The Role Of Helicobacter Pylori And Cag A Antibody Titers In The Pathology Of Chronic Gastritis

N Moorchung, A Srivastava, N Gupta, S Bandopadhyay, B Achyut, B Mittal

Citation

Abstract
Chronic gastritis is a multifactorial disorder, which is influenced primarily by the bacterium Helicobacter pylori. In addition to the density of the organism, the CagA pathogenicity island (PAI) of H pylori is thought to be a primary factor contributing to the antral inflammation. Endoscopic findings are believed to be non contributory in the diagnosis. In this study, we have shown that endoscopic features like erythema, erosions and nodularity maybe contributory in predicting the presence of H pylori in the biopsy specimen. The primary factor influencing the degree of inflammation in gastritis appears to be the density of H pylori in the antrum. The CagA pathogenicity island does not appear to have a role in influencing the severity of the antral inflammation. The role of other H pylori antigens remains undetermined.

INTRODUCTION
Although gastritis was first interpreted to be due to ageing and lifelong exposure to various insults, it is now clear that the most common cause of this inflammatory condition is infection with Helicobacter pylori. It has been shown that this organism is strongly associated with chronic active gastritis as well as gastric adenocarcinoma and MALT (Mucosal Associated Lymphoid Tissue) lymphomas.

Although H pylori infection is extremely common, only a small proportion of those infected develop chronic gastritis. Thus the clinical outcome is dependant on a complex interplay between the bacterium and the host. Several bacterial virulence factors have been implicated in influencing the severity of gastritis. The CagA island which encodes a high molecular weight antigen (CagA) is believed to stimulate gastric mucosal cells to produce high levels of interleukin 8 that has a pivotal role in the inflammatory responses to infection. H pylori strains that express the CagA protein are considered to be endowed with increased pathogenicity. Studies have shown that the prevalence of antibodies to the CagA protein is higher in patients with peptic ulcer disease than in H pylori gastritis without ulcer. However it is not clear if increased titres of Cag A antibodies are associated with a severe phenotype of chronic gastritis.

The endoscope has been used extensively in visualizing antral gastritis but histopathological examination remains the gold standard for the diagnosis. Various gastroscopic features may be interpreted as signs of gastritis. These include erythema (diffuse, spotty, linear), erosions, absence of rugae in the gastric corpus, and presence of visible vessels. It remains uncertain if any of the endoscopic features are a predictor for the presence of H pylori or any particular histological features of gastritis.

The Sydney system for grading and classifying chronic gastritis was devised to provide a standardised approach to the histological interpretation of biopsies and it was later upgraded in 1994. Several parameters are assessed in this system including the density of H pylori. The parameters are graded on a visual analogue scale as normal, mild, moderate and marked. This system remains an excellent predictor for evaluating the presence of H pylori and the severity of gastritis.

In this study, we gauged if the density of H pylori in biopsy specimens and the presence of anti CagA antibodies were significantly associated with the severity of gastritis as evaluated by histopathology. We also studied the role of endoscopy in predicting the severity of gastritis. The aim of the study was two fold; Firstly, we wished to evaluate the role of H pylori and its main antigen in the pathogenesis of the disease. Secondly, we attempted to assess if a careful endoscopic examination would obviate the need of
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MATERIALS AND METHODS

PATIENTS

Between August 2004 and August 2005, 120 patients with non ulcer dyspepsia who underwent upper gastrointestinal endoscopy were studied. The duration of the illness and history of tobacco and alcohol consumption were recorded. Dietary habits noted included a vegetarian or non-vegetarian diet and history of excessive consumption of spices. Vegetarians included patients who consumed only grain, vegetables and milk or milk products with the total exclusion of meat, fish or eggs in their food. A non-vegetarian diet included patients who consumed meat, fish or eggs regularly in addition to grain, vegetables and milk products. Exclusion criteria were present or past history of gastric neoplasm's or gastric surgery, long term therapy with nonsteroidal anti-inflammatory drugs, liver disease and previous treatment with antibiotics or bismuth salts. All subjects had given informed consent for the study and the local ethics committee had approved the protocol.

