Biological markers in *Helicobacter pylori*-associated gastritis and carcinoma: the value of a scoring system

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**Background:** *Helicobacter pylori*-associated gastritis has been linked to the pathogenesis of gastric adenocarcinoma (GA), especially when associated with intestinal metaplasia (IM) and atypia/dysplasia (A/D). We examined p53 expression, ploidy and proliferative activity and assessed *H. pylori* infection in relationship to IM and/or A/D in cases of gastritis not associated with GA and in cases of GA.

**Methods:** We examined 53 gastric biopsies from patients with gastritis not associated with GA, including patients with gastritis not associated with IM and/or A/D (n=35) and with gastritis associated with IM and/or A/D (n=21). Thirty-six distal gastrectomy specimens from patients with GA constituted a third group of patients. A scoring system that encompassed the presence or absence of *H. pylori*, degree of gastritis, IM and/or A/D, p53, MIB-1 proliferative index (MPI) and ploidy was estimated in the cases of gastritis and in cancer-associated mucosa (CAM) and the adenocarcinoma from patients with GA.

**Results:** Patients with GA had a higher median age than those with gastritis without IM and more were males (ratio, 2.2:1). *H. pylori* was detected in 75% (40/53) of gastritis specimens and in 55% (20/36) of GA cases. There was a statistically significant difference between the incidence of gastritis without IM and/or A/D and CAM (P<0.01). p53 expression was seen in 67% of cases (14/21) of gastritis with IM and/or A/D and in only 5% (2 cases) of gastritis without IM (P=0.0005). A statistically significant difference in MPI was seen between CAM and GA (P<0.01) and gastritis without IM and/or A/D and gastritis with IM (P=0.004). Cases of gastritis without IM and/or A/D had a median score of 8 while cases of gastritis with IM and/or A/D had a median score of 12 (P=0.0003). CAM had a median score of 13, which was significantly different than gastritis without IM and/or A/D (P=0.0003).

**Conclusions:** The presence of IM and/or A/D can be used in *H. pylori*-associated gastritis as a starting point to further investigate high-risk lesions. Those showing p53 expression, high proliferative activity and aneuploidy require closer follow up and perhaps additional biopsies. Although aneuploidy is commonly seen in GA, its presence in cases of gastritis as an isolated finding should not indicate a high-risk lesion.

**Key words:** Gastritis, *Helicobacter pylori*, protein p53, adenocarcinoma, ploidies, proliferative activity

*Helicobacter pylori*-associated gastritis has been linked with the development of gastric adenocarcinoma. This association is more commonly seen with cases exhibiting incomplete intestinal metaplasia and epithelial atypia/dysplasia. Grading of gastric dysplasia has not been as universally reproducible as it has been in other parts of the gastrointestinal tract. Additionally, incomplete intestinal metaplasia requires special histochemical stains for its conclusive diagnosis. The identification of unequivocal precursors for malignant transformation in *H. pylori*-associated gastric cancer has thus been an illusive issue.

Mutation of the tumor suppressor gene p53 is one of the most common mutations associated with carcinogenesis. The mutant gene has a longer half life than the normal gene, making detection of the mutant gene easier by immunohistochemistry. Immunohistochemical detection of p53 does not necessarily correspond to gene mutation, but it does imply genetic instability in some cases. Although aneuploidy is not always associated with neoplastic transformation, it is more often seen in neoplasms than in reactive processes. It is believed that *H. pylori* induces proliferative activity in gastritis that may be decreased when the organism is eradicated.

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The purpose of this study was to evaluate ploidy, proliferative activity and p53 expression, *H. pylori* infection and epithelial atypia in cases of gastritis and gastric adenocarcinoma. These variables were also assessed in the cancer-associated mucosa (CAM) adjacent to the carcinomas. An attempt was also made to evaluate a scoring system in identifying gastritis lesions possibly associated with an increased risk of gastric adenocarcinoma.

