The Role of Histochemical and Immunohistochemical Methods for The Detection of Helicobacter Pylori in Chronic Gastritis

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ABSTRACT:

BACKGROUND:
Helicobacter pylori represents one of the most common pathogen worldwide. Infection with it can cause medical problem and establishe as etiologic factor in the development of gastritis, So the accurate detection is important for proper patient management.

OBJECTIVE:
This study was conducted to assess the detection of H pylori by Hematoxylin & Eosin, special stain (Modified Giemsa stain) and immune stain in gastritis biopsies and to assess the sensitivity and specificity of each method use in detection.

MATERIAL AND METHODS:
A retrospective and prospective (case series) study, from October 2019 to October 2020, 100 gastric biopsies with gastritis referred to histopathology department, in AL-Khansaa Teaching Hospital and some private labs in Mosul city, were examined.
All biopsy specimens examined histologically to assess H. pylori infection, by Hematoxylin & Eosin , modified Giemsa and immunohistochemical stains.

RESULTS:
Patients age range 17 to 78 years with a mean SD of 44.5+6.11 years. H. pylori positive with H&E /MGS in 79% of cases, while with IHC in 88% of cases. The sensitivity and specificity of the H&E /MGS comparing with the IHC stain were 90% and 100% respectively.

CONCLUSION:
We have concluded from this research that H& E is a reliable way to detect H. Pylori infection. As well as we recommend the usage of IHC in special cases.

KEYWORLDS: Gastritis, Helicobacter pylori, Modified Giemsa stain, immunohistochemistry.

INTRODUCTION:

Helicobacter pylori which is a gram negative bacterium has helical shaped help it to penetrate the gastric lining epithelium and establish infection [1] which firstly discovered by Dr. Barry Marshall and Robin Warren in 1982 [2]. It's morphology is curved rod bacterium measure 3 um in long and 0.5 um in diameter and it can form biofilm [3] and the motility is high because it has six flagella at same site [4] it also can convert from helical form to viable but non cultured coccoid form [5].

It is wide present infection in the world occur about 50% in developing countries, but lower than that in developed countries [6] because it associated with socioeconomic state [1]. There is data suggest that high rate of infection acquired in childhood [7]. In Mosul /Iraq the relative frequency of H. pylori was found in about 67% of patient suffering from gastritis [24].

The transmission can occur from person to person by oral-oral or feco-oral rout which is most likely[1].It used it 's flagella to penetrate the superficial layer of epithelium and reach the deep layer which is less acidic than the lumen of stomach [8], also the H. pylori can sense the gradient of pH in gastric mucosa and chemotaxis to the less acidic area [9]. And another mechanism to avoid acid environment that H. pylori neutralized the acid by produce urease, which convert the urea present in stomach to ammonia and carbon dioxide[10].

The person who is infected with it carries life risk about 20% to develop peptic ulcer [11], and the presentation of acute infection usually as acute gastritis with abdominal pain [12], therefore, many investigations were used to prevent H. pylori-related diseases by eradicating the bacterium during an early stages of infection by using antibiotic regimens [13], and many efforts make by pathologists to detect it by using sensitive and specific methods such as special stain and immunohistochemistry.

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MATERIAL AND METHODS:

A retrospective and prospective (case series) study, was performed during a twelve months' period, from October 2019 to October 2020, 100 Endoscopic biopsies specimens with gastritis were fixed in 10% formalin overnight then processed and embedded in paraffin wax. At first, 4 micron-thick tissue sections were taken and stained with H&E and examined under light microscope.

In all patients, biopsy specimens were taken to assess H. pylori infection, and degree of colonization by three methods: H&E, Modified Giemsa (MGS), (SHEEHAN'S MODIFIED MAY) and immunohistochemical stain (IHC) using a rabbit polyclonal antibody against H. pylori.

The studied cases of chronic gastritis were reviewed according to the updated Sydney system for classification of gastritis.

A subjective assessment was done of the overall staining quality of organism morphology, taking into account the staining intensity and clarity of demonstration of the various morphological variants of the organism (spiral, rod-shaped, angulated and coccoid) forms.

1+ represents poor morphology clarity and weak intensity.

2+ represents moderate morphology clarity and moderate intensity.

3+ represents good morphology clarity and strong intensity.

A Chi-square test was used to compare positive and negative cases as determined by H&E/MGS and IHC for pathologic features. A significance level of $p < 0.05$ was used for comparison of cases.

RESULTS:

The age of studied cases ranged from 17 to 78 years with a mean +SD of 44.5+6.11 years. There were 57 (57%) males and 43 (43%) females with a male to female ratio of 4:3. The frequency of H pylori was higher in male and with young age group, as shown in (Table1). The presence of H. pylori was associated significantly with male and with young age group (p=0.009).

Table 1: H.pylori density in relation to age and sex of the sampled cases.

H pylori	Age ≤ 40	Age > 40	Male	Female	Total
Positive	48	31	42	37	79
Negative	5	16	15	6	21
Total	53	47	57	43	100

P value =0.009   Highly significant value

Gastric biopsies from the antrum in 79 (79%) cases and the corpus in 21 (21%) cases. The presence of H. pylori was higher in the antrum than corpus, as shown in Table (2).