Endoscopy was performed after an overnight fast with standard upper gastrointestinal endoscopes (Olympus Optical Co Ltd., Tokyo, Japan) and biopsy specimens were obtained with standard biopsy forceps. On endoscopy, the features noted were the presence or absence of erythema, nodularity, exudates, mucosal edema and excessive friability of the mucosa. A visible vascular pattern, intramucosal bleeding and the presence of erosions were also recorded. A special mention was made of the presence of rugal atrophy or hypertrophy. The endoscopic evaluation was done by an experienced endoscopist (NKG) who had performed more than 15,000 endoscopies.

Two to three antral biopsies were taken for histological examination from the distal lesser and greater curvature, 2 to 3 cm from the pylorus. The biopsies were immediately fixed in formalin. They were processed by routine techniques and stained by the haematoxylin and eosin stain, the modified Giemsa stain for the detection of H pylori and the Cookes stain for the detection of mast cells (15). The modified Giemsa stain was used for the evaluation of H pylori because it has been reported to be a reliable, cheap and reproducible method for the detection of the organism (16). The slides were evaluated by three pathologists (NM, ANS and SB) according to the Updated Sydney System (14). The pathologists were unaware of the clinical, endoscopic and serological data. A score of 0 to 3 (absent, mild, moderate, marked) was assigned to each of the morphological variables: H pylori density, density of neutrophils, lymphocytes, plasma cells, eosinophils and mast cells. Glandular atrophy, glandular shortening, intestinal metaplasia and the presence or absence of fibrosis was also noted and graded into the above three categories. The presence of micro ulceration, activity, and foveolar hyperplasia was noted and graded as present or absent. The grading of the presence of lymphoid follicles was done as per the histological scoring index as proposed by Wotherspoon et al (17).

For each variable, the highest score was given among the antral biopsies. To minimize the interobserver variability in grading the histopathological features, we used the scoring system as proposed by Aydin et al (18).

SEROLOGY

Serum samples stored at -20°C were tested for the presence of IgG antibodies against the CagA antigenic fraction of H pylori using standard ELISA (Genesis diagnostics, UK). Eight controls were run with each set of samples. In addition to the positive and negative control, six serial dilutions of the CagA antigen were included. The dilutions were 0, 6.25, 12.5, 25, 50 and 100 U/ml. A standard curve was constructed based upon the OD of the controls. The OD of the test samples were then read off the curve. Repeat ELISA's were done as and when required.

STATISTICAL ANALYSIS

The association between the clinical, endoscopic and histological findings was assessed by means of the $\chi^2$ test for trend (19). A probability value of $p\leq0.05$ was considered statistically significant.

RESULTS

DEMOGRAPHIC DATA

69 males and 51 females were included in the study. The
mean age of the patients was 36.14 yrs with a range of 16 yrs to 70 yrs. The mean age of the male patients was 37.55 yrs with a range of 16 yrs to 67 yrs. The mean age of the female patients was 35.55 with a range of 16 yrs to 70 yrs. There was no significant difference in age and sex with regard to the colonization by H pylori or the severity of gastritis as evaluated by histopathological examination.

There were fifteen smokers and five reformed smokers in the study. One of the female patients was a smoker. The rest of the smokers were males. Of the reformed smokers, one patient was a female and four were males. Ten patients gave a history of alcohol consumption. All the ten patients were male. Five patients were reformed drinkers, which included one female and four male patients. The numbers of the smokers and drinkers was too small to reach a statistical conclusion.

Fifty patients were non-vegetarians. There was no significant difference between the vegetarian and non-vegetarian group with any of the endoscopic or histopathological features.

### ENDOSCOPIC FEATURES

The commonest endoscopic finding noted was the presence of erythema, which was seen in 49 patients. The presence of erythema was associated with the presence of H pylori (p = 0.03). It was also associated with the presence of lymphoid follicles (p = 0.038) and micro ulceration (p = 0.028). At a p value of p \leq 0.1, erythema was associated with the density of neutrophils (p = 0.071) and active lesions (0.081). (Table 1)

Figure 1

Table 1: Prevalance of and other histological features in patients with erythema on endoscopy

