Original Article

*Helicobacter pylori*-induced apoptosis in pathogenesis of gastric carcinoma

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Background: Despite a possible role of *Helicobacter pylori* in gastric carcinoma (GC), its pathogenesis is not clear. There is scanty data on apoptosis in GC in relation to *H. pylori* and CagA antibody. Therefore, we studied gastric epithelial apoptosis in GC and non-ulcer dyspepsia (NUD) with or without *H. pylori* infection, and the degree of apoptosis in relation to CagA antibody status.

Methods: 20 patients each with GC and NUD were investigated for *H. pylori* using rapid urease test (RUT), IgG anti-*H. pylori* and anti-CagA antibodies, histology of endoscopically normal-looking mucosa for *H. pylori*, intestinal metaplasia (IM), and apoptosis using TUNEL assay. Positivity to one tissue-based (RUT or histology) and one serology based (anti-*H. pylori* or CagA IgG) test was taken as diagnostic of active *H. pylori* infection, and negative result in both tissue-based tests suggested its absence.

Results: Patients with GC more often had anti-*H. pylori* IgG (16 of 20 vs. 8 of 20; p=0.02) and a trend towards higher apoptotic index (AI) (48.6 [19.2 to 71.7] vs. 41.4 [11.7 to 63.6]; p=0.06) than NUD. AI was higher in GC (66.7 [57.5 to 71.7] vs. 32.6 [19.2 to 39.8]; p <0.0001) and NUD (58.6 [50.7 to 63.6] vs. 24.4 [11.7 to 32.2]; p<0.0001) infected with *H. pylori* than in those without infection. AI was also higher in GC than in NUD with *H. pylori* infection (66.7 [57.5 to 71.7] vs. 58.6 [50.7 to 63.6]; p= 0.01). Four of the 20 patients with GC and none with NUD had IM (p=ns). There was no difference in AI in relation to CagA antibody. AI positively correlated with patients’ age in presence of *H. pylori* infection (correlation coefficient = 0.5, p=0.03) but not in its absence.

Conclusion: Exaggerated apoptosis may play a role in *H. pylori*-mediated gastric diseases including carcinogenesis. AI increases with aging in patients infected with *H. pylori*. [Indian J Gastroenterol 2005;24:193-196]

Gastric cancer (GC) has been etiologically linked to infection with *Helicobacter pylori*.1 The mechanism of *H. pylori*-induced gastric carcinogenesis, however, is not clear.

Integrity of tissues, including that of gastric epithelium, is maintained by a balance between cell death by apoptosis or necrosis and regeneration.2 This balance may be altered by *H. pylori* infection,2 since it induces apoptosis of gastric epithelial cells both in vivo and in vitro.2,3 In vivo data on this aspect in GC are scant.3,4

Though studies from the developed world suggest that CagA-bearing strains of *H. pylori* are more often associated with gastroduodenal diseases than CagA-negative strains,5 this has not been substantiated from developing countries with higher prevalence of *H. pylori* infection.5,6,7 A few studies on gastric epithelial apoptosis, which is believed to be an important mechanism of cellular injury resulting from *H. pylori* infection, have shown contradictory results with respect to CagA antibody status.7

We prospectively evaluated gastric epithelial apoptosis in patients with GC and non-ulcer dyspepsia (NUD) with or without gastric *H. pylori* infection, and determined the degree of apoptosis in these patients in relation to CagA antibody status.

Methods

Twenty patients with GC and 20 with NUD diagnosed using standard criteria,8 who had not received anti-secretory drugs within seven days and anti-*H. pylori* treatment ever, were studied. Informed consent was obtained from patients and approval by the Ethics Committee of the Institute.

At esophagogastroduodenoscopy using a video endoscope (Pentax, Japan or Xion GmbH, Munich, Germany) six gastric biopsies each were obtained from tumor margins and normal looking areas (both antrum and body); two from normal area (antrum and body) were used for in-house rapid urease test (RUT), whose sensitivity and specificity have been validated previously.9 The remaining biopsy specimens were paraffin embedded and sectioned for histological examination and evaluation of apoptosis.

