Research Article

A comparative study of endoscopic findings, Rapid Urease Test and Conventional Histopathology in diagnosis of Helicobacter Pylori infection

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Abstract

Background: *Helicobacter pylori (H pylori)* related gastritis is a major health ailment in developing nations. There is high morbidity and mortality ranging from chronic gastritis to gastric malignancies. Prevalence of *H pylori* infection varies markedly from country to country and in a country, region to region. **Aim of the study:** To evaluate the commonly available diagnostic methods, Rapid Urease Test (RUT) and conventional histopathology in diagnosis of *H pylori* gastritis and analyse the association of *H Pylori* with the development of gastrointestinal complications. **Settings and design:** Cross sectional prospective study conducted during the period of May 2012 to September 2014. **Material and method:** The study was carried out in a tertiary medical college hospital in southern India. Patients presenting with dyspeptic symptoms were subjected to endoscopy and investigated for *H pylori* infection through histopathological examination and RUT of biopsy specimen. Diagnosis of H pylori was made if one or both diagnostic test results were positive. **Results:** Out of 530 patients analyzed, diagnosis of *H pylori* was made in 329 patients (62.0%). There were significant statistical correlations of presence of endoscopic abnormalities and serious gastrointestinal complication (peptic ulcer and dysplasia/cancer) with H pylori infection**.** RUT had comparable predictive values to histopathology in diagnosis of *H Pylori* infection. **Conclusion:** H Pylori is implicated with significant morbidity and mortality due to associated gastrointestinal complications. Early and precise detection by multiple cost-effective methods, bearing good patient compliance and prompt treatment is essential for prevention of serious complications.

Keywords: H pylori infection, Histopathological examination, Rapid urease test, Gastrointestinal complications

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Introduction

Chronic gastritis and gastric ulceration are prevalent in a high magnitude throughout the world [1]. *H pylori* gastritis is the principal cause of chronic active gastritis and has major complications like gastric adeno-carcinoma and mucosa associated lymphoid tissue lymphoma (MALT lymphoma) [2]. *H pylori* is a Gram negative flagellated bacilli that usually colonizes gastric pits under the mucus layer and in close association to gastric epithelial cells. Approximately 50% of normal population across the world harbour *H pylori*, though only 10-20% of them become symptomatic [3, 4]. There is association of H pylori infection with the hygiene related conditions, life style and economy with annual incidence rate of *H pylori* infection ≈4-5% in developing nations compared to that of ≈0.5% in developed and industrialized countries [5].

There are many other etiological factors such as smoking, non steroidal antiinflammatory drugs (NSAIDS), reflux of gastric juice (chemical gastritis) that are also implicated to cause chronic gastritis. *H pylori*, though is regarded as primary cause of gastritis, it can act as synergist in addition with other etiological factors [6].

A wide range of laboratory investigations are available for diagnosis of *H pylori*. The tests belong to non

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invasive group and invasive group. Non invasive tests include urea breath test (UBT), serological IgG and IgM detection, saliva and urinary antibody test and stool antigen test [7]. The invasive tests are endoscopy based tests which include histopathological examination, RUT and polymerase chain reaction (PCR). Whereas invasive tests carry high sensitivity and specificity of >90% [8], the role of non invasive tests such as serology is limited in areas of high prevalence, because of non distinction between previous and current infection. *H. pylori* infections like other major chronic infectious diseases (i.e., syphilis and tuberculosis) are associated with a long latent period before presenting clinically.

As such, many infections will be discovered during this latent period. A number of methods to *H. pylori* infection have been developed and they are generally grouped as being "invasive" meaning that they require gastric tissue or mucus, or "non-invasive" requiring only blood, breath or stool or analysis. Here, we discuss the rapid urease test (RUT) or RUT which is an invasive test in that it requires sampling of the gastric mucosa. The test provides indirect evidence of the infection by identifying the presence of a non-mammalian enzyme, urease, in or on the gastric mucosa. This study emphasized on finding out the predictive values of RUT and histopathology in detection of *H Pylori* infection. Association of endoscopy related changes and gastrointestinal complications with *H Pylori* infection was also computed.

