Original Article

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A Comparative Study of Diagnosis Methods for Detection of Helicobacter Pylori in Gastro-Duodenal Diseases In Human

Fiaz Ahamed[1] **N.R. Chandrappa**[2] **Emad M. Abdallah**[3]

[1]*Pathology and Laboratory medicine, Al-Rass General Hospital, Saudi Arabia* [2]*Microbiology, J.J.M. Medical College, Davangere, Karnataka, India* [3]*Laboratory Sciences, College of Sciences and Arts at Rass, Qassim University, Saudi Arabia*

Background: Helicopacter pylori play an important role in the pathogenesis of gastroduodenal disease. Appropriate diagnosis of H.pylori infection in the laboratory results in relief of the symptoms and many also be cured without surgical intervention.

Method: 76 males and 24 females who underwent diagnostic endoscopy formed the study group. Microscopic examination, culturing, rapid urease test and ELISA test were carried out.

Results: Among 100 cases (study group) suffering from gastroduodenal diseases, *H. pylori* isolated from 18 cases, identified in 54 grams stained smears, rapid urease test was positive in 62 cases, IgG immunoglobulins against *H. pylori* was detected in 62 cases.

Conclusion: No single test can be considered sensitive or specific to detect or rule out *H. pylori* infection and it is necessary to use a combination of tests

Key words: Helicopacter pylori, diagnosis, gram staining, rapid urease test, ELISA

Introduction

Helicobacter pylori (H. pylori) infection is found in 50% of humans and can result in a group of disorders of the upper gastrointestinal tract involving principally stomach and the most proximal portion of the duodenum. Marshall and Warren in 1983, first reported the presence of gram negative spiral bacillus from a patient suffering from gastritis, since then over whelming evidence has become available that *H. pylori* plays an important role in the pathogenesis of gastrodudenal disease (1).

Since that time, this bacterium has been hotly debated both in the medical press and by the media. Later, in 1975 Steer showed the association of this spiral bacteria in 80% of patients suffering from gastritis, isolated from gastric resection specimens (2). Pathologically, it is claimed that there are etiologic association of *H. pylori* infection with an increasing number of some associated disorders such as cardiovascular diseases, metabolic syndrome and as an independent carcinogen (3).

The international agency for research on cancer working group WHO defined *H. pylori* as a grade-1 or definite human carcinogen (4). Helicobacter pylori bacterium (Helicobacter: a spiral rod, pylori: gate keeper), is S-shaped or curved gram negative rod, measuring 0.5-0.9 µm wide by 2-4 µm long with rounded ends and spiral periodicity, micro-aerophilic, non-sporing, with 4-7 sheathed, unipolar flagella with rapid drating motility, coccal transformation occurs when exposed to air for about 2 hours (5, 6). The biochemical testing showed that it is non-saccharolytic, oxidase positive, catalase positive

Corresponding Author: Emad Mohamed Abdallah; Pathology and Laboratory medicine, Al-Rass General Hospital, Saudi Arabia. **E-mail**: *drfiazahamed@hotmail.com*

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and urease positive. Growth was seen after 3-5 days incubation at 37oC under microaerophilic conditions with sufficient humidity (1). Interestingly, Sensitivity pattern of *H. pylori* to antibiotics in vitro differs from that in vivo due to the effects of the conditions into gastric mucus epithelium.

The most successful used antibiotics in vivo are amoxicillin, clarithromycin and tinidazole, but these antibiotics are subject to lose their effectiveness due to mutations of *H. pylori* since plasmids have been detected inn about 50% of *H. pylori* strains (7,8). The major modes of transmission of *H. pylori* are still uncertain, with oral-oral,gastro-oral, and faecal-oral routes all possibilities (9, 10). The prevalence of *H. pylori* in the world vary widely based on the geographical differences, However the majority of patients suffering from *H. pylori* infection were found living in poor hygienic conditions as well as poor countries (11). There are different of method for diagnosis of *H. pylori* infection, they are generally grouped as being "invasive" meaning that they require gastric tissue or mucus, or "non-invasive" requiring only blood, breath or stool analysis (12).

