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Original article

Comparison of Histopathology and Serological Testing for *Helicobacter pylori* Infection in Paediatric Dyspeptic Patients

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Helicobacter Pylori, Serology, Endoscopy, Prevalence, Dyspeptic, Children.

A B S T R A C T

Helicobacter pylori (H. pylori) infection remains a major health problem in developing countries because of its high prevalence. *H. pylori* colonizes the stomach of children and causes local inflammation. Accurate diagnosis is essential to ensure appropriate treatment and reduce the risk of long-term complications. In Libya, limited data are available regarding *H. pylori* diagnostic methods. The Objective of this study was to compare the diagnostic performance of invasive and non-invasive methods for detecting *H. pylori* infection among pediatric dyspeptic patients (histopathology method and serology method). A gastric biopsy and a blood sample were collected from 120 dyspeptic children attending the gastroenterology units at Tripoli Children's Hospital. The overall prevalence of *H. pylori* infection was 84% using the invasive method, and 51% using the non-invasive method. The histopathology-based diagnostic method is the gold standard diagnostic tool for *H. pylori* detection. However, additional research with a higher number of patients and in other areas of Libya should be conducted in the future to confirm the study's findings.

Introduction

Helicobacter pylori (H. pylori) infection is acquired mainly in childhood, but the majority of infected children are asymptomatic as it colonizes the gastric mucosa and passes asymptomatically in most patients, where it will remain in the gastric cavity throughout life in the absence of eradication therapy [1]. Gastric colonization with *H. pylori* may induce various human pathological illnesses, varying from superficial gastritis and peptic ulcer disease to MALT lymphoma, and gastric adenocarcinoma and its precursors [2]. *H. pylori* is a spiral, S-shaped, gram-negative organism measuring (2 to 4µm) in length and (0.5 to 1µm) in width that lives within and under the human mucosal layer of the stomach [3]. The bacterial infection conventionally occurs during childhood, with a prevalence in children ranging from less than 10% in developed countries to more than 80% in developing countries [4]. *H. pylori* was identified for the first time in children undergoing upper gastrointestinal endoscopy as having histological features of chronic active gastritis [5]. This was followed by other studies, which described the association of the organism with gastroduodenal inflammation as well as the presence of the organism in the gastric mucosa of children with antral gastritis and duodenal ulcers. Moreover, subsequent studies in 1986 demonstrated the healing of duodenal ulcers in two patients following the eradication of the organism [6]. These findings strongly support *H. pylori* as an important pathogen in pediatric gastroenterology.

There are multiple methods for diagnosing *H. pylori* infection, each with varying levels of sensitivity and specificity. However, the primary diagnosis of the infection in children should be conducted using the invasive method, and the evaluation of the response should be conducted using non-invasive methods [7]. Invasive tests are considered the gold standard for the detection of *H. pylori* infection in both children and adults because of their high diagnostic accuracy in comparison to non-invasive methods. Invasive methods include histology, rapid urease test, microbial culture, and polymerase chain reaction (PCR). They all require gastric biopsies [8]. Conversely, non-invasive methods do not require biopsy and are represented by serology, breath test, and stool antigen testing. This study was conducted to determine the prevalence of *H. pylori* infection in pediatric dyspeptic patients attending the endoscopy unit of Tripoli Children's Hospital in Tripoli using Histopathology testing and Serological Testing, and to compare the results obtained by the two techniques (invasive and non-invasive methods).

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Methods

Study Population

A cross-sectional study was conducted to find out the prevalence of *H. pylori* among 120 pediatric dyspeptic patients (49 males and 71 females) aged (<1-14 years) attending the gastroenterology unit in Tripoli Children's Hospital with recurrent abdominal pain, unexplained weight loss, vomiting, and dyspeptic complaints, and in whom endoscopy was indicated. The appropriate permission was obtained from the concerned authorities for sample collection, parents were informed about the purpose of this research study, and a written agreement was given to them before any sample collection.

Exclusion Criteria

To rule out possible false-negative results, patients who have used antibiotics in the last month, proton-pump inhibitors (PPIs) in the last 14 days, H2 receptor antagonists or antacids in the last 24 hours, and those with chronic gastrointestinal diseases were excluded.

Sample Collection

Over the study period, 3 Biopsies were obtained (gastric antrum and corpus biopsies) from the patients in whom esophagogastroduodenoscopy was performed following at least eight hours of fasting. The biopsies were placed in a tube containing 1mL of 10% neutral formalin solution and transferred to the pathology laboratory. Gastric biopsies were submitted for histology, placed in slides, stained with hematoxylin and eosin stain (Abbey Colour, Philadelphia, PA, USA), and assessed by a pathologist. The pathologist organized a report about the findings. He looked for the presence of gastritis and *H. pylori* according to the updated Sydney classification [9]. Before admission to the endoscopy unit, three milliliters (3mL) of venous blood were drawn from the child and put into serum separator tubes with gel for serological tests. The serum was frozen at -20 °C for longer storage before being tested for the presence of *H. pylori*-specific IgG using ELISA antibody kits. Samples with a concentration lower than 5arbU/ml are considered negative for *H. pylori*-Ag IgG. Samples with a concentration higher than 5arbU/ml are considered positive for *H. pylori* -Ag IgG.

