Photon Journal of Pediatrics

Comparative Characteristics of Different Methods for Diagnosis of *Helicobacter pylori* in Children

Sorokman Tamila Vasylivna[a*], Andriychuk Denys Romanovych[a], Sokolnyk Snizhana Vasylivna[b], Popeluk Nataliya Olexandrivna[c].

[a] Pediatrics and Medical Genetic of Bucovinian State Medical University, Ukraine
[b] Pediatrics and Medical Genetic of Bucovinian State Medical University, Ukraine
[c] Pediatrics, Neonatology and Perinatal Medicine of Bucovinian State Medical University, Ukraine

Corresponding Author:
Sorokman T.V.*, Ph.D, MD
Head of the Department
Professor
Email: t.sorokman@gmail.com
Phone: +380506642667

Andriychuk D.R., Ph.D, MD
Assistant of the Department
Email: andreychuk_denis@mail.ru
Phone: +380506785365

Sokolnyk S.V., Ph.D, MD
Docent of the Department
Email: t.sorokman@gmail.com
Phone: +380509368747

Popeluk N.O., Ph.D, MD
Docent of the Department
Email: t.sorokman@gmail.com
Phone: +380506028493

Abstract

Comparative evaluation of the results of different methods of infection *H. pylori*, the study involved 120 patients from 7 to 18 years of pathology of the upper gastrointestinal tract. Fibrogastroduodenoscopy was conducted with fence biopsies from the body, antrum and duodenum bulb. Verification of *H. pylori* was performed by the following methods: express methods (industrial sets CLO-test the company Delta (Australia), De-Nol-test firm Yamanouchi (Japan)) and laboratory tests (Campy-test (Russia), Helikotest (Russia) and rapid urease test (Russia)), histological investigation, PCR detection ureC, CagA, VacA gene pathogenicity islands microorganism, biological, enzyme-linked immunosorbent assay (ELISA) for the detection of specific immunoglobulin classes M, A and G to CagA *H. pylori* antigen in serum. There was established that all express tests have a high sensitivity and specificity. To improve the accuracy of diagnosis helicobacter infection it is recommended to use at least two, and preferably three, research methods, preferably a combination of express urease test or De-Nol-test with histological methods (biopsy of the antrum of the stomach) or PCR (gene detection ureC, CagA and VacA). Helic testis recommended as an exact noninvasive method for assessing the effectiveness of eradication therapy, especially by children.

1. Introduction

1.1

Helicobacter pylori infection is one of the most serious problems of gastroenterology due to the fact that the prevalence of infection with *Helicobacter pylori* (*H. pylori*) is progressively increasing, the disease is often detected in young working age and this microorganism is recognized cancerogene of first order (Maastricht-3 Guidelines for *Helicobacter pylori* infection, 2005). Thus, the development of algorithms for early and exact diagnostics of *Helicobacter pylori* infection will improve quality of treatment these patients. In addition, more attention is paid to the problem of re-infection, and therefore the necessity clarification of terms of control tests for *H. pylori* for differentiation reinfection and failure of eradication therapy.

1.2 Review of the literature

During numerous comparative studies found that the results of different methods are not always identical, so to avoid getting a false-negative or false-positive results, more exact diagnostics of the presence of infection must use at least two methods and the result is considered positive or negative in the case of coincidence indices of both methods of investigation (Isakov, 2011). Some authors even recommend the use of three methods in order to talk about absence of infection (Pacifico, 2010; Moya, 2012; Kindermann, 2009).

1.3

A comparative analysis of the effectiveness of different methods for diagnostics of *H. pylori* in adult patients with *H. pylori-* associated diseases (Isakov, 2011), it was found that according to the rapid urease and breath test and *H. pylori* was detected in 100 % of patients, histological examination determined 70 %, by polymerase chain reaction (PCR) - 70% of patients. We found that the results of bacteriological methods in 25 % of cases were negative with the positive results of other methods of investigation, due to the complexity of culturing *H. pylori*, therefore, only the data bacteriological method is not recommended to navigate to avoid false negative results (Hirschi et al., 2007).

