Detection of *Helicobacter pylori* Infection by *Helicobacter pylori* IgG Serology Test in Pediatric Patients at the Philippine General Hospital

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ABSTRACT

Objective. To determine the validity of serum *H. pylori* IgG in the detection of *H. pylori*-associated gastroduodenitis in patients with gastrointestinal symptoms.

Methods. Cross-sectional study which included consecutive patients 1-18 years old with upper gastrointestinal symptoms who underwent esophagogastroduodenoscopy. *H. pylori* infection was diagnosed by positive tests for both rapid urease test (RUT) and Giemsa stain of gastric biopsies. *H. pylori* IgG (ELISA) serology was also performed.

Results. Twenty-five patients [Mean (SD) age: 12 (4.5) years, 68% females] were included. Majority presented with epigastric pain (64%) and had endoscopic gastritis (84%). Four patients had ulcers (1 antral, 3 duodenal). Giemsa stain was positive in 16 (64%) patients and RUT in one. Prevalence of *H. pylori* infection was 4%. Serum *H. pylori* IgG test was positive in two; borderline in three with a 100% sensitivity, 80% specificity, and a positive and negative likelihood ratio of 10.9 and 0.6.

Conclusion. The present study showed a low prevalence of *H. pylori* infection, thus, the validity of the *H. pylori* serology could not be adequately evaluated. We presently could not recommend the serum IgG in the detection of *H. pylori* gastroduodenitis in our setting.

Keywords: H. pylori, Serology ELISA IgG, Rapid Urease Test

INTRODUCTION

Helicobacter pylori (H. pylori) is a spiral-shaped, gram-negative bacterium isolated in 95% of gastric cancer and in 20-40% of gastritis, gastric and duodenal ulcers.[1,2,3] It is primarily transmitted by fecal-oral route, although gastro-oral and oral-oral transmission are also possible. Several studies indicated that in children, abdominal pain is the most common symptom seen in 25%.[4]

The prevalence of *H. pylori* infection in children based on serological detection of IgG ELISA, varies by country from 20% to 90%, with higher prevalence rates of >60% observed in developing countries. In 1,055 Tunisian children, the prevalence was reported to be 51.4% using enzyme-linked immunosorbent assay (ELISA) for IgG antibodies and 25% of these patients had abdominal pain.[4]

There are different diagnostic methods used to detect *H. pylori*.[5] Histology and/or culture of gastric tissue obtained by endoscopy are generally considered the gold standard for

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diagnosis.[6] The ESPGHAN and NASPGHAN in 2011, recommend that the initial diagnosis should be based on examination of the gastric mucosa that is *H. pylori* positive on culture or positive for both histopathology and rapid urease test (RUT). This recommendation however, will require invasive endoscopic procedure to obtain gastric biopsies that is not always acceptable for children. Endoscopy is costly with risk of possible anesthesia-induced side effects from sedation. Hence, different non-invasive tests for detecting *H. pylori* infection have been investigated as it is simpler, cheaper and easier to perform, and more convenient for pediatric patients. In a local study involving dyspeptic and non-dyspeptic children, rapid qualitative immune-chromatographic test for antibodies against *H. pylori* from serum and stool samples showed good specificity from 88 to 100% but with a sensitivity of only 30 to 50%.[7] They were therefore not considered as a reliable, non-invasive screening test for *H. pylori*.

Studies conducted in Saudi Arabia [8] and Egypt [9] investigated the use of ELISA to detect the presence of *H. pylori* antibodies in children. Using histopathology and urease test as gold standard, the test was reported to have a sensitivity of 70 and 93% and specificity of 89 and 96%. In these countries, the prevalence of *H. pylori* infection were 88% and 57%, respectively.

Despite the availability of the ELISA-based test, no local study has investigated its validity as a diagnostic tool. Knowledge of this is important so that proper recommendations could be made in our setting. The objective of this study is to determine the validity of serum *H. pylori* IgG in the detection of *H. pylori*-associated gastroduodenitis and/or ulcers in patients with varied upper gastrointestinal symptoms. The prevalence of the disease will also be reported.

