

Diagnostic performance of gastric imprint smear for determination of *Helicobacter pylori* infection

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BACKGROUND: Despite the availability of several methods (invasive and noninvasive) for the diagnosis of *Helicobacter pylori* infection, no test is considered to be the 'gold standard'. Endoscopy-based tests are regarded as the reference method in most studies.

OBJECTIVE: To evaluate the diagnostic performance of imprint cytology smears of antral biopsies compared with Gram-stained smears, the rapid urease test and culture methods, separately and in combination.

METHODS: Antral biopsies were obtained from consecutive patients undergoing upper gastrointestinal endoscopy at a single centre. The biopsies were examined for the presence of *H pylori* by Gram-stained smear, the rapid urease test, culture methods and imprint cytology smear.

RESULTS: A total of 273 biopsies were studied. All tests were positive in 36% of the patients. Of 252 biopsies tested, 73% were positive using the imprint cytology technique. Using Gram-stained smear, the rapid urease test and culture methods individually, the sensitivity and specificity of imprint cytology smears for the detection of *H pylori* were found to be 92.7% and 50%; 92.7% and 49%; and 92.4% and 38.5%, respectively. Combining the three microbiological methods resulted in a sensitivity of 92.1%, a specificity of 51.0% and an efficiency of 71.7% for imprint cytology smears.

CONCLUSIONS: Endoscopic examination provides useful clinical information. Imprint gastric cytology can be used as a rapid test to establish the diagnosis of *H pylori* infection at the time endoscopy is performed, enabling the endoscopist to start treatment with immediate effect.

Key Words: Endoscopy; *Helicobacter pylori*; Imprint cytology

Helicobacter pylori has now become a generally accepted cause of dyspepsia, regardless of ulcer disease in the upper gastrointestinal tract. The organism has been associated with many intestinal diseases including chronic gastritis, peptic ulcer disease, gastric carcinoma and low-grade gastric mucosa-associated lymphoid tissue lymphomas (1-3). Antimicrobial chemotherapy directed against *H pylori* results in its eradication and the relief of symptoms associated with the multiple gastrointestinal diseases caused by this organism (4). Therefore, it is essential to accurately diagnose gastric colonization of *H pylori* in symptomatic individuals.

Several diagnostic procedures, both invasive and non-invasive, are currently available for the diagnosis of *H pylori* infection. These include culture methods, the rapid urease test, histology, polymerase chain reaction assays, serology, urea breath tests using either carbon-13 or carbon-14, and tests for *H pylori* antigens in stool specimens (4-9). However, because

Le rendement diagnostique du frottis gastrique par empreinte directe pour déterminer la présence d'une infection à *Helicobacter pylori*

HISTORIQUE : Malgré l'existence de plusieurs méthodes (effractives ou non) pour diagnostiquer l'infection à *Helicobacter pylori*, aucun test n'est considéré comme la norme de référence. Dans la plupart des études, les tests endoscopiques servent de référence.

OBJECTIF : Évaluer le rendement diagnostique des cytologies par empreinte directe des biopsies antrales par rapport à la coloration de Gram, au test rapide à l'uréase et aux méthodes de culture, seuls et combinés.

MÉTHODOLOGIE : Les chercheurs ont obtenu les biopsies antrales dans un seul centre, auprès de patients consécutifs qui subissaient une endoscopie du transit œsogastroduodénal. Ils ont examiné les biopsies pour déceler la présence d'infection à *H pylori* au moyen de la coloration de Gram, du test rapide à l'uréase, des méthodes de culture et de la cytologie par empreinte directe.

RÉSULTATS : Au total, les chercheurs ont étudié 273 biopsies. Tous les tests étaient positifs chez 36 % des patients. Des 252 biopsies testées, 73 % étaient positives selon la technique de cytologie par empreinte directe. Selon la coloration de Gram, le test rapide à l'uréase et les méthodes de culture pris individuellement, la sensibilité et la spécificité de la cytologie par empreinte directe pour déceler le *H pylori* s'élevaient à 92,7 % et à 50 %; à 92,7 % et 49 %; et à 92,4 % et 38,5 %, respectivement. L'association des trois méthodes microbiologiques favorisait une sensibilité de 92,1 %, une spécificité de 51,0 % et une efficacité de 71,7 % de la cytologie par empreinte directe.

