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RAPID COMMUNICATION

Helicobacter pylori infection and expression of DNA mismatch repair proteins

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Abstract

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 84.14 ± 7.32% in *H pylori*-negative patients, while it 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 (81.16 ± 8.32% in *H pylori*-negative *versus* 78.24 ± 8.71% in *H pylori*-positive 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.

Key words: *Helicobacter pylori*; DNA mismatch repair; hMLH1, hMSH2

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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]. 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[9,10]. 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[11]. 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 the gastric barrier function[12,13] and stimulates gastric cell proliferation[14-19], which leads to mucosal repair[20], but which can also induce cellular DNA damage[18-22].

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

MATERIALS AND METHODS

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[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-*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 immunohisto-chemical staining of hMLH1 and hMSH2. Patients were considered to be *H pylori*-positive when histological demonstration of the bacterium was positive. Fifty *H pylori*-positive and 50 *H pylori*-negative patients were enrolled. The updated Sydney system was used to evaluate pathological findings, such as gastritis severity, gastritis activity, intestinal metaplasia, gastric atrophy and dysplasia[26].

Immunohistochemical staining

Immunohistochemical staining was performed following the Envision method on the gastric biopsy specimens from *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[24]. The slides were evaluated by two pathologists who were blinded regarding *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 I analysis was performed by measuring the total number of cells and the positive-staining epithelial cells. The percentage positivity was then calculated[24].

Statistical analysis

The χ^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, IL, USA).

RESULTS

Fifty *H pylori*-positive patients with a mean age of 41.78 ± 15.21 years and 50 *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 *H pylori*-positive group *versus* 23/27 in the *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 *H pylori*-negative patients; while it was 73.34 ± 10.10% in *H pylori*-positive patients ($P < 0.0001$). No

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			0.45
1	8 (16)	13 (26)	
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			0.54
Positive	7 (14)	5 (10)	
Negative	43 (86)	45 (90)	
Dysplasia			0.31
Positive	3 (6)	1 (2)	
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

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; $P = 0.09$). As shown in Table 2, the immunohistochemical staining in the corpus and antrum was relatively similar for hMLH1 or hMSH2.

Intensity of immmunohistochemical 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 immmunohistochemical 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]. Previous studies have shown that active *H pylori* infection neither was more frequently seen in patients who had MSI-positive gastric carcinomas or intestinal metaplasia nor attach to carcinoma cells *in vivo* It is possible that during chronic gastritis, *H pylori* is physically in direct contact with gastric epithelial cells, and disturbs them at the molecular level. Studies on cytokine induction by *H pylori* support this hypothesis[28,29]. 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 in sporadic and hereditary human cancers[9,10]. In humans, MMR is mediated by at least six genes, including hMLH1, hMSH2, hMSH3, hMSH6, hPMS2 and hPMS1[30]. Germline mutations in hMSH2 and hMLH1 account for approximate 90% of all reported MMR gene mutations, whereas hPMS2 and hMSH6 account for the remainder[31]. Several studies have shown that hMLH1 and hMSH2 are the two main MMR proteins and the other MMR proteins including hPMS2, hPMS1, and hMSH6 seem to be unstable in the absence of the main MMR proteins[32,33].

Our findings indicate that decreased levels of hMLH1 proteins are seen in gastric epithelial cells in *H pylori*-positive patients. Although the level of hMSH2 proteins was lower in *H pylori*-positive patients, 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*[28] 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*[27] 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[23]. Lack of an efficient DNA

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 that the 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 oncogenes 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 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-positive 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* might 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[12,13] and stimulates gastric cell proliferation[14-19], which leads to mucosal repair[20], 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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