METHODS

Definition of Terms

H. pylori gastroduodenitis was considered if both histopathology and RUT were positive on gastric tissue.[10]

Study Design and Setting

This is a cross-sectional study conducted from January 2017 to January 2018 at the Section of Pediatric Gastroenterology, Hepatology and Nutrition, Philippine General Hospital (PGH), Taft Avenue, Manila, Philippines. The subjects were referred from the pediatric emergency room, out-patient department and wards.

This research was approved by the Ethics Review Board of the PGH. Parental consent and patient's assent were sought prior to inclusion.

Patients

Included were all patients aged 1-18 years old with gastrointestinal symptoms based on ROME IV Criteria (May 2016) (Table 1)[11] who underwent an esophagogastroduodenoscopy (EGD). Also included were those with: (1) ulcer-like epigastric pain, gnawing or burning in character, lasting for at least two weeks in duration and not relieved by histamine 2 blocker or proton pump inhibitor; and (2) history of hematemesis in which an underlying liver or any other medical illness is not present.

Excluded were patients with hematemesis due to a liver disease and those with history of consuming known caustic substances.

Procedure and Data Collection

Once the patient fulfills the inclusion and exclusion criteria, a written informed consent and assent (for patients ≥15 years old) were obtained.

Table 1. ROME IV Criteria (May 2016)

	Definition	Duration
Functional Dyspepsia	One or more of the following symptoms: 1. Postprandial fullness 2. Early satiation 3. Epigastric pain or burning not associated with defecation 4. After appropriate evaluation, the symptoms cannot be fully explained by another symptoms of medical condition	Symptoms for at least 4 days per month for at least 2 months before diagnosis
Epigastric pain syndrome *(subtype of functional dyspepsia)*	Severe enough to interfere with normal activities or burning localized to the epigastrium and the pain is not generalized or localized to other abdominal or chest regions and is not relieved by defecation or passage of flatus. Supportive criteria includes: 1. Burning quality of the pain but without a retrosternal component and 2. The pain commonly induced or relieved by ingestion of a meal but may occur while fasting	Symptoms for at least 4 days per month for at least 2 months before diagnosis
Functional abdominal pain - *NOS (not otherwise specified)*	1. Episodic or continuous abdominal pain that does not occur solely during physiologic events like eating or menses 2. Insufficient criteria for irritable bowel syndrome, functional dyspepsia, or abdominal migraine 3. After appropriate evaluation, abdominal pain cannot be fully explained by another medical condition	Fulfilled at least 4 times per month for at least 2 months

On admission, the medical history with emphasis on the presence and duration of any upper gastrointestinal symptoms were gathered. The socio-demographic data such as age, sex, number of household members, history of drug or alcohol intake, smoking, and household members with similar upper gastrointestinal complaints were noted.

All patients underwent complete physical examination. Physical findings included nutritional status based on the measurements of the weight, height, and mid-upper arm circumference (MUAC), and the computation of the body mass index with their corresponding WHO z-scores.

Esophagogastroduodenoscopy (EGD) and Biopsy

All patients underwent EGD using an Olympus GIFXP 20 or XP 10 endoscope after six to eight hours fasting (Appendix 1). Patients <15 years old were referred to an anesthesiologist for sedation, while patients 16 years old and above were sedated with Midazolam (0.1mg/kg) and Nalbuphine (0.1mg/kg). EGD was performed by the principal investigator at the hospital's gastrointestinal clinic or OPD endoscopy center. The appearance of the stomach and duodenum were reported as presence of erythema, erosions and/or ulcers and were graded using the Forrest's Classification (Appendix 2).[12] During the procedure, a biopsy specimen from the antrum and body was collected for histopathology and RUT [Gastrex-Pronto Dry (Manufacturer Gastrex, France)]. The Pronto Dry is the only available test in PGH (Appendix 3). Both the Pronto Dry and the CLO tests were highly accurate for the diagnosis of *H. pylori* infection.[13] The result of RUT was interpreted within 24 hours by the principal investigator and a change in color from yellow to magenta was considered positive.

All specimens for histopathology were sent to PGH histopathology section for H&E and Giemsa stain. All the slides were collected and reviewed by a single pathologist at the end of the study blinded to the history and EGD results. The presence of curved or spiral-shaped rods in the mucus-secreting epithelium was indicative of *H. pylori* infection.

