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The Role of Serological Tests in Diagnosis of *Helicobacter pylori* Infection in Children

Mohammad Hadi Imanieh [1,2]; Seyed Mohsen Dehghani [1,2,*]; Amin Masjedi [1]; Abbas Rezaianzadeh [1]; Mahmood Haghighat [1]

[1]Gastroenterohepatology Research Center, Department of Pediatric Gastroenterology, Nemazee Teaching Hospital, Shiraz University of Medical Sciences, Shiraz, IR Iran
[2]Shiraz Transplant Research Center, Nemazee Teaching Hospital, Shiraz University of Medical Sciences, Shiraz, IR Iran

*Corresponding author: Seyed Mohsen Dehghani, Gastroenterohepatology Research Center, Nemazee Teaching Hospital, School of Medicine, Shiraz University of Medical Sciences, P. O. Box: 71937-11351, Shiraz, IR Iran. Tel: +98-7116125849, Fax: +98-7116474298, E-mail: dehghanism@sums.ac.ir

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Background: *Helicobacter pylori* infection is a common infection that affects human beings. This infection also affects children. Different diagnostic methods such as serology, stool antigen detection, rapid urease test and histology are used to detect this microorganism.
Objectives: The aim of this study was to determine the correlation between serology and histology/rapid urease test.
Patients and Methods: In this study, two groups (case and control) were selected and matched for age and sex. The case group comprised of 77 children with confirmed *H. pylori* infection, as they had positive rapid urease test and histology results. The control group included 77 healthy children. Both case and control groups were checked serologically for detection of anti *H. pylori* IgM, IgG and IgA antibody titers. Receiver operating characteristic (ROC)-analysis software was used for data analysis.
Results: Amongst the case group 54.6% were female and 45.4% were male. The most common complaints were abdominal pain (96%) and anorexia (82%). Using ROC-analysis method three cut-off points for IgG, IgM and IgA were obtained. These points were 3.3 U/ML for IgA, 6.4 U/ML for IgM and 9.9 U/ML for IgG. Considering cut-off points for each antibody, higher levels were considered as positive and lower levels as negative. Antibody titers were compared with gold standard methods including histologic and rapid urease tests. IgA level had a sensitivity of 64%, specificity of 58%, accuracy of 59.3%, positive predictive value of 31.5% and negative predictive value of 76.9%. IgM level had a sensitivity of 76%, specificity of 36.1%, accuracy of 74.2%, positive predictive value of 31.5% and negative predictive value of 76.9%. IgG level had a sensitivity of 58.6%, specificity of 61.3%, accuracy of 60.6%, positive predictive value of 36.9% and negative predictive value of 79.3%.
Conclusions: Therefore, these antibodies have a relatively high negative predictive value and a low positive predictive value. Thus, their negative results are more valuable. The most sensitive antibody is IgM and most specific antibody is IgG. However, the performances of all serological tests for *H. pylori* are poor in children and these tests should not be used for diagnosis of *H. pylori* infection and treatment decisions for the pediatric age group.

Keywords:Helicobacter pylori; Serology; Children

1. Background

Helicobacter pylori infection is a common infection that affects human beings. Its incidence is estimated to be 0.5% per year in developed countries and 3 to 10% per year in developing countries. Also, its incidence throughout the world is higher in children than in adults. *H. pylori* are capable of causing a number of gastrointestinal diseases including: acute and chronic gastritis, atrophic gastritis, gastric and duodenal ulcer, intestinal metaplasia, lymphoma and even gastric adenocarcinoma. High prevalence of the infection and its curable pattern and also the importance of problems caused by this infection make its diagnosis very important. Moreover, many complications of the chronic *H. pylori* infection could be prevented by ideal treatment (1-5). There are several ways to diagnose *H. pylori* and they are divided into two main groups: invasive methods including culture, histological evaluation and rapid urease test and non-invasive methods including urea breath test, serology and evaluation of stool antigens (6-11).