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Grade</th>
<th>Erythema present</th>
<th>Erythema absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. pylori</td>
<td>Nil</td>
<td>19</td>
<td>30</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>31</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>8</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Marked</td>
<td>10</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Lymphoid</td>
<td>Nil</td>
<td>40</td>
<td>35</td>
<td>120</td>
</tr>
<tr>
<td>Follicles</td>
<td>Grade 2</td>
<td>12</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grade 3</td>
<td>15</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grade 4</td>
<td>11</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Micro</td>
<td>Absent</td>
<td>49</td>
<td>46</td>
<td>120</td>
</tr>
<tr>
<td>Uceration</td>
<td>Present</td>
<td>19</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Neutrophil</td>
<td>Nil</td>
<td>18</td>
<td>21</td>
<td>120</td>
</tr>
<tr>
<td>density</td>
<td>Mild</td>
<td>31</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>11</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Marked</td>
<td>8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Activity</td>
<td>Present</td>
<td>33</td>
<td>17</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>35</td>
<td>35</td>
<td></td>
</tr>
</tbody>
</table>

The presence of nodularity was also associated with the density of H pylori (p = 0.045). It was also associated with the density of neutrophils (p = 0.039), plasma cells (p = 0.030), eosinophils (p = 0.00), micro ulceration (p = 0.00) and activity (p = 0.037). (Table 2). It was also was associated with the density of lymphocytes (p = 0.097) and the presence of lymphoid follicles (p = 0.098) at a p value of p \leq 0.1. The presence of erosions was the third endoscopic feature, which was associated strongly with the presence of H pylori (p = 0.02) (data not shown).

Figure 2

Table 2: Prevalance of severe histological features in patients with nodularity on endoscopy

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Grade</th>
<th>Nodularity present</th>
<th>Nodularity absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. pylori density</td>
<td>Nil</td>
<td>6</td>
<td>99</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>3</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>0</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Marked</td>
<td>3</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Neutrophil</td>
<td>Nil</td>
<td>0</td>
<td>39</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>2</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>2</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Marked</td>
<td>2</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Plasma cell</td>
<td>Nil</td>
<td>0</td>
<td>25</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>0</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>2</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Marked</td>
<td>6</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Eosinophil</td>
<td>Nil</td>
<td>0</td>
<td>50</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>2</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>2</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Marked</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Micro Ulceration</td>
<td>Present</td>
<td>5</td>
<td>20</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>1</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>Activity</td>
<td>Present</td>
<td>6</td>
<td>45</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>1</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Lymphocyte density</td>
<td>Nil</td>
<td>0</td>
<td>26</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>0</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>0</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Marked</td>
<td>6</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Lymphoid Follicles</td>
<td>Nil</td>
<td>1</td>
<td>74</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Grade 2</td>
<td>3</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grade 3</td>
<td>2</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grade 4</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

The presence of edema, friability, mucosal exudates, rugal atrophy and hypertrophy was seen in an insignificant population of patients to have any statistical significance.

### HISTOPATHOLOGICAL PARAMETERS

67 antral biopsies showed H pylori on histopathological examination. The density of H pylori as visualized by histopathology (Fig 1) was strongly associated with the density of neutrophils (p = 0.00), lymphocytes (p = 0.050), plasma cells (p = 0.01), eosinophils (p = 0.08)(Fig 2), ulceration (p = 0.04) and active lesions (p = 0.00). In this study, the number of cases of intestinal metaplasia was too small to reach a statistical conclusion. Mast cells did not show any significant association with the density of H pylori (Fig 3).
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**DISCUSSION**

The use of endoscopy in the diagnosis of gastritis is debatable. Redeen et al. (12) studied 488 patients with gastritis and attempted to correlate the endoscopic features. They concluded that the absence of rugae and the presence of visible vessels correlated with histological gastritis in the corpus and the antrum. No endoscopic features showed a sensitivity of more than 57% for H. pylori infection. They

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**Figure 3**

Figure 3: Box Plot showing the lack of a relationship between density and the density of Mast cells as visualized by the Cookes stain.

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**Figure 4**

Figure 4: Scatter plot showing a lack of correlation of anti CagA antibodies with the density of as visualized on histopathology.

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**Figure 5**

Figure 5: Box Plot showing the lack of a relationship between density and the density of Mast cells as visualized by the Cookes stain.

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Serological association - No association between the CagA antibody levels and the density of H pylori as visualized by the Giemsa stain was noted. The scatter plot of the CagA antibody levels is shown in Fig 4.

