The Evaluation of Diagnostic and Predictive Values of *Helicobacter pylori* Stool Antigen Test in Iranian Patients with Dyspepsia

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KEYWORDS	ABSTRACT
	Background and Objectives*:** Iran, as a developing country, is experiencing high burdens of *Helicobacter pylori* (Hp)-associated non-communicable diseases. Hp stool antigen test (HpSA) is widely used as an inexpensive and feasible noninvasive method to diagnose Hp infection, instead of invasive approaches. The current study aimed at evaluating the diagnostic and predictive values of HpSA test for Hp infection in Iranian patients with dyspepsia. ***Methods: The current cross sectional study was performed on 100 patients with dyspepsia. Gastric mucosal specimens were taken, processed, and examined according to the standard protocols. Simultaneously, stool samples were obtained and sent to laboratory for further analyses. Hp stool antigen titers were assessed using enzyme-linked immunosorbent assay (ELISA) technique. ***Results:*** Stool antigen titers were not associated with gender (*P*-value=0.284), but correlated to age (r=0.213, *P*-value=0.034). Considering 0.385 as a cutoff point, the HpSA test had 80.4% sensitivity and 85.7% specificity. ***Conclusion:*** Based on cost-effectiveness of HpSA test, the current study findings corroborated the use of HpSA test to detect and follow-up patients with Hp infection, as an alternative method to detect Hp rather than invasive procedures.

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Introduction

Helicobacter pylori (Hp) infection is a well-known step in gastric cancer susceptibility and other chronic gastric diseases in adults and children (1-3). Prevalence of Hp infection varies by geographical regions, genetic background, and socioeconomic status (4-6). Reliable and accurate detection of this infection is of importance (7). Hp can be detected either invasively or non-invasively. Invasive methods, such as histopathology-based, culture, or rapid urease test (RUT), need gastric mucosa specimens obtained through endoscopy. Although invasive methods are commonly used as gold standards, studies demand less invasive or noninvasive methods such as urea breath test (UBT) and Hp stool antigen (HpSA) test (8-10).

There is not a definite method as a gold standard to detect Hp infection (8, 11, 12). Diagnostic and predictive values and the cost-effectiveness of tests help to choose the optimal approach (13, 14). Both the UBT and HpSA tests can detect an active Hp infection, and their sensitivity is affected by the recent medications, which limits the use of noninvasive tests for a larger population (14). UBT

is sensitive, specific, and accurate; however, it is expensive and needs trained operators. HpSA test is widely used as a simple, inexpensive, and feasible noninvasive method to diagnose and follow-up Hp infection, as an alternative method to invasive and other noninvasive approaches such as UBT (15, 16).

Iran, as a developing country, is experiencing high burdens of Hp-associated non-communicable diseases (17, 18). The current study aimed at evaluating the diagnostic and predictive values of HpSA test for Hp infection in Iranian patients with dyspepsia.

Materials and Methods
Subjects

The current cross sectional study was performed on 100 patients with dyspepsia within the age range of 20 to 70 years referred to Imam Reza Specialized Polyclinic affiliated to Arak University of Medical Sciences, Arak, Iran, from March to October 2015. All participants signed the informed consent prior to enrollment. The data pertaining demographics, medications, and medical history of patients were recorded using a questionnaire. Exclusion criteria were age under 20 years, pregnancy, post-Hp eradication, or recent use of antibiotics (within the last 2 months) or antacid medications such as proton pump inhibitors (within the last 2 weeks), and contraindications for endoscopy and biopsy such as coagulopathies. Histopathological diagnosis of Hp was considered as a gold standard.

Endoscopy and histopathology

Patients, sedated by a trained nurse using midazolam, underwent upper gastrointestinal endoscopy; and 2 gastric mucosal biopsies were taken from antrum and corpus according to the standard protocols. Specimens were processed and stained, using hematoxylin-eosin and Giemsa, and examined by an expert pathologist. The pathologist reported Hp colonization on an ordinal scale ranging from 0 (no colonization) to 3 (severe colonization).

Stool samples and antigen detection

Fresh stool samples provided in the sterile containers were immediately sent to the polyclinic laboratory. Exclusion criteria of the stool samples were diarrhea, inadequate amount and delayed delivery after collection. Examination of the stool samples to detect Hp antigen was performed using *H. pylori* Ag stool enzyme-linked immunosorbent assay (ELISA) kits (ACON, San Diego, USA). The manufacturer of the kit suggested the index values above 1.1 as positive.

