Role of Proton Pump Inhibitors on Rapid Urease Test (PYLO DRY) and Histopathological Examination

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Abstract: In the diagnosis of Helicobacter pylori infection, rapid urease test (RUT) plays an important role. According to previous studies, if there was a low density of Helicobacter pylori in the stomach, then rapid urease test requires longer time to become positive. Density of Helicobacter pylori could be reduced by recent use of PPIs, hence rapid urease test may require longer reading time as well. The main objective was to determine the effect of PPI on the results of rapid urease test (PYLO DRY) and histopathology. Two antral biopsies were obtained from 35 dyspectic patients, who underwent gastro intestinal endoscopy from March 2015 to July 2015. An informed consent from all patients and approval from the institutional ethical committee were obtained before proceeding. Antral biopsies were subjected for rapid urease test (PYLO DRY from HALIFAX RESEARCH LABORATORY KOLKATA, INDIA) and histopathologic examinations. The results of Rapid urease test were observed after 1 hour and 24 hours. The histopathologic examinations were used as the gold standard. PYLO DRY was positive in 17.6% (6) and 53 % (18) when observed after one hour and 24 hours respectively. Eight were positive during histopathological examination. In our study, 24 hours Rapid urease test yeilded higher sensitivity but lower specificity compared to RUT at 1 hour for diagnosis of H.pylori infection in patients who were on PPI.

Keywords: Proton pump inhibitors (PPIs), Rapid Urease test (RUT), Helicobacter pylori, Antral biopsy

I. Introduction

Infection with *H.pylori* is worldwide and is a potential cause of many gastroduodenal diseases such as Peptic ulcer diseases, Non ulcer dyspepsia, MALT lymphoma and gastric cancer (1,2, 3). Invasive and non-invasive methods are available for the diagnosis of *H.pylori* infection. Obtaining biopsy through endoscopy is an invasive method, where biopsies are subjected to rapid urease test, culture, histopathology and DNA amplification, whereas urea breath test, serology and stool antigen test comes under non-invasive methods (4). Rapid urease test is the common diagnostic tool used for the detection of *H.pylori* infection. In Rapid urease test bacterial density plays a very important role. The density of *H.pylori* should be high for the RUT to be positive. If any agent leads to the reduction of bacterial load then there are high chances of getting false –negative results. To obtain a better results of rapid urease test, it is advised to go for more number of biopsies and also to increase the number of sites from the stomach (5). If the patients are on PPI, antibiotics or bismuth compounds, sensitivity of RUT is decreased (6,7). There will be reduction in the number of bacteria when the patients are on antibiotics (8) .The presence of drugs which are known to decrease the acid in the stomach, thereby increasing the pH affects the site of the stomach to be biopsied. Hence the site of the stomach from which biopsy is obtained plays an important role in determining the results of RUT (9). The main objective of this study was to determine the effect of PPI on the results of rapid urease test (PYLO DRY) and histopathology.

II. Materials and Methods

Biopsies were obtained from 35 dyspectic patients, who underwent gastro intestinal endoscopy 2015 at Department of Gastroenterology, JSS Hospital Mysore from March 2015 to July2015. All patients had used PPI. Clinical symptoms at the time of presentation and diagnosis were noted with endoscopic findings. All adult patients of both genders, undergoing upper gastrointestinal endoscopy with features of peptic ulcer disease, Non ulcer dyspepsia and gastric carcinoma were included in this study. Patients with present or past history of gastric surgery or long term therapy with non-steroidal anti-inflammatory drugs were excluded from this study.An informed consent from all patients and approval from the institutional ethical committee were obtained before proceeding.

Biopsy Specimens:

Two antral biopsies were obtained from each patient. Biopsy specimens were evaluated by Rapid urease test and histopathological examination.

Rapid Urease Test:

PYLO DRY (a commercially manufactured Rapid urease test from HALIFAX RESEARCH LABORATORY KOLKATA, INDIA) was performed according to the manufacturer's instructions. PYLO DRY consists of a dry filter paper containing urea, phenol red (a pH indicator), buffers and bacteriostatic agent in a sealed plastic slide. If the urease enzyme of *H.pylori* is present in an inserted tissue sample the resulting decomposition of urea causes the pH to raise and the colour of the dot turns from yellow to pink or red. Antral biopsy from each patient was placed into the test dots. After releasing the test the label was pressed over the test dot with the finger to squeeze the tissue fluid out of the specimens. The tissue fluid was absorbed by the filter paper. Results were monitored at room temperature after one hour and 24 hours. A positive result was defined as the colour change on the test from yellow to pink-magenta, and no colour change as negative for PYLO DRY

Histopathological Examination for H.pylori Infection

Antral biopsies were fixed in 10% formalin, before embedding in paraffin wax, the sections were stained with haematoxylin and eosin (H&E) and Giemsa (in doubtful cases) for light microscopy to detect *H.pylori* infection. The presence of spiral organism on the slide was considered as positive for *H.pylori* infection. Histopathologist was kept blinded about the results of PYLO DRY

Statistical Analysis

Data was analysed using SPSS software. The sensitivity, specificity, positive predictive value and negative predictive value of PYLO DRY at one hour and 24 hours were calculated. The histopathologic findings were used as the gold standard for diagnosis of *H.pylori* infection.