Methods

Study design: Cross sectional prospective study conducted during the period of May 2012 to September 2014. **Inclusion criteria:** The patients were selected on the basis of chief complains of dyspepsia and the age of patients ranged from 14 to 86 years. **Exclusion Criteria:** Patients undergone proton pump inhibitor (PPI) therapy or any antibiotic therapy within last one month. **Participants:** The age of patients ranged from 14 to 86 years. **Study Size:** The study was conducted over 530 patients from out patients and in-patients.

Data analysis: Data analysis was done using statistical package for social sciences, version 16.0 (SPSS 16). Categorical variables were compared with Chi square test. p value <0.05 was taken as statistically significant. Endoscopy was carried out using "Pentax'' forward viewing oesophago gastro duodenoscope. The endoscopy was considered normal on visualizing mucosa which is pink in colour, smooth and lustrous. Two endoscopic biopsy fragments were obtained from each patient from antrum. One biopsy fragment was sent to histopathology department in formalin container. Two sections of 4µ thickness were cut from each block and mounted on two slides, one each on a slide. Slides were stained with normal Haematoxylin and Eosin (H and E) stain and Giemsa stain. Histopathological assessment of gastric mucosa was done by pathologist and grading was done for mononuclear cell infiltration, neutrophilic infiltration, atrophy, intestinal metaplasia and density of H pylori according to the visual analogue of updated Sydney grading system [9] of reporting gastric biopsies. Microscopic assessment of slides was carried out using labomed microscope vision 2000, India. In positive cases, *H pylori* appeared as light bluish rods in H and E stained slides with varying sizes (3-6µ) on the luminal surface of mucosal cells.

In Giemsa stain, H pylori appeared dark blue in a light background. Another biopsy fragment was sent to microbiology department for RUT. RUT was performed by following method: Urea (2 g) was dissolved in 20 ml double distilled water. 20 drops of phenol red was added to the solution and pH was adjusted between 6.8 and 6.9 by adding a drop of N/10 HCl, if pH was greater or N/10 NaOH, if pH was less. Solution was faint yellow tint at this stage. This was transferred to sterile vial each containing 2 ml in each vial. Biopsy material was added and the temperature was kept constant at 35-37°C. Test was considered positive, if colour changed within 30 minutes and weekly positive, if the change occurred after 2 hours.

The study was approved by scientific research committee of the institution. The study involved the data analysis of routine procedure carried out in institution and informed consent was obtained from each patient before the procedure. Positivity for one or more methods- histopathology by any of the stains (H&E/Giemsa) or RUT was considered as true positive. Sensitivity, specificity, PPV and NPV of different methods were computed and compared.

Sensitivity = True positive/ (True positive + False negative); Specificity = True negative/ (True negative + False positive); PPV = True positive/ (True positive + False positive); NPV = True negative/ (True negative + False negative). Youden's index = Sensitivity + Specificity – 100.

Results

Data of 530 patients were analyzed, out of which 328 (61.9%) were males and 202 (38.1%) were females. The mean age was 48.8 ± 16.68 years with age ranging from 14-86 years. Histopathological examination showed H pylori positivity in 315 cases and rapid urease test showed H pylori positivity in 306 cases. Diagnosis of H pylori infection was made if both or either of the tests was positive. 329 cases were found to be *H pylori* positive, on combining both test results.

Endoscopic abnormalities when compared with *H pylori* presence, yielded significant results, with 294 out of 329 H pylori positive cases revealing any of the abnormalities, with p value <0.001 (Table 1). The most common endoscopic abnormality was gastritis (69%), followed by duodenitis (17%), oesophagitis (12%), duodenogastric reflux (7%), hiatal hernia (6%) gastric ulcer (GU) (2%), duodenal ulcer (DU) (2%) and Barrett's oesophagus (2%). Serious gastrointestinal pathology (GU, DU and carcinoma) was only seen in 42 patients (7.9%). Patients showed varied presentation of clinical symptoms such as abdominal pain (61%), gastric fullness (17%), vomiting (12%), fatty food intolerance (9%), bloating (7%), belching (6%), melena (5%), early satiety (5%) and weight loss (4%).

