Original Article

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Microbiology Section

Validation of a Rapid Stool Antigen Test for the Diagnosis of *Helicobacter pylori* Infection in Dyspeptic Patients-A Study from Central Kerala

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ABSTRACT

Introduction: Accurate diagnosis of *Helicobacter pylori* infection in dyspeptic patients is highly essential to institute eradication therapy and to prevent complications. The introduction of 'test and treat strategy' using validated rapid stool antigen tests can significantly reduce the burden of *H.pylori* infection in developing countries.

Aim: To validate a rapid monoclonal immunochromatographic stool antigen test, Epituub® fecal *H.pylori* antigen test kit for the diagnosis of *Helicobacter pylori* infection among dyspeptic patients.

Materials and Methods: Stool samples were collected for Epituub® fecal *H.pylori* antigen test from randomly selected patients undergoing upper gastrointestinal endoscopy for the evaluation of dyspepsia. Gastric biopsy samples were also collected for urease test and histopathology. The diagnostic criteria for *H.pylori* infection was defined as a positive test result for both rapid urease test and histopathology examination. All other combination of the results was considered as negative. The test performance was assessed by determining sensitivity, specificity, positive predictive value and negative predictive value with reference to histopathology examination and rapid urease test.

Results: Based on the reference criteria, 31% (35/113) patients were diagnosed as *H.pylori* infected and 41% (46/113) were rapid stool antigen test positive. The sensitivity, specificity, positive predictive value and negative predictive value of the Epituub® fecal *H.pylori* antigen test kit were 88.5%, 80.76%, 67.3% and 94.02% respectively.

Conclusion: Epituub® fecal *H.pylori* antigen test can be used as a valid alternative to invasive tests for the diagnosis of *H.pylori* infection. It is also relatively cheap, fast and easy to perform.

Keywords: Gastrointestinal endoscopy, Histopathology, Sensitivity, Specificity

INTRODUCTION

Helicobacter pylori infection remains a major global health problem causing peptic ulcer disease and gastric neoplasia with more than 50% of the world's population infected. The World Health Organisation has identified this Gram negative gastric bacterium as a Class I human carcinogen and has proposed *H.pylori* eradication as a strategy for preventing gastric cancer [1,2]. The World Gastroenterology Organisation reported a prevalence of 88% *H.pylori* infecton among Indian adults in August 2010 which was very high compared to other Asian countries [3]. A large meta-analysis published in 2017 showed a prevalence of 63.5% in Indian population [4]. This has become a big public health issue in India.

There are several diagnostic tests for *H.pylori* infection which are classified into non invasive and invasive. Invasive methods include Rapid Urease Test (RUT), histopathology, culture and molecular tests done on gastric biopsies obtained during endoscopy. The main non invasive tests available are Urea Breath Tests (UBT), serology and Stool Antigen Tests (SAT). Maastricht V/Florence Consensus Conference 2015 recommends a 'test and treat' strategy involving non invasive methods for uninvestigated dyspepsia in populations where *H.pylori* prevalence is high with certain exceptions [5].

Among the non invasive tests UBT has excellent sensitivity and specificity. However, UBT is a highly expensive test and requires trained staff which makes it a less acceptable choice in present population. Serology based tests are poor differentiators of current and past infection and often yields misleading results in areas of high prevalence [6]. Thus, pathogen-specific stool antigen tests that detect active infection are a valid choice of non invasive tests to detect *H.pylori* infection. Both European and Japanese guidelines endorsed the use of SATs using monoclonal antibodies for primary diagnosis as well as for the assessment of eradication therapy [7].

Stool antigen tests are available in two formats: ELISA and Immunochromatography (ICT). Faecal *H.pylori* antigen tests based on immunochromatographic reactions are more widely used nowadays due to their ease of use and cost benefits in addition to their rapidity. As the accuracy of the test may vary in different populations due to the difference in the antigenicity of *H.pylori* strains, a local validation of SAT is essential before use as a diagnostic test [8]. There are only few published data from India regarding the validation of these newer stool antigen test kits [9].

