

## RELATION BETWEEN HELICOBACTER PYLORI, INFLAMMATORY (NEUTROPHIL) ACTIVITY, CHRONIC GASTRITIS, GASTRIC ATROPHY AND INTESTINAL METAPLASIA

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### ABSTRACT

**Background:** To determine the relation of Helicobacter pylori infection with chronic inflammation, atrophy, activity level and intestinal metaplasia.

**Patients and Methods:** A cross-sectional study of 100 consecutive patients with dyspepsia. These patients were fasted for 12 hours and gastroscopic biopsy specimens were obtained from their gastric mucosae. The specimens were histologically evaluated for H. pylori, inflammatory activity, chronic inflammation, gastric atrophy and intestinal metaplasia.

**Results:** There were 50(50%) females and 50(50%) males. The average ages of women and men were 36.3±11.5 and 42.9±12.8 respectively. Helicobacter pylori was found in 79%. Neutrophil activity was observed in 83%. Inflammation was found in 95%, glandular atrophy in 38%, intestinal metaplasia in 28% of the cases. Incidental (early gastric) cancer was found in 3%, dysplasia in 2% and reactive gastropathy in 7% of the cases.

A statistically significant relationship was found between Helicobacter pylori colonization intensity and the degrees of neutrophil activity, chronic inflammation and intestinal metaplasia.

**Conclusion:** We concluded that Helicobacter pylori infection results in neutrophil activation and chronic gastritis, and that it has a role in the development of intestinal metaplasia. The greater the intensity of Helicobacter pylori infection, the greater the degrees of neutrophil activation, chronic gastritis and intestinal metaplasia.

**Key Words:** Helicobacter pylori, Gastritis, atrophy, Intestinal metaplasia, Gastric cancer, Jos (Accepted 7 May 2007)

### INTRODUCTION

The relation of H. pylori to various gastric lesions has been the subject of many studies. In most of these studies, the correlation between H. pylori and the development of these lesions has been established.<sup>1,2,3,4</sup> H. pylori infection varies among countries and even among different regions in one country. In Africa, it varies from 74% in Rwanda,<sup>5</sup> 84% in Northeastern Nigeria<sup>6</sup> to 85% in Kenya.<sup>7</sup> Helicobacter pylori infection is the most common bacterial infection of humans worldwide.<sup>8,9</sup> An estimated 50% of the world's population is infected by Helicobacter pylori.<sup>10</sup> It is a Gram-negative, curved motile bacterium which is able to live in acidic environment.

Although at the time of its discovery, there was initial controversy over whether it was an opportunistic commensal bacillus, today it is agreed that H. pylori plays an important role in the aetiology of duodenal and gastric ulcers, gastric atrophy, intestinal metaplasia, gastric adenocarcinoma and mucosa

associated lymphoid tissue (MALT) lymphoma.<sup>10,11,12</sup> About 20% of those infected are symptomatic while the remaining 80% are asymptomatic.<sup>13</sup> Histological gastritis is a universal feature of H. pylori infection whether the patients have symptoms or not.<sup>10</sup> Although chronic gastritis (with lymphoid follicles) is the hallmark of H. pylori infection<sup>14</sup>, the later clinical manifestations are variable. Since the risk of gastric cancer increases in people with H. pylori infection compared to those who are not H. pylori infected, and because the incidence of gastric cancer would be high over time in individuals with H. pylori infection, the relation between H. pylori infection and gastric cancer has been investigated. Furthermore, the World Health Organization (WHO) has recently classified H. pylori as a "class 1 carcinogen".<sup>15</sup> Other factors such as genetic and nutritional have been suggested as explanations for why gastric cancer develops in only a small proportion of those infected with H. pylori. The sequence of events in gastric carcinogenesis is assumed to progress from chronic gastritis, gastric atrophy, intestinal metaplasia, dysplasia and finally to gastric carcinoma.

It is known that H. pylori can be involved in the chain

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of these chronic phenomena, at least early in its evolution, but it is not certain whether the continuous presence of *H. pylori* is required for progression to cancer.<sup>14</sup>

The aim of this study was to investigate the relation of *H. pylori* with chronic atrophic gastritis and intestinal metaplasia.