2. Objectives

Since Iran is a developing country with a high prevalence of *H. pylori* infection, accurate and economic diagnostic methods are needed. Diagnosis of this infection in our center is now based on the combination of histological evaluation and rapid urease test on samples obtained from gastroscopy however these methods are invasive and difficult in children. As there is not a cut-off point for antibodies in children and the serological method is a simple, economic and non-invasive method, we aimed to evaluate the efficacy of this method for diagnosis of *H. pylori* infection in children and if possible define a cut-off

point for related antibodies in order to use them in diagnosis of this infection.

3. Patients and Methods

During a two-year period, 104 children younger than 18 years old with clinical suspicion of *H. pylori* infection were entered in the study. Suspicion to *H. pylori* infection was defined as having dyspepsia, pain or discomfort in the upper abdomen, fullness, early satiety, anorexia, eructation, nausea and vomiting. All cases underwent upper gastrointestinal (GI) endoscopic evaluation and tissue samples were taken from the antrum. All samples were evaluated for *H. pylori* histologically using hematoxylin and eosin and the modified Giemsa method. Accompanied with endoscopic evaluation rapid urease test was also done. During the 24 hours after the test, if peripheral color changed to pink, the test results were interpreted as positive or presence of *H. pylori* was assumed.

We considered these children infected with *H. pylori* if they had both positive histology and rapid urease test as the gold standard. Overall, 77 patients (74%) had positive rapid urease test and histology for *H. pylori* and were considered as the *H. pylori* infected group. Of the 27 reminders 16 patients (59.3%) had positive histology but negative rapid urease test and 11 patients (40.7%) had positive rapid urease test but negative histology and all of them were excluded from the study. Controls were also younger than 18 years, without any gastrointestinal (GI) symptoms and clinical suspicion of *H. pylori* infection. Controls and their first-degree families were also free of any GI symptom during the study period and their entire life, before the study. Neither cases nor controls received antibiotics during the last week of the study.

It was supposed that all controls were free of *H. pylori* and they did not undergo endoscopic evaluation due to ethical issues. However, blood samples were obtained from all subjects (cases and controls) and checked for three antibodies against *H. pylori*. The IBL Hamburg kits for each antibody were obtained from Germany. Using the enzyme-linked immunosorbent assay (ELISA) (sandwich principle), titers of IgA, IgM and IgG antibodies were detected. Therefore, 77 cases were evaluated by the rapid urease test, histology and measuring IgM, IgG, and IgA antibody titers against *H. pylori*. However, controls were only checked for IgM, IgG, and IgA antibody titers against *H. pylori*. All data were entered into Microsoft Excel 2003 and were analysed by the ROC-analysis method and finally three cut-off points for IgM, IgG, and IgA were obtained.

4. Results

There were 42 (45.4%) boys and 35 (54.6%) girls in the case group. According to age, 6 patients (7.7%) were between 1 and 5, 34 patients (44.1%) between 6 and 10, 26 patients (33.7%) between 11 and 16, and 11 patients (14.2%) were between 16 and 18 years. The most common clinical symptoms were abdominal pain (96%), anorexia (82%), nausea (57%), heartburn (31%) and vomiting (23%). Sixty-two patients (80.5%) had used medicines such as antacid, cimetidine, ranitidine and omeprazole. Four patients (5.2%) had undergone an endoscopic examination in the past and one of them had a history of GI bleeding. Due to GI problems, 27.2% of the parents of cases underwent endoscopic examinations and 2.4% reported a history of GI bleeding; also, 52% reported a history of use of gastric acid suppressor medications.

Using ROC-analysis, three cut-off points for IgM, IgA and IgG were obtained. The cut-off point for IgA was 3.3 U/ML, for IgM 6.4 U/ML and for IgG 9.9 U/ML. To calculate sensitivity, specificity and accuracy, positive and negative predictive values for each of these antibody titers above these points were supposed positive and those below these points as negative. With 3.3 U/ML as the cut-off point for IgA, sensitivity, specificity, accuracy, positive and negative predictive value for this antibody was 64, 58, 59.3, 36.7 and 81.1%, respectively. IgM had 76% sensitivity, 36.1% specificity, 74.2% accuracy, 31.5% positive predictive value, and 76.9% negative predictive value with 6.4 U/ML as the cut-off point. Furthermore, 58.6% sensitivity, 61.3% specificity, 60.6% accuracy, 36.9% positive predictive value and 79.3% negative predictive value was obtained for IgG with the cut-off point equal to 9.9 U/ML.

