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Detection of virulent strains of *Helicobacter pylori* and its relation to symptoms and signs in children

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ABSTRACT

Objective: To detect the prevalence of *Helicobacter polori* (*H. pylori*) in a group of pediatric patients complaining of gastrointestinal disorders, and to identify the virulent strains of *H. pylori* and its relation to peptic disease. **Methods**: The study included 60 patients (41 males and 19 females), aged from 6 months to 14 years, with complaining of different gastrointestinal troubles. Anti-*H. pylori* IgG antibodies were used. Thorough examination of the oesophagus, stomach and duodenum was done in all patients using a fibreoptic endoscopy. In addition, gastric antral biopsies were taken for diagnosis of *H. pylori* infection in the following test: rapid urease test, histopathologic examination and culture. And cytotoxin assay of virulent strains was employed. *H. pylori* infection was diagnosed if 3 or more tests for diagnosis were found to be positive. **Results**: 33 out of 60 patients (55%) was positive. 12 (20%) patients had positive toxin producing strains (virulent strains). Virulent strain had a significantly closer relationship with recurrent abdominal pain, while no difference was found in other symptoms. Age of patients showed a highly significant positive correlation with virulent *H. pylori* strain. Histopathological finding revealed that oesophagitis was significantly common in patients with negative *H. pylori*. Endoscopy and histopathological findings revealed that virulent strain had a significantly higher gastritis and oesophagitis rates. **Conclusion**: Infection by virulent strains of *H. pylori* in children is not uncommon: It is associated with endoscopic and histopathologic changes. All cases infected with virulent strain showed positive results by using rapid urease test, culture and IgG antibodies.

1. Introduction

Helicobacter pylori (*H. pylori*), a spiral, shaped microaerophilic bacterium infects more than 50% of the world´s population, with higher rate of infection in developing countries[1]. *H. pylori* is a major etiological agent in several gastroduodenal diseases, such as functional dyspepsia, peptic ulcer disease, gastric cancer and mucosal-associated lymphoid tissues lymphoma. The clinical outcome following infection with this pathogen has been related to environmental conditions, host immunological factors and microorganism virulence[2,3].

Vacuolating cytotoxin (Vaca), cytotoxin associated gene A (cagA) and blood adhesion binding antigen (babA) are the most commonly studied virulence markers of *H. pylori*. However, there are other bacterial proteins with pathologic potential, such as sialic acid binding adhesion (SabA), outer inflammatory protein (oipA), and duodenal ulcer promoting gene (dupA). The influence of these proteins on *H. pylori* pathogenesis is still under study[4].

H. pylori VacA is a secreted performing toxin that is comprised of two domains, designated as p33 and p55. The p55 domain has an important role in the binding of VacA to eukaryotic cell surfaces. A total of 111 residues at the amino terminus of p55 (residues 312 to 422) are essential for the intracellular activity of vacA, which suggests that this region may constitute a subdomin with an activity distinct from cell binding[5].

H. pylori is a highly successful human specific gastric pathogen that colonizes more than half the world´s population. Infection with this bacterium can induce gastric pathologies ranging from chronic gastritis to peptic ulcer.

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Virulent *H. pylori* isolates harbour the cytotoxic -associated genes (cag) pathogenicity island, a 40 kb stretch of DNA that encodes components of a type Ⅳ secretion system[6].

The *H. pylori* virulence factors cagA and VacA are implicated in the development of gastroduodenal diseases. Most strains possessing cagA also psossess the more virulent vacuolating form of VacA[7].

We are aiming to detect the prevalence of *H. pylori* in a group of pediatric patients complaining of gastrointestinal disorders, also to identify the virulent strains of *H. pylori* and its relation to peptic disease.

2. Materials and methods

The study included 60 patients (41 males and 19 females), with age ranged from 6 months to 14 years in Pediatric Endoscopy Units, Al-Zahraa and Al-Hussein Hospitals, Faculty of Medicine, Al-Azhar University. They all complained of different gastrointestinal troubles, such as heart burn, persistent vomiting, recurrent abdominal pain, abdominal distension and hematemesis.

All patients were subjected to careful history taking and complete physical examination.

2.1. Laboratory investigation

2-3 mL blood were taken from each patient for serologic detection of anti-*H. pylori* antibodies by the enzyme-linked immunosorbent assay (ELISA) technique using GAP-IgG kit (Biomerica, USA).

2.2. Upper gastrointestinal endoscopy

Thorough examination of the oesophagus, stomach and duodenum was done in all patients using a fibreoptic endoscopy (Pentax FG-27 with diameter of 0.9 mm insertion tube diameter).