Diagnosis of *H. pylori* infection
Anti-*H. pylori* IgG and anti-CagA IgG antibodies were
tested using commercially available ELISA kits with known sensitivity and specificity\(^1\) (Genesis Diagnostics, Cambridgeshire, UK; positive ≥ 6.25 IU/mL [cut-off value to classify positive and negative as per manufacturer’s instruction]). Typing of tumor was done in H&E-stained biopsies. H&E, periodic acid-Schiff, alcin blue and Giemsa-stained sections from normal looking areas were evaluated for intestinal metaplasia (IM) and \(H.\ pylori\), respectively.

Active \(H.\ pylori\) infection was diagnosed if at least one tissue-based test (RUT or histology) and one serology-based test (anti-\(H.\ pylori\) IgG or anti-CagA IgG) were positive. Absence of active \(H.\ pylori\) infection was based on negative result in both the tissue-based tests irrespective of result of serology.

**Assay for apoptosis**

Terminal deoxynucleotidyl nick-end labeling (TUNEL) assay was performed using a commercially available kit (\textit{In Situ Cell Death Detection Kit, Roche, Manheim, Germany}) on 5-6 μ thick sections of formalin-fixed paraffin-embedded tissues mounted on poly-L-lysine coated slides (Sigma, USA) following standard protocol. Sections pre-treated with DNase (Bangalore Genei, India; 1 mg/mL) and those without treatment with dUTP were considered as positive and negative controls, respectively, during each assay.

A total of 300 cells (100 in each) were counted in three different high power fields (100 X) by three independent observers. All slides were coded and the mean count of the three observers was taken as the final count. Apoptotic index (AI) was calculated as the number of apoptotic cells per 100 cells.

**Statistical analysis**

Continuous and categorical variables were compared using Mann-Whitney U test and chi-squared test, respectively. Continuous variables were correlated using Pearson correlation.

**Results**

The Table shows demographic, clinical and endoscopic parameters of the patients and controls. Patients with GC were comparable to patients with NUD with respect to age and gender (p=ns).

**Histology:** 12 patients had intestinal type tumor, 5 had diffuse and in 3 it was unclassified. \(H.\ pylori\) was detected in 8 of 20 patients with GC and 10 of 20 with NUD (p=ns). Intestinal metaplasia was detected in 4 of 20 (20%) patients with GC and none with NUD (p=ns).

Of the 10 patients with GC with \(H.\ pylori\) infection, 3 were positive by all four tests, 2 were negative by histology but positive by RUT and serology, 2 were negative for anti-CagA antibody but positive to all other tests, one was negative for both serology tests (anti-\(H.\ pylori\) IgG and anti-CagA IgG) but positive to RUT and histology, and 2 were negative for anti-\(H.\ pylori\) IgG antibody but positive by RUT, histology and anti-CagA IgG. Eight of 10 patients with NUD with \(H.\ pylori\) infection were positive for all the four tests; the other two were positive to all tests except anti-\(H.\ pylori\) IgG.

Of the 10 patients with GC without \(H.\ pylori\) infection, 9 were positive by serology (anti-\(H.\ pylori\) IgG and CagA IgG antibody) but negative by RUT and histology, and one was positive for anti-CagA IgG but negative to all other tests. In the \(H.\ pylori\)-negative NUD group, one was negative by all four tests and one was positive to anti-CagA IgG but negative by RUT and histology, while 6 were positive to anti-CagA IgG antibody and 2 were positive to anti-\(H.\ pylori\) IgG.

**Apoptotic indices:** AI was similar in patients with GC and NUD (48.6 [19.2 - 71.7] vs. 41.4 [11.7 - 63.6]; p=0.06). Patients with GC infected with \(H.\ pylori\) had higher AI than those without \(H.\ pylori\) infection (66.7 [57.5 - 71.7] vs. 32.6 [19.2 - 39.8]; p<0.0001) despite comparable age (57 y [36 - 74] vs. 52 [29 - 73], respectively; p=ns). Patients with NUD infected with \(H.\ pylori\) also had higher AI than those without infection (58.6 [50.7 - 63.6] vs.