Serology test

H. pylori test was done using *H. pylori* IgG (ELISA) test (Demeditec *H-pylori* IgG). Five ml of blood was drawn and transferred on a dry tube from each patient. The specimen was centrifuged and serum was extracted. The ELISA was performed by a single medical technologist blinded to the clinical details of the patient, using standard techniques following the procedure of the manufacturer. Based on the 450nm ELISA reader, the results were interpreted as follows: <0.9, no detectable antibody; 0.9-1.1, borderline positive; and >1.1, positive for antibody to *H. pylori* IgG.

Sample size and Data Analysis

Sample size calculation was based on the prevalence[10] of *H. pylori* of 57% and specificity of *H. pylori* (ELISA) IgG serology test of 93% with absolute precision set at 0.10 in the nomogram. A sample size of 25 was needed. Chi square or Fischer exact test, as appropriate, were used to determine the association between variables.

RESULTS

Clinical features

A total of 25 patients were included in this study (Table 2). The mean (SD) age of the patients was 12.36 (4.5) years, with the youngest at 5 years and the oldest at 18 years old. A female predominance (68%) was observed. The mean (SD) duration of gastrointestinal symptoms before consult was 22.0 (27.2) months. Of the 25, 16 (64%) patients presented with epigastric pain, three of whom had concomitant dyspepsia or heartburn; five (20%) consulted for prolonged history of vomiting; and four patients (16%) with gastrointestinal bleeding (melena in three and hematochezia in one). Only one case (patient #1) had a family history of similar symptoms with a sibling with epigastric pain and vomiting. All patients had no known smoking, alcohol or drug intake.

Twenty-three of the 25 patients had normal physical examination and two (patients #13, #24) were noted to have splenomegaly secondary to portal hypertension from umbilical catheterization.

Endoscopic findings and RUTs

EGD revealed features of endoscopic gastritis in 21 (84%) patients (14 with erythema, six with erosions and one with edematous pylorus); one had non-erythematous pyloric nodule; another had antral ulcer and two patients had normal findings of the gastric area.

Twenty-two (88%) patients had normal duodenal findings and three had ulcers at the duodenum noted at the duodenal bulb (patient #13) and at the 2nd part of the duodenum (patients #15, #21). These were all classified as Forrest III.

In addition, eight (32%) subjects had a hiatal hernia and two (patients #13, #24) had 4-column esophageal varices secondary to pre-hepatic portal hypertension.

Only one of the 25 (4%) patients was positive for RUT. This 17-year-old female (patient #15) presented with epigastric pain and melena. The EGD finding showed generalized erythema, hematin stains and one flat-based ulcer at the duodenum.

Histopathology and Giemsa stain

Histopathology showed that 24 had mild and one had severe chronic gastritis. The Giemsa stain was positive for *H. pylori* bacilli in 16 (64%) patients (Figure 1).

Of the four patients with endoscopic ulcers, three showed non-specific duodenitis on histology and one had mild chronic gastritis. Three (patients #15, #21, #24) of the four patients were positive on Giemsa stain.

Table 2. Clinical features of the 25 patients investigated for *H. pylori* infection

Patient (Px)	Age (years)	Chief complaint	Duration of symptoms before consult (months)	Gastrointestinal symptoms prior to admission				
				Abdominal pain*	Dyspepsia	Hematemesis	Melena	Vomiting
1	18	Epigastric pain	6	(+)	(+)			(+)
2	17	Epigastric pain	120	(+)		(+)		(+)
3	13	Epigastric pain	8	(+)			(+)	(+)
4	8	Generalized abdominal pain	48	(++)			(+)	
5	9	Vomiting	36	(+)				(+)
6	6	Vomiting	12	(+)				(+)
7	12	Vomiting	36	(+)				(+)
8	13	Vomiting	9	(+)				(+)
9	9	Hematemesis	12	(+)		(+)		(+)
10	12	Vomiting	72	(+)				(+) Blood streaked
11	18	Epigastric pain	11	(+)				(+)
12	12	Epigastric pain	2	(+)			(+)	
13	6	Epigastric pain	6	(+)		(+)		
14	17	Epigastric pain	36	(+)				(+)
15	17	Melena	12	(+)			(+)	
16	18	Epigastric pain	12	(+)				(+)
17	5	Epigastric pain	3	(+)				(+)
18	18	Epigastric pain	48	(+)	(+)			
19	15	Epigastric pain	12	(+)				(+)
20	7	Epigastric pain	24	(+)				(+)
21	12	Epigastric pain	3	(+)				(+)
22	17	Hematochezia, melena, Right upper quadrant pain	1	(+)			(+) (with hematochezia)	
23	8	Epigastric pain	6	(+)				(+)
24	6	Melena, Epigastric pain	12	(+)		(+)	(+)	
25	16	Epigastric pain	4	(+)	(+)	(+)		

