

Original Research Article**Evaluation of Role of Touch Cytology in Diagnosis of Helicobacter Pylori in Non-Ulcer Dyspepsia Cases****Udita Singhal^a, C.L. Pandey^b, Sangeeta Lamba^c, Annu Nanda^d**

^aAssistant Professor, ^dProfessor and Head, Department of Pathology, ESIC Dental College & Hospital, Rohini, Sector - 15, Delhi 110089, India. ^bEx-Head of the Department, Bhagwan Mahavir Cancer Hospital, Jaipur, Rajasthan 302017, India. ^cSenior Specialist and Head, Department of Pathology, ESIC Hospital, Sector 15A, Rohini, Delhi 110089, India.

Abstract

H. pylori has emerged as an undisputed cause of chronic active gastritis, however the relationship between H. pylori and non-ulcer dyspepsia is still a matter of controversy. Various tests are available for diagnosis of H. pylori. Research in several directions has brought forward no general consensus about the most suitable method to diagnose H. pylori. Hence this study was done to evaluate the role of touch cytology diagnosis of H. pylori infection in patients of non-ulcer dyspepsia and to compare it with histology and urease test.

Corresponding Author:**Udita Singhal,**

Assistant Professor,
Department of Pathology,
ESIC Dental College & Hospital,
Rohini, Sector - 15, Delhi 110089,
India.

E-mail:

druditasinghal@gmail.com

(Received on 18.01.2018,

Accepted on 09.02.2018)

Material And Methods: Touch smears were made from antral biopsies obtained from patients (125) undergoing upper GI endoscopy. Rapid urease test was performed. Touch smears were stained with Giemsa stain and histological sections with Haematoxylin and Eosin stain. The gastric biopsies were classified as per Sydney system.

Observations and Results: In this study touch cytology was positive for H pylori in 53 out of 83 cases (62.8%). Organisms were better appreciated on smears than on the biopsies, smears took lesser time to screen and report was available to the patient and the clinician the same day. Urease test was positive in 57.8% cases making it a reliable and rapid test for diagnosis. The percentage positivity of it is comparable to touch cytology results (62.8%) in our study. Thus touch smear cytology appears to complement histopathology.

Conclusions: Touch cytology is a quick and simple sensitive test for diagnosis of H. pylori infection in patients of non-ulcer dyspepsia. It complements histology and urease test.

Introduction

The discovery of Helicobacter pylori from gastric biopsies by Marshall and Warren in 1982 opened the flood gates to the new era of understanding of gastro-duodenal pathology [1]. Studies have shown that the presence of H.pylori is associated with a variety of gastrointestinal diseases including gastritis, gastric and duodenal ulcers,

gastric adenocarcinomas and lymphomas [2,3]. However, its role in non-ulcer dyspepsia remains controversial [4]. The removal of the organism by antimicrobial therapy is correlated with the resolution of symptoms and cure of disease [5].

The tests available for the diagnosis of H. pylori can be categorized as direct and indirect. Cytology, histopathology, rapid urease test and culture method

constitute direct evidence of its presence in a biopsy. Indirect tests include serological diagnosis, stool antigen test and urea breath test (UBT), PCR, and even in situ hybridization. A test should be sensitive and specific, inexpensive, easy, rapid with good patient acceptance and minimally invasive. One such test is touch cytology [6].

Role of touch cytology is already established in diagnosis of various benign and malignant lesions of lymph nodes [7,8], its use in diagnosing non-neoplastic lesions of GIT is increasing.

The characteristic histological appearance of H. pylori is 3x0.5um, spiral rod, haematoxyphilic on H&E stain. It is located adjacent to the gastric epithelium. Since these organisms browse on the mucosa, mucosal samples can be taken through endoscope, touch imprints can be made and the same biopsy be subjected for histo-pathological examination. Because histologic gastritis may be present in the absence of mucosal abnormalities, histological examination permits better correlation between presence of bacteria and its pathological result.

An important characteristic of H. pylori is its ability to produce urease [9]. The presence is indicated by color change. Urease test is dependent on density of bacteria and therefore most sensitive when performed on antral tissue.

The prevalence of H. pylori infection varies between developed and developing countries, being 30% and more than 80% respectively [10]. A recent report from India indicates almost 80% of the population is infected with H. pylori [10].

The discovery of H. Pylori and its close association with gastritis has led to speculation that it may have a role in causing symptoms "non-ulcer dyspepsia". It is defined as upper abdominal pain or epigastric pain, discomfort, heartburn, nausea, vomiting or other symptoms referable to the upper GIT and lasting for more than four weeks, unrelated to exercise and for which no focal lesion or systemic disease can be found responsible [11].

