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Imprint Cytology of Gastric Mucosa Biopsy – Fast, Simple and Reliable Method for Detection of Helicobacter Pylori Infection

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A B S T R A C T

The aim of the study was to determine the value of gastric mucosa imprint cytology in the detection of Helicobacter *pylori infection. A total of 182 biopsy specimens, from 182 randomly selected patients undergoing gastroscopy with gastric mucosa biopsy, were analyzed. Specimens were first submitted to slide imprinting and then formalin fixed for further routine histopathology. One-hundred and fifty-five specimens proved adequate for definitive comparison of the methods used for detection of* Helicobacter pylori *infection.* Helicobacter pylori *was detected by histopathology in 51 specimens and by cytology in 54 specimens. Agreement between the findings obtained by the two methods was recorded in 130 of 155 (83.1%) specimens. Positive cytology and negative histology findings were obtained in 14, and vice versa in 11 specimens. Gastric mucosa imprint cytology provides a useful method for the detection of* Helicobacter pylori *infection. The method is advantageous for being fast, simple and inexpensive. When the sample is obtained exclusively for confirmation of the presence of* Helicobacter pylori *infection, cytology reduces the time and cost of the procedure, at the same time providing data on morphological changes of gastric mucosa. Every finding suspect of malignant transformation of the mucosa can also be verified by histopathology because imprint manipulation causes no damage to the sample.*

Key words*: imprint cytology,* Helicobacter pylori*, gastric mucosa biopsy*

Introduction

Helicobacter pylori is spiral, wound or straight unbranched gram-negative rod which have been successfully infecting humans for thousands of years since *H. pylori* antigens have been found in fecal specimens from 3000-year–old Andean mummies[1]. Although *H. pylori* was first isolated in 1982, and in 1983 Warren and Marshall pointed to the association between spiral bacteria and gastric mucosa infiltration with polymorphonuclear leukocytes[2–3], first hypothesis that the bacteria caused ulcer disease was made in 1875 by Botcher and Letulle[4]. The diagnostic and therapeutic approach to diseases of the upper gastrointestinal tract has considerably modified since these initial concepts on *H. pylori*. The importance of *H. pylori* is best illustrated by the Nobel Prize for physiology and medicine 2005 being awarded to Warren and Marshall for their discovery of the bacterium *H. pylori* and its role in gastritis and gastric ulcer[5].

The advent of endoscopic methods, i.e. endoscopic biopsy, has brought a breakthrough in the concepts on the role of *H. pylori* in the upper gastrointestinal tract diseases, as it has allowed for sampling of adequate specimens that could be analyzed on time. Previously, analyses could only be done on the material obtained on autopsy or by surgical resection, where autolysis of the samples precluded any thorough analysis of the mucosal surface, now known as the site of *H. pylori* invasion[2,5–7].

H. pylori infection occurs all over the world, however, its prevalence varies among countries as well as among different population groups in a particular country. The prevalence of *H. pylori* infection is closely related to the socioeconomic status. Once acquired, the infection may persist for years, while spontaneous eradication is extremely rare[8]. In Croatia, the mean prevalence of *H. pylori* infection has been estimated to range between

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60.4 and 68.0% in the 20–70 age groups. The infection is most commonly transmitted by the gastro-oral route, and less frequently by the oro-oral or feco-oral routes[2].

The clinical course of *H. pylori* infection may greatly vary. In addition to being responsible for the majority of duodenal and gastric ulcers, there is strong evidence for *H. pylori* to increase the risk of gastric carcinoma and gastric MALT lymphoma[9–17]. In 1994 International Agency for Research on Cancer classified *H. pylori* as a group I carcinogen[18]. Humans can also be infected with *Helicobacter (H.) heilmannii*, another member of the family *Helicobacter* adapted to gastric mucosa. It is a spiral bacterium that is otherwise found in pigs, cats, dogs and primates. In humans, the prevalence of *H. heilmannii* infection is about 0.5%. According to literature data, *H. heilmannii* infection causes mild forms of gastritis; however, its association with gastric MALToma has also been reported[11,19,20].

