Correlation of Serum Antibody Titres with Invasive Methods for Rapid Detection of Helicobacter Pylori Infections in Symptomatic Children

KHALED A. IBRAHIM, M.D. 1; SABRY M. GHANEM, M.D. 2; ABEER M. EL-MAHALAWAY, M.D. 3; AMER ABDELHAMEED, M.D. 4 and ALSAYED A. ABDELWAHAB, M.D. 5

The Departments of Pediatrics 1,2, Histology & Cell Biology 3, Tropical Medicine 4 and Clinical Pathology 5, Faculty of Medicine, Benha and Al-Azhar Universities, Egypt.

Abstract

Background: Helicobacter pylori (H pylori) are causally associated with peptic ulcer disease and gastric carcinoma. Typically children get infected during the first decade of life but diseases associated with H. pylori, are seen mainly in adults. Multiple diagnostic methods are available for the detection of Helicobacter pylori infection, but at present no single one can be used as the gold standard.

Objective(s): To evaluate the correlation and diagnostic accuracy of invasive methods (rapid urease test (RUT), Histology and bacterial culture) and non invasive one (IgG antibodies serologic test) for diagnosis of Helicobacter pylori infection in symptomatic children.

Patients and Methods: This is a prospective cohort study was done on 50 children between 2 and 18 years of age suffering from dyspeptic symptoms. Endoscopy with gastric biopsies was done for rapid urease test, culture and histopathological examination, respectively. Blood sample for IgG antibodies by using commercially available enzyme-linked immunosorbent assay–(ELISA) based technology for detection of Helicobacter pylori infection.

Results: The ages of the 50 patients ranged from 3y5m to 18y [mean±SD (10.6±6.8y)], of whom 64% (32/50) were over 6 years old and 36% (18/50) under 6 years old; 64% (36/50) were male and 36% (14/50) were female. Recurrent abdominal pain was present in 96% (48/50), vomiting was present in 70% (35/50) hematemesis was in 16% (8/50) and chronic diarrhoea was in 10% (5/50). RUT and positive H pylori IgG antibodies agreed in 88% (44/50) of patients, of which both were negative in 32% (16/50) and both were positive in 56% (28/50). Disagreement occurred in 12%: three patients (6%-3/50) presented negative H pylori IgG antibodies tests and three patients (6%-3/50) negative RUT.

Conclusion: Association of the rapid urease test with non-invasive method of serology constituted the best choice for diagnosis of Helicobacter pylori infection in symptomatic children, and bacterial culture alone could not be used as the gold standard due to its low sensitivity.

Key Words: Helicobacter pylori infections – Children – Antibody titres.

Introduction

HELICOBACTER pylori infection is almost always acquired in early childhood and usually persists throughout life unless a specific treatment is given [1]. Helicobacter pylori infection is common in both developed and developing countries. In developed countries 30-50% of the adult population is infected. In developing countries, the prevalence of H. pylori infection is noted even higher-approximately 80% [2].

Helicobacter pylori are important human pathogens, responsible for most peptic ulcer diseases, gastritis, gastric adenocarcinomas and gastric mucosa-associated lymphoid lymphoma, even in the pathogenesis of some extra gastric diseases. In most persons, H pylori infection is largely restricted to the gastric antrum [3].

The vast majority of GI causes of iron deficiency anaemia (IDA) affect the upper GI tract and, in particular, there is a high prevalence of conditions associated with iron malabsorption such as Helicobacter pylori (H pylori) related-pan gastritis [4]. Helicobacter pylori are a slowly growing, microaerophilic, highly motile, gram-negative spiral organism with 4-6 flagella at one end. The organism has the striking biochemical characteristic of abundant urease enzyme production. This enzyme is important for colonization and is an indirect marker.
of the organism’s presence, as it is the basis of rapid urease test (RUT), the urea breath test and as an antigen for a serological test [5,6]. Diagnosis of H pylori can be made with both invasive and non-invasive tests. Invasive tests, including rapid urease, histology and bacterial culture, require endoscopy to obtain biopsies of the gastric mucosa. Non-invasive tests include detection of the bacterial urease by the 13C- or 14C-urea breath tests and serologic testing of the patient’s serum to detect IgG and IgA antibody response to the organism. Recently, a rapid enzyme immune assay (EIA) to detect H pylori antigen in stool has also become available. At present there is no single test for H pylori that can be used as the gold standard [7].

