**Helicobacter pylori** infection and expression of DNA mismatch repair proteins

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AIM: To determine the expression of DNA (MMR) proteins, including hMLH1 and hMSH2, in gastric epithelial cells in the patients with or without Helicobacter pylori (H pylori)-infected gastritis.

METHODS: Fifty H pylori-positive patients and 50 H pylori-negative patients were enrolled in the study. During endoscopy of patients with non-ulcer dyspepsia, two antral and two corpus biopsies were taken for histological examination (Giemsa stain) and for immunohistochemical staining of hMLH1 and hMSH2.

RESULTS: The percentage of epithelial cell nuclei that demonstrated positivity for hMLH1 staining was 73.34 ± 10.10% in H pylori-positive patients (P < 0.0001). No significant difference was seen between the two groups regarding the percentage of epithelial cell nuclei that demonstrated positivity for hMSH2 staining (78.24 ± 8.71% in H pylori-negative patients; P = 0.09).

CONCLUSION: This study indicates that **H pylori** might promote development of gastric carcinoma at least in part through its ability to affect the DNA MMR system.

Abstract

**INTRODUCTION**

*Helicobacter pylori* (H pylori) infection affects about half of the world's population, and gastric carcinoma is one of the most frequent malignancies despite a decrease in incidence and mortality in recent decades[1,2,3]. The association of H pylori with gastric cancer is supported by epidemiological studies that show odds ratios for gastric cancer up to nine-fold greater in H pylori-infected individuals[3]. Chronic H pylori infection can cause chronic gastritis, which often progresses to gastric atrophy and intestinal metaplasia, which are premalignant lesions of the stomach[4]. Although many epidemiological studies that address the association of *H pylori* infection and gastric cancer have been carried out, fewer advances have been made to understand how long it takes for *H pylori* infection to induce the development of gastric cancer.

The main molecular mechanisms that underlie cancer development include the overexpression of genes, including oncogenes and growth factors or their receptors, and impaired expression of tumor suppressor genes that results from mutation or allelic losses[5,6] and deficiencies of the DNA mismatch repair (MMR) system[7,8].

Impairment of the DNA MMR system is a known mechanism of carcinogenesis and tumor progression.
of both sporadic and hereditary human cancers\textsuperscript{[18-22]}. The MMR deficiency leads to the accumulation of base-base mismatches, and short insertion/deletion mispairing during DNA replication, which results in widespread mutation generated as a consequence of DNA replication errors\textsuperscript{[23]}. Most cells deficient in MMR display a high level of genomic instability characterized by changes in simple repetitive sequences, so-called microsatellite instability (MSI). Chronic \textit{H pylori} infection damages the gastric barrier function\textsuperscript{[12,13]} and stimulates gastric cell proliferation\textsuperscript{[14-19]}, which leads to mucosal repair\textsuperscript{[20]}, but which can also induce cellular DNA damage\textsuperscript{[19,22]}.

\textit{H pylori} gastritis occurs more frequently in individuals with MSI-positive than MSI-negative gastric cancers, which raises the possibility that \textit{H pylori} infection affects the DNA MMR system\textsuperscript{[23]}.  

\section*{MATERIALS AND METHODS}

\subsection*{Patients}

We examined dyspeptic patients who were referred for endoscopic evaluation to Taleghani hospital, a tertiary hospital in Tehran, Iran. Dyspepsia was defined as persistent or recurrent abdominal pain or discomfort, centered in the upper abdomen, with a duration of at least 3 mo. Abdominal discomfort was characterized by early satiety, fullness, nausea, retching, upper abdominal bloating and anorexia\textsuperscript{[24-25]}. We recruited consecutive patients with non-ulcer dyspepsia upon upper gastrointestinal (GI) endoscopy. Patients were examined using an Olympus GIF-Q30 endoscope (Olympus, Tokyo, Japan). One experienced endoscopist participated in the study, which allowed the inclusion of patients. Patients with duodenal ulcer (circumscribed break of > 5 mm depth in the mucosa, covered with exudate, present in the prepyloric, pyloric, or duodenal bulb region), gastric ulcer (with the above-described mucosal defect located at the angulus or above it), gastric polyps or cancers, bleeding complications, previous gastric resection and those who had been treated with anti-\textit{H pylori} treatment, aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs) or antibiotics 2 wk before the study were excluded.

