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BRIEF ARTICLE

Prevalence of *Helicobacter pylori* virulence genotypes among children in Eastern Turkey

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Abstract

AIM: To identify the virulence genotypes of *Helicobacter pylori* (*H. pylori*) if present in children in Eastern Turkey and if those genotypes are mostly associated with severe clinical presentations.

METHODS: A total of 49 *H. pylori* positive Turkish children (42 with antral nodularity and 7 with peptic ulcer) who underwent upper gastrointestinal endoscopy with abdominal symptoms during the period from March 2011 to September 2012 were enrolled in this study. Antral nodularity was diagnosed endoscopically by two of the authors. We determined for the presence of *cagA*, *vacA*, *cagE*, *iceA* and *babA2* genotypes of *H. pylori* isolates in DNA obtained directly from frozen gastric biopsy samples by polymerase chain reaction test using specific primers.

RESULTS: Of the 49 *H. pylori* isolates studied, 61.2%, 91.8%, 22.4%, 28.6%, 57.1% and 40.8% were positive for the *cagA*, *vacA* s1, *cagE*, *iceA1*, *iceA2* and *babA2* genes, respectively. We showed that the most common *vacA* subtype was s1a (79.6%). However, the s2 gene was found less frequently with an isolation rate of 8.2% of the *H. pylori* isolates. The genotypes *iceA2* and *vacA s1m2* were the most frequently found types in children with antral nodularity. In addition, the genotypes *iceA1*, *babA2* and *vacA s1m1* were found in similar ratios in all the *H. pylori* isolates obtained from children with peptic ulcer. The genotypes *vacA s2m1* and s1c were not observed in any of isolates studied.

CONCLUSION: This study showed that *vacA s1m2*, *cagA* and *iceA2* were the most common genotypes, and no association between antral nodularity and genotypes was observed.

Key words: *Helicobacter pylori*; Children; Genotype; Polymerase chain reaction

Core tip: In this research we have attempted to determine the prevalence of some genotypes of *Helicobacter pylori* (*H. pylori*) among children in Eastern Turkey and to investigate the relationship between these genotypes with antral nodularity. Identifying the virulence genes among *H. pylori* isolates in children would allow for the development of new treatments and eradication policies in adults. The study results suggest that there was no significant association between antral nodularity and the presence of genotypes ($P > 0.05$).

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INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is generally acquired

during childhood, persists throughout life unless treated with antibiotics, and the infection is usually associated with the development of several gastroduodenal diseases such as gastritis, peptic ulcer, gastric carcinoma and mucosa-associated lymphoid tissue lymphoma[1-3].

The cytotoxin associated gene A (*cagA*) being a marker for the presence of the cag pathogenicity island (*cag* PAI) was the first recognized virulence gene in the *H. pylori* genome in both adults and children[3,4]. Cytotoxin associated gene E (*cagE*) is also a member of the *cag* PAI, and has been described as a potential virulence factor associated with duodenal ulcer in children[5]. The vacuolating cytotoxin (*vacA*) gene exists in different subtypes, varying in the signal (s1 or s2) and middle (m1 or m2) regions[6,7]. *H. pylori vacA* alleles differ in their ability to express an active toxin[7]. Inducement occurs *via* contact with the epithelium gene (*iceA*), has two allelic variants (*iceA1* and *iceA2*), and has been determined through its upregulation after adherence of *H. pylori* to the gastric epithelium[8,9]. The blood adhesion binding antigen A (*babA*) adhesion of *H. pylori*, encoded by the *babA2* gene is an outer membrane protein that binds to the fucosylated histo-blood group antigens on the surface of gastric epithelial cells[10].

Despite a high prevalence of *H. pylori* infection among children and adults in Turkey, the published data on geographic distribution of the virulence genes in *H. pylori* strains among Turkish children are very limited, and relatively few studies have been reported on the prevalence of the *cagA* gene of *H. pylori* in Turkish children[11-13]. This study was performed to determine the prevalence of some virulence genes of *H. pylori* which were not previously reported among children in Eastern Turkey, and to investigate the association between these genotypes with clinical disease.

MATERIALS AND METHODS

A total of 49 *H. pylori* isolates were investigated for the presence of virulence genotypes. These isolates [by polymerase chain reaction (PCR)] were recovered from 101 Turkish children (53 girls and 48 boys, ranging between 4 and 18 years old, average 12 years) who underwent upper gastrointestinal endoscopy with abdominal symptoms at the clinic of the Pediatric Gastroenterology Department at the Firat University Hospital between March 2011 and September 2012. Antral nodularity was defined as being endoscopically characterized by the irregular appearance of the mucosa as like that of a "cobblestone pavement" [14]. Also, the presence of ulcers was determined by endoscopic examination.

Our study was approved by the Medical Ethics Committee of Firat University. All patients received informed consent that was signed by their parents before endoscopic procedures.