*Legend: (+) - present; *Abdominal pain: (+) epigastric pain; (++) generalized abdominal pain*

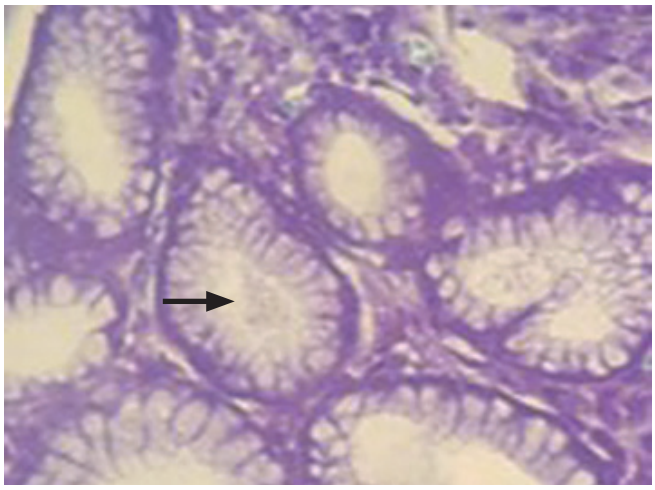

Figure 1. *H. pylori* bacilli *(black arrow)* in the gastric mucosa on histopathology.

H. pylori IgG (ELISA) serology test

Two cases were positive and three had borderline *H. pylori* IgG serology test. The first positive case was a 12-year-old female (patient #7) with abdominal pain and vomiting. Endoscopy showed a hiatal hernia and histopathology revealed mild chronic gastritis with negative RUT. The second patient was a 17-year-old female (patient #15) with abdominal pain and melena who had a flat-based ulcer at the second part of the duodenum which was confirmed histologically as moderate chronic gastritis and duodenitis with positive RUT.

The three cases with borderline results on *H. pylori* serology test presented with epigastric pain with endoscopic findings of erythema at the cardia in one (patient #2) and presence of duodenal ulcers (patients #13, #21) in two cases. All three patients had mild chronic gastritis and duodenitis on histopathology and were negative on RUT.

Table 3. Prevalence of *H. pylori* as determined by the serology test results

Serology test	*H. pylori* (+)	*H.pylori* (-)
IgG positive	1 (4%)	4 (16%)
IgG negative	0	20 (80%)

Validity of the *H. pylori* IgG test

Overall, there was only one patient who was positive both on histology and RUT, giving a prevalence of *H. pylori* infection of four percent (Table 3). Validity test showed a sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios of 100%, 80%, 20%, 100%, 10.9 and 0.6, respectively. The accuracy of the test is 84%.

DISCUSSION

The present study showed a low prevalence of *H. pylori* gastroduodenitis in our setting, thus, the validity of the serum IgG *H. pylori* serologic test could not be properly evaluated among children presenting with gastroduodenal symptoms.

There are several factors that may account for our findings. First, only one of the 25 (4%) children investigated have *H. pylori* gastroduodenitis, using both a positive histopathology and RUT to diagnose the disease, based on 2016 recommendations by NASPGHAN. In previous local studies, the prevalence of *H. pylori* infection among children with gastrointestinal symptoms was between 71 to 77% using histopathology alone as gold standard.[7,14] Had we limited ourselves to histopathology alone, the prevalence would have been 64% with 16 of our 25 cases demonstrating a positive Giemsa stain on gastric tissue. We had used a lower prevalence of 57% to compute the sample size based on the study in Egypt on serum IgG *H. pylori* test, but with a low prevalence observed, the sample size should have been bigger.

