ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 01, pp.45770-45773, January, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

EVALUATION OF INVASIVE AND NONINVASIVE DIAGNOSTIC METHODS OF *HELICOBACTER PYLORI* INFECTION IN ENDOSCOPIC BIOPSY SAMPLES OF DYSPEPTIC PATIENTS: A RURAL HOSPITAL BASED STUDY

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ARTICLE INFO

Article History:
Received 27th October, 2016
Received in revised form
15th November, 2016
Accepted 12th December, 2016
Published online 31st January, 2017

Key words:

H. pylori,
Dyspepsia,
Rapid urease test,
Brucella broth,
Brucella chocolate agar.

ABSTRACT

Introduction: The identification of the clinical consequences of *Helicobacter pylori* infection is certainly one of the major discoveries within the past 20 years in medicine. *H. pylori* was the first formally recognized bacterial carcinogen and is one of the most common human pathogen. It has been etiologically associated with gastritis and gastric associated diseases, peptic ulcer, gastric adenocarcinoma and primary gastric carcinoma. Dyspepsia is a very common group of symptoms referring upper gastrointestinal tract for which patients consult the physician, accounting for about 4% to 14% of their consultations. Annual Incidence of *Helicobacter pylori* is 6%-14% in developing countries with faeco-oral route of transmission. In this context this study has been undertaken to look for the association of *H. pylori* in adult patients of dyspepsia with evaluation of diagnostic test for detection of *H. pylori* in dyspeptic patients visiting in a tertiary hospital in a rural setup of central India.

Material and Methods: It was a cross sectional studies involving 52 patients. After obtaining history and written informed consent, 4 antral mucosal biopsy specimens were collected along with 5 ml blood sample. Biopsy specimens were subjected to Rapid urea test (RUT), Culture, Gram staining and Histopathological examination. Subculture from Brucella broth was done on Brucella chocolate agar with & without antibiotics. Identification was done by standard tests. Serum was separated and used for ELISA test for *H. pylori* IgG antibody.

Results: Out of the 52 patients studied, 25 (48.07%) were positive by Rapid urease test, 20(38.46%) by Gram staining, 17(32.69%) by Culture, 21(40.38%) by Histopathology and IgG 28(53.84%) by ELISA test. RUT had 100% sensitivity and 77.14% specificity, while histopathology had 94.11% sensitivity and 85.14% specificity and IgG had 94.11% sensitivity and 65.71% specificity in reference to culture.

Conclusion: In our study, we have revealed that for diagnosing *H. pylori* either one can use RUT for rapid diagnosis in the endoscopic room itself and confirm diagnosis by culture or histopathology.

Citation: Kumar, R., Chaudhuri, S.J., Deotale, V., Jain, J., Gangane, N., Mendiratta, D.K. and Narang, P., 2017. "Evaluation of invasive and noninvasive diagnostic methods of helicobacter pylori infection in endoscopic biopsy samples of dyspeptic patients: a rural hospital based study", *International Journal of Current Research*, 9, (01), 45770-45773.

INTRODUCTION

The identification of the clinical consequences of *Helicobacter pylori (H. pylori)* infection is certainly one of the major discoveries within the past 20 years in medicine. *H. pylori* was the first formally recognized bacterial carcinogen and is one of the most common human pathogen. It has been etiologically associated with gastritis and gastric associated diseases, peptic ulcer, gastric adenocarcinoma and primary gastric carcinoma (Davood, 2009).

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H. Pylori is a small, curved and motile Gram negative bacillus which only infects the mucus layer of the human stomach (Marshall, 1984; Jones et al., 1984). Dyspepsia is a very common human experience for which there are numerous causes. Dyspepsia is the group of symptoms which refers to the upper gastrointestinal tract for which patients consult the physician, accounting for about 4% to 14% of their consultations. However, the prevalence of dyspepsia in the general population is much higher, with 20%-30% patients experiencing dyspeptic symptoms and with many of them self medicating themselves (Chiba, 1998). Until the discovery of *H. pylori,* the physicians and microbiologists believed that the stomach was likely to be sterile because of the presence of its acid 'milieu'.

