Original Article

DOI: 10.7860/NJLM/2016/21674:2158

Microbiology Section

Comparison of Different Diagnostic Methods of *Helicobacter pylori* in Dyspeptic Patients of a Tertiary Care Hospital of Uttarakhand, India

SHIWANI SHARMA, GARIMA MITTAL, R K AGARWAL, VIVEK AHUJA, ROHIT GUPTA, SOHAIB AHMAD

ABSTRACT

Introduction: *Helicobacter pylori* (*H.pylori*) infection is very common worldwide. A reliable diagnosis is crucial for better treatment of the patients. However, there is no single diagnostic method that can meet the criteria in identification of *H.pylori*.

Aim: To detect *H.pylori* from endoscopic biopsies in dyspeptic patients and to compare the sensitivity and specificity of different diagnostic methods for *H. pylori* infection.

Materials and Methods: This observational and cross-sectional study was conducted in the Department of Microbiology and Medicine, Himalayan Institute of Medical Sciences (HIMS), Swami Ram Nagar, Dehradun, over a period of 12 months. Biopsies of gastric antrum from 100 patients with dyspepsia were studied for the detection of *H.pylori* by various methods like bacterial culture, Rapid Urease Test (RUT) and Christensen's Tube Urease (CTU) test. Stool samples from all the patients were also screened for *H.pylori* stool antigen (HpSA) test. Bacterial culture was considered as gold standard in this study and other diagnostic tests were compared with the gold standard.

Results: Out of 100 patients *H.pylori* was detected by bacterial culture, RUT, CTU and HpSA in 34%, 61%, 53% and 28% cases respectively. Sensitivity of RUT, CTU and HpSA were 100%, 91% and 73.5% respectively and specificity of RUT, CTU and HpSA were 59%, 66.7% and 95.3% respectively. Thus, RUT was the most sensitive (100%) and HpSA (95.3%) was the most specific test, when culture was being considered as gold standard.

Conclusion: RUT is best considered as a screening test and not as the gold standard for *H. pylori*. The HpSA test is also rapid, simple and non-invasive test with acceptable results that can be used for monitoring.

Keywords: Bacterial culture, Christensen's tube urease test, Rapid urease test, Stool antigen test (HpSA)

INTRODUCTION

Helicobacter pylori (*H.pylori*) is a spiral shaped, microaerophilic, motile, flagellated gram negative bacterium which has been recognised as the most common cause of chronic human bacterial infection affecting up to 50% of the world's population [1,2]. The discovery of *H.pylori* in 1984 by Warren and Marshall represents one of the most important developments in medicine of the past century [3].

Infection results in persistent chronic gastritis lasting for many years possibly life long, such gastritis is thought to be involved in the possible sequence of gastric mucosal atrophy, intestinal mucosal metaplasia, Mucosa Associated Lymphoid Tissue (MALT) lymphoma and gastric carcinoma. Most *H.pylori* infections are probably acquired in childhood and adolescence [4]. The prevalence of *H.pylori* infection is 25%-50% in developed countries and 70%-90% in developing countries [5]. The most probable mode of transmission is person-to-person spread but oral-oral and faecal-oral transmissions have also been reported [6].

There are several invasive and non-invasive techniques used to diagnose *H. pylori* infection, each having its own advantages and disadvantages. Invasive methods require biopsy samples from stomach and duodenum and can be tested by various methods such as histology, Rapid Urease Test (RUT), microbiological culture and Polymerase Chain Reaction (PCR) whereas non-invasive tests include stool antigen test, serology and Urea Breath Test (UBT). The choice of the test is governed by several factors like clinical condition of the patient, cost of the test and its sensitivity and specificity [7]. But, all these tests have their own limitations due to faulty technique in collecting biopsy samples, observer related variations, distribution of *H. pylori* in stomach and type of stain used. These factors may sometimes give false results [8].

