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Imprint cytology – a cheap, rapid and effective method for diagnosing *Helicobacter pylori*

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Summary: To compare the efficacy of imprint cytology, histology and CLO-test (for biopsy urease) in detecting *Helicobacter pylori* infection, antral biopsies were taken from 239 patients undergoing upper gastrointestinal endoscopy. Both imprint cytology and histology showed the presence of *H. pylori* in 215 (90%) patients. The sensitivity and specificity of imprint cytology *vis-à-vis* histology was noted to be 100%. The CLO-test was performed in 165 patients and was positive in 130 (79%) patients. The sensitivity and specificity of the CLO-test were 89% and 95%, respectively. The median time required for the CLO-test to become positive and for imprint was 60 minutes for each. The sensitivity of the CLO-test was reduced further in patients receiving colloidal bismuth subcitrate. Of the 27 patients receiving the drug the sensitivity of the CLO-test was only 9% after 4 weeks of therapy. However, the specificity was 100%. The sensitivity and specificity of imprint cytology were unaffected by the antimicrobial therapy and after 4 weeks of treatment were still 100%.

It is concluded that the CLO-test has a lower sensitivity and specificity for diagnosing *H. pylori* infection compared to imprint cytology, which had a sensitivity and specificity equal to that of histology. Imprint cytology may be prepared as an adjunct to histology in patients in whom antral biopsies are taken as it offers a relatively quick diagnosis of *H. pylori* infection, is considerably cheaper than the CLO-test and does not require additional biopsy material.

Introduction

There are several methods to detect the presence of *Helicobacter pylori*. The most frequently used are the biopsy urease test,[1,2] of which a commercial test kit (CLO-test;[2-5] Delta West, Bently) is available; culture,[6] histology,[7] ^{14}C-urea breath test,[8-9] ^{13}C-urea breath test[10,11] and serology.[12] Most of these tests have high sensitivity and specificity. Although culture of the bacterium has been used as a gold standard in many studies,[11,13] it is difficult because the organism is microaerophilic and fastidious in its growth requirements, and its yield has not been good at several centres.[14,15] Similarly, the ^{14}C-breath test requires radioactive material and the ^{13}C-breath test needs costly equipment which may not be available at many centres, especially in developing countries. Recently, a few investigators have noted that direct smear[14] and brush cytology[16] give good results. One study has noted touch cytology of gastric biopsies to have a good sensitivity.[15] However, to the best of our knowledge, there has been no comparison of imprint cytology, CLO-test and histology in diagnosing *Helicobacter pylori* infection and the effect of treatment with colloidal bismuth subcitrate on their yield, and the present study was designed to answer this issue.

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Patients and methods

Two hundred and thirty-nine patients undergoing oesophago-gastroduodenoscopy were studied. Two to four biopsies were taken from the antrum within 4 cm of the pylorus. Imprint was prepared by keeping the antral biopsies on a slide and lightly pressing them with the help of a hypodermic needle. Thereafter the biopsies were fixed in 10% formal saline for histopathological examination. Imprints were fixed in absolute alcohol and thereafter were stained by the modified Giemsa method.[17]

Three micrometre thick paraffin wax embedded sections were cut and stained with haemotoxylin and eosin and the modified Giemsa stain.[17] CLO-test (Delta West Ltd, Bently) was performed on 165 patients. Two antral biopsy specimens were embedded at opposite ends of the gel. Change in the colour of the gel was observed at 20, 60, 120, 180 and 240 minutes and thereafter at 24 hours after taking the biopsy.

The pathologist evaluating the imprint cytology and histology was given coded slides and was not aware of the clinical diagnosis of the patients or the results of the CLO-test.

To see the effects of antimicrobial treatment on these tests, 27 patients received 240 mg of colloidal

bismuth subcitrate (Denol, Elders Pharmaceuticals, Bombay) twice daily, half an hour before meals. These patients were interviewed at 0, 2, 4 and 8 weeks and antral biopsies were taken at each visit for the CLO-test, imprint cytology and histology.

H. pylori was graded in imprint smears as well as histology as – Grade 0: no bacteria detected; Grade 1: sporadic bacteria seen; Grade 2: many bacteria seen in most microscopic fields at high power magnification; and Grade 3: bacteria seen in clusters in all fields examined.

Statistics

Kappa statistics, the χ^2 (chi-square) test and the Kolomogorov–Smirnov two sample test were used wherever appropriate.

