© 2013,
Scienceline Publication
www.science-line.com

ISSN 2322-4789
Asian Journal of Medical and Pharmaceutical Researches
Asian J. Med. Pharm. Res.3(4): 105-110, 2013

AJMPR

Comparison of Conventional Diagnostic Modalities with PCR for Detection of *Helicobacter Pylori* Infection in Symptomatic Patients

Jayesh R. Ruparelia[1], Nilofar R. Sodagar[2], Jagdish S. Patel[3*], Himani B. Pandya[2], Navneetkumar R. Singh[2], Karishma B. Naik[2], Heta B. Prajapati[1]

[1] *Shree P. M. Patel Institute of P.G. Studies & Research in Science, Anand, Gujarat, 388001-India*

[2] *Shree P. M. Patel College of Paramedical Science and Technology, Anand, Gujarat, 388001-India*

[3] *Department of Biochemistry, P. D. Patel Institute of Applied Science, Charotar University of Science and Technology (CHARUSAT), Changa-388421; Petlad, Anand, Gujarat, India*

* Corresponding author's Email: jagdishpatel.biochem@ecchanga.ac.in

ABSTRACT: Introduction: *Helicobacter Pylori* are currently implicated in the pathogenesis of various gastric and duodenal disorders and also a risk factor in gastric carcinomas. *H. Pylori* infection is detected by various invasive & noninvasive methods and each method has its own pits & falls. Out of all the techniques PCR is considered to be most rapid, accurate and sensitive method. Purpose: The present study is an attempt to evaluate and compare the efficiency of conventional diagnostic techniques with PCR. Methods: Four fragments of antral biopsies were collected from 100 patients & were processed for PCR, RUT, Culture and Gram staining. Serum sample was processed for determination of IgG antibodies (indirect ELISA).Results: The detection rate *H. Pylori* infection of various tests were as follows serology 54%, PCR 44%, RUT 36%, Gram staining 24% and Culture 22%. Sensitivity of Serology and PCR was found higher 100% than RUT (81.81%), Gram's staining (54.54%) and culture (50%). The specificity of PCR, RUT, Gram's staining and culture were 100%, and serology was 82.14%. Among the five diagnostic methods PCR was most accurate with 100% accuracy and the highest agreement of PCR was found with RUT. Conclusions: Our study suggestes that association of PCR & Serology constitutes the best choice for confirming the diagnosis due to its high concordance rate followed by RUT and serology if PCR is not possible due to limited resources.

Key words: *Helicobacter Pylori*, PCR, Serology, RUT, Culture.

ORIGINAL ARTICLE

INTRODUCTION

Helicobacter Pylori is a small, spiral shaped, highly motile, Gram-negative, microaerophillic bacterium that resides beneath and within the mucous layer of gastric mucosa and colonizes mucosa of human stomach of more than half of the world's population (Blaser. 1997; Neale and Logan , 1995; Udaya et al., 2000). Its infection is almost always associated with non-ulcer dyspepsia, histologic chronic (type B) gastritis, gastric and duodenal ulcer, gastric adenocarcinoma and mucosa associated lymphoid tissue lymphoma (Buck, 1990; Graham, 1989; Brooks et al., 2004). In Developing Countries most infection occurs in childhood with prevalence rate exceeding 50% at age 10 and 80% in adulthood (Graham et al., 1988). Accurate diagnosis is essential for the effective treatment and management of infections that are cause by this organism. (Shiotani et al., 2000; Brooks et al., 2004). Different invasive and noninvasive diagnostic tests are available for the diagnosis of *H. Pylori*. A reliable test to detect this infection is crucial, but none of the tests available is suitable for all clinical situations, each having its own pros & cons. it has been difficult to establish a gold standard (Brooks et al., 2004). The routine invasive diagnostic assays consist of culture, rapid urease tests, histological examination of gastric biopsy sections and Gram's staining requiring endoscopy. (Coudron and Kirby, 1989; Dooley and Cohen, 1988; Glupczynski, 1994).On the other hand noninvasive tests especially serological testing are cheaper and more convenient as these tests detect the global presence of *H. Pylori* in the stomach even when the bacteria are irregularly distributed on gastric mucosa and thus serological tests should be preferred in situation where the additional information yielded by an endoscopy is not needed.