The aim of this study was to compare and to know if this bacterium could be identified by at least two of the four diagnostic methods used which are: Rapid urease test, direct gram's stain smear examination, culturing and IgG ELISA.

Study Design

Samples were collected at Department of Gastro-enterology, JJM medical College Hospital, India. Prior of sample collection, the ethical Considerations were followed Purpose and benefits of the survey were explained to patients and consent has obtained. Patients suffering from gastroduodental diseases were endosopied after 12 hours of fasting. The endoscope with biopsy forceps were rinsed thoroughly with water and soaked in 2% glutaraldehyde for 20 minuets, then rinsed with physiological saline just before insertion. Three samples from the ulcer site was taken from each patient under aseptic precautions. The specimen from the biopsy forceps was picked up by sterile disposable needle (about 1mm in diameter of tissue). The first specimen was taken in stuarts transport medium for culturing. The second specimen was inoculated into urease broth. The third specimen was used for imprint smear by placing it on sterile slide and pressing it gently with another slide. Also, serum was collected from the patient for IgG estimation by ELISA.

Rapid Urease test

Urear broth was impregnated with the biopsy specimen by inoculating a tube of broth with heavy suspension of the tested organism, incubated at 37 ^{o}C and observed at 15, 30, 60 minutes and up to 4 hours for a change in color to pink or red (positive) (13).

Gram staining

The biopsy specimen was transferred to sterile slide and compressed with another sterile slide. Smear was prepared by gentle pressing, air dried and the usual gram stain was carried out and examined by light microscope (13). *H. pylori* is gram negative spiral "S" or "U" Shaped bacilli arranged in groups and single.

Culturing

The biopsy specimen was transported in stuarts medium was immediately inoculated onto the freshly poured media. The media used were selective and non-selective. The selective media was Brain Heart Infusion Agar (BHI agar), plus 10% lysed de-fibriginated sheep blood supplemented with the following antibiotics Vancomycin (10mg), Trimethoprim (20 mg) and Nalidixic acid (20 mg). The non-selective media was BHI agar plus 10% sheep blood. The biopsy specimen was gently rubbed on the medium, incubated under micro-aerophilic condition in glass jar at 37 ^{o}C for 4-5 days (13).

Biochemical tests

Some in vitro biochemical tests were performed on the cultured bacterium, which are oxidase,catalase and urease tests (13).

Serology

Serum sample was collected from patient undergoing endoscopy and IgG anti-helicobacter pylori antibodies in serum was detected using Helicobacter IgG ELISA kit and ELISA reader and following the manufacturer's instructions (RIDASCREEN® Gliadin).

Results

A total of 100 cases (study group) suffering from gastroduodenal disease were endoscopied and specimen were collected and another 20 cases from apparently healthy individuals endoscopied and served as control group, the maximum number of cases located between age 26 to 50 years and males, male and female ratio is approximately 3:1, respectively. Findings of different laboratory tests are summarized in (*Table-1*), showing that rapid urease test recorded high positive result (62%), compared to gram staining and culturing.

Table-1: Detection of *H. pylori* in study group

Group	M	F	Rapid urease test	Gram staining	Culture	Serology
Study group	76	24	62	54	18	64
Control group	10	10	–	–	–	–

*****Control group had normal endoscopy and were negative for all investigations. *Abbr*. M: male, F: female.

According to *Table-2*, it can be inferred that *H. pylori* infection was observed in more number of antral gastritis cases and duodenal ulcers, followed by gastric ulcers, gastroduodenitis and carcinoma stomach, respectively.