**Aim of the study:**

To evaluate the correlation and diagnostic accuracy of 3 invasive methods (rapid urease test (RUT), Histology and bacterial culture) and non invasive one (IgG antibodies serologic enzyme linked immunosorbent assay test (ELISA) for Helicobacter pylori infection diagnosis in a symptomatic children.

**Patients and Methods**

Fifty children with dyspeptic symptoms (Recurrent abdominal pain–vomiting - diarrhoea - anorexia- failure to thrive–iron deficiency anaemia) selected from Pediatric and Tropical Medicine Departments, Al-Jedaany Hospitals, Jeddah, KSA, were enrolled in this study in the period from November 2007 to April 2009. The age of children ranged from 2-18 years. Patients who had taken antimicrobial agents, antacids, H 2 blockers, a proton-pump inhibitor, or bismuth subsalicylate within 4 weeks before the endoscopy were excluded from the study. Patients were also excluded if they had a past history of infection with H pylori or had a known bleeding disorder or previous endoscopy.

Our study was approved by the clinical research committee of the hospital and performed according to ethical procedures. A written consent was obtained from parents for the participation in our study. A structured questionnaire was administered to the child's parents to collect information on the presenting symptoms. All cases were subjected to full history taking, thorough clinical examination in addition, weight and height measurements.

For detection of Helicobacter pylori infection, endoscopy with gastric biopsies will be done for (rapid urease test, histopathological evaluation and bacterial culture) respectively and blood sample for anti H pylori IgG antibodies by ELISA test.

As there is no single gold standard test for H pylori, an operational gold standard definition of H pylori infection was used. The definition was as follows: Patients with positive culture result were considered as infected. In the case of a negative culture, patients' positive by both RUT and histology were considered as infected. Patients negative by all three gastric biopsy specimen based tests were considered as non-infected. Patients negative by culture and positive by either RUT or histology were considered as indeterminate or when Serological tests were positive were considered as infected [8].

**Gastric biopsy:**

Endoscopy was performed under general anesthesia or conscious sedation (midazolam {0.2 mg/kg} and meperidine {1 mg/kg}). Three antral biopsies within about 2 cm of the pyloric channel were collected by using an Olympus (Center Valley, PA, USA) ZIF XQ230 fiber-optic endoscope: a rapid urease test was performed on the first biopsy, the second biopsy was sent for histopathological evaluation, and the third biopsy was sent for culture.

**Blood collection:**

A 5 mL blood sample was collected within 3 days of the endoscopy and before initiation of any therapy against H pylori. After collection, the blood was kept at room temperature for 1 hour, followed by centrifugation at 1500 rpm for 10 minutes. The serum was aliquot into cryovials and stored at -70°C.

**Rapid urease test:** Immediately after collection, one biopsy was tested for urease activity by using the "hpfast" test (GI Supply, Camp Hill, PA, USA) according to manufacturer instructions. In brief, the biopsy was placed in an agar gel containing urea and 2 pH dye indicators: bromthymol blue and methyl red. Change of the agar colour from yellow to dark green or blue within 24 hours was interpreted as a positive test.

**Histopathological evaluation:** All biopsies were interpreted by the histopathologist. The histopathologist was unaware of the clinician's prediction.

**Light microscopic studies:** Tissue specimens were fixed in 10% neutral buffered formalin, embedded in paraffin and cut in sequential 4-5 µm sections. These sections were stained with Giemsa stain [9] for detection of helicobacter pylori, hematoxylin and eosin stain (Hx&E) [10] for histopathological examination of gastric mucosa and with Genta stain [11] for goblet cells staining.
Scanning electron microscopic study [12]: Biopsy specimens were washed in saline, fixed in a mixture of 2.5% gluteraldehyde in (0.2M) cacodylate buffer (pH.7,4) for 24 hours, then washed in two changes cacodylate buffer, then post fixed for 2 hours in osmium tetroxide, specimens were dehydrated in ascending grade of ethyl alcohol and dried, then fixed on scanning electron microscopy specimen holder, coated with gold and examined by JEOL Model JSM 35 scanning electron microscope, Ltd company, Tokyo, JAPAN.