The purpose of this study was to detect *H.pylori* from endoscopic biopsies in dyspeptic patients and to compare the sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of different diagnostic methods for *H. pylori* infection.

MATERIALS AND METHODS

This observational and cross-sectional study was conducted in the Department of Microbiology and Medicine, Himalayan Institute of Medical Sciences (HIMS), Swami Ram Nagar, Dehradun, over a period of 12 months. A total of 100 adult dyspeptic patients who had undergone upper gastrointestinal endoscopy from July 2014 to June 2015 were enrolled in this study.

Inclusion criteria: Adult patients (>18 years) who presented with dyspeptic symptoms to the Gastroenterology OPD requiring upper GI endoscopy.

Exclusion criteria: Patients who had taken antibiotics during past two weeks, active GI bleeding, pregnancy and history of gastrectomy.

History of risk factors like alcohol intake, smoking, excessive intake of tea or coffee and stress were taken to see whether any statistical significance with *H. pylori* infection was there or not.

Written informed consent was obtained from all the patients before endoscopy and sample collection. Approval from Institutional ethical committee was taken prior to initiation of this study.

Endoscopy and biopsy sampling: Endoscopy was carried out using Olympus GIF Q150 and KARL STORZ 13801 PKS on patients after an overnight fast. Three biopsy specimens from mucosa of the gastric antrum were obtained by endoscopy and were placed in a small screw capped bottle containing 0.2 mL sterile normal saline to maintain humidity. Out of these three biopsy samples, one was sent to bacteriology laboratory for bacterial culture, second was used for rapid urease test and last one for Christensen's tube urease test.

Bacterial culture: Culture was done by direct plating from biopsy samples. The media used was BBLTM Brucella agar (Becton, Dickinson and Company, USA), with 5% defibrinated sheep blood. The inoculated plates were incubated at 37ºC for three days in a microaerophilic environment which was provided by Campypak (BD Gas Pack). The isolated bacteria were identified by its colony morphology, microscopy and biochemical tests i.e. positive catalase, oxidase and urease test [9].

Rapid Urease Test (RUT): One gastric biopsy sample was screened by commercial rapid urease kit i.e. RUT DRY Test kit (Gastro Cure System, Kolkata, WB, India) available at pharmacy store of our HIMS hospital. Here the biopsy sample was directly put into the well of kit. The urease enzyme produced by *H.pylori* rapidly hydrolyses urea in the well, producing ammonia. The rise in the pH of the medium by ammonium ions can be detected with a pH indicator [9]. Immediately, there was change in colour to pink, in case of positive samples and in case of negative it remained yellow. This test was read after four hours for positive and negative results.

Christensen's Tube Urease Method (CTU): Christensen's urease medium was prepared in our Microbiology laboratory using distilled water, phenol red as indicator, glucose and urea. It was inoculated directly with the crushed biopsy material and incubated at room temperature. A positive control *Proteus mirabilis* (ATCC 29906) and a negative control *E.coli* (ATCC 25922) were put up for each test. The test was read after half hour, 1 hour, 4 hour, 6 hour and 12 hours of incubation. The test was considered positive when the colour changed from yellow to red [9].

***H. pylori* Stool Antigen Test (HpSA):** Stool specimens were analysed using a commercially available kit i.e. SD BIO LINE H.pylori Ag detection kit (Standard Diagnostic, Inc., Republic of Korea) according to manufacturer's protocol. It is a non-invasive method for the detection of *H.pylori* infection. Test device and stool sample were allowed to settle to room temperature. Assay diluent was taken in the sample collection tube and with the help of swab, a portion of faeces about 500 mg was taken and inserted into the sample collection tube containing assay diluent, three drops of this mixture (assay diluent and stool sample) were added into the sample well of the test device. Interpretation of test result was done within 10-15 minutes. The presence of two colour bands as test band (T) and control band (C) within the result window indicated a positive result. The presence of only control band (C) within the result window indicated a negative result as per the kits instruction.