**Methods**

Three groups of patients were studied. The first group had antral gastritis not associated with intestinal metaplasia and/or A/D or malignancy (n=32); the second group had antral gastritis associated with intestinal metaplasia and/or aplasia/dysplasia (n=21); the third group had distal gastric adenocarcinoma treated by gastrectomy (n=36). Patients that had adenocarcinoma of the proximal stomach were excluded from the study. The modified Sydney system was used to grade gastritis.14 We attempted to identify cytolological atypia/dysplasia.15 When the changes were significant, a grade II was assigned to the case. When the changes were mild, a grade I was assigned. One pathologist reviewed the slides *H. pylori*-like organisms were identified using H&E stains. All sections were routinely stained with alcian blue for the identification of intestinal metaplasia. Sections from all cases were obtained for p53, MIB-1 and Feulgen staining. All cases of gastric adenocarcinoma were graded. Sections encompassing the tumor and the cancer-associated mucosa were selected for p53, MIB-1 and Feulgen staining.

Sections from gastric biopsies and gastrectomy specimens from areas of gastritis and gastric adenocarcinoma were mounted on aminoalkyl silane-coated slides for immunochrometry for p53 and MIB-1. The antibodies used were the DO7 clone for p53 (Novacstra, New Castle upon Tyne, UK) and the MIB-1 antibody (Immunotech, Westbrook, Maine, USA) for the proliferative index. Positive control slides included normal tissues and tumor tissues known to express p53. Negative control slides included PBS in lieu of antibody and non-immune IgG.16-19 Tonsils were used as controls for MIB-1. Feulgen staining was performed according to a standard technique, using a CAS quantitative DNA staining kit (Becton-Dickinson, CAS, Cellular Imaging Systems). Tissue sections were stained in batches with one calibration slide of unstained rat hepatocytes (Becton-Dickinson cellular imaging systems).

All Feulgen stained slides were analyzed on a CAS200 cell image analyzer with version 3.05 QDNA software (Becton-Dickenson, CAS cellular imaging systems, San Jose, California, U.S.A).19 Immunostained slides for p53 and MIB-1 were examined with the same analyzer with version 4.0 QNA software and a 40X Plan Achro (n=0.17) microscope objective (X400 total magnification). The CAS200 QDNA software calculated the amount of nuclear DNA picogram (pg) in accordance with the Beer-Lambert law. Cases falling outside of 10% of the diploid control were considered aneuploid.20 The QDNA software calculated the positive nuclear immuno-stained area relative to the total nuclear area based upon background staining and immuno-staining thresholds.21,22 Boundaries for areas of carcinoma and cancer-associated mucosa were delineated to avoid overlap. Expression of p53 and MIB-1 was calculated as the percentage of cells expressing the antigen in 400 nuclei. The expression was calculated for the epithelial nuclei in cases of gastritis, cancer-associated mucosa and gastric adenocarcinoma. The expression was also plotted in a histogram format (Figure 1).

All parameters observed, including the grade of gastritis, presence or absence of *Helicobacter*, atypia/dysplasia, p53 expression, MIB-1 labeling index and ploidy were scored. Gastritis was given a score of 1 to 3 depending on the degree of inflammation. p53 was given a semiquantitative score of 1 to 3: A score of 1 was given when there was no immunostaining, a score of 2 when the staining was focal and a score of 3 when the staining was extensive. Atypia was given a score of 1 to 3. A score of 1 was given when there was no atypia/dysplasia, a score of 2 when the changes were mild and a score of 3 when the changes were significant. MIB-1 labeling was given an arbitrary score of 1 when the MIB-1 labeling index was ≥10% and a score of 2 when the labeling index was >10%. The total combined score ranged from 7 to 18.