The diagnosis of *H. pylori* infection can be made by a number of methods, the choice of method depending primarily on the clinical presentation in individual patient[7,16]. Noninvasive methods include urea breath test, serologic tests and stool antigens, the former being of greatest value in daily clinical routine[19]. Invasive methods are esophagogastroduodenoscopy with gastric mucosa biopsy for histopathology, rapid urease test, culture of *H. pylori* and slide imprinting[7,21]. Gastroscopy with biopsy sampling is indicated in patients with dramatic symptoms (anemia, weight loss, gastrointestinal bleeding, age over 50)[16]. In this case, the test of choice to demonstrate *H. pylori* infection is rapid urease test on antral mucosa biopsy. The test is characterized by 72– 100% sensitivity and 92–100% specificity[6]. Test results can be corrected by additional sampling. If a false negative finding is suspected (recent antibiotic therapy, bleeding, etc.), more biopsies should be obtained for additional histopathology and imprint cytology[6,7]. When the rapid urease test is unavailable, another as fast and inexpensive method is required to demonstrate *H. pylori* infection. The value of imprint cytology in demonstrating *H. pylori* infection has been reported in a number of studies to date[22–25]. We embarked upon the present study to show the value of this method as compared with the classic histopathologic analysis.

Subjects and Methods

A total of 182 gastric mucosa biopsy specimens obtained from randomly selected patients undergoing gastroscopy with gastric mucosa sampling between November 1, 2003 and April 30, 2004 were analyzed. The aim of the study was to compare the value of gastric mucosa cytology and histology in the detection of *H. pylori* infection.

Each specimen was first submitted to slide imprinting and then formalin fixed for further routine histopathology. The imprints were air dried, stained according to May-Grünwald-Giemsa (MGG) (Figure 1), and analyzed under a light microscope. (Figure 2 and 3) Then the specimens were fixed in 10% buffered formalin, paraffin embedded, cut into 4- to 5-μm sections, stained with hema-

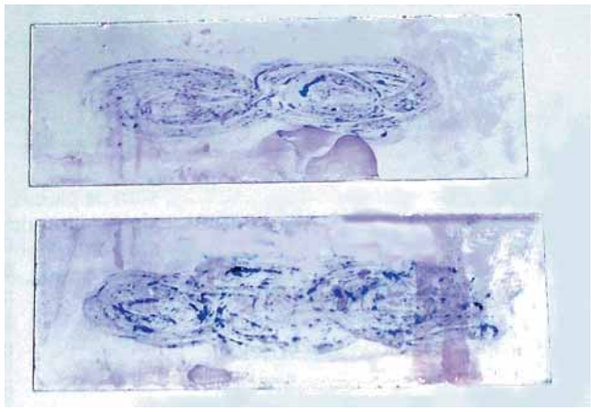

Fig. 1. Imprint smear stained by Mäy-Grünwald-Giemsa (macroscopic view).

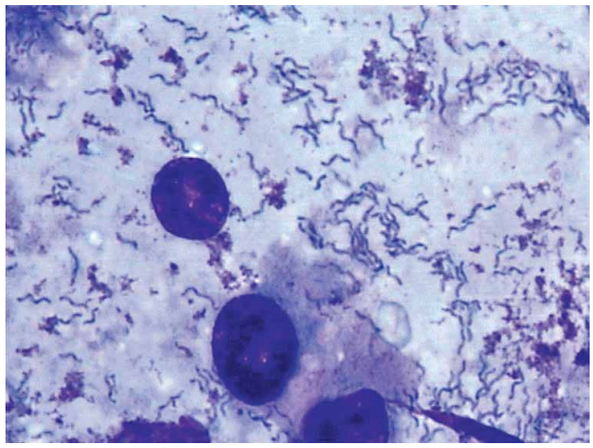

Fig. 2. Helicobacter heilmanii *in gastric mucus, with degenerative changes of epithelial cells of gastric mucosa, MGG × 1000.*

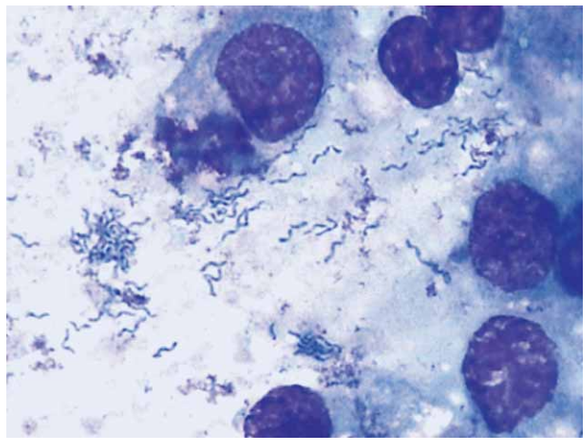

Fig. 3. Helicobacter pylori *in gastric mucus and degenerative changes of epithelial cells of gastric mucosa, MGG × 1000.*

toxylin-eosin (HE) and analyzed under a light microscope.