RESULTS

Out of the 100 enrolled patients in this study, 75 were males and 25 were females. Out of the 34 culture positive patients, 28 (82.4%) were males and 6 (17.6%) were females. This was not found to be statistically significant (p-value=0.22). The age of the patients ranged from 18 to 70 years with a mean age of 40.34±12.35 years. Maximum numbers of patients were in the age group 31-40 years (29%).

The patients presented with various symptoms, the commonest being pain abdomen (77%), nausea and vomiting (75% and 65%) and dyspeptic symptoms (68%). Loss of appetite was seen in 40%, alteration in bowel habits in 20% and weight loss in 12% of total patients [Table/Fig-1]. Risk factors like alcohol intake (p-value=0.502), smoking (p-value=0.61), excessive intake of tea or coffee (p-value=0.48), and stress (p-value=0.33), did not show any statistical significance with *H. pylori* infection.

Seventy-one percent patients had gastritis on endoscopy followed by gastric ulcers (3%) and gastric erosions (2%). Normal findings were seen in 24% dyspeptic patients. 32 out of 71 (45.1%) were found to be culture positive, which was statistically significant with odds ratio of 11.07 (95%CI: 2.4-50.2) and p-value of 0.0006.

The prevalence of *H. pylori* infection came out to be 34% by bacterial culture. Out of which 21 (61.8%) showed pure

Symptoms	Out of total patients (N=100) No. (%)	Out of culture positive patients (N=34) No. (%)
Pain abdomen	77(77)	26(76.5)
Nausea	75(75)	29(85.3)
Dyspeptic symptoms	68(68)	22(64.7)
Vomiting	65(65)	29(85.3)
Loss of appetite	40(40)	19(55.9)
Alteration in bowel habits	20(20)	10(29.4)
Weight loss	12(12)	5(14.7)

[Table/Fig-1]: Clinical features of the study subjects.

growth and rest 13 (38.2%) showed mixed growth with other bacteria.

Out of 100 patients, *H. pylori* were detected by RUT in 61% cases, by CTU test in 53 % cases and by stool antigen test (HpSA) in 28% cases. Comparison of various diagnostic tests when culture was considered as gold standard is shown in [Table/Fig-2].

DISCUSSION

The discovery of *H.pylori* by Warren and Marshall in 1983 has changed the conventional concept of gastroduodenal ulcer disease [10]. It has now been recognised as a definite gastroduodenal pathogen with its role in causation of chronic gastritis, peptic ulcer disease and gastric carcinoma [11]. The accurate method of detection of *H. pylori* is required for treatment of infected patients and for eradicating the bacteria.

Currently there are several diagnostic methods for detection of *H.pylori* infection, each having its own advantages, disadvantages and limitation in terms of indication, sensitivity, specificity, cost and time.

Isolation rate of *H. pylori* was 34% on brucella agar and bacterial contamination of the medium was frequently seen. The contaminant bacteria were *Pseudomonas* spp., *Proteus* spp. and *Klebsiella* spp. the source of which could be contaminated biopsy forceps. The other reason could be contamination during obtaining, transporting and preparing of defibrinated sheep blood added to the brucella agar. Various other studies have reported isolation rates varying from 22.5% – 52% as shown in [Table/Fig-3][12-19]. Though, culture from biopsy samples is considered as gold standard for diagnosis of *H. pylori* infection but it cannot be routinely used because it is time consuming and very fastidious conditions are required for the growth of bacteria.

The most common identifiable lesion at endoscopy in this study was gastritis i.e. 71% which is comparable to the study done by Nanivadekar et al., who studied 200 patients with dyspepsia showing gastritis (81.5%) as the predominant finding [20]. Out of these 71% patients, 45.1% patients showed culture positivity. A study by Tzeng et al., showed that out of 48 endoscopically diagnosed gastritis patients, 93% were diagnosed with *H.pylori* infection [21].