Transmission electron microscopic study [12]: Biopsy specimens were fixed in a mixture of 2.5% gluteraldehyde in (0.2M) cacodylate buffer (pH.7,4) for 24 hours, then washed in two changes cacodylate buffer, then post fixed for 2 hours in osmium tetroxide, specimens were dehydrated in ascending grade of ethyl alcohol and embedded in Epon 812. The semithin section (1um) were cut by ultramicrotome and stained with toluidine blue and examined by light microscope to show the tissue and for good selection and localization of the needed part to be examined in thin section. The ultrathin section (100nm) were prepared and stained with uranyl acetate and lead citrate and examined by JEOL model XC100 transmission electron microscope.

Bacterial culture:

Immediately after collection, the biopsy sample was placed into 0.1mL of sterile 0.85% saline. Half of the biopsy homogenate was placed onto a Columbia blood agar plate and the other half onto a Columbia blood agar plate with supplement (trimethoprim, vancomycin, and polymyxin B) and tetroxide, specimens were dehydrated in ascending grade of ethyl alcohol and embedded in Epon 812. The semithin section (1um) were cut by ultramicrotome and stained with toluidine blue and examined by light microscope to show the tissue and for good selection and localization of the needed part to be examined in thin section. The ultrathin section (100nm) were prepared and stained with uranyl acetate and lead citrate and examined by JEOL model XC100 transmission electron microscope.

Serum anti –H pylori antibodies ELISA testing:

Serum samples were tested for the presence of anti–H pylori IgG antibodies to specific H pylori antigen (high molecular –weight- cell associated protein (HM-CAP) by using the HM-CAP enzyme-linked immunosorbent assay (ELISA) kit (EZ-EM, Inc, Westbury, New York) according to manufacturer directions. Samples with an ELISA value of <1.8 ELISA units were considered negative, and samples with an ELISA value of >2.2 ELISA units were considered positive. Samples with values between 1.8 and 2.2 ELISA units were considered indeterminate and were retested. A sample that was still indeterminate after the repeat test was recorded as negative.

Analytical plan:

Descriptive statistical tests were expressed as mean ± standard deviation. The differences between the groups were evaluated using the non-parametric Mann-Whitney U test with the threshold of significance set at p<0.05. Cut off values of >2.2 U/ml H pylori IgG were used, as these gave the best sensitivity, specificity, positive and negative predictive value and likelihood ratio.

Results

The present study was carried out on 50 children with dyspeptic symptoms (36 males (64%) & 14 females (36%) & their ages ranged between 2-18 years). The demographic and clinical characteristics of the studied patients are summarized in Table (1) and shows that, there were no statistical differences in patient’s demographic and clinical characteristics at time of admission. The ages of the 50 patients ranged from 3y 5m to 18y [mean 10y 6m (SD±2y8m)], of whom 64% (32/50) were over 6 years old and 36% (18/50) under 6 years old; 64% (36/50) were male and 36% (14/50) were female. Recurrent abdominal pain was present in 96% (48/50), vomiting was present in 70%. (35/50), hematemesis in 16% (8/50) and Chronic diarrhoea was in 10% (5/50).

Table (1): Demographic and clinical characteristics of all studied cases.

