EVALUATION OF INVASIVE METHODS TO DIAGNOSIS *Helicobacter pylori* INFECTION IN CHILDREN AND ADOLESCENTS WITH DYSPEPSIA INVASIVE METHODS TO DIAGNOSE Hp INFECTION

AVALIAÇÃO DE MÉTODOS INVASIVOS PARA DIAGNÓSTICO DA INFECÇÃO POR Helicobacter pylori EM CRIANÇAS E ADOLESCENTES COM SINTOMAS DISPÉPTICOS

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OGATA SK; KAWAKAMI E & REIS FPS. Evaluation of invasive methods to diagnosis *helicobacter pylori* infection in children and adolescents with dyspepsia invasive methods to diagnose Hp infection. **Medicina, Ribeirão Preto, 35**: 24-29, jan./march 2002.

Abstract: In our country there are few studies evaluating the accuracy of invasive methods in children, although the usual practice of endoscopic gastric biopsies to diagnose *Helicobacter pylori* infection. Methods: We evaluated prospectively 120 patients submitted to endoscopy for investigation of dyspeptic symptoms. Six antral biopsies were taken to detect *Helicobacter pylori* by rapid urease test, histology and culture. Patients were considerate infected if culture was positive or if histology and rapid urease test were positive. Results: The age ranges from 3mo to 17y (Median: 10y1mo); 54% were female and 46% were male; 44% (53/120) were infected. The endoscopic examination was normal in 54% (65/120) and abnormal in 46% (55/120). The diagnosis was gastritis - 69% (38/55), esophagitis - 13% (13/55), duodenal ulcer - 13% (7/55) and duodenitis - 5% (3/55). The 3 methods agreed in 72.5% (87/120), and were all negative in 48% (58/120) and all positive in 24.5% (29/120). The best agreement occurred between histology and rapid urease test (91.7%), followed by culture and histology (78.3%) and finally culture and rapid urease test (75%). The sensitivity of the rapid urease test was 100%, followed by histology (98.1%) and culture (56.6%). The specificity of histology was 97%, followed by rapid urease test (89.5%). Conclusions: The histology and rapid urease test combination was the most accurate to identify *Helicobacter pylori* infection in account of high sensitivity of rapid urease test and high specificity of histology, besides they were low cost and practicable. The culture alone must not be considered gold standard due to its low sensitivity.

UNITERMS: Child. Adolescence. Dyspepsia. Diagnosis. *Helicobacter pylori*.

1. INTRODUCTION

Helicobacter pylori (Hp) infection can be diagnosed through invasive methods that need endoscopic gastric biopsies to detect the bacteria (rapid urease test, culture, microbiology methods, polimerase chain reaction) or through non-invasive methods (serology, C^{13} or C^{14} urea breath test, fecal antigen)[1,2,3]. Invasive tests have been considerated the gold standard, but biopsy-based methods may suffer from sampling error, because the patchy nature of the infection and low concentration of bacteria in fragments[4,5,6]. Culture has low sensitivity, then no single test can be used as gold standard and the tendency has been to use a

combination of tests in adult and pediatric studies[7,8,9]. In Brazil, the *Helicobacter pylori* infection has high prevalence but there are few pediatric studies about the accuracy of invasive methods to diagnose *Helicobacter pylori* infection[10,11,12].

The aim of this study was to evaluate the accuracy of 3 invasive methods: rapid urease test, histology and culture in gastric biopsies of children and adolescents to identify Hp.

2. PATIENTS AND METHODS

During 36 months, 120 patients with dyspeptic symptoms submitted to diagnostic upper GI endoscopy with gastric biopsies were prospectively evaluated. Medical Ethic Committee of our University approved the study and the informed consent was obtained from responsible for each patient. Patients with chronic extra digestive or immunosuppressive disease and patients in use of immunosuppressive or chemotherapy drugs, anti-inflammatory drugs, H2 receptor antagonist, antimicrobians and/or nitroimidazoles and/or bismuth compounds, at least 3 months prior the examination, were excluded.

3. METHODS

Endoscopy was performed under general anesthesia or conscientious sedation (midazolan – 0.2 mg/kg and meperidin – 1 mg/kg) using a pediatric videoendoscopy (PENTAX EG 2430), after overnight fasting. Topic anesthesia and dimeticone were not used. Antral biopsies were taken from the antrum within 2 cm of pyloric channel for histology (2 fragments), rapid urease test (2 fragments) and bacterial culture (2 fragments).

4. RAPID UREASE TEST

We used a homemade solution containing 1 ml of distilled water, 2 drops of 1% red phenol and 0.1g of urea. This solution was prepared by endoscopist in the same day of the examination and maintained in environment temperature. The test was considered positive when color changes from yellow to red and it was observed until 24 hours[13].