1.4 Objective of research

Main aim - to compare different methods of diagnostic of *Helicobacter pylori* in children.

1.5

Main problem - different methods of diagnostic of Helicobacter pylori have different sensitivity and specificity especially in pediatric gastroenterology.

1.6

We want to compare different methods of diagnostic of Helicobacter pylori in children in our region.

2. Materials and Methods

2.1

We performed a comparative evaluation of the results of different methods of infection *H. pylori* with further development of optimization algorithm diagnosis of *pyloric Helicobacter* infection.

2.2

There were examined 120 patients from 7 to 18 years of pathology of the upper gastrointestinal tract (37 % of patients with peptic ulcer disease, 63 % - with chronic gastroduodenitis). The study involved the observance of the concept of informed consent on the basis of ethical principles in relation to children who are the subject of research (World Medical Association Declaration of Helsinki 1964; 2000; 2008).

2.3

Patients underwent fibrogastroduodenoscopy with biopsy from the body and antrum and duodenal bulb (Sydney-Houston System, 1996). Verification of *H. pylori* was performed by the following methods: express methods (industrial sets CLO-test the company Delta (Australia), De-Nol-test firm Yamanouchi (Japan)) and laboratory tests (Campy-test (Russia), Helikotest (Russia) and express urease test (Russia)), histological investigation, PCR with detection ureC, CagA, VacA gene pathogenicity islands microorganism, bacterilogical, enzyme-linked immunosorbent assay (ELISA) for the detection of specific immunoglobulin classes M, A and G to CagA *H. pylori* antigen in serum by conventional method using diagnostic test-system HelioBest-antibodies (series D-3752) and a set of reagents company BEST Vector (Novosibirsk, Russia). Results evaluated with a spectrophotometer by measuring the optical density at a wavelength of 450 nm.

2.4

Results of the study are the number of observations in the group, percentages or average and standard deviation, the exact value was determined by the correlation coefficient (r). Reliability difference between the relative values determined by Fisher's angular transformation «Pφ».

3. Justification of Research

This research is very important in pediatrics and child gastroenterology, because when patient have *H. pylori* we must administrate eradication therapy. To diagnose this infection doctors must use highly informative test for indication *H. pylori*. We try to find most specific and sensitive method of diagnostic of H. pylori. That means, the doctor can choose method of diagnostic of Helicobacter pylori and not administrate of all methods. It saves time, money and recourses.

4. Results

4.1

By comparison of the results, it was found that the maximum number of positive results was determined using the express urease test, the minimum amount - when seeding biopsies (Fig. 4.1).

Figure 4.1: Comparative description of the results of various diagnostic methods H. pylori (U - urease test, H - helicon-test, Hyst - histology, PCR - polymerase chain reaction ureC, IEA - ELISA , Bact - bacteriological study). The vertical axis - research methods, X-axis - the number of positive results, %
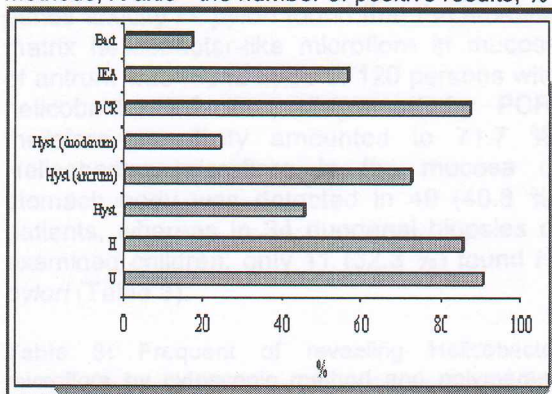
Bact.	17 %
IEA	56 %
PCR	87 %
Hyst (duodenum)	24 %
Hyst (antrum)	72 %
Hyst	45 %
H	85 %
U	90 %

4.2

In addition, correlation analysis on match performance of different methods of diagnostics, according to which established a correlation between the results of the urease test and PCR (gene ureC) (r=0.67, p<0.01), urease test and histological examination (H. pylori in the stomach) (r=0.59, p<0.05), helic-test and histological examination (H. pylori in the stomach) (r=0.70, p<0.01). On this basis, it can be argued that the use of combinations of these methods will be the most informative for the diagnostics of infection.