Helicobacter pylori is found in 43 to 87% of subjects of NUD. Wide variation in subject's frequency represent both differences in criteria to diagnose NUD and different populations evaluated [12].

With this background the present study was done to evaluate the role of touch cytology in diagnosis of H. pylori in patients of non-ulcer dyspepsia and compare it with histology and urease test.

Material and Methods

The present study was conducted in department of pathology and endoscopy room of the gastro enter ology

department of the hospital, over a period of 1 year. All the patients presenting with symptoms of dyspepsia like nausea, vomiting, belching, epigastric pain, requiring upper GI endoscopy were included. The total number of patients were 125. The biopsy was obtained with help of punch biopsy forceps (Olympus). 2-4 biopsies were taken from the antrum. The first biopsy was put in 10% W/V of the urease solution to detect for the presence of H.pylori. It was considered positive if the color changed to pink in one hour.

The second and the third biopsies were used to make touch smears. The biopsies were gently rolled on the two slides and were stained with MGG stain. The biopsies were then put into 10% buffered formalin for histological correlation, stained with H and E. They were graded according to The Sydney system 1994 [13].

Results

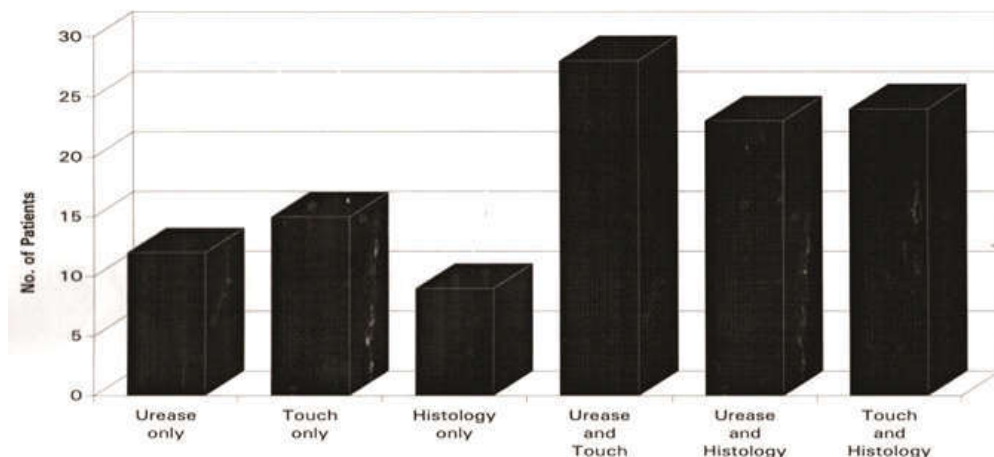
The study group comprised of 125 patients with 31-40 years as the most common age group (40%)(50). (Table 1). Out of total 83 cases positive for H. pylori by either urease test, touch cytology or histology, maximum cases were positive in age group of 31-40 years (40.9%)(34) (Table -2). 72%(90) were males and 28% were females (35). Most common symptom was pain (26.4%), followed by pain and belching (21.6%).

Out of 83 positive cases 26n experienced only pain (31.3%) while 18 patients (21.6%) had both pain and belching. The most common finding on endoscopy was antral gastritis 60n (48%). 29.6% (37) had normal study, while 10 (8%) had antral hyperemia, 7(5.6%) had erosive antral gastritis, 8 (6.4%) had duodenitis, 3 had antral ulcer (Table 3). Maximum percentage positivity fell in the group of antral gastritis 42 out of 60 (50.6%) and out of 37 patients with normal study, 23 were positive for H.pylori. All 3 patients with antral ulcer on endoscopy were positive for H. pylori by either of the methods.