4.1. Histology

The specimens were oriented in filter paper, fixed in 10% formaldehyde solution and stained by hematoxylin and eosin, and modified Giemsa[14]. An expert pathologist characterized as positive the presence of spiral bacteria in mucosal layer or in the surface of epithelial cells[1].

4.2. Culture

The fragment was inoculated in "Brain Heart Infusion" solution and maintained for 3 hours at 4° C then we gently scraped biopsy samples in "Brain Heart Infusion" agar containing 5% sheep blood and selective medium with nalidixic acid (2.5 mg/ml), vancomycin (2.5 mg/ml) and amphotericin B (0.25 mg/ml). The plates were incubated at 37°C for 7 days under microaerophilic conditions[15]. Bacterial culture was considerate positive when small translucent colonies were spiral Gram-negative bacteria and positive for oxidase, catalase and urease tests[16,17].

5. STATISTICAL ANALYSIS

Sensitivity, specificity, positive and negative predictive value was calculated considereting the positivity of culture or of histology and rapid urease test as gold standard. Cochran's G test was used to analyze the agreement among positive and negative results and McNemar's test to analyze disagreement between 2 tests.

6. RESULTS

The age of 120 patients ranged from 3m to 17y (Median=10y1m ± 2y10m), 54% (65/120) were female and 46% (55/120) were male. Abdominal pain was evaluated in 89 patients aged from 6 to 17 years, and was present in 99% (88/89), identified in epigastric region in 82% and characterized as burning in 75%. Family history of peptic disease was present in 57% of patients.

Endoscopy was normal in 54% (65/120) and abnormal in 46% (55/120). *Helicobacter pylori* infection was present in 44% (53/120). Abnormal endoscopy showed gastritis in 69% (38/55), esophagitis in 13% (7/55), duodenal ulcer in 13% (7/55) and duodenitis in 5% (3/55). Active chronic gastritis was observed in 86% (103/120).

All methods agreed in 72.5% (87/120), all negative in 48% (58/120) and positive in 24.5% (29/120). Disagreement occurred in 33 (27.5%) patients. In 23 patients only culture was negative; in 7 patients only rapid urease test was positive; in 2 only histology was positive and 1 patient presented only histology nega-

tive. The analysis of all concordant tests presents G calc = 45.82 (G crit = 5.99) (Table I), showing that there was large concordance among these three invasive tests. However culture presents the major disagreement, in 23 patients only culture was negative.

Analyzing the disagreement between each two tests, we observed that major agreement occurred between histology and rapid urease test (91.7%), followed by culture and histology (78.3%) and finally, culture and rapid urease test (75%) (Table II).

The major sensitivity was achieved by rapid urease test (100%); the specificity was 89.5%. Histology presented sensitivity of 98% and specificity of 97%. Culture presented lower sensitivity (56.6%) and 100% of specificity (Table III).

Table I: Evaluation of 3 diagnostic methods for Hp infection.

	Rapid urease test	Histology	Culture	Total (%)
	-	-	-	58 (48)
	+	-	-	7 (5.8)
	-	+	-	2 (1.8)
	+	-	+	1 (0.9)
	+	+	-	23 (19)
	+	+	+	29 (24.5)
Total of positive tests (%)	60 (50%)	54 (45%)	30 (25%)	120 (100%)
Cochran's G Test		G calc = 45.82	G crit = 5.99	

Table II. Agreement and disagreement analysis.

Methods	Agreement (%)	Disagreement (%)	p
Histology and rapid urease test	91.7	8.3	0,114
Culture and histology	78.3	21.7	< 0,001*
Culture and rapid urease test	75	25	< 0,001

McNemar's test
* Statistical significant test

Table III: Sensivity, specificity, positive and negative predictive value, accuracy and confidence interval.

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Accuracy (%)	CI(95%) SENS	CI (95%) SPEC
Rapid urease test	100	89.5	88.3	100	94.2	100	84-95
Histology	98.1	97	96.3	98.5	97.5	95.6-100	94-100
Culture	56.6	100	100	74.4	80.8	47.7-65.5	100

7. DISCUSSION

This study shows Hp infection in 44% of 120 symptomatic patients submitted to endoscopy, higher rate than observed in developed countries[18,19] and higher than observed in study of seroprevalence in asymptomatic children in our Country (34%)[10].