Many commercial RUTs are available like gel-based tests, paper-based tests and liquid based tests, which give results in varying time, depending on the procedure of the test and the bacterial load in the biopsy specimen [22]. RUTs available commercially have specificities above 95%-100%; and sensitivity ranging from 85%-95% [23]. Sensitivity of rapid urease test in our study was 100%, specificity was 59%. Another study showed that commercial RUT kits have

Diagnostic method	Culture positive	Culture negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Odds ratio	p-value
RUT (+ve)	34	27	100	59	55.7	100	Infinity	<0.0001
RUT (-ve)	0	39						
CTU (+ve)	31	22	91	66.7	58.5	93.6	20.67	<0.0001
CTU (-ve)	3	44						
HpSA (+ve)	25	3	73.5	95.3	89.3	87.3	57.4	<0.0001
HpSA (-ve)	9	62						
Total	34	66/65*						

[Table/Fig-2]: Comparison of different diagnostic methods (RUT, CTU, and HpSA) by gold standard.
**in case of stool antigen detection, only 99 patients gave the stool sample.

S. No	Studies	Year/ Place	Culture positivity
1	Yoosuf H et al., [12]	1995/India	22.5%
2	Arora U et al., [13]	2003/India	52%
3	Khashei R et al., [14]	2008/Iran	34.8%
4	Kargar M et al., [15]	2011/Iran	31.94%
5	Redeen S et al., [16]	2011/Sweden	33.2%
6	Aktepe OC et al., [17]	2011/Turkey	42.4%
7	Abdalsadeg NAO et al., [18]	2012/Sudan	48%
8	Siavoshi F et al., [19]	2015/Iran	40%
9	Present study	2015/India	34%

[Table/Fig-3]: Culture positivity of *H. pylori* as seen in various studies.

the sensitivity of 85-90% and specificity >95-100% [24].

In our study 53% of the samples were Christensen's tube urease positive with 32 (60.36%) being positive within an hour. Other studies showed Christensen's urease positivity in varying numbers ranging from 36.9%22 to 72% [25]. We noted sensitivity of 91% and specificity of 66.7% in our study.

Many studies have shown that the stool antigen test is useful for the primary diagnosis and follow up after treatment of *H. pylori* infection [22]. Also, prior preparation of the patient is not necessary unlike in upper gastrointestinal endoscopy. Premier platinum HpSA (*H.pylori* stool antigen) is the first and the most valid *H.pylori* stool antigen test used [26]. In our study, we used HpSA kit of SD BIOLINE showing sensitivity of 73.5% and specificity of 95.3%. Out of all the diagnostic tests used in the study it showed highest specificity. A study done by Krogfelt et al., revealed that the global sensitivity and specificity of stool antigen tests are 94% and 97% respectively [27]. Recent studies also suggest that the specificity of the faecal antigen test is reduced in the presence of bleeding peptic ulcer disease and should not be the sole diagnostic test [28]. Though stool antigen test is simple to perform however due to unpleasantness of handling and storing stool and the decreased compliance of the patients to give the stool specimen and limited availability of this test are the factors slowing its widespread use [22].

LIMITATION

Firstly, the study subjects were recruited from a hospital setting and thus do not represent the true prevalence of *H.pylori* infection among general population. Secondly, due to financial constraints, PCR test for the confirmatory diagnosis of *H. pylori* cannot be performed in this study.

CONCLUSION

The best test for the detection of *H.pylori* infection has been defined as the test that has the greatest combination of sensitivity and specificity. In our study none of the method showed good combination of sensitivity and specificity. However out of all the test, rapid urease test was the most sensitive (100%) and stool antigen test (95.3%) was the most specific test, when culture was being considered as gold standard.

REFERENCES

[1] Tan VP, Wong BC. *Helicobacter pylori* and gastritis: Untangling a complex relationship 27 years on. *J Gastroenterol Hepatol.* 2011;26(Suppl 1):42-45.

