ORIGINAL RESEARCH ARTICLE

Effectiveness of rapid urease, serum antibody, and histopathological detection of helicobacter pylori infection in children

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Abstract

This was an original study, aimed to explore the efficacy of rapid urease, serum antibody and histopathological detection of Helicobacter pylori (Hp) infection in children. One hundred and forty (140)children admitted to Liaoning Cancer Hospital & Institute due to gastrointestinal symptoms who underwent electronic gastroscopy from June 2020 to June 2022 were included. Among the 140 children, 72 were positive for Hp infection by rapid urease, 71 with serum antibody, and 67 with histopathology. The specificities of Hp infection detected by the three methods – rapid urease, serum antibody and histology were 88.7%, 90.6% and 91.7%, respectively. We conclude that the combination of three detection methods of rapid urease, serum antibody and histopathology can improve the sensitivity, specificity, and the accuracy of Hp detection. *(Afr J Reprod Health 2025; 29 [10]: 100-108).*

Keywords: Helicobacter pylori infection; children; rapid urease, serum antibody; histopathological detection

Résumé

Il s'agit d'une étude originale visant à évaluer l'efficacité de l'uréase rapide, des anticorps sériques et de la détection histopathologique de l'infection à Helicobacter pylori (Hp) chez l'enfant. Cent quarante (140) enfants admis Liaoning Cancer Hospital & Institute pour symptômes gastro-intestinaux et ayant subi une gastroscopie électronique entre juin 2020 et juin 2022 ont été inclus. Parmi ces 140 enfants, 72 étaient positifs pour l'infection à Hp par l'uréase rapide, 71 par les anticorps sériques et 67 par l'histopathologie. Les spécificités de l'infection à Hp détectée par les trois méthodes – uréase rapide, anticorps sériques et histologie – étaient respectivement de 88,7 %, 90,6 % et 91,7 %. Nous concluons que la combinaison de ces trois méthodes de détection – uréase rapide, anticorps sériques et histopathologie – peut améliorer la sensibilité, la spécificité et la précision de la détection de Hp. *(Afr J Reprod Health 2025; 29 [10]: 100-108).*

Mots-clés: : Infection à Helicobacter pylori, enfants, uréase rapide, anticorps sériques, détection histopathologique

Introduction

Helicobacter pylori (Hp), is a slow-growing, gram-negative, spiral-shaped microbe that colonizes the human stomach mucosa and is a common cause of digestive diseases. Since Marshall and Warren successfully isolated the microbe from the gastric mucosa biopsy in 1983, it has attracted extensive attention from clinical scholars[1,2]. According to the 2011 "Clinical Guidelines of the World Organization of Gastroenterology-Helicobacter pylori infection in developing countries"[3], there are differences in the infection rate of Hp among children of different ages. About 10% of children under the age of 10 are infected, with geographical differences. According to available data[4], China is one of the countries with high incidence of Hp, with up to 50% of personsaround the age of 20 years old being affected. This has become a major public health problem.

The only source of Hp infection is within the population, and it is transmitted through oral cavity and feces, which is closely related to poor dietary hygiene, bad dietary habits, and unsafe drinking water. Among them, adult Hp infection mostly begin in childhood. Hp in children mostly comes from older family members, with significant family clustering[5,6]. A study has pointed out that

children infected with Hp who are not treated in time may suffer from growth, development, and nutritional challenges [7]. In addition, they may suffer from gastrointestinal diseases such as chronic active gastritis, gastrointestinal ulcers along with gastric cancer in adulthood, and may also affect the body's blood system, immune system, and nervous system, seriously threatening their quality of life. Therefore, early screening and intervention for Hp-infected diseases in children is crucial.

