

The bacterium Helicobacter pylori colonize the gastric mucosa of approximately half the world’s population. This gastric colonization induces chronic gastric inflammation in all infected individuals, but only 15-20% of infected patients develop gastric or duodenal ulcer (DU) and <1% develop gastric adenocarcinoma39). The prevalence of H. pylori infection in a population generally does not predict the incidence of serious clinical sequel, suggesting that host and pathogen genetic variation, as well as dietary and other environmental factors, play an important role. These factors analyzed in isolation have failed to provide adequate explanations for the variability in infection outcomes. However, Experience with other bacterial pathogens suggests that H. pylori strain-specific factors may influence the pathogenicity of different H. pylori isolates. This study have primarily focused on two groups of putative bacterial virulence factors, the cag pathogenicity island (for which cagA is a marker) and the iceA2.

Several studies have suggested that cagA is a useful marker for the most virulent strains that are associated with peptic ulcer, atrophic gastritis and adenocarcinoma2. The cag pathogenicity island (PaI) encodes a type IV secretory system and delivers cagA into the host cytosol where becomes phosphorylated on tyrosine residue. Phosphorylated cagA interacts with the phosphatase SHP-2 causing dephosphorylation of cortactin and cytoskeletal rearrangements forming the “hummingbird” phenotype40). 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 outcome41-43).
Recently, a novel putative virulence factor has been identified; the iceA (for induced by contact with epithelium) was suggested to have an association with peptic ulcer. The iceA gene has two main allelic variants, iceA1 and iceA2. The expression of iceA1 is up-regulated on contact between H. pylori and human epithelial cells, and may be related with peptic ulcer disease. Van Doorn reported that the iceA allelic type was independent of the cagA and vacA status, and there was a significant association between the presence of the iceA1 allele and peptic ulcer disease. Those researchers proposed that genotyping of iceA and cagA might offer an effective combination for identification of patients with peptic ulcers. Their results were obtained from patients in Tabriz, northwest of Iran, and the search for virulence factors related to outcome of infection has been hampered by the fact that there appear to be differences in the predominant strain in circulation in different geographic regions. Thus, conclusions derived from data from a single geographic region may not be true for other geographic regions.

In this study, we examined the iceA allele type in stains from our region and its relation with cagA status genotypes and clinical outcome.

Materials and methods

Patients

A total of one hundred two H. pylori isolates were obtained from gastric biopsies of patients with gastritis, peptic ulcer and gastro esophageal reflux diseases undergoing endoscopy. This study was approved by the ethical committee of regional Medical Research of Tabriz University of Medical Sciences and all patients provided written informed consent for this research.

H. pylori Culture and extraction of Genomic DNA

Briefly gastric biopsy samples were homogenized and cultured onto Brucella agar supplanted with 5% sheep blood and antibiotics (Vancomycin, Amphotericin B and Trimethoprim). Culture plates were incubated at microaerophilic condition, 37 °C and high humidity for 5-7 days. Organisms were identified as H. pylori based on colony morphology, gram staining and positive oxidase, catalase and urease tests. Genomic DNA of total H. pylori isolates was extracted by using CTAB method and stored at -20 °C.

Detection of cagA and iceA genes

In this study PCR was used to detect the H. pylori specific ureC gene for confirmation of H. pylori isolates, the virulence-associated cagA structure and the presence of iceA gene. All primer sets were selected from the published literatures (Table 1). PCR reactions were performed in a volume of 50 µL containing 10 mmol/L Tris-HCl, 1.5 mmol/L MgCl2, 0.2 mmol/L of each deoxynucleotide, 25 pmol of each primer and 2.5 units of Taq polymerase (Geneone, Germany). Thermal cycler program consisted the following steps; initial denaturation at 94 ºC for 3 min followed by 35 cycles repetitions of 30 seconds at 94 ºC (denaturation), 30 seconds at 58 ºC for cagA and glmM, 57 ºC for iceA1 and 48 ºC for iceA2 (annealing) and 30 seconds at 72 ºC (extension) and final extension step was 3 min at 72 ºC.

Statistics analysis

Data were analyzed by SPSS version 16. The Pearson X2 test was used to evaluate the relationship between individual genotypes and a variety of diseases.

Results

Of the 102 patients infected with H. pylori, 84 patients with non-ulcer diseases, 16 patients with peptic ulcer disease and 2 patients with gastric cancer. The mean age of the patients was 34±19 years (gender ratio M/F: 1.05). There was no significant difference between the mean age of patients with and without ulcers.