CONCLUSIONS : L'examen endoscopique fournit de l'information clinique utile. La cytologie gastrique par empreinte directe peut être utilisée comme test rapide pour poser un diagnostic d'infection à *H pylori* au moment de l'endoscopie, ce qui permet à l'endoscopiste d'amorcer un traitement aux effets immédiats.

none of these tests have been universally accepted as the 'gold standard', the use of more than one method and equivocal results can lead to diagnostic dilemma. There is, therefore, a definite need for a reliable and veracious test to diagnose *H pylori* infection. Comparative evaluations of currently available and newer diagnostic methods have been studied for their relative merits.

Previous reports (10-12) have suggested cytology of gastric brushings and imprint smears as reliable methods for the detection of *H pylori*. The more widely used staining procedures for imprint smears, such as Papanicolaou and May-Grünwald Giemsa, require approximately 30 min to complete (13).

In the present study, we evaluated imprint cytology of gastric biopsy samples using the Diff-Quik (DIFF-3, Gainland Chemical Co, United Kingdom) staining method for the diagnosis of *H pylori* infection because it is a simple, easy to perform test that provides results within minutes inside the endoscopy

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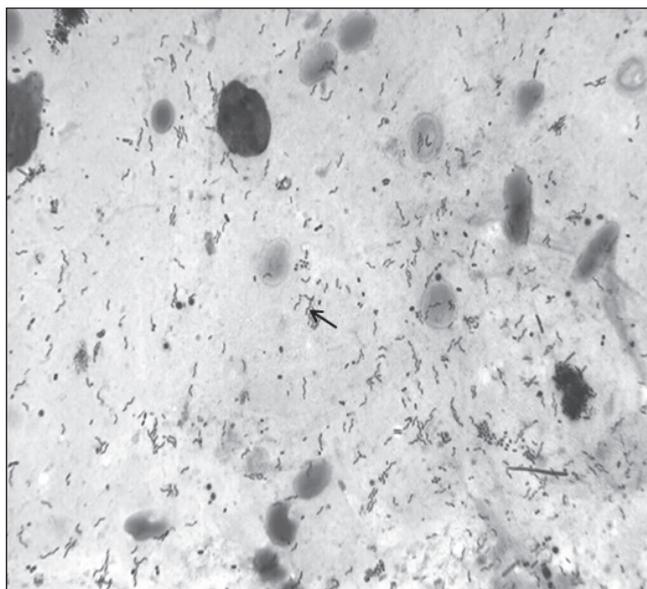


Figure 1) Diff-Quik (Gainland Chemical Co, United Kingdom) stain showing *Helicobacter*-like organisms (arrow) within mucus

suite. We compared the results of this technique with currently used methods (direct Gram staining, the rapid urease test and culture of biopsy specimens) in our laboratory.

METHODS

Upper gastrointestinal endoscopy was performed on 310 consecutive unselected patients for related symptoms. Two biopsy specimens were obtained from each patient – one from the antrum, the other from the body of the stomach. One of the biopsy samples was placed in normal saline, while the other was used to prepare an imprint smear by gently rolling it on a clean glass slide.

The smears were air dried and subsequently stained using the Diff-Quik method, requiring approximately 2 min to complete the entire procedure. After air drying the slides following staining, the smears were mounted and examined for *H pylori* under high-power magnification. The bacterium was identified by its spiral or S-shaped morphology and purple/violet colour adjacent to epithelial cells and within the well-preserved gastric mucus (Figure 1). The imprint cytology was evaluated and graded by the cytopathologist as follows: grade 0, no *Helicobacter*-like organisms (HLO) seen; grade 1, sporadic HLO seen; grade 2, many HLO detected in most microscopic fields; and grade 3, HLO seen in clusters in all fields examined.

The biopsy specimen placed in normal saline was processed in the microbiology laboratory. The tissue was minced with a sterile scalpel blade and a portion of the material was inoculated into a vial for the rapid urease test (Jatrox-HP-Test, Röhm Pharma GmbH, Germany), a portion was used for preparing a smear that was stained by Gram's method and the remaining material was inoculated onto chocolate agar, which was subsequently incubated at 37°C for one week under microaerophilic conditions. The agar plates were checked for growth on days 3, 5 and 7. The isolates were identified to be *H pylori* when the Gram-stained smear revealed highly curved Gram-negative bacilli, which demonstrated a positive reaction with the catalase, oxidase and urease reagents.