During endoscopy, two antral and two corpus biopsies were taken and fixed in 10% buffered formalin and then embedded in paraffin for histological examination (Giemsa stain) and for immunohistochemical staining of hMLH1 and hMSH2. Patients were considered to be \textit{H pylori}-positive when histological demonstration of the bacterium was positive. Fifty \textit{H pylori}-positive and 50 \textit{H pylori}-negative patients were enrolled. The updated Sydney system was used to evaluate pathological findings, such as gastritis severity, gastric atrophy, intestinal metaplasia, gastric atrophy and dysplasia\textsuperscript{[20]}.

\subsection*{Immunohistochemical staining}

Immunohistochemical staining was performed following the Envision method on the gastric biopsy specimens from \textit{H pylori}-positive and -negative patients. Four-micrometer-thick sections were obtained from formalin-fixed paraffin-embedded tissue blocks. The tissue sections were deparaffinized in xylene and rehydrated in graded concentrations of alcohol. Endogenous peroxidase activity was blocked by treating the sections with blocking solution. For antigen retrieval, the sections were treated while boiling in citrate buffer pH 9.0 in a microwave oven. Then sections were incubated with primary antibodies against hMLH1 (BD Biosciences Pharmingen, clone G168-15, 1:100 dilution) and hMSH2 (Calbiochem, Oncogene Sciences, clone FE11, 1:100 dilution). After each step, slides were washed with TBS buffer for 3 min. Then, slides were treated with Envision (DAKO, REAL Envision) for 20 min. To visualize immunoreactivity, 3,3′-diaminobenzidine was used and samples were counterstained with hematoxylin. Intramucosal lymphocytes were used as positive controls. Samples from patients with hereditary non-polyposis colon cancer were used as a negative control. These samples have loss of nuclear staining in tumoral cells\textsuperscript{[20]}. The slides were evaluated by two pathologists who were blinded regarding \textit{H pylori} status.

A case was considered positive for expression of hMLH1 or hMSH2 in the presence of nuclear staining of epithelial cells; however, it was considered negative when there was a complete absence of nuclear staining of the epithelial cells in the presence of an unquestioned internal positive control. The staining intensity was divided into three grades. We counted more than 500 epithelial cells (including glandular neck, foveolar and surface epithelium) in each case at × 200 magnification. Quantitative 1 analysis was performed by measuring the total number of cells and the positive-staining epithelial cells. The percentage positivity was then calculated\textsuperscript{[20]}.

\subsection*{Statistical analysis}

The \( \chi^2 \) test and an unpaired Student’s \( t \) test were used, when appropriate. \( P < 0.05 \) was considered statistically significant. All data were analyzed by SPSS version 13.0 (SPSS, Chicago, II., USA).

\section*{RESULTS}

Fifty \textit{H pylori}-positive patients with a mean age of 41.78 ± 15.21 years and 50 \textit{H pylori}-negative patients with a mean age of 46.58 ± 13.41 years were studied (\( P = 0.1 \)). There was no significant difference in the male to female ratio between the two groups (28/22 in the \textit{H pylori}-positive group versus 23/27 in the \textit{H pylori}-negative group; \( P = 0.32 \)). Table 1 outlines the characteristics and pathological data of both groups. As shown in Table 1, pathological findings such as gastritis severity, gastritis activity, intestinal metaplasia, gastric atrophy and dysplasia were not significantly different between the two groups.

The percentage of epithelial cell nuclei that demonstrated positivity for hMLH1 staining was 84.14 ± 7.32\% in \textit{H pylori}-negative patients; while it was 73.34 ± 10.10\% in \textit{H pylori}-positive patients (\( P < 0.0001 \)). No
significant difference was seen between the two groups regarding the percentage of epithelial cell nuclei that were positive for hMSH2 staining (81.16 ± 8.32% in H pylori-negative patients versus 78.24 ± 8.71% in H pylori-positive patients; P = 0.64). As shown in Table 2, the immunohistochemical staining in the corpus and antrum was relatively similar for hMLH1 or hMSH2.