Table-1: Correlation of endoscopic abnormalities with H pylori infection:

		Endoscopic abnormalities		
		Present	Absent	Total
H pylori	Positive	294(89.4%)	35(10.6%)	329(100%)
	Negative	149(74.1%)	52(25.9%)	201(100%)

df-1, chi square-21.100, p-.000

Table-2: Types of histopathological reporting:

Types of pathological reporting	% Total biopsy cases examined
H Pylori gastritis	(329/530) 62.0%
Reactive gastritis	(56/530) 10.5%
Non-specific gastritis	(58/530) 10.9%
Ulcer(Gastric, Duodenal)	(21/530) 3.9%
Dysplasia/carcinoma	(21/530) 3.9%
Normal gastric mucosa	(45/530) 8.4%

Table-3: Categorisation of cases based on results of diagnostic tests

Cases	Histopathology	RUT	Final result
292	P	P	P
23	P	N	P
14	N	P	P
201	N	N	N
Total no. 530	**P-**	**P- 306/530**	**P-329/530**

RUT-Rapid urease test, N-Negative, P-Positive, No.-Number

Table-4: Predictive value of different diagnostic tests

Diagnostic methods	Sensitivity	Specificity	PPV	NPV	YI
Histopathology	95.7	100	100	93.4	95.7
RUT	93.0	100	100	89.7	93.0

RUT-Rapid urease test, PPV-Positive Predictive value, NPV-Negative predictive value, YI-Youden's index

Table-5: Pathological diagnosis and associated histopathological features.

Pathological diagnosis	H.Pylori% Positivity	% with acute inflammation	%with chronic inflammation	% with intestinal metaplasia	%with glandular atrophy
Gastritis (n=443)	(292/443) 65.9%	(180/443) 40.6%	(359/443) 81.0%	(36/443) 8.1%	(83/443) 18.7%
Peptic ulcer (n=21)	(18/21) 85.7%	(21/21) 100%	(18/21) 85.7%	(7/21) 33.3%	(11/21) 52.3%
Dysplasia or cancer (n=21)	(19/21) 90.5%	(15/21) 71.4%	(17/21) 80.9%	(12/21) 57.1%	(15/21) 71.4%

df-1, chi square-13.11, p-.000

Histopathological features when analyzed, 56 of 530 patients (10.5%) were found to have reactive gastritis (Table 2). *H pylori* positivity was seen in 292 of 443 patients (65.9%, Table 5) of patients with diagnosis of gastritis. Normal gastric mucosa was evident in 45 of 530 cases. Histological features such as intestinal metaplasia and glandular atrophy were seen in 8.1% and 18.7% respectively in patients with gastritis, whereas these entities were seen in 57.1% and 71.4% of cases respectively in patients with dysplasia/cancer. The correlation of gastrointestinal complications (Peptic ulcer and dysplasia/cancer) was statistically highly significant with *H. pylori* infection, with p value of <0.001, whereas correlation of gastritis *with H pylori* infection was statistically not significant, with p value of 0.092.

Discussion

RUT is the most frequently performed test during routine gastroendoscopy practice. It is extremely valuable because it gives a positive result for *H. pylori* infection before the patient leaves the endoscopic suite. Histological diagnosis of *H. pylori* infection is usually reserved for patients with a negative biopsy urease test or when histology was required for another reason such as exclusion of malignancy. In an earlier study rapid urease test, said et al [10] had the sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy were 98%, 100%, 100%, 98% and 99%, respectively.

Gastritis, gastric ulceration and gastric malignancies have many etiological factors, among which H pylori infection is the principal cause. H pylori infection is dependent upon many variables such as age, sex, socioeconomic status, dietary habits, genetic and immunological factors. In the present study the commonest identifiable lesion at endoscopy was gastritis (69%). The correlation of endoscopic abnormality with H pylori infection was statistically highly significant with a p value of <0.001, proving endoscopic changes to be a sensitive indicator of H. pylori infection. This is in contrast to the observation laid by Jemilohun et al [11] in which the correlation was not statistically significant. This can be attributed to lower number of cases (86) being evaluated in their study compared to the present study (530).

