IDENTIFICATION OF HELICOBACTER PYLORI IN GASTRIC BIOPSIES: A COMPARISON OF HAEMATOXYLIN AND EOSIN STAINING WITH IMMUNOHISTOCHEMISTRY

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ABSTRACT

Helicobacter pylori remains widely prevalent today despite modern medical advances.[1] It is responsible for an ever increasing number of gastrointestinal and extra-intestinal diseases.[2] Objective was to compare the efficacy of H&E staining with immunohistochemistry in the detection of H. pylori in gastric biopsies. It was a descriptive study and was carried out in the Pathology Department, Post Graduate Medical Institute, Lahore – Pakistan during the period of 1.2.211 to 30.10. 2011.

Material and Methods: Seventy gastric biopsies were collected from Lahore General Hospital, Lahore. The tissue sections were stained with routine haematoxylin and eosin (H&E) stain and also with H. pylori immunostain. Histopathological examination of the sections was carried out under an optical microscope. The results of histological examination (H&E as well as immuno – staining), and clinical data were subjected to statistical analysis.

Results: Helicobacter pylori was detected by H&E staining in 27.2% of our 70 cases while 72.8% were negative for this. In 20% cases there was mild colonisation where in 7.1% this was moderate. Immunohistochemistry carried out with Helicobacter pylori immunostain yielded 31.4% positive cases while 68.6% were negative. Among the 70 cases there were 64.3% males and 35.7% females. The maximum number of cases were found to be in the age group of 20 – 29 years and was 18 or 25.71%. This was followed by age groups 30 – 39 years and 50 – 59 years each of which had 12 (71.14%) patients. Age was compared in male and female patients. There was no significant age difference among males and females. A comparison between mean age in H. pylori positive and negative cases was also carried out. There was no significant correlation between age and H. pylori detection. A comparison between sex distribution and H. pylori positive and negative cases did not yield any significant difference between males and females.

Conclusion: Histopathological examination of gastric biopsies is still an accurate and efficient means of diagnosis. Immunostaining increases the diagnostic yield.

Key words: Helicobacter pylori, gastritis, immunohistochemistry.

INTRODUCTION

Helicobacter pylori remains widely prevalent today despite modern medical advances.[1] It is responsible for an ever increasing number of gastrointestinal and extra-intestinal diseases.[2] Over 50% of the world's population is infected with H. pylori. The infection was acquired in childhood and often persisting throughout life. This makes it one of the most prevalent infectious diseases in the world.[3] Incidence is high in developing countries while it is decreasing in Western countries.[4]

In Pakistan, the majority of patients having undergone endoscopy for dyspepsia are positive for H. pylori; this was particularly so in patients belonged to lower socio-economic groups in a study in Karachi.[5] In a study carried out in Peshawar in 2008, 100% patients with duodenal ulcer were positive for H. pylori while 84% of those with chronic gastritis were H. pylori positive.[6]

Numerous gastro-intestinal diseases have been found to be caused by / associated with H. pylori.[7] This organism has been implicated in gastritis, peptic ulcer, non-ulcer dyspepsia; gastric adeno-carcinoma and non-Hodgkin lymphoma arising in MALT mucosa associated lymphoid tissue (Leontiadis et al 1999). Now studies have also linked it to gastro-oesophageal reflux disease (Khan et al 2010). H. pylori is being held responsible for an ever – increasing number of benign, pre-malignant and malignant disease. In this scenario the accurate diagnosis / identification of H. pylori becomes imperative so that prompt treatment of H. pylori positive patients can be instituted. Eradication of this pathogen can avert considerable morbidity while preventing potential

malignancy.[9]

Diagnostic methods for H. pylori identification have been divided into two broad groups: Invasive and Non-invasive.[10] The invasive methods are based mainly on tissue obtained by gastric biopsy. Despite the emergence of non-invasive tests, gastric biopsy with histology, culture, and rapid urease test remains the gold standard for H. pylori.[11]

Although providing good histopathological detail, haematoxylin and eosin staining can miss H. pylori when they are present in low numbers.[12] Using H&E staining there is insufficient contrast between the background and the microorganism since the sensitivity is low; specificity is also low due to non-specific staining of the commensal gut bacteria.[13]

Hence the use of special stains is recommended to facilitate detection of H. pylori.[14] A vast number of stains are available. These include Warthin Starry, Modified Giemsa, Acridine orange, Cresyl violet, Giemenez, H. pylori silver stain and Modified Genta stain.[15] Silver stains such as Warthin Starry are useful for H. pylori identification since they give better results than H&E; however, interpretation might be difficult due to their tendency to precipitate.[14] Hence in current laboratory practice H. pylori immunohistochemistry is considered to be the gold standard for tissue identification of H. pylori.[16]

