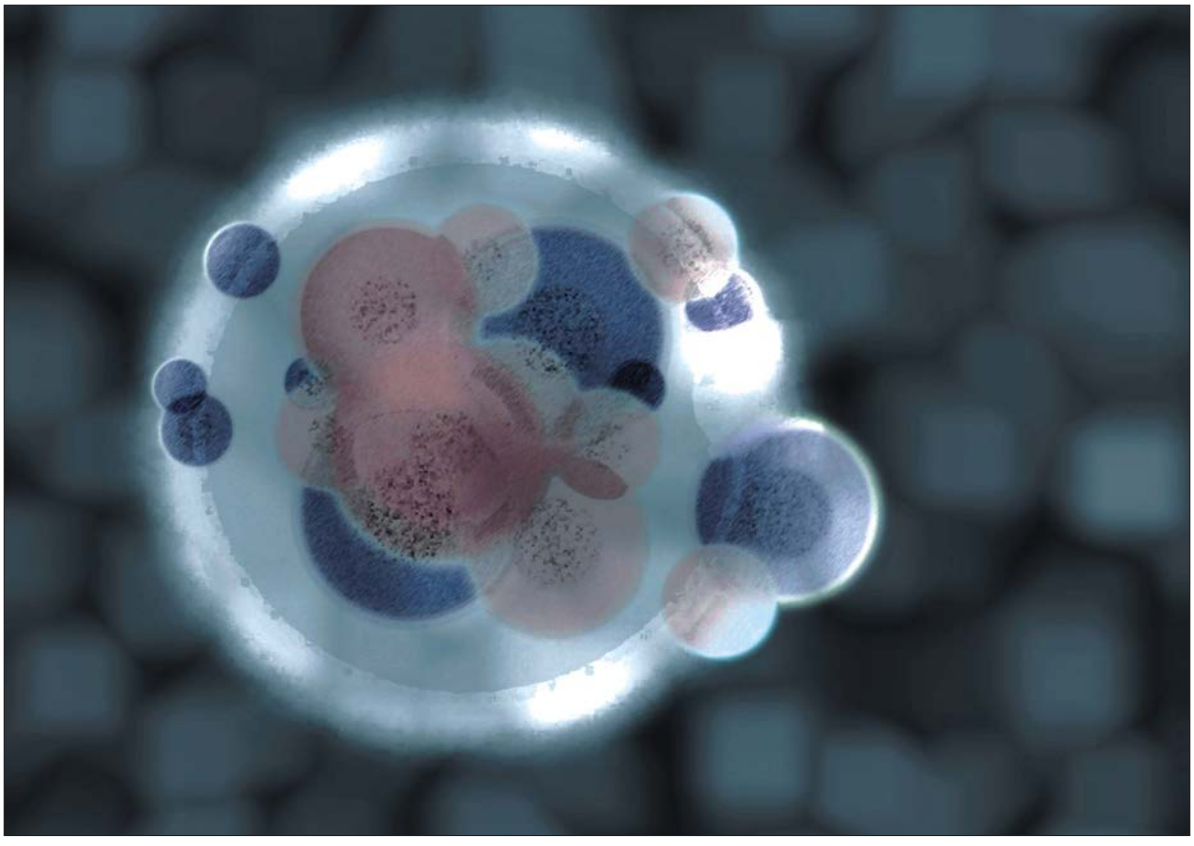

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Comparison of Different Laboratory Methods for Diagnosis of *Helicobacter pylori*

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Abstract: The aim of the present study was to compare four laboratory methods for diagnosis of Helicobacter *pylori*. 101 sets of four antral biopsies were collected from 101 patients, one of the biopsies in each set were tested by rapid urease test, other three biopsies were used for culture, direct staining and histopathology, respectively. By culture method in 62 cases (61.3%), *Helicobacter pylori* were isolated. Among several primary media that tested in this study, Brucella agar supplemented with 10% whole sheep blood supported relatively good growth of *H. pylori*. In present study 65 cases (64.3%) were positive by rapid urease test. Of 101 biopsy specimens in 68 cases (67.3%) were obtained positive result by histopathology method (Gimsa Staining). Sensitivity and specificity of direct staining test were 89.7 and 96.9%, respectively. We found histopathology method was the best method for diagnosis of *H. pylori* and it can be selected as gold standard in detection of *Helicobacter pylori*.