Pathological reporting of 530 cases showed only (42/530) 7.2% of cases having serious gastrointestinal complications like peptic ulcers (GU and DU) and dysplasia/carcinoma. The association of these lesions with H. pylori infection was found to be statistically significant with 18 of 21 (85.7%) patients with peptic ulcer and 19 of 21(90.5%) with dysplasia/carcinoma. Cotran et al [12] narrates the international association of H pylori with gastric ulceration to be more than 70%. The present study showed 10.5% of cases having

reactive gastritis. There is rising incidence of reactive gastritis in rural and suburban population of developing nations like India due to increased duodenogastric reflux associated with changing life style of the population, injudicious and rampant use of drugs like NSAID which are easily available and often being prescribed for musculoskeletal ailments.

The present study showed that dysplasia and carcinoma develop only in few of the cases having predisposing factors such as intestinal metaplasia and glandular atrophy. High H pylori prevalence, 19 of 21(90.5%) in cases having dysplasia and carcinoma in this study, implies that majority of gastric adenocarcinomas can be prevented with early stage detection of *H. pylori* and *H pylori* eradication therapy.

This is in concordance with the findings documented by International agency for research on cancer, stating that 60% or more of gastric cancers worldwide can be prevented by absence of *H pylori* infection [13].

As noted above, the RUT is a test for the presence of the urease enzyme. The actual results will however depend on the gastric disease and the likelihood of atrophic changes or exogenous factors that reduce the bacterial load and thus produce false negative results. False positive results can occur if other urease containing organisms are present in sufficient quantity or if one allows contact of the specimen and the media for a prolonged period, typically longer than 24 hours. Approximately 10^5 bacteria must be present in the biopsy sample for a positive result [14].

The two most common reasons for false negative results are the recent use of proton pump inhibitors and the presence of intestinal metaplasia. It is unlikely that a false negative RUT will also be accompanied by histologically uninflamed and normal gastric mucosa. When in doubt and the result is important, it is best to obtain a noninvasive test (urea breath test or stool antigen) after discontinuation of the PPI.

False-positives are rare and when present may be due to the presence of other urease containing organisms such as Proteus mirabilis, Citrobactor freundii, Klebsiella pneumonia, Enterobactor cloacae and Staphylococcus aureus [15]. However unless the patient has achlorhydria or hypochlorhydria, non-*H. pylori* organisms are unlikely to be present in sufficient concentration to produce a positive test unless the RUT substrate lacks an inhibitor to bacterial growth in which they may possibly overgrow during the 24 hours observation period.

Generally speaking, upper endoscopy is an expensive test associated with a small but definite risk and unless there are specific contraindications, biopsy for examination of the mucosal histology is generally indicated. RUT testing can also be done and is especially helpful in difficult diagnostic situations when the physician would like to start treatment soon. The tissue sample contained in the agar of an RUT test can be used for molecular testing for *H. pylori* and/or for the presence of clarithromycin resistance

Conclusion

In conclusion there is high prevalence of H pylori infection in rural and suburban population of South India. Though the prevalence of H. Pylori gastritis and associated abdominal symptoms is high in number, serious gastrointestinal complications develop in few. Absolute prevention of these complications and relief from the distressing abdominal symptoms can be achieved through early detection by conventional and affordable diagnostic methods and empirical treatment with anti H pylori therapy.

RUT should be used as an informal assessment of the accuracy of the pathology laboratory and discrepancies between the RUT and histology especially a positive RUT and negative histology should lead to prompt review of the histopathology. The positive tests, should be correlated with endoscopy findings and histological assessment of gastric mucosa should be done, where ever feasible, to gather additional information on architecture.

Contribution

Study concept, Data collection, Manuscript writing, Final review and approval- Dr Shashikant Adlekha
Data collection, Data Compiling, Manuscript editing, Final review and approval- Dr Tandra Chadha.

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