## MATERIALS AND METHODS

This study was a cross-sectional survey carried out at the Jos University Teaching Hospital, Jos. Endoscopic biopsy specimens from the gastric mucosa of 100 consecutive patients were histologically evaluated for the intensity of *H. pylori* colonization, the degrees of inflammatory (neutrophil) activity, chronic inflammation, gastric atrophy and intestinal metaplasia. These patients were recruited from the gastroenterology clinic between the months of February and August 2004, and who had not been treated with eradication therapy for *H. pylori*, or had previous gastric procedures (endoscopy or surgery), were included in the study. Three biopsies were taken from each patient under aseptic conditions; one each from the antrum, the incisura angularis and the corpus. Wide channel Olympus GIF-P30 endoscope and the fenestrated biopsy forceps Olympus FB-21K-1 were used to take the biopsies. The biopsy samples were fixed immediately in 10% buffered formalin in separate containers and labeled accordingly and then delivered to the histopathology laboratory. The tissues were subjected to routine procedures of tissue processing and afterward embedded in paraffin wax. Cross sections 3-4 microns thick were taken from paraffin blocks and then deparaffinized by heating at 60°C for ten minutes followed by three washes in xylene. They were rehydrated in graded alcohol concentrations. They were stained with haematoxylin and eosin. The slides were evaluated under light microscope. Giemsa stain and Periodic-Acid-Schiff/Alcian Blue stains were applied where necessary to determine the presence of *H. pylori* and intestinal metaplasia respectively.

The histopathological examination was done using the criteria of the updated Sydney system (Table I).<sup>7</sup> All the variables were graded independently on a 4-point semiquantitative scale: nil, mild, moderate and marked. The relation of *H. pylori* with neutrophil activity, chronic inflammation, atrophy and intestinal metaplasia was determined. The statistical data were analyzed using EPI Info 2002 computer software program. Chi-square test was employed for test of significance. A value of  $P < 0.05$  was accepted as significant.

## RESULTS

The sex distribution showed that there were 50 (50%) males and 50 (50%) females. The mean

ages of females and males were  $36.3 \pm 11.5$  S.D and  $42.9 \pm 12.8$  S.D respectively. The age distribution showed that the females were significantly younger than the males. ( $P < 0.008$ ).

## The Relation Of *H. Pylori* With The Degree Of Neutrophil Activity

*Helicobacter pylori* was detected in 79 (79%) of all the cases; the remaining 21(21%) were *H. pylori* negative. Eighty three (83%) cases had neutrophil activity with 4 (4.8%) being negative for *H. pylori*.

Among the 79 *H. pylori* positive cases, 37 (46.8%) had mild, 34 (43%) had moderate, and 8 (10.2%) had marked *H. pylori* colonization respectively.

Of the 37 cases with mild colonization, mild and moderate neutrophil activity was found in 21 (56.8%) and 16 (43.2%) cases respectively.

Of the 34 cases with moderate *H. pylori* colonization, 4 (11.8%), 25 (73.5%) and 5 (14.7%) had mild, moderate and marked neutrophil activity in that sequence.

Among the 8 cases with marked *H. pylori* colonization, 1 (12.5%) and 7 (87.5%) had moderate and marked neutrophil activity respectively.

The correlation between the intensity of *H. pylori* colonization and the degree of neutrophil activity was highly significant. ( $P < 0.0001$ ), Table II.

## The relation of *H. pylori* with chronic inflammation

Chronic inflammation was found in 95 (95%) of all cases. Seventy-nine (83.2%) were positive for *H. pylori* while 16 (16.8%) were *H. pylori* negative. Of the 79 *H. pylori* positive cases, 37 (46.8%), 34 (43%) and 8 (10.2%) had mild, moderate and marked *H. pylori* colonization respectively. Among the 37 cases with mild *H. pylori* colonization, 5(13.5%), 24 (64.9%) and 8 (21.6%) had mild, moderate and marked chronic inflammation in that order.

Of the 34 cases with moderate *H. pylori* colonization, 1 (3%), 10 (29.4%) and 23 (67.6%) had mild, moderate and marked chronic inflammation in that sequence. The 8 cases with marked *H. pylori* colonization had 1(12.5%) moderate and 7 (87.5%) marked chronic inflammation respectively.