With the development of medical technology, there are various methods for the diagnosis of Hp infection, which can be separated into invasive and non-invasive ones. Invasive diagnosis involves the in-vivo detection of diseased tissues under electronic gastroscope, including rapid urease test, histological examination, and genetic examination, with certain trauma[8]. Non-invasive screening mainly includes breath test, serum antibody test, and fecal Hp antigen test[9,10]. Therefore, how to quickly and sensitively detect the presence of Hp infection is essential for the diagnosing and treating upper digestive diseases. At present, studies in China and abroad mostly focus on the comparison of two detection methods for Hp infection in adults, and few studies analyze the comparison of rapid urease, serum antibodies, and histopathological detection of Hp infection in children[11,12].

Given this background, this study explored the comparative observation of rapid urease, serum antibody and histopathological detection of Hp infection and makes a recommendation on the most effective ways to diagnosis Hp in children.

Methods

In this study, 140 children admitted to Fujian Children's Hospital due to gastrointestinal symptoms who underwent electronic gastroscopy from June 2020 to June 2022 were selected. These included 77 boys and 63 girls, with the average age of 10.75 ± 2.47 years old. The gastroscopy and pathological biopsy confirmed that there were 34 cases of chronic superficial gastritis, 7 cases of erosive gastritis, 12 cases of bile reflux gastritis, 28 cases of nodular gastritis, 18 cases of duodenal bulbar inflammation and 41 cases of peptic ulcer. All patients underwent rapid urease, histopathological, and serum antibody tests.

Inclusion and exclusion criteria

The inclusion criteria were: Children with gastrointestinal diseases such as upper abdominal pain, xiphoid process discomfort, malignant vomiting, anorexia, belching, and hematochezia; Patients confirmed to be infected with Helicobacter pylori by gastroscopy and pathological biopsy; Age ≤15 years old; Children who received rapid urease, histopathological examination and serum antibody test; Children had no cognitive disorder or mental disorder and with normal communication; All children and their families knew the content of the study and signed the informed consent form voluntarily.

The exclusion criteria were: Children had unintentional, severe vascular and liver and kidney tissue disorders or malignant tumors; Children with diseases of the haematological system or autoimmune system and severely infected persons; Patients who had taken antibiotics for inhibiting Helicobacter pylori, H2 receptor blockers, bismuth, and proton pump inhibitors recently; Patients with incomplete clinical data; Patients who failed to cooperate with the completion of the test; Patients with allergies and contraindications to the therapeutic drugs used in this study.

Research methods

Rapid urease test: Two gastric mucosa samples were taken 5 cm from the gastric antrum to Youmen Gate by forceps under electronic gastroscope, and then put into gastric Hp detection reagent (Shanghai Huitai Medical Technology Co., Ltd.) for detection. The results were reflected at room temperature (15-30℃) for 1-3 min and observed by visual inspection. The positivity was that the mucosa sample around the tissue had a light yellow to red color; If it changed to red within 1min, it was strongly positive; If it turned red within 1–3 min, it was weakly positive. The tissue surrounding mucosa sample was negative if there was no color change after 3 min[13]. Wherein that more red change, the more helicobacter pylori in gastric mucosa.

Serum antibody detection: Two mL fasting venous blood was obtained , and the serum was

separated. Fresh samples were used as much as possible. If not detected in time, the samples would be stored at 2-8 ℃ for 3 d. The specific operation procedures were as follows: that reagent was taken out from the original aluminum box, place on a clean and tidy platform, serum samples were sucked by a plastic suction tube, about 3 drops of serum were added with dropwise and placed in the sample adding hole of the reagent, and the results were interpreted within 10–20 min by visual inspection at room temperature. The positive result showed two red bands, while the negative result showed only one red band in the control area[14].