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>All ages</th>
<th>&lt;6 years</th>
<th>&gt;6 years</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD (years)</td>
<td>10.6±2.8</td>
<td>10.6±2.8</td>
<td>11±2.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Sex:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>32 (64%)</td>
<td>12 (38%)</td>
<td>20 (62%)</td>
<td>0.2</td>
</tr>
<tr>
<td>Female</td>
<td>18 (36%)</td>
<td>6 (33%)</td>
<td>12 (67%)</td>
<td>0.4</td>
</tr>
<tr>
<td>Clinical characteristics:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrent abdominal pain</td>
<td>48 (96%)</td>
<td>17 (94%)</td>
<td>31 (97%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Vomiting</td>
<td>35 (70%)</td>
<td>12 (67%)</td>
<td>23 (72%)</td>
<td>0.3</td>
</tr>
<tr>
<td>Hematemesis</td>
<td>8 (16%)</td>
<td>3 (17%)</td>
<td>5 (16%)</td>
<td>0.6</td>
</tr>
<tr>
<td>Chronic diarrhoea</td>
<td>5 (10%)</td>
<td>3 (17%)</td>
<td>2 (6%)</td>
<td>0.3</td>
</tr>
<tr>
<td>Others</td>
<td>17 (34%)</td>
<td>6 (33%)</td>
<td>11 (33%)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

p>0.05.
Table (2) showing RUT and positive H pylori Ig G antibodies agreed in 88% (44/50) of patients, of which both were negative in 32% (16/50) and both were positive in 56% (28/50). Disagreement occurred in 12% (6/50), of which both were negative in 32% (16/50) and both were positive in 56% (28/50). Disagreement occurred in 12% (6/50): three patients {6% (3/50)} presented positive RUT and negative H pylori Ig G antibodies tests and the other 3 patients {6% (3/50)} were negative RUT and positive H pylori Ig G antibodies tests. Culture and H pylori Ig G antibodies agreed in 80% (40/50) of patients, of which both were negative in 28% (14/50) and both were positive in 52% (26/50). Disagreement occurred in 20% (10/50); 5 patients {10% (5/50)} presented positive culture and negative H pylori Ig G antibodies tests and the other 5 patients {10% (5/50)} were negative culture and positive H pylori Ig G antibodies tests. Culture and RUT methods agreed in 76% (38/50) of patients, of which both were negative in 26% (13/50) and both were positive in 50% (25/50). Disagreement occurred in 24% (12/50); 7 patients {14% (7/50)} presented positive culture and negative RUT tests and the other 5 patients {10% (5/50)} were negative culture and positive RUT tests.

<table>
<thead>
<tr>
<th>Test</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive RUT and H pylori Ig G antibodies</td>
<td>28 (56%)</td>
</tr>
<tr>
<td>Negative RUT and H pylori Ig G antibodies</td>
<td>16 (32%)</td>
</tr>
<tr>
<td>Positive RUT and negative H pylori Ig G antibodies</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Negative RUT and positive H pylori Ig G antibodies</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Positive culture and H pylori Ig G antibodies</td>
<td>26 (52%)</td>
</tr>
<tr>
<td>Negative culture and H pylori Ig G antibodies</td>
<td>14 (28%)</td>
</tr>
<tr>
<td>Positive culture and negative H pylori Ig G antibodies</td>
<td>5 (10%)</td>
</tr>
<tr>
<td>Negative culture and positive H pylori Ig G antibodies</td>
<td>5 (10%)</td>
</tr>
<tr>
<td>Positive culture and RUT</td>
<td>25 (50%)</td>
</tr>
<tr>
<td>Negative culture and RUT</td>
<td>13 (26%)</td>
</tr>
<tr>
<td>Positive culture and negative RUT</td>
<td>7 (14%)</td>
</tr>
<tr>
<td>Negative culture and positive RUT</td>
<td>5 (10%)</td>
</tr>
</tbody>
</table>

The endoscopic findings in Table (3) was normal in 32% (16/50) and abnormal in 68% (34/50). In 16 patients with a normal endoscopic examination, 75% (12/16) were Helicobacter pylori-negative and 25% (4/16) were Helicobacter pylori-positive. In 34 patients with an abnormal endoscopic examination, 82% (28/34) were Helicobacter pylori-positive and 18% (6/34) were Helicobacter pylori-negative. Cases with abnormal endoscopy showed gastritis in 50% (17/34), esophagitis in 35% (12/34), duodenal ulcer in 18% (6/34) and duodenal erosions in 3% (1/34).

Table (3): The endoscopic findings among studied cases.

