Original Article

Iran J Pediatr
Oct 2013; Vol 23 (No 5), Pp: 551-556

Association between *Helicobacter pylori*, *cagA*, and *vacA* Status and Clinical Presentation in Iranian Children

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Received: *Jan 19, 2013*; Accepted: *Jun 29, 2013;* First Online Available: *Aug 15, 2013*

Abstract

Objective: Seroprevalence of *H. pylori* infection in Iran exceeds 65% of pediatric population. In this study, we intended to find association between the virulence genes (*cag*A and *vac*A) and clinical presentations.

Methods: *H. pylori* isolates were achieved from the gastric mucosa of children. In each case, the gastric biopsy specimens were cultured and the organisms identified. Detection of different genotypes was carried out by PCR method.

Findings: A total of 106 biopsy specimens were cultured and 33 *H. pylori* isolates obtained. Among these 33 *H. pylori* strains 24 (73%) were *cagA*-positive. Genotypes of *vacA* s1m2, s1m1, s2m2, and s2m1 were 45.5%, 30.3%, 21.2%, and 3%, respectively. Most female patients were infected with genotype s1m2. The *vacA*-m1 strains were significantly more common in patients with nodular gastritis. There were no statistical differences between the *vacA* and *cagA* genotypes and clinical outcomes.

Conclusion: The frequency of *cagA* genotype was high. In this study, nodular gastritis was a common finding and was rather significantly associated with m1 allele of *vacA*.

Iranian Journal of Pediatrics, Volume 23 (Number 5), October 2013, Pages: 551-556

Key Words: Helicobacter Pylori; CagA Protein; VacA Protein; Peptic Ulcer

Introduction

The frequency of *H. pylori* is high in developing countries[1]. Its seroprevalence in Iran exceeds 65% of pediatric population[2-3]. A recent study revealed an early colonization/infection of infants with *H. pylori* with a prevalence of 67% at 9 months of age in Northwest and West Iran[4-5]. The prevalence varies among countries with existing evidence suggesting that the diversity in disease outcome may be recognized by variations in infecting strains[6-7]. Histological gastritis is essentially universal among *H. pylori* infected individuals, but only a minority develops a clinically main outcome, such as peptic ulcer disease, lymphoma or gastric cancer[8]. *H. pylori* strain-specific factors may influence the pathogenicity of different *H. pylori* isolates. *H. pylori* studies have primarily focused on two groups of bacterial virulence factors as the *cag* pathogenicity island and the *vacA*[9-10]. The presence of an intact *cag* pathogenicity island is associated with increased interleukin-8 production and mucosal inflammation[9]. Overall, the data support the idea that infection with a *cagA* positive isolate increases the risk but does

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not predict the presence of a clinically significant outcome[10]. Differences in the *vacA* generation of signal and middle region allelic types have been identified, and attempts have been made to associate specific *vacA* genotypes especially s1m1 type with different outcomes, especially with duodenal ulcer disease[11]. There are several fresh studies inspecting the association between *H. pylori* virulence factors and clinical presentations in Iranian adult patients[12-13]. In this study, we intended to find the association between virulence genes and clinical outcome.

Subjects and Methods

Patient population and endoscopic evaluation:

H. pylori isolates were achieved from the gastric mucosa of children who underwent endoscopy at Pediatric Hospital, Tabriz University of Medical Sciences. Gastric biopsies were obtained during a diagnostic fiberoptic upper endoscopy performed at the discretion of the pediatric gastroenterologist because of the subjects' persistent gastrointestinal symptoms during one year. Disease diagnosis was defined as follows: normal gross appearance, erosions, ulcers, and nodularity. Patients who received *H. pylori* therapy within 4 weeks prior to the study were excluded. Informed consent was obtained from parents of participants, and the protocol was approved by the ethics committee of Tabriz University of Medical Sciences.

Histopathology evaluation:

One biopsy from the antrum and/or fundus was fixed in formalin and processed for pathologic evaluation. Hematoxylin-eosin, and Giemsa stained slides were graded using the visual analog scale of the Sydney classification, which guided analysis of the density of *H. pylori*, and the amounts of neutrophils, mononuclear inflammatory cells, and intestinal metaplasia[12].

***H. pylori* culture and genotyping**:

In each case, the gastric biopsy specimens were cultured and the organisms identified as *H. pylori* as previously described[15]. DNA extraction was obtained by CTAB reagent method[16]. The extracted DNA was eluted in 50 µl of 1 × TE buffer {10 mMTris -HCl, 1 mM EDTA (pH 8.0)} and stored at -20°C until used. The *glmM* (*ureC*) gene was used as a positive control for detecting *H. pylori* DNA. The genotypes of *vacA* s-region (s1 or s2) and m-region (m1 or m2), and the presence of *cagA* genes were carried out by PCR as previously described[15].