The present study conducted at Government Medical College, Thrissur, Kerala, India, was an attempt to validate a locally available rapid SAT for diagnosing *H.pylori* infection among dyspeptic patients. The *H.pylori* infection among dyspeptic patients is diagnosed primarily by endoscopic biopsy and histopathological examination in present institution. The availability of locally validated SAT can not only reduce the endoscopy workload but also provide an acceptable non invasive diagnostic method.

MATERIALS AND METHODS

A prospective diagnostic validation study was carried out among randomly selected 113 patients above 14 years of age who were undergoing upper gastrointestinal endoscopy for evaluation of dyspepsia at the Department of Gastroenterology, Government Medical College, Thrissur, kerala, India. The study was conducted over a period of one year from March 2015 to February 2016 after Institutional Ethics Committee approval (Reg. No 170/2). The sociodemographic and clinical data were collected after a written

informed consent. Patients who were treated with antibiotics or proton-pump inhibitors within two weeks prior to endoscopy or with history of gastric surgery were excluded from the study.

During endoscopy three gastric biopsy samples (two from antrum and one from body) were collected from each patient. Sampling from antrum as well as corpus biopsy from greater curvature was done to avoid false negative results due to patchy distribution of *H.pylori* in the stomach. One antral biopsy sample was used for RUT and other two were used for histopathology examination. The RUT was done using 0.5 mL of RUT broth (Himedia M1828). The test was considered as positive when the colour of the medium changed from yellow to pink on incubation at 37°c for maximum upto 18 hours [10]. Histopathology examination was carried out in the Department of Pathology using Haematoxylin and Eosin stain and Giemsa stain as per standard protocols.

Stool Antigen Test

Stool samples were collected in sterile wide mouthed containers and rapid SAT was done immediately using Epituub® fecal *H.pylori* antigen test kit, a commercially available kit based on ICT assay according to manufacturer's (Epitope Diagnostics, Inc. Sandiego, USA) instructions. Stool samples were transferred to the collection tube containing extraction solution to extract *H.pylori* antigens from faeces. The sampling tube was mixed vigorously to ensure a good liquid suspension and then the sampling tube was positioned upside down in vertical allowing the stool particles to sediment for about one minute. Then the test strip was removed from the sealed foil pouch and screwed in a vertical position into the sampling tube by breaking into the bottom seal of the sampling tube. Thus, the solution was allowed to flow into the bottom space of the test strip. The test result was observed at 10 minutes. If two red/pink coloured bands were visible at the test area and control area within 10 minutes, the test result was considered positive and valid. If test area has no coloured band and the control area displays a red/pink coloured band, the test result was negative. If a coloured band was not formed in the control area regardless there is any band in the test area, the test result was considered invalid.

The gold standard diagnostic criteria for *H. spylori* infection in the present study was defined as a positive test result for both rapid urease test and histopathology examination. Positive test result for only one of these tests was considered as negative.

STATISTICAL ANALYSIS

Data were analysed by descriptive analysis using Epiinfo software. Quantitative variables were expressed as means±standard deviation while qualitative variables were expressed as percentages. Chi-square test was used to compare categorical data. The test performance was assessed by determining sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy.

RESULTS

The age of patients ranged from 15 to 79 years and mean age±SD was 45.8±14.7 years. Out of the 113 patients with dyspepsia, 54% (61/113) were females and 46% (52/113) were males. Based on the on the gold standard diagnostic criteria, 35 (31%) patients were diagnosed as *H.pylori* infected and 78 (69%) as uninfected. Histopathology showed *H.pylori* positivity in 61% of the cases (69/113) where as RUT showed positivity in only 38% (43/113) of the cases. Of 113 patients, 41% (46/113) were rapid SAT positive.

