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EDITORIAL

Evaluation of Imprint Cytology of Endoscopic Gastric Mucosa Biopsy in the Diagnosis of Helicobacter pylori Infection

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Abstract

Background:*Helicobacter pylori* colonization of the gastric mucosa is associated with the pathogenesis of gastritis, peptic ulcer disease, and gastric malignancy. There are several methods to detect the presence of *Helicobacter pylori*.These tests include noninvasive method (serology, urea breath test, or stool antigen test) and invasive methods, such as, culture, histological examination, and rapid urease test.

Method: This descriptive prospective cross sectional study was conducted in Gezira state in Wad Madeni from March - August 2016;it aimed to determine the sensitivity, specificity, positive (PPV), and negative predictive values (NPV) of imprint cytology in the detection of *H. pylori* compared with stool Ag test. *H.pylori* stool Ag test was done for 50 clinically suspected patients for *H.pylori* infection and one gastric biopsy from each patient was collected during endoscopy. Air-dried imprint smears of gastric biopsies were stained by the Diff-Quik method and examined for *H.pylori*. The presence of inflammation and intestinal metaplasia were documented.

Results: The *H. pylori* prevalence was 38% by stool Ag test and 42% by imprint cytology. The sensitivity and specificity of imprint cytology in the detection of *H. pylori* were 89.5% and 87.1% respectively. The PPV and NPV were 80.1% and 93.1%, respectively. The accuracy of the test was 88.0%.

Conclusion: This study concludes that gastric imprint smears stained with Diff-Quik method is a rapid, cheap, and reliable method for the detection of *H.pylori* infection.It recommends the use of Imprint cytology for detection of *Helicobacter pylori* inpatients undergoing upper gastrointestinal endoscopy.

Key words: H. pylori, Imprint cytology, Chronic Gastritis.

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Introduction

Helicobacter pylori (H. pylori) are gram negative, spirally shaped, unipolar multiflagellate fastidious bacterium, firstly discovered by Warren and Marshall in 1983 [1, 2, 6].This micro-organism colonizes the antrum and cardia area in the gastric mucosa, or lives freely on the gastric surface [6]. *H. pylor* is urease positive which allow it to survive for a short period in the highly acidic gastric lumen, while motility allows it to rapid movement toward the neutral pH of the gastric mucosa[8, 17].Half of the world's population is infected with H. pylori, and the prevalence varies between developed and developing countries; in various developing countries, more than 80% of the population is H.pylori positive, even at young ages. The prevalence in industrial countries generally remains under 40% and is considerably lower in children and adolescents than in adults. Within geographical areas, the prevalence of H.pylori inversely correlates with socioeconomic and hygiene status[11, 8].Most infected persons were asymptomatic; however H. pylori cause chronic gastritis, gastroduodenal ulcers and have risk of developing gastric cancer and mucosal-associated-lymphoid-type (MALT) lymphoma[14, 15, 17].It has been classified by the International Agency for Research on Cancer (IARC) as a grade-I carcinogen[1].The accurate detection of *H. pylori* is very important, mainly for managing infected patients and for eradicating the bacteria. [4].Several tests may be used to confirm the presence of H. pylori.A universally accepted "gold standard" for the diagnosis of H. pylori infection is not available, and the choice of test depends on the specific clinical case. These tests include noninvasive methods (stool antigen test, urea breath test or serology) and invasive methods requiring endoscope evaluation such as, culture, histological examination and rapid urease test, which require upper gastrointestinal endoscopy to collect the specimens(gastric biopsy specimens) [10, 16].. More recently, immunohistochemistry, in situ hybridisation and the polymerase chain reaction (PCR) have been suggested as alternative specific detection methods. PCR- based methods have been developed to detect the bacteria directly in clinical specimens.

Imprint cytology is one of cytological technique which is a touch preparation in which tissue is touched on the slide and it leaves behind its imprint in the form of cells on glass slide.[5].This study aimed to evaluate the endoscopic gastric imprint smears stained by Diff-Quick in the detection of Helicobacter pylori infection in clinically suspected patients compared with stool Ag test.

