

DIAGNOSTIC ACCURACY OF UREA BREATH TEST FOR *HELICOBACTER PYLORI* INFECTION IN CHILDREN WITH DYSPEPSIA IN COMPARISON TO HISTOPATHOLOGY

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ABSTRACT - Background - *Helicobacter pylori* infection is the gram negative bacillus with the close association with chronic antral gastritis. **Objective -** In this study, we evaluate the accuracy of urea breath test (UBT) with carbon isotope 13 in comparison with histopathology of gastric antrum for detection of *H. pylori* infection in children with dyspepsia. **Methods -** This cross-sectional study was performed at specialized laboratory of Shiraz Gastroenterohepatology Research Center and Nemazee Hospital, Iran, during a 12-months period. This study investigated the sensitivity, specificity, and positive and negative predictive values of UBT in comparison with biopsy-based tests. We included a consecutive selection of 60 children who fulfilled Rome III criteria for dyspepsia. All children were referred for performing UBT with carbon isotope 13 (C13) as well as endoscopy. Biopsies were taken from antrum of stomach and duodenum. The pathologic diagnosis was considered as the standard test. **Results -** The mean age of the participants was 10.1±2.6 (range 7-17 years). From our total 60 patients, 28 (46.7%) had positive UBT results and 32 (53.3%) had negative UBT results. Pathologic report of 16 (57.1%) out of 28 patients who had positive UBT were positive for *H. pylori* and 12 (42.9%) ones were negative. Sensitivity and specificity of C13-UBT for detection of *H. pylori* infection were 76.2% and 69.2% respectively. **Conclusion -** Sensitivity and specificity of C13-UBT for detection of *H. pylori* infection were 76.2% and 69.2% respectively. Another multicenter study from our country is recommended.

HEADINGS - Child health. Dyspepsia. Gastritis. *Helicobacter* infections. Peptic ulcer. Urease.

INTRODUCTION

One of the most common discovered infections of the human being is a gram-negative spiral-shaped bacillus called *Helicobacter pylori* (*H. pylori*)⁽⁷⁾. It is proved that this bacteria has a close association with chronic antral gastritis as well as duodenal and gastric ulcers both in adults and children⁽¹⁶⁾. This further underlines the necessity of a reliable diagnosis of *H. pylori* infection both before and after eradication therapy⁽²⁸⁾.

Nowadays, there are some diagnostic techniques for detection and identifying *H. pylori* infection⁽²²⁾. They are classified as invasive and non-invasive methods^(6,12). It is obvious that non-invasive methods are mostly preferred especially in pediatric age-group. Non-invasive methods include serologic evaluation of body fluids (blood or urine), checking stool for specific *H. pylori* antigens, and the last technique is Urea Breath Test (UBT). The later method identifies the "urease" enzyme activity of the *H. pylori*. If the organism is present in the stomach, it will dissolve isotope carbon-13 urea (which was eaten

by patient) and produce CO₂ with labeled isotope carbon-13 that can be detected by special equipment. The UBT has been reported to have an excellent sensitivity and specificity in both adults and pediatric age-group (reported sensitivity and specificity for UBT is 81%-100% and 80%-98%, respectively)^(6,21,22).

Nevertheless, it was indicated that the test specificity and sensitivity will decrease in young children. As a result, it is currently being preferred to apply for children older than 6 years. But it can be practically performed in all ages⁽¹²⁾.

UBT is used by some centers in our country. By using Medline, but there is no published report about sensitivity and specificity of UBT in children in our country. The purpose of this cross-sectional study is to evaluate the value of UBT with Carbon-13 for prediction of *H. pylori* infection in patients with Rome III criteria for dyspepsia. We set the histopathology report of antral biopsies as the standard for the diagnosis of *H. pylori* in this survey and compare the results of UBT with the pathology.

Declared conflict of interest of all authors: none

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METHODS

Study population

This cross-sectional study was carried out during a 12-months period from May 2011 to May 2012. All the referred children (7-18 years old) to the Pediatric GI Special Clinic of Motahari and Imam-Reza Poly-clinics of Shiraz University of Medical Sciences, Shiraz, Iran. Children with primary diagnosis of dyspepsia were examined thoroughly by a trained second-year resident of pediatrics. Complete history taking and interview with both parents were also done by the same physician. Those children who fulfilled Rome III criteria for dyspepsia were included in the study. According to these criteria, children with no anatomical disorder should have at least one of these symptoms including bothersome postprandial fullness, early satiation, epigastric pain and epigastric burning. Endoscopy was done in cases who did not respond to initial proton pump inhibitor.