Histopathological examination: One to two mucosal tissues such as gastric antrum and body were clamped under the gastroscope for histopathological examination. The tissue samples were immobilised in 10% neutral formalin for 12 h, dehydrated with different concentrations of ethanol and made transparent in xylene, embedded in paraffin, frozen on a microtome and sectioned into sections with a thickness of 3 m, and stained by dehydration, dewaxing and toluidine blue staining. The staining operations were as follows: the paraffin sections were deparaffinized with xylene and stained with 1% toluidine blue solution for 5-8 min, followed by water washing, dehydration with 95% ethanol, transparentizing with xylene, and the sections were sealed with neutral gum. The staining results and morphological characteristics of bacteria were then observed under a microscope. The slides were read by two experienced pathologists in a double-blind method, and the presence of Hp infection was judged following the consensus opinion of the National Symposium on Chronic Gastritis and the pathological judgment criteria of gastric and duodenal mucosal biopsy[15], with the positive being brown or brown curve.

Clinical diagnosis criteria for Hp infection

Hp infection can be judged according to the "Expert Consensus" on the diagnosis and treatment of Helicobacter pylori infection in children in 2015[16] if one of the following four conditions is met: Both histopathological examination and rapid urease test are positive; If the histopathological examination and rapid urease test results are inconsistent, further non-invasive examinations such as serum antigen test shall be conducted; If the patient suffers from peptic ulcer bleeding, one of the histopathological examination and rapid urease test shall be positive.

Observed indices

1. Positive rates of Hp infection detected by the three detection methods were compared.
2. With the total detectable rate of Hp infection as the gold standard, we compared the diagnostic efficacy of three methods for detecting Hp infection, including true positive, true negative, false positive, false negative, sensitivity along with specificity. Sensitivity = number of true positive / (number of true positive + number of false negative) × 100%. Specificity = number of true negative / (number of true negative + number of false positive) × 100%.
3. The detection rates of Hp infection in digestive diseases of children, including chronic superficial gastritis, erosive gastritis, bile reflux gastritis, nodular gastritis, duodenal bulb inflammation along with peptic ulcer, were compared.

Statistical analysis

SPSS 24.0 statistical software was employed for data analysis. The data conforming to the normal distribution were exhibited as ($\bar{x}\pm s$), followed by comparison using t test. Enumeration data were exhibited as case number (N) and percentage (%), followed by comparison using χ^2 test, and $P<0.05$ indicated that the difference was statistical significance.

Ethical consideration

Our study was approved by the ethics committee of Liaoning Cancer Hospital & Institute, and the approval date was January 2020.

Results

*Positive rate of Hp infection detected by three detection method***s**

Among the 140 children, 72, 71 and 67 cases were positive for Hp infection by rapid urease, serum antibody and histopathological examination, respectively, with the positive rates of 51.4%%, 50.7% and 47.9%, respectively ($P>0.05$).

Table 1: Comparison of the positive rate of Hp infection detected by three detection methods

Group	Number of cases	Positive	Negative	Positive detection rate	χ^2 value	*P* value
Rapid urease test	140	72	68	51.4%	6.2	0.0
Serum antibody detection	140	71	69	50.7%		
Histopathological examination	140	67	73	47.9%		

Table 2: Comparison of diagnostic efficiency of three methods for detecting Hp infection

Group	Number of cases (n)	True positive (n)	True negative (n)	False positive (n)	False negative (n)	Sensitivity (%)	Specificity (%)
Rapid urease test	140	65	55	7	13	83.3	88.7
Serum antibody detection	140	69	60	2	9	88.5	96.7
Histopathological examination	140	70	59	3	8	89.7	95.2

Table 3: Comparison of the positive rate of Hp infection detected by three detection methods (cases, %)

Group	Number of cases (n)	Rapid urease test (+)	Serum antibody detection (+)	Histopathological examination (+)	Hp infection (+)
Chronic superficial gastritis	34	19 (55.9)	16 (47.1)	18 (52.9)	18 (52.9)
Erosive gastritis	7	2 (28.6)	2 (28.6)	2 (28.6)	2 (28.6)
Bile reflux gastritis	12	3 (25.0)	2 (16.7)	2 (16.7)	2 (16.7)
Nodular gastritis	28	21 (75.0)	23 (82.1)	20 (71.4)	23 (82.1)
Duodenal bulb inflammation	18	4 (22.2)	4 (22.2)	3 (16.7)	4 (22.2)
Peptic ulcer	41	23 (56.1)	24 (58.5)	22 (53.7)	29 (70.7)