Statistical Analysis

Descriptive statistics were used to describe the demographic data. For categorical variables, frequencies and percentages were reported. Comparisons of categorical variables were carried out using Pearson's chi-square test. An a priori two-tailed level of significance was set at 0.05. Agreement between the results of the *H. pylori* diagnostic tests was evaluated by calculating the Cohen's kappa coefficient. Kappa result can be interpreted as follows: values ≤ 0 indicate no agreement, 0.01–0.20 indicate slight, 0.21–0.40 indicate fair, 0.41–0.60 indicate moderate, 0.61– 0.80 indicate substantial, and 0.81–1.00 indicate almost perfect agreement. Statistical analyses were conducted using Statistical Package for the Social Sciences version 24 (SPSS Inc., Chicago, IL, USA).

Results

At Tripoli Children's Hospital, a total of 120 biopsies were collected, of which 50 were excluded as the biopsy results were indicative of other chronic diseases. The overall prevalence of *H. pylori* among pediatric dyspeptic patients using the histopathology technique (invasive method) was 84% (101/120) (Figure 1).

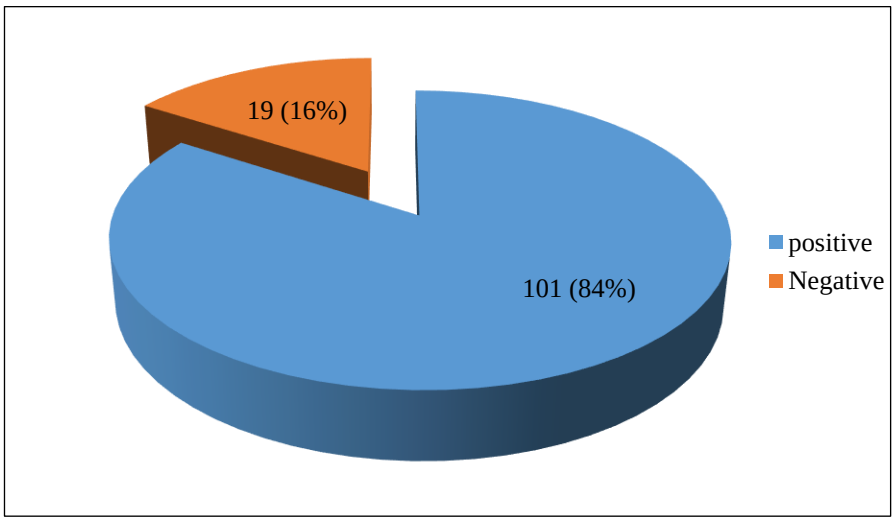

Figure 1. Prevalence of Helicobacter pylori among Children with Dyspeptic Patients using the histopathology technique.

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As shown in (Figure 2), the overall prevalence of *H. pylori* was 51% among pediatric dyspeptic patients using the non-invasive method (Serology). Sensitivity and specificity of the serology test were 56.4% and 73.7%, respectively.

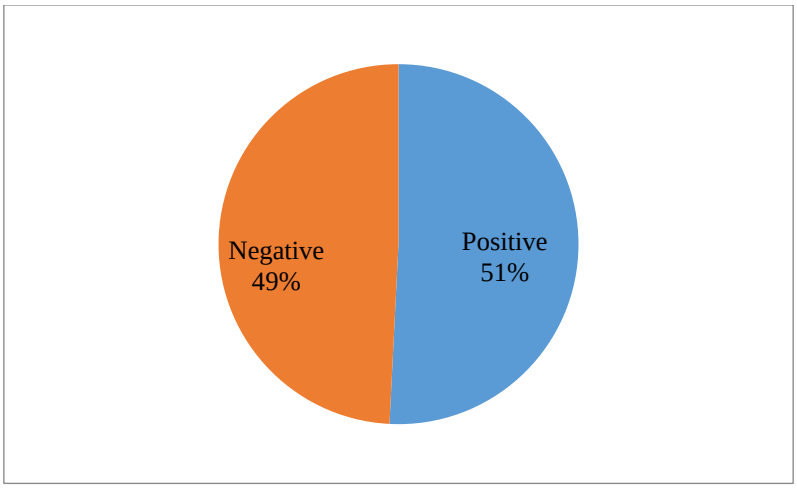

Figure 2. Sero-prevalence of Helicobacter pylori among Children with Dyspeptic Patients.