Materials and Methods:

This was a descriptive prospective cross sectional study carried out in Wad Medani Teaching Hospital, unit of endoscopy from March to August 2016.A total of 50 clinically suspected patients for *H. pylori* infection who underwent upper gastrointestinal endoscopy were included in the study.

A prior approval was obtained from the ethics committee of this institution. Exclusion criteria included patients who had taken antibiotics (e.g. Metronidazole, Amoxicillin and Clarithromycin), omeprazole or bismuth compounds less than three weeks prior to

endoscopy. Patients who take alcohol, non-steroidal anti-inflammatory drugs (NSAIDs) and aspirin drugs were excluded.

Sample collection

In this study two types of specimens were collected:

Feces specimens (about 1-2 ml or 1-2 g) were collected in clean dry water proof containers with screw- cap lids before endoscopy.

During endoscopy, one gastric biopsy from the patients was taken, preferably from the antral region. This process was done by physician by inserting sterile forceps through the endoscopy device.

Imprint technique

On a clean glass slide using forceps, the biopsy was firstly placed on a clean slide and then repeatedly moved using light pressure in serial adjacent areas with the cut surface of the tissue. The smears were air-dried. Then stained by the Diff- Quick method and took an average of 2 minutes. The stained smears were then air-dried, mounted and examined for H. pylori blinded to the stool Ag test results to avoid bias. The cytomorphological change associated with H. pylori infection was also documented. In Diff-Quick stained smears the bacteria were identified by their spiral or curved rod morphology and the purple-violet color within the well-preserved gastric mucus. The presence of neutrophils or large numbers of plasma cells, lymphocytes, lymphoid aggregates and goblet cells were documented when present reflecting the grade of the active or chronic inflammation and intestinal metaplasia. The results of the two methods with regard to detection of H. pylori infection were compared and the Sensitivity, specificity, PPV, NPV and accuracy of the Imprint cytology were calculated using statistical evolutions (ROC curve on SPSS for windowsV18.0)

Results

Among the 50 patients who underwent the study; 21 patients were males (42%) and 29 were females (58%). The mean age was 42 years with an age range of 14-70 years. Recently residence 30 (60.0%) patients were from rural areas and 20 (40%) patients from urban. The prevalence of *H.pylori* infection among the study population by Imprint cytology was (42%) when the Diff-Quick imprint smears showed presence of H. pylori curved bacilli. Fig 1

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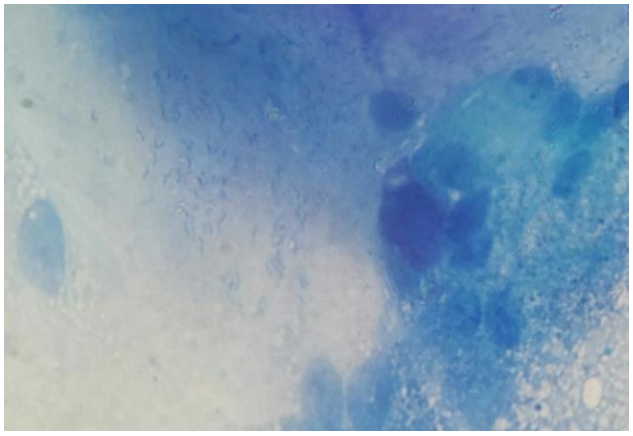

Fig 1: showing the presence of *H. pylori* by Diff-Quick method

Imprint cytology and Stool Ag test Comparison:

When we compared the results obtained by stool Ag test with that obtained by imprint cytology we found the following: 17(34%) patients were positive for *H. pylori* by imprint method were also positive by stool Ag test. 27(54.0%) patients were negative for *H. pylori* by both methods. There were 2 negative cases on imprint cytology that were positive on stool Ag. There were 4(8.0%) positive cases on imprint cytology that were negative on stool Ag test.