Results

Of the 239 patients studied, 215 (90%) showed presence of *H. pylori* by imprint method (Figure 1). All of these patients were positive for *H. pylori* on histological examination of their antral biopsy specimens (Figure 2). All 24 patients who tested negative by the imprint method were found to be negative by histopathology ($K = 1$, $P < 0.001$). The sensitivity, specificity, positive and negative predictive value and the overall diagnostic accuracy of imprint cytology *vis-à-vis* histology were each 100%.

A comparison of the CLO-test, imprint cytology and histology is shown in Table I. In one patient the CLO-test was positive while imprint and histopathology did not show the presence of *H. pylori*. The sensitivity, specificity, positive and negative predictive value and the overall diagnostic accuracy of the CLO-test were 89%, 95%, 99%, 54% and 90%, respectively. There was a good association between the CLO-test and imprint cytology and histology ($K = 0.64$, $P < 0.001$). There were 16 patients with a false negative CLO-test. All patients with a false negative CLO-test showed grade 1 or 2 bacterial colonization at histology. None of these patients showed grade 3 colonization (Table II). A good association was noted between the grade of bacteria seen at histology and the CLO-test ($\chi^2 = 65.4$, $P < 0.001$).

The median time required for the CLO-test to become positive was 60 minutes. It became positive

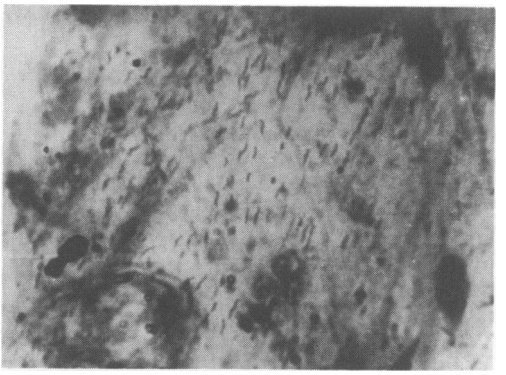

Figure 1 Imprint cytology showing presence of *Helicobacter pylori* (modified Giemsa, × 660).

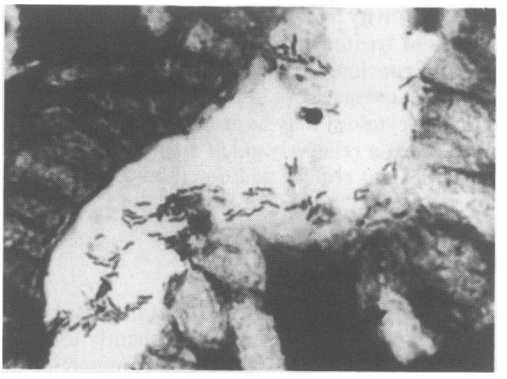

Figure 2 *H. pylori* seen at histopathology (modified Giemsa, × 660).

Table I Comparison of the CLO-test, histology and imprint cytology ($n = 165$)

	Histology			*Imprint cytology*		
	+ ve	*– ve*	*Total*	*+ ve*	*– ve*	*Total*
CLO-test						
+ ve	129	1	130	129	1	130
– ve	16	19	35	16	19	35
Total	145	20	165	145	20	165

Table II Comparison of the CLO-test with grade of *H. pylori* seen at histology (figures in parentheses show percentage)

Histology (grade)	*CLO test* + ve	− ve
0	1 (5)	19 (95)
1	10 (48)	11 (52)
2	58 (91)	5 (9)
3	61 (100)	0 (0)

Table III Comparison of the CLO-test, imprint cytology and histology in patients receiving colloidal bismuth subcitrate 240 mg twice daily for 4 weeks ($n = 27$)

	CLO-test +	*Imprint cytology* +	*Histology* +
0 weeks	27	27	27
2 weeks	6	27	27
4 weeks	2	22	22
8 weeks*	25	25	25

*4 weeks after completion of therapy.

within 20 minutes in 57 (44%) and by one hour in 94 (72%) patients. No association was noted between the time it took for the CLO-test to become positive and the number (grade) of bacteria seen at histopathology. The CLO-test became positive within 60 minutes in 49 (72%) patients showing grade 1 and 2 colonization by *H. pylori* compared to 45 (74%) patients with grade 3 colonization. The difference between the two groups was statistically not significant ($\chi^2 = 0.05$, $P > 0.05$). The median time required for imprint cytology was 60 minutes and that for histology was 48 hours.