TABLE 1
Comparison of results obtained by bacteriological methods and imprint cytology for evidence of *Helicobacter pylori* infection in 273 gastric antral biopsies

Cytological test method to detect the presence of <i>H pylori</i>	n (%)	Result of bacteriological methods, n (%)		
		Positive	Negative	Not performed
Direct smear				
Positive	149 (54.6)	127 (46.5)	10 (3.7)	12 (4.4)
Negative	121 (44.3)	56 (20.5)	56 (20.5)	9 (3.3)
Not performed	3 (1.1)	1 (0.4)	2 (0.7)	–
Rapid urease test				
Positive	149 (54.6)	127 (46.5)	10 (3.7)	12 (4.4)
Negative	115 (42.1)	54 (19.8)	52 (19.0)	9 (3.3)
Not performed	9 (3.3)	3 (1.1)	6 (2.2)	–
Culture				
Positive	114 (41.8)	97 (35.5)	8 (2.8)	9 (3.3)
Negative	147 (53.8)	83 (30.4)	52 (19.0)	12 (4.4)
Not performed	12 (4.4)	4 (1.4)	8 (2.8)	–

The sensitivity, specificity, and positive and negative predictive values were calculated in accordance with standard methods.

RESULTS

Of the 310 consecutive patients from whom biopsy samples were collected, results of 273 samples were included for evaluation. The results of 21 biopsies were excluded from cytological analysis while three, nine and 12 biopsies were not analyzed by direct smear, rapid urease test or culture methods, respectively, because of diagnostic test failures or missing values. Smears for cytological examination were available for 252 patients and HLO were identified in 184 (67.4%) of the cases examined. Direct smear was prepared for 270 samples; Gram-stain examination revealed the presence of HLO in 55.2% of cases. Of 261 biopsies that were cultured, 43.7% yielded growth of *H pylori*. The rapid urease test was performed on 264 biopsy samples, with 56.4% testing positive. These results were compared with the results of imprint cytology for the evidence of *H pylori* infection (Table 1). Of 109 samples that tested positive by all three microbiological methods, 92% were positive by cytomorphology. Thirty-five samples that tested negative by culture methods were found to be positive by imprint cytology, direct smear and the rapid urease test. This may, therefore, reflect a lack in culture method sensitivity rather than poor cytomorphology specificity (Table 2). The sensitivity, specificity, and positive and negative predictive values for imprint cytology in the diagnosis of *H pylori* infection are summarized in Table 3.

DISCUSSION

Ever since the discovery of *H pylori* approximately three decades previously, a plethora of literature now exists describing its epidemiology and its role in the pathogenesis of gastritis, gastric and duodenal ulcer, and gastric cancer as well as antimicrobial therapy for its eradication (14-16). It has been estimated that up to 60% of the adult population is chronically infected with *H pylori*, probably making it the most common chronic bacterial infection (14,17,18). The rate of infection depends on age, socioeconomic class and country of origin, affecting as many as 85% of the population in the Middle East (19,20).

TABLE 2
Comparison of combined bacteriological methods and cytological results for demonstration of *Helicobacter pylori* infection in gastric antral biopsies

Bacteriological method/result			Imprint cytology result, n			
Direct smear	Rapid urease test	Culture	n	Positive	Negative	NP
Positive	Positive	Positive	109	92	8	9
Negative	Negative	Positive	2	2	0	0
Positive	Negative	Positive	1	1	0	0
Positive	Positive	Negative	35	31	1	3
Negative	Positive	Negative	4	4	0	0
Positive	Negative	Negative	3	2	1	0
Negative	Negative	Negative	107	48	50	9
NP	Positive	NP	1	0	1	0
NP	Negative	NP	2	1	1	0
Negative	NP	NP	7	1	6	0
Positive	NP	NP	1	1	0	0
Negative	NP	NP	1	1	0	0
Total	–	–	273	184	68	21

