

Study of 111 Consecutive Cases of Gastric Biopsies Considering Age, Sex, Degree of Inflammation and Effects of Different Staining Methods for Helicobacter pylori Infection

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SUMMARY

The objective of this study was to determine the prevalence of Helicobacter pylori infection in different age and sex group with associated degree of inflammation and effects of different staining methods in our settings. We studied 111 consecutive endoscopic biopsies over a period from March 1996 to August 1996. The presence or absence of H. pylori was tabulated according to age and sex of the patients. Thirty-Nine (35%) of 111 patients were positive for H. pylori. There were 56 males, among whom 17 (43.58%) were positive. There were 55 females, among whom 22 (56.42%) were positive for H. pylori. The presence of H. pylori in various age group was as follows: 21-30 years, 27.77%; 31-40 years, 38.88% 41-50 years, 27.77% and above 51 years, 13.88%. Reading the degree of inflammation, 8 (20.15%) patients showed mild degree of inflammation, 27 (69.23%) showed moderate degree of inflammation and 4 (10.25%) showed severe degree of inflammation. Regarding staining methods for evaluation of H. pylori, 33 patients (84.61%) showed positivity for H. pylori on Haematoxylin and Eosin staining and 39 (100%) showed positivity for H. pylori on Giemsa staining method.

INTRODUCTION

Helicobacter pylori is now well recognized as the major cause of peptic ulcer disease and as an important risk factor for the development of gastric adenocarcinoma and lymphoma¹. The presence of spiral-shaped microorganisms in stomach mucosa was described almost 100 years ago. Their presence was not really taken seriously until late 1970s, when Jhon Warren, a pathologist in Perth, Western Australia, noted the appearance of spiral bacteria overlying gastric mucosa, and chiefly over inflamed tissue². Warren and Barry Marshall cultured these organisms in 1982 from 11 patients with gastritis; the story of the early part of the discovery of H. pylori is related to Marshall and is worth reading because of that³.

Originally called Campylobacter pyloridis, the name was changed to Campylobacter pylori and then later to Helicobacter pylori as specific

morphologic, structural and genetic features indicate that it should be placed in a new genus³. This organism is a motile, gram-negative, curved rod which is oxidase, catalase and urease positive.

Marshall and Warren were able to demonstrate a strong association between the presence of H. pylori and the finding of inflammation in gastric biopsy³. People who did not have gastritis did not have the organism, a finding confirmed in a number of studies⁴. Marshall elegantly fulfilled Koch's postulate for the role of H. pylori in antral gastritis with self-administration of H. pylori, and also showed that it could be cured by use of antibiotics and bismuth salts⁵.

A wide body of evidence has indicated that chronic gastritis is linked to the development of adenocarcinoma of the stomach, the most important gastric malignancy in the world⁶, but the causation of the gastritis was then unknown. In 1991, four reports first showed associations between H. pylori

infection and the presence⁷ or development⁸ of gastric cancer. In 1994, the International Agency for Cancer Research, an arm of the World Health Organization, reviewed the available evidence and declared that *H. pylori* was a carcinogen of humans⁹.

H. pylori infection also has been associated with the development of gastric non-Hodgkin's lymphomas⁶ and with lymphoproliferative disorder, gastric mucosa-associated lymphoid tissue (MALT) lymphoma (MALToma)¹⁰.

Importantly, treatment of gastric MALToma patients with antibiotics that eradicate *H. pylori* often leads to regression of the tumor¹¹. Thus in total, *H. pylori*, a previously obscure organism, has now been associated with many of the most important diseases involving gastro duodenal tract.

Histological examination of tissue biopsy samples (usually four, taken from different parts of the stomach lining) permits detection of the bacterium together with evaluation of tissue damage. Most infection can be detected with hematoxylin & eosin (H&E) stain of gastric tissue, but special stains like Giemsa or Silver Stain can be used if H&E results are not conclusive¹².

This article elaborates and evaluates the study of 111 consecutive gastric biopsies considering age, sex, degree of inflammation and effects of different staining methods.