Table 4: Analysis of results of the three detection method for detecting the digestive tract diseases

Group	Rapid urease test (+) Histopathological examination (-) Serum antibody detection (+)	Rapid urease test (-) Histopathological examination (+) Serum antibody detection (+)	Rapid urease test (+) Histopathological examination (+) Serum antibody detection (-)
Chronic superficial gastritis	1	0	1
Erosive gastritis	0	0	0
Bile reflux gastritis	1	0	0
Bodular gastritis	3	2	0
Duodenal bulb inflammation	0	1	0
Peptic ulcer	7	4	5
Total	12	7	6

A total of 50 of 56 children who met the criteria for histopathological examination and rapid urease test were positive for serum antibody, and 21 of 27 patients who had inconsistent results of histopathological examination and rapid urease test were positive for serum antibody. At last, 78 cases of Hp infection were diagnosed, and 62 cases were negative, with the positive rate of 55.7%, as shown in Table 1.

Diagnostic efficiency of three methods for detecting Hp infection

The outcomes of rapid urease, serum antibody and histopathological examination of Hp infection in 140 children exhibited no significant difference ($P>0.05$). Taking the total detection rate of Hp infection as the gold standard, the sensitivities of rapid urease, serum antibody and histopathological examination of Hp infection were 83.3%, 88.5% and 89.7%, respectively. The sensitivity of serum antibody for Hp infection was higher than that of rapid urease ($P<0.05$). The specificity of the three methods for the detection of Hp infection was 88.7%, 96.7%, and 95.2%, respectively, but with no significance ($P>0.05$, Table 2).

Positive rate of Hp infection detected by three detection methods

In 140 cases, 34 cases were chronic superficial gastritis, with a Hp infection positive rate of 52.9%; Seven cases were erosive gastritis, with a Hp infection positive rate of 28.6%; 12 cases were bile reflux gastritis, with a Hp infection positive rate of 16.7%; 28 cases were nodular gastritis, with a Hp infection positive rate of 82.1%; 18 cases were duodenal bulbitis, with a Hp infection positive rate of 22.2%; 41 cases were peptic ulcer, with a Hp infection positive rate of 70.7%. It could be seen that the Hp infection rate in the nodular gastritis presented higher than that in the chronic superficial gastritis and peptic ulcer ($P<0.05$, Table 3).

Results of the three detection methods for detecting the digestive tract diseases

Using the detection of Hp infection in digestive tract diseases of children as the gold standard, 25 cases showed the results inconsistent with the three tests, including 12 cases with rapid urease positive, histopathological negative and serum antibody positive tests, 7 cases with rapid urease negative, histopathological positive and serum antibody positive tests, and 6 cases with rapid urease positive, histopathological positive and serum antibody negative tests, as shown in Table 4.

Discussion

Under normal circumstances, the gastrointestinal system has perfect self-protection mechanisms, including gastric acid, gastric protein secretion function and the protection of mucus layer, which can effectively resist the invasion of foreign microorganisms[17]. As the only Gram-negative and microbial species that can survive in the stomach and duodenum, Hp can penetrate the natural barrier of the mucus layer and settle on the surface of the gastrointestinal epithelial cells, and can also destroy the gastrointestinal epithelial cells, cause various inflammatory factors and reactions, etc., promote the gastrointestinal environment disorder, and lead to various types of acute and chronic gastrointestinal inflammation, even gastric cancer, etc.[18,19].