Table 1: Primers used in this study.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Nucleotide sequence</th>
<th>Size (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ureC</td>
<td>Bp-F</td>
<td>GGATAAGGTTTACGCTGTTAGGGG</td>
<td>294</td>
<td>Ko et al., 2008</td>
</tr>
<tr>
<td></td>
<td>BP-R</td>
<td>TTTGCTTTTATCCAGGCTGAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cagA</td>
<td>cagA-F</td>
<td>ACGCTGGCTAAGAAATCTGCTG</td>
<td>352</td>
<td>Van Doorn et al., 1998</td>
</tr>
<tr>
<td></td>
<td>cagA-R</td>
<td>CAGAGAGTGCTGCTGCTGCTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iceA</td>
<td>iceA1-F</td>
<td>TTCTGGTCTCGCTGCTGCTG</td>
<td>247</td>
<td>Ko et al., 2008</td>
</tr>
<tr>
<td></td>
<td>iceA1-R</td>
<td>TTTGCTTTTATCCAGGCTGAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>iceA2-F</td>
<td>GCAAGATGATGATGATGATG</td>
<td>229</td>
<td>Ko et al., 2008</td>
</tr>
<tr>
<td></td>
<td>iceA2-R</td>
<td>TTTGCTTTTATCCAGGCTGAA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Amplified products of iceA1 gene by PCR

Lane 1: 100-3000 bp DNA ladder, Lane 2-7 clinical isolates of iceA1 positive, Lanes 8: negative control.
In this study the distribution of cagA and clinical outcome was analyzed statistically and it was observed that the frequency of cagA-positive isolates in PUD, NUD and GC patients was 81.25% and 65.5% and 100%, respectively (Table 2).

Overall, iceA1 was detected in 41 isolates and iceA2 in 13 isolates. Seven isolates (6/9%) were positive for both iceA1 and iceA2, while 56 isolates (54/9%) did not yield any PCR product for iceA.

The iceA1 allele was identified in 2 (100%) GC patients but iceA2 allele was not detected in these patients. As shown in Table 2 the iceA1 allele was observed in PUD patients (43.75%) and in NUD patients (38.1%) while the prevalence of the iceA2 allele was observed in PUD (12.5%) cases and in NUD patients (13.1%); however, these differences were not statistically significant.

### Table 2: Relationship between clinical outcome and status of cagA and iceA

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Number (%) of isolates</th>
<th>Total (n=102)</th>
<th>P&lt;sub&gt;v&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>iceA1</td>
<td>22 (68.1%)</td>
<td>7 (23.3%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>iceA2</td>
<td>31 (31%)</td>
<td>2 (12.5%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>cagA</td>
<td>31 (31%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
</tbody>
</table>

**Discussion**

Gastric mucosa colonization by *H. pylori* leads to chronic gastritis, atrophic gastritis and is associated with several diseases such as peptic ulcers, gastric carcinoma, and MALT lymphoma<sup>(12)</sup>. However, there is an obvious difference between the number of infected and those who patients with clinical outcome. Although environmental and host factors are important, also previous studies show that the specific genotype of bacteria play an important role in the development of clinical symptoms<sup>(13)</sup>. Thus infection with *H. pylori* specific genotypes such as cagA and iceA is related to more severe conditions, while other strains occur less pathogenic<sup>(14)</sup>. This study was designed to characterize the genotype of *H. pylori* from gastric biopsy specimens from patients with upper gastrointestinal diseases and the relationship with clinical outcome in northwest of Iran. The presence of the cagA, iceA1 and iceA2 genes were detected in *H. pylori* isolates.

Survey of previous studies showed that the cagA prevalence is different around the world<sup>(15)</sup>. As the prevalence of cagA gene in this study was 68%. Our result is in agreement with reports from Western countries<sup>(16)</sup>, but lower than the East Asian countries where the cagA are present in more than 90% of cases<sup>(17)</sup>. The results of our study showed that cagA-positive isolates compared to cagA-negative isolates were more frequently isolated from PUD patients. While in NUD patients was the opposite, while this finding was not statistically significant (P<sub>v</sub> > 0.05). These findings are supported by previous studies<sup>(17, 18)</sup> and suggest that colonization with cagA-positive *H. pylori* strains associated with developing peptic ulcer disease.

Our results show that the prevalence of iceA1 and iceA2 genes in isolates was 39% and 13%, respectively. These results are in agreement with previous studies that the iceA1 gene was found to be prevalent in Japan, Korea and Netherlands patients<sup>(19-21)</sup>. However, several studies have reported different results, as the iceA2 gene was detected to be predominant genotype in these studies<sup>(22, 23)</sup>. It was found that iceA1 was significantly associated with peptic ulcer disease in polish and the USA<sup>(23, 24)</sup>. However, these reports have not been confirmed in other countries such as Korea and India<sup>(25, 26)</sup>. In our study of total patients infected with *H. pylori*, two patients had gastric adenocarcinoma. The genotypes of strains isolated from these patients were cagA and iceA1 positive while these strains were negative for the iceA2 gene.

In conclusion, this study was show the prevalence of virulence genes cagA iceA1 and iceA2 in Northwest Iran. The cagA gene and iceA1 genotype was found to predominate in gastric adenocarcino-
ma patients, and also iceA2 genotype was also associated with PUD. It may be the size of sample in our study insufficient to predict of clinical outcome relationship with virulence genes in H. pylori infection. Despite of the results of some studies have shown that the iceA2 genotype was frequently found in patients with gastric carcinoma or duodenal ulcer. However, it is not easy to declare that iceA2 gene is considered as a protective factor in some area and that is associated with more severe diseases in other countries. This virulence gene could be used a molecular marker for bacterial pathogenesis.

Conclusions

The cagA gene and iceA1 genotype was found to predominate in gastric adenocarcinoma patients, and also iceA2 genotype was also associated with PUD.

References


Acknowledgements

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Corresponding author

KIANOOSH DADASHZASDEH
Department of Laboratory Sciences, Marand Branch, Islamic Azad University
Marand (Iran)