Intensity of immunohistochemical staining for hMLH1 did not differ significantly between the groups (1.99 ± 0.41 in H pylori-negative patients versus 1.95 ± 0.47 in H pylori-positive patients; P = 0.64). For hMSH2, intensity of immunohistochemical staining was 1.93 ± 0.46 in H pylori-negative patients and 1.99 ± 0.42 in H pylori-positive patients; however, the difference was not statistically significant (P = 0.50).

### DISCUSSION

The relationship between H pylori infection, gastric mucosal damage, and cell proliferation rate is a matter of debate. One hypothesis regarding how H pylori causes gastric carcinoma is through impairment of DNA repair in the gastric epithelium. This results in the accumulation of mutations and a genomic imbalance in the epithelium, which increases the risk of gastric carcinoma[27]. However, there was no significant difference. Results were the same as the study of Halling et al[34], which found that MSI-positive gastric carcinoma is usually associated with a lack of hMLH1 and rarely with a lack of hMSH2. Leung et al[35] have demonstrated that active H pylori infection is more frequently found in individuals with MSI-positive than in those with MSI-negative gastric cancer, which suggests that H pylori infection affects the DNA MMR system during the stepwise progression of gastric carcinogenesis. Park et al have studied the expression of hMLH1 and hMSH2 in patients with chronic H pylori infection before and after bacterial eradication. They have found that the expression of DNA MMR proteins increases in the gastric mucosa after H pylori eradication, which indicates that H pylori is associated with impairment of the DNA MMR system. Kim et al[36] cocultured gastric cancer cell lines with H pylori and then determined MutL and MutS DNA MMR protein and RNA levels. All cell lines showed decreased levels of MutL and MutS DNA MMR proteins in a dose-dependent manner[37]. Lack of an efficient DNA

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**Table 1** Demographic and pathological findings of H pylori-positive and -negative patients n (%)

|                | Positive group | Negative group | P value |
|----------------|---------------|----------------|---------|
| Age (mean ± SD, yr) | 41.78 ± 15.21 | 46.58 ± 13.41 | 0.1     |
| Male:female     | 28:22         | 23:27          | 0.32    |
| Gastritis severity |              |                |         |
| 1              | 8 (16)        | 13 (26)        | 0.45    |
| 2              | 34 (68)       | 29 (58)        |         |
| 3              | 8 (16)        | 8 (16)         |         |
| Gastritis activity |              |                | 0.23    |
| 0              | 8 (16)        | 15 (30)        |         |
| 1              | 6 (12)        | 9 (18)         |         |
| 2              | 28 (56)       | 20 (40)        |         |
| 3              | 8 (16)        | 6 (12)         |         |
| Intestinal metaplasia |        |                | 0.45    |
| Positive       | 8 (16)        | 11(22)         |         |
| Negative       | 42 (84)       | 39(78)         |         |
| Atrophy        | 7 (14)        | 5 (10)         | 0.54    |
| Positive       | 43 (86)       | 45 (90)        |         |
| Negative       |              |                |         |
| Dysplasia      | 3 (6)         | 1 (2)          | 0.31    |
| Positive       |              |                |         |
| Negative       | 47 (94)       | 49 (98)        |         |