Majority of the patients studied belonged to the age group 50-59 years with maximum *H.pylori* positivity. *H.pylori* positivity was also more in females, 23 (37.7%) as compared to males, 12 (23%). There was no statistically significant association between female sex and *H.pylori* positivity. [Table/Fig-1] depicts the age distribution and [Table/Fig-2] shows the age and sex characteristics of the study population.

Age Group	Frequency (Percentage)	*H.pylori* infected (Percentage)
15-19	3 (2.65%)	0
20-29	17 (15.04%)	5 (29.4%)
30-39	17 (15.04%)	4 (23.5%)
40-49	24 (21.24%)	8 (33.3%)
50-59	27 (23.89%)	10 (37%)
60-69	22 (19.47%)	7 (31.8%)
70-79	3 (2.65%)	1 (33.3%)
Total	113 (100.00%)	35

[Table/Fig-1]: Age distribution among the study population.

		No of patients, n (%)	*H.pylori* infection n (%)	Chi-square	p-value
Age	≤45 years	55 (48.7)	15 (27.2)	0.6864	**0.4**
	>45 years	58 (51.3)	20 (34.4)		
Sex	Males	52 (46)	12 (23)	2.8	**0.09**
	Females	61 (54)	23 (37.7)		

[Table/Fig-2]: Age and sex characteristics of the study population and their association with *H.pylori* infection.

Endoscopic abnormalities were observed in 75 out of 113 patients (66.3%). Gastric ulcers were present in 11 (9.73%) patients where as 9 (8%) patients were suffering from duodenal ulcer. Gastritis was observed in 18 patients. Other endoscopic findings were hiatus hernia, proximal gastropathy, polyp, oesophagitis, telengectasia etc. One patient had carcinoma stomach. [Table/Fig-3] shows the characterisation of *H.pylori* infected population based on endoscopic diagnosis. One patient was having both hiatus hernia and gastric ulcer. Another patient was having both proximal gastropathy and hiatus hernia. Patients with other findings were not having *H.pylori* infection.

	Endoscopic finding	Total No n (%)	*H.pylori* Infection No (%)	X^2	p-value
0	Normal endoscopy	38 (33.63)	12 (31.57)	0.0098	0.9
1	Gastritis	18 (16)	5 (27.7)	0.1023	0.74
2	Hiatus hernia	13 (11.5)	4 (30.8)	0.0003	0.986
3	Gastric ulcer	11 (9.73)	6 (54.5)	3.16	0.07
4	Proximal gastropathy	10 (8.8)	7 (70)	7.815	0.005
5	Duodenal ulcer	9 (8)	3 (33.3)	0.0255	0.87

[Table/Fig-3]: Characterization of *H.pylori* infected population based on endoscopic diagnosis.
Note: One patient was having both hiatus hernia and gastric ulcer. Another patient was having both proximal gastropathy and hiatus hernia

Only proximal gastropathy was statistically associated with *H.pylori* infection (p-value=0.005). Histopathological examination of gastric biopsies obtained from 113 patients showed chronic gastritis in 88 patients, *H.pylori* was demonstrated by staining in 68 out of these 88

Histopathological diagnosis	no	*H.pylori* infected No (%)	Chi-square	p-value
Chronic gastritis	88	34 (38.6)	10.92	0.001
Polyp	2	0		
Carcinoma	1	1		
Erosive gastritis	3	0		
No pathology	19	0		

[Table/Fig-4]: Correlation of *H.pylori* infection and histopathological diagnosis.

Histopathological diagnosis	No	*H.pylori* positivity by staining	Chi-square	p-value
Chronic gastritis	88	68	43.96	0.001
Others	6	1		
No pathology	19	0		

[Table/Fig-5]: Correlation of *H.pylori* staining and histopathological diagnosis.

patients and this association was significant (p<0.001). Based on the diagnostic criteria 34 out of 88 chronic gastritis patients (38.6%) were *H.pylori* infected (p<0.001) [Table/Fig-4,5] respectively.