Isolation of H. pylori DNA and PCR detection of its genotypes

H. pylori DNA was prepared using the QIAamp DNA mini kit (Qiagen, Germany) following the manufacturer's instructions. The extracted DNA was kept at -20 ℃ until tested.

PCR was carried out using oligonucleotide primers targeting the 298 bp fragment of the *cagA* gene; the fragment 259 bp or 286 bp in size for type s1 or s2; the 190, 187 and 213 bp fragments for s1a, s1b, and s1c; the 567 bp and 642 bp fragments for m1 and m2; the 508 bp fragment of the *cagE* gene; the 247-bp fragment of *iceA1*; the 229 or 334-bp fragments of *iceA2*; and the 271 bp fragment of the *babA*2 gene, in order to amplify the *cagA, vacA, cagE, iceA* and *babA2* genes of the *H. pylori* isolates[7,15-20]. Ten μL of each PCR product was subjected to electrophoresis in a 1.5% (w/v) agarose gel.

All reactions were performed with positive controls containing the DNA of the HP 26695, HP J99, and some clinical isolates supplied by Dr. Yoshio Yamaoka, along with negative controls containing all PCR components with distilled water to substitute the DNA sample.

Statistical analysis

Statistical analysis was performed by statistical software program SPSS for Windows version 12.00 (SPSS, Chicago, IL, United States). The correlation between *H. pylori* genotypes and antral nodularity was assessed by Fischer's exact and χ^2 tests. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Table 1 summarizes the prevalence of *cagA, vacA, cagE, iceA* and *babA2* genes with antral nodularity and peptic ulcers. The number of children with peptic ulcers in the present study was low; therefore further analysis was not carried out.

The *cagA* gene was found in 30 of the 49 isolates (61.2%). In our study, the *vacA* genes were observed in all isolates. The most predominant subtype was s1a (79.6%), followed by s1b (12.2%), then s2 (8.2%). The genotype *s1m2*, which was predominant in this study, was observed in 28 (57.1%) isolates. However, the genotypes *s1m1* and *s2m2* were detected in 17 (34.7%) and 4 (8.2%) isolates, respectively. Furthermore, the genotype *vacA s2m1* and subtype s1c were not found in any of the isolates. The prevalence of *cagE* gene in children with antral nodularity and peptic ulcer was 8 out of 42 (19%) and 3 out of 7 (42.9%) isolates, respectively. The *iceA* gene was not observed in 4 of the 49 isolates. The *iceA2* gene was positive in 28 (57.1%) isolates, while *iceA1* was detected in 14 (28.6%) isolates. Three isolates (6.1%) were positive for both *iceA1* and *iceA2*. The prevalence of *iceA1* was higher in patients with peptic ulcers (57.1%), with no significance difference observed compared to patients with antral nodularity (23.8%). The *babA2* gene was detected in 20 (40.8%) samples. The *babA2* showed a higher proportion (57.1%) in patients with peptic ulcer compared to patients with antral nodularity (38.1%).

We emphasized no significant association between antral nodularity and the presence of the genotypes ($P > 0.05$).

Table 1 The prevalence of the virulence genotypes of *Helicobacter pylori* from children with antral nodularity and peptic ulcer *n* (%)

Virulence factor genes	Antral nodularity (*n* = 42)	Peptic ulcer (*n* = 7)	Total (*n* = 49)
cagA	25 (59.5)	5 (71.4)	30 (61.2)
vacA s1	38 (90.5)	7 (100)	45 (91.8)
vacA s1a	32 (76.2)	7 (100)	39 (79.6)
vacA s1b	6 (14.3)	0 (0)	6 (12.2)
vacA s2	4 (9.5)	0 (0)	4 (8.2)
vacA m1	13 (31)	4 (57.1)	17 (34.7)
vacA m2	29 (69)	3 (42.9)	32 (65.3)
vacA s1/m1	13 (31)	4 (57.1)	17 (34.7)
vacA s1/m2	25 (59.5)	3 (42.9)	28 (57.1)
vacA s2/m2	4 (9.5)	0 (0)	4 (8.2)
cagE	8 (19)	3 (42.9)	11 (22.4)
iceA1	10 (23.8)	4 (57.1)	14 (28.6)
iceA2	25 (59.5)	3 (42.9)	28 (57.1)
Both *iceA1* and *iceA2*	3 (7.1)	0 (0)	3 (6.1)
Non *iceA1* and *iceA2*	4 (9.5)	0 (0)	4 (8.2)
babA2	16 (38.1)	4 (57.1)	20 (40.8)

DISCUSSION

Although only one study on virulence genes of *H. pylori* has been performed in adults in the Elazig Province in Eastern Turkey[21], there is no data related to the prevalence of *H. pylori* genotypes among children in this region. However, there are a few studies on determining the prevalence of the *cagA* gene of *H. pylori* in Turkish children[11-13].