NP Not performed

Several diagnostic procedures currently used for the diagnosis of *H pylori* infection can be categorized as direct or indirect (6-9,19,21,22). The direct demonstration of the presence of the organism by histological staining or by culture of gastric biopsy specimens is based on endoscopy, which, although invasive, is a definitive procedure. On the other hand, indirect techniques, which are invariably noninvasive, include carbon-13 and carbon-14 breath tests, and the measurement of serum antibody levels for *H pylori*. These tests rely on detecting a phenotypic characteristic of the organism (eg, the ability to hydrolyze urea) or the appearance of specific antibodies directed against certain bacterial antigens. Although these tests have the advantage of being inexpensive and simple to perform, there are some limitations (3,23). The distinction between positive and negative values may not be definitive in the breath tests, while serology is invariably inconclusive because it is difficult to distinguish between a current and previous infection. In contrast, endoscopy, which is an invasive procedure, allows for the direct observation of the lesions present in the gastrointestinal tract and the opportunity to take biopsies from different anatomical sites in the stomach. The biopsy material can be used for preparing smears for bacteriological or cytological studies, testing for urease enzyme produced by *H pylori*, culture methods, polymerase chain reaction testing and histopathological assessment (5,24). However, the most popular and practical diagnostic method for *H pylori* in most centres remains histopathological examination of antral biopsy specimens (17).

The purpose of the present study was to evaluate the utility and reliability of imprint cytology for the determination of *H pylori* infection in gastric biopsies from an unselected series of patients who underwent endoscopy.

Excluding the unlikely event of contamination, culture of *H pylori* from stomach biopsy samples is definitive proof of infection. Unfortunately, failure to grow *H pylori* does not exclude the possibility of infection because organisms tend to lose their viability during collection, transport or processing,

TABLE 3
Sensitivity, specificity, and positive and negative predictive values of imprint cytology when compared with direct smear, rapid urease test and culture methods for the diagnosis of *Helicobacter pylori* infection

Bacteriological method	Isolates with results, n/isolates tested by imprint cytology, n (%)			
	Sensitivity	Specificity	Predictive value	
			Positive	Negative
Direct smear	127/137 (92.7)	56/112 (50.0)	127/183 (69.4)	56/66 (84.8)
Rapid urease test	127/137 (92.7)	52/106 (49.0)	127/181 (70.2)	52/62 (83.9)
Culture	95/103 (92.2)	52/137 (38.0)	95/180 (52.8)	52/60 (86.7)
Direct smear, rapid urease test and culture	92/100 (92.0)	50/98 (51.0)	92/140 (65.7)	50/58 (86.2)

or as the result of overgrowth of other organisms in the specimen (4,15). Our results demonstrated a sensitivity of 92% for the culture method, which is consistent with reports from other centres (15). Two of the samples that were positive only by culture had positive cytomorphology for *H pylori* by imprint smear. The rapid urease test demonstrated a sensitivity of 93%, whereas low sensitivity has been reported by other investigators using the *Campylobacter*-like organism test. The results vary because the tests are dependent on the pH of the media (4,5). Of four specimens that tested positive only by the rapid urease test, all were imprint smear test positive for *H pylori*.

The Diff-Quik staining procedure is simple, rapid and highly comparable with histological methods, with a sensitivity and specificity of 82% and 100%, respectively (25). According to a previous study (13), imprint cytology had a sensitivity of 87.7% and a specificity of 60.7% when compared with histological positivity for *H pylori*.

Previous reports (15,17,18,24) have suggested imprint cytology as a useful and cost-effective method for the diagnosis of *H pylori* infection. In our experience, imprint smears provided good cellularity, with one smear per patient found to be adequate. It requires minimal manpower, with a turnaround time of 10 min, enabling the surgeon to commence therapy on the same day as the endoscopic procedure. Furthermore, if HLO are detected in an imprint cytology smear, pre-emptive therapy may be initiated based on the predetermined antimicrobial susceptibility pattern in a particular population (21). For situations in which a strong clinical suspicion of *H pylori* infection exists, but the organism is not visible in the smear, the biopsy sample may be processed by culture methods and confirmed with antimicrobial susceptibility testing.

CONCLUSIONS

Several rapid detection techniques have been developed as alternatives to culture methods and other labour-intensive and cost-ineffective approaches. As an office-based procedure, we believe that imprint cytology can be a useful aid in helping to accurately diagnose *H pylori* infection rapidly and enable immediate initiation of specific antimicrobial therapy.

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