The successful isolation of spiral Gram negative bacilli by them has changed the focus from a non-infectious to an infectious etiology i.e. from pH to Hp (Unidentified curved bacilli on gastric epithelium in active chronic gastritis, 1983). *H. pylori* infection is now a particular concern in developing countries. It colonizes 70% to 90% of the population whereas it is 50% in developed countries (Davood, 2009). The prevalence of infection varies both between and within countries in relation with race, ethnicity and geographical area of the population. The pattern of infection is an early childhood acquisition of *H. pylori,* 30-50% that reaches over 90% during adulthood in developing countries. Unless treated, colonization persists lifelong. This has been attributed to the poor socioeconomic status, unhygienic practice, overcrowding condition The accepted routes of its transmission are the faeco-oral route in the developing countries and the Oro-oral route (through the saliva) in the developed countries (Dunn et al., 1997). Today, the role of *H. pylori* has been established in chronic antral gastritis, duodenal ulcers, chronic gastric ulcers, dyspepsia, gastric cancer and gastric lymphoma.

MATERIALS AND METHODS

This cross sectional study was carried out in the dept. of microbiology, MGIMS, Sewagram, Wardha from January 2011 to June 2012 after obtaining permission from the institutional ethical committee. Patients of both sexes and age group of 15 yrs or above, diagnosed to have dyspepsia as per clinical history were recruited as the study subjects. Exclude the patients with history of PPI, H2 receptor antagonist, warfarin, fluoxetin, steroid use within one week before endoscopy, antibiotic use within four weeks before endoscopy, severe medical illness and active gastrointestinal bleeding. After obtaining the informed written consent, the patient's details were noted down according to the case record form, including duration of symptoms, socioeconomic status, education and residence.

Collection and transportation of Biopsy sample

Four antral biopsies were collected from each patient with an Olympus Fibro-optic Video-gastro-endoscope under local anesthesia (10% Xylocain). The patients were instructed to report to the endoscopic unit on an empty stomach. Upper gastrointestinal endoscopy was performed under aseptic precautions

- One biopsy sample was put into urea broth.
- One biopsy was transported in Brucella broth for culture and Gram staining.
- Two biopsy samples were submitted for histopathological analysis in 10% formal saline.

Collection of blood for serum: 2-3 ml blood samples were collected by taking all the aseptic precaution. The separated serum was divided into small aliquots and these were properly labeled and stored at -70^0 C to be processed collectively for IgG against *H. pylori* later.

Rapid urease test: A biopsy tissue from antrum is placed in 0.5ml to 1ml 10% fresh urease broth with positive and negative control and incubated at 37^0 C. The broth was examined after every 30 minutes for 4hrs, a color change from yellow to red or magenta was read as positive.

Culture

The biopsy sample was collected and transported to the laboratory in Brucella broth. Samples were processed within 1hr. Subculture from Brucella broth was done on Brucella chocolate agar with antibiotics (Polymixin- B, Amphotericin B & Vancomycin) and without antibiotics. Incubated plates and Brucella broth vials were incubated in candle jar with burning of candle at 37^0 C. Lawn culture of ATCC *E. coli* on Mackonkey agar was used to maintain microaerophilic condition. Sterile cotton soaked in sterile water was used to create 90-100% humidity. The culture plates and broth culture vials were first examined for *H. pylori* after 72 hrs, followed by every 24hrs for 7 days. Purity of the growth was checked by Gram staining, Oxidase, Catalase, rapid urease test and sub culture on solid medium (Brucella agar) and incubating in aerobic and micro-aerobic conditions.

Direct Gram Staining

The prepared smears were stained by using Jensen's modification of Gram staining, but instead of using Saffranin, dilute Carbol fuschin (0.2 %) was used as a counter stain, which helped in the better visualization of Gram negative, spiral or sea gull wing shaped bacteria.

Histopathology: The biopsy specimens which were collected in 10 % formal saline as a fixative were transported to the pathology laboratory after proper labeling and with the requisition form for the Toulidine blue staining.

ELISA *H. pylori* IgG: The Demeditec *H. pylori* IgG ELISA DEEHLAK08 kit was used. All the serum samples which were stored at -70^{0}C were thawed and all the test procedures were carried out according to the manufacturer's instructions. Titres above the cut off value of 10 U/ml were noted as positive.

RESULTS

The present study was conducted in the department of Microbiology in collaboration with Pathology and Medicine in a tertiary hospital in a rural setup of central India.

Table 1. The number of samples which were processed by various diagnostic methods for the diagnosis of *H. pylori* and their overall positivity

Tests performed	No. of case	Positive	Percentage
Culture	52	17	32.69%
Direct Gram smear	52	20	38.46%
RUT	52	25	48.07%
Histopathology	52	21	40.38%
H. pylori IgG	52	28	53.84%

Table 2. Comparison of the tests in reference to Culture

Tests	Sensitivity	Specificity	PPV	NPV
Direct Gram stain	94.11%	88.57%	80%	96.87%
RUT	100%	77.14%	68%	100%
Histopathology	94.11%	85.14%	76.19%	96.77%
H. pylori IgG	94.11%	65.71%	57.16%	95.83%

Amongst the enrolled patients, 29/52 males (55.76%) were predominant for dyspepsia. Majority of the study populations were range from 25- 55 years of age groups, with 53.84% patients were belonging to lower socioeconomic class and 59.6% patients were belonging to nearby village i.e rural area.