In practice, endoscopic tests are best for the primary diagnosis of *H. Pylori* infection because endoscopy allows assessment of treatment. But due to patchy distribution of *H. Pylori* all biopsy based test may theoretically fail to diagnose the infection. There are also molecular techniques that are based on nucleic acid hybridization and polymerase chain reaction (PCR) which target different *H. Pylori* genes (Clayton et al., 1992; Foxall et al., 1992; Lin et al., 1996). The PCR can amplify minute quantities of nucleic acid with the use of absolute specific primers. Though PCR tests are usually highly sensitive, specific and fast and accurate for *H. pylori* detection (Dunn et al., 1997; Linpisam et al., 2003; Brooks et al., 2004), it cannot

To cite this paper:Ruparelia1 J.R..,Sodagar N.R.., Patel J.S., Pandya H.B., Singh N.R..,Naik K.B., Prajapati H.B.2013. Comparison of Conventional Diagnostic Modalities with PCR for Detection of *Helicobacter Pylori* Infection in Symptomatic Patients. *Asian J. Med. Pharm. Res.*, 3 (4): 105-110.

be used routinely in the all microbiology lab of country like India due to lack of resources. So the study was an attempt to find out the most accurate method for detection of *H. Pylori* infection in comparison with PCR.

MATERIALS AND METHODS

Patients:

100 patients (age range 15-75 yrs) with the complaint of dyspeptic symptoms, with the clinical indication of endoscopy of upper gastrointestinal tract during November 2008 to June 2009 were studied. Informed consent was obtained from each patient before endoscopy, Approval of Ethical Review Committee of Pramukh swami medical college (HREC), Karamsad, was taken prior to initiation of the work. As per the exclusion criteria decided for the present study, patients with prior *H. Pylori* eradication therapy, or those treated with antibiotics, proton pump inhibitors or H2 receptor blocker within the last four weeks were excluded.

Sample:

Oesophagogastro-duodenoscopy was performed on each patient using an Olympus fibreoptic endoscope. Four fragments of antral biopsies/lesion biopsies were taken from each patient for RUT (1 fragment), Gram's staining (1 fragment), PCR (1 fragment) and culture (1 fragment). Samples for bacteriological examination were collected in sterile Brain Heart infusion broth tubes and transported and processed within 2 hours and 1biopsy is directly placed in to Sterile urea broth at the time of endoscopy and time was noted. Endoscopic flush fluids were checked monthly for bacterial contaminants by routine culture and for *H. Pylori* DNA by PCR as described later. After endoscopy 3 ml of venous blood was collected from each patient and serum was separated and was immediately processed for serological test.

Definition of *H. pylori* status:

A positive *H. pylori* status was defined as positive culture or in case of culture negative, positive results for both PCR and RUT/Gram's staining/ high titer of serology. A negative *H. pylori* status was confirmed when all invasive tests performed gave concordant negative results.

1. Rapid urease test: we used 0.5 ml of urea broth with 10% urea and 0.002% phenol red (pH 6.5). Biopsy was immediately placed in the sterile urea broth at the time of endoscopy. Tube was then incubated at 37°C and the test was considered positive if the color changes from yellow to pink.

Time taken was noted (Berry and Sagar, 2006; Dunn et al., 1997; Kazoo et al., 2001; Kumala et al., 2006).

2. Culture: Tissue homogenate was inoculated on Brucella blood agar, Modified chocolate agar and Belo horizonte media with TTC (Dulciene and Rocha, 1987; Dunn et al., 1997; Glupczynski, 1998) and supplemented with 10% human blood with antibiotics Vancomycin, Polymyxin and Trimethoprim (Skirrows supplement) (Pronovast and Rose, 1994) and Amphoteriein B. The plates were inoculated microaerophically at 37°C in an anaerobic jar (Dunn et al., 1997; Glupczynski, 1998; Kazoo et al., 2001; Pronovast and Rose, 1994) with gas pack (Oxoid) system for 3-5 days. Isolates were considered *H. Pylori* if they grew as a 0.5-1.0 mm translucent grayish colonies on Brucella and modified chocolate agar and golden yellow on Belo Horizonte media (Dulciene and Rocha, 1987; Dunn et al., 1997; Glupczynski, 1998).