There is a high significant relationship with p value <0.001 between the histochemical and IHC for detection of H. pylori and the location of gastric biopsy.

Table 2: Correlation between histochemistry and IHC for detection of H. pylori in relation to the location of the gastric biopsy.

H. pylori presence	Corpus (n=21)	Antrum (n=79)	Total (n=100)	P value
Histochemistry Positive Negative	9(43 %) 12(57%)	70(89%) 9(11%)	79 21	<0.001
IHC Positive Negative	13(62%) 8(38%)	75(95%) 4(5%)	88 12	

According to the updated Sydney system, cases of gastritis were classified and graded as follows: chronic inflammation, activity, glandular atrophy, intestinal metaplasia and H. pylori density, in this study we added gastric dysplasia, as shown in Table (3). Of the total of 100 cases of chronic gastritis the chronic active gastritis was found in 74 (74 %) of cases of gastritis, glandular atrophy was detected in 25(25%) cases, Intestinal metaplasia was present in 3 (3%) cases of gastritis, as shown in Photo (1a),

The dysplasia was identified in 2 (2%) cases only of gastritis, as shown in Photo (1b).

The detection rate of H. pylori was different with different stains used. The bacilli were positive with H&E/MGS in 79 (79 %) of cases, raised to 88 (88%) by IHC stain, as shown in Table (4), Photo (1c, d & e). The modified coccoid form and the presence of a single or few bacteria stained obviously by IHC were negative by H&E/ MGS, as shown in Photo (1f). The degree of colonization by H. pylori was graded into mild, moderate and marked, there were 16

(16 %) cases of mild colonization detected by IHC, of these 16 cases only 7 cases were positive by the H&E/MGS. On the other hand, all moderate and marked cases were positive by all stains. The sensitivity and specificity of the H&E/MGS were measured comparing with the recommended standard sensitive and specific IHC test; they were 90% and 100% respectively. Finally, this study noticed a statistically no significant difference between IHC on one hand and H&E/MGS on the other hand with P value (0.112) for detection of H. pylori.

Table 3: H. pylori colonization and histological grades of cases with gastritis by updated Sydney system.

Sydney system	H pylori +	H pylori -	Total	P value
Chronic inflammation 1 2 3	32 29 18	7 13 1	39 42 19	P = 0.061
Activity 0 1 2 3	11 8 51 9	15 3 3 0	26 11 54 9	P <0.001
Glandular atrophy 0 1 2 3	59 14 6 0	16 2 2 1	75 16 8 1	P=653
Intestinal metaplasia 0 1 2 3	79 0 0 0	18 2 0 1	97 2 0 1	P=0.008
Dysplasia 0 1 2 3	77 2 0 0	21 0 0 0	98 2 0 0	P=0.461

Table 4: H. pylori colonization in correlation to histochemistry and IHC.

H pylori grade	H&E/MGS	IHC
0 None	21	12
1 Mild	7	16
2 Moderate	45	45
3 Severe	27	27
Total	100	100

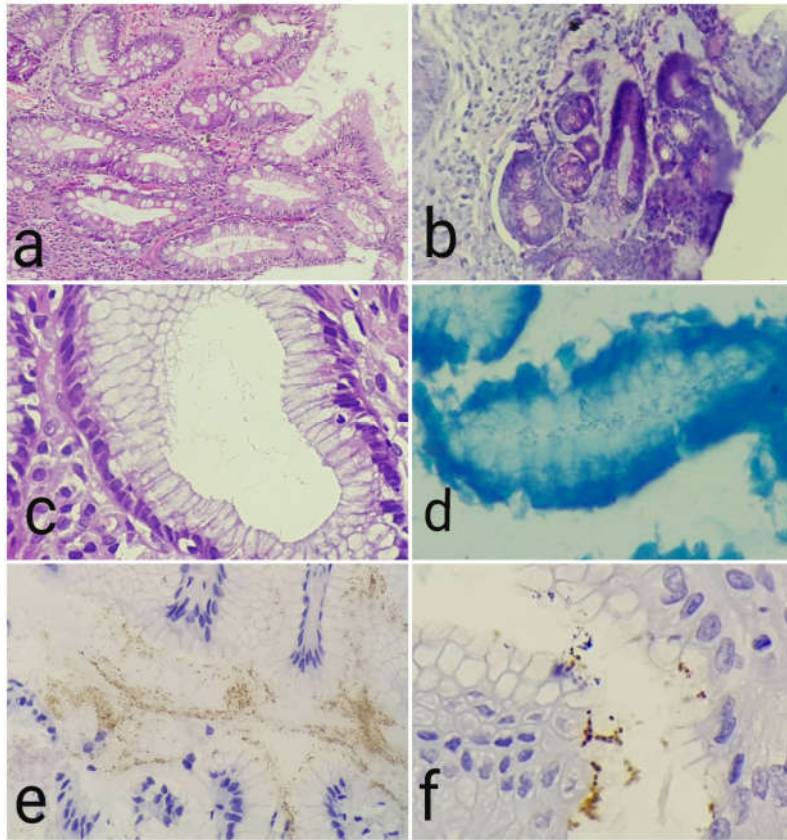**Photo 1: Chronic gastritis with intestinal metaplasiain H&E in (a),and dysplasia in H&E in (b), typical H. pylori in H&E in(c), MGS in (d), IHC in(e), coccoid form of H. pylori, IHC in (f).**