The Kruskall-Wallis analysis of variance test was used to compare p53 expression, proliferative activity and ploidy in cases of gastritis exhibiting intestinal metaplasia and/or atypia/dysplasia and in those cases that did not. Additionally, a comparison of the total scores of cases with gastritis exhibiting intestinal metaplasia and/or atypia/dysplasia and those not exhibiting those changes was made. The total score of the cancer-associated mucosa was also compared with cases of gastritis not exhibiting intestinal metaplasia and/or atypia/dysplasia. Adjustment for multiple comparisons was done using Bonferroni's correction.23

**Results**

The patient ages in the first group (gastritis without intestinal metaplasia) ranged from 5 to 79 years with a mean of 43 years. In the second group (gastritis with intestinal metaplasia), ages ranged from 27 to 75 years with a mean of 49 years. The difference in the mean age between the two groups was not statistically significant (P=0.08). The patients with gastric adenocarcinoma had an age range of 29 to 91 years with a mean of 63 years. The difference in age distribution between the first group and patients with gastric adenocarcinoma was statistically significant (P=0.03). The male to female ratio in the first group was 1.5:1, in the second group, 1:1, and in the carcinoma group, 2:2:1.

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Helicobacter pylori-like organisms were identified in 40 of the 53 cases of gastritis (75%). This included 23 cases of gastritis without intestinal metaplasia (65%) and 17 cases of gastritis associated with intestinal metaplasia (80%). The organisms were identified in 20 cases of gastric adenocarcinoma (55%).

Complete intestinal metaplasia was seen in 18 of 53 cases of gastritis (34%) (Table 1). Atypia and/or dysplasia were seen in 14 of the 18 cases (77%). Complete intestinal metaplasia and atypia/dysplasia was seen in 12 cases (66%). Atypia and/or dysplasia without complete intestinal metaplasia were seen in only two cases (10%). Atypia/dysplasia was mostly moderate. Intestinal metaplasia was seen in the cancer-associated mucosa of the patients with gastric adenocarcinoma in 33 cases (92%) (Table 1) The difference in incidence of gastritis without IM and/or AD was significantly different from that in cancer-associated mucosa ($P=0.01$), but not between gastritis with IM and/or A/D and CAM with IM and A/D ($P=0.117$) (Table 1). Atypia/dysplasia was seen in 29 cases (88%) of CAM. All cases of atypia/dysplasia were associated with intestinal metaplasia. The epithelial atypia/dysplasia was severe in 5 of the 29 cases (17%).

Cases of gastritis showing no evidence of intestinal metaplasia had MIB-1 proliferation indices of 9.9 to 48.3% (median, 21%) (Table 2). Cases of gastritis showing intestinal metaplasia had proliferative indices ranging from 15.3 to 54.6% (median, 29%). The difference between the proliferative indices was statistically significant ($P=0.004$). In the patients with gastric adenocarcinoma, the proliferation indices in the cancer-associated mucosa ranged from 0.9 to 44% (median 12%). Proliferation indices in gastric adenocarcinoma ranged from 3.2 to 62% (median, 24%). The difference between proliferation indices in the adenocarcinoma and the cancer-associated mucosa was statistically significant ($P=0.01$).

In patients with gastritis, p53 was expressed in 16 cases (30%). The expression was mostly moderate (Figure 2). The antigen expression was seen in 14 of the 18 cases exhibiting intestinal metaplasia and/or atypia/dysplasia (77%) and in only 2 of the 35 cases (6%) not exhibiting these changes ($P=0.0005$) (Table 2). In the patients with gastric adenocarcinoma, p53 expression was seen in 15 cases in the cancer-associated mucosa (41%) whereas it was seen in 19 cases in gastric adenocarcinoma (52%).

Aneuploidy was seen in 13 of the 18 cases of gastritis exhibiting intestinal metaplasia and/or atypia/dysplasia (72%) whereas 16 of the 35 cases (45%) of gastritis not exhibiting these changes were aneuploid ($P=0.113$) (Table 2). In the patients with gastric adenocarcinoma, aneuploidy was seen in 22 cases in the cancer-associated mucosa (62%) whereas it was seen in 29 cases in gastric adenocarcinoma (80%).