<table>
<thead>
<tr>
<th>Normal</th>
<th>Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>No (%)</td>
<td>16/50 (32%)</td>
</tr>
<tr>
<td>H pylori +ve</td>
<td>4/16 (25%)</td>
</tr>
<tr>
<td>H pylori –ve</td>
<td>12/16 (75%)</td>
</tr>
<tr>
<td>Gastritis</td>
<td>0.0</td>
</tr>
<tr>
<td>Esophagitis</td>
<td>0.0</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>0.0</td>
</tr>
<tr>
<td>Duodenal erosions</td>
<td>0.0</td>
</tr>
</tbody>
</table>

In Table (4), shows the sensitivity, specificity, positive predictive value, negative predictive value, the likelihood ratio for positive results (LR+), the likelihood ratio for negative results (LR-) and accuracy of serum H pylori antibodies levels with Cut off values of >2.2 U/ml H pylori IgG were used. (70%, 89%, 76%, 68%, 8.0, 0.8 & 85% respectively), while RUT (98%, 89%, 88%, 98%, 11.1, 1.0 & 92% respectively), histology (92%, 100%, 100%, 90%, 9.2, 0.9 & 95% respectively), and bacterial culture (90%, 100%, 100%, 75%, 9.0, 0.9 & 86% respectively), for diagnosis of Helicobacter pylori infection in symptomatic children. We observed that the greatest sensitivity was achieved by the rapid urease test (98%), followed by histology (92%), serology (70%) and lastly by culture (90%). The highest specificity was obtained by histology (100%) and culture (100%) followed by the rapid urease test (89%) and serology (89%).

Histopathological findings: A section of antrum of stomach in helicobacter pylori infected patient showing rod shaped organism which stain blue by Giemsa stain along the luminal surface and in the luminal mucosa (Fig. 1). In (Fig. 2) showing chronic inflammatory cells as lymphocytes, neutrophils and plasma cells infiltrate foveolar epithelium and superficial lamina propria. In (Fig. 3) showing lymph follicle with germinal cap present in lower portion of mucosa. In (Fig. 4) showing heavy infiltration of full thickness of mucosa with chronic
inflammatory cells as lymphocytes, neutrophils and plasma cells and glands are compressed and separated by inflammatory cells. In (Fig. 5) showing intestinal type of columnar epithelium with goblet cells replaced atrophic gastric mucosa and mild infiltration of lamina propria by chronic inflammatory cells. In (Fig. 6) showing intestinal type of columnar epithelium with goblet cells stained blue with alcian blue stain replaced atrophic gastric mucosa and mild infiltration of lamina propria by chronic inflammatory cells.

**Scanning electron microscopic results:** A section of antrum of stomach in helicobacter pylori infected patient showing ruffle formation of helicobacter pylori on the gastric epithelial cells (Fig. 7). In (Fig. 8) showing degeneration and necrosis of gastric epithelial cells by helicobacter pylori.

**Transmission electron microscopic results:** A section of antrum of stomach in helicobacter pylori infected patient showing gastric lumen showing spiral bacteria adherent by filament like structure to gastric epithelial cells which contain numerous electron dense secretory granules (Fig. 9). In (Fig. 10) showing intestinal metaplastic columnar absorptive cells with surface microvilli and intracytoplasmic helicobacter pylori.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (CI 95%)</th>
<th>Specificity (CI 95%)</th>
<th>Positive predictive value (%)</th>
<th>Negative predictive value (%)</th>
<th>Likelihood ratio positive</th>
<th>Likelihood ratio negative</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H pylori antibody</td>
<td>70 (54.2-80.3)</td>
<td>89 (73.4-96.1)</td>
<td>76</td>
<td>68</td>
<td>8.0</td>
<td>0.8</td>
<td>85</td>
</tr>
<tr>
<td>RUT</td>
<td>98 (90.1-99.2)</td>
<td>89 (70.4-89.8)</td>
<td>88</td>
<td>98</td>
<td>11.1</td>
<td>1.0</td>
<td>92</td>
</tr>
<tr>
<td>Histology</td>
<td>92 (81.4-96.2)</td>
<td>100 (92.0-100)</td>
<td>100</td>
<td>90</td>
<td>9.2</td>
<td>0.9</td>
<td>95</td>
</tr>
<tr>
<td>Culture</td>
<td>90 (78.6-94.4)</td>
<td>100 (92.0-100)</td>
<td>100</td>
<td>75</td>
<td>9.0</td>
<td>0.9</td>
<td>86</td>
</tr>
</tbody>
</table>

Table (4): Performance characteristics of H pylori IgG, rapid urease test (RUT), histology and culture among studied cases.