Sensitivity, specificity, predictive values and accuracy of the stool antigen test kit were calculated in relation to the diagnostic criteria. The rapid stool antigen test detected *H.pylori* antigen in 31 of the 35 *H.pylori*-infected patients (sensitivity 88.5%; 95% Confidence Interval (CI): 85.4-91.6%), and there were four false-negatives. A total of 63 patients showed negative results out of 78 *H.pylori*-negative patients

Stool antigen test	*H.pylori* infection		Total	Chi-square	p-value
	Present	Absent			
	1	0			
Positive	31 (67.39%)	15 (32.61%)	46	48.12	0.001
Negative	4 (5.97%)	63 (94.03%)	67		
TOTAL	35	78	113		

[Table/Fig-6]: Performance of Epituub® fecal H.pylori antigen test kit.

Validity	Percent	95% CI	Chi-square	p-value
Sensitivity	88.57	85.4-91.6	48.12	0.001
Specificity	80.77	76.9-84.61		
PPV	67.39	62.8-71.9		
NPV	94.03	91.7-96.3		
Accuracy	83.1%			
LR +	4.585			
LR-	0.142			

[Table/Fig-7]: Validation of Epituub® fecal *H.pylori* antigen test in relation to gold standard.
PPV: Positive predictive value; NPV: Negative predictive value; LR: Likelihood ratio

(specificity 80.76; 95% CI: 76.9-84.61%), and there were 15 false-positives. The positive predictive value of the stool antigen test was 67.3 (95% CI: 62.8-71.9%) and the negative predictive value of the stool antigen test was 94.03 (95% CI: 91.7-96.3%) [Table/Fig-6,7].

DISCUSSION

The worldwide prevalence of *H.pylori* infection varies widely. A study conducted by Adlekha S et al., in Central Kerala detected a prevalence of 62% among dyspeptic patients (urease test and histopathology) whereas, Paul N et al., reported a prevalence of 36% from South Kerala (IgG antibody and urease test) [11,12]. Sodhi JS et al., detected 58% prevalence in Kashmir (RUT and histopathology) [13]. A study conducted by Rastogi M et al., showed a prevalence of 50.51% by SAT [14]. High prevalence of infection in present population justifies the use of 'test and treat approach' with non invasive tests [4]. Diagnostic tests with both high sensitivity and specificity, exceeding 90% are advisable for accurate diagnosis of *H.pylori* infection in clinical practice [15].

Differences in the antigenicity of *H.pylori* strains can affect the accuracy of SATs in different populations [16]. Also, the detection limit of bacterial antigen varies among different kits. Therefore, sensitivity and specificity of SATs should be tested in each population before use in the management of *H.pylori* infection [8]. Meta-analyses have shown that monoclonal antibody based assays are better compared to polyclonal antibody based assays [17]. Among the SATs ICT based tests are easy to perform and are useful for in-office rapid diagnosis of *H.pylori* infection [18]. In comparison to ELISA technique, ICT based tests do not require specialised equipment, technical expertise or a laboratory set up [19]. Validation of any test requires its comparison to a gold standard. In present study, a combination of two invasive methods, RUT and HPR are used as the diagnostic criteria for *H.pylori* infection which has served as the gold standard.

Poor socio-economic status and overcrowded conditions in developing countries have been attributed to the early childhood acquisition of *H.pylori* infection (30%-50%) which peaks during adulthood (over 90%) [20]. However, present study did not show any specific trend in the distribution of *H.pylori* infection among various age groups. Maximum *H.pylori* infection was in the age group 50-59 years (37%) and the number of patients with dyspepsia was also maximum in the same age group. There was no statistically significant difference in *H.pylori* infection above and below 45 years. Increasing trend of prevalence with age is not uniformly reported in all Indian studies. In a study of 500 adults, Ahmed KS et al., noticed increasing prevalence with age which peaked in the 70-79 year age group (90%; p<0.01) [21] while Khan S et al., noticed a maximum (74%) between 16-30 years and thereafter showing a decline [22].

