Detection of H. Pylori by Different Conventional Staining Methods and Immunohistochemistry in Sudanese Patients with Chronic Gastritis

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Abstract

Background*:* *H.pylori* is a major cause of chronic gastritis, peptic ulcer and gastric cancer**,** is found in half of the population of the world. Different special stains and immunohistochemisty stain for detection of *H.pylori* were adopted .This study was conducted to compare haematoxylin and eosin (H&E), Warthin - starry silver with immunohistochemisty for detection of H.pylori.

Methods: paraffin embedded sections were collected from Military and Bahry hospitals in Khartoum state in Sudan .H&E, Warthin - starry silver and immunohistochemisty were done in 50 samples of patients with chronic gastritis for detection of H.pylori.

Results: *H.pylori* was detected with the immunohistochemisty used as the gold standard, then by Warthin- starry silver impregnation the sensitivity is (72%), and H&E the sensitivity is (27.8%), of the 50 positive and negative cases, 18 were positive by the immunohistochemisty, 13 by silver impregnation method, and 6 by H&E. However there was one false positive case with the H&E method.

Conclusions: to detect H. *pylori* in tissue sections, suitable staining procedure and careful examination will increase sensitivity. The immunohistochemisty is method of choice.However, *silver stain can be used as alternative.*

Key words: H.pylori, Chronic Gastritis, Immunohistochemisty

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Introduction

Gastritis considering vast range of potentially damaging agents to which the stomach is exposed (either accidentally or deliberately).The commonest type of gastritis is type B chronic gastritis tends to increase in prevalence with advancing years even through it can arise at any age, while any part of stomach may be affected, the lesions are usually more pronounced in the antrum or functional zone. Histological, the predominant change is an intense plasma cells infiltration of the foveolar zone of the mucosa. In most cases minute curved bacillary organism can be formed in close opposition to the surface of the epithelium and can be visualized even in sections stained by H & E, although their demonstration is facilitated by special stains such as warthin - starry silver. The pathogenesis role of this bacterium , now referred to as H.Pylori[(1)] , spiral- shaped gram negative bacterium[(2)] one of the most common bacterial infection throughout the world , involving 50% of the population in developed countries[(3)], and up to 80 -90 % of the population in developing countries[(4)] . H. pylori are present in over 80% of active chronic gastritis and are selective in that they inhabit especial gastric -type epithelium the prevalence of H.pylori increase also with age. Serological testing for H.pylori specific Abs suggests that the infection is generally acquired in early adult hood. However, H.pylori gastritis can also occur in children. This observations are of considerable important since this type of chronic gastritis is not only

though to be responsible for dyspeptic symptoms but may be involved in the pathogenesis of peptic ulcer and possibly gastric carcinoma [(1)] . There are number of tests for detecting H.pylori , including the breath test , urease test , culture and PCR but the histological detection in a gastric biopsy is the commonest and Among the most sensitive[(5)] . The routine stain H& E and special stain warthin – starry silver and more recently, the immunohistochemistry are needed for diagnosis and detection of H.pylori. In this study we have compared traditional detection methods using H & E and warthin – starry silver stained sections with immunohistochemistry using anti *H. pylori* antibody.

Materials and Methods

Study population:
This study was carried out on samples of Formalin fixed paraffin wax embedded tissue from chronic gastric endoscopy samples that showed chronic gastritis (50cases). These samples were taken from military and Bahry hospitals in Khartoum state in Sudan recorded in histopathology department, all sections were stained with H & E, Warthin-starry silver, and immunohistochemistry.

Immunohistochemistry is the agreed “gold standard” for histology, being a highly sensitive and specific staining method [(6)].

Immunohistochemistry procedure was done as the follows; sections (3 microliter) from formalin fixed embedded were cut and mounted on to salinized slides (dako). Following deparaffinization in xylene, slides were rehydrated through a graded series of alcohol and were placed in running water. Samples were steamed for antigen retrieval for H.pylori using PT link. briefly , slides were placed in the PT tank containing enough tris buffer (PH 9.0) to cover the section, then the machine was turn on at 20 mints to start heating from 65C° until reach 95C° and then boiled at high temp (95 C°) for 20 mints then allow sections to cool to 65 C° Endogenous peroxidase activity was blocked with peroxidase blocking reagent (3% hydrogen peroxide and methanol) for 10 mint , the slides were incubated with (100 – 200micro liter) of primary antibodies for 20 mint at room temp in moisture chamber , and then rinsed in phosphate Buffer saline . The primary

antibody for H.pylori was concentrated then diluted 1 micro ml of primary antibody to 250 micro liters of antibody diluents (Dako, carpintera). After washing with PBS for 3 min, binding of antibodies was detected by incubating for 20 minutes with poly dextran labeled polymer (Dako – Envision TM Flex kit) .Finally, the sections were washed in three changes of PBS, followed by adding 3, 3 diaminobenzidine tetra hydrochloride (DAB) (Dako) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/ enzyme complex for up to 5 min. Slides were counterstained with haematoxylin.