These symptoms should be present for the last 3 months after symptom onset and/or at least 6 months prior to diagnosis of dyspepsia. Children who consume any kind of Proton pump inhibitors (PPI), H2 blocker or antibiotic during the past 4 weeks were also excluded. Children whose father and/or mother and/or sibling smoke cigarettes, water-bubble or pipe (passive smokers) or inhaler addicts were excluded.

Study protocol

Those children who fulfilled our inclusion and exclusion criteria (after through history taking and physical examination) were prepared for performing UBT and upper GI endoscopy. UBT test was performed by well-trained laboratory technicians at Specialized Laboratory of Shiraz Gastroenterohepatology Research Center affiliated with Shiraz University of Medical Sciences, Shiraz, Iran. All upper GI endoscopies were done by the same pediatric gastroenterologist.

On the day of visit, first the patient underwent UBT which was done by an experienced personnel. All the patients were fasting from at least 8 hours prior to beginning the UBT.

Afterwards, upper GI endoscopies were performed and samples from antrum of stomach were sent to pathology Laboratories of Nemazee Hospital and Motahari Poly-clinic. The study protocol was approved by the Institution Ethics Committee. Parents of all children gave their informed written consent.

Urea breath test; technical performance

The software used for automatic measurements is combined into the HeliFAN plus[®] device (FANci (PRG), version 2003; using windows XP[®]). All samples were measured and analyzed by using the software, according to cut off value of 4% for predicting positive and negative test. The number which was indicated by the instrument (on the monitor) was considered as the UBT score of the patient.

Upper GI endoscopy

In order to do esophagogastroduodenoscopy, at first, the pediatric gastroenterologist (attending physician) described the whole procedure to the parents, and answered any questions they might have. A local anesthetic (Lidocaine spray 0.1%) was applied to the mouth of all children in order to prevent coughing or gagging when the endoscope was inserted. A mouth guard was inserted to protect your teeth and the endoscope.

Midazolam with the dose of 0.1 mg/kg intravenously were used for children who had intractable crying and/or not cooperated for endoscopy and/or their parents requested sedation. The vital signs as well as level of consciousness of the children were monitored before, during the whole procedure and a while after finishing the endoscopy by a trained anesthesiology technician. Tissue samples were taken from antrum and duodenum and sent to an experienced pathologist.

Statistical analysis

The statistical software package SPSS for Windows, version 16.0 (SPSS, Chicago, IL, USA) was used for data analysis. The accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of UBT were calculated. Receiver-operating characteristics (ROC) curve was prepared (plot of sensitivity vs. 1-specificity) and the areas under the curves (AUC) estimated. AUC=1 indicates a perfect test, AUC>0.9 indicates high accuracy and AUC between 0.7 and 0.9 indicates moderate accuracy (Lindsetmo et al., 2008). Data are reported as means \pm standard deviation (SD) for 95% confidence interval (CI).

RESULTS

A total of 60 children with different signs and symptoms of dyspepsia that fulfilled Rome III criteria for dyspepsia by history taking and physical examination were included in this study. All the patients as well as their parents interviewed completely in order to reach precise diagnosis. The mean age of the patients was 10.15 ± 2.6 (ranging from 7 to 17) years.

Thirty patients (50%) were male and the rest were female. Figure 1 indicates signs and symptoms of patients regarding dyspepsia in our patients. According Rome III Diagnostic Criteria for Gastrointestinal Disorders, the four following sign and symptoms were evaluated; Post-prandial fullness, Early satiation, Epigastric pain or burning sensation, Regurgitation.

Regurgitation alone was not present in any patients and it always accompanied epigastric pain or burning sensation ($n=5$, 8.3%). Sixteen (26.7%) children had more than one sign and symptoms and the rest ($n=44$, 73.3%) complained of just one item. Various degrees of gastritis were observed in 27 (45%) patients through histopathologic evaluation.

