

Effect of *Helicobacter pylori* Eradication According to the IL-8-251 Polymorphism in Koreans

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Received: 16 November 2011
Accepted: 30 July 2012

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This study was supported by a grant from the Seoul National University Hospital Research Fund, Republic of Korea (No. 03-2008-005).

Previous studies suggested that polymorphisms of proinflammatory cytokine genes are important host genetic factors in *Helicobacter pylori* infection. The present study evaluated whether IL-8-251 polymorphism affected *H. pylori* eradication rate and to investigate the effect of *H. pylori* eradication on angiogenesis and the inflammatory process according to the IL-8-251 polymorphism. A total of 250 *H. pylori*-positive patients treated by endoscopic resection of the gastric neoplasm were classified into 3 groups (134 *H. pylori*-eradicated group, 19 *H. pylori*-eradication failure group, and 97 *H. pylori*-infected group). *H. pylori* status, histology, and angiogenic factor levels were evaluated at baseline, 6 months, and 18 months. *H. pylori* eradication rate was 92.9% in AA genotype, 85.7% in AT genotype and 88.4% in TT genotype (P value = 0.731). Elevated IL-8 and matrix metalloproteinase-9 concentrations in *H. pylori*-infected gastric mucosa were reversible by successful eradication of *H. pylori*, independent of the IL-8-251 polymorphism. It is suggested that elevated IL-8 and MMP-9 concentrations in *H. pylori*-infected gastric mucosa are altered significantly after successful eradication and these conditions continue for 18 months. However, IL-8-251 polymorphism does not affect *H. pylori* eradication rate and the sequential changes of related angiogenic factors after *H. pylori* eradication in Koreans.

Key Words: *Helicobacter pylori*; Interleukin-8; Polymorphism

INTRODUCTION

Helicobacter pylori infection plays a major role in gastric carcinogenesis, commencing with chronic gastritis and leading to a sequence of atrophic gastritis, metaplasia, dysplasia, and subsequently, cancer (1, 2). Host genetic factors, bacterial virulence, and environmental factors are associated with gastric carcinogenesis in *H. pylori*-infected hosts. Recent reports have suggested that polymorphisms of proinflammatory cytokine genes are important host genetic factors in *H. pylori* infection (3-8).

Interleukin-8 (IL-8), a potent chemoattractant for neutrophils and lymphocytes, has been reported as a strong stimulator of angiogenesis in gastric adenocarcinoma (9-12). In Korea, the IL-8-251 A allele has been associated with higher IL-8 production, more severe inflammation, mucosal atrophy, and intestinal metaplasia when compared with the IL-8-251 TT genotype of *H. pylori*-infected patients. Recent studies suggested a possible association of the IL-8-251 A allele with angiogenesis and inflammation in gastric carcinogenesis in *H. pylori*-infected Koreans (13, 14). *H. pylori* infection induces several angiogenic factors and proteases, including vascular endothelial growth factor (VEGF), angiopoietin and matrix metalloproteinase-9

(MMP-9) (11, 15).

There has been no data to show the association of IL-8-251 polymorphism and *H. pylori* eradication in gastric neoplasm. The primary aim of this study was to evaluate whether IL-8-251 polymorphism affects *H. pylori* eradication rate. The secondary aim was to evaluate the effect of *H. pylori* eradication on angiogenesis and the inflammatory process in Koreans according to the IL-8 polymorphism, after endoscopic resection of gastric neoplasms.

MATERIALS AND METHODS

Patients and tissue collection

From April 2005 to June 2008, patients who underwent endoscopic resection of gastric adenoma or early gastric cancer (EGC) at Seoul National University Hospital were prospectively enrolled and retrospectively reviewed. The presence of *H. pylori* infection was confirmed with rapid urease test and/or histology prior to endoscopic resection. Patients with a history of gastric surgery, *H. pylori* eradication therapy, or systemic disease requiring chronic medication were excluded. Patients who had used proton pump inhibitors (PPI), nonsteroidal anti-inflammatory

drugs, or antibiotics within 4 weeks of enrollment were also excluded.

Patients received either *H. pylori* eradication treatment or were followed-up without eradication after endoscopic resection of gastric neoplasm. Patients in the eradication group received omeprazole 20 mg twice daily, amoxicillin 1,000 mg twice daily, and clarithromycin 500 mg twice daily for a week, whereas patients in the follow-up group received standard care without eradication of *H. pylori*.

Patients underwent an endoscopic follow-up examination at 3 months, 6 months, 12 months, and 18 months after endoscopic resection to evaluate gastric histology and angiogenic factor levels. During the follow-up period, biopsy samples were taken for evaluation of *H. pylori* status, histology, and angiogenic factors.

IL-8-251 A/T polymorphism

One piece of gastric mucosal tissue was taken from a non-lesional area of the antrum, and genomic DNA was isolated using a standard proteinase K digestion and phenol/chloroform extraction. The IL-8-251 A/T polymorphism was identified using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis, as previously described (16). For PCR-RFLP, the genomic region containing the IL-8-251 polymorphism was amplified with the forward (5'-TCA TCC ATG ATC TTG TTC TAA-3') and reverse (5'-GGA AAA CGC TGT AGG TCA GA-3') primers, using the PerkinElmer model 9600 (PerkinElmer Co., Norwalk, CT, USA), programmed at 95°C for 5 min, 35 amplification cycles (95°C for 30 sec, 54°C for 1 min and 72°C for 1 min), followed by 72°C for 5 min. Following PCR, the *MfeI* (New England Biolabs Inc., Beverly, MA, USA) restriction enzyme was used, and the enzyme-digested products were applied to a 2% agarose-gel running electrophoresis system. IL-8-251 AA, AT and TT genotypes were determined according to the expected band patterns visualized on the agarose gel; the AA genotype showed 92 and 450 base-pair bands, whereas the TT genotype showed a 542 base-pair band, and the AT genotype showed 92, 450, and 542 base-pair bands (13, 16).

