Simple Stain for *Helicobacter Pylori*

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ABSTRACT

Several methods are available for the detection of *Helicobacter pylori* in the gastric antral biopsies. Most of these are technically demanding, slow and expensive with varying sensitivity. Aim of the present study was to find out the prevalence of *Helicobacter pylori* in various gastroduodenal diseases and to evaluate the advantages of Loeffler's methylene blue stain for crushed biopsy smear.

A total of 236 (male 176, female 60) randomly selected patients with endoscopically proved chronic duodenal ulcer (180), gastroduodenitis (42) and gastric ulcer (14) formed the study group. Four gastric antral biopsies were taken from each patient. Biopsies were subjected to histology, rapid urease test, Gram's and Loeffler's methylene blue (LMB) stains for crushed smears. Evidence of H. pylori infection was found in 62.71% considering histopathology as the gold standard. H. pylori was more common in males (65.90%) and in the age group of 31-40 years (77.50%). In duodenal ulcer, gastric ulcer and gastroduodenitis, 68.88%, 57.14% and 38.09% were positive for *H. pylori* respectively. A smear stained with LBM was found to be rapid, simple, reliable, and cost effective with good sensitivity and specificity for detecting H. *pylori.*

Key Words: *Helicobacter pylori*, Loeffler's methylene blue stain

INTRODUCTION

Helicobacter pylori is an important pathogen in humans causing chronic gastritis & playing a major role in the development of peptic ulcers & gastric cancer [1]. The present study was undertaken to find out the presence of *H.pylori* in various gastroduodenal diseases & to evaluate the advantages of Loeffler's methylene blue (LMB) stain for crushed biopsy smears. The study was conducted in the department of Microbiology and Gastroenterology of B and L.C. Hospitals Bangalore. The study period was from July 2000 to dec2003.

MATERIALS AND METHODS

A total of 236 randomly selected, symptomatic patients (male 176, female 60) in the age group of 16-65 years, endoscopically diagnosed as duodenal ulcer (180), gastroduodenitis (42) and gastric ulcer (14) formed the study group. Ethical clearance from the institution and consent of patients was taken prior to endoscopy.

Four endoscopic antral biopsy specimens from each case were transported in vials containing normal saline, urease medium & 10% formalin. From the specimens in normal saline, two crushed smears were made and fixed with methanol.Of this one smear was stained by Gram's using carbol fuchsin as counter stain [Table/Fig-1]. 2nd smear was stained with LMB for 30 seconds [Table/Fig-2. Specimens in urease medium were incubated at 37°C for 60 minute which turns pink if positive [2]. Histopathology sections were stained by over night Giemsa stain,which is considered as the gold standard for the detection of H.pylori [Table/Fig-3].

RESULTS

Of the 236 cases studied 176 were males & 60 females. The age varied from 16-65 years. A total of 148 (116 males, 32 females) were positive for *H.pylori* infection, considering histopathology section stained by Giemsa as the gold standard.
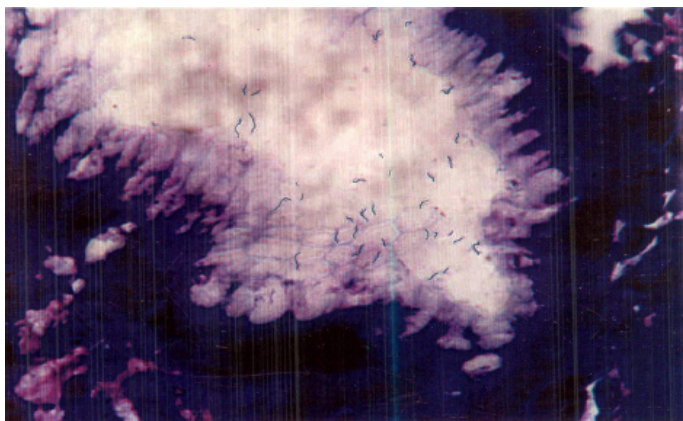
In duodenal ulcer, gastric ulcer and gastroduodenitis, 68.88%, 57.14% and 38.09% were positive for *H. pylori* respectively.
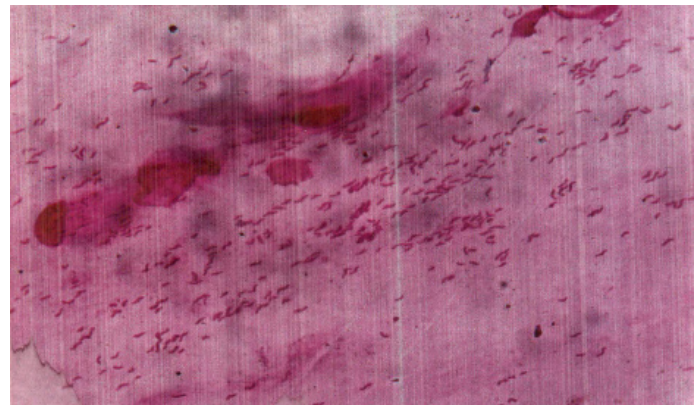
[Table/Fig-1]: Gram's stain- Helicobacter pylori (100x)
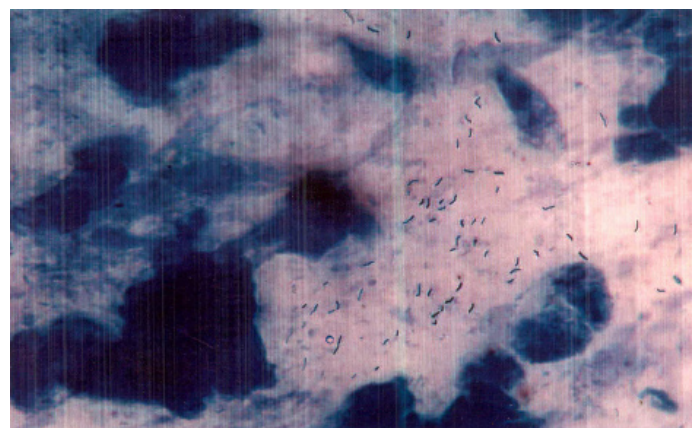
[Table/Fig-2]: LMB stain-- Helicobacter pylori (100x)