Table 3. Comparative study by various authors

Authors (year)	Sensitivity (%)				Specificity (%)			
	RUT	Histology	Gram's stain	Culture	RUT	Histology	Gram 's stain	Culture
Personnet et al. (1988)	-	-	81	100	-	-	-	-
Coudron et al. (1989)	59		62	100				
Srinivas et al. (1998)	91	100	91	-	-	100	73	-
Goel et al. (2003)	83	67	67	67	100	100	-	100
Present study	100	94.14	94.14	100	77.14	85.14	88.57	100

DISCUSSION

The prevalence of *H. pylori* infection has varied in different countries depending upon the environmental, host, laboratory detection, patient's selection, socioeconomic status and age (Rothenbacher *et al.,* 1998). A total of 52 patients were included in this study, with a mean age 38 years [range 18-70 yrs]. Among these patients, 55.76% were males while 44.24% were females. Similar findings were found in a study which was done in India by Ahmed et al. (2006) and Navin chandra Motiram Kaore et al. (2012) who also observed the active age group of 20 to 40 yrs to be more vulnerable, as was also observed in our study. Out of 52 dyspeptic patients, 53.84% belonged to lower socioeconomic class, 26.93% to middle class and only 19.23% belonged to higher class. About 60% study populations belonged to rural area. High rates of infection have been associated with low socioeconomic status Rajesh *et al*. (2006) and Fernando *et al*. (2002). The method of detection of *H. pylori* infection varies from hospital to hospital. It depends on the various factors which add to the isolation of organism. In the present study, we had lower isolation of *H. pylori* compared to other hospitals studies as we used the criteria for *H. pylori* isolation was culture positive (Ramakrishna, 2006). Various authors have shown the positivity of presence of *H. pylori* associations in dyspeptic patients are as:-

The sensitivity of culture varied from 90% to 97% reported by various workers like Marshall et al. (1984) and Goodwin et al. (1985). They had used Trypticase Soya agar, Brain Heart Infusion agar, Skirrow agar and Brucella agar supplemented with 10 % whole sheep blood. The Indian studies had reported using these media sensitivity varies from 1.09% to 63% (Arora et al., 2003; Prasad et al., 1991). In the present study, 17 (32.69%) of patients were positive by Culture, in spite of using Brucella chocolate agar with selective agents like (Polymyxin, Amphoterecin and Vancomycin). Our culture sensitivity was in accordance with these results. The low rate of isolation may be because of the fastidious nature of *H. pylori* and because of a number of other factors like the patchy distribution of the organism, inadequate mincing of the biopsy material, the presence of oropharyngeal flora, the loss of viability of the specimen during transportation, etc. These factors are difficult to control. All these factors together, result in low sensitivity and a low negative predictive value. Considering culture as the gold standard, the sensitivity of the RUT is 100%, specificity 77.14%, Positive predictive value 68% and negative predictive value 100%. In comparison to Javed Yakoob et al. (2005) Vandana Berry et al. (2006) our sensitivity was higher but specificity lower. 28/52 samples were positive (53.48%) for IgG against *H. pylori* by this ELISA based test. Kullavanijaya et al. (2004) reported 120/191 patients to be positive i.e. 62.8 %, with a sensitivity of 96.8% and a specificity of 96.8 %, while Abida Malik et al. (1999) reported a seropositivity of 58.3 %. Our positivity rate of 53.48 % was comparable to that which was reported by all the authors. Histological evidence of *H. pylori* was recorded in 21(40.38%) of patients. It has been observed that histology yields quite variable results reported as 67% and 100% in study conducted by Goel et al. (1984) (2003), Sadeghifard et al. (2006) 2006. Variation may be due to improper collection of mucosal biopsy specimens.

Conclusion

In our study, we have revealed that for diagnosing *H. pylori* either one can use RUT for rapid diagnosis in the endoscopic room itself and confirm diagnosis by culture or histopathology.

Limitation of study

For this research study we have used the most common easily available methods to diagnose *H. pylori* due to money constraints. We could not evaluate many expansive media to grow the *H. pylori* for better isolation. We were unable to do Urea breath test in our study though it was highly specific due to money constraints.

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