[2] McColl K. *Helicobacter pylori* infection. *N Engl J Med*. 2010; 362:1597-604.

[3] Buckley MJM, O'Morain CA. Helicobacter biology - discovery. *British Medical Bulletin*. 1998;54(1):07-16.

[4] Ahmad F, Jaffar R, Khan I. *Helicobacter pylori* detection in chronic gastritis: a comparison of staining methods. *J Ayub Med Coll Abbottabad*. 2011;23(2):112-14.

[5] Kabir S. Detection of *Helicobacter pylori* in faeces by culture, PCR and enzyme immunoassay. *J Med Microbiol*. 2001;50:1021-29.

[6] Allaker RP, Young KA, Hardie JM. Prevalence of *Helicobacter pylori* at oral and gastrointestinal sites in children: evidence for possible oral-to-oral transmission. *J Med Microbiol*. 2002; 51(4):312-17.

[7] Peng NJ, lai KH, Lo GH, Hsu PI. Comparison of noninvasive tests for *Helicobacter pylori* infection. *Med Princpract*. 2009;18(1):57-61.

[8] Dunn BE, Cohen H, Blaser MJ. *Helicobacter pylori*. *Clin Microbiol Rev*.1997;10(4):720-41.

[9] Old DC. *Vibrio, Aeromonas, Plesiomonas, Campylobacter, Arcobacter, Helicobacter, Wolinella*. In: Collee JG, Fraser AG, Marmian BP, Simmons A. Mackie and McCartney practical medical Microbiology. 14th ed. Churchill Livingstone; 1996.425-28.

[10] Warren JR, Marshall BJ. Unidentified curved bacillus on the gastric epithelium in active chronic gastritis. *Lancet.* 1983:1:1273-75.

[11] Zhang W, Lu H, Graham DY. An Update on *Helicobacter pylori* as the cause of gastric cancer. *Gastrointest Tumors*. 2014;1(3):155–65.

[12] Yoosuf H, Rao UA, Thyagarajan SP. A comparative study between Rapid urease (Modified), CLO test, Culture and Histopathological examination of *Helicobacter pylori* in patients with acid peptic diseases. *Indian J Pathol Microbiol*. 1995;38(4):349-54.

[13] Arora U, Aggarwal A, Singh K. Comparative evaluation of conventional methods and ELISA based IgG antibodies detection for diagnosis of *Helicobacter pylori* infection in cases of dyspepsia. *Ind J Med Microbiol*. 2003; 21:46-48.

[14] Khashei R, Shojaei H, Adibi P, Shavakhi A, Aslani MM, Naser AD. Genetic diversity and drug resistance of *Helicobacter pylori* strains in Isfahan, Iran. *Iranian Journal of Basic Medical Sciences*.2008;11(3):174-82.

[15] Kargar M, Baghernejad M, Doosti A, Ghorbani-Dalini S. Clarithromycin resistance and 23S rRNA mutations in *Helicobacter pylori* isolates in Iran. *Afr J Microbiol Res*. 2011; 5(8) :853-56.

[16] Redeen S, Petersson F, Tornkarntz E, Levander H, Mårdh E, Borch K. Reliability of diagnostic tests for *Helicobacter pylori* infection. *Gasteroenterol Research and practice*. 2011;10:1155-61.

[17] Aktepe OC, Ciftci IH, Safak B, Uslan I, Dilek FH. Five methods for detection of *Helicobacter pylori* in the Turkish population. *World J Gastroenterol*. 2011;17(47): 5172-76.

[18] Abdalsadeg NAO, Adam A, Abdul-Aziz H, Omer WH, Osman HA, Bolad AK. Comparison of different diagnostic methods of *Helicobacter pylori* infection in Sudanese patients. *NMJ*. 2012:1858-6279.