Control: for each run of staining, positive and negative control slides were also prepared. The positive control slides contain the antigen under investigation and the negative control slides were prepared from the same tissue block, but incubated with PBS instead of the primary antibody.

Each slide was evaluated with investigator then the results were confirmed by consultant histopathologist.

All histological sections showed fair staining quality and all quality control measures were considered throughout study procedures.

Statistical analysis

Data were analyzed using SPSS software package (version 16 for windows 7)

Results

In this study, 30 male and 20 female patients were included. Fig (1) the age ranges from 25-82 years.

IHC stains showed that 18 (36%) cases were positive for *H. pylori* and 32 (64%) were negative. *H. pylori* was identified in 6(12%) sections with H&E and 44(88%) were negative.

Warthin starry silver impregnation technique permitted *H. pylori* identification in 13(26%) cases and 37(74%) were negative in Table (1).

Table (1) Staining results

Technique	Positive	negative	total
immunohistochemistry	18(36%)	32(64%)	**50**
Warthin and starry silver impregnation	13(26%)	37 (74%)	**50**
Haematoxylene and Eosin	6(12%)	44(88%)	**50**

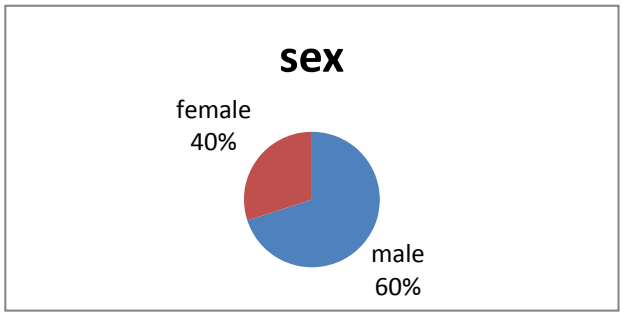

Figure (1) shows the percentage of sex in the study population included.

Immunohistochemistry is the agreed "gold standard" for histology, being a highly sensitive and specific staining method. According to equation:

Sensitivity =number of true positive/number of true positive +number of false negative. X100 %

The sensitivity of H&E=5/5+13x100%=27.8%

The sensitivity of warthin and starry =13/13+5x100%=72.2% and this is depicted in fig (2).

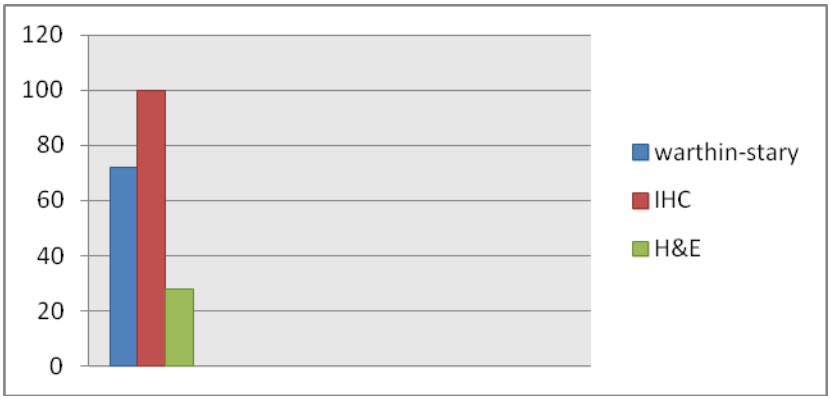

Figure (2) shows the sensitivity of staining methods of Warthin-starry, IHC, and H&E.

Discussion

Infection with Helicobacter pylori has been established as an etiological factor in development of gastritis, peptic ulcer, gastric adenocarcinoma and Mucosal-Associated Lymphoid tissue lymphoma. [7]

In view of this pathogenic importance and prevalence, accurate detection of H. pylori is essential for management of patients and for eradication of the bacterium following treatment. [8]

Immunohistochemistry stains are the most sensitive histological method to detect H. pylori in tissue sections. [9]

In current study, the value of H&E and Warthin & starry silver impregnation for detection of H. pylori in comparison with specified immunohistochemisty stain were evaluated. In our study the sensitivity of H&E for detection of H.pylori organism was 27.8% which is in consistence with study done by Raziye Tajalli et al [10], their work found 41% sensitivity of H &E for detection of H.pylori, also Pity et al. [11] concluded that, H&E had no clinical value in detection of H. pylori in gastric biopsies. And against study done by wang et al [12], their work publication showed that routine H&E staining method was sufficient for identification of the organism. In this study also the Warthin-starry and silver related techniques showed sensitivity of 72% and this is in consistent with M Ashton-Key et al [9] which their study showed that Warthin-starry is method of choice for H.pylori, but there is difficulty in reproducibility.

Conclusion

In this study we concluded that, Warthin-Starry is relatively sensitive, inexpensive and it is more reliable than H&E and can be used careful as alternative to immunohistochemistry in detection of *H. pylori*. But the disadvantage of this method is difficulty in application of the procedure

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