Fig. (1): A photomicrograph of a section of antrum of stomach in helicobacter pylori infected patient showing rod shaped organism (arrow) which stain blue along the luminal surface and in the luminal mucosa. (Giemsa stain X1000).

Fig. (2): A photomicrograph of a section of antrum of stomach in helicobacter pylori infected patient showing chronic inflammatory cells (C) as lymphocytes, neutrophils and plasma cells infiltrate foveolar epithelium and superficial lamina propria. (Hx & E X100).
Fig. (3): A photomicrograph of a section of antrum of stomach in helicobacter pylori infected patient showing lymph follicle with germinal cap (F) present in lower portion of mucosa. (Hx & E X100).

Fig. (4): A photomicrograph of a section of antrum of stomach in helicobacter pylori infected patient showing heavy infiltration of full thickness of mucosa with chronic inflammatory cells (C) as lymphocytes, neutrophils and plasma cells and glands (G) are compressed and separated by inflammatory cells. (Hx & E X400).

Fig. (5): A photomicrograph of a section of antrum of stomach in helicobacter pylori infected patient showing intestinal type of columnar epithelium with goblet cells (arrow) replaced atrophic gastric mucosa and mild infiltration of lamina propria by chronic inflammatory cells (C). (Hx & E X200).

Fig. (6): A photomicrograph of a section of antrum of stomach in helicobacter pylori infected patient showing intestinal type of columnar epithelium with goblet cells (arrow) stained blue with alcian blue stain replaced atrophic gastric mucosa and mild infiltration of lamina propria by chronic inflammatory cells (C). (Genta stain X 200).

Fig. (7): Scanning electron micrograph of a section of antrum of stomach in helicobacter pylori infected patient showing ruffle formation of helicobacter pylori (arrow) on the gastric epithelial cells. (X100).

Fig. (8): Scanning electron micrograph of a section of antrum of stomach in helicobacter pylori infected patient showing degeneration and necrosis of gastric epithelial cells (E) by helicobacter pylori. (X600).
Fig. (9): Transmission electron micrograph of a section of antrum of stomach in helicobacter pylori infected patient showing gastric lumen (L) and spiral bacteria (one arrow) adherent by filament like structure (two arrows) to gastric epithelial cells and its cytoplasm contain numerous electron dense secretory granules (E). (X 20000).

Fig. (10): Transmission electron micrograph of a section of antrum of stomach in helicobacter pylori infected patient showing intestinal metaplastic columnar absorptive cells with surface microvilli (M) and intracytoplasmic helicobacter pylori (arrow) (X 17,000).

Discussion

Since its discovery many tests have been designed for diagnosis of H pylori. But no test is accurate enough to be the ‘gold standard. Invasive tests have been considerate the gold standard due to high specificity, but biopsy-based methods have disadvantages as risk of anaesthesia, discomfort, inconvenient for the patient, expensive and available only at specialized centres, also may suffer from sampling error, because of the patchy nature of the infection and low concentration of bacteria in fragments. Culturing has low sensitivity, and so no single test can be used as the gold standard and the tendency has been to use a combination of tests in adult and pediatric studies [14].

Non-invasive tests for the diagnosis of H pylori, which are based on analysis of samples of breath, stool, or blood, have been developed. Among the non-invasive tests, the urea breath test (13C urea breath test) is certainly the best, but it is more expensive, not always available (poor feasibility limit), and difficult to apply to the noncompliant child. In addition, its cut off value in pediatric patients remains unsettled [15]. Another non-invasive test is the stool antigen ELISA test. In adults, the test has a sensitivity and specificity of 90%. However, it has the disadvantage of stool samples being difficult to collect and to handle [16,17]. Polymerase chain reaction is another test for detection Helicobacter pylori from stool and is limited in their use due to the presence of inhibitors of H pylori DNA amplification in faeces. Also, H pylori is difficult to culture from stool [18].

