SHORT COMMUNICATION

High frequency of cagA and vacA s1a/m2 Genotype among Helicobacter pylori Infected Gastric Biopsies of Pakistani Children

Faisal Rasheed1*, Tanvir Ahmad1, Muhammad Ali2, Salman Ali3, Sahifa Ahmed4 and Rakshanda Bilal1

1Life Science Group, Isotope Application Division (IAD), Pakistan Institute of Nuclear Science and Technology (PINSTECH), Nilore, Islamabad, Pakistan.
2Department of Biotechnology, Quaid-I-Azam University, Islamabad, Pakistan.
3Department of Pediatrics, Army Medical College, Rawalpindi, Pakistan.
4Department of Microbiology, Quaid-I-Azam University, Islamabad, Pakistan.
E-mail: frohpl@gmail.com

Received 30 November 2010; received in revised form 2 February 2011; accepted 20 February 2011

ABSTRACT

The vacuolating cytotoxin VacA and cytotoxin associated gene product CagA, encoded by vacA and cagA are major virulence determinants associated with pathogenesis of Helicobacter pylori. The presence and prevalence of two major H. pylori virulence associated genes among gastric biopsies of Pakistani children were investigated in the current study. Fifty one gastric biopsy specimens of children were analysed for 16S rRNA, vacA and cagA genes using PCR. The results showed that 21 (41.2%) biopsies were positive for H. pylori as determined by 16S rRNA PCR. In the 21 H. pylori positive gastric biopsies, 19 (90.5%) showed vacA s1a, 1 (4.75%) was vacA s1b and 1 (4.75%) was vacA s2 whereas, 5 (23.8%) were vacA m1 and 16 (76.2%) were vacA m2. None of the H. pylori positive biopsies carried vacA s1c subtype. The cagA gene was found in 13 (61.9%) of H. pylori infected biopsies and different vacA combinations were found with or without cagA gene. H. pylori was detected with high frequency of cagA while vacA s1a and vacA m2 regions with vacA s1a/m2 genotype were predominant in H. pylori infected gastric biopsies of children.

Keywords: H. pylori, Frequency, Children, vacA, cagA, PCR

INTRODUCTION

Helicobacter pylori is the major infectious agent of chronic superficial gastritis and plays a central role in the development of peptic ulcer disease and adenocarcinoma (Al-Shamahy, 2005), hence classified as a class I carcinogen by the World Health Organization (International Agency for Research on Cancer, 1994). The outcome of infection depends on the genetic makeup of the host, the pathogen and the environment. Many virulent factors have been identified which enable H. pylori in invading, surviving and colonizing the hostile acidic environment of the human stomach. The two most important factors that determine the outcome of infection include vacA and cagA genes. Evidence suggests that H. pylori infection is a risk for development of gastric carcinoma whereas, vacA and cagA genes can be used as indicators of its virulence level (Cittelly et al., 2002).

DNA analysis has revealed that vacA has a mosaic structure comprising allelic variations in the signal and mid region, each having two different alleles (s1a, s1b, s1c/s2 and m1/m2) with different biological activities (Atherton et al., 1995; Ahmad et al., 2009). In general, vacA type s1/m1 and s1/m2 strains produce high and moderate levels of toxin, whereas s2/m2 strains produce little or no toxin (van Doorn et al., 1998).

There are reports that cagA is present in more than 50% of the H. pylori strains and it is a marker for the presence of cag-pathogenicity island and is associated with peptic ulcer and gastric malignancy (Kidd et al., 2001). The basic purpose of the current study was to determine the status of vacA and cagA genes in H. pylori infected gastric biopsies of Pakistani children.

MATERIALS AND METHODS

Subjects

This was a prospective study and all children appearing at KRL General Hospital, Islamabad and Military Hospital, Rawalpindi, Pakistan with symptoms suggestive of upper gastrointestinal endoscopy were enrolled. The protocols were approved by The Research Ethics Committee of Pakistan Institute of Nuclear Science and Technology (PINSTECH) and written informed consent was obtained from parents/guardians of the subjects. Gastric biopsies collected from 51 children (age range: 3 to 18 years) were studied to determine the presence of H. pylori and to test the distribution of its two major virulence determinants, vacA and cagA.