Table-2: Positive *H. pylori* infection among various gastroduodenal diseases

Endoscopic diagnosis	Num. of cases	Number of positive tests			
		Rapid urease test	Gram's staining test	Culture & Biochemical test	Serology
Antral gastritis	52	36	32	6	38
Duodenal ulcers	22	17	14	6	14
Gastric ulcer	14	6	6	4	8
Gastroduodenitis	10	2	2	2	4
Carcinoma stomach	2	1	–	–	–

As shown in *Table-3*, there is gradual increase in the seropositivity with increasing age and gradual decline is seen after 50 years of age. Interestingly, in comparison between non-invasive and invasive tests, it can be inferred that seropostitive cases also give maximum positive number (non-invasive), while the maximum positive number of invasive tests was rapid urease, followed by gram's stain and culture, respectively.

Table-3: Comparison between positive *H. pylori* cases of non-invasive and invasive tests among gastroduodenal diseases for different age groups

Age groups	Non-invasive serology	Invasive tests		
		Rapid urease test	Gram's staining test	Culture
21-25	4	4	3	1
26-30	8	8	5	1
31-35	15	11	6	2
36-40	14	10	6	2
41-45	9	7	5	1
46-50	6	5	4	1
51-55	4	3	2	1
56-60	2	1	1	0
61-65	1	1	–	0
66-70	1	1	0	0
Total num. of positive cases	64	51	32	9

Discussion

The discovery of *H. pylori* by Warren and Marshall in 1982 not only introduced a new group of bacteria but also revolutionized our concept of gastroduodenal pathology. This study highlights the role of *H. pylori* in chronic antral gastritis, duodenal ulcer, gastric ulcer, gastroduodenitis and gastric cancer. Now, *H. pylori* infection is the leading cause of gastric cancer worldwide (14). Currently, the diagnosis of *H. pylori* is based upon endoscopic biopsy and/or rapid urease, gram's staining and culture. For invasive tests, not every patient complaining from syptoms of gastroduodenal disease are subject to these tests, particularly in developing countries. On the other hand, non–invasive serology based test detecting IgG antibodies are available commercially. The findings of current study assumed that none of them (non-invasive or invasive) are 100% accurate.

The findings of this study correlates with that of Maimooma et al. (15) who stated that rapid urease test was the best choice, detects *H.pylori* highly and accurately. Rapid urease test is cheap and simple test that is frequently used in clinical practice (12). The rapidity with which the test becomes positive was probably related to the number of *H.pylori* percent in biopsy material (16). Due to patchy distribution of *H.pylori* in gastric mucosa, false negativity can also be possible (17). The gram's staining is an important test for detection of *H. pylori* in biopsy material which is 100% sensitive and 96% with low false positivity (18).

In our study, the use of diluted carbolfuchsin as counter stain facilitated easy identification of this organism under microscope. The current study suggests that detection of *H. pylori* by culturing is highly specific but it is relatively expensive, time consuming, technically demanding and may be unnecessary unless antibiotic sensitivity required. Accordingly, the easiest was to diagnose *H. pylori* infection is by serological testing for IgG antibodies using ELISA (*Table-3*). Notably, The maximum number of intection occurred between age groups 26 to 50 years old and less common among children and elder people.

More studies should be carried out regarding the prevalence of *H. pylori* among different ages. May be some conditions may play role in spreading of this disease such as low socio-economic status, housing tenure, overcrowding, occupation, area of residence and geographical variation. It was published that the prevalence of *H. pylori* varies widely by age,

race, ethnicity and geographic area, the developing countries appeared higher rates, with most of the infections occurring during childhood, and decreasing of this infection is associated with the improvements in hygiene practices (19).

Conclusion

Based on the findings of the present study, it may be appropriate to conclude that for detection of *H. pylori* infection among symptomatic individuals it is necessary to use more than one test. The usage of rapid urease test with detection of *H. pylori* antibodies stands better chance in establishing laboratory diagnosis.

Conflict of Interest

The authors declare that no conflict of interest exists in publishing this article.

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