We evaluated three different methods to diagnosis Hp infection, using as gold-standard the positivity of culture, that was incontestable proof of bacterial presence, however presents high rate of false negative results that became difficult its use as gold standard[7]; we also used the positivity of histology and rapid urease test, this point designed the patient as infected or not infected, then the methods were evaluated using this artifice as gold standard. Unfortunately, at present, no single test can be relied upon to detect definitely Hp infection[3,5] and a combination of tests is recommended as gold standard [3,8,9,20,21].

Agreement of 3 methods (72.5%), was lower than observed in similar studies (87%)[9]. The Cochran's G test presents G calc = 45.82 (G crit = 5.99) (Table I), it appointed to a large concordance among these three invasive tests. The highest agreement between two tests occurred in histology plus rapid urease test combination (91.7%) similar to observed in other study[21].

Low sensitivity of culture was expected in account of its high number of false negative results related in literature, however our sensitivity (56,6%) was lower than observed in others studies (86 a 100%)[8,22]. Culture, although considerate gold standard, is recommended only in research and to evaluate antibiotics sensitivity in retreated patients.

Our results show rapid urease test as the most sensitivity (100%), in opposition to others studies that report lower sensitivity in children[18,23] and adults[21,24,25]. Specificity (89,5%), however, were lower when compared to these studies. This can occur by contamination by oral bacteria or by others urease producer bacteria[26], or by biopsy grasp or fragment contamination[27,28]. A homemade rapid urease test is routinely used in our service, this test allows quickly evaluation of patient's infectious status (first 20 minutes) and has low cost (US$ 0,01/test)[29].

Histology presented high sensitivity (98%) and specificity (97%) similar to observed in literature [18,20,21,24,25]. As biopsy based method, it is possible to obtain a fragment without Hp due to its patchy distribution on gastric mucosa[6] or by low quantity of bacteria in children[30]. False positive result can occur if contamination occurred by others Hp like bacteria (Ex. C. jejuni var. doylei)[31] or colonization by others Hp species[26]. We used Hematoxylin-eosin and modified Giemsa stain to detect Hp on antral biopsies, because this association increased the sensitivity of histology[14]. Modified Giemsa stain become Hp more evident and allows better visualization of Hp [1,32]. Sometimes, Hp can locate profoundly in the crypts, so the fragment orientation in a filter paper can become the exam better[33].

The histology and rapid urease test association, presented the highest agreement tax among evaluated methods (91,7%), similar results were observed in adults (Lido) and allows rapid evaluation of patient's infectious status (Rapid urease test) and microscopic analysis of gastric mucosa (Histology).

We conclude that histology and rapid urease test association was the most accurate to identify Hp infection, with high agreement tax, high sensitivity (Rapid urease test) and high specificity (Histology). Our results suggest that culture alone may not be used as gold standard due to its low sensitivity.

OGATA SK; KAWAKAMI E & REIS FPS. Avaliação de métodos invasivos para diagnóstico da infecção por *Helicobacter pylori* em crianças e adolescentes com sintomas dispépticos **Medicina, Ribeirão Preto, 35**: 24-29, jan./mar. 2002.

RESUMO: Apesar do uso rotineiro de biópsias endoscópicas para o diagnóstico da infecção por *Helicobacter pylori*, em nosso meio, há poucos estudos pediátricos, avaliando a acurácia dos métodos invasivos. **Métodos:** Foram avaliados prospectivamente 120 pacientes submetidos à endoscopia para investigação de sintomas dispépticos. Foram obtidos seis biópsias de região antral para detecção do *Helicobacter pylori* através do teste rápido da urease, histologia e cultura. Os pacientes foram considerados infectados, se a cultura ou a histologia e o teste rápido da urease resultaram positivos. **Resultados:** A idade variou de 3m a 17anos (média: 10a1m); 54% do sexo feminino e 46% masculino; 44% (53/120) estavam infectados. O exame endoscópico foi normal em 54% (65/120) e anormal em 46% (55/120). O diagnóstico foi gastrite - 69% (38/55), esofagite - 13% (13/55), úlcera duodenal - 13% (7/55) e duodenite - 5% (3/55). Os 3 métodos concordaram em 72,5% (87/120), foram todos negativos em 48% (58/120) e todos positivos em 24,5% (29/120). A melhor concordância ocorreu entre a histologia e o teste rápido da urease (91,7%), seguido pela cultura e histologia (78,3%) e, finalmente, a cultura e o teste rápido da urease (75%). A sensibilidade do teste rápido da urease foi 100%, seguido pela histologia (98,1%) e pela cultura (56,6%). A especificidade da histologia foi de 97%, seguida pelo teste rápido da urease (89,5%). **Conclusão:** A associação histologia e o teste rápido da urease mostraram maior acurácia na detecção da infecção por *Helicobacter pylori* devido a sua alta sensibilidade (teste rápido da urease) e alta especificidade (histologia), além do baixo custo e praticidade. A cultura isolada não deve ser utilizada como padrão-ouro devido à baixa sensibilidade.