Key words: Helicobacter pylori, rapid urease test, histopathology

INTRODUCTION

Helicobacter pylori is a small, curved and highly motile gram negative bacillus which only infects the mucus layer of the human stomach (Marshall *et al*., 1989; Jones *et al*., 1984). Since the discovery in 1984 that this bacterium was associated with gastritis and peptic ulcer, it is recognized as being highly prevalent but this varies with age and socio-economic status (Logan and Walker, 2001; Whitaker and Dubiel, 1993). Infection is mainly acquired in childhood and is usually asymptomatic (Neale and Logan, 1995). However, in about 15-20% of subjects long-term infection can lead to peptic ulcer or gastric cancer. The outcome of infection depends mainly on the severity and topography of histological gastritis. Infection in infancy leads to pan-gastritis, whilst later acquisition leads to an antral predominant gastritis. With the latter an undamaged gastric corpus secretes a high acid load, which on reaching the duodenum causes duodenal gastric metaplasia. This is then colonized by *H. pylori* leading to duodenitis and duodenal ulcer. In contrast, pangastritis, with an inflamed corpus, is associated with the loss of acid secretion which leads to atrophic gastritis and an increased risk of gastric ulcer or cancer (Blaser, 1992; Baron and Logan, 1994). *H. pylori* generally requires 3 to 7 days to grow on solid primary media. Rapid identification techniques, such as the rapid urease test and various staining procedures, have been described (Buck and Smith, 1987; Marshall *et al*., 1987; Westblom *et al*., 1988). Because different strains of *H. pylori* may show variable growth patterns on solid media, it is important that all media to be tested with primary media. We tested several different solid media for the support of primary growth and isolation of *H. pylori*. In addition, we tested rapid methods for identification of *H. pylori*, including urease test and staining techniques and compared these results with results obtained from culture and histopathology methods (Coudron and Kirby, 1989).

MATERIALS AND METHODS

101 sets of four antral biopsies were collected from 101 patients who had upper abdominal complaints

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consistent with peptic disease. Biopsy specimens were collected with an Olympus fiberoptic endoscope. Two biopsies from each patient were transported to the laboratory in saline buffer and processed in the following manner. One biopsy was thoroughly ground with a mortar and pestle in 1 mL of saline. To ensure equal distribution of tissue fragments, the homogenate was flushed up and down in a sterile, disposable plastic pipette and then evenly distributed among 4 plates of solid media. These media including Trypticase Soy agar, Brain Heart Infusion agar, Skirrow agar and Brucell agar. Inoculated media were incubated at 37°C under microaerophilic condition. After 5 to 7 days of incubation, isolates that were urease, catalase, oxidase and phosphatase positive and showed characteristic cell morphology when stained with the modified Gram stain, were identified as *H. pylori* (Logan and Walker, 2001). The second biopsy was coincidently prepared with culture for direct staining by modified Gram stain (Blaser, 1992). Slides were examined by light microscopy for up to 10 min for characteristic curved, S, or U-shaped bacterial cells. The third biopsy from each patient placing in urea broth medium for rapid identification by rapid urease method. The test was read as positive when a red or pink color developed around the biopsy specimen. Finally the last biopsy was studied by Histology method. Biopsy was transported in formalin to Dep of Pathology, then embedded in paraffin and cut into 4 mm sections and stained with two different stains including Hematoxylin and Eosin (H & E) and Giemsa stain. Sections that were stained by H & E used for detection pathology findings including Chronic Gastritis, Active Chronic Gastritis, Lymphoid follicular and Adenocarcinoma. Gimsa stained sections were scored positive or negative for *H. pylori* depending on the presence or absence of stained *H. pylori* organisms in the sections and also the density of bacteria observed in the sections was classified as low, moderate or extensive.