Colonies were identified by spiral Gram negative and positive for catalase, Oxidase and urease test (Dunn et al., 1997; Glupczynski, 1998; Kazoo et al., 2001; Pronovast and Rose, 1994). *H. pylori* NCTC 11637 was used as a positive control (Brooks et al., 2004).

3. Gram Staining: Biopsy sample was crushed and smear was prepared on clean glass slide and stain by Gram's staining. Presence of spiral Gram negative microorganism embedded in the tissue cells was diagnostic for *H. Pylori* (Dunn et al., 1997; Kazoo et al., 2001; Kumala et al., 2006; Pronovast and Rose, 1994).

4. Serology: For detection of *H. Pylori* IgG antibodies, an indirect solid phase enzyme immunoassay (EIA) test kit (Immunocomb II, organics, Israel) was used. Assay was performed according to the manufacturer's instruction. A sample was considered positive if a spot had an intensity equal to or greater than positive control (anti*H. Pylori* IgG), indicating the presence of IgG antibody to *H. Pylori* (>20units/ml). Negative results were indicated by spot with intensity less than that of positive control.

5. DNA Extraction from biopsy sample: DNA extraction from biopsies was done by the method of Marais et al. (Marais et al., 1999). Briefly, the biopsy samples were ground and centrifuged for 5 min at 10 000×g. The pellet was resuspended in 300 µL extraction buffer (20 mmol/L Tris-HCl, pH 8.0; 0.5% Tween 20) and proteinase K (0.5 mg/mL final concentration). The mixture was incubated at 56°C for one hour after which the enzyme was inactivated by boiling for 10 min. five µL of DNA was used as the template for each PCR.

6. PCR Assay: PCR was carried out with primers specific to the *H. Pylori*. The primer were selected were as follows: cag A gene (cg1-GAT AAC AGG CAA GCT TTT GAGG 3, cg 2-CTG CAA AAG ATT GTT TGG

CAGA) (Misra et al., 2006); vac A gene (vc 1-ATG GAA ATA CAA CAA ACA CAC, vc 2-CTG CTT GAA TGC GCC AAAC) (Misra et al., 2006) and 16s rRNA (Hp 1-CTG GAG AGA CTA AGC CCT CC, Hp 2- ATT ACT GAC GCT GAT TGT GC) (Ho et al., 1991). PCR was performed under the following conditions. 1µl of each oligonucleotide primer was placed (50 pmol/µl for each primer) in an eppendorf tube, and 5 µl of extracted DNA, 5µl of 10x PCR buffer (500 mM KCl, 100 mM Tris-Cl, 15 mM MgCl2, 0.1% Gelatin [pH 8.3]), 8 µl of deoxynucleoside triphosphate mixture (final concentration, 1.25 mM each dATP, dCTP, dGTP, and dTTP; B'Genei, India), 2.5 U of Taq DNA polymerase (B'Genei) and molecular biology-grade distilled water were added to make a final reaction volume of 50 µl. Briefly spin in a microcentrifuge, and placed in a thermal cycler. Thermal cycler (2720) of applied biosystem was used for amplification. Forty cycles were employed and each cycle consisted of 5 minute pre-denaturation at 95 ˚C, 1 minute denaturation at 95 ˚C, 1 minute annealing at 60 ˚C and 1 minute extension at 72 ˚C. After 35 cycles, the reaction mixture was further extended for 7 minutes at 72 ˚C and the mixture was subsequently refrigerated at 4°C before analysis.

The PCR products were analyzed by agarose gel electrophoresis. Eight microlitres of PCR product were run on a 2.5% agarose gels, containing ethidium bromide. A 109-bp, 286-bp and 349-bp band was considered a positive PCR result respectively 16s rRNA, VacA and CagA gene. Negative and positive controls were Campylobacter jejuni ATCC 33560 and *H. Pylori* NCTC 11637, respectively. (Brooks et al., 2004).

Statistical analysis:

Sensitivity, specificity, PPV, NPV and accuracy was calculated for each method. Chi square method and Mc Nemar's test was used to analyze disagreement between two tests.

RESULTS

Out of 100 dyspeptic patients, 58 were males and 42 were females, age range from 15 to 75 yrs. 44 patients were positive for *H. Pylori* with the prevalence rate of 44%. Infection ratio for male: female was 60%: 40%.

