Research Article

A comparative study of rapid urease test and dilute carbol fuchsin staining technique for diagnosis of *Helicobacter pylori* infection

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ABSTRACT

Background: *Helicobacter pylori* (*H.pylori*) is a gram negative spiral bacilli associated with acid peptic disease (APD) and gastric carcinoma. A rapid urease test is the most common test performed for a quick clinical diagnosis with or without histopathological examination of antral biopsy specimens.
Methods: In this study we evaluated the utility of a simple dilute carbol fuchsin stain (DCF) for identifying the spiral or curved forms of H.pylori in the direct smears of antral biopsy samples for diagnosis of H.pylori infection and compared the results with rapid urease test (RUT).
Results: Sums of 100 cases were included in the study from which 61 (61%) were positive for urease production and shown typical spiral or curved bacilli by D.C.F stain.
Conclusions: DCF stain was found to be an excellent stain for direct microscopic evaluation and compared well with RUT.

Keywords: *H. pylori*, Rapid urease test, Dilute carbol fuchsin

INTRODUCTION

Helicobacter pylori a gram negative curved or spiral bacterium has been implicated in the aetio pathogenesis of a variety of gastric duodenal disorders like duodenal ulcer, gastric ulcer acute and chronic dyspepsia, gastric cancer and gastric lymphoma.[1] The world health organisation has listed *H. pylori* in the list of known carcinogens.[2] A variety of tests are available for diagnosis of *H. pylori* infection viz endoscopic biopsy based tests like RUT, culture, Gram staining and Histopathology, PCR, imprint cytology and non-invasive tests like serological test for detection of IgG antibodies, urea breath test.[3] A variety of staining techniques viz Gram's, Giemsa, Warthin starry and Genta stain have been reported for demonstration of the bacteria in clinical specimen with varying rates of sensitivity. In the present study we have evaluated the utility of dilute carbol fuchsin as a simple staining procedure for demonstration of *H. pylori* in biopsy samples.

METHODS

A total of 100 patients with signs & symptoms of Acid peptic disease who were subjected to diagnostic endoscopy are the subjects for the study. An informed & written consent was obtained from all the patients and institutional ethics committee approval was also obtained. A detailed history was obtained in a well-designed proforma. The endoscopy procedure was done by the consulting surgeon at our hospital. A pair of biopsy samples approx. measuring 5 mm in size was collected from the gastric antrum and the bit was placed in approx 1.5 ml of a Christenson urea broth culture solution

(Himedia) prepared freshly and checked with known urease positive and negative strains of bacteria and the other bit in a vial containing 0.5 ml of sterile normal saline. The samples were sent to the Microbiology lab within one hour of collection. The urease broth samples were incubated at 37°C along with uninoculated urea broth as control for 24 hours observing the broth after every one hour till the close of the working hours and the following morning i.e. approx after 18 hours. Broth showing a change of color to pink was considered as positive for urease test.

The second bit was rubbed over a couple of new glass slides making a smear of approx 1 cm oval in shape, air dried and fixed with methanol for 3 minutes. The fixed smear was stained by dilute carbol fuchsin (1 in 10 dilution) freshly prepared on the day of use for 10 minutes, washed with water and air dried. The smears were examined under oil immersion (100x) objective examining at least 50 fields for the presence of spiral or comma shaped bacilli. Presence of such bacilli was assumed to be positive for *H. pylori*. The results of rapid urease test and smear examination were recorded. One fifth of urease positive cases were also stained by Giemsa staining from the reserve smears prepared earlier. A freshly prepared Giemsa stain (1in 30 dilutions) from the stock on the day of staining was used for staining. The smears were left overnight in the staining jar before examining the slides.

RESULTS

The age and sex wise distribution of the cases is shown in Table 1. Out of 100 cases studied by endoscopy based urease test 61(61%) were positive for urease production. All the cases that were urease positive showed typical spiral or curved bacilli by D.C.F stain as well as the inflammatory cells. Giemsa stained slides showed typical purple colored curved or spiral forms of the bacteria and also the inflammatory cells as shown in Figure 1 and Figure 2.

Table 1: Age and Sex wise distribution of cases.

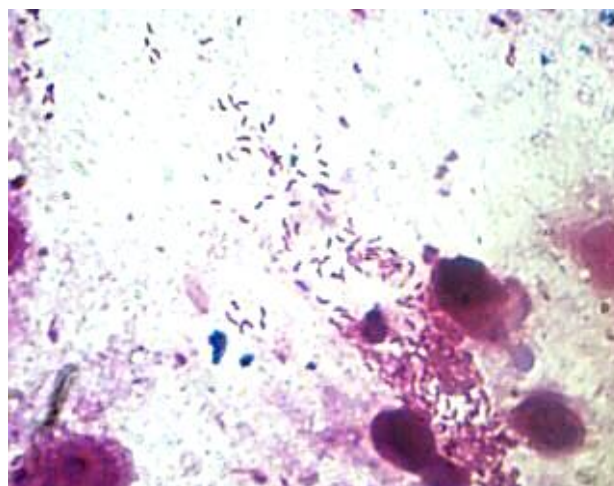| Age	Male	Female
0-10	-	-
11-20	6	1
21-30	5	5
31-40	22	8
41-50	9	13
51-60	9	13
61-70	7	1
71-80	1	-
Total	59	41

Figure 1: Dilute carbol fuchsin stain shows curved bacilli *H. pylori*.

Figure 2: Giemsa stain.

DISCUSSION

H. pylori is a fastidious, microaerophilic, motile gram negative curved bacilli found in individuals of all age groups with a prevalence rate of 20% to 80%.[4] The prevalence rates in our study was 61%. Approximately 80% of gastric ulcer and 95% of duodenal ulcer were caused by *H. pylori*.[5] An array of invasive and non-invasive tests like endoscopy biopsy based tests viz RUT, HPE, PCR, Giemsa, Warthin starry staining and serological tests for demonstration of antibodies are available for diagnosis of *H. pylori* infection each with its own advantage and disadvantage. A combination of more than one test usually gives a higher rate of sensitivity and specificity for diagnosis. RUT is a simple and rapid test but sensitivity is influenced by the bacterial density and the morphological form of the bacteria in the biopsy.6 However the low cost speed and simplicity of the technique gives an edge over the culture which requires complex media & long duration as also is the histopathological examination (HPE).[7-9] Giemsa stain is a simple, highly sensitive and economical staining

technique for demonstration of *H. pylori*.[10,11] However dilute carbol fuchsin stain (1 in 10 dilutions) which is easy to prepare from strong carbol fuchsin and available in most of the laboratories is found to be a useful simple stain for demonstration of the typical morphological forms of the bacteria in smear of biopsy tissues obtained during endoscopy. This simple stain imparts a distinct red color to the organisms as well as the inflammatory cells which are well stained and is very economical easy and very quick (5-10 min) with sensitivity on par with Giemsa stain, which was done in one fifth of the RUT positive cases.

The study is based on morphology and similar morphological forms of other species of helicobacter are known to be present in the stomach. False positive RUT was also known to occur with a few other species colonizing the stomach. The study has not been compared with a gold standard test like PCR or isolation in culture. Since most of the studies are based on RUT and HPE or staining. We have considered RUT and DCF stain a novel method as significant and sufficient for *H. pylori* infections.

CONCLUSION

Staining with dilute carbol fuchsin stain is found to be a very simple, economical & rapid supplementary test for diagnosis of *H. pylori* infection from biopsy specimens on par with Giemsa stain.

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