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IMMUNOHISTOCHEMISTRY ON HELICOBACTER PYLORI: An institutional diagnostic hands-on review

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ABSTRACT

BACKGROUND:
H.Pylori infection is chronic &the prime risk factor for peptic ulcer disease, gastric cancer & primary B-cell lymphomas. It is classified recently by WHO as a CLASS I carcinogen.

OBJECTIVE:
1.To review the use of anti H.pylori antibody
2.To compare the efficacy of IHC & special stains in patients with H.pylori associated chronic gastritis.

METHODS:
A retrospective study involving 55 cases of H.pylori associated chronic gastritis. Under microscopy, the density of H.pylori,efficacy and sensitivity of the immunohistochemical staining were assessed.

RESULTS:
Antibody against H.pylori showed higher sensitivity on comparison with routine H&E and modified Giemsa.

CONCLUSION:
Use of H.pylori antibody along with the routine H&E and Modified Giemsa would increase the sensitivity, specificity and decrease the false positive results.

KEYWORDS : H.pylori, gastritis, H.pylori antibody, diagnosis, immunohistochemistry

INTRODUCTION:

Helicobacter pylori (H.Pylori) is seen in significant number of dyspeptic patients with endoscopically normal stomach. A strong relationship is also documented between H.pylori and peptic ulcer disease. It has been classified recently by WHO as a CLASS I carcinogen because it is involved in the development of Atrophic gastritis with Intestinal metaplasia (pre-neoplastic gastric lesion).

Inflammation with this micro aerobic, gram negative, spiral, motile bacterium has become major prime cause for chronic active gastritis. It resides in the gastric pits and the overlying mucus. H.pylori colonization of stomach is associated with a spectrum of gastro duodenal disease. H.pylori can receive signals from gastric epithelium, allowing the host and bacteria to participate in a dynamic equilibrium. However, there are biological effects to this long term relationship.

Chronic inflammation induced by bacteria causes the loss of normal architecture of gastric mucosa with destruction of glandular gastric cells and their replacement by the intestinal type epithelium , pyloric type glands , & fibrous tissue (Atrophic gastritis). There is an increased risk of developing gastric cancer with ATROPHY – METAPLASIA – DYSPLASIA sequence. H.pylori colonisation usually triggers an inflammatory reaction in the *lamina propria*. The grading of activity in gastritis is done according to *MODIFIED SYDNEY SYSTEM*.

There are a number of methods for detection of H.pylori, including the Breath test, Urease test & Culture. But the histological detection in gastric biopsy is the commonest and more sensitive of all methods. This spiral shaped organism can be seen in routine H&E stains, but are more easily detected with special stains such as *Giemsa&, Warthin – Starry silver stain* .None of these are more specific for the organism and the *coccoid forms and* indolent forms are missed out easily in routine stains. More recently, *IMMUNOHISTOCHEMISTRY, IN SITU HYBRIDISATION & PCR has* been proposed as alternative & more specific modalities in detection.

Few studies have been done towards this, and found out that *I H C* against H.pylori is more specific (66% positivity) followed by *Warthin Starry silver* (61% positivity). Hence, *I H C* is more reliable and accurate in the cases of *coccoid* forms and those negative in Genta stains. To extend further, this study is focussed , to *compare* the efficacy of basic staining techniques ,I H C & the histological tissue changes in H.pylori associated Chronic gastritis.

MATERIALS AND METHODS:

In this study, we included gastric biopsies taken from the patients presenting with complaints of dyspepsia, or clinically suspected Helicobacter pylori infection. Sample size is 55, for a period of 1 year duration. Overall there were around 700 endoscopy guided biopsy samples received, of which about 250 were from the ***stomach***, followed by small intestine and esophagus. We maintained a routine confidentiality protocol and proper ethics approval to access the ***ARCHIVES*** OF ***DEPARTMENT OF PATHOLOGY*** in our institution.The samples were taken out randomly and numbered in an order so as to maintain the data for this study.

Once the slides were prepared, we placed them separately segregating them into three stains for each case. They were accompanied by positive controls for the special stain (Modified Giemsa) and for H.pylori Antibody. The controls were used to compare the morphology of bacterium to term them as "positive". The slides were reviewed in the following order: ***H&E, Modified Giemsa and H.pylori Antibody.***

In H&E we looked for the features suggesting the H.pylori infection, inflammation grade (Modified Sydney System) and other reactive changes of the gastric mucosa. Whereas Modified Giemsa was used to correlate the diagnosis along with the supplementation by use of immunohistochemistry.

The slides were observed using LABOMED VISION 2000 microscope.

The representative photomicrographs were taken using microscope (LEICA) and Leica Application Suite.

RESULTS& ANALYSIS:

Using H.pylori antibody, there was ***MARKED*** intensity in staining

and it was easy to pick up these spiral organisms compared to routine stains. The sensitivity and specificity was much higher than the routine H&E and histochemical studies. The time consumption was less and diagnosis was more accurate than the routine stains .Thereby it reduces the risk of reporting false positive cases. This antibody also elicits the organisms residing in deeper mucus glands, situated within the debris and in scant colonization. There are some cases in patients who are on treatment with PPI, the spiral organism, takes up a different shape and structure to hide from the host response; the common being ***coccoid*** forms. The IHC, provokes an **Antigen – Antibody** reaction and lightens up all the H.pylori organisms residing over the gastric mucosa irrespective of the clinical status of the patient.

To concise, 55 clinically suspected and histopathologically diagnosed cases were taken for this study.

We found there is **MALE** predominance, with the age group being 4th to 5th decade, and common site being **GASTRIC ANTRUM.**

In the sections studied, on H&E, there was **MILD** activity in 31 cases out of 55 followed by Moderate activity in around 20 cases.