**Table 2** Immunohistochemical staining of gastric biopsy specimens from H pylori-positive and -negative patients

|                | Positive group | Negative group | P value |
|----------------|---------------|----------------|---------|
| hMLH1 Body Area | 73.80 ± 11.77 | 85.28 ± 7.71 | 0.000   |
| Intensity      | 2.02 ± 0.65   | 2.08 ± 0.63   | 0.64    |
| Antrum Area    | 72.44 ± 11.35 | 82.36 ± 9.63 | 0.000   |
| Intensity      | 1.88 ± 0.59   | 1.92 ± 0.63   | 0.75    |
| Overall Area   | 73.34 ± 10.10 | 84.14 ± 7.32 | 0.000   |
| Intensity      | 1.95 ± 0.47   | 1.99 ± 0.41   | 0.64    |
| hMSH2 Body Area | 77.24 ± 11.36 | 81.28 ± 10.58 | 0.07   |
| Intensity      | 1.96 ± 0.57   | 1.96 ± 0.64   | 1.00    |
| Antrum Area    | 78.76 ± 11.24 | 80.62 ± 10.89 | 0.40   |
| Intensity      | 2.04 ± 0.53   | 1.90 ± 0.65   | 0.24    |
| Overall Area   | 78.24 ± 8.71  | 81.16 ± 8.32  | 0.09    |
| Intensity      | 1.99 ± 0.42   | 1.93 ± 0.46   | 0.50    |
MMR system can potentially have dramatic effects on the cell genome by allowing the accumulation of mutations in critical regulatory genes.

In this study, immunohistochemical staining of the corpus and antrum was similar for hMLH1 and hMSH2. This indicates that H pylori affects DNA MMR stems of gastric epithelium regardless of its location. In conclusion, this study indicates the that oncogenic bacterium H pylori might promote development of gastric carcinoma, at least in part through its effect on the DNA MMR system. Impairment of the DNA MMR system represents a novel mechanism of infection-associated cancer promotion.

**COMMENTS**

**Background**
Cancer arises from the accumulation of inherited polymorphism (i.e. SNPs) and mutation and/or sporadic somatic polymorphism (i.e. non-germline polymorphism) in cell cycle, DNA repair, and growth signaling genes. Main molecular mechanisms underlying cancer development include the overexpression of genes, including oncopogens and growth factors or their receptors, and impaired expression of tumor suppressor genes resulting from mutation or allelic losses and deficiencies of the DNA mismatch repair (MMR) system.

**Research frontiers**
During chronic gastritis the mucosa undergoes rapid turnover and increased cell proliferation may permit an increased number of uncorrected mutations that may be induced by inadequate DNA MMR activity. Impairment of the DNA MMR system is a known mechanism of carcinogenesis and tumor progression of both sporadic and hereditary human cancers. Fewer advances have been made to understand how long it takes for Helicobacter pylori (H pylori) infection to induce the development of gastric cancer. The MMR deficiency leads to the accumulation of base-base mismatches, and the short insertion/deletion mispairs during DNA replication resulting in widespread mutation generated as a consequence of DNA replication errors. Most cells deficient in MMR display a high level of genomic instability characterized by changes in simple repetitive sequences so-called microsatellite instability (MSI).

**Innovations and breakthroughs**
Chronic H pylori infection damages gastric cell barrier function and stimulates gastric cell proliferation, which leads to mucosal repair, but which can also induce cellular DNA damage. H pylori gastritis occurs more frequently in individuals with MSI-positives than those with MSI-negative gastric cancers, raising the possibility that H pylori infection affects the DNA MMR system. The present study confirmed H pylori may promote development of gastric carcinoma at least in part through the ability to affect the DNA MMR system.

**Applications**
This study indicates that impairment of the DNA MMR system represents a novel mechanism of infection-associated cancer promotion.

**Terminology**
The association of H pylori with gastric cancer is supported by epidemiological studies showing that the odds ratios for gastric cancer is up to nine-fold greater in H pylori-infected individuals. Impairment of the DNA MMR system is a known mechanism of carcinogenesis and tumor progression of both sporadic and hereditary human cancers. Most cells deficient in MMR display a high level of genomic instability characterized by changes in simple repetitive sequences, so-called microsatellite instability (MSI). Chronic H pylori infection damages gastric barrier function[2,12] and stimulates gastric cell proliferation[15,16], which leads to mucosal repair[2,15], but which can also induce cellular DNA damage.

**Peer review**
Interesting paper but needed some amendment. This paper is adequately presented. The investigation is useful for the progress in the knowledge of this topic.

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