5. Discussion

In this study histological evaluation and rapid urease test were considered as the gold standard and results of serological tests in *H. pylori* infected group were compared with the control group. IgA, IgM and IgG have a high negative predictive value that could be used (scale) for a screening and diagnostic test. For all three antibodies, the negative predictive value was about 80%. These findings show that negative results have a higher value compared to positive results. When the test is negative with 80% probability, the person is not infected by *H. pylori*, while when it is positive, IgG has the highest (60.6%) and IgM the lowest (42.2%) accuracy. IgA accuracy is about 59.3% and the most specific antibody is IgG and the most sensitive is IgM.

Since Iran is a developing country with high prevalence of *H. pylori* infection, it is ideal to evaluate all controls by endoscopy to see how many of them were infected by *H. pylori* and not suppose that all are free of *H. pylori*. Therefore, the number of false positive and negatives would decrease and sensitivity, specificity, accuracy and positive and negative values of antibodies would increase. However, because of ethical issues this was impossible. Predictive value of a test is dependent on sensitivity, specificity and prevalence. In this study we could not estimate the exact prevalence of *H. pylori* with the small sample size. Thus, a study with a large sample size should be performed in Shiraz city to estimate the prevalence and predictive values.

A great deal of studies has been conducted in different countries. In an adults study, serum IgG with cut-off point

of 15.2 U/mL had 94.1% sensitivity and 97.9% specificity for diagnosis of *H. pylori* infection (12). In another adult study, IgG antibody titers higher than 10 U/mL were considered positive for *H. pylori* infection; cut-off point of 3 U/mL had 100% sensitivity and 99% specificity for eradication of *H. pylori* (13). A study in Spain reported sensitivity, specificity, positive and negative predictive values for IgG as 81, 97, 89, and 93% and for IgA as 90, 76, 36, and 96%, respectively, in children. However, IgM was not evaluated in this study (2). Another pediatric study in Italy showed sensitivity, specificity, positive and negative predictive values for IgG to be 86, 80, 72 and 90%, respectively (14).

In Brazil it was shown that sensitivity, specificity, positive and negative predictive values and accuracy for IgG was 64, 83.7, 82, 66.6 and 73.1% and for IgA this was 72, 65.9, 72, 67.4 and 69.8%, respectively (15). Different values for sensitivity, specificity, positive and negative predictive values have been reported by various studies. This could be due to differences in prevalence of *H. pylori* between populations because of the variety in socioeconomic status, health status, crowding and other factors. Differences in prevalence have an obvious impact on statistical indexes.

A serological assay for *H. pylori* infection may be useful in epidemiological survey of prevalence, transmission mode and spontaneous clearance of the *H. pylori* infection, while it also may be helpful in the development of preventive measures for *H. pylori* infection. In Asia, the strains of *H. pylori* are different from those that are prevalent in other continents. Thus, the specificity, sensitivity and positive and negative predictive values of one kind of serology kit may differ in various geographic or ethnic populations.

We concluded that these antibody tests have a relatively high negative predictive value and a low positive predictive value. Thus, serological tests are not useful on their own, for diagnosis of *H. pylori* infection because the sensitivity and specificity of these tests for detection of antibodies against *H. pylori* in children vary widely and they have not been recommended for clinical practice in pediatric patients. More researches with a larger sample size are needed in the future to confirm the findings of this study.

Authors' Contributions

Mohammad Hadi Imanieh, Seyed Mohsen Dehghani, Mahmood Haghighat and Abbas Rezaianzadeh were involved in the study concept and design, drafting of the manuscript, critical revision of the manuscript and study supervision. Mohammad Hadi Imanieh, Seyed Mohsen Dehghani, Amin Masjedi and Abbas Rezaianzadeh were in charge of data acquisition, analysis and interpretation of data and drafting of the manuscript. The present article was extracted from the thesis written by Amin Masjedi.

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