Cases of gastritis exhibiting intestinal metaplasia and/or atypia/dysplasia had a total score ranging from 10 to 13 with a median of 12 whereas cases of gastritis not exhibiting those changes had scores ranging from 7 to 10 with a median score.
Table 1. Incidence of intestinal metaplasia and/or atypia/dysplasia in patients with gastritis and in patients with gastric adenocarcinoma.

|                                      | Gastritis (n=53) | Cancer-associated mucosa (n=36) | P value |
|--------------------------------------|-----------------|---------------------------------|---------|
| Intestinal metaplasia without atypia/| 18/35 (34%)     | 33/36 (92%)                     | 0.01    |
| dysplasia                            |                 |                                 |         |
| Intestinal metaplasia with aplasia/  | 14/18 (77%)     | 29/36 (88%)                     | 0.117   |
| dysplasia                            |                 |                                 |         |
| Aplasia/dysplasia without intestinal | 2/53 (4%)       | -                               |         |
| metaplasia                           |                 |                                 |         |

*from patients with adenocarcinoma

Table 2. Markers in patients with gastritis with and without intestinal metaplasia and/or atypia dysplasia.

|                                      | Gastritis without intestinal metaplasia (n=35) | Gastritis with intestinal metaplasia (+/- A/D) (n=18) | P value |
|--------------------------------------|-----------------------------------------------|-----------------------------------------------------|---------|
| MIB-1 P1 (%) (median)                | 9.9-48.3 (21)                                 | 15.3-54.6 (27)                                      | 0.004   |
| p53+                                 | 2/35 (6%)                                     | 14.21 (66%)                                        | 0.0005  |
| Aneuploidy                           | 16/35 (45%)                                   | 13/21 (62%)                                        | 0.113   |
| Total score (median)                 | 7-19 (8)                                      | 10-13 (12)                                         | 0.0003  |

A/D= aplasia/dysplasia , PI = proliferation index
of 8 ($P=0.0003$) (Table 2). Cancer-associated mucosa cases had a score ranging from 10 to 17 with a median of 13. The difference between the scores of gastritis without intestinal metaplasia and/or atypia/dysplasia and cancer-associated mucosa was statistically significant ($P=0.0003$) (Figure 3).

**Discussion**

*Helicobacter pylori* has been linked to the pathogenesis of gastric adenocarcinoma. The exact mechanism is not completely understood. It is however believed that some strains of the organism are more associated with malignancy than others. It is also believed that *H. pylori* induces the proliferation of gastric epithelium in vivo. Whether the organism acts as a triggering agent in the process of carcinogenesis or whether it remains as the disease progresses to invasive tumors is not known, but from our results, it appears that the organism is more likely found in cases of gastritis than in gastric adenocarcinoma. This may suggest that the organism acts as a triggering agent in the process of oncogenesis. This triggering may be followed by other factors involved in the process of transformation of the epithelial cells.

Gastric dysplasia grading is more difficult than grading dysplasia seen in other parts of the gastrointestinal tract. Inter-observer disagreement in determining the degree of dysplasia is seen more frequently in assessing gastric epithelial dysplasia than in the esophagus and colon. It has however been shown that higher grades of dysplasia are less likely to be reversible and are more frequently associated with the development of malignancy. Dysplasia is however not an obligate precursor of malignancy. Some lesions may regress. There are therefore other factors at the molecular level that may be more indicative of malignant transformation than simple phenotypic abnormalities.

In our study we did not attempt to subclassify dysplasia into several grades. We subdivided the morphologic changes into atypia/dysplasia and high-grade dysplasia. We included all cases showing atypia/dysplasia as well as intestinal metaplasia into one group and the other cases of gastritis without those changes into another group. Gastritis without any changes of intestinal metaplasia and/or atypia/dysplasia represented one extreme of the spectrum of the disease; gastric adenocarcinoma represented the other. Cancer-associated mucosa came close to the same end of the spectrum while gastritis with intestinal metaplasia and/or atypia/dysplasia was intermediate in the spectrum. At the same time, we looked for more objective parameters to help us determine the possible risk of developing cancer in cases of gastritis. These included ploidy, proliferative activity and mutation of the p53 gene.