Statistical analysis:

Variables such as gender, age and the presence of each candidate gene were evaluated. The univariate association between each genotype and the clinical presentations were quantified by the Chi-square or Fisher's exact tests. Independent samples t and one way ANOVA tests were used to compare quantity variables. A *P* value of less than 0.05 was accepted as statistically significant. The SPSS statistical software package version 16.0 was used for all statistical analyses.

Findings

The patients' demographic characteristics, endoscopic presentation, and histological scores are presented in Table 1. A total of 106 specimens of biopsy mucosa were available for histological analysis and the results also are summarized in Table 1. The mean age of patients was 8.28 (±1.59) years (rnge: 2-17). After processing for culture only 33 patients had *H. pylori* positive cultures. Most common finding on the basis of endoscopy was nodular gastritis. As expected, the mean age in peptic ulcer diseases (PUD) patients was not significantly higher than in non ulcer dyspepsia (NUD) group (*P*=0.9). Demographic factors such as gender and education did not show any statistical differences in PUD and NUD.

Overall, 24 (72.7%) patients were infected with *cagA*-positive strains (Table 2). The mean age of these patients was 8.33 (±1.87) years. The *cagA* gene was present in PUD and NUD patients, in 50% and 74.2% of strains, respectively. There were also no statistical differences between the *cagA* status and endoscopic or pathologic and clinical presentations irrespective of the peptic disease. This study showed that the presence of *cagA* gene was independent of the PUD risk,

Table 1: Demographic characteristics of patients, endoscopic and histopathology findings (n=106)

Variables	Levels	Frequency (%)
Gender	Male	52 (49.1)
	Female	54 (50.9)
Endoscopic finding	Ulcer	4 (3.8)
	Erythema	104 (98.1)
	Congestion	10 (9.4)
	Erosion	8 (7.5)
	Nodular gastritis	53 (50)
Pathologic finding	Acute gastritis	15 (14.2)
	Chronic gastritis	81 (76.4)
	Duodenitis	82 (77.4)
	Atrophy	3 (2.8)
	Esophagitis	95 (89.6)
***H. pylori* density**	Negative	62 (58.5)
	Mild positive	39 (36.8)
	Moderate positive	4 (3.8)
	Severe positive	1 (0.9)
Chief complaint	Recurrent epigastric pain	88 (83)
	Vomiting	24 (22.6)
	Chronic diarrhea	5 (4.7)
	Failure to thrive	22 (20.8)
	GI bleeding	8 (7.5)

adjusted by age (*P*=0.8), sex (*P*=0.2) and other demographic data.

Thirty-three (100%) *H. pylori* isolates were carrying *vacA* gene. Genotypes of *vacA,* s1m2, s1m1, s2m2, and s2m1 were 45.5%, 30.3%, 21.2% and 3%, respectively. There were also no statistical differences between the *vacA* genotypes and clinical outcomes both by univariate analyses and when adjusted by age, sex and other demographic data (Table 3). The *vacA*-m1 strains

Table 2: Relation between *cag*A and endoscopic and pathologic findings (n=24)

Variables	Level	*cag*A+ (%)	*P*-value
Gender	**Male**	10 (41.7)	0.2
	Female	14 (58.3)	
Endoscopic finding	**Congestion**	1 (4.2)	0.5
	Erosion	2 (8.3)	0.4
	Ulcer	1 (4.2)	0.4
	Erythema	2 (100)	0.09
	Nodular gastritis	15 (62.5)	0.7
Pathologic finding	**Chronic gastritis**	16 (66.7)	0.5
	Acute gastritis	7 (29.2)	0.4
	Duodenitis	16 (66.7)	-
	Esophagitis	22 (91.7)	0.8
	Atrophy	0 (0)	0.9
***H. pylori* density**	**Negative**	9 (37.5)	0.4
	Mild positive	14 (58.3)	
	Moderate positive	1 (4.2)	
	Severe positive	0 (0)	
Chief complaint	**Epigastric pain**	22 (91.7)	0.3
	Vomiting	8 (33.3)	0.2
	Chronic diarrhea	0 (0)	0.09
	Failure to thrive	2 (8.3)	0.01
	GI bleeding	3 (12.5)	0.22