Most adult Hp infections begin in childhood and show dyspepsia, abdominal pain and diarrhea, which are difficult to attract the attention of parents and medical staff and miss the optimal treatment time. However, once Hp infection in childhood is not diagnosed and treated in time, it will carry pathogenic bacteria for the whole life, causing a series of digestive system diseases in adulthood[20,21]. It has been verified by several studies[22] that the harm done by Hp infection is not only limited to the gastrointestinal and digestive system, but also damages the nutritional mechanism, growth and development, nervous system diseases of the body, and even causes gastric cancer, affecting the life quality and safety of patients, etc. At present, triple therapy (proton pump inhibitor + clarithromycin + amoxicillin or metronidazole) is adopted for the disease caused by Hp infection after early diagnosis, and the individual risk of cancer transformation can be reduced by adopting the treatment scheme as soon as possible[23].

Therefore, there is a need for a safe, efficient, and child-friendly detection of Hp infection, which is important for the prevention and

treatment of upper gastrointestinal disease. With the continuous innovation and progress of medical detection level, the commonly used detection methods for Hp infection are mainly rapid urease, histopathological examination and serum antibody examination, etc., but there are few studies comparing the three methods for the detection of Hp infection in children[24]. Therefore, in this research, we compared the sensitivity and specificity of three different detection methods for Hp infection in children, so as to provide reference for disease diagnosis and treatment.

Hp, as a bent, spiral or S-shaped gram-negative, microaerophilic bacillus, can decompose urea and generate ammonia through urease on that surface of a bacterial biofilm, form an ammonia cloud, surround the thalli, and generate catalase and superoxide dismutase to avoid being killed by neutrophils and then colonize in stomach and duodenum of the human body; In addition, Hp can tightly connect with epithelial cells through adhesin, and promote massive secretion of gastrin, causing increase of gastric acid concentration and injury of gastric mucosa to form gastric ulcer. At the same time, it secretes a variety of cytotoxins, inflammatory mediators, acid secretion factors, and so on, thus causing gastrointestinal diseases and finally inducing gastrointestinal tumors[25].

Therefore, early diagnosis and treatment of Hp infection exert an important function in preventing and controlling disease. Bacterial culture, as the gold standard for diagnosing Hp infection, cultivates Hp flora under specific conditions with high specificity and sensitivity, but it is difficult to widely carry out Hp culture because of the need of nutrient-rich medium, special gas environment, demanding pathological tissue specimen, and the long culture time and high cost[26]. Compared with the bacterial culture method, the rapid urease, serum antibody, and histopathological detection has the advantages of simple operation, convenient sampling, and short detection time, which is more suitable for the detection of Hp infection in clinical practice.

The results of this study indicate that the positive rate of Hp infection detected by rapid urease, serum antibody and histopathology in 140 children was not statistically significant, and 78 cases of Hp infection along with 62 cases of negative Hp infection were finally diagnosed. The difference in the results of HP infection detection among the three groups was not statistically significant. Taking the total detectable rate of Hp infection as the gold standard, the sensitivities of rapid urease, serum antibody, and histopathology for detecting Hp infection were 83.3%, 88.5% and 89.7%, respectively, and the sensitivity of serum antibody for detecting Hp infection was higher than that of rapid urease. There was no difference in the specificity of the three methods for the detection of Hp infection. Rapid urease, serum antibody, and histopathological detection of Hp infection each had advantages and disadvantages. Among them, the rapid urease detection principle is that Hp can generate urease with high activity, urease can hydrolyze urea to generate ammonia and carbon dioxide, and the synthesis of ammonia promotes the alkalinity in gastric mucosa tissue to increase, which can cause pH reagent pHenol to change from colorless to red or purple, so whether Hp infection exists can be judged according to the color change of pH reagent paper, and the method possesses the advantages of fast reaction speed, convenient operation along with low price.