The statistical analysis by chi-square test showed a highly significant relationship between the intensity of *H. pylori* colonization and the degree of chronic inflammation. ( $P < 0.0001$ ), Table III.

## The relation of *H. pylori* with glandular atrophy

Overall, 38 (38%) of the cases had chronic atrophic gastritis and 72 (72%) were negative for atrophy. Of the 38 cases with glandular atrophy, 31 (81.6%) were positive for *H. pylori* while 7 (18.4%) were negative for *H. pylori*. Among the 31 *H. pylori* positive cases,

13 (41.9%), 14 (45.2%) and 4 (12.9%) had mild, moderate and marked *H. pylori* colonization intensity respectively. Of the 13 cases with mild *H. pylori* colonization, 8 (61.5%) and 5 (38.5%) had mild and moderate glandular atrophy respectively. Among the 14 cases with moderate *H. pylori* colonization, 5 (35.7%) and 9 (64.3%) had mild and moderate atrophy in that order. Of the 4 cases that had marked *H. pylori* colonization, 1 (25%), 2 (50%) and 1 (25%) had mild, moderate and marked atrophy in that sequence.

The statistical test by chi-square test suggested no significant relationship between the intensity of *H. pylori* colonization and degree of atrophy. ( $P < 0.4306$ ), Table IV.

Table 1: The Updated Sydney System

Histologic Feature	Definition	Grade		
		mild	moderate	marked
<i>H. pylori</i>	H.p intensity in Epithelium	+	++	+++
Activity	Neutrophil infiltration In lamina propria or Superficial epithelium	<1/3	1/3-2/3	>2/3
Chronic inf.	Lymphocytes & plasma Cells in the lamina propria	+	++	+++
Gland atrophy	Loss of corpus and antral Glands	+	++	+++
I. M	I.M in mucosal epithelium	<1/3	1/3-2/3	>2/3

H. p = *Helicobacter pylori*

I. M = Intestinal metaplasia

Table II: The Relation of the Intensity of *H. Pylori* Colonization with Degree of Neutrophil Activity.

H. Pylori	Neutrophil Activity				Total
	Nil	Mild	Moderate	Marked	
Nil	17	4	0	0	21
Mild	0	21	16	0	37
Moderate	0	4	25	5	34
Marked	0	0	1	7	8
Total	17	29	42	12	100

( $X^2 = 144.00, P < 0.00001$ )

Table III: Relation of the Intensity of *H. Pylori* Colonization with the Degree of Chronic Inflammation.

H. PYLORI	CHRONIC INFLAMMATION				Total
	Nil	Mild	Moderate	Marked	
Nil	5	11	4	1	21
Mild	0	5	24	8	37
Moderate	0	1	10	23	34
Marked	0	0	1	7	8
Total	5	17	39	39	100

( $X^2 = 53.15, P < 0.00001$ )

Table IV: Relation of *H. Pylori* Colonization Intensity and Degree of Glandular Atrophy.

H. Pylori	Glandular Atrophy				Total
	Nil	Mild	Moderate	Marked	
Nil	14	2	3	2	21
Mild	24	8	5	0	37
Moderate	20	5	9	0	34
Marked	4	1	2	1	8
Total	62	16	19	3	100

( $X^2 = 9.07, P < 0.43$ ).

Table V: Relation of *H. Pylori* with Intestinal Metaplasia.

H.Pylori	Intestinal Metaplasia				Total
	Nil	Mild	Moderate	Marked	
Nil	12	7	8	6	33
Mild	23	3	1	1	28
Moderate	31	2	0	0	33
Marked	6	0	0	0	6
Total	72	12	9	7	100

( $X^2 = 14.82, P < 0.02$ )

## DISCUSSION

Non-human reservoirs for *H. pylori* have been suggested since its first description in 1983 by Warren and Marshall.<sup>16,17,18</sup> However, it was only recently that isolation of gastric *helicobacter*-like organisms (GHLOs) from inflamed gastric mucosa of domestic cats and farmyard animals and the ability to experimentally infect cats has raised the possibility of zoonotic infection. GHLOs commonly noted with cats and dogs are associated with approximately 0.08%-1% of gastritis in humans.<sup>15</sup> These GHLOs often infect patients who own pets suggesting a zoonotic link.<sup>19</sup> *H. pylori* is believed to be transmitted from person to person by oral-oral, gastric-oral or faeco-oral routes.