Table 3: Comparison of demographic data and *vac*A subgroups

Variables	Level	S1 (%)	S2 (%)	M1 (%)	M2 (%)	*P.* value
Age(years)	**Mean(SE)**	9.36 (1.21)	8.09 (1.09)	9.63 (1.89)	8.72 (1.45)	0.5
Gender	**Male**	8 (36.4)	8 (72.7)	1 (12.5)	15 (60)	0.01
	Female	14 (63.6)	3 (27.3)	7 (87.5)	10 (40)	
Endoscopic finding	**Congestion**	0 (0)	1 (9.1)	0 (0)	1 (4)	0.5
	Erosion	2 (9.1)	0 (0)	1 (12.5)	1 (4)	0.4
	Ulcer	1 (4.5)	1 (9.1)	0 (0)	2 (8)	0.4
	Erythema	21 (95.5)	11 (100)	8 (100)	24 (96)	0.5
	Nodular gastritis	13 (59.1)	7 (63.6)	7 (87.5)	13 (52)	0.07
Pathologic finding	**Chronic gastritis**	15 (68.6)	6 (54.5)	6 (75)	15 (60)	0.4
	Acute gastritis	6 (27.3)	5 (45.5)	2 (25)	9 (36)	0.6
	Duodenitis	13 (59.1)	9 (81.8)	6 (75)	16 (64)	0.6
	Esophagitis	19 (86.4)	11 (100)	7 (87.5)	23 (92)	0.7
	Atrophy	0 (0)	1 (9.1)	0 (0)	1 (4)	0.6
H. pylori density	**Negative**	9 (40.9)	3 (27.3)	4 (50)	8 (32)	0.8
	Mild positive	12 (54.5)	7 (63.6)	4 (50)	15 (60)	
	Moderate positive	0 (0)	1 (9.1)	0 (0)	1 (4)	
	Severe positive	1 (4.5)	0 (0)	0 (0)	1 (4)	
Chief complaint	**Epigastric pain**	18 (81.8)	11 (100)	8 (100)	21 (84)	0.2
	Vomiting	8 (36.4)	1 (9.1)	6 (75)	3 (12)	<0.001
	Chronic diarrhea	1 (4.5)	0 (0)	0 (0)	1 (4)	0.6
	Failure to thrive	4 (18.2)	2 (18.2)	1 (12.5)	5 (20)	0.6
	GI bleeding	2 (9.1)	1 (9.1)	0 (0)	3 (12)	0.3
cagA		19 (80.4)	5 (45.5)	7 (87.5)	17 (68)	0.7

were rather significantly more common (*P*=0.07) in patients with nodular gastritis. In this study s1m2 *vacA* genotype was more frequently detected in females than in males.

Discussion

There is ongoing interest in identifying *H. pylori* virulence factors that might predict the risk for clinical presentation. It has been proposed that *cagA* genes are such markers and can identify patients with peptic ulcer[15].

The present study investigated the *cagA* and *vacA* genotypes of *H. pylori* isolated from pediatric population living in Azerbaijan, Iran. Because the strains were attained from symptomatic patients, the results reproduce the findings in these groups of patients rather than in the whole population. The current study confirms the distinctive difference in *H. pylori* genotypes in Iranian pediatric groups residing within the same region. In some researches, the prevalence of *H. pylori* was reported to vary among different countries, regions, and patient groups, and it was reported as 82% in Brazil[17], 78 to 80% in Turkey[18,19], 82% in Japan[20]. The majority of these studies indicated that in patients with duodenal ulcer, the *cagA* positivity rate is relatively higher than in patients with gastritis or gastric ulcer and ranges from 80 to 100%[17-20]. In another study, the *cagA* positivity in Iranian isolates has been reported to vary from 44% to 91%[11,21-23]. In the present study, 72.7% of the patients were infected with *cagA* positive strains; similar to other Iranian reports[21,22,24,25]. However, this is different from studies in East and South Asian countries where more than 90% of the strains have the *cagA* gene irrespective of clinical outcome[26-28]. Excitingly, many authors[29, 30] have found a significant correlation between the severity of histological alterations and the presence of the *cagA* gene in the *H. pylori* genome, whereas others[22,25,31,32] have been unable to support this relationship. The present study did not reveal associations between the *cagA* status and clinical presentation.

Our results revealed that *cagA* and *vacA* subtype m1 *H. pylori* strains were especially associated with nodular gastritis in Azerbaijan province. Hosseini et al found no correlation of *cagA* genotype and disease status, whereas *vacA* was demonstrated as a useful marker in predicting the disease outcome[33]. van Doorn et al examined 94 gastric biopsy samples from patients in the Netherlands and reported that *cagA* positivity and *vacA* s1 genotype were associated with peptic ulcer disease[15]. The present study did not reveal any association between the *vacA* and *cagA* status and PUD. This finding is in agreement with some reports from Iran[13], but was different from other Iranian studies[25,34] and many studies from Western countries where *vacA* s1 and *cagA*-positive strains are more often isolated from patients with PUD than with NUD[30]. In our study the number of patients is relatively small, so it is necessary that additional studies with large numbers of samples be studied to clarify the role of *cag*A, *vac*A in clinical presentation.

Conclusion

In conclusion, *cag*A and *vac*A-s1m1 genotypes are the predominant genotypes of *H. pylori* isolated from the northwestern Iranian pediatric population. However, we could not reveal clear association of *cag*A, and *vac*A genotypes with clinical presentation in pediatric age groups living in Azerbaijan province.

Acknowledgment

This study has been founded by Tabriz University of Medical Sciences.

Conflict of Interest: None

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