However, the method is easily affected by factors such as sampling site, tissue size, environmental temperature, and the like, and higher false negative and false positive results exist[27]. The detection of serum antibody is mainly due to that presence of flagellum, a plurality of antigen component and the like on the surface of Hp thalli, which induce the body to produce an inflammatory reaction and stimulates the antibody level to be significantly increase, and different antibody types are produced by different bacterial types, thereby judging the severity of gastropathy, drug resistance and the like. the qualitative detection of the anti-Hp antibody in serum by enzyme linked immunosorbent assay has the advantages of non-invasiveness, high speed, high sensitivity and the like, but the detection result of most diagnosed children is negative, Moreover, the test results turned positive after follow-up for several months, probably because the corresponding antibody was not produced until several weeks after the body was infected with Hp, but the antibody could still be maintained in the body for six months or even one year after anti-Hp treatment, which could not

accurately reflect the current infection situation[28]. There are many histopathological detection methods. Once there is Hp infection in the body, there will be pathological changes in the related gastric mucosa tissues. After a series of operations such as fixation, embedding, fixation and staining, the collected pathological tissues can be observed for the distribution of Hp flora and infection under the microscope, with high specificity and sensitivity. However, this detection method is tedious to operate, takes a long time, and is vulnerable to the limitation of sampling. Moreover, it belongs to invasive examination and is not suitable for reexamination and general survey[29]. Therefore, each of the above three methods has advantages and disadvantages. It is necessary to select the appropriate test protocol according to the wishes of patients and examination needs, which is of great value for disease screening and improving the therapeutic effect.

Hp infection has an important role in the occurrence of gastrointestinal diseases, especially in chronic gastritis as well as peptic ulcer. Among the patients with chronic gastritis, nodular gastritis and superficial gastritis have relatively high Hp infection, and nodular gastritis mostly presents granular or nodular pathological changes with uniform color in the gastric area with moderate to severe inflammatory reactions[30]. The outcomes of this study manifested that among the 140 children, the Hp infection rate in patients with nodular gastritis presented higher than patients with chronic superficial gastritis and peptic ulcer through rapid urease positive, histopathological negative and serum antibody positive tests. Taking the detection of Hp infection in digestive tract diseases of children as the gold standard, 25 cases showed that the results of the three tests were not consistent, including 12 cases with rapid urease positive, histopathological negative and serum antibody positive tests, 7 cases with rapid urease negative, histopathological positive and serum antibody positive tests, and 6 cases with rapid urease positive, histopathological positive and serum antibody negative tests. To analyze the reasons for the inconsistent results of rapid urease and histopathological examination, it was possible that the distribution of Hp in the stomach was different under normal conditions, and the density in the gastric antrum was relatively high. However, rapid urease sampling mainly included the selection of a tissue in the gastric antrum and around the pylorus, and histopathological sampling mainly included the selection of a local section from a tissue at the bottom of the stomach. In other patients, taking anti-acid drugs could lead to the decrease in the density and disorder of distribution structure of Hp colonies in the stomach, leading to false-negative rapid urease imaging. In addition, the existence of digestive tract condition in the body or the existence of Helicobacter, vibrio and other flora in histopathological examination may also cause the inconsistency between the two test results. Therefore, when the conditions permit, the combination of multiple detection methods can improve the accuracy and specificity of the test results[31].

Study strengths and limitations

The main strength of our study was to explore the sensitivity and specificity of rapid urease, serum antibody and histopathological detection of Hp infection in children, which provide the clinical reference for Hp detection in children. The single-center study and limited sample size were the limitations of our study.

Conclusion

There is no significant difference in the positive detection rate and specificity of rapid urease, serum antibody and histopathological examination in the detection of Hp infection. However, serum antibody examination has the advantages of convenient operation, no trauma, high sensitivity and so on, which can be taken as the preferred choice. For patients with gastrointestinal diseases that cannot be determined by serum antibody testing, rapid urease and histopathological examination can be combined to improve the reliability of the test results.

Conflicts of interest

We have no conflicts of interest to declare in this study.

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Data availability

Original data generated/analyzed in this study can be obtained from the corresponding author on reasonable requirements.

Authors contributions

Yang D drafted the manuscript and performed data analysis, Mo TR, Chen Q collected data and assisted in data analysis, Liang L revised the manuscript, and concepted and supervised the study.

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