Those four positive cases on imprint smear that were diagnosed as negative by stool Ag test, showed a low density of H. pylori (grade 1) on the imprint smears. Table 1

Table (1): Showing the comparison between Imprint cytology and stool Ag test

		Stool Ag test		Total
		Positive	Negative	
Imprint cytology	Positive	17	4	21
	Negative	2	27	29
Total		19	31	50

ROC curve for Imprint cytology technique

The sensitivity of imprint cytology in the diagnosis of *H.pylori* infection was 89.5%, while the specificity of the test was 87.1%. Fig 2

The positive predictive value (PPV) of the test was (17/21) ´ 100 = 80.1% and the negative predictive value of the test was (27/29) ´ 100 = 93.1%.

The accuracy of the test was (17+27) ´ 50 = 88.0%. Fig 2

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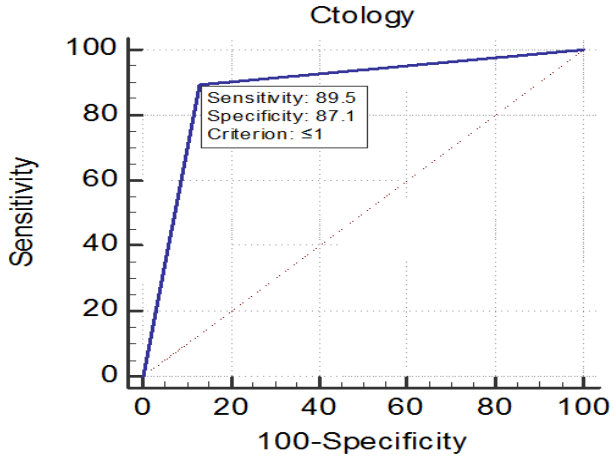

Fig (2): Showing the sensitivity and specificity of Imprint cytology technique

Imprint cytology and stool Ag test correlation:

The area under the ROC curve (AUC) was 0.883 with Standard error 0.0474 and the P-value <0.0001 that means the imprint cytology technique is a highly sensitive method for diagnosis of H. pylori infection. Table 2

Table (2): Showing Imprint cytology and stool Ag test correlation

Area under the ROC curve (AUC)	0.883
Standard Error [a]	0.0474
95% Confidence interval [b]	0.760 to 0.956
z statistic	8.081
Significance level P (Area=0.5)	<0.0001

Cytomorphological changes associated with *H. pylori infection*

Imprint smears produced good cytological preparations. The gastric mucus stained a light violet. *H. pylori* were easily visualized within the gastric mucus. Of the 21 patients with *H. pylori* infection on imprint smears, only 4 smears had poor cellularity. About 6 smears showed mucosal infiltration of neutrophils on a background of chronic inflammation that indicated for presence of chronic active gastritis. Only 2 smears showed the presence of enlarged glandular nuclei and spindly cells which correspond to chronic gastric peptic ulcer. 5 smears showed dysplastic epithelial cells and features of intestinal metaplasia. Only one smear showed degenerative changes and feature of malignancy in epithelial cells. 3 smears showed normal looking epithelia with abundant mucus in the background. (Fig 3, 4, 5,6)

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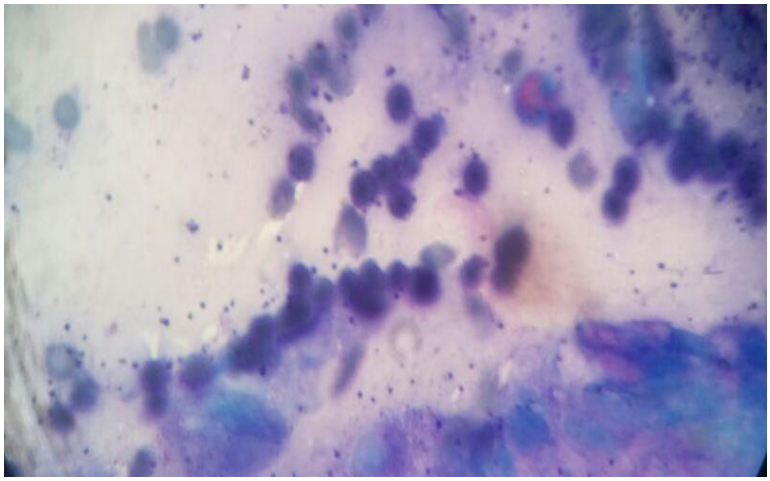

Fig (3): chronic active gastritis: mucosal infiltration of neutrophils, lymphocytes and plasma cells that indicated the presence of chronic active gastritis.