Serological tests are widely available and cheap, and may be helpful in screening populations or in confirming the presence of H pylori infection in case of equivocal results of the other diagnostic methods due to bleeding ulcers, antibiotic and/or antisecretory treatments [19]. In our study we evaluate the correlation and diagnostic accuracy of 3 invasive methods {rapid urease test (RUT), Histology and culture} and one non invasive (IgG antibodies serologic ELISA test) for diagnosis of Helicobacter pylori infection in symptomatic children.

Our histopathological findings revealed rod shaped organism which stain blue by Giemsa stain along the luminal surface and in the luminal mucosa, chronic inflammatory cells as lymphocytes, neutrophils and plasma cells infiltrate foveolar epithelium and superficial lamina propria. Our findings also demonstrated by Hala (2006) [20] who reported that helicobacter pylori are found throughout the stomach, in the early stages of disease, H pylori-associated inflammation is often mild, superficial, or even absent in the gastric corpus. Pylori are gram-negative rods that have the ability to colonize and infect the stomach. The bacteria survive within the mucous layer that covers the gastric surface epithelium and the upper portions of the gastric foveolae. The infection is usually acquired during childhood. Once the organism has been acquired, has passed through the mucous layer, and has become established at the luminal surface of the stomach, an intense inflammatory
response of the underlying tissue develops and associated with tissue damage and the histological finding of both an active and chronic gastritis [21].

Our histopathological finding also revealed lymph follicle with germinal cap present in lower portion of mucosa, heavy infiltration of full thickness of mucosa with chronic inflammatory cells as lymphocytes, neutrophils and plasma cells and glands are compressed and separated by inflammatory cells. Ricuarte and his colleagues [22] reported that the presence of a higher density of mucosal mononuclear cells was a predictor for the presence of gastric atrophy in Colombian children with atrophy, suggesting that a high density of mononuclear cells that infiltrate deep into the lamina propria may be a precursor to atrophy. The host response to H pylori and bacterial products is composed of T- and B-cell lymphocytes, denoting chronic gastritis, followed by infiltration of the lamina propria and gastric epithelium by polymorphonuclear leukocytes that eventually phagocytise the bacteria. The interaction of H pylori with the surface mucosa results in the release of proinflammatory cytokine interleukin (IL)-8, which leads to recruitment of polymorphonuclear cells and may begin the entire inflammatory process. Gastric epithelial cells express class II molecules, which may increase the inflammatory response by presenting H pylori antigens, leading to further cytokine release and more inflammation [23,24]. Johanna and his colleagues [25] reported that Lymphocytic gastritis is commonly associated with Helicobacter pylori infection which is characterized by a marked increase in the number of intraepithelial lymphocytes (IELs), most being CD8+ or CD3+ cytotoxic T-cells. Lymphocytic gastritis may represent an atypical host immune response to the infection.

Our histopathological finding also revealed intestinal type of columnar epithelium with goblet cells replaced atrophic gastric mucosa and mild infiltration of lamina propria by chronic inflammatory cells. Atrophy was defined as the loss of normal glands and some glands units develop an intestinal-type epithelium, and intestinal metaplasia eventually occurs in multiple foci throughout the gastric mucosa, while other glands simply are replaced by fibrous tissue, resulting in an expanded lamina propria. There is tight link between H pylori infection, atrophic gastritis and intestinal metaplasia in the stomach of Japanese, H pylori infection may provide the proper environment for atrophic gastritis and intestinal metaplasia to occur [26]. Furthermore, we found a glandular atrophy and intestinal metaplasia more frequent and severe in angulus and antrum. One possibility is that with continued inflammation, antral atrophy may lead to a sufficient destruction of gastrin producing cells to produce and fall in acid secretion, which would allow the development of corpus gastritis.