The mean UBT scores was found to be $7.31 \pm 9.78\%$ (ranging from 0.00 to 50.5). Overall the UBT score was found to be over 4% in 28 (46.7%) subjects and normal in 32 (53.3%) subjects.

From our total 60 patients, 28 (46.7%) participants had positive UBT test and 32 (53.3%) patient had negative UBT results.

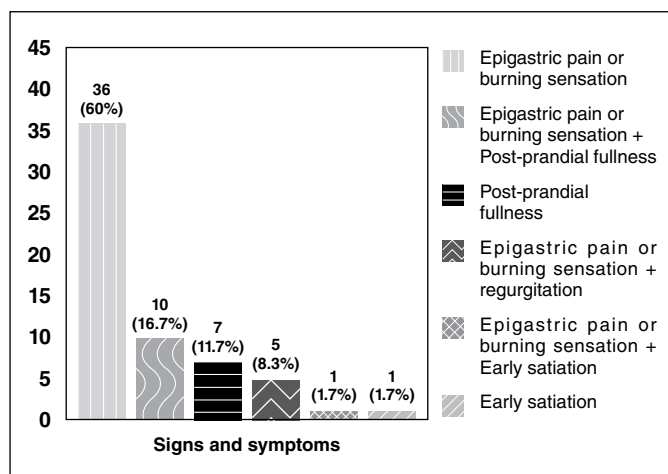


FIGURE 1. Frequency (%) of four Rome III criteria for dyspepsia by physical examination and history taking in 60 children

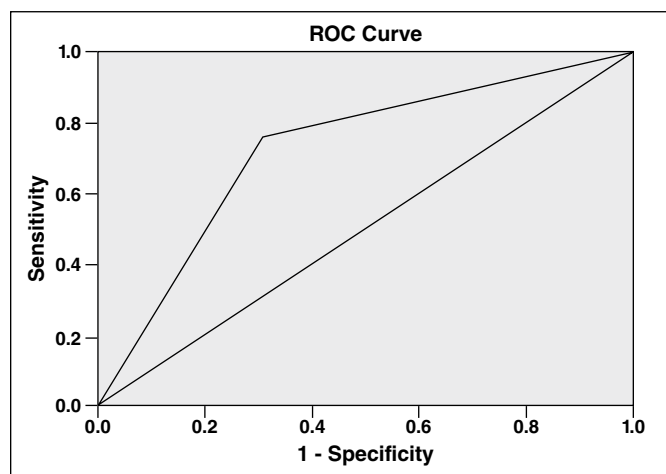


FIGURE 2. Receiver-operating characteristics (ROC) curve for the relationship between UBT and *H. pylori* infection in 60 children. Area under curve (AUC) = 0.727 (CI 95%: 0.59 - 0.86) suggestive of moderate accuracy

Histopathologic report of 16 (57.1%) out of 28 patients who had positive UBT tests were positive for *H. pylori* and the remained 12 (42.9%) ones were negative (Table 1).

Thirty two (53.3%) had negative UBT results and out of which, 27 (84.4%) children had negative histopathologic reports for *H. pylori* and the rest of them (n=5; 15.6%) were positive for *H. pylori* based on histopathology. In our survey, the histopathologic was considered as the standard for diagnosing of *H. pylori* infection. So the results of other alternative test were compared to the standard.

In our study, the Sensitivity and specificity of Urea Breath Test by using Carbon 13 isotope in detection of *H. pylori* infection were 76.2% (CI 95%: 52.4 - 90.9) and 69.2% (CI 95%: 52.3 - 82.5) respectively. Sixteen patients out of 28 patients (positive UBT) were positive regarding pathology which indicates 57.1% (CI 95%: 37.4 - 74.9) as PPV while 27 ones out of 32 (negative UBT) patients were negative according to histopathologic report which indicates 84.4% (CI 95%: 66.4 - 94.1) as NPV. Finally the accuracy of UBT for detection of *H. pylori* in our study was 71.1%.

Positive likelihood ratio and negative likelihood ratio were 2.48 (1.46-4.20) and 0.34 (0.16-0.76) respectively.

The ROC curve was drawn and the AUC was calculated by considering the histopathologic report as the standard test. We found the AUC to be 0.727 (P=0.004, CI 95%: 0.59 - 0.86) indicating a moderate accuracy for UBT (Figure 2).