*Corresponding author
DNA Extraction

DNA was extracted by using ethanol precipitation method from gastric biopsies of patients. Gastric biopsies were crushed manually in an eppendorf tube. The crushed material was suspended in a medium consisting of 20 µL of 10% SDS, 80 µL of proteinase K buffer (0.5 M EDTA and 4 M NaCl, pH 7.5) and 40 µL of proteinase K (10 mg/mL). The mixture was incubated at 55 °C for 5 h, then 100 µL of 6 M NaCl was added and vortexed for 1 min followed by centrifugation at 14,000 rpm for 1 min. The nucleic acids were precipitated by adding chilled absolute ethanol and harvested by centrifugation. After centrifugation DNA pellet was washed with 70% ethanol and resuspended in 100 µL of TE buffer (10 mM Tris and 1 mM EDTA, pH 7.5). Samples were stored at −4 °C till further use.

PCR

All PCR reagents were supplied by Promega Corporation (Madison, USA) and primers by Alpha DNA (Montreal, Canada) and Gene Link (Hawthorne, NY, USA). Each PCR reaction mixture consisted of 1× green reaction buffer, 1.5 mM MgCl₂, 200 µM each dNTP, 10 pmol of each primer, 1.5 U Taq DNA polymerase and 5 µL DNA templates of H. pylori strains G27 and SS1 were used as positive controls. PCR Basic Thermocycler and 72 °C for 30 sec, annealing (see Table 1 for annealing temperatures and conditions) and final polymerization at 95 °C for 3 min followed by 30 cycles of denaturation at 95 °C for 30 s, annealing (see Table 1 for annealing temperatures specific to each primer pair used) for 30 sec, polymerization at 72 °C for 30 s and final polymerization at 72 °C for 5 min. PCR was performed on T Professional Basic Thermocycler and DNA templates of H. pylori strains G27 and SS1 were used as positive controls. PCR mixture was subjected to gel electrophoresis (1.5% agarose) with 100 bp DNA ladder and ethidium bromide staining for detection of amplified products.

Statistical Analysis

The chi-square test was used for analysis of categorical data. Significance was defined as p value of less than 0.05.

RESULTS

The study subjects consisted of 51 children [29 (56.9%) males and 22 (43.1%) females] with age range of 3 to 18 years. Based on 16S rRNA PCR, 21 (41.2%) gastric biopsies were positive for H. pylori DNA. Out of the 21 subjects positive for H. pylori, 14 were male (48.3%) and 7 were female (31.8%).

The polymorphism in signal sequence and middle region of vacA was typed by PCR in all H. pylori positive gastric biopsies. Among H. pylori positive gastric biopsies, 19 (90.5%) were positive for vacA s1a, 1 (4.75%) for vacA s1b and 1 (4.75%) for vacA s2 whereas, 5 (23.8%) were vacA m1 and 16 (76.2%) were vacA m2 positive. All of these positive H. pylori biopsies were negative for vacA s1c subtype.

Of the 21 gastric biopsy specimens that showed positive amplification in 16S rRNA gene, 13 (61.9%) were positive for cagA gene. All negative biopsies for 16S rRNA gene were also negative for cagA and vacA genes. vacA s1a/m1 and s1a/m2 genotypes with cagA+ were found in 4 (30.8%) and 8 (69.2%) respectively but the correlation was not significant (p = 0.516), while the vacA s1a/m1, s1a/m2 genotypes with cagA+ were detected in 1 (12.5%) and 6 (75.0%) of the 21 cases, respectively. vacA genotypes s1b/m2 and s2/m2 were found in two cagA+ strains whereas these could not be seen in cagA− strains.