Proliferative activity is increased in *H. pylori*-associated gastritis, according to several studies, but the investigators do not seem to agree on whether eradication of the organism would lead to a return of proliferative activity...
to normal values. Some studies have shown that proliferative rates return to normal after eradication of *Helicobacter*. Others have shown that the rates are maintained at higher levels even after removal of the organism. This may suggest that *H. pylori* induces both reversible and irreversible changes in the epithelial cells of the stomach. Our study has shown a stepwise increase in the proliferative activity in cases of gastritis. We also showed that the proliferative activity in cases of carcinoma was similar to those cases of gastritis exhibiting intestinal metaplasia and/or atypia/dysplasia. It is very difficult to explain the reason for low proliferative indices in the cancer-associated mucosa. p53 mutation is one of the most common and early events involved in the process of carcinogenesis. Almost 60% of cases of gastric adenocarcinoma show p53 mutation. This mutation is manifested on either the immunohistochemical or the molecular level. The molecular mutation does not always correspond to immunohistochemical expression of the p53 protein. There is, however, no agreement in the literature on whether p53 gene abnormalities are seen in cases of gastritis. Some studies have shown that there are abnormalities in p53 expression in cases of gastritis, especially those associated with *Helicobacter*. Other investigators have failed to show any evidence of p53 mutation in cases of gastritis. There is also disagreement in the literature on whether p53 mutation is an early or late molecular event in the process of carcinogenesis. Our study has shown that abnormal p53 expression is seen in a significant number of cases of gastritis exhibiting intestinal metaplasia and/or atypia/dysplasia. Considering that the great majority of these cases would not develop gastric cancer, the results of our study suggest that p53 mutation may be an early oncogenic event that would, for the most part, be reversible. This was also confirmed in a study of p53 in cases of *Helicobacter*-associated gastritis before and after treatment, where p53 expression disappeared after treatment.

Ploidy can be viewed as an indicator of gross genetic instability. Aneuploidy is usually seen in neoplastic processes and is thought to predict the biological behavior of many neoplasms, but this is not universally true. Most cases of gastric adenocarcinoma are aneuploid. One study showed that aneuploidy is irreversible, even after eradication of the organism. Studies have shown that cases of gastritis with atrophic changes and atypia can exhibit genetic instability in the form of aneuploidy and other oncogene expression, including p53 and c-myc mutation. The changes may sometimes be reversible. Another study suggested that *H. pylori*-associated gastritis represents an intermediate but important step in the process of carcinogenesis if the aneuploidy is significant. Our study has shown that aneuploidy can be seen in both gastritis and gastric adenocarcinoma. Ploidy should be considered significant only if other parameters suggest malignancy.

It seems that the process of oncogenesis in the stomach, as in other organs, is a multi-factorial process involving the sequence of chronic gastritis, mucosal atrophy, intestinal metaplasia, dysplasia and finally invasive carcinoma. The process may involve *Helicobacter pylori* as a triggering agent, which is followed by genetic instability manifested by oncogene mutation, such as p53 and c-myc mutation and aneuploidy. This would also be manifested by an increase in proliferative activity. None of the cases of carcinoma had the maximum total score, emphasizing the need for multi-parametric measurements. Our study has also shown that there is a stepwise increase in the total score from gastritis without atypia to gastric adenocarcinoma. It is therefore imperative to assess several parameters when an attempt is made to assess the possible malignant potential of cases of *H. pylori*-associated gastritis. Our findings also suggest that cases of *H. pylori*-associated gastritis exhibiting intestinal metaplasia and/or dysplasia should be further investigated for p53 and other oncogene mutations, ploidy and proliferative activity. Cases showing significant mutations, aneuploidy and increased proliferative activity may represent high-risk lesions requiring closer follow up and even more frequent and extensive biopsies. Our study also suggests that aneuploidy as an isolated finding is not an indicator of high-risk lesions.

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