On gastric biopsy, 3–6 samples *per* patient were obtained to analyze the diagnosis indicated by histopathology or cytology in terms of the presence or absence of *H. pylori* infection. All specimens collected from a particular patient were analyzed as a sample pool to reach definitive diagnosis.

Statistical analysis was performed by use of the Analyse-it software (General + Clinical Laboratory Statistics Version 1.73, Analyse-it Software Ltd.). The specificity, sensitivity, positive and negative predictive value of imprint cytology in the detection of *H. pylori* infection were determined.

Results

The study included 182 patients, 86 (47.3%) male and 96 (52.7%) female, mean age 57.5 (range 20–85) years. The diagnoses made by endoscopy were analyzed to elucidate the reasons for gastric mucosa biopsy. Biopsy specimens were obtained from the antral region mucosa in 12 patients; macroscopic lesions indicative of gastritis were diagnosed in 82 patients (chronic gastritis in 53 and erosive gastritis in 29 patients), ulcers in 32 patients (gastric ulcer in 19 and duodenal ulcer in 10 patients, pyloric ulcer in 2, and one ulcer on anastomosis after resection of malignant tumour), ventricular polyps in eight patients, and reflux esophagitis was diagnosed in five patients.

As previously mentioned, from each patient 3–6 samples were obtained, and all specimens were analysed as a sample pool to reach definitive diagnosis.

One hundred and fifty-five specimens were found to be appropriate for definitive method comparison. Out of the remaining 27 patients, a diagnosis of malignant disease was made in nine patients. In seven of these nine patients, the presence of malignancy was confirmed by both methods, whereas in two patients the material was inadequate for cytological analysis. The material proved inadequate for cytology in another 11 specimens, probably due to inappropriate slide imprinting; one of these specimens proved inappropriate also for histopathology. Another five specimens were found to be inadequate for histopathology, most likely due to inappropriate sampling procedure. In the remaining two specimens gastric polyps were indicated by histopathology, whereas the presence of *H. pylori* was not tested.

Comparison of the definitive diagnoses made by both methods in 155 specimens showed agreement between the two methods in 130 patients. Positive findings of *H. pylori* infection were obtained by both methods in 40 patients, and negative findings in 90 patients. Positive imprint cytology findings and negative histopathology findings were recorded in 14 patients, and negative imprint cytology findings with positive histopathology findings in 11 patients.

Statistical analysis showed the imprint method to have an accuracy of 83.9%, sensitivity of 78.4%, specificity of 86.5%, and positive and negative predictive value in the detection of *H. pylori* infection of 74.1 and 89.1%, respectively, with a note that histology was used as the »gold standard«, i.e. the histopathology finding was always considered accurate. (Table 1)

TABLE 1
STATISTICAL ALALYSIS OF THE DATA

	Histology findings		Total
Cytology findings	HP+	HP–	
HP+	40	14	54
HP–	11	90	101
Total	51	104	155
		95% CI	
Sensitivity	78.4%	64.7%	to 88.7%
Specificity	86.5%	78.4%	to 92.4%
Positive predictive value	74.1%		
Negative predictive value	89.1%		
Efficiency	83.9%		

HP – *Helicobacter pylori*

Discussion and Conclusion

H. pylori infection has been associated with the development of gastric and duodenal ulcer[9,10], and there is strong evidence that it increases the risk of gastric carcinoma[11–15]. In subjects with *H. pylori* infection, the risk of peptic ulcer development has been estimated to 3–25% (these results refer to the USA and Japan)[6]. Literature data also show the malignant disease recurrence following endoscopic resection of early gastric carcinoma to be prevented by eradication of *H. pylori* infection[6,26]. The more so, the risk of MALT lymphoma has been shown to significantly increase in patients with *H. pylori* infection, while this bacterial infection is present in 72–98% of patients diagnosed with gastric MALToma[13]. Eradication of *H. pylori* infection leads to regression of gastric MALToma in 70–80% of patients[6]. Thus, an accurate diagnosis and eradication of *H. pylori* infection are of paramount importance, requiring a rapid, simple and inexpensive method of detection. Gastric brush sampling and cytological analysis has been described as a highly specific, sensitive and accurate method[24–27].