Statistical analysis

Receiver operating characteristic (ROC) curve was drawn and the Youden J statistic was used to determine the optimal cutoff point. Area under curve (AUC) was analyzed. Sensitivity, specificity, accuracy, positive predictive value (PPV), negative predictive value (NPV), and diagnostic ratios were calculated for 4 cutoff points. Correlation between the age and HpSA results was evaluated using the Pearson correlation. The results of HpSA test were compared among the categories using the nonparametric Kruskal-Wallis test. P-value <0.05 was regarded as significant. Data were analyzed using IBM SPSS Statistics v. 22.

Ethical considerations

The study was approved by the Ethics Committee of Arak University of Medical Sciences and all patients signed the informed consent before enrollment. Researchers were committed to the principles of the Declaration of Helsinki and had no conflict of interest.

Results

The patients within the age range of 20 to 75 years (mean age= 47.51) were enrolled in the study. Forty-nine patients (49%) were male. The mean HpSA test result (index value) was 1.82 (standard deviation (SD) =2.02). Stool antigen titer was significantly correlated with age (r=0.213 [95%CI: 0.012-0.394], P-value=0.034). There was no significant difference in HpSA results between the 2 genders (P-value=0.284). Hp stool antigen titer was associated with severity of epithelial changes, particularly in males (Table 1, 2).

Table 1- *Helicobacter pylori* Stool Antigen Test Results Based on Gender and Epithelial Changes

Variables			Stool Antigen (Index Value) [Mean ±SD]	P-value*
Name	Categories	Count		
Gender	Male	49	1.59 ±1.95	0.284
	Female	51	2.03 ±2.08	
Chronic gastritis[†]	No	3	0.17 ±0.09	<0.001
	Mild	32	0.36 ±0.90	
	Moderate	30	2.35 ±2.05	
	Severe	35	2.83 ±1.99	
Intestinal metaplasia[†]	No	89	1.66 ±1.95	0.161
	Focal mild	6	2.90 ±2.22	
	Focal moderate	2	4.87 ±0.18	
	Extensive	3	2.35 ±2.60	
Hp colonization[†]	No	49	0.60 ±1.20	<0.001
	Mild	17	2.60 ±2.28	
	Moderate	17	3.13 ±1.74	
	Severe	17	3.25 ±1.87	
Total		**100**	**1.82 ±2.02**	

CI: Confidence interval; Hp: *Helicobacter pylori*; SD: standard deviation

[†] Based on the histopathological findings (gold standard)

[*] The nonparametric Kruskal-Wallis one-way ANOVA test

Table 2- *Helicobacter pylori* Stool Antigen Test Results Based on Epithelial Changes in Males and Females

Gender	Epithelial Change	Categories	Count	Stool Antigen (Index Value) [Mean ±SD]	P-value*
Male	Chronic gastritis[†]	No	2	0.19 ±0.11	<0.001
		Mild	15	0.16 ±0.06	
		Moderate	14	2.04 ±2.09	
		Severe	18	2.60 ±2.02	
	Intestinal metaplasia[†]	No	41	1.19 ±1.64	0.043
		Focal mild	5	2.87 ±2.49	
		Focal moderate	2	4.87 ±0.18	
		Extensive	1	- [a]	
	Hp colonization[†]	No	24	0.44 ±1.05	<0.001
		Mild	8	2.45 ±2.46	
		Moderate	11	2.73 ±1.85	
		Severe	6	2.98 ±1.82	
	Total		49	1.59 ±1.95	
Female	Chronic gastritis[†]	No	1	- [a]	0.001
		Mild	17	0.54 ±1.22	
		Moderate	16	2.62 ±2.05	
		Severe	17	3.08 ±1.98	
	Intestinal metaplasia[†]	No	48	2.06 ±2.12	0.822
		Focal mild	1	- [a]	
		Focal moderate	0	- [b]	
		Extensive	2	0.91 ±1.01	
	Hp colonization[†]	No	25	0.75 ±1.33	<0.001
		Mild	9	2.74 ±2.20	
		Moderate	6	3.86 ±1.36	
		Severe	11	3.39 ±1.97	
	Total		51	2.03 ±2.08	

CI: Confidence interval; Hp: Helicobacter pylori; SD: standard deviation

[†] Based on the histopathological findings (gold standard)

[*] The nonparametric Kruskal-Wallis one-way ANOVA test

[a] One sample, no means were calculated.

[b] No samples, no means were calculated.

According to the gold standard, 51 patients had the grades of Hp colonization and 49 had no signs of Hp infection. First, the study used 1.100 (manufacturer's protocol) as a cutoff index value to detect Hp antigen in stool. Then, the ROC curve was drawn for HpSA test (Figure 1).

AUC was 0.851 (95%CI: 0.772-0.931) and significant (P-value <0.001). Using the ROC curve, the cutoff point was 0.385 (the Youden J=0.661). The study also tried 0.110 and 5.855 as the most sensitive and the most specific cutoff points, respectively. The positive and negative results for each cutoff point were cross-tabulated against the gold standard, ie, histopathology (Table 3).