Significant damage associated with the release of bacterial and inflammatory toxic products is inflicted on the gastric epithelial cells, resulting in increasing cell loss or gastric atrophy over time [20]. George and Frederic [27] reported that gastric atrophy and intestinal metaplasia exist in children and sometimes in young children.

Scanning electron microscopic examination of our tissue sections showing a degeneration and necrosis of gastric epithelium due to adhere of H pylori to epithelium and also ruffle formation of H pylori on the epithelium. Many previous reports have described the ability of H pylori to adhere to epithelial cells by interaction of bacteria with mucosal cell receptors and cytotoxin is released by H pylori cause severe damage to gastric epithelium [28,29]. Finally and Cossart [30] who observed the present of the ruffle formation of H pylori to gastric epithelium by using scanning electron microscopic. The transmission electron microscopic examination of our tissue sections showing a spiral bacteria adherent by filament like structure to gastric epithelial cells and its cytoplasm contain numerous electron dense secretory granules. Adhesion is considered to be an important aspect of bacterial pathogenicity. Adhesion was defined as a close attachment between bacteria and epithelial cells, such that virtually no space was visible between them using transmission electron microscopy. Adherence affords advantage for toxin producing organisms and induces degeneration of microvilli, degeneration of the cytoskeleton with actin polymerisation, depletion of mucus granules, and an increase in sialic acid-rich glycoproteins in the apical part of the cytoplasm [31,32]. In the present study H pylori observed in the cytoplasm of metaplastic columnar absorptive cells. Transmission electron microscopic studies have been performed to demonstrate the morphologic features of H pylori and its ability to invade epithelial cells. This organism has invasive potential and can be demonstrated in the epithelial cells and intercellular tight junctions [33].

Finally electron microscopic finding confirmed the histopathological finding at cellular level.

Our study demonstrated that, invasive tests particularly the rapid urease test are highly sensitive (98%) but low specific (89%) in diagnosis of H
pylori. The specificity is lower due to false positive results. These findings are in accordance with the studies done by many authors [6,7,34]. But the sensitivity of rapid urease test is disagree with Elitsur and his colleagues (1998) [35] who reported that sensitivity of test are low in children. The association of rapid urease test and histology together produces a better accuracy for confirming diagnosis of infection because of high sensitivity of rapid urease test and high specificity of histology methods. In the present study the bacterial culture has low sensitivity (90%) in comparison to other studies [36,37]. However, its specificity was high (100%): incontestable proof of the presence of bacteria [38,39]. Difficulties in isolation and culturing, technical requirements and its relative low availability do not allow this method to be used as a single gold standard.

Our study demonstrated that the sensitivity and specificity results of IgG antibodies by ELISA assay test in children were (70% and 89% respectively). Our results in agreement with other studies who reported a lower sensitivity for serological H. pylori tests in children compared to adults [7,40]. Other studies reported a higher sensitivity for serological H. pylori tests in children [41,42] and a higher specificity [43,44].

Serological tests are commercially available, easy to perform and inexpensive and therefore have been recommended for the diagnosis of H. pylori infection in adults [45,46]. Many serological tests, mainly IgG based, have been validated in adult populations against invasive methods with acceptable sensitivity and specificity for clinical use [13,47,48]. Studies of children showed controversial results, with a large sensitivity range of 50 to 96% and specificity ranging from 83 to 100% [40]. Both the sensitivity and specificity of serological test kits depend on the antigen preparation used [40]. The differences in sensitivity of the same test in children of different ages or geographical origins [50] may be explained by different immune responses of the populations under investigation. There are differences in the antigenicity of the multiple H. pylori strains and even of the different antigens of the same strain. Besides the differential immune responses at different ages, duration of infection may play a role. Since the primary infection occurs most commonly in infants and toddlers, younger children are expected to have a shorter duration of infection than older children and adults [51].

Conclusion: Association of the rapid urease test and histology with non-invasive method of serology constituted the best choice for diagnosis of Helicobacter pylori infection in symptomatic children, and bacterial culture alone could not be used as the gold standard due to its low sensitivity.

References