H. Pylori infection detected by PCR, serology, RUT, Gram staining and culture were found 44 (44%), 54 (54%), 36 (36%), 24 (24%) and 22 (22%) respectively (Table 1).

Sensitivity of Serology and PCR were found highest (100%) than RUT (81.81%), Gram's staining (54.54%) and culture (50%). The specificity of PCR, RUT, Gram's staining and culture was 100% and serology was 82.14% (Table 2). Positive Predictive Value of PCR, Gram staining, RUT and Culture was found 100%, and of serology was found 81.48%. Negative Predictive Value of PCR and Serology were found 100%, RUT 87.50%, Gram staining 73.68% and culture 71.79%. Among the five diagnostic methods, PCR was found to be most accurate with 100% accuracy compared to that of Serology 90%, RUT 92%, Gram staining 80% and culture 78% (Table 2).

Analyzing the agreement between the two tests, we observed that the major agreement occurred between PCR and RUT, followed by PCR and serology, Gram's Staining and Culture, RUT and Culture, RUT and Serology, PCR and Gram's Staining and then PCR and Culture (Table 3).

Table1: *H. Pylori* detection rate

Total Number	PCR	Serology	RUT	Gram staining	Culture
100	44(44%)	54(54%)	36(36%)	24(24%)	22(22%)

Table 2: Sensitivity, Specificity, Positive and Negative predictive values and Accuracy of methods used for diagnosis of *H. Pylori*

Method	Sensitivity	Specificity	Predictive value		Accuracy (%)
			Positive	Negative	
PCR	100%	100%	100	100	100
Gram staining	54.54%	100%	100	73.68	80
RUT	81.81%	100%	100	87.50	92
Serology	100%	82.14%	81.48	100	90
Culture	50%	100%	100	71.79	78

Table 3: Agreement and disagreement analysis.

Methods	Agreement	Disagreement	Kappa statistics (p value)
PCR+ Gram's staining	80%	20%	0.573
PCR+ RUT	92%	8%	0.8344
PCR+ Serology	90%	10%	0.8019
PCR+ Culture	76%	24%	0.9583
Gram staining + RUT	76%	24%	0.4382
Gram staining + Serology	70%	30%	0.424
Gram staining + culture	90%	10%	0.718
RUT + Serology	82%	18%	0.648
RUT + Culture	86%	14%	0.668
Serology + Culture	68%	32%	0.387

DISCUSSION

Early detection of *H. Pylori* is critical in preventing serious consequences. There is no single standard test for early detection of *H. Pylori* infection. Therefore, a standard definition of *H. Pylori* infection was used for this study is that patients with positive *H. pylori* status was defined as positive culture or in case of culture negative, positive results for both PCR and RUT/Gram's staining/ high titre of serology are considered infected with *H. pylori*. In case of negative PCR, patient positive by any other two tests considered to be infected. Patient negative by all the five tests were considered to be non-infected.

Present study shows prevalence rate of 44% which in comparison with other studies was high (Clayton et al., 1992; Dooley and Cohen, 1988; Pandya et al., 2009). The prevalence of *H. Pylori* infection has varied in previous studies from different countries depending on the environment, host, lab detection methods, socioeconomic status, age range and period of study (Foxall et al., 1992; Linpisam et al., 2003; Pandya et al., 2009). An insight into the gender ratio we found the male female ratio was 60:40. Rate was higher in males than females which is consistent with the studies done by Kumar et al. (Graham, 1989; Pandya et al., 2009) [64.13%in males], is due to abusive habits like tobacco, alcohol and smoking (Kazoo et al., 2001; Kumala et al., 2006; Pandya et al., 2009). Various standard methods for the detection of *H. Pylori* infection in human gastric mucosa were evaluated. Like other researches different methods yielded different rates of detection of *H. Pylori* infection (Dunn et al., 1997; Foxall et al., 1992; Pandya et al., 2009). Our study showed that *H. Pylori* detection rate by PCR, Serology, RUT, Gram's staining, and Culture were 44%, 54%, 36%, 24% and 22% respectively.