Antral gastritis, gastric and duodenal ulcers, gastric cancer and mucosa associated lymphoid tissue (MALT) lymphoma are included in the group of diseases in which *H.pylori* plays a definite role. *H. pylori* has also been cited in some studies as playing a possible role in non-ulcer dyspepsia, retardation in children, coronary heart disease and Menetrier's disease.<sup>10</sup>

It is assumed that the sequence of events in gastric cancer is as follows: chronic gastritis, atrophy, intestinal metaplasia, dysplasia and carcinoma and that *H. pylori* is involved in the chain of these chronic phenomena.

According to our data, neutrophil activity was found in 100% of the *H. pylori* infected group and none in the non-infected group. However, 4 patients with

neutrophil activity were found to be negative for *H. Pylori*. Neutrophils are a very sensitive indicator of the presence of *H. pylori* and the hallmark of its diagnosis. They disappear within days of the cure of infection. If neutrophils are detected in the lamina propria and intraepithelial and *H. pylori* is not seen, sampling error may have occurred due to the spotty nature of the infection or the organism has recently been eradicated. Neutrophil activity is linked with tissue damage due to the release of neutrophil-derived reactive oxygen radicals and proteases.

A statistically significant relation was found between the degree of *H. pylori* colonization and the intensity of neutrophil activity ( $P < 0.00001$ ). This finding is concordant with that of Sasa *et al.*<sup>20</sup>

We also found that the degree of chronic inflammation was directly related to the intensity of *H. pylori* colonization ( $P < 0.00001$ ). This result is found concordant with the results of two different studies performed by Kenji *et al.*<sup>21</sup> and Sipponen *et al.*<sup>22</sup> *Helicobacter pylori* infection is always associated with chronic gastritis. The normal stomach mucosa lacks organized lymphoid tissue, so the presence of a marked infiltrate of chronic inflammatory cells usually indicates the presence of *H. pylori* or lymphoma, although chronic inflammatory cells may persist for up to one year after *H. pylori* eradication.

In our study, we did not find a significant relationship between *H. pylori* and atrophy ( $P < 0.4306$ ). This finding is concordant with that of Sasa *et al.*<sup>20</sup> but not with the findings of Kenji *et al.*,<sup>21</sup> Alaska *et al.*<sup>23</sup> and Topal *et al.*<sup>10</sup> Nevertheless, atrophy was found more frequent in the *H. pylori*-positive than *H. pylori*-negative cases. It might be suggested that *H. pylori* causes atrophy but does not colonize atrophic areas. In our study, *H. pylori* colonization was not observed in glands with atrophy but *H. pylori* colonization was focally observed in non atrophic glands within the same histologic sections. This may explain the lack of correlation in our study and that of Sasa *et al.*<sup>20</sup> It is known that atrophic glands are depleted of mucus secretion and thus might provide no suitable environment for the continuous colonization of *H. Pylori*.

A significant relationship was however found between *H. pylori* and intestinal metaplasia ( $P < 0.0216$ ). This finding agrees with the findings of two studies that were stated previously<sup>21,23</sup> but not concordant with the results of Sasa *et al.*<sup>20</sup> and Topal *et al.*<sup>10</sup> who found a negative correlation between the degree of *H. pylori* colonization and the frequency of intestinal metaplasia.

As with atrophy, *H. pylori* causes intestinal metaplasia but does not colonize metaplastic epithelium, but it may colonize sections of the same biopsies without intestinal metaplasia. All of the 28 cases of intestinal metaplasia were seen in a background of chronic atrophic gastritis.

When atrophy was related to intestinal metaplasia in our study, a significant relationship was found ( $P < 0.0001$ ), suggesting that the presence of *H. pylori* is an important factor in the development of atrophy and intestinal metaplasia.

We therefore conclude that *H. pylori* causes neutrophil activity, chronic inflammation, gastric atrophy and intestinal metaplasia, all intermediate lesions in the process of gastric carcinogenesis. However, due to its paucity in atrophic and metaplastic glands, its continuous presence in the gastric mucosa may not be necessary for progression to cancer once significant atrophy has occurred. Larger multicentre prospective studies may be required to prove these assumptions.

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