Contents

Editorials

Measuring hepatic functional reserve using MEGX still a mirage! S K Sarin, Manoj Kumar 203

Gastrointestinal stromal tumor - paradigm for successful targeted therapy Susy Kurian 207

Original Articles

Impact of shorter duration of treatment on virological response rate in genotype 2 or 3 chronic hepatitis C virus infection Ioannis S Elefshiniotis, Konstantinos D Pantazis, Dimitrios Dimitroutopoulos, Sotirios Koutsounas, Antonios Moulaakakis, Emmanouel Paraskevas 209

Gastrointestinal stromal tumors: a demographic, morphologic and immunohistochemical study F Rauf, Y Bhurgri, S Pervez 214


Analysis of Helicobacter pylori antimicrobial susceptibility and virulence genes in gastric mucosal biopsies in the United Arab Emirates Mubarak S Alfaresi, Adeel Islam Abdulsalam, Abida A Elkoush 221

Gastrointestinal stromal tumors: a single institution experience of 50 cases Senthil Rajappa, Krishna Mohan Muppavarapu, Shantveer Uppin, Ragunadharao Digumarti 225

Review

Celiac disease in India Surender Kumar Yachha, Ujjal Poddar 230

Case Series

Hydatidiarrhea Suyash Mohan, Ashish Verma, Sanjaya Saran Baijal 238

Clinico-pathology conference

A treated case of follicular lymphoma presenting with fever and diarrhea Kim Vaiphei, Pankaj Malhotra, Nidhi Sharma, Anil Kumar Narasiyappah, Subhash Chander Varma 240

Case Snippets

Endovascular management of hepatic hemorrhage and subcapsular hematoma in HELLP syndrome Chandan Jyoti Das, Deep Narayan Srivastava, Jyotindu Debnath, Vijay Ramchandran, Sujoy Pal, Peush Sahni 244

Visceral leishmaniasis: acute liver failure in an immunocompetent Asian-Indian adult G Malatesha, Nishith K Singh, Vinay Gulati 245

Endoscopic removal of chicken bone that caused gastric perforation and liver abscess R J Mukkada, A P Chettupuzha, V J Francis, P G Mathew, S P Chirayath, Abraham Koshy, Philip Augustine 246

Letters


Colonoscopic and ileoscopic biopsies increase yield of diagnosis in chronic large bowel diarrhea with normal colonoscopy S Khanna, R Talukdar, N Saikia, S Mazumdar, S Kulkarni, J C Vij, A Kumar 250

Delta hepatitis infection in northeast India Biswa Jyoti Borkakoty, Dipankar Biswas, J agadish Mahanta 251


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Indian Journal of Gastroenterology 2007 Vol 26 September - October i
Contents (contd.)

Hepatitis C virus infection and risk behaviors among injection drug users of Nagaland
Hiranya Kumar Das, Biswa Jyoti Borkakoty, Jagadish Mahanta,
Gojendra Kumar Medhi, Pradeep Kumar Chelleng 253

Endoscopic removal of giant colonic lipomas Georgia Lazaraki, Dimitrios Tragiannidis,
Anestis Tarpagos, Dimitrios Tzilves, Ioannis Pilpilidis, Ioannis Katsos 255

Rectal bleeding due to leech bite in a young child Vincent Ho, Peter Boyd 256

Cecal web causing neonatal intestinal obstruction Sushil Budhiraja 256

Images

Colonic leiomyoma with huge ulceration Akihiko Takeda, Shinichi Ban, Akihiro Yasumoto,
Keiko Ishikawa, Hiroyoshi Iseki, Hideki Takeuchi, Norio Takahashi, Isamu Koyama 213

Gastric cancer presenting with cutaneous metastasis
George Barreto, Shailesh Shrikhande, Parul Shukla 237