Among the five diagnostic methods sensitivity of PCR was found (100%) with 100% specificity. In study of Kathleen M.B et al, sensitivity and specificity of PCR were found 100% and 94.6% respectively which are comparable to present study (Coudron and Kirby, 1989). To exclude false positive result by contaminated endoscope, we checked endoscopic flush fluids monthly for bacterial contaminants by routine culture and for *H. Pylori* DNA by PCR, which lead to high specificity of PCR in this study. The PCR could detect *H. Pylori* DNA concentration of 0.4 pg or approximately 70 bacterial cells were detectable (Hammar et al., 1992; Ho et al., 1991).

Sensitivity & specificity of Gram staining was 54.54% and 100% respectively, which is lesser than those of PCR. 20% false negative results were obtained by Gram staining due to patchy distribution of organisms and due to limitation of visualization techniques. The sensitivity and specificity of rapid urease test was 81.81% and 100% respectively, in study of Lea Veijola et al (Lea et al., 2006), sensitivity and specificity of RUT were found 91% and 93% respectively which are higher than present study. 08% false negative results were obtained by RUT that because of also patchy distribution of organisms. The sensitivity of Gram's staining and RUT is greatly affected by the quality and accuracy of sampling and also affected by the presence of other urease producing bacteria in the gastric sample such as *H. heilmanni* and Proteus spp which may also reduce the specificity (Hammar et al., 1992). The sensitivity of Culture was 50% with 100% Specific. Bacterial culture presented the highest rate of false negative results which decreased the sensitivity to 20% in comparison to other studies (Glupczynski, 1998; Pandya et al., 2009; Pronovast and Rose, 1994). Success rate of culture depends on technical expertise of the microbiological laboratory, patchy distribution of organisms and viability of the organisms during transportation (Kumala et al., 2006; Marais et al., 1999;

Pandya et al., 2009). Sensitivity and specificity of serology was found 100% & 82.14% respectively. Out of 100 samples 10 false positive results were obtained by serology may be due to past infection of *H. Pylori*. Positive serology does not indicate active current infection when results were negative by PCR, RUT, Gram's staining and Culture (Pandya et al., 2009). The presence of IgG antibody has been reportedly present at different titers shortly after the primary infection and lasts in the blood for many years after infection.

Agreement and disagreement analysis of the result shows that the highest concurrence was found between PCR and RUT (92%) followed by PCR & serology (90%), PCR & Gram staining (80%), PCR & Culture (76%) and RUT & serology (82%) and highest discrepancy was found between serology and culture (32%).

PCR was found to be the most rapid, sensitive, and accurate method and has opened a new era of rapid *Helicobacter Pylori* Laboratory Diagnosis, though it is recommended to use PCR as a complimentary test rather than substituting the standard microbiological analysis. Our study suggested that association of PCR & Serology constitutes the best choice for confirming the diagnosis due to its high concordance rate.

REFERENCES

Berry V and Sagar V (2006). Rapid urease test to diagnose *Helicobacter Pylori* infection. JK Sci 8:86-88.

Blaser MJ (1997). Ecology of *Helicobacter Pylori* in the human stomach. J Clin Invest. 100:759–62.

Brooks HJL, Ahmed D, McConnell MA, Barbezat GO (2004). Diagnosis of *Helicobacter Pylori* infection by polymerase chain reaction: is it worth it? : Diagnostic Microbiology and Infectious Disease 50: 1-5.

Buck GE (1990). *Campylobacter pylori* and gastroduodenal disease. Clin Microbiol Rev 3:1-12.

Clayton CL, Kleanthous H, Coates PF, Morgan DD, Tabaqchali S (1992). Sensitive detection of *Helicobacter Pylori* by using polymerase chain reaction. J Clin Microbiol 30:192-200.

Coudron PE, Kirby DF (1989). Comparison of rapid urease tests, staining techniques, and growth on different solid media for detection of *Campylobacter pylori*. J Clin Microbiol; 27:1527-30.

Dooley CP and Cohen H (1988). The clinical significance of *Campylobacter pylori*. Ann Int Med 108:70-79.

Dulciene M and Rocha A (1987). Indicator medium for the isolation of *Campylobacter pylori*. J of Clin Microbial 25:2378-2379.

Dunn BE, Cohen H, Blaser MJ (1997). *Helicobacter Pylori*. Clin. Microbial Rev 720-741.

Foxall PA, Hu LT, Mobley HLT (1992). Use of polymerase chain reaction-amplified *Helicobacter Pylori* urease structural genes for differentiation of isolates. J Clin Microbiol 30:739-41.