UNITERMOS: Criança. Adolescência. Dispepsia. Diagnóstico. *Helicobacter pylori*.

BIBLIOGRAPHIC REFERENCES

1 - BROWN KE & PEURA DA. Diagnosis of *Helicobacter pylori* infection. **Gastroenterol Clin North Am 22**:105-114, 1993.

2 - MAKRISTATHIS A; PASCHING E; SCHUTZE K; WIMMER M; ROTTER ML & HIRSCHL AM. Detection of *Helicobacter pylori* in stool specimens by PCR and antigen enzyme immunoassay. **J Clin Microbiol 36**:2772-2774, 1998.

3 - VAIRA D; HOLTON J; MENEGATTI M; RICCI C; GATTA L; GEMINIANI A & MIGLIOLI M. Invasive and non-invasive tests for *Helicobacter pylori* infection. **Aliment Pharmacol Ther, 14**:13-22, 2000.

4 - FENNERTY MB. *Helicobacter pylori*. **Arch Intern Med 154**:721-727, 1994.

5 - MÉGRAUD F. Advantages and disadvantages of current diagnostic tests for the detection of *Helicobacter pylori*. **Scand J Gastroenterol 31**:57-62, 1996.

6 - MORRIS A; ALI MR; BROWN P; LANE M & PATTON K. *Campylobacter pylori* infection in biopsy specimens of gastric antrum: laboratory diagnosis and estimation of sampling error. **J Clin Pathol 42**:727-732, 1989.

7 - CUTLER AF; HAUSTAD S; MA CK; BLASER MJ; PEREZ-PEREZ GI & SCHUBERT TT. Accuracy of invasive and non-invasive tests to diagnose *Helicobacter pylori* infection. **Gastroenterology 109**:136-141, 1995.

8 - THIJS JC; VAN ZWET AA; THIJS WJ; OEY HB; KARRENBELD A; STELLAARD F; LUIJT DS; MEYER BC & KLEIBEUKER JH. Diagnostic tests for *Helicobacter pylori*: A prospective evaluation of their accuracy, without selecting a single test as the gold standard. **Am J Gastroenterol 91**:2125-2129, 1996.

9 - VANDENPLAS Y; BLECKER U; DEVREKER T; KEPPENS E; NIJS J; CADRANEL S; PIPELEERS-MARICHAL M; GOOSSENS A & LAUWERS S. Contribution of the 13 C-urea breath test to the detection of *Helicobacter pylori* gastritis in children. **Pediatrics 90**:608-611, 1992.

10 - OLIVEIRA AMR; QUEIROZ DMM; ROCHA GA & MENDES EN. Seroprevalence *of Helicobacter pylori* infection in children of low socio-economic level in Belo Horizonte, Brazil. **Am J Gastroenterol 89**:2201-2204, 1994.

11 - SOLARI CA; ARARUNA RP; REIS EM; HOFER E; DIAS G; MORAES G; BASILIO CA; RODRIGUEZ CM & LUNA LL. *Helicobacter pylori* in dyspeptic children and adults: endoscopic, bacteriologic and histologic correlations. **Mem Inst Oswaldo Cruz 89**:581-586, 1994.

12 - CARVALHO; AST; QUEIROZ; DMM; MENDES; EN; ROCHA, GA & PENNA, FJ. Diagnosis and distribution of *Helicobacter pylori* in the gastric mucosa of symptomatic children**. Braz J Med Biol Res 24**:163-166, 1991.

13 - MCNULTY CAM & WISE R. Rapid diagnosis of *Campylobacter*-associated gastritis. **Lancet 1**:387, 1986.

14 - PRICE AB. The histological recognition of *Helicobacter pylori*. In: LEE A & MÉGRAUD F, eds, ***Helicobacter pylori*: Techniques for clinical diagnosis and basis research**. W.B. Saunders, London, p. 33-49, 1995.

15 - QUEIROZ DMM; MENDES EN & ROCHA GA. Indicator médium for isolation of *Campylobacter pylori*. **J Clin Microbiol 25**:2378, 1987.

16 - BARTHEL JS; WESTBLOM TU; HAVEY AD; GONZALEZ F & EVERETT D. Gastritis and *C. pylori* in healthy, asymptomatic volunteers. **Arch Intern Med 148**:1149-1151, 1988.