H. pylori infection was suspected if there was gastritis especially antral nodularity. In addition, gastric antral biopsies were taken for diagnosis of *H. pylori* infection by the following tests:

-Rapid urease test: One antral biopsy was used for rapid urease testing using Clo test (Delta west Ltd., Bentley, Australia)[8].

-Histopathologic examination: One biopsy was placed in formalin solution until it was sent to the pathology laboratory for histopathologic examination. Gastritis was graded from 0 to 3 on the basis of the Sydney system[9].

2.3. Culture

One biopsy was put in sterile saline tube and transported within 2 hours to microbiology laboratory for culture of *H. pylori* colonies. The main reagents included:

-Dent´s media used for isolation of *H. pylori*.

-Sloppy blood agar (6% sheep blood and 0.4% agar in nutrient broth No 2) used for preservation of isolated *H. pylori* strains.

H. pylori was diagnosed on the basis of positive catalase, oxidase and urease test and by gram staining.

2.4. Cytotoxin assay of virulent strains

Main reagents included 6% sheep blood agar and sterile brucella broth (Merk) supplemented with 10% fetal calf serum (FCS) (Gibco, Grand Island, NY, USA).

Gastroesophageal reflux is diagnosed if there was one or more of the following: hiatus hernia, incompetent cardia and gross esophagitis.

2.5. Statistical methods

Statistical package for social science (SPSS) version 9.0 was used for analysis of the data. Chi-square test was used for analysis of the qualitative data. Pearson´s correlation was also done.

3. Results

H. pylori infection was diagnosed if 3 or more tests for diagnosis were found to be positive. It was positive in 33 out of 60 patients (55%). 12 (20%) patients had positive toxin producing strains (virulent strains). Endoscopic findings revealed that 15 (25%) patients had gastritis and oesphagitis, 2 (3.3%) patients had gastritis, 34 (56.7%) had oesophagitis and 9 (15%) patients were free. Virulent strain had a significantly closer relationship with recurrent abdominal pain ($P<0.05$), while no difference was found in comparison of other symptoms ($P>0.05$) (Table 1). Age of patients showed a strong highly significant positive correlation with virulent *H. pylori* strain ($r= 0.7$, $P= 0.0001$). Table 2 showed that all patients with toxin producing strains had a positive result in rapid urease test, culture and IgG *H. pylori* antibodies test. Histopathological finding revealed that oesophagitis is significantly higher in patients with negative *H. pylori* (Table 3). Endoscopy and histopathological findings revealed that virulent strain had a significantly higher gastritis and oesophagitis (Table 4).

Table 1
Comparison between symptoms of patients in relation to toxin producing strains (Virulent strains).

Symtoms	Positive toxin (n=12)		Negative toxin (n=12)	
	No.	%	No.	%
Recurrent abdominal pain	8	66.7	14	29.2*
Vomiting	7	58.3	22	45.8
Diarrhea	1	8.3	7	14.6
Haematemesis	2	16.7	2	4.2
Heart burn	6	50.0	14	29.2
Abdominal distension	3	25.0	11	22.9

*$P<0.05$

4. Discussion

The result showed that 33 out of 60 patients (55%) was positive, higher than the report of Tam et al (53.3%)[10], Fayed *et al* (55.7%)[11] and Fayed *et al* (51.7%)[12], and lower than report of Marie (67%)[13] and Alfaresi *et al* (60%)[14].

Patients infected by virulent strains constitute 20% of the study group. All patients with toxin producing strains had a positive result in rapid urease test, culture and IgG *H. pylori* antibodies test. It was found to be lower than report of Hussein *et al*[15]. The study of Go *et al*[16] showed that the result was 20% of *H. pylori* strains isolated from

Table 2
Different diagnostic tests of *H. pylori* infection in relation to toxin producing strains.

	Urease test		Culture		*H. pylori* IgG	
	Positive	Negative	Positive	Negative	Positive	Negative
Positive toxin	12(36.4%)	0(0.0%)	12(46.2%)	0(0.0%)	12(35.3%)	0(0.0%)
Negative toxin	21(63.6%)	27(100.0%)	14(53.8%)	34(100.0%)	22(64.7%)	26(100.0%)
P-value	0.0001		0.0001		0.0001	

Table 3
Comparison between *H. pylori* in relation to histopathological finding.