Immunohistochemistry (IHC) makes use of anti H. pylori antibody which reacts with somatic antigens of the whole bacteria and have been found to correlate well with the presence of the bacteria.[14] In all current studies IHC has the highest sensitivity and specificity, usually 100%.[16]

In biopsy specimens negative for H. pylori by H&E and special stains, H. pylori can be demonstrated by IHC.[17] IHC can be used in treated MALT lymphoma patients to confirm eradication of H. pylori.[18] H. pylori organisms that remain despite triple – therapy are usually not seen by special stains; these can be visualized with IHC.[19] IHC is able to detect low numbers of H. pylori and can also identify its coccoid forms.[18] A semi-quantitative assessment of H. pylori colonisation can be made by IHC.[15]

MATERIALS AND METHODS

Tissue specimens were collected in properly labelled jars containing 10% formalin solution. Proforma was attached to each case. Detailed gross examination of the specimens was carried out and recorded on the proforma.

Tissue Processing: The gastric biopsy specimens were placed in an automated tissue processor. After processing, embedding of tissues was done in paraffin wax. 3 – 4 μm thick tissue sections were cut by a rotary microtome.

Microscopic Examination: H&E staining was used on the tissue sections for histological examination. The prepared slides were examined using CX 31 Olympus microscope by two observers and the results were entered in the proforma.

H. Pylori Immuno-staining

Immuno-staining for H. pylori was done according to the ABCAM – company instructions and following the DAB method.

Principle and Procedure: Antigen retrieval was carried out by boiling tissue sections in 10 mM Citrate buffer, pH 6.0 for 10 – 20 minutes followed by cooling at room temperature for 20 minutes. The tissue sections were incubated with H. pylori primary antibody (SPM 526), biotinylated linking antibody and labeling reagent (streptavidin – biotin complex). A chromogen reagent (DAB) was added to the tissue sections. The chromogen reagent was converted to insoluble dark brown and product by the labelling reagent. This made the entire antibody complex visible in the tissue sections.

Microscopic Examination: The prepared slides were examined using a CX 31 Olympus microscope by two observers and the results were noted in the Proforma. The results of H. pylori immuno-staining were based on the distinct brown staining.

RESULTS

Helicobacter pylori was detected by H&E staining in 27.2% of our 70 cases while 72.8% were negative. In 20% cases there was mild colonisation whereas in 7.1% this was moderate. Immunohistochemistry carried out with Helicobacter pylori immunostain yielded 31.4% positive cases while 68.6% were negative (Table 1). Among our 70 cases there were 64.3% males and 35.7% females. Age was compared in male and female patients (Table 2). There was no significant age difference among males and females. A comparison between mean age in H. pylori positive and negative cases was carried out (Table 3). There was no significant correlation between age and H. pylori detection. A comparison between sex distribution and H. pylori positive and negative cases ac-

Table 1: *Comparison of H. pylori immunohistochemistry and H&E staining.*

	IHC Positive	*IHC Negative*	*Total*
H&E positive	18	1	19
H&E negative	5	46	51
Total	23	47	70

Sensitivity: 78.3%, Specificity: 97.9%, PPV: 94.7%
NPV: 90.2%, Accuracy: 91.43%

cording to Table 4 did not yield any significant difference between males and females.

Table 2: *Comparison of age in males and females.*

Variable	*Number of Cases*	*Age in Years Mean ±SD*
Male	45	44.0 ± 17.92
Female	25	41.0 ± 20.50

P = not significant

Table 3: *Comparison of mean age in H. pylori IHC positive and negative patients.*

Variable	*Number of Cases*	*Age in Years Mean ±SD*
H. pylori positive	22	45.9 ± 17.60
H. pylori negative	48	41.7 ± 18.00

P = not significant

Table 4: *Comparison of sex distribution in H. pylori IHC positive and negative patients.*

	H. pylori Positive	*H. pylori Negative*	*Total*
Male	13	22	35
Female	9	16	25
Total	22	38	70

P = not significant

DISCUSSION

The role of H. pylori as a Grade I carcinogen in the gastro-intestinal tract has been established. Numerous studies have elucidated the role of H. pylori in the causation of gastric adenocarcinoma and gastric MALT lymphoma making it imperative to identify the presence of this organism in gastric tissue.[20]

A myriad of invasive and non-invasive tests are available for the detection of H. pylori and its antibodies in various specimens like blood, saliva, urine and stool. Despite these advances, histo-pathological examination of gastric biopsy remains the gold standard against which other tests can be compared.[16,21-23] H. pylori can be visualized in a well – stained routine H&E (H&E section, however detection becomes easier and more accurate using H. pylori immuno – stain.[17] Found in their study that the sensitivity and specificity of H. pylori detection by H&E was 97% and 80% respectively whereas by immunostaining both these parameters became 100%.[16]