Gastroenterology Elsewhere 257
India Elsewhere 258
Announcements
Indian Journal of Gastroenterology Mitra Memorial Award 206
New and Notices 216
Index to Advertisers 220
Instructions to Contributors 259
Aim: To determine the virulence attributes (presence of cagA and vacA genes) of Helicobacter pylori, and presence of clarithromycin resistance genes in gastric mucosal biopsy samples obtained in the United Arab Emirates. Methods: DNA was extracted from antral gastric biopsy samples from 91 dyspeptic patients. Real-time PCR and melting curve analysis were used to identify patients infected with H. pylori and to further identify strains containing the A(2142/43)G or the A(2142)C mutations that are associated with clarithromycin resistance. PCR was also used to identify cagA- and vacA-positive strains. Results: Real-time PCR analysis detected the presence of H. pylori in 55 (60%) samples. Thirty-six pathogen-positive samples contained at least one of three point mutations associated with clarithromycin resistance. The vacA gene was present in 40 (72.7%) and cagA was present in 41 (74.5%) of the positive samples. Both genes were present in 36 (65%) of the positive samples. The presence of each clarithromycin-inducing mutation was largely independent of the others. Mutation at one position, A(2142/43)G, was strongly associated with the presence of both the vacA gene and the cagA gene. Conclusions: A high proportion of gastric mucosal biopsies obtained in the UAE is positive for genes associated with clarithromycin resistance. This may have implications for treatment of the infection. [Indian J Gastroenterol 2007;26:221-224]

Helicobacter pylori chronically infects more than half of the world’s population, with a prevalence ranging from 25% in developed countries to more than 90% in developing areas. It is associated with chronic superficial gastritis, peptic ulceration and gastric cancer. The reasons for such clinically diverse outcomes as a result of infection remain unclear, but may include host and environmental factors as well as differences in the prevalence or expression of bacterial virulence factors such as cytotoxin-associated (cagA) gene and vacuolating cytotoxin (vacA). The cagA gene is considered to be a marker for the presence of a pathogenicity island (cag PAI), and individuals infected with cagA-positive H. pylori strains have a higher risk of developing peptic ulcers and gastric cancer. The vacA gene encodes a vacuolating toxin excreted by H. pylori that has been associated with epithelial cell damage. This gene is present in all strains and comprises two variable parts, the s-region (signal) and the m-region (middle). The production of vacuolating cytotoxin is related to the mosaic combination of s- and m-region allelic types, which in turn has been associated with specific genotypes and clinical outcomes.

Treatment failure rates for most H. pylori eradication regimens remain high in some parts of the world. Many factors have been implicated in treatment failure, including ineffective penetration of antibiotics into the gastric mucosa, antibiotic inactivation by low gastric pH, lack of compliance and emergence of acquired antibiotic resistance by H. pylori. Three point mutations in the 23S rRNA gene have been associated with clarithromycin resistance, leading to treatment failure. It has been suggested that eradication rates among patients without peptic ulcer are lower than among those with peptic ulcer, with the causes of this phenomenon still a subject of speculation.

This study examined biopsies from patients in the United Arab Emirates (UAE) for H. pylori presence, vacA and cagA genes and clarithromycin resistance genes.

Methods

Samples

Gastric biopsy samples from 91 dyspeptic patients (mean age 36 years [range 16-65]; 64 [70%] men) undergoing endoscopy in the Endoscopy Department of our institute were received in our department. For each patient, one endoscopic biopsy specimen was taken from the antrum of the stomach. The biopsy was immediately placed in normal
saline, transported to our laboratory and frozen at -80°C. The research was conducted according to the appropriate ethical guidelines.

**DNA isolation**

DNA was isolated from 25-50 mg thawed biopsy tissue using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Germany, Cat. No. 1796828) according to the manufacturer’s directions. The isolated DNA was eluted in 200 µL elution buffer and stored at -80°C until further analysis.

**Detection of point mutations in the 23S rRNA gene of *H. pylori***

Real-time PCR-based hybridization assay was performed directly on DNA obtained from the gastric biopsy samples to detect point mutations that confer resistance to clarithromycin. The method includes amplification of a fragment of the 23S rRNA gene of *H. pylori* coupled with simultaneous detection of the product by probe hybridization and analysis of the melting curve using real-time PCR.11,12 Using the Light Cycler thermocycler (Roche Diagnostics), the PCR and hybridization reactions were carried out in glass capillaries in a volume of 20 µL comprising 3 µL of template DNA, 1.6 µL of MgCl₂ (25 mM), 0.4 µL of forward and reverse primers (20 µM each), 0.2 µL of sensor and anchor probes (20 µM each) and 2 µL of FastStart DNA master hybridization probes (Roche Diagnostics). PCR amplification comprised an initial denaturation step at 95°C for 10 minutes, followed by 50 amplification cycles (with a temperature transition rate of 20°C/s) consisting of 95°C for 0 s, annealing at 60°C for 10 s, and extension at 72°C for 17 s. After amplification, a melting step was performed, consisting of 95°C for 0 s, cooling to 45°C for 30 s (with a temperature transition rate of 20°C/s), and finally a slow rise in the temperature to 85°C at a rate of 0.1°C/s with continuous acquisition of fluorescence decline. Any presence of any point mutation was considered as resistant for clarithromycin. Bacterial culture and antibiotic sensitivity were not done.