DISCUSSION

Helicobacter pylori is a microorganism which can be found on the gastric mucosa^(10,26,28). *H. pylori* infection has been proved to as one of the major etiologies of gastritis, peptic ulcer disease, gastric cancer⁽²⁶⁾, and even some non-gastrointestinal manifestations such as iron deficiency anemia⁽⁸⁾. As *H. pylori* infection is generally acquired early in life⁽²⁾ and can last during life if not treated. The important aspect is that *H. pylori* infection in children might affect the onset of disease in adults⁽⁴⁾.

The urea breath test with ¹³C-carbon is a non-invasive method for diagnosis of *H. pylori* infection. The test has been used worldwide in both adults and children and is known to be a reliable method showing high sensitivity and specificity in adults. Nevertheless, relatively few validation studies have been performed in children^(14,18,27).

In this cross-sectional study, we tried to determine the value of UBT with Carbon-13 for prediction of *H. pylori* infection in patients with Rome III criteria for dyspepsia. We found that UBT possesses a sensitivity and specificity of 76.2% and 69.2% respectively. The PPV and NPV were also 57.1% and 84.4% respectively. But they can only be measured if the actual disease status of the person undergoing the test is known.

TABLE 1 Summary of Statistical results of Urea Breath Test and pathology for detection of *H. pylori* in 60 children

	Sensitivity	Specificity	PPV	NPV
Calculator	TP/TP+FN	TN/TN+FP	TP/TP+FP	TN/TN+FN
Our numbers	16/16+5	27/27+12	16/16+12	27/27+5
Final results	76.19 (52.83-91.78)	69.23 (52.43-82.98)	57.14 (37.18-75.54)	84.38 (67.21-94.72)

PPV: positive predictive value; NPV: negative predictive value; TP: true positive; TN: true negative; FP: false positive; FN: false negative. Positive Likelihood ratio: 2.48 (1.46-4.20). Negative Likelihood Ratio: 0.34 (0.16-0.76).

Positive likelihood ratio and negative likelihood ratio were 2.48 (1.46-4.20) and 0.34 (0.16-0.76) respectively.

In our survey, UBT with carbon-13 referring to our center had a sensitivity and specificity rate of 76.2% and 69.2%, respectively, which can be compared to results from other studies. Rowland et al. reported sensitivity and specificity of 100% and 97.6% respectively⁽²⁷⁾. Kalach et al. found 100% and 98.3% for specificity and sensitivity, respectively⁽¹⁵⁾. Findings of Vandenplas et al. showed 96% and 93% for sensitivity and specificity⁽²⁹⁾. Delvin et al. indicated both sensitivity and specificity of 100% in their study⁽⁹⁾. In Belgium survey by Cadranet et al. Sensitivity of 95.7% and specificity of 95.2% was reported⁽⁵⁾.

Kuloğlu et al. performed a survey in Turkey with C-14 isotope. Their results showed 92.5% and 85.5% respectively⁽¹⁹⁾. Yang et al. from Korea, indicated sensitivity and specificity of 97.1% and 96.4% correspondingly⁽³⁰⁾. In another study on 129 children age 2.1-19 years, sensitivity and specificity of 13 C-UBT were more than 90%⁽²⁵⁾. In the study by Kawakami et al. on 75 children aged 6 months to 8 years, sensitivity and specificity of UBT were 96.8% and 93.2% respectively⁽¹⁷⁾. In another study by Machado et al. on 68 children aged 6 months to 5 year 11 months, sensitivity and specificity of UBT was 93.3% and 96.2% respectively⁽²³⁾. In the current study 60 children aged 7-17 years were included which had difference compared to Kawakami et al.⁽¹⁷⁾ and Machado et al. study⁽²³⁾.

In 2011, a systematic review and meta-analysis by Leal et al. declared sensitivity of 95.9% and specificity 95.7% for all age groups⁽²⁰⁾. The authors insisted that all results of UBT have to be adjusted for cutoff value, pretest meal, and urea dose. By accommodating these factors, the accuracy of the test can be improved⁽²⁰⁾.