In present study population, both dyspepsia and *H.pylori* infection were more in females (54% and 37% respectively) compared to males (46% and 23%) which were statistically non significant. The female preponderance in *H.pylori* positivity is contrary to other studies from India where male preponderance was reported without statistical significance [11,22]. It is well known that *H.pylori* infection is dependent upon many variables such as age, sex, socio-economic status, dietary habits, genetic, and immunological factors [3].

The test used in present study was Epituub® fecal *H.pylori* antigen Test kit which employs dye-conjugated monoclonal antibody against *H.pylori* antigen, and solid-phase/membrane coated specific anti-*H. pylori* monoclonal antibody. The detection limit of *H.pylori* is about 4-8 ng/mL. This rapid SAT had a sensitivity of 88.5% (95% CI: 85.4-91.6%), and specificity of 80.76% (95% CI: 76.9-84.610) in relation to gold standard. To the best of present knowledge, this is the first prospective study to examine the efficacy of the fecal *H.pylori* antigen test in the diagnosis of *H.pylori* infection from Kerala. The performance characteristics of different assays against similar gold standards have been published from across the world [23-26] [Table/Fig-8]. The sensitivity of kits range from 51% to 87% and

Authors (Year)	Country	Test	Patients	Gold standard	Sensitivity	Specificity
Rastogi M et al., [9]	India	Immunocard STAT HpSA test	78	HP, RUT	95.5	81.1
Trevisani L et al., [23]	Italy	ImmunoCard STAT	105	RUT, HP	85	93
Kesli R et al., [24]	Spain	*H.pylori* Fecal Antigen Test	168	RUT, HP	81	92
Korkmaz H et al., [25]	Turkey	ImmunoCard STAT	198	RUT, HP	68.9	92.6
		One step *H.pylori* antigen	198	RUT, HP	86.7	88.9
		H.pylori Fecal Antigen Test	198	RUT, HP	78.9	87
Korkmaz H et al., [26]	Turkey	Genx *H.pylori* CARD test	162	RUT, HP	51.1	95
Present study	India	Epituub® fecal *H.pylori* antigen test	113	HP, RUT	88.57	80.77

[Table/Fig-8]: Performance characteristics of different assays [9,23-26].

specificity ranged from 87% to 95%.

When compared to different rapid stool antigen methods, performance of present test kit was quite acceptable. A low rate of false negatives i.e., 11.5% may be due to factors like mild gastrointestinal bleed, poor bacterial colonisation of the stomach [25], possible use of bismuth containing antacids and a rare possibility of inadequate period of abstinence of PPI before SAT. Interference with other *Helicobacter* species may have caused false-positive test results [27,28].

The availability of validated non invasive SAT will facilitate the introduction of 'test and treat strategy' in the present study population. Easy and early detection of *H.pylori* infection and treatment may reduce the prevalence of associated complications such as chronic gastritis and carcinoma stomach in the long run.

LIMITATION AND FUTURE RECOMMENDATION

One major limitation of the study was the small sample size and hence larger studies are required to draw a conclusion regarding the utility of present method as a non invasive diagnostic test. A detailed analysis of sociodemographic factors is also required. Such studies will definitely help us to introduce the rapid SAT as an effective diagnostic or screening tool in present population.

CONCLUSION

In conclusion, this rapid ICT *H.pylori* SAT appears to have reasonable diagnostic accuracy in the pre-treatment setting, and could represent a valid alternative to the invasive tests. It is relatively easy, fast to perform and can reduce the endoscopic work load. Larger studies should be planned to confirm present results, and its use in the childhood population and in the post treatment setting. Large scale use of non invasive methods will make the tests cheaper and they can be introduced in primary care setting for early detection and treatment of *H.pylori* infection.

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