III. Results

34 patients were recruited, of these 21 were males and 13 were females. The mean age was 46 years with a range of 21 to 90 years. The endoscopic findings were shown in Table 1. The most common endoscopic findings were Non ulcer dyspepsia, Gastric ulcer, duodenal ulcer and adenocarcinoma. Abdominal pain was present in 35% (12), APD 26.4% (9), Dyspepsia 8.8% (3), GERD 8.8% (3), and Hematemesis 5.8% (2) (TABLE 1).

Rapid Urease Test (PYLO DRY) and Histopathology

PYLO DRY was positive in 17.6% (6) and 53 % (18) when observed after one hour and 24 hours respectively. Among 8 patients who had positive histopathology for *H.pylori*, 3 patients had positive RUT at one hour and 5 patients had positive RUT at 24 hours. There were 12 Patients who had negative RUT at one hour and positive results at 24 hours.

Comparison of Rapid urease test and Histopathology

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of PYLO DRY at one hour were 50%, 82%, 37.5% and 88% respectively. AT 24 hours they were 27%, 81%, 62.5% and 50% respectively (TABLE 2 and 3).

Table 1: showing demographic data, symptoms, endoscopic findings, results of rapid urease test and histopathology

Factors	Patients (%)
Gender	
Male	21(61%)
Female	13(38%)
Age (year) +/- SD	
Clinical symptoms	
Pain abdomen	12(35%)
APD	9(26.4)
Dyspepsia	3(8.8%)
GERD	3(8.8%)
Hematemesis	2(5.8%)
Anorexia	2(5.8%)
GI bleed	2(5.8%)
Anaemia	1(2.9%)
Endoscopic findings	
Gastric ulcer	8(23.5%)

Duodenal ulcer	8(23.5%)
Non ulcer	16(47%)
Adenocarcinoma	2(5.8%)
Rapid urease test	
At one hour	
Positive	6(17%)
Negative	28(82%)
After 24 hours	
Positive	18(52%)
Negative	16(47%)
Histopathology	
Positive	8(23.5%)
Negative	26(76.4%)

Table2: The results of rapid urease test at one and 24 hours compared with histopathologic examinations

Diagnostic test	PYLO DRY at one hour		PYLO DRY at 24 hours	
Histopathologic examination	Positive(n)	Negative(n)	Positive(n)	Negative(n)
Positive(n)	3	5	5	3
Negative(n)	3	23	13	13

Table 3: The sensitivity, specificity, PPV and NPV of rapid urease test at one and 24 hours compared with histopathologic examinations

Test	Sensitivity	Specificity	PPV	NPV
Urease test at one hour	50%	82%	37.5%	88%
Urease test at one 24 hours	27%	81%	62.5%	50%

PPV = Positive Predictive value
NPV = Negative Predictive value

IV. Discussion

Generally, PPIs are prescribed to dyspeptic patients for a specific duration, if the condition of the patient is not improving then they are advised to stop PPI before recruiting to endoscopy. According to previous studies, the recent usage of PPIs were known to reduce the sensitivity of diagnostic tests for H.pylori (10). In India the prevalence of H.pylori infection is around 80% (11). In our study the prevalence of H.pylori is less because patients had self-prescribed PPIs before upper GI endoscopy though the physicians advised to avoid PPIs. Previous studies suggest that, the density of H,pylori was reduced by PPIs and there by shifting their distribution (7), resulting in reduced sensitivity of several diagnostic tests available for H.pylori infection like RUT, Histopathology, culture, urea breath test, PCR and stool antigen test (10, 12). Our study shows that the sensitivity of PYLO DRY test at one hour in diagnosis of H.pylori infection was lower than the sensitivity at 24 hours, but specificity was more. 12 patients had negative results at 1 hour and showed positive results after 24 hours. Among these patients, 8 patients had positive histopathological findings. This indicates that the sensitivity of RUT increases at 24 hours and also increases the chances of getting false positive results. Earlier studies suggest that if density of H,pylori was less then RUT requires longer time to turn positive (13, 14). The load of H.pylori in the stomach can be reduced by PPIs. RUT may require longer time to detect H.pylori in patients who had PPI. In our study, we found that 28 patients had negative PYLO DRY test at 1 hour and negative histopathological examination for H.pylori infection. In contrast, 12 out of these 28 samples were positive at 24 hours with PYLODRY. PYLO DRY results obtained at 24 hours might be false positive as patients who were on PPIs might develop achlorhydria with subsequent superficial colonization by other urease producing organisms like Klebseilla or *Proteus mirabilis* (15,16). These results might be false negative as histopathological examination was considered as gold standard. Chey WD et al. intheir study found that the sensitivity, specificity and Histopathologic examination was high in patients who were not on PPIs, whereas the sensitivity was reduced in patients who are on PPIs (10). Histopathologic examination may not be sensitive enough to analyse the results of PYLO DRY at 24 hours as true positives or false positives, in case of patients who were on PPI.

V. Conclusion

Consumption of PPI affects the results of rapid urease test as there high chances of ending up with false negative results. Hence it is very important to know weather PPI has been used or not before recruiting to endoscopy. Physicians should create an awareness against the self prescription of PPIs if the patient is recruited for endoscopy. if it is very difficult to avoid PPI, multiple biopsies from different parts of the stomach should be taken. In this study, 24 hours Rapid urease test yeilded higher sensitivity but lower specificity compared to RUT at 1 hour for diagnosis of *H.pylori* infection in patients who were on PPI. Further studies are required to determine the proper diagnostic method for *H.pylori* infection in patients who are on PPIs.

Acknowledgements

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