[Table/Fig-3]: Giemsa stain- Helicobacter pylori (100x)

DISCUSSION

Various techniques are available for detection of *H.pylori*, but the aim of modern research is to find a method, which is rapid, cost effective and accurately identifies the *H. pylori*. The sensitivity of the test depends on the number of bacteria present in the specimen. In experienced hands every test has good sensitivity & specificity [3].

Finding	Total
All test positive (Histology+Grams+LMB+Urease)	146
3 test positive (Histology+LMB+Urease)	2
2 test positive (LMB+Urease)	4
One test positive (urease)	2
All test negative	82

[Table/Fig-4]: Results of various test methods

Out of 236 cases 148 (62.71%) were positive and 88 (37.29%) negative for *H.pylori*. *H.pylori* was more common in males (65.90%) than females (53.66%). *H.pylori* positivity was found in all the age group (16-65 years), as exposure to *H.pylori* occurs early in India & is widespread [4]. Maximum positivity was found in age group of 31-40 years (77.50%). In duodenal ulcer, gastric ulcer and gastroduodenitis, 68.88%, 57.14% and 38.09% were positive for *H.pylori* respectively. Nair D, Ayyagari in their studies have found similar prevalence of *H.pylori* in all three conditions mentioned [5,6]. This prevalence has not increased as seen from the report presented from this department in 1999 [7].

The rapid urease test has the advantage of being simple, inexpensive, rapid & positive in 60 minutes, as evidenced in other studies also [2,8]. False positives could occur in urease test due to its high sensitivity(100%).

Crushed smears stained with Gram's showed sensitivity of 98%, specificity of 100% can provide rapid diagnosis. This is in correlation with the study of VanHorn [9]. No false positive will be found in an experienced hand. False negative may be due to, organisms hidden in proteinaceous material.

H.pylori take up deep blue and mucus faint blue colour in LMB stain and stood very well against the background. Staining time was only one minute. Smears stained with LMB showed high sensitivity and specificity, is similar to the study of others [10,11]. LMB staining is technically simple, rapid, reliable, inexpensive, with high positive and negative predictive value ,hence found to be excellent stain for the detection of *H.pylori* as it can pick up a very light load of infection, compared to technically demanding, expensive, slow histopathology and/or culture method [10,11].

CONCLUSION

Authors are of the opinion that smear stained with LMB has high specificity (95.45%)and PPV(97.36), will be sufficient for the detection of *H.pylori* in an ordinary set up.

Age Years	Duodenal ulcer 180		Gastroduodenitis 42		Gastric ulcer 14		
	Male 135	Female 45	Male 30	Female 12	Male 11	Female 3	
	+ -	+ -	+ -	+ -	+ -	+ -	Total+ %
16-20 Total 26	7 5	4 6	1 0	1 2	0 0	0 0	13 50
21-30 Total 34	15 10	1 1	2 2	0 0	0 1	1 1	19 55.88
31-40 Total 40	20 6	8 2	2 1	0 0	0 0	1 0	31 77.50
41-50 Total 59	31 10	5 2	2 3	2 2	1 1	0 0	41 69.49
51-65 Total 77	25 6	8 8	5 12	1 4	5 3	0 0	44 57.14
Total % +ve	98 37 72.59	26 19 57.77	12 18 40.00	4 8 33.33	6 5 54.54	2 1 66.66	
Positives %	124 68.88%		16 38.09%		8 57.14%		148 62.71

[Table/Fig-5]: Helicobacter pylori positivity in relation to age, sex and disease.

Test	Total +ve	True +ve	False +ve	False –ve	True -ve	Sensitivity	Specificity	PPV	NPV
Grams	146	146	0	2	88	98.64	100	100	97.77
LMB	152	148	4	0	84	100	95.45	97.36	100
Urease	154	148	6	0	82	100	93.18	96.10	100

[Table/Fig 6]: Analysis of the various diagnostic tests, considering histopathology as gold standard.

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