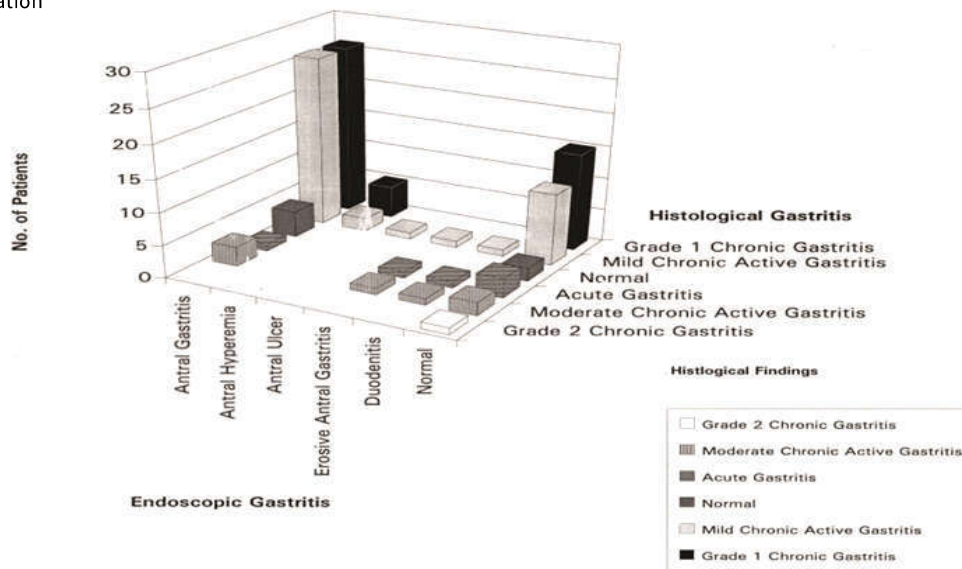
On correlating endoscopic diagnosis with histologic gastritis as per Sydney classification, out of 125 cases only 6 had normal histology others had evidence of acute, chronic and chronic active gastritis.

In our study, out of 83 positive cases, 53 were positive by touch smears (63.8%) 48 by urease test (57.8%) and 41 by histology (49.3%). Positive concordance rates was 12.8% (16) and negative concordance rate was 33.6% (42) (Table 4).

In our study 50.9% (28 out of total 55) were positive by cytology and urease test. 52.2% (23 out of total 44) were positive by histology and urease test. Out of 48 cases 24 showed positivity by both histology and touch cytology (50%). The range of percentage positivity by two tests was in range of 50 to 52.2% (Table 5).



Graph 1: Respective contributions of these Different test for Diagnosis of Helicobacter pylori alone and in combination



Graph 2: Correlation endoscopic gastritis with histologic gastritis in patients of non-ulcer dyspepsia

Table 1: Helicobacter pylori positive in various age groups

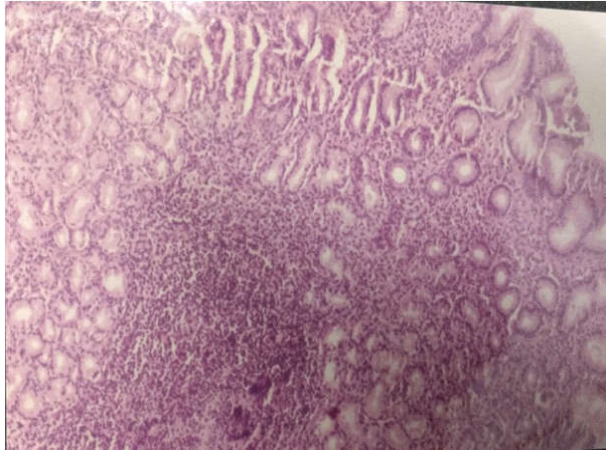
S. No	Age in Years	Number	H. Pylori Positive	Percentage
1	0–10	0	0	0
2	11–20	4	2	2.6
3	21–30	49	30	36.1
4	31–40	50	34	40.9
5	41–50	19	14	16.8
6	51–60	3	3	3.6
	Total	125	83	100

Table 2: Association of symptoms with helicobacter pylori infection

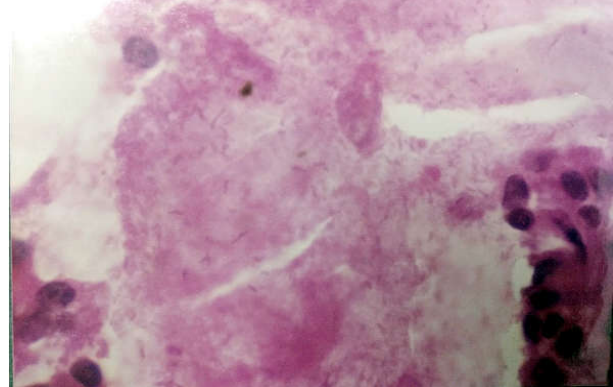
S. No	Symptoms	Number	Percentage	H. Pylori Number and %
1	Pain and Belching	27	21.6	18 21.6
2	Pain and Nausea	21	16.8	11 13.2
3	Pain Only	33	26.4	26 31.3
4	Belching Only	3	2.4	3 3.6
5	Belching Pain And Nausea	16	12.8	10 12
6	Belching and Nausea	15	12	10 12
7	Nausea, Vomiting and Pain	4	3.2	2 2.4
8	Nausea And Vomiting	2	1.6	2 2.4
9	Others	4	3.2	1 1.2
	Total	125	100%	83 100%