As a comparison between Invasive and non-invasive techniques used in our study to diagnose *H. pylori* infection (Table 1), a total of 57 patients were positive for *H. pylori* with both techniques. While (44) patients were positive using the invasive method, but negative for the non-invasive method, with a remarkable significance difference (with no or slight agreement) between the two techniques (p= 0.02, kappa 0.16).

Table 1. Comparison Between Invasive and Non-invasive Methods.

Histopathology Results	Sero +ve	Sero -ve	Total	X2	DF	*P*-value	kappa
Positive	57	44	101	5.81	1	0.02	0.16
Negative	5	14	19				

Discussion

The interest in *H. pylori* infection among the paediatric population has been expanding in recent years, and gastric function disorders due to infectious disease have led to studies in this direction. The prevalence of infection in paediatric age varies from country to country and in the same geographical areas, influenced by factors of the host's gastric mucosa, environmental factors, and bacterial virulence [10]. In Libya, this is the first microbiological and histology-based study about *H. pylori* prevalence and impact on Libyan dyspeptic children, and to compare with a non-invasive method (serology).

Histopathology has historically been considered the gold standard diagnostic tool for *H. pylori* detection in suspicious patients with upper gastrointestinal symptoms or in highly prevalent areas [11]. The results of this study are consistent with the view, demonstrating an infection prevalence of 84% among dyspeptic children when assessed by histopathology (invasive method) [12]. Nevertheless, serological testing (Non-invasive method) detected a lower prevalence of 51%.

Concordance between the two diagnostic tests carried out in this study, according to Cohen's kappa analysis, was 0.164, which indicates that there is a slight agreement between the tests. The sensitivity and specificity of the serology test were 56.4% and 73.7%, respectively. Referring to this study, the results of the comparison between the two diagnostic methods (invasive and non-invasive method) were supportive of other studies which concluded that the diagnosis of infection should be based on upper-digestive endoscopy with biopsy-based methods (invasive methods) which is the gold standard method for the diagnosis of the bacterium while eradication control after treatment should be based on validated non-invasive tests [13]. A comparative Iranian study between the different invasive and non-invasive diagnostic techniques was conducted on 91 patients and revealed that for a more accurate diagnosis, it is advisable not to solely rely on non-invasive methods of *H. pylori* diagnosis [14]. Another study from Nepal aimed to compare the accuracy of different diagnostic techniques for the detection of *H. pylori* infection among dyspeptic patients, and the accuracy for histology and serology methods was 91.1% and 80%, respectively [15]. The findings in our study were also in line with a large population-based Chinese study of 7,241 subjects, which reported 70.41% *H. pylori* positive cases by the histopathology method, and 41.87% positive cases by the *H. pylori*-ELISA method [16]. Alternatively, a study from Islamabad revealed that 40 cases (41.67%) were positive for *H. pylori* via histopathology and rapid urease testing, while 46 subjects tested positive for IgA and IgG

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antibodies via ELISA [17]. One of the reasons that led to these differences is that the various serological tests use different antigens, some partially purified, some recombinant, and others a mixture of purified antigens. For this reason, the results of different serological tests may vary even in the same sample populations. Overall, there is a trade-off with antigen purification between loss of sensitivity and increased specificity [18]. However, the practice guidelines from the European and North American Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN and NASPGHAN) advise against using serology testing for initial diagnosis of *H. pylori* infection in a clinical setting. This recommendation is based on the low performance of serology testing compared to the histology-based technique [7].

Conclusion

This study is the first in Libya to detect *H. pylori* infection among pediatric dyspeptic patients using an invasive method and to compare the results obtained with the ELISA technique (non-invasive method). The study concluded that the prevalence of *Helicobacter pylori* infection among 120 pediatric dyspeptic patients was 84% when diagnosed using the histopathology technique, an invasive method, compared to only 51% when assessed through serology, a non-invasive method. These findings indicate and confirm that histopathology is the gold standard diagnostic tool for detecting *H. pylori* in patients with suspected upper gastrointestinal symptoms. In contrast, serological tests are not reliable for confirming infection. Therefore, when upper endoscopy is not feasible, alternative non-invasive diagnostic methods with higher sensitivity are recommended to ensure an accurate diagnosis.

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Competing interests
The authors have declared that no competing interests exist.

Authors' Contributions
This work was carried out in collaboration between the authors.

Disclaimer
The article has not been previously presented or published.

Conflict of Interest
There are no financial, personal, or professional conflicts of interest to declare.

Ethical approvals
The appropriate permission was obtained from the concerned authorities for sample collection, parents were informed about the purpose of this research study, and a written consent agreement was given to them before sample collection.

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