4.3

Express-diagnostics of pyloric helicobacter pylori based on the properties of H. pylori in large numbers (compared to other microorganisms) secrete urease that breaks down urea, which is part of a diagnostic test for carbon dioxide and ammonia. As a result, the pH shifted to the alkaline side and recorded by changing the color of diagnosticum. Exposure time of biopsy transfer in environment is different. Thus, by performance CLO-test and Campy-test results obtained after 24 hours, conducting Helicotest allowed to conclude that the presence of H. pylori for 2 hours. The most express tests were De-Nol-test and urease quick test, in which the outcome was assessed for 5-20 min. All express tests had different sensitivity and specificity (Table 1).

Table 1: Comparative characteristics of express-methods for diagnostic H. pylori

Express-tests	Sensitivity	Specificity
CLO-test	95,1%	100%
Campy-tesy	92,1%	94,7%
Helico-test	96,3%	100%
De-Nol-test	96,8%	100%
Urease test	96,5%	100%

4.4

Rate of change of color express diagnosticums depend on the degree of sowing mucosa by microorganisms, confirmed following histological investigation. The most expressed, this pattern was observed during the De-Nol test and urease test. With a high degree sowing mucosa of pyloric bacteria changing color of these diagnostics do not exceed 5 minutes if the color change occurred within 5-15 minutes, the degree of sowing was mainly middle and low level at sowing came the reaction after 15-30 minutes. If the response time of the test exceeded 30 minutes, the result was considered doubtful.

4.5

Genotyping H. pylori in individuals studied by PCR performed with primers specific for locus of genes responsible for the synthesis of CagA and VacA. The results of the study are presented in Table 2.

Table 2: Results of genotyping of Helicobacter pylori

Strain of Helicobacter pylori									
H. pylori (tox+), n=86								H. pylori (tox -), n=34	
CagA+VacA+		CagA+VacA-		CagA-VacA+		Total		CagA-VacA-	
Abs.	%	Abs.	%	Abs.	%	Abs.	%	Abs.	%
46	53,4	24	27,9	16	18,6	86	71,7	34	28,3

4.6

The gravity of all strains with the presence of gene toxicity (tox +) is 71.7 % of all strains of H. pylori. Spectrum of gene toxicity pylori was distributed as follows: CagA (+) identified in 70 persons (81.3 %), VacA (+) - 62 people (72.1 %). Thus among strains of *H. pylori was* detected heterogeneity with natural increase biochemical activity of bacteria in the presence of these gene CagA.

4.7

When comparing the results of cytoscopic investigation smears-matrix and DNA typing of genes toxicity *H. pylori* found that the smears-matrix helicobacter-like microflora in mucosa of antrum was found in 86 of 120 persons with helicobacter infection, diagnosed by PCR, therefore sensitivity amounted to 71.7 %. Helicobacter microflora in the mucosa of stomach body was detected in 49 (40.8 %) patients, whereas in 34 duodenal biopsies of examined children, only 11 (32.3 %) found *H. pylori* (Table 3).

Table 3: Frequent of revealing Helicobacter microflora by cytoscopic method and polymerase chain

Biopsy sampling location	Cytoscopic method, (%)	Polymerase chain reaction method, (%)
Antrum, n=120	71.7*	100
Body of the stomach, n=120	40.8*	100
Duodenum, n=34	32.3*	100

Mark. * - difference is reliable (p<0.01).