Table 3: Endoscopic findings in patients of non ulcer dyspepsia and correlation with helicobacterpylori

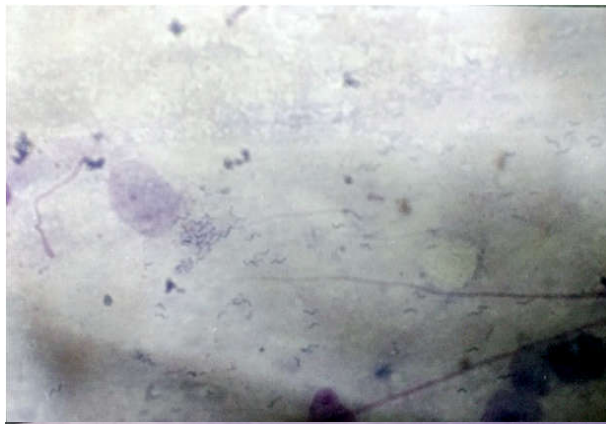
Endoscopic Findings	Number	Percentage	H. Pylori Positive	Percentage
Antral Gastritis	60	48	42	50.6
Antral Hyperemia	10	8	7	8.4
Antral Ulcer	3	2.4	3	3.6
Erosive Antral Gastritis	7	5.6	4	4.8
Duodenitis	8	6.4	4	4.8
Normal	37	29.6	23	27.8
Total	125	100%	83	100%



Photomicrograph 1: Showing lymphoid follicles in a biopsy H &E



Photomicrograph 4,5: Showing H.pylori in H&E sections



Photomicrograph 2,3: Showing presence of spiral shaped H.pylori in imprint ,touchsmearsMGG stain



Fig. 1: Helicobacter pylori on touch smear

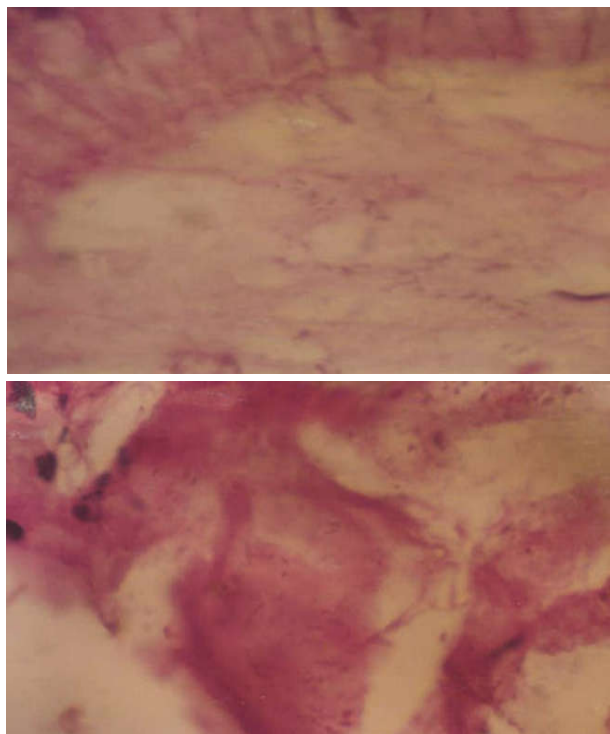


Fig. 2: Helicobacter pylori in gastric pits on H & E

Lymphoid follicles were seen in 22 out of 125 patients of non-ulcer dyspepsia, 12 were positive for H.pylori infection accounting for 54.5%.

Discussion with Fast Forward 21 years

The association of H.pylori with gastrointestinal pathology has generated intense interest in developing methods for diagnosis of H.pylori infection.

Various stains have been recommended by different authors from time to time. These include H & E by Taylor, Giemsa by Gray, acridine orange by Walters, War thin starry stain by Marshall and Brown, Hop's stain by Westblom. Genta RM suggested the use of a new stain for simultaneous visualization of H.pylori and gastric morphology [14].

More refined methods are immunohistological methods based on monoclonal or polyclonal antibodies against H.pylori by Perez [15]. Cerqueira L proposed the use of in situ to identify the bacteria when the conventional methods pose a difficulty [16]. Clayton et al found PCR to be a useful method, its sensitivity being comparable to histology and a culture method [17]. Luzzo F used salivary specific immunoglobulin G in diagnosis of H.pylori infection in dyspeptic patients [18]. However, the above methods have not gained popularity owing to their cost, special requirements and non-availability at every center. Zullo analyzed the clinical practice view point to culture or not

to culture H.pylori, and found culture to be time consuming, low sensitivity test and it added significantly to the cost when antibiotics are tested [19]. Thus culture has its limitations viz. requirement of proper standard, reduced viability of H.pylori, and proper specimen transport. H.pylori is a fastidious organism and not all microbiological labs are well equipped for its isolation.