H. pylori can be detected in the surface mucous layer as well as in the foveolae. When the bacterial density is high routine H&E staining is sufficient to demonstrate them.[12] However when micro-organisms are sparse special stains may be required. It has been found in many studies that immuno-histochemistry using H. pylori immuno-stain is superior to H&E as well as special stains for identifying these bacteria.[14]

In our study 18 (25.71) cases were positive for H. pylori by H&E staining and 23 (32.9%) were positive by immuno-staining. In research done by[17] as early as it was found that H. pylori was detected in 38% patients by H&E and in 58% patients by immuno-histochemistry. In the study of H. pylori was identified by H&E in 70% of biopsies and by immunohistochemistry in 78%.[24] It was also seen that the bacteria were more prominent and much easier to identify in the immunostained sections. In our study the sensitivity of H. pylori detection by immunohistochemistry was 78.3% and specificity was 97.9%. Found a sensitivity of 100% and a specificity of 100% using immunostaining.[16]

Our study showed a positive H. pylori infection rate of 23 (32.9%) by immunostaining. Wabinga reported H. pylori immunopositivity to be 67%.[14] The study of Afzal et al., showed a figure of 70%.[21] Khan et al.,[6] found 100% H. pylori in all their cases of chronic gastritis while Langner et al.,[25] reported this bacterium in 97.7% cases. Nwokediuko and Okafur[26] observed H. pylori to be positive in 36.3% patients and also reported that although some studies show a higher prevalence of H. pylori in patients with gastritis and non-ulcer dyspepsia the results are not consistent. However they went on to mention that dyspepsia and gastritis always occur after experimental ingestion of H. pylori.

H. pylori detection rate in our study is lower than most reported studies. It has been seen that the use of proton pump inhibitor drugs reduces the diagnostic yield of H. pylori.[27] Most of the patients in this study were illiterate and did not know the treatment they had received. Although they did not give history of proton pump inhibitor intake this was not reliable and consequently could account for the low detection rate in our study.

There was no significant age difference between males and females (Table 1). This was similar to the findings of El-Sayed et al.[28]

In this study of 70 cases, the maximum number of patients fell in the age group 20 – 29 years and was 18 or 25.71%. Kacar et al.,[16] found the maximum number of cases in the age group 60 – 69 years. In our study the difference in age distribution among males and females was not statistically significant (Table 2). This is in accordance with the findings of Nwokediuko and Okafur.[26] Our study showed that H. pylori positive patients had a mean age of 45.9 years. This was greater than the mean age of H. py-

lori negative patients who were 41.7 years (Table 4).

No statistically significant correlation was found between age and H. pylori detection in our cases by making a comparison between mean age in H. pylori positive and negative patients. Similarly Khan found that there was no statistically significant difference in H. pylori detection between younger and older patients. A comparison of sex distribution between H. pylori positive and negative cases was carried out in our study (Table 4) but there was no statistically significant difference among males and females. In a study carried out by Khan in Saudi Arabia it was found that both sexes were equally affected but Khan also reports that studies in developed countries found a higher incidence in females.

Our study however showed Helicobacter pylori detection to be 25.7% with H&E staining and 32.9% with immunostaining. Immunohistochemistry improves the diagnostic yield by making visualisation of bacteria easier so that they can even be detected in low numbers.[18] It is important to accurately detect H. pylori in gastric tissue since it is predicted that eradication of this organism will lead to reduction in the incidence of gastric carcinoma. There will also be a decrease in the incidence of gastric MALT lymphoma.[30] Several effective regimens against H. pylori are available. Their efficacy depends on precise identification of these organisms.[31] Histological identification of Helicobacter pylori in gastric biopsy sections remains the gold – standard.[23]

This study *concludes* that among the 70 gastric biopsies. The histopathological examination of gastric biopsies is still an accurate and efficient means of diagnosis. Although it is an invasive method, its advantages make it justifiable. In some patients with chronic gastritis who are negative for H. pylori with routine staining, this organism can be detected with immunostaining. The use of H. pylori immunohistochemistry hence allows precise and unequivocal diagnosis of gastric biopsies. It also improves the diagnostic yield. The precise identification of Helicobacter pylori allows the institution of specific treatment directed at eradication of this organism. Furthermore, vaccines are being developed that prevent the acquisition of Helicobacter pylori infection. It is to be anticipated that eradication and vaccination against potentially carcinogenic Helicobacter pylori will markedly reduce the incidence of both gastric adeno-carcinoma and gastric MALT lymphoma in the future.

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