RESULTS

Among 101 biopsy specimens that cultured for organism, *H. pylori* were isolated in 62 cases (61/3%). Sensitivity, specificity, Positive predictive Value (PPV) and Negative Predictive Value (NPV) of culture were 91.2, 100, 100 and 84/6%, respectively (Table 1). Among the media that tested for support growth of *H. pylori*. Brucella agar supplemented with 10% whole sheep blood, supported better growth of *H. pylori* than Skirrow agar, Tryptic Soy agar and Brain Heart Infusion agar. In this study, we used Histopathology method stained by Gimsa as gold standard and the results other methods were compared with this method. Sensitivity, Specificity, positive predictive Value and Negative Predictive Value for all of methods are shown in Table 1. According to result of Histopathology stained by H & E, there was significant relationship between Active Chronic Gastritis and *H. pylori* infection, so that 41 patients with *H. pylori* infection had Active Chronic Gastritis, in the other hand none of patients without *H. pylori* infection did not have Active Chronic Gastritis ($p<0.05$) (Table 2).

Table 1: Comparison of results of Culture, rapid Urease test and direct staining with Histopathology stained by Gimsa as gold standard method

		Results of histopathology Stained by Gimsa		Performance characteristics (%)			
Test methods		Positive	Negative	Sensitivity	Specificity	PPV*	NPV†
Culture	Positive	62	0	91.2	100	100	84.6
	Negative	6	33				
	Total	68	33				
Urease test	Positive	65	0	95.6	100	100	91.7
	Negative	3	33				
	Total	68	33				
Direct staining	Positive	61	1	89.7	97	98.4	82
	Negative	7	32				
	Total	68	33				

* Positive Predictive Value, † Negative Predictive Value

Table 2: Results obtained from Histopathology stained by H & E

Type of gastritis	Number of patients with *H. pylori* infection	Number of patients without *H. pylori* infection
Active chronic Gastritis	41	0
Active Gastritis with Lymphoid follicular	16	0
Chronic Gastritis	9	11
Chronic Gastritis with Lymphoid follicular	2	2
Normal	0	20
Total	68	33

DISCUSSION

In the clinical setting, a rapid and cost-effective detection method for diagnosis of *H. pylori* infection is desirable. *H. pylori* infection can be detected by a variety of methods (Taj *et al*., 2003). In the routine clinical diagnostics, the urease test, histological examination of specimens stained by Giemsa method as well as bacterial culture and direct staining are valuable methods of detecting *H. pylori* infection. There are many studies conducted about evaluating several methods in diagnostic of *H. pylori*. Correct and reliable histological diagnosis of *H pylori* gastritis has a great influence on clinical practice as an indicator for therapy. Reliability in assessing intestinal metaplasia and atrophy in histological specimens was especially important because these changes are associated with an increased risk of gastric cancer (Tepes *et al*., 1999; Correa, 1992; Meining and Stolte, 2002). Histological technique described in this study, used as the gold standard for detection of *H. pylori* infection and other methods like rapid urease test, direct staining and culture were compared with this method. (Andrew *et al*., 1994). Tepes held that histopathology was a reliable diagnostic method for *H pylori* gastritis based on their results (Tepes *et al*., 1999). In our study the sensitivity of rapid urease test was 95.6% that is very close with results other authors (Marshall *et al*., 1987; Thijs *et al*., 1996; Taj *et al*., 2003; Jarczyk *et al*.,1996; Aguilar-Soto *et al*., 2004). Also the specificity of the urease test is the same that have been reported by other workers but there was not any false positive result by rapid urease test that is in opposite with their results (Aguilar-Soto *et al*., 2004; Thillainayagam *et al*., 1991; Zaitoun, 1993). In the other hand we observed three false negative samples with rapid urease test. The possible cause of false negative urease tests is the complete absence of *H. pylori* in antral biopsy specimens due to the patchiness of organisms in patients who have *H. pylori* infection. In our study, direct staining did not have a sensitivity similar to the histology method of Barrett (Barrett *et al*., 1988) who reported that the sensitivity of the direct Gram stain is generally poor and that the procedure is tedious, time consuming, and distinctly inferior to culture. Although *H. pylori* is a fastidious organism requiring special culture conditions in vitro, good growth is now achieved in many complex media (Albertson *et al*., 1998). In this study, Brucella agar supplemented with 10% whole sheep blood proved to be the best and most sensitive medium of those tested for the primary isolation of *H. pylori* from gastric mucosal biopsy specimens. We recommend that freshly made Brucella agar supplemented with 10% whole sheep blood medium is the best medium for primary isolation of *H. pylori*. Although commercially available Skirrow agar and Brain Heart Infusion agar were less sensitive in compare with Brucella agar for isolation of *H. pylori*, but they were better than Trypticas Soy agar.

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