Histopathological finding	Positive *H. pylori*(n = 33)	Negative *H. pylori*(n = 27)	*P*-value
Gastritis and oesophagitis	11 (33.3%)	4 (14.8%)	0.09
Gastritis	48 (36.4%)	5 (18.5%)	0.1
Oesophagitis	0 (0.0%)	13 (48.1%)	0.0001
Free	10 (30.3%)	5 (18.5%)	0.2

Table 4
Comparison between endoscopic and histopathological findings in relation to virulent strains.

		Positive toxin (n = 12)	Negative toxin (n = 48)	*P*-value
Endoscopic findings	Gastritis and oesophagitis	8(66.0%)	7(14.5%)	<0.01
	Gastritis	1(8.3%)	1(2.1%)	>0.05
	Oesophagitis	3(25.0%)	31(64.5%)	>0.05
	Free	0(0.0%)	9(18.8%)	<0.01
Histopathological findings	Gastritis and oesophagitis	6(50.0%)	9(18.8%)	<0.05
	Gastritis	4(33.3%)	13(27.1%)	>0.05
	Oesophagitis	2(16.7%)	21(43.7%)	<0.05
	Free	0(0.0%)	5(10.4%)	<0.01

children and 42% of strains isolated from adults expressed vacuolating activity.

Age of patients showed a strong highly significant positive correlation with virulent *H. pylori* strain. This might be due to more exposure of the population to the organism. This is in agreement with Tam *et al*[10] and Marie[13]. Falsafi *et al*[17] reported that infection occurs mainly in childhood and infected individuals usually carry it for life unless treated[18, 19]. Epidemiology of infection by *H. pylori* has been characterized by a linear increase with age in western industrial countries and by a large number of children and juveniles being infected in developing countries[20].

Endoscopic examination revealed that *H. pylori* associated gastritis and oesophagitis was found in 25% of cases, gastritis in 3.3% and oesophagitis seen in 56.7% of cases. These results were confirmed with Misiewicz *et al*[21] and Mahony *et al*[22].

Virulent strain had a significantly closer relationship with recurrent abdominal pain, while no difference was found in comparison of other symptoms. This is coincide with those reported by Marie[13] and Treiber *et al*[23]. On the contrary, Tam *et al*[10] found that thirty *H. pylori* positive patients (70%) presented with acute gastrointestinal bleeding, whereas only 19 (30 %) had a history of epigastric pain.

Histopathological findings revealed that patients with positive *H. pylori* had higher incidence of gastritis and oesophagitis (18.3%). 20% patients had gastritis only, but it was not statistically significant. On the other hand, positive *H. pylori* patients had significantly lower oesophagitis rate.

H. pylori infection in humans is associated with gastritis, gastric ulcer, and gastric cancers[17-24].

The prevalence of gastroesophageal reflux disease (GERD) ranges from 2.5% to 7.1% in most population-based studies in Asia. There is evidence that GERD and its complications are rising, coinciding with a decline in *H. pylori* infection. Asian GERD patients share similar risk factors and pathophysiological mechanisms with their Western counterparts. Possible causes for the lower prevalence of GERD include less obesity and hiatus hernia, a lesser degree of esophageal dysmotility, a high prevalence of virulent strains of *H. pylori*, and low awareness[25]. On the contrary, Bayrak ç i *et al*[26] reported that no impact of *H. pylori* on the severity of esophagitis or symptoms was shown. All patients infected by virulent strains showed endoscopic and histopathological findings changes. Gastritis and oesophagitis was significantly higher in patients infected by virulent strains in both endoscopy and histopathology test. This is because infection by vacuolating cytotoxic *H. pylori* strains is considered to constitute increased risk for chronic gastritis and subsequent development of peptic ulcer.

CagA-positive *H. pylori* isolates are associated with a higher rate of gastric inflammation and damage, when compared with cagA- negative strains[27,28]. Several epidemiological studies have shown the correlation between cagA-positive strains and a higher risk of developing peptic ulceration, gastric atrophy and gastric cancer[27,28].

It is concluded that infection by virulent strains of *H. pylori* in children is not uncommon: Infection by virulent strains is associated with endoscopic and histopathologic changes. All cases infected with virulent strain showed positive results by using rapid urease test, culture and IgG antibodies.

Besides, it is recommended as following:

-Combination of more than one test is recommended for diagnosis of *H. pylori* infection.

-Patients with virulent strains of *H. pylori* infection must be treated with complete eradication of infection due to associated symptoms, endoscopic and histopathologic changes.

–Promising vaccine for control of *H. pylori* infection is needed due to complex therapy regimen and large number of infected persons are asymptomatic.

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