[19] Siavoshi F, Saniee P, Khalili-Samani S, Hosseini F, Malakutikhah F. Evaluation of methods for *H. pylori* detection in PPI consumption using culture, rapid urease test and smear examination. *Ann Transl Med*. 2015;3(1):11.

[20] Nanivadekar SA, Sawant PD, Patel HD, Shroff CP, Popat UR, Bhatt PP. Association or peptic ulcer with *Helicobacter pylori* and the recurrence rate. A three year follow-up study. *J Assoc Physicians India*. 1990;38(Suppl 1):703-06.

[21] Tzeng JE, Lin YL, Chung SM, Chu YT. Comparison of Four Diagnostic methods for *Helicobacter pylori*. *Tzu Chi Med J*. 2005; 17:339-43.

[22] Lopes AL, Vale FF, Oleastro M. *Helicobacter pylori* infection - recent developments in diagnosis. *World Journal of Gastroenterology*. 2014;20(28):9299-13.

[23] Tseng CA, Wang WM, Wu DC. Comparison of the clinical feasibility of three rapid urease tests in the diagnosis of *Helicobacter pylori* infection. *Dig Dis Sci*. 2005;50: 449-52.
[24] Lin MH, Cheng HT, Chuang WY, Yu LK, Tsou YK, Lee MS. Histological examination of ulcer margin for diagnosing *Helicobacter pylori* infection in patients with gastric ulcers. *Ann Diagn Pathol*. 2013;17(1):63-66.
[25] Arora U, Aggarwal A, Singh K. Comparative evaluation of conventional methods and elisa based IgG antibodies detection for diagnosis of *Helicobacter pylori* infection in cases of dyspepsia. *Indian J Med Microbiol*. 2003;21:46-48.
[26] Alikhani MY, Sadeghifard N, Farajnia S, Hajia M, Aslani MM, Zamani AR. Evaluation of selective and non selective media for isolation of *Helicobacter pylori* from gastric biopsy specimens. *Pakistan Journal of Biological Sciences*. 2007;10:4156-59.
[27] Krogfelt KA, Lehours P, Mégraud F. Diagnosis of *Helicobacter pylori* Infection. *Helicobacter.* 2005;10(Suppl 1):5-13.
[28] Gisbert JP, Morena F, Abraira V. Accuracy of monoclonal stool antigen test for the diagnosis of *H. pylori* infection: a systematic review and meta-analysis. *Am J Gastroenterol*. 2006;101: 1921-30.

AUTHOR(S):
1. Dr. Shiwani Sharma
2. Dr. Garima Mittal
3. Dr. R K Agarwal
4. Dr. Vivek Ahuja
5. Dr. Rohit Gupta
6. Dr. Sohaib Ahmad

PARTICULARS OF CONTRIBUTORS:
1. Post Graduate student, Department of Microbiology, Himalayan Institute of Medical Sciences, SRH University, Jolly Grant, Dehradun, India.
2. Associate Professor, Department of Microbiology, Himalayan Institute of Medical Sciences, SRH University, Jolly Grant, Dehradun, India.
3. Professor and Head, Department of Microbiology, Himalayan Institute of Medical Sciences, SRH University, Jolly Grant, Dehradun, India.
4. Assistant Professor, Department of Medicine, Himalayan Institute of Medical Sciences, SRH University, Jolly Grant, Dehradun, India.
5. Assistant Professor, Department of Medicine, Himalayan Institute of Medical Sciences, SRH University, Jolly Grant, Dehradun, India.
6. Professor, Department of Medicine, Himalayan Institute of Medical Sciences, SRH University, Jolly Grant, Dehradun, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Garima Mittal,
Associate Professor,
Department of Microbiology,
Himalayan Institute of Medical Sciences,
SRH University, Jolly Grant, Dehradun,
Uttarakhand-248140, India.
E-mail: garimamittal80@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Publishing: **Oct 01, 2016**