4.8

Thus, most often *H. pylori* is detected in the antrum (71.7 %), the least - in duodenal bulb (32.3 %). The difference between the frequencies of detection *H. pylori* in different parts of the gastroduodenal region is reliable (n=120, p<0.01).

5. Discussion

5.1

All express tests have a fairly high level of sensitivity and specificity. For express diagnostics for the presence of Helicobacter pylori bacteria can use any of these, however, the advantage, in our opinion, should be given to De-nol test or urease test, which allows not only to carry out a qualitative response, but also to assess the degree of sowing mucous membrane of these bacteria.

5.2

However, we performed express-tests is quite informative, but they are qualitative reactions, recording only the waste products of *H. pylori*. Positive results were obtained in the course of urease and helic-test with negative results obtained by histological methods or PCR, can not explain false-positive results, and the fact that during the urease test and helic-test determined the waste products of *H. pylori*, rather than the microrganism that can not get into the biopsies studied using histological methods or PCR.

Research Highlights

All express tests have a fairly high level of sensitivity and specificity. The evaluation methods of diagnosing *H. pylori* in formativeness was found that the bacteriological method is characterized by the lowest level of detection of the microorganism and we also do not recommend to rely on this method to avoid false negative results.

Limitations

There was no limitation. Different methods for diagnostic of *Helicobacter pylori* in different countries.

Recommendations

Individual diagnostic program to all patients and use in clinical practice more informative method for diagnostic H. pylori.

Funding and Policy Aspects

To make one standard algorithm for diagnostic of *H. pylori* in all countries.

Justification of Research

This research is very important in pediatrics and child gastroenterology, because when patient have *H. pylori* we must administrate eradication therapy. To diagnose this infection doctors must use highly informative test for indication H. pylori. We try to find most specific and sensitive method of diagnostic of H. pylori. That means, the doctor can choose method of diagnostic of *Helicobacter pylori* and not administrate of all methods. It saves time, money and recourses.

Conclusion

Furthermore, the evaluation methods of diagnosing *H. pylori* informativeness was

found that the bacteriological method is characterized by the lowest level of detection of the microorganism and we also do not recommend relying on this method to avoid false negative results.

Author's Contribution and Competing Interests

Authors make clinical examination of patients, all this methods of diagnostic of *H. pylori*, worked all information, write this article.

Conclusions

To improve the accuracy of diagnosis *Helicobacter pylori* infection it is recommended to use at least two, and preferably three research methods, preferably a combination of express urease test or De-nol-test with histological methods (biopsy of the antrum of the stomach) or PCR (gene detection ureC, CagA and VacA). For express-diagnostics for the presence of Helicobacter pylori bacteria it can be used any of them. Helic-test is recommended as an exact noninvasive method for assessing the effectiveness of eradication therapy, especially by children.

References

Europe, Ukraine, Chernivtsy region, Chernivtsy, Bukovinian State Medical University, Teatralna square, 2, 58000

Hirschi A.M., Makristathis A., 2007. Methods to detect *Helicobacter pylori*: from culture to molecular biology. Helicobacter. 12(2), 6-11.

Isakov V.A., 2011. New Projects in diagnostic technology *H. pylori*, 344 p.

Kindermann A., Lopez A., 2009. *Helicobacter pylori* Infection in Pediatrics. Helicobacter. 14(1), 52-57.

Maastricht-3 Guidelines for *Helicobacter pylori* infection, 2005. 13 United European Gastroenterology Week. Copenhagen.

Moya D.A., Crisinger K.D., 2012. *Helicobacer pylori* persistence in children: distinguishing inadequate treatment resistant organism and reinfection. Current Gastroenterology Reports. 14, 236-242.

Pacifico L., Anania C., Osborn J., 2010. Consequences of *Helicobacter pylori* infection in children. World Journal of Gastroenterology. 16(41), 5181-5194.