**Determination of *H. pylori* cagA and vacA genotypes**

Primers (previously published5,13) were obtained from TIB MOLBIOL (Germany). The 20 mL PCR reaction mixture contained 2 µL of each primer (final concentration 0.5 mM), 2 µL of genomic DNA, 2.4 µL MgCl₂ (4 mM) and 2 µL of FastStart SYBR Green dye solution, according to the recommendations of the LightCycler-DNA Hot start SYBR Green I Kit (Roche Molecular Biochemicals, Germany). The dye solution included nucleotides, Taq DNA polymerase and the reaction buffer in a reaction mixture of 5 µL volume in the LightCycler 1.5 Instrument. After an initial denaturation step at 94°C for 30 s, amplification of cagA gene was performed with 40 cycles of denaturation (94°C, 2 s, with slopes of 20°C/s), annealing (46°C, 4 s with slopes of 20°C/s) and extension (72°C, 12 s with slopes of 5°C/s). For amplification of the vacA gene, we used 40 cycles with 94°C denaturation for 5 s, a 45.5°C annealing for 3 s and 72°C extension for 10 s with slopes of 20°C/s, 20°C/s and 5°C/s, respectively. Fluorescence was detected at the end of the extension phase by F1/L filter. After the amplification, melting point analysis was performed by raising the temperature slowly (0.1°C/s) from 56 to 94°C for cagA and from 55 to 94°C for vacA. Fluorescence signals were detected continuously.8,9

The presence of *H. pylori* was confirmed by PCR of the 16S rRNA and glmM genes.14,15

**Statistical analyses**

Chi-square was used to test for differences in proportions between different groups; p value less than 0.05 was reported as statistically significant. Logistic regression was used to assess the roles of the vacA and cagA genes and the A(2142/43)G and A(2142)C mutations in explaining the dichotomous dependent variable of presence of resistance genes versus absence of resistance genes to clarithromycin. A logistic regression using a forward stepwise variable selection procedure using vacA, cagA, presence of the A(2142/43)G mutation and presence of the A(2142)C mutation to explain clarithromycin sensitivity only selected the A(2142/43)G mutation as an explanatory factor.

**Results**

Seventy-six (74%) patients were UAE nationals. The urease test was positive in 40 samples (44%). The presence of *H. pylori* was confirmed by PCR of the 16S rRNA and glmM genes in 55 samples (60%).

One or more of three mutations that are associated with *H. pylori* resistance to clarithromycin were detected by real time PCR in 36 of 55 samples positive for *H. pylori* by PCR. From those samples which were real-time PCR positive, 26 were found to have the A(2142/43)G mutation alone, one had the A(2142)C mutation alone and 9 had both.

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*H. pylori antimicrobial susceptibility and virulence genes*
Clarithromycin resistance mutations were identified in equal proportions of UAE nationals (26 of 39, 66.7%) and non-UAE nationals (10 of 16, 62.5%). Clarithromycin resistance genes were identified in 13 of 29 biopsies from female patients compared to 23 of 69 biopsies from male patients (p=ns). The vacA gene was present in 40 (72.7%) and cagA in 41 (75.5%) of the 55 samples that were positive for H. pylori by PCR. Both genes were present in 36 (65%) of the positive samples. There was a strong association between the presence of the vacA and cagA genes (r = 0.653, p < 0.001) in the same biopsy sample. In contrast, the presence of each clarithromycin-inducing mutation was largely independent of the other, as evidenced by their low correlation of 0.257 (p = 0.057). The incidence of the A(2142/43)G mutations were strongly associated with the presence of both the vacA gene (chi-square = 22.553, p < 0.001) and the cagA gene (chi-square = 9.553, p = 0.002). In contrast, the incidence of the A(2142)C mutation exhibited no association with the presence of either the vacA gene (p = 0.175) or the cagA gene (p = 0.772).