Effect of treatment with colloidal bismuth subcitrate

A comparison of the CLO-test, imprint cytology and histopathology for detecting *H. pylori* in patients receiving colloidal bismuth subcitrate (CBS) ($n = 27$) is shown in Table III. The sensitivity of the CLO-test was reduced further in patients receiving CBS. After 4 weeks of therapy the sensitivity of the CLO-test was only 9%. However, the CLO-test was negative in all five patients with negative imprint cytology and histology (Table IV) and the specificity of the CLO-test was noted to be 100%. The sensitivity and specificity of imprint cytology when compared to histology were each 100%. All 20 patients with a false negative CLO-test showed only grade 1 or 2 bacterial colonization at histology. The two patients with positive CLO-test showed grade 1 and 2 colonization, respectively. After 4 weeks of therapy with CBS, all the three tests were negative in only 5 patients. Three of these five patients were positive again for *H. pylori* one month after completing treatment. Eradication of the bacteria was thus achieved in only two of 27 (7.4%) patients.

Discussion

Histology is one of the reliable methods for detection of *H. pylori* and in expert hands it has been noted to be as good as microbiology.[18,19] Several studies have used histology alone for the diagnosis of *H. pylori* infection.[20–22]

In the present study imprint cytology was noted to have a sensitivity and specificity equal to that of histology, which was taken as the gold standard. Imprint cytology was also noted to be a relatively easy and quick method for detection of *H. pylori*. We therefore suggest that imprint cytology may be performed in patients undergoing endoscopy and biopsy for detection of *H. pylori* as an adjunct to histology as it provides a rapid diagnosis and does not require additional biopsies. Moreover imprint cytology is much cheaper than the CLO-test.

The sensitivity of the CLO-test was found to be lower in this study compared to that reported by Morris *et al.*[2] and Marshall *et al.*[3] However, in a

Table IV Comparison of the CLO-test, histology and imprint cytology in patients receiving colloidal bismuth subcitrate after 4 weeks of therapy

	Histology			*Imprint cytology*		
	+ ve	*− ve*	*Total*	*+ ve*	*− ve*	*Total*
CLO-test						
+ ve	2	0	2	2	0	2
− ve	20	5	25	20	5	25
Total	22	5	25	22	5	25

recent study, Dill *et al.*[11] reported the sensitivity of the CLO-test similar to that noted in this study. A much lower sensitivity of only 59% was reported by Vaira *et al.*[5]

The sensitivity of the CLO-test was found to be very low in patients who received treatment with CBS. Other workers have also noted similar findings.[11,22-24] This is probably because of a decrease in urease production as a consequence of decrease in the number of the bacteria, which is evident from the cytological and histological results of this study. Yet another possibility is that during and immediately after treatment, the bacteria loses its potential to produce urease or that urease production is markedly reduced and therefore the negative CLO-test despite positive imprint and histology. Even the urea breath tests have been noted to have decreased sensitivity in detecting *H. pylori* immediately after treatment with tri-potassium dicitrato bismuthate.[11,22] Whether the decreased urease output, during and immediately after treatment, is caused entirely by the decrease in the number of the bacteria, by the decrease in the urease production, or by a combination of the two is not clear.

Although the CLO-test is simple to interpret and can be performed by anyone and the non-invasive tests such as serology and the breath-urea tests are easy to perform and repeat, histology provides much more information, especially the relationship between *H. pylori* and gastritis. It allows for grading of the severity of colonization by *H. pylori* in a specimen or consecutive specimens and monitoring the effect of drug therapy on *H. pylori* as well as gastritis.

In the present study we did not find a significant association between the time required for the CLO-test to be positive and the number of bacteria seen on histological examination of the antral biopsies. This is in agreement with Marshall *et al.*[4] and probably reflects the patchy distribution of the bacteria. However, other workers[3,25] have noted a good association between urease activity and the number of *H. pylori*.

Different clearance rates have been reported by various investigators using different dosage schedules of bismuth compounds. *H. pylori* is cleared in about 18–100% of patients receiving bismuth salts alone.[11,26-29] However, eradication of the bacteria, which is defined as a negative test one month or more after stopping the treatment, has been observed to be extremely low in patients receiving bismuth salts as monotherapy.[11,28,30-31] The clearance rate of 18.5% and eradication rate of 7% noted in this study are very similar to that noted in a recent study.[11]

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