When H.pylori like organisms was visualized on H&E, Modified Giemsa and Immunohistochemistry we graded the colonization of bacilli on each stain separately.

The Routine stains (H&E and Mod. Giemsa) showed **MILD** colonization in around 37 cases. Whereas there was **MARKED** colonization in majority of cases stained with **antibody**. (34 cases).

The other pathological features were noted, and we found there was an increased lymphoid follicle in around 41 cases, followed by Intestinal metaplasia. There were also mixed overlap in features, many lesions having lymphoid follicles in common with other features in permutations.

CONCLUSION:

Though there are number of methods for detection of H.pylori, Histopathology is always the gold standard. To add it on, use of Immunohistochemistry would increase the specificity and also reduces the false negative results.

Thus by using the H.pylori antibody in routine practice along with Modified Giemsa, the diagnosis could be made easily, more specifically and quickly. In the ***developing countries***, though the feasibility is low due to cost, we would suggest that implementation of ***IHC*** as a routine in near future would help in providing an ***early diagnosis*** and appropriate treatment for H.pylori associated chronic gastritis.

AGE (RANGE)	No. of cases clinically / endoscopically suspected	Positive for H.pylori associated gastritis
10 – 20 yrs	04	04
21 – 30 yrs	03	03
31 – 40 yrs	11	11
41 – 50 yrs	21	21
51 – 60 yrs	14	14
61 – 70 yrs	02	02

Table1. Age predominance

Average predominance of age group is **4th to 5th Decade** and it is about 21 out of 55 cases studied.

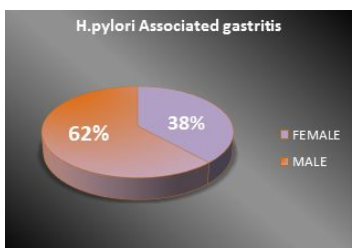

Table2. In the cases studied, there is MALE predominance.

H.PYLORI ANTIBODY	No. Of Cases	Percentage (%)
MILD	03	5.45%
MODERATE	18	32.72%
MARKED	34	**61.81%**

Table3. Grading of gastritis using H.pylori antibody

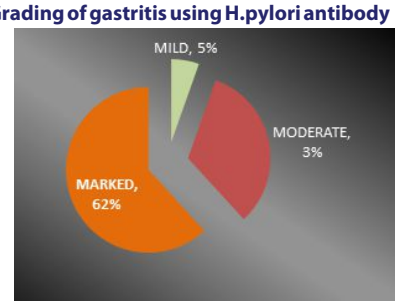

Table 4. COMPARISON OF INTENSITY ON H&E, MODIFIED GIEMSA AND H.PYLORI ANTIBODY

BACTERIAL COLONIZATION	H & E	MODIFIED GIEMSA	H.PYLORI ANTIBODY
MILD	**42 (76.3%)**	**37 (67.2 %)**	**03 (5.4%)**
MODERATE	**09 (16.3%)**	**13 (23.6 %)**	**18 (32%)**
MARKED	**04 (7.27%)**	**05 (9.09%)**	**34 (61.8%)**

Table5. Comparison of the Intensity of staining Histopathological Changes associated with H.pylori infection

Pathology of Gastritis	No. of Cases	Percentage (%)
Lymphoid follicles	**41**	**74.5%**
Intestinal metaplasia	33	60%
Villiform transformation	09	16.3%
Atrophy	nil	nil

Table 6.Histopathological Changes associated with H.pylori infection

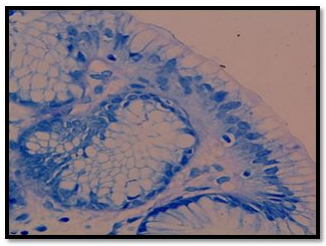

Figure1. H&E (40 x) comma shaped H.pylori like organisms (arrow)

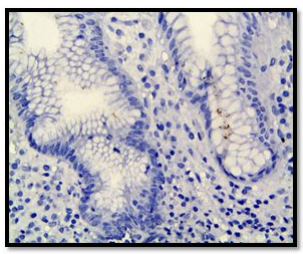

Figure2. H.pylori Ab (40x). Mild colonization

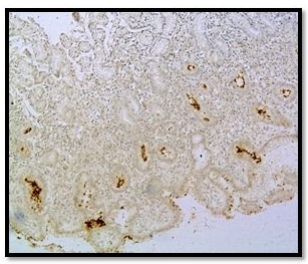

Figure4. H.pylori Ab (10 x) strong, marked (++++) positivity

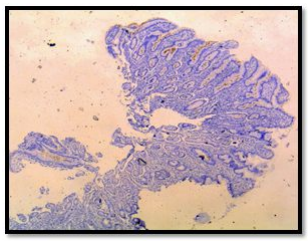

Figure 5. H.pylori Ab (5 x) Shows positivity for H.pylori and villiform transformation

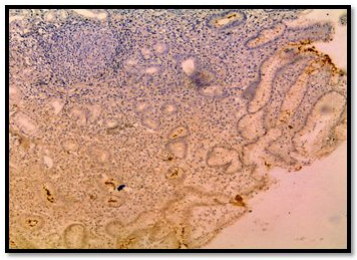

Figure 6. Sensitivity of H.pylori antibody

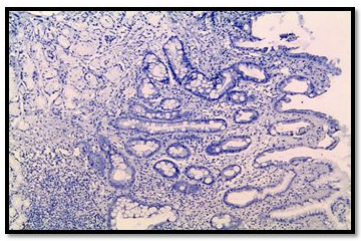

Figure 7. Villiform transformation and Intestinal metaplasia

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