Calvet reappraised the accuracy of diagnostic test for H.pylori, he found 94% sensitivity of rapid Urease test [20]. Similar results of reliability of RUT have been in the literature from time to time. Masahiro Kawanishi, found rapid urease test for identification of *Helicobacter pylori* in comparison with histological and culture studies to be better and very rapid test [21]. Redeen et al also found urease test to be a reliable test for diagnosis of H. pylori, and Tokunga et al found MRU test has high sensitivity, specificity, low cost, shorter incubation time and combination with Giemsa stain for Touch cytology is cost efficient in clinical settings [22,23]. Roy A, suggests use of RUT to confirm H. pylori and recommends it for early diagnosis and treatment of H.pylori associated diseases [24].

Saxena et al on evaluation of endoscopic based diagnostic methods found the specificity, sensitivity of urease test, touch cytology and histopathology, concluded that when single test is to be used touch cytology is best [25]. In our present study also, the highest number of positive cases were seen with touch cytology alone 63.8%, and combination with urease test (57.8%) and or histology (49.3%) gave comparable result. Similar studies by Fakhjou. A, et al however found diagnostic value of RUT to be lower than Touch cytology and histopathology. He found Touch cytology to be most sensitive and histology to be most specific in outpatient setting [26]. L Trevisani et al found Touch cytology to be a reliable and cost effective method for diagnosis of H.pylori infection. He suggested that rapid urease test is cheapest, touch cytology is faster and cheaper than histology [27].

Cubukcu A, et al studied Imprint cytology in diagnosis of H.pylori and found it to be simple rapid, cheap method and imprints do not affect the quality of biopsy [28]. Yamamoto T et al evaluated the usefulness of touch smear cytology for diagnosis of H.pylori infection and cytology is a very accurate, convenient rapid, low cost test and has stability [29].

Kaur G et al studied diagnosis of H.pylori infection in gastric imprint smears and found out sensitivity, specificity, and PPV and NPV of Touch cytology and compared it with histology, best results are seen when complemented with histology [30].

Hashemi MP et al Touch cytology in diagnosing H.pylori infection, comparison of four staining methods RUT should still be acknowledged as primary test in diagnosis of

H.pylori. If RUT is negative, Wright stained TC is a safe substitute [31]. Rahabar M et al, Adlekha S et al studies also found imprint cytology to be fast and reliable [32,33].

In a study by Rami I, who evaluated diagnostic methods in gastric biopsies of non-ulcer dyspepsia, found that in comparison to histology and urease test, the RUT and PCR is 100% sensitive and RUT with culture is 100% specific [34]. Khalifghogli M compared five diagnostic methods for H.pylori; according to his study, the accuracy of the tests for H. pylori diagnosis can be arranged in order as follows: RUT>PCR>histology>stool antigen test>serology, however he suggested that utilization of biopsy-based and non-invasive methods is useful for H. pylori infection confirmation [35].

Lee JY et al recommended that histology is an excellent method for detecting H. pylori as it provides additional information on the mucosa [36]. In our study also histology was helpful as it provided the status of the mucosa as per Sydney system of classification, but combined with touch cytology the results were superior to just histology, 50% as compared to histology alone in 18%. Uotani also recommends that urease test can be used for initial screening, but for confirmation at least two tests should be done [37].

On reviewing the vast literature on invasive and non-invasive tests for diagnosis of H. pylori, we found touch cytology and rapid urease test to be the most practical, rapid and cheap methods among all for the diagnosis of Helicobacter pylori infection; histopathology complements these two tests as is seen in our study too.

It is surprising that the gastroenterologists are not using this simple technique as an office procedure to detect H.pylori infection. It calls upon to ponder the reasons why this test has not gained popularity, when the report is available within few hours in the endoscopy room itself.

Conclusions

Various tests are available for diagnosis of Helicobacter pylori ranging from simple urease test to FISH and PCR. Research in several directions has brought no general consensus about the best method for its diagnosis.

With this view the study was carried out and we found Touch cytology to be simple, rapid cost effective method for diagnosis. The organisms are better appreciated morphologically in smears than biopsies, smears took lesser time than histology, they were even positive in cases of fragmented biopsies, however histology gives additional information on gastric mucosa, hence we recommend touch cytology as simple fast screening test and report can be given on the same day. We suggest its role complementary to histological examination.

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