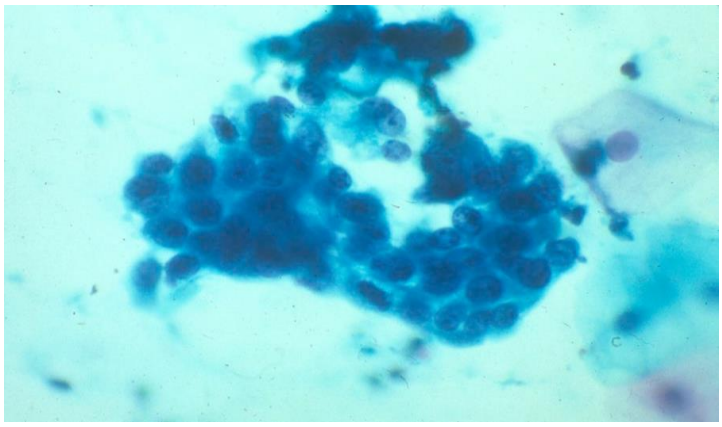

Fig (4): Gastric peptic ulcer. The epithelial cells showed uniform enlarged glandular nuclei, and have vesicular chromatin and well-developed nucleoli.

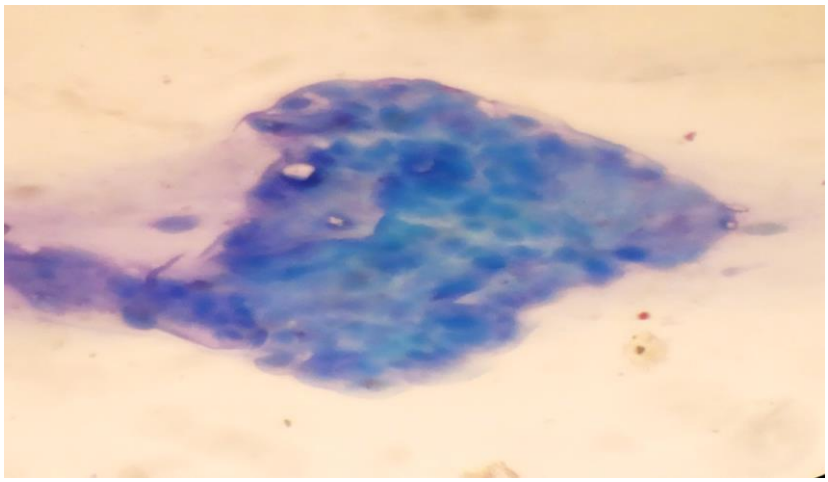

Fig (5): Intestinal metaplasia: Smears contain cohesive aggregates of dysplastic epithelial cells and variable numbers of mixed inflammatory elements.

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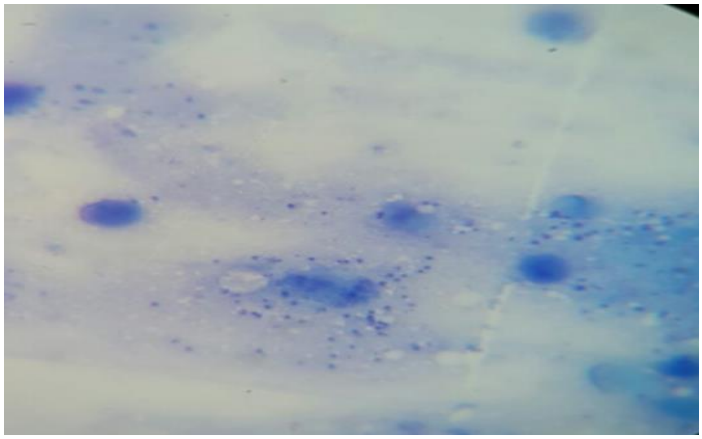

Fig (6): Gastric carcinoma: degenerative changes and feature of malignancy in epithelial cells.