17 - PETTROSS CW; APPLEMAN MD; COHEN H; VALENZUELA JE; CHANDRASOMA P & LAINE LA. Prevalence of *Campylobacter pylori* and association with antral mucosal histology in subjects with and without upper gastrointestinal symptoms. **Dig Dis Sci 33**: 649-653, 1988.

18 - HELDENBERG D; WAGNER Y; HELDENBERG E; KEREN S; AUSLAENDER L; KAUFSHTEIN M & TENEBAUM G. The role of *Helicobacter pylori* in children with recurrent abdominal pain. **Am J Gastroenterol 90**:906-909, 1995.

19 - ROWLAND M & DRUMM B. Clinical significance of *Helicobacter* infection in children. **Br Med Bull 54**:95-103, 1998.

20 - LIDO CV; PALOMBO FP; FERRARI GO & MAGRINI E. Estudo comparativo entre os teste de histologia, cultura e urease para diagnóstico de *Helicobacter pylori*. **J Bras Med 79**:85-92, 2000.

21 - WONG BCY; WONG WM; WANG WH; TANG VSY; YOUNG J; LAI KC; YUEN ST; LEUNG SY; HU WHC; CHAN CK; HUI WM & LAM SK. An evaluation of invasive and non-invasive tests for the diagnosis of *Helicobacter pylori* infection in Chinese. **Aliment Pharmacol Ther 15**:505-511, 2001.

22 - VELDHUYZEN SJO; TYTGAT K; DEGARA CJ; GOLDIE J; RASHID FA & BOWEN BM. A prospective comparison of symptoms and five diagnostic tests in patients with *Helicobacter pylori* positive and negative dyspepsia**. Eur J Gastroenterol Hepatol 3**:463-468, 1991.

23 - ELITSUR Y; HILL I; LICHTMAN SN & ROSENBERG AJ. Prospective comparison of rapid urease tests (Pyloritek, CLOTest) for the diagnosis of *Helicobacter pylori* infection in symptomatic children: A pediatric multicenter study. **Am J Gastroenterol 93**:217-219, 1998.

24 - MORAIS M; MACEDO EP; SILVA JR MR; ROHR MRS; FERRAZ MLG CASTRO RRO; DELLA LIBERA E; SIQUEIRA ES; BRANT CQ & FERRARI JR AP. Comparação entre testes invasivos para o diagnóstico da infecção pelo *Helicobacter pylori*. **Arq Gastroenterol 34**:207-11, 1997.

25 - PERRI LG; CROSSARA EMM; AKITI T & PENTEADO JF. Diagnóstico do *Helicobacter pylori* pelo exame endoscópico. **Folha Med 102**:203-206, 1991.

26 - ANDERSEN LP; BOYE K; BLOM J; HOLCK S; NORGAARD A & ELSBONG L. Characterization of a culturable "*Gastropirillum hominis*" (*Helicobacter heilmannii*) strain isolated from human gastric mucosa. **J Clin Microbiol 37**:1069-1076, 1999.

27 - FANTRY GT; ZHENG QX & JAMES SP. Conventional cleaning and disinfection techniques eliminate the risk of endoscopic transmission of *Helicobacter pylori*. **Am J Gastroenterol 90**:227-232, 1995.

28 - TYTGAT GNJ. Endoscopic transmission of *Helicobacter pylori*. **Aliment Pharmacol Ther 9**:105-110, 1995.

29 - OGATA SK; KAWAKAMI E; PATRÍCIO FRS; PEDROSO MZ & SANTOS AM. Evaluation of invasive and non-invasive methods for the diagnosis of *Helicobacter pylori* infection in symptomatic children and adolescents. **São Paulo Med J 119**:67-71, 2001.

30 - DRUMM B; SHERMAN P; CUTZ E & KARMALI M. Association of *Campylobacter pylori* on the gastric mucosa with antral gastritis in children. **N Engl J Med 316**:1557-1561, 1987.

31 - MASSUDA HK & BOYD EJS. Diagnóstico da infecção por *H. pylori* através de material obtido por biópsia endoscópica. In: CASTRO LP; SAVASSI-ROCHA PR & COELHO LGV, eds, **Tópicos em gastroenterologia 4**. Medsi, Rio de Janeiro, p. 259-261, 1993.

32 - KILLBRIDGE PM; DAHMS BB & CZINN SJ. *Campylobacter pylori* associated gastritis and peptic ulcer disease in children. **Am J Dis Childhood 142**:1149-1152, 1988.

33 - ZAITOUN AM. Histology compared with chemical testing for urease for rapid detection of *Helicobacter pylori* in gastric biopsy specimens. **J Clin Pathol 46**:684-685, 1993.

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