The prevalence of the *cagA* gene in children among European countries varies from 22.4% to 76%[2,22]. Earlier studies performed in Turkish children showed the prevalence of the *cagA* gene was 55%-74.4%[11-13]. In this study, we detected the prevalence of 61.2% of the *cagA* gene among Turkish children. The inconsistent findings may be due to adaptation of *H. pylori* to the environment in different geographic regions[23]. Some studies had confirmed a significant correlation between the severity of histological changes and the presence of the *cagA* gene in the *H. pylori* genome[23-26], whereas others[11,27-29] have not emphasized this association.

It has been demonstrated that the geographic distribution for *vacA* alleles differs in many countries around the world[30]; *s1c* is the common strain in East Asia, while s1a is the prevalent strain in Northern Europe, and s1b in Portugal and Spain[19]. The majority of *H. pylori* isolates identified as s1a; however, no subtypes s1c and s2 were found in this study. The *vacA s1m1, s1m2,* and *s2m2* genotypes were found in 34.7%, 57.1%, and 8.2%, respectively. No *s2m1* genotype was detected in the present study. Our data is consistent with the results reported in Poland[31] and Shanghai[32] where the *s1m2* was the most prevalent genotype. In contrast, other predominant *vacA* genotypes were reported in Brazil, Slovenia, the Midwestern United States (*s1m1*), and Spain (*s2m2*)[23,25,33,34].

The prevalence of the *iceA1* genotype was found to be 14% in Brazil[24], 37% in Israel[28], 44% in North America[27], and 62% in Slovenia[23]. The prevalence (28.6%) of the *iceA1* gene in this study was similar to the Brazilian population (14%)[24], but lower than in Korea (76%)[35]. Although it has been shown that the *iceA1* gene is associated with ulcer disease in adults[19], no significant association between the *iceA1* subtype and disease severity was found which is concordant with other studies[23,27,28]. We found that the *iceA2* gene (57.1%) was the predominant genotype, supporting the findings of pediatric studies in Brazil (68.9%), Israel (52%), and the Midwestern United States (84%)[25,28,33].

The prevalence of *cagE* was found in 24.5% of *H. pylori* isolates in Israel[28], 59% in Canada[5], and 41.7% in Bulgaria[26]. The *cagE* gene was detected in 11 (22.4%) out of 49 isolates, and no significant association was found between the *cagE* and peptic ulcers in children in this study, consistent with a study by Benenson *et al*[28]. However, another study showed just such an association[5]. Furthermore, we observed that the *cagE* gene was predominantly detected in *H. pylori* isolates from children with peptic ulcers. Because of the relatively low number of children with peptic ulcers, statistical analysis was not carried out.

The prevalence of *H. pylori babA2* was 17.2% in Portugal, 36% in the Midwestern United States, 84.4% in Brazil, and 66.7% in Bulgaria[2,25,26,33]. In the present study, the *babA2* gene was detected in 40.8% of the *H. pylori* isolates. The low prevalence of *babA2* in children can also be explained by the fact that *H. pylori* strains exhibit different patterns of adherent to gastric mucosa cells in adults and children, pointing out the importance of host characteristics in the selection of determinants of the infecting strain[36,37].

In conclusion, we feel that the clinical presentations observed are not correlated with the presence of the virulence genotypes because of small numbers of *H. pylori* isolates. However, the identification of virulence genotypes in this study will be important for future policies for the eradication of *H. pylori* in order to prevent severe diseases in adults.

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COMMENTS

Background

There are a few studies on the virulence genotypes of *Helicobacter pylori* (*H. pylori*) in Turkish children and the correlation of these genotypes with clinical outcome. The present study aimed to describe the prevalence of *cagA*, *vacA*, *cagE*, *iceA* and *babA2* genotypes of *H. pylori* in children in Eastern Turkey and to assess the association between these virulence genotypes and antral nodularity.

Research frontiers

In this study, the authors investigated the prevalence of *cagA*, *vacA*, *cagE*, *iceA* and *babA2* genotypes of *H. pylori* among children in Eastern Turkey and evaluated the association between these genotypes with antral nodularity. There was no significant association between virulence factor genes with antral nodularity.

Innovations and breakthroughs

This is the first study on the prevalence of the *vacA*, *cagA*, *cagE*, *iceA* and *babA2* genes among children in Eastern Turkey and the correlation of these virulence factor genes with antral nodularity. This research is useful not only in developing future strategies to control and eradicate *H. pylori* infection but also to contribute a better understanding of the epidemiology of *H. pylori* infection. In this study, they examined small numbers of *H. pylori* isolates. More large population and genotyping studies are needed for the development of the future policies to eradicate *H. pylori* infection.

Applications

The data obtained from this study will be useful in developing the future policies for the eradication of *H. pylori* in order to prevent severe diseases in adults.

Peer review

The authors studied the prevalence of *H. pylori* virulence genotypes among children in Eastern Turkey. This is a useful paper on a topic for which there is, as yet little information. It will certainly contribute to knowledge on the issue.

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