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ORIGINAL ARTICLE

Effect of Helicobacter pylori infection on the expression of DNA Mismatch Repair Protein

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ABSTRACT

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

Background: Impairment of DNA mismatch repair (MMR) system is a known mechanism of carcinogenesis and tumor progression in both sporadic and hereditary human cancers.

Patients and methods: Fifty H.pylori-positive patients and 50 H.pylori-negative subjects were enrolled in the study. During endoscopy of patients with non-ulcer dyspepsia, 2 antral and 2 corpus biopsies were taken for histologic examination (Giemsa stain) and immunohistochemical staining on hMLH1 and hMSH2.

Results: The percentage of epithelial cell nuclei demonstrating positivity for hMLH1 staining was 84.14±7.32 in H.pylori-negative subjects in comparison of 73.34±10.10 in H.pylori-positive patients ($p<0.0001$). The percentage of epithelial cell nuclei demonstrating positivity for hMSH2 staining did not differ significantly (81.16±8.32 in H.pylori-negative versus 78.24±8.71 in H.pylori-positive patients; NS).

Conclusion: Study indicates that H.pylori might promote development of gastric carcinoma at least in part through the ability to affect the DNA MMR system.

Keywords: *Helicobacter pylori, DNA mismatch repair (MMR), hMLH1, hMSH2*.

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INTRODUCTION

Helicobacter pylori (H. pylori) infection affects about half of the worldwide 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 epidemiologic studies showing odds ratios for gastric cancer up to 9-fold greater in H.pylori-infected individuals (3). Chronic H.pylori infection can causes chronic gastritis, which often progresses to gastric atrophy and intestinal metaplasia which are premalignant lesions of the stomach (4). Although many epidemiologic studies have addressed the association of H.pylori infection and gastric cancer, fewer advances have been made to understand how long it takes for H.pylori infection to induce the development of gastric cancer.

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

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losses (5,6) and deficiencies of the DNA mismatch repair (MMR) system (7,8).

Impairment of DNA mismatch repair (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 the short insertion/deletion mispairs during DNA replication resulting in widespread mutation (11) 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). Chronic H pylori infection damages gastric barrier function (12,13) and stimulates gastric cell proliferation (14-19) which leads to mucosal repair (20), but can also induce cellular DNA damage (18-22).

H. pylori gastritis occurs more frequently in individuals with microsatellite instability-positive than those with microsatellite instability-negative gastric cancers, raising the possibility that H. pylori infection affects DNA mismatch repair (MMR) system (23).

The aim of this study was to determine the expression of DNA MMR proteins, including hMLH1 and hMSH2, in gastric epithelial cells of patients with or without H. pylori infected gastritis.

PATIENTS and METHODS

We examined dyspeptic patients who referred for endoscopic evaluation to Taleghani hospital in Tehran. Dyspepsia was defined as persistent or recurrent abdominal pain or abdominal discomfort, centered in the upper abdomen, with duration of at least 3 months. 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 in upper 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 considerable depth [>5 mm] in the mucosa, covered with exudate, present in the prepyloric, pyloric, or duodenal bulb region), gastric ulcer (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 on H.pylori treatment, aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs) or antibiotics 2 weeks prior to the study, were excluded. During endoscopy, 2 antral and 2 corpus biopsies were taken and fixed in 10% buffered formalin and then embedded in paraffin for histologic examination (Giemsa stain) and for immunohistochemical staining on hMLH1 and hMSH2.

Patients were considered to be H.pylori positive when histologic demonstration of H.pylori was positive. 50 patients with H.pylori positive and 50 subjects with H.pylori negative were enrolled in the study. The updated Sydney system was used to evaluate pathologic findings such as gastritis severity, gastritis activity, intestinal metaplasia, gastric atrophy and dysplasia (26).

Immunohistochemical staining was performed following Envision method on the gastric biopsy specimens of 50 patients of both H.pylori-positive and -negative groups. Four micron-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. Then sections were incubated with primary antibodies 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 minutes. Then, slides were treated with Envision (DAKO, REAL Envision) for 20 minutes. To visualize immunoreaction, 3, 2′-diaminobenzidine was used and samples were counterstained with hematoxylin. Intramucosal lymphocytes were used as positive controls. Samples of patients with HNPCC (hereditary non-polyposis colon cancer) were used for negative control. The slides were evaluated by two pathologists who were blinded to the H.pylori status.

A case was considered positive for expression of hMLH1 or hMSH2 in the presence of nuclear staining of the 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 using 200 magnifications. 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).