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**Figure 6**

Figure 6: Scatter plot showing a lack of correlation of anti CagA antibodies with the density of as visualized on histopathology.

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Section showing a gland filled with spiral H pylori (modified Giemsa x 1000)
concluded that except for the absence of rugae and visible vessels, macroscopic features as observed during gastroscopy are of very limited value in the evaluation of gastritis.

In our study, the presence of erythema, nodularity and erosions were statistically associated with the H pylori density and other inflammatory parameters. Our findings suggest that a careful endoscopic examination maybe a useful predictor for the presence of H pylori although further studies are needed to confirm our findings.

A strong association has been reported between the H pylori density and inflammatory activity on gastritis (20). It has been noted that the presence of H pylori in a biopsy specimen was associated with acute and chronic inflammation (21, 22). However, the same studies have reported that there was no correlation between the density of H pylori and the degree of inflammation (23). In our study, there was a strong association between the degree of infiltration by various inflammatory cells and H pylori density in chronic gastritis. The neutrophilic infiltrate, activity and micro ulceration showed a significant association with the H pylori density. This is in concordance with the study by Sobala et al who have noted that some degree of neutrophilic infiltration is an almost constant companion of persisting infection (24).

There was also a significant association of the H pylori density with the chronic inflammatory cell infiltrate. However, there was no correlation of the density of the organism with parameters like glandular atrophy and glandular shortening. Atrophy maybe a consequence of auto destructive products such as neutrophils and monocyte activation such as reactive oxygen metabolites and proteases (25). Glandular atrophy and intestinal metaplasia are probably not hospitable environments for the bacteria (26) and are hence associated with a decrease in the density of the organism.

In Caucasian populations it has been reported that strains that contain the CagA pathogenic island are associated with a more severe disease than strains that lack CagA (27, 28). CagA positive isolates are associated with a higher grade of antral polymorphonuclear inflammation. (29). It has also been reported that there is a strong correlation between the presence of serum antibodies to CagA and the isolation of CagA positive strains from a patient (30). In our study, although there was a strong association between the density of H pylori and the density of various inflammatory cells, there was no association between the CagA antibody levels and the degree of inflammation. This suggests that the presence of the organism is the primary factor influencing the inflammatory response and not the presence of the CagA pathogenic island.

It has been reported that in Asian populations, the association of CagA positivity and disease risk is much weaker or not present (31, 32). This finding was also seen in this study. The existence of distinct variants of certain genes of the Cag pathogenicity island may offer an explanation for this discrepancy. Analysis of H pylori strains from East Asian patients suggested that in some strains, only a part of the Cag pathogenicity island is present (31, 32). Maeda et al (33) reported that 6% of the H pylori strains they had studied lacked the CagI or CagII islands although all of them retained the CagA gene. All the patients who lacked the complete pathogenicity island presented with non ulcer dyspepsia. 41 of 59 patients with the complete Cag pathogenic island presented with peptic ulcer or gastric cancer. It is possible that the absence of a part of the Cag pathogenic island may confer less pathogenicity than in patients who harbor the complete pathogenic island. Ikenoue et al (34) have reported similar findings. In their patients, the strains containing CagA but lacking CagE and/or CagT were less pathogenic than the strains with the complete pathogenic island. The CagA protein is strongly immunogenic and most patients colonized with a CagA positive strain show a high titre of anti CagA antibodies (35). Truncated proteins which do not contain the CagE or CagT components are likely to confer a strong immunogenicity but low pathogenicity. This would explain why the antibody titres against CagA have no relationship to the H pylori density or the degree of the inflammatory response. The existance of variant specific antibodies remains unknown.

In conclusion, we suggest that endoscopic features like the presence of erythema, erosions and nodularity maybe a useful predictor for the presence of H pylori in the antrum. The degree of infiltration by H pylori is the primary mediator of the degree of inflammation in chronic gastritis and not the presence or absence of the CagA pathogenicity island. In the Indian context, it appears that the presence of the CagA pathogenicity island does not have a role in influencing the severity of gastritis. It fuctions more as an immunogenicity island rather than a pathogenicity island. Other host or bacterial factors need to be evaluated in order to define the primary factor leading to severe gastritis.
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