Glupczynski Y (1994). The diagnosis of *Helicobacter Pylori* infection: a microbiologist's perspective. Rev Med Microbial 5:199-208.

Glupczynski Y (1998). Microbiological and serological tests for *Helicobacter Pylori*: an overview. Br Med Bull 54:175-186.

Goodwin CS, Mendall MM (1997). Northfield TC. *Helicobacter Pylori* infection. Lancet 349:265-269.

Graham DY (1989). *Campylobacter pylori* and peptic ulcer disease. Gastroenterol 96:615-625.

Graham DY, Alpert LC, Smith JL, Yoshimura HH (1988). An iatrogenic *Campylobacter pylori* infection is a cause of epidemic achlorohydria. Am J Gastroenterol 83:974-980.

Hammar M, Tyszkiewicz T, Wadström T, O'Toole PW (1992). Rapid detection of *Helicobacter Pylori* in gastric biopsy Material by polymerase chain reaction. J Clinic Microbiol 30(1):54-58.

Ho SA, Hoyle JA, Lewis FA, Secker AD, Cross D, Mapstone NP, Dixon MF, Wyatt JI, Tompkins DS, Taylor GR (1991). Direct polymerase chain reaction test for detection of *Helicobacter Pylori* in humans and animals. J Clinic Microbiol 29:2543-2549.

Kazoo S, Mario A, Kawakami E (2001). Evaluation of invasive and none invasive method for the diagnosis of *H. Pylori* infection in symptomatic children and adoleseents. Sao Paulo Med J 119(2):67-71.

Kumala W and Aziz R. (2006). Pattern of *H. Pylori* isolate resistant to fluoroquinolones, Amoxicillin, Clarithromycin and Metronidazoles. S E Asian J Trop Med Pub Health 37:970-974.

Lea V, Anna S, Hilpi R, Timo KU, Pentti S, Hannu H, Reijo T, Seppo S, Arkkila PE, Kari S (2006). Clinical Significance of Widespread Gastric Metaplasia in the Duodenal Bulb. J Clin Gastroenterol 40(6):510-514

Lin TT, Yeh CT, Yang E, Chen PC (1996). Detection of *Helicobacter Pylori* by polymerase chain reaction assay using gastric biopsy specimens taken for CLO test. J Gastroenterol 31:329-332.

Linpisarn S, Prommuangyong1 K, Steger HF, 0Lertprasertsuke N, Phornphutkul K (2003). Detection of *Helicobacter Pylori* in Gastric Biopsy Samples by Polymerase Chain Reaction with a Simple DNA Extraction Method. CMU J 2 (1):7-13.

Marais A, Monteiro L, Occhialini M, Pina M, Lamoliatte H, Megraud F (1999). Direct detection of *Helicobacter Pylori* resistance to macrolides by a polymerase chain reaction/DNA enzyme

immunoassay in gastric biopsy specimens. Gut 44:463–467.

Misra RN, Bhagat M, Ahmed N (2006). *Helicobacter Pylori* in Dyspepsia – Antibiotic Sensitivity and Virulence Patterns. Med J Armed Forces India 62:22-26.

Neale KR, Logan RP (1995). The epidemiology and transmission of *Helicobacter Pylori* infection in children. Aliment Pharmacol Ther. 9: 77–84.

Pandya HB, Patel JS, Sodagar NR, Singh NR, Salvi PN, Patel SB, Naik KB, Agravat HH (2009). Comparison of invasive tests with serology to diagnose *H. Pylori* infections in symptomatic patients. J Cell and Tissue Res 9(3):2019-2022.

Pronovast AD and Rose SL (1994). Evaluation of a New Immunodiagnostic assay for *Helicobacter Pylori* antibody detection: Correlation with Histopathological and Microbiological Results. J Clinic Microbiol 32:46-50.

Raymond J, Kalach N, Bergeret M, Barbet JP, Benhamou PH, Gendrel D, Dupont C (1996). Evaluation of a serological test for diagnosis of *Helicobacter Pylori* infection in children. Eur J Clin Microbiol Infect Dis 15(5):415-7.

Udaya BS, Prakash MD, Thomas MH and Aberman MD (2000). Myo internal medicine board review. 4th ed. Lippincott Williams and Wilkins Publications. New york.