Chi-square and unpaired Student's t-test were used, when appropriate. P-values less than 0.05 were considered statistically significant. All data were analyzed by SPSS program (version 13.0, SPSS Inc., Chicago, IL)

RESULTS

Fifty H.pylori-positive patients with mean (±standard deviation) age of 41.78±15.21 years and 50 H.pylori-negative patients with mean age of 46.58±13.41 were studied (NS). There was no significant difference between male to female ratio among two groups. (28/22 in H.pylori-positive versus 23/27 in H.pylori-negative group). Table 1 demonstrated characteristic and pathologic data of both groups. As shown in table 1, pathologic finding such as gastritis severity, gastritis activity, intestinal metaplasia, gastric atrophy and dysplasia were not significantly different between groups.

Table 1. *Demographic and pathologic findings among H.pylori-positive and H.pylori-negative patients*

	H.pylori positive group (n=50)	**H.pylori negative group (n=50)**	**p-value**
Age(years)	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%)	
2	34 (68%)	29 (58%)	0.45
3	8 (16%)	8 (16%)	
Gastritis activity			
0	8 (16%)	15 (30%)	
1	6 (12%)	9 (18%)	0.23
2	28 (56%)	20 (40%)	
3	8 (16%)	6 (12%)	
Intestinal metaplasia			
Positive	8 (16%)	11(22%)	0.45
Negative	42 (84%)	39(78%)	
Atrophy			
Positive	7 (14%)	5 (10%)	0.54
Negative	43 (86%)	45 (90%)	
Dysplasia			
Positive	3 (6%)	1 (2%)	0.31
Negative	47 (94%)	49 (98%)	

The percentage of epithelial cell nuclei demonstrating 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 ($p<0.0001$). However, there was a non-significant difference between groups regarding to the percentage of epithelial cell nuclei demonstrating positivity for hMSH2 staining (81.16±8.32 in H.pylori-negative versus 78.24±8.71 in H.pylori-positive patients; $p=0.09$). As shown in table 2, the results of immunohistochemical staining in body and antrum was relatively similar for hMLH1 or hMSH2. Intensity of immmunohistochemical staining for hMLH1 was not significantly differed between groups (1.99±0.41 in H.pylori-negative 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 versus 1.99±0.42 in H.pylori-

positive patients; however their difference did reach a significant level.

Table 2. *Results of immunohistochemical staining among H.pylori-positive -negative patients*

	H.pylori positive group (n=50)	H.pylori negative group (n=50)	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
hMLH2			
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

* mean±SD

DISCUSSION

The relation between H pylori infection, gastric mucosal damage, and the cell proliferation rate is a matter of debate. One hypothesis explains that H.pylori causes an impairment of DNA repair in the gastric epithelium. This results in accumulation of mutations and a genomic imbalance in the epithelium, increasing 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, disturb epithelial cell molecular pathway. 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 DNA mismatch repair (MMR) system is a known mechanism of carcinogenesis and tumor progression of both 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 about 90% of all reported MMR gene mutations, whereas *hPMS2* and *hMSH6* account for the reminders (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 were seen in gastric epithelial cells in H.pylori positive patients. Although the level of *hMSH2* proteins was lower in H.pylori positive patients, their difference did not reach a statistically significant level. Results are in agreement with Halling KC et al. in which they found that microsattelite instability (MSI)- positive gastric carcinomas are usually associated with lack of hMLH1 and rarely with lack of hMSH2 (34). Leung et al. demonstrated that active H.pylori infection was more frequently found in individuals with MSI-positive than in those with MSI- negative gastric cancers, proposing that H.pylori infection affects the DNA MMR system during the stepwise progression of gastric carcinogenesis (28).

Park et al studied the expression of hMLH1 and hMSH2 in patients with chronic H.pylori infection

before and after eradication of the H.pylori. They found that the expression of DNA MMR proteins increased in the gastric mucosa after H.pylori eradication, indicating that H.pylori may be associated with a reduced DNA MMR system (27). Kim JJ et al 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 after coculture with H.pylori strains (23). Lack of efficient DNA MMR system can potentially have dramatic effects in the cell genome by allowing the accumulation of mutations in critical regulatory genes.

In this study, the results of immunohistochemical staining of body and antrum were similar for hMLH1 and hMSH2. It indicates that H.pylori affects DNA MMR stems of gastric epithelium regardless of its location.

In conclusion, this study indicates that the oncogenic bacteria H.pylori might promote development of gastric carcinoma at least in part through the ability to affect the DNA MMR system. Impairment of the DNA MMR system represents a novel mechanism of infection-associated cancer promotion.

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