Discussion:

From this study the imprint cytology method is highly sensitive method for diagnosis of *H.pylori* infection (the P-value <0.0001).

Previously reported studies used various combinations of techniques and staining methods. Histology, culture, urease test and touch imprints were common, while the stains used included Papanicolaou, May-Grunwald-Giemsa and Diff-Quick reagents.

In previous studies the sensitivity of *H. pylori* detection by imprint cytology using various stains has been reported to be between 83% to 100%, while the specificity was 51% to 100% [9, 13, 7, 6, 1 and 3].

In this study the sensitivity was 89.1% which was significantly higher than that obtained by Kaur*et al* in 2004, where sensitivity was 83.3% compared with histology method. The low prevalence of *H.pylori* infection in Kaur et al study population may be justifying this variation in sensitivity.This study sensitivity was similar to that obtained by Ahsanet al study (sensitivity was 89.3%). Also the sensitivity from this study was relatively lower than that obtained by both Fakhrjuo.study and Al-Ali J. *et al* studies (sensitivity was 100% and 92.7% respectively).

The specificity obtained from this study was 87.1% which was significantly lower when compared with Kaur*et al.*,Fakhrjuo*et al* and Ahsanet al studies (specificity was 100%, 90% and 92.6%).

Only two previous studies calculated the positive predictive value (PPV) and the negative predictive value (NPV) of imprint cytology for diagnosis of *H. pylori*. In this study the PPV was 80.1% and NPV was 93.1%. There is agreement in (NPV) when compared with Kaur *et al* and Rahba r*et al* studies (NPV was 98.6% and 90.1% respectively) and disagreement in (PPV) (it was 100% and 96.8% respectively).

In the current study the accuracy of the imprint cytology was 88.0% which was significantly lower than that obtained by Fakhrjuo *et al* in 2011. (Accuracy was 98%).

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No previous study has found on agreement between the imprint cytology method and stool Ag method in the diagnosis of *H.pylori* infection.

In 21 positive patients by imprint cytology, the Diff-Quick imprint smear showed the presence of scattered tiny curved rod like structures associated with features of active and chronic inflammation (gastritis), chronic ulcer, intestinal metaplasia and feature of malignancy in epithelial cells with a dirty mucus background. This finding was in agreement with Kaur *et al* study, who has documented the presence of inflammation and intestinal metaplasia in *H.pylori* infected patients by gastric imprint smears as compared with histology.

From this study the Diff-Quik stain has proved to be reliable quick, and easy for identifying *H. pylori* in smears, requiring no additional staff besides the pathologist who stained and interpreted the imprint smears. This agrees with Zaitoun study which was aimed to check the accuracy of the Diff-Quik stain for identifying *H.pylori* in gastric biopsy specimens of patients with ulcer and non-ulcer dyspepsia and compared with Giemsa stain. Results obtained by the Diff-Quick stain correlated 100% with those obtained using the Giemsa stain. However, the advantage of the Diff-Quick stain over the Giemsa stain is that it is a rapid procedure which takes less than one minute compared with 30 minutes using Giemsa stain. [(18)].Kaur et in 2004 has also reported that, the Diff-Quick staining imprint cytology was simple, rapid and produced good cytological preparations.

Although stool Ag test is a reliable, simple and rapid method for detection of *H. pylori* and several studies have been done for the diagnosis of *H. pylori* infection, the Imprint cytology was also noted to be a relatively easy method for detection of *H. pylori*.