UBT has a advantage over the endoscopy for evaluation of *H. pylori*, because it is non invasive and there is no sampling error associated with endoscopic biopsy because of patchy infiltration of *H. pylori*⁽²⁴⁾.

Genetic variety of *H. pylori* strains and genetic specifications of the host can be important in this view. It has been

shown by Atherton et al. that the *H. pylori* load in a tissue sample is associated with specific genotypes called "cagA" and "vacA", in that cagA/vacA s1 strains infect more densely and severely than other strains⁽¹⁾. The proportion of *H. pylori*-infected subjects who are infected with different strains varies throughout the world. For instance, high prevalence of cagA strains was found in Northern Europe and in Taiwan⁽³¹⁾.

Though, till now few data are available about the ethnic or racial alterations in *H. pylori* prevalence or in the distribution and spreading of different strains within specific populations and countries⁽¹³⁾.

False positive and false negative of UBT test are unlikely and may be noted in patients who are not compliant⁽¹¹⁾.

It is also possible that the children consumed PPIs and/or antibiotics and their parents did not give the precise past drug history or the children take PPIs and/or antibiotics unintentionally. In other aspects, some pathologic variations may affect results. In our study, only histopathology was applied for detection of the organism which has some false negative results by itself. The host-organism interaction which is varied among races can affect the urease activity of organism which finally is able to change the outcomes. In the study by Bode et al., Turkish children had a higher 13C-UBT value than German children⁽³⁾. There was no study about 13C-UBT value in Iranian children compared to other countries.

Sensitivity and specificity of UBT in our study was lower than most of the studies. Another prospective multicenter study is recommended to evaluate specificity, sensitivity, positive predictive value and negative predictive value in our country.

Authors' contributions

Honar N and Haghghat M: concept/design, acquisition of data and critical revision of the manuscript. Saki F, Minazadeh A and Shakibazad N: data collection, analysis and manuscript preparation. Javaherizadeh H: writing and revision of the manuscript. All authors approved of the final version of the article.

Honar N, Minazadeh A, Shakibazad N, Haghghat M, Saki F, Javaherizadeh H. Sensibilidade diagnóstica do teste respiratório da urease para infecção por *Helicobacter pylori* em crianças com dispepsia em comparação com histopatologia. Arq Gastroenterol. 2016;53(2): 108-12.

RESUMO - Contexto - A infecção por *Helicobacter pylori*, bacilo gram negativo, tem estreita associação com gastrite antral crônica. **Objetivo** - Neste estudo, avaliou-se a precisão do teste respiratório da urease (UBT) com isótopos de carbono 13 em comparação com a histopatologia do antro gástrico para detecção da infecção por *H. pylori* em crianças com dispepsia. **Métodos** - Estudo transversal realizado no laboratório especializado no Centro de Pesquisa Gastroenterológica de Shiraz e do Hospital de Nemazee, Iran, durante um período de 12 meses. Este estudo investigou a sensibilidade, a especificidade e valores preditivos positivos e negativos da UBT em comparação com testes baseados em biópsia. Incluímos uma seleção consecutiva de 60 crianças que preencheram os critérios de Roma III para dispepsia. Todas as crianças foram encaminhadas para a realização de UBT com isótopos de carbono 13 (C13) assim como endoscopia. Biópsias foram tiradas do antro do estômago e duodeno. O diagnóstico patológico era considerado o teste padrão. **Resultados** - A idade média dos participantes foi 10.1±2.6 (intervalo de 7 a 17 anos). Do nosso total de 60 pacientes, 28 (46,7%) tiveram resultados positivos UBT e 32 (53,3%) tiveram resultados negativos de UBT. Dezesesseis (57,1%) de 28 pacientes que tiveram UBT positiva foram *H. pylori* positivo e 12 (42,9%) foram negativos. A sensibilidade e especificidade do C13-UBT para detecção da infecção por *H. pylori* foi de 76,2% e 69,2%, respectivamente. **Conclusão** - A sensibilidade e especificidade do C13-UBT para detecção da infecção por *H. pylori* foi de 76,2% e 69,2%, respectivamente. Recomenda-se outro estudo multicêntrico de nosso país.

DESCRITORES - Saúde da criança. Dispepsia. Gastrite. Infecções por *Helicobacter*. Úlcera péptica. Urease.

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