Resistance or sensitivity to clarithromycin was almost completely accounted for by the presence of the A(2142/43)G mutation.

There was no difference in the presence of vacA or cagA gene in the biopsy samples in patients with peptic ulcer, non-ulcer dyspepsia or gastroesophageal reflux disease (Table).

Discussion

Real-time PCR analysis detected the presence of H. pylori in 55 samples (60%); further PCR analysis found that 36 of the pathogen-positive samples (65.5%) contained at least one of three point mutations associated with clarithromycin resistance. Patients from the UAE had the same mutation incidence as non-UAE patients. The vacA gene was present in 72.7% and cagA was present in 75.5% of the positive samples. The 55 H. pylori-positive patients were clinically categorized as having non-ulcer dyspepsia (19 patients), peptic ulcers (20 patients) or gastroesophageal reflux disease (16 patients).

H. pylori infection is the major causative agent of chronic active gastritis, which plays a central role in the etiology of peptic ulcer disease and is a risk factor for development of gastric carcinoma. H. pylori eradication therapy consists of combination of antibiotics, imidazole derivative, proton pump inhibitor and bismuth; the first line regimen consists clarithromycin, amoxicillin and a proton pump inhibitor for one week. Treatment failure is increasing worldwide.1-5

The wide use of clarithromycin worldwide is most likely responsible for the development of H. pylori strains that are resistant to clarithromycin: the prevalence of clarithromycin resistance varies from 1% in Norway, to 18% to 29% in Japan.16 Recent data from Western Europe indicate that greater than 60% of failed clarithromycin-based treatments are associated with clarithromycin-resistant H. pylori isolates.1-5 Resistance to clarithromycin is associated with point mutations within the peptidyltransferase-encoding region of the 23S rRNA gene [20]. Three point mutations in 2 positions have been described in which an adenine residue is replaced by guanine or a cytosine residue at adjacent positions: A2142C, A2142G and A2143G.17

Thirty-six of the 55 samples in this study that were PCR-positive for H. pylori also contained the A2142G, A2143G or A2142C point mutations (65.5%). The A2142/43G mutations were more common than the A2142C mutation, while the combination of both mutations was present in 16.3% of the positive samples. The prevalence of the point mutation A2142/43G and A2142C among our patients is higher than figures reported from various other countries such as Japan (29%).17 The present study did not examine cultured isolates of H. pylori from biopsies. Rather it examined the biopsies directly using molecular techniques and therefore a correlation with the antibiotic resistance of the organism could not be examined.

In the present study, the cagA gene and the vacA gene could only be detected each in approximately three fourths of biopsy samples. Several studies have shown that the prevalence of the cagA gene in H. pylori isolates ranges from 80% to 100%.18,19,20 However, geographical differences in

### Table: Presence of the vacA and cag A genes in biopsy samples from patients with specific clinical diagnoses

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>PU</th>
<th>NUD</th>
<th>GERD</th>
</tr>
</thead>
<tbody>
<tr>
<td>vacA Negative</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Positive</td>
<td>14</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>cagA Negative</td>
<td>6</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Positive</td>
<td>13</td>
<td>17</td>
<td>10</td>
</tr>
</tbody>
</table>

Values are as number of patients. Chi-square for vacA= 0.188, p = 0.910, for cag A 1.615, p = 0.446

PU: Peptic ulcer; NUD: Non ulcer dyspepsia; GERD: Gastroesophageal reflux disease
prevalence have been found. It has been reported in Western countries that the cagA gene is present in 95% of *H. pylori*-infected patients. The same was published for Eastern countries, where the cagA gene-expressing *H. pylori* was found in 89.3% of the 28 and in 92.3% of the 24 isolates.\(^{18,19,20}\)

In conclusion, this study showed that clarithromycin resistance genes are present in almost two-thirds of gastric biopsies positive for *H. pylori*. Further study is required to correlate these findings with clinical response to antimicrobial therapy in patients with *H. pylori* infection. These data may provide the basis for treatment recommendations for *H. pylori* eradication in the UAE.

References


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