DISCUSSION:

H pylori is, a gram negative, spiral pathogen, which infection is associated with gastritis, and proper detection is important for early patient management. Several methods can use in detection of it but no one has sensitivity and specificity enough to be a gold standard [14]. In most cases, the pathologist can be recognized H. pylori by H&E stain but the sensitivity of this routine stain may be low if there is mild colonization and small number of bacilli or after treatment. Therefore, most laboratories use another stain in addition to H&E to give a good identification of the organism and correct diagnosis.

In the present study the mean age was 44.5 years which is slightly higher than a study done in Duhok\Iraq [15] and study done in Iran [16], while lower than a study done in Mosul [14]. In this study, the H. pylori infection was significantly associated with young age group, a finding similar to that observed in the developing countries by previous study [22]. In the current study the male to female ratio was 4:3. with

males predominant affected. This finding compatible to the other studies in Mosul [14] and Iran [16], and this different in the gender may be attributed to some habits, like smoking, alcohol drinking, and diet factors that may cause damage to gastric mucosa and changes.

The antral site of gastritis with high prevalence of H. pylori colonization are the commonest in this current study which is similar to most other studies [17,14,18] because the antrum is the common site for endoscopic biopsy and the body mucosa may be more resistant to inflammatory reaction to H. pylori.

In the present study, for detection of H. pylori we dependent on three different stains (H&E, MGS and IHC).

On H&E/MGS 79% cases of gastritis were positive for H. pylori bacilli, by using IHC this rate raised to 88% cases of mild grade. The sensitivity and specificity of the H&E/MGS were calculated, they were 90% and 100% respectively. The false negative results occur if there is scattered single organism easily missed

by routine stain, in addition, many H. pylori morphology alter to coccoid and short bacilli forms after antibiotic and proton pump inhibitor administration but obviously identified by IHC.

Regarding IHC results, it is higher than those reported in Mosul[14] (75%), India [20] (62%) but lower than that in a study from UK [19], in which the result of IHC reach to 98%.

In the present study all the 100 cases had chronic inflammation (100%), which is similar to other studies [14,15,17] but H. pylori was detected in only 79% of cases and this result comparable with the result of other study (77%) [17].

In the current study the activity of inflammation which represent by neutrophilic infiltration was observed in 74% of cases, this result go with the studies in Duhok by Azad M, 2011 [15] and Mosul by Aziz ZW, 2019 [14], there is a high significant relationship between the presence of H. pylori and the activity of inflammation, so we can consider that the neutrophilic infiltration is important indicator for the presence of H. pylori.

Atrophic changes that occur in mucosa were found in 25% of cases lower than two studies in Iraq by Azad M, 2011 (38%) [15] and Aziz ZW, 2019 (48%) [14]. most of them were mild and this also go with the result of previous study in Mosul [14].

Intestinal metaplasia was found in only 3% of cases but higher rate result were found in previous study by AL-Nuaimy et al [21] which was found in 23% of cases with chronic gastritis, this difference may be due to use special stain of mucin which can improve detection of intestinal metaplasia [21].

Dysplasia is important histological finding which was noted in this study because it may lead to carcinoma with the time, in this study only 2% of cases show dysplasia with mild degree which is lower than that noted by Hassan TM,2016 [23] and Hussein HA,2019[17] 6.4% and 9% respectively. While disagree with AL-Nuaimy et al, 2019 [21] where no dysplasia was reported. Despite that these studies were done in the same locality. The reason of this discrepancy may be due to compromised immunity of people in those periods of time due to poor conditions they passed through in the previous period which made them vulnerable to H. pylori infection and chronic inflammation that could initiate this dysplastic change. The sensitivity and specificity of the H&E/ MGS were calculated, they were 90% and 100% respectively. The false negative results occur if there is scattered single organism easly missed by routin stain, in addition, many H. pylori morphology alter to coccoid and short

bacilli forms after antibiotic and proton pump inhibitor administration but obviously identified by IHC. The results of the present study were shown that, in the majority of cases the H. Pylori can be relatively easily seen with H&E staining regardless to the pathologists training level and in our result we found that ancillary staining methods is not indicated in our practice to diagnosis H. pylori gastritis. However, immunohistochemical particularly useful in a small number of cases like patient receiving eradication treatment to prevent false-negative results.

CONCLUSION:
The routine H& E (with or without MGS) is a reliable way to a great extent for the detection of H- Pylori infection in gastric biopsy samples; As well as we recommend the usage of another stain like IHC detection (with H& E) in special cases and considerations, like: In biopsy specimens from previously treated patient, in cases of unexplained gastritis. In addition, the IHC is easier to detect organism, highly sensitive and specific.

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