Second, the demographic characteristics of patients that we had seen were different from previous reports. *H. pylori* infection increases with age with a 50% prevalence in those >12 years of age[15], yet in our study, only one of the 12 patients more than 12 years old was infected. We also did not observe an intrafamilial infection. Only one patient had a household member with abdominal pain and diarrhea, but she likewise was negative for *H. pylori* infection. This finding is similar to a previous local report showing no significant association between *H. pylori* infection and household size, crowding and similar complaints of dyspepsia among family members.[8] This is in contrast with a study in Japan where person-to-person transmission between family members, with mother-to-child, parent-to-child, intra-spousal and sibling(s)-to-sibling transmissions have been reported. [16]

Third, only one of the 25 patients in our investigation was positive on RUT, an indirect test of the presence of *H. pylori* on the gastric mucosa as the bacteria produces abundant urease. All our patients were not on antibiotics, bismuth containing compounds or proton pump inhibitors that may result in false negative results on RUT. A positive test requires approximately 10^5*H. pylori* in the antral and gastric mucosa, thus with a low bacterial load and a patchy distribution, the amount of enzyme might not be sufficient to be detected in the tissue sample. This might explain why 16 patients were positive on histopathology but only one on RUT. The occurrence of transient infection and spontaneous clearance has also been reported in children.[17] *H. pylori* has been shown to be abundantly present in gastric and duodenal ulcers, but even among the four patients in our study who had ulcers, only one was noted to be positive on RUT. The sensitivity of various RUT tests as primary diagnostic tests is high and has been reported to vary between approximately 80% and 100% and specificity between 97% and 99% in adults[18] but in a local study, the accuracy of RUT is only 40% using histology alone as a reference for detection of *H. pylori* infection.[14]

In conclusion, the present study showed a low prevalence of *H. pylori* gastroduodenitis among children with gastrointestinal symptoms. Only one patient out of the 25 tested was positive for *H. pylori* IgG serologic test. We are unable to presently recommend the use of the test in our setting.

To evaluate the validity of the serum IgG *H. pylori*, a retrospective study may be done in children who has satisfied the gold standard for the diagnosis of *H. pylori* gastroduodenitis, as this assay is not affected by treatment.

Ethical Considerations

The protocol was sent and approved by the Technical Review Board and EHRO. An informed consent and/or assent was taken and explained to the parents or guardian of the child. Consent was voluntary. The risks of bleeding, pain and possible infection at blood extraction site was indicated in the form and was explained. No monetary compensations were given to patients, relatives or guardians. All information was kept confidential including the result of the study. All specimens and patient information were disposed properly. No serum was kept.

Statement of Authorship

All authors participated in data collection and analysis, and approved the final version submitted.

Author Disclosure

All authors have no conflict of interest.

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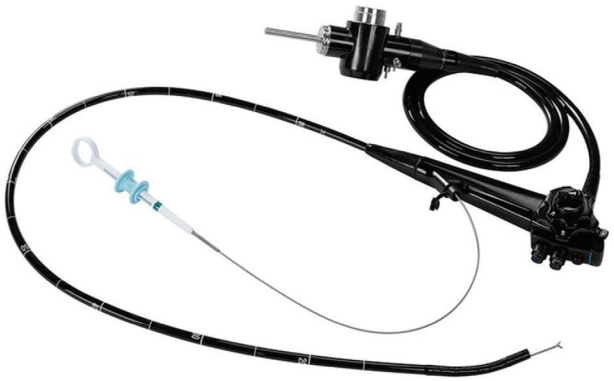
APPENDICES



Appendix 1. EGD scope.

Appendix 2. Forrest's Classification for PU Bleeding

Stage	Characteristics	Rebleeding
Ia	Jet arterial bleeding	90%
Ib	Oozing	50%
IIa	Visible vessel	25-30%
IIb	Adherent clot	10-20%
IIc	Black spot in ulcer crater	7-10%
III	Clean base ulcer	3-5%

Appendix 3. Gastro-Prex Pronto Dry.