MATERIAL AND METHODS

From March 1996 to August 1996, the Histopathology laboratory of Sheikh Zayed Hospital received 111 endoscopic biopsies for documentation of *H. pylori*. These patients were referred to gastroenterologists for upper abdominal pain, dyspepsia, or previous history of peptic ulcer disease. Two / Three endoscopic specimens were taken from the gastric antrum of each patient. All the endoscopic biopsies were fixed in 10% formalin and were routinely stained with hematoxylin and eosin. When the organism could not be identified by the routine stains, additional special stain was performed, such as Giemsa stain. The histopathology reports of the age and gender of the patients and presence or absence of *H. pylori*. Degree of inflammation was considered and the

comparison of both H&E and Giemsa stain were made. The results were statistically analyzed by the chi-squared method.

RESULTS

Of 111 cases. 39 patients were positive, giving an overall frequency of *H. pylori* infection of 35%. There were 56 males and 55 females in total, out of which 17 males (43.58%) and 22 females (56.42%) were positive for *H. pylori* (Table 1). Regarding age distribution, the ages of the patients were divided in four categories. Among the 21- 30 years age group 10 patients were positive for *H. pylori*, in 31-40 years age group 14 in 41-50 years age group 10 and in 51 above 50 patients showed positivity for *H. pylori*. The percentage frequencies in various age group were thus as follows: 21-30 years, 27.77%; 31-40 years, 38.88%; 41-50 years, 27.77% and above 51 years, 13.88% (Table 2).

Table 1: Sex distribution for total positive cases (n=39)

Sex	Number	Percent
Males	17	43.58
Females	22	56.42
Total	39	100

Table 2: Age distributaries for total positive cases (n=39)

Age (Years)	Number	Percent
21-30	10	27.77
31-40	14	38.88
41-50	10	27.77
50 >	05	13.88
Total	39	100

Regarding the degree of inflammation, 27 (69.23%) showed moderate degree of inflammation and 4 (10.25%) showed severe degree of inflammation (Table 3). Regarding staining methods evaluation of *H. pylori*, 33 patients (84.61%) showed positivity for *H. pylori* on Haematoxylin and Eosin staining and 39 (100% showed positivity for *H. pylori* on giemsa staining method (Table 4).

Table 3: Degree of inflammation for total positive cases (n=39)

Degree of Inflammation	Number	Percent
Mild	08	20.15
Moderate	27	36.23
Severe	04	10.25
Total	39	100

Table 4: Effects of histochemical stains.

Histochemical Stains	Number	Percent
H & E	33	84.61
Giemsa	39	100

DISCUSSION

The gold standard for diagnosis of *H. pylori* infection is considered to be culture or histologic assessment of the organism's presence. Infection with *H. pylori* increases the risk for gastric, adenocarcinoma up to 9-fold and is the major cause of low-grade gastric mucosa associated lymphoid tissue (MALT) lymphomas.

Our study evaluated the frequency of *H. pylori* infection with associated factors. Highest numbers of positive patients were seen in the 31 to 40 years of age category. In one study done abroad the highest risk age group was above 50. however, it is known that *H. pylori* infection occurs mainly in early childhood and the link between the infection and risk factors such as socioeconomic status and living conditions in childhood is well documented¹³. Thus, there is a marked difference in the prevalence of *H. pyloric* infection between developing and developed countries during early childhood¹⁴.

However, every where in the world, serological data have shown that the prevalence of the infection increases with age¹⁵. In particular in Greece, according to a study carried out in the early '90s¹⁶ sero-prevalence increased about 10% per 10 years, from 40% in people aged 21-40 years to 77% in those older than 60 years¹⁷.

More females were affected than males in our study. However in most studies done abroad, *H. pylori* infection affects both sexes to the same extent¹⁵. Most of the patients showing moderate

degree of inflammation were positive for *H. pylori*.

When *H. pylori* are present, careful examination will almost always reveal these, whichever of these stains is used. However, the modified Giemsa stain is the method of choice because it is sensitive, cheap, easy to perform, and reproducible. In our study, Giemsa proved to be more reliable staining methods than H&E. However new staining methods are being evaluated, the modified McMullen's, Helicobacter Pylori silver stain HpSS methods and anti-*H. pylori* antibody immuno-stain are also being implicated for *H. pylori* staining^{18,19}.

The true prevalence appears to be increasing as more sensitive and specific screening techniques and developed.

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