Conclusion:

This study concluded that, Imprint cytology technique for diagnosis of *Helicobacter pylori* infection is very simple, inexpensive, easier to perform, less time consuming and provides more information, especially the grading of the severity of colonization by *H.pylori* in a specimen and cytological feature associated with infection**.**

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References

1. Ahsan, K,;Hossain, MZ. andUddin MR.Role of crush cytology for the detection of the Helicobacter pylori in gastroduodenal disease.J Dhaka Med Coll 2008; 17 (2): 89-91.
2. Akhter J, Shrestha HG. Rapid detection of helicobacter pylori by endoscopic brush cytologyand comparison with histopathology.Kathmandu University Medical Journal 2007; Vol. 5, No. 3, Issue 19, 387-390
3. Al-Ali, J.; Al-Asfar, F.; Dhar, R.;Dhar, PM. and Kapila, k. Diagnostic performance of gastric imprint smears for determination of Helicobacter pylori infection. *Can J Gastroenterol* 2010; 24(10): 603-606.
4. Ashton-Key M, T C Diss, P G Isaacson.Detection of Helicobacter pylori in gastric biopsyand resection specimens.J7 ClinPathol 1996;49:107-111
5. Bal, MS.Kahlon, SK. and Bidani, R.Comparative evaluation of aspiration cytology and preoperative imprint cytology in breast lesions.J of Cytology 2000; 17:27-32.
6. Fakhrjou, A.; Somi, MH.;Fattahi, E.; Koohbanani, SS. and Shadravan, S.Rapid urease test, touch cytology and Histological assessment in determining infection by Helicobacter Pylori in outpatient setting. Pakistan journal of biological sciences 2011; 14(12): 698-702.
7. Jafari, M.; Khalilian, A. and Rostampour, F. Comparison the Sensitivity of Stomach Mucus Touching Cytology and Urea's Rapid Test in Helicobacter Pylori Diagnosis in Endoscopies Patients with Gastritis or Peptic Ulcer.Jundishapur J Microbiol 2013; vol 6(4): 1-4.
8. Johannes, G.; Kusters,;Arnoud, HM.; Van Vitel, and Kuipers, JE.Pathogeneses of Helicobacter Pylori infection. Clinical Microbiology Review 2016; Vol 29, issue 3: 449-490.
9. Kaur,G.; Madhavan, M.; Hakim, AB.; Mat, SA.; Hussain, M S.; Yatiban, M K., *et al*. Rapid diagnosis of Helicobacter pylori infection in gastric imprint smear.Southeastasian J trop MED public health 2004; Vol 35 No.3 :677-680
10. Lee, YJ. and Kim, N.Diagnosis of Helicobacter pylori by invasive test: histology. Ann TranslMed2015 ;3(1):10-19
11. Malatey HM, Hoda N.Helicobacter pylori infection:genetic and environmental influences. BestPractice& Research ClinGasteroenterol 2007;21(2): 205-14.
12. McColl, KE. Clinical practice.Helicobacter pylori infection.NEngl J Med 2010 ;362:1597-604
13. Rahbar, M.; Mardanpur, K. and Tavafzadeh R.Imprint cytology: a simple, cost effectiveness analysis for diagnosing Helicobacter pylori, in west of Iran. MJIRI 2011; Vol. 26, No. 1: 12-16
14. Sipponen, P. and Hyvarinen, H.Role of Helicobacter pylori in the pathogenesis of gastritis, peptic ulcer and gastric cancer.Scand J Gastroenterol 1993; 28: 3-6.
15. Talukder SI.Association of Helicobacter pyloriwith gastric cancer and other gastroduodenal lesions. Mymensingh Med J 2000; 12(1): 33-5.
16. Versalovic J.Helicobacter pylori: pathology anddiagnostic strategies. Am J ClinPathol 2003;119: 403-12.
17. .Warren JR, Marshall B. Unidentified curved bacilli ongastric epithelium in active chronic gastritis. Lancet 1983; 127: 3-5.

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18. Zaitoun, MA. Use of Romanowsky type (Diff-3) stain for detecting Helicobacter pylori in smears and tissue sections.*JClinPathol*1992; 45: 448-449.