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**Table: Demographic, clinical and endoscopic parameters of patients with gastric carcinoma and non-ulcer dyspepsia**

<table>
<thead>
<tr>
<th></th>
<th>Gastric carcinoma (n=20)</th>
<th>Non-ulcer dyspepsia (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>55.5 (21-74)</td>
<td>40.5 (41-61)</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>Clinical presentation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyspepsia+</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>Gastric outlet obstruction</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>GI bleed</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Obstructive jaundice</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Malignant ascites</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Tumor location</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antrum</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>Body</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Body extending to fundus</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Positive (H.\ pylori) tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RUT</td>
<td>10 (50%)</td>
<td>10 (50%)</td>
</tr>
<tr>
<td>Histology</td>
<td>8 (40%)</td>
<td>10 (50%)</td>
</tr>
<tr>
<td>IgG antibody</td>
<td>16 (80%)</td>
<td>10 (50%)*</td>
</tr>
<tr>
<td>Anti-CagA IgG antibody</td>
<td>17 (85%)</td>
<td>17 (85%)</td>
</tr>
</tbody>
</table>

Data expressed as median and range. *p<0.05; chi-squared test. +These patients had anorexia and weight loss as well
Discussion

Our study showed that patients with GC and NUD infected with *H. pylori* had higher AI than those without this infection. Patients with GC with *H. pylori* infection had higher AI than *H. pylori*-infected NUD. Apoptotic index was comparable in patients with anti-CagA IgG antibody and in those without it. Apoptotic indices positively correlated with patients’ age in those with *H. pylori* infection but not in those without.

Our finding may suggest that apoptotic cell death could be a factor contributing to the development of GC. Though a possibility of age-related difference in AI between patients with GC than NUD remains, it is unlikely as the difference in age was not significant. In the proposed model of gastric carcinogenesis, more severe the gastritis more is the cell loss, resulting in gastric atrophy, which is followed by IM, a precursor of GC. Our results suggest that such excessive cell death might be related to exaggerated apoptosis in these patients compared to patients with NUD. The mechanism of such exaggerated apoptotic cell death needs further study.

CagA-bearing strains have been shown to have increased risk of developing severe gastric inflammation, atrophic gastritis, and non-cardia gastric adenocarcinoma as compared with CagA-negative strains, in studies from developed countries. A study from India showed that antibodies to CagA protein are not predictive of serious gastroduodenal disease, which is contradictory to the studies from developed countries on this issue. Our study, however, had a small sample size and a small number of patients with negative CagA antibody test.
Though the frequency of positive results on RUT and histology in patients with GC was comparable to that in NUD, anti-\textit{H. pylori} IgG antibody was more often detected in the former group. This is not entirely unexpected as IM and hypochlorhydria, frequently found in patients with GC, result in reduced density of \textit{H. pylori}, which may be associated with false-negative results to tissue-based methods such as RUT and histology.\textsuperscript{14} Since IgG anti-\textit{H. pylori} antibody remains positive even after eradication\textsuperscript{15} or reduction in density of \textit{H. pylori}, patients with GC frequently have positive result to this test despite showing negative results to the two tissue-based tests.\textsuperscript{15}

One interesting finding was the higher frequency of anti-CagA IgG antibody than anti-\textit{H. pylori} IgG antibody. Previous studies showed that antibody response to CagA is more intense and persists longer than antibody response to \textit{H. pylori}.\textsuperscript{16} Also, IgG antibody to \textit{H. pylori} disappears with time.\textsuperscript{15,16} Some authors, therefore, suggested that anti-CagA antibody is a more sensitive serological test for diagnosis of \textit{H. pylori} infection than anti-\textit{H. pylori} IgG.\textsuperscript{15}

Before the discovery of \textit{H. pylori}, workers from India reported development of atrophic chronic gastritis in old age,\textsuperscript{17} which is known to predispose to GC. Later studies have shown that age-related atrophy of the gastric mucosa might be related to \textit{H. pylori} infection.\textsuperscript{16} Our results substantiate this; moreover, our data show that \textit{H. pylori}-induced age-related gastric mucosal atrophy might result from increasing apoptotic cell death with aging.

The frequency of IM was low in our patients with GC. A possibility of type II error cannot be excluded due to small sample size. We took multiple biopsies in order to avoid the chance of missing IM due to sampling error as these are focal lesions. However, the possibility of true infrequency of IM in Indian patients\textsuperscript{18} cannot be entirely excluded.

References


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