The present study was performed to demonstrate the value of imprint cytology in the diagnosis of *H. pylori* infection. Unlike the method of brush sampling, the imprint method does not prolong the time needed for examination, esophagogastroduodenoscopy; does not increase the cost of examination because it does not require any additional equipment besides common accessories, and does not require specific sampling. The specimen used in this method can subsequently be submitted to histopathology or rapid urease testing. Slide imprinting entails no damage to the specimen, thus it can subsequently be

used for analysis by any other method, as shown by our own experience and reported in the literature[22,27].

In our study we compared imprint cytology only with histopathology in diagnostic process of *H. pylori* infection detection. Comparison of all other previously mentioned methods should be done in order to establish advantages and disadvantages of imprint cytology in *H. pylori* infection detection. In our opinion the biggest advantage of imprint cytology is that method is less expensive and time consuming than histopathology. In some cases when rapid urease test is not available, imprint cytology might replace it. Additionally, it has been shown that rapid urease test may result in false negative results when mild infection is present[23,26], and therefore combination of rapid urease test and imprint cytology, in our opinion might reduce possibility of false negative results. Another very reliable method for detection of *H. pylori* is culturing, but it is expensive, time consuming and not always available. This test should be employed in case of failure of second-line eradication therapy, to identify the right treatment option.

Prevalence of *H. pylori* of general population in Croatia has been estimated to range between 60.4 and 68.0% while in our study it has been identified in only 34.8% by imprint cytology and 32.9% by histopathology. It can be explained with the number of patients investigated. The number of 155 is probably small and it cannot represent the rate of infection in general population. Additionally, patients included in this study were mostly from city areas were infection rate is usually lower than in rural areas.

Accordingly to our results from this preliminary study it can be pointed out that gastric mucosa imprint cytology is a useful rapid, simple and inexpensive method for the detection of *H. pylori* infection, characterized by acceptable sensitivity and specificity as compared with histopathology. Further investigations should be made, with more patients included, and more than two methods compared, to establish the right place of imprint cytology in detection of *H. pylori* infection as well as in screening for malignancies.

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CITOLOŠKA ANALIZA PREPARATA OTISKA BIOPSIJE SLUZNICE ŽELUCA – BRZA JEDNOSTAVNA I POUZDANA METODA ZA OTKRIVANJE HELICOBACTER PYLORI INFEKCIJE

SAŽETAK

Cilj ovog rada bio je ustanoviti vrijednost citološke analize preparata otiska bioptata želučane sluznice u otkrivanju infekcije *Helicobacter pylori (HP).* Analizirano je 182 bioptata od 182 slučajno odabrana bolesnika kojima je učinjena

gastroskopija sa biopsijom želučane sluznice. Od svakog uzetog uzorka najprije je učinjen preparat otiska na staklo, a zatim je uzorak fiksiran u formalinu za daljnju rutinsku patohistološku obradu. Preparat otiska sušen je na zraku, bojan metodom po Mäy-Grünvald-Giemsi i analiziran svjetlosnim mikroskopom. Za konačnu usporedbu metoda u otkrivanju *H. pylori* infekcije adekvatno je bila 155 uzoraka. Patohistološkom analizom *HP* nađen je u 51 uzorku, a citološki u 54 uzorka. Nalazi obje pretrage podudarali su se u 130 od 155 pregledanih uzorka (83,1%). Pozitivan nalaz *HP* citološkim pregledom, a negativan histološkim nađen je u 14 uzoraka, dok je obrnuta situacija nađena u 11 uzoraka. Citološka analiza preparata otiska bioptičkog materijala sluznice želuca zadovoljavajuća je u otkrivanju *HP* infekcije. Najveća prednost metode je brzina, jednostavnost i mala cijena. Kada se uzorak uzima samo u svrhu potvrde prisutnosti *HP* infekcije, citološka analiza ubrzava i pojeftinjuje postupak, a istovremeno može dati i podatak o morfološkim promjenama sluznice. Svaki suspektan nalaz, u smislu zloćudne promjene sluznice, može se provjeriti i patohistološkom analizom, jer manipulacija uzorkom pri pripremanju preparata otiska ne oštećuje uzorak.