Table 1: Primer details of 16S rRNA, vacA and cagA genes

<table>
<thead>
<tr>
<th>Target site</th>
<th>Amplicon size (bp)</th>
<th>Primer names and sequences</th>
<th>Annealing temperature</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rRNA</td>
<td>138</td>
<td>HPF (5’ GCGACCTCGCTGGAACATTAC 3’)</td>
<td>60 °C</td>
<td>GramLey, et al., 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HPR (5’ CTGTTAGCTCATTACTGGAGA 3’)</td>
<td>56 °C</td>
<td>Atherton et al., 1995</td>
</tr>
<tr>
<td>vacA s1a</td>
<td>190</td>
<td>AA1F (5’ CTGCTTTGAATCAGCACAAC 3’)</td>
<td>56 °C</td>
<td>Yamaoka et al., 1995</td>
</tr>
<tr>
<td>vacA s1b</td>
<td>187</td>
<td>SS3F (5’ AGCGCCATACCGCAAG 3’)</td>
<td>56 °C</td>
<td>Yamaoka et al., 1995</td>
</tr>
<tr>
<td>vacA s1c</td>
<td>213</td>
<td>S1CF (5’ TCTGGTTTAACTGGGGCTA 3’)</td>
<td>56 °C</td>
<td>Atherton et al., 1995</td>
</tr>
<tr>
<td>vacA s2</td>
<td>199</td>
<td>S1CR (5’ TCTGGTTAACTGGGGCAAC 3’)</td>
<td>56 °C</td>
<td>Yamaoka et al., 1995</td>
</tr>
<tr>
<td>vacA m1/m2</td>
<td>570/645</td>
<td>SS2F (5’ GCTAACGCGCATAATGCTCC 3’)</td>
<td>56 °C</td>
<td>Atherton et al., 1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SS2R (5’ CTGCTTTGAATCAGCACAAC 3’)</td>
<td>56 °C</td>
<td>Yamaoka et al., 1995</td>
</tr>
<tr>
<td>cagA</td>
<td>189</td>
<td>CAGAF (5’ TTGACACACACAACACACACAG 3’)</td>
<td>57 °C</td>
<td>van Doorn et al., 1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CAGAR (5’ CTGCCCTTAGTCCGAGATTCC 3’)</td>
<td>62 °C</td>
<td>van Doorn et al., 1998</td>
</tr>
</tbody>
</table>
DISCUSSION

There is geographical variation in distribution of *H. pylori* genotypes and very little information about frequency of *H. pylori* vacA genotypes and cagA in Pakistan is available. In the present study, we determined the vacA and cagA status among *H. pylori* infected gastric biopsies of children.

All the strains of *H. pylori* have vacA gene but its structure varies, mainly in the region encoding the signal sequence (type s1a, s1b, s1c or s2) and the mid-region (type m1 or m2), (Atherton et al., 1995). A strain’s vacA structure determines its in-vitro cytotoxic activity, with vacA type m1 being more active than type m2, type s1a being more active than type s1b and type s2 vacA not producing detectable activity. Recently, Ahmad et al., (2009) found vacA s1b and vacA m2 as main subtypes in gastric biopsies of adult patients from Rawalpindi while vacA s1a and vacA m1 as predominant subtypes were reported mainly in adult patients of Karachi, Pakistan (Yakoob et al., 2009). Our results showed that the predominant vacA genes were s1a and m2 in *H. pylori* infected gastric biopsies of children which were similar to the report of Hou et al., (2000) who observed that s1a and m2 were more prevalent vacA genes in China while Mishra et al., (2002) in India found vacA s1a and vacA m1 were major subtypes in gastric biopsies of patients referred for endoscopy.

More prevalent vacA genotype in a recent report from Pakistan was s1b/m2 (54.5%) in adult dyspeptic patients (Ahmad et al., 2009), while in India predominant vacA genotype was s1a/m1 (53.2%), (Mishra et al., 2002). This is in contrast to current study where vacA genotype s1a/m2 was detected in 66.7% of *H. pylori* infected gastric biopsies of children, which was similar to the same vacA genotype reported in China with the frequency of 90.5% (Hou et al., 2000). The vacA genotype s1b/m1, s1c/m1, s1c/m2 or s2/m1 was not detected in this study.

Ahmad, et al., (2009) described a 24.2% prevalence of cagA gene in gastric biopsies obtained from adult dyspeptic patients from Rawalpindi area and Yakoob et al., (2009) found 56% frequency of cagA in adult patients from Karachi, while our data involved gastric biopsy specimens collected from children and reported a higher prevalence of cagA viz. 61.9%. This frequency of cagA is low as compared to our neighboring countries where the frequency of cagA was 93.2% in China (Hou et al., 2000), 76% in Iran (Jafari et al., 2008) and 96.2% in India (Mishra et al., 2002).

In the study presented here, we have reported high frequency of cagA (61.9%) with predominant vacA s1a/m2 genotype in *H. pylori* infected gastric biopsies of Pakistani children, which to our knowledge has not been previously published. We investigated frequency of cagA and vacA genotype among Pakistani children which was different from the information provided by authors of same region who reported the frequencies in adults. Our investigation set here a question: Is there a different genetic makeup of *H. pylori* strains infecting children and adults of this region? Nevertheless, this study provides a basis for future detailed investigations to understand genetic diversity of *H. pylori* among Pakistani population.

ACKNOWLEDGMENT

This study was financially supported by Pakistan Science Foundation through project no. PSF/res/C-PINSTECH/Med(207). Thanks to Musharraf Jellani for his technical assistance during this study and we are also thankful to Muhammad Azfal Javed for his comments and suggestions in writing of this paper.

REFERENCES