Table 4 shows sensitivity, specificity, predictive values, and diagnostic ratios of HpSA test for each cutoff points.
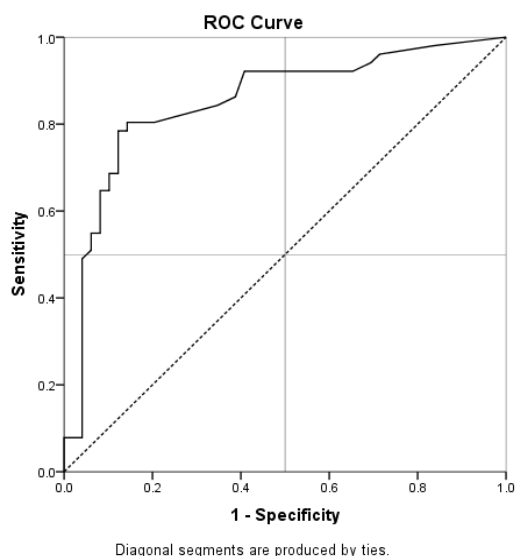

Fig 1. Receiver Operating Characteristic Curve for Helicobacter pylori Stool Antigen Test

Table 3- Positive and Negative Results for Four Cutoff Points Stool Antigen Index Values to Detect Helicobacter pylor

		Test Cutoff Point (Stool Antigen Index Value)								Total
		0.110		0.385		1.100		5.855		
		+	-	+	-	+	-	+	-	
Hp colonization (pathology) [a]	+	50	1	41	10	40	11	1	50	51
	-	41	8	7	42	7	42	0	49	49
Total		91	9	48	52	47	53	1	99	100

Hp: *Helicobacter pylori*
[a] Gold standard

Table 4- *Helicobacter pylori* Stool Antigen Test Diagnostic and Predictive Values for Four Cutoff Points Stool Antigen Index Values

Test Characteristic	Cutoff Point (Stool Antigen Index Value)			
	0.110 [Most Sensitive]	0.385 [Selected]	1.100 [Predefined]	5.855 [Most Specific]
Sensitivity (%)	98.04	80.39	78.43	1.96
Specificity (%)	16.33	85.71	85.71	100.00
Accuracy (%)	58.00	83.00	82.00	50.00
PPV (%)	54.95	85.42	85.11	100.00
NPV (%)	88.89	80.77	79.24	49.49
LR+	1.17	5.63	5.49	- [a]
LR-	0.12	0.23	0.25	0.98
DOR	9.75	24.48	21.96	- [a]

PPV: positive predictive value; NPV: negative predictive value; LR+: positive likelihood ratio; LR-: negative likelihood ratio; DOR: diagnostic odds ratio
[a] Unable to compute (division by 0)

Discussion

Consistent with the former studies (16, 19-25), the current study found that HpSA test was sensitive, specific, and accurate to diagnose Hp infection (sensitivity: 80.4%, specificity: 85.7%). To avoid false negative results, which would be achieved if there was a history of chronic liver diseases (eg, cirrhosis), gastrointestinal bleeding, and antacid medications, the patients undergoing proton pump inhibitor or bismuth therapy were excluded. However, a few patients with asymptomatic gastrointestinal bleeding may have been included in the study, which may drive the results into the false negative HpSA tests (21). Besides, mild Hp colonization in the gastric mucosa accompanied by low concentrations of stool antigens could cause false negative results. Other *Helicobacter* species may cause false positive results (26). The condition of stool samples should be considered, too. Unformed or watery stool samples lead to less accurate test results due to diluted antigens (27).

It is challenging to define an appropriate cutoff point and test validations are important to find the optimal cutoff index for a distinct population (28, 29). The current study employed the manufacturer's cutoff index value (1.1) coming with a lower sensitivity (78.4%) and diagnostic odds ratio (DOR) of 21.96, compared with using the new cutoff index (0.385) with sensitivity and DOR of 80.4% and 24.48, respectively.

The HpSA test is quick, easy, patient-friendly, and inexpensive. Based on the rising trend of Hp-associated non-communicable diseases in Iran and the cost-effectiveness of HpSA (13), the current study findings corroborated the use of HpSA test to detect and follow-up patients with Hp infection, as an alternative to the invasive procedures.

Conclusion

According to the current study findings, the new cutoff point of 0.385 was useful when a highly sensitive test (eg, in screening) or a test with low false negative rates (eg, during follow-up) is employed to diagnose Hp infection in Iranian patients with dyspepsia. The use of other non-invasive methods can confirm the HpSA test results.

Recall bias and small sample size were the potential source of limitations in the current study.

Conflicts of interests

The authors declared no conflict of interest.

Acknowledgements

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