H. pylori tests and grading of gastritis

H. pylori infection was defined if results from either the rapid urease test (CLOtest; Kimberley-Clark, Draper, UT, USA) or modified Giemsa staining of the antral and/or body mucosa were positive. *H. pylori* eradication was defined if both results from follow-up biopsy were negative. Two pieces of gastric tissue, one from the non-lesional mucosa of the lesser curvature side of the antrum and the other from the midbody, were taken for histological evaluation using the updated Sydney system (17). The degree of *H. pylori* density, neutrophil infiltration, mononuclear cell infiltration, atrophy, and intestinal metaplasia were assessed and scored as 0 = none, 1 = mild, 2 = moderate, and 3 = marked. Specimens were examined in a blinded manner by

a single pathologist.

Determination of IL-8, MMP-9, VEGF and Ang-1 concentrations

Two pieces of mucosal tissue were obtained from the non-lesional gastric antrum during endoscopic examination. For analysis, the tissues were homogenized using a tissue homogenizer (Thomas scientific, Swedesboro, NJ, USA); supernatants were taken after centrifugation at 10,000 g for 10 min. The total protein amount was measured using a modified Lowry method. Commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems Inc., Minneapolis, MN, USA) were used according to manufacturer instructions for immunological quantification of IL-8, VEGF, MMP-9, and Ang-1. All assays on samples and controls were performed in duplicate, and the two results were averaged. Mucosal concentrations of cytokines were expressed as picogram or nanogram of cytokine per milligram of total protein (pg/mg or ng/mg protein).

Statistical analysis

Baseline characteristics were compared using the chi-square test and the Student's t-test for categorical and continuous variables, respectively. Following endoscopic resection, changes in histological scores and angiogenic factor levels were compared in relation to IL-8-251 genotypes (A carriers, including AA and AT vs TT) using the one-way ANOVA. A *P* value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS software (version 19.0; SPSS Inc., Chicago, IL, USA).

Ethics statement

The institutional review board of the Seoul National University Hospital (Institutional Review Board Number: H 0603-122-170, 0608-023-180) approved this study. Written informed consent was obtained from all patients.

RESULTS

Characteristics of patients and *H. pylori* eradication rate

Data were collected prospectively from consecutive patients who underwent endoscopic resection of the gastric neoplasm, and then retrospectively reviewed. Among a total of 250 *H. pylori*-positive patients treated by endoscopic resection, 153 patients (61.2%) received eradication therapy, and 97 patients (38.8%) were followed-up without eradication. *H. pylori* was successfully eradicated in 134 patients (eradication rate 87.6%). A total of 19 patients were classified as eradication failure group who were treated with *H. pylori* eradication treatment but had persistent *H. pylori* infection. Clinical and pathological findings were compared among the 3 groups (134 *H. pylori*-eradicated group, 19 *H. pylori*-eradication failure group, and 97 *H. pylori*-

infected group). No significant differences were noted among the 3 groups in terms of mean age, gender ratio, gastric disease, and IL-8-251 polymorphism (Table 1).

Successful eradication rate of *H. pylori* was compared depending on the IL-8-251 A/T polymorphism. *H. pylori* eradication

rate was 92.9% in AA genotype, 85.7% in AT genotype and 88.4% in TT genotype (P value = 0.731). A comparison between A carriers (*H. pylori* eradication rate 86.9%) and TT genotype also showed no significant difference (P value = 0.779).

Table 1. Characteristics of patients

	All (N = 250)	<i>H. pylori</i> eradicated (N = 134)	<i>H. pylori</i> infected (N = 97)	<i>H. pylori</i> eradication failure (N = 19)	<i>P</i> value
Age (mean ± SD)	60.5 ± 8.0	59.7 ± 8.2	61.6 ± 7.6	60.4 ± 8.1	0.207
Sex (male %)	65.6	70.9	61.9	47.4	0.079
Disease (N, %)					0.244
Adenoma	108 (43.2%)	64 (47.8%)	38 (39.2%)	6 (31.6%)	
Cancer	142 (56.8%)	70 (52.2%)	59 (60.8%)	13 (68.4%)	
IL-8-251 (N, %)					0.457
AA	24 (9.6%)	13 (9.7%)	10 (10.3%)	1 (5.3%)	
AT	124 (49.6%)	60 (44.8%)	54 (55.7%)	10 (52.6%)	
TT	102 (40.8%)	61 (45.5%)	33 (34.0%)	8 (42.1%)	
IL-8-251 (N, %)					0.213
A carrier	148 (59.2%)	73 (54.5%)	64 (66.0%)	11 (57.9%)	
TT	102 (40.8%)	61 (45.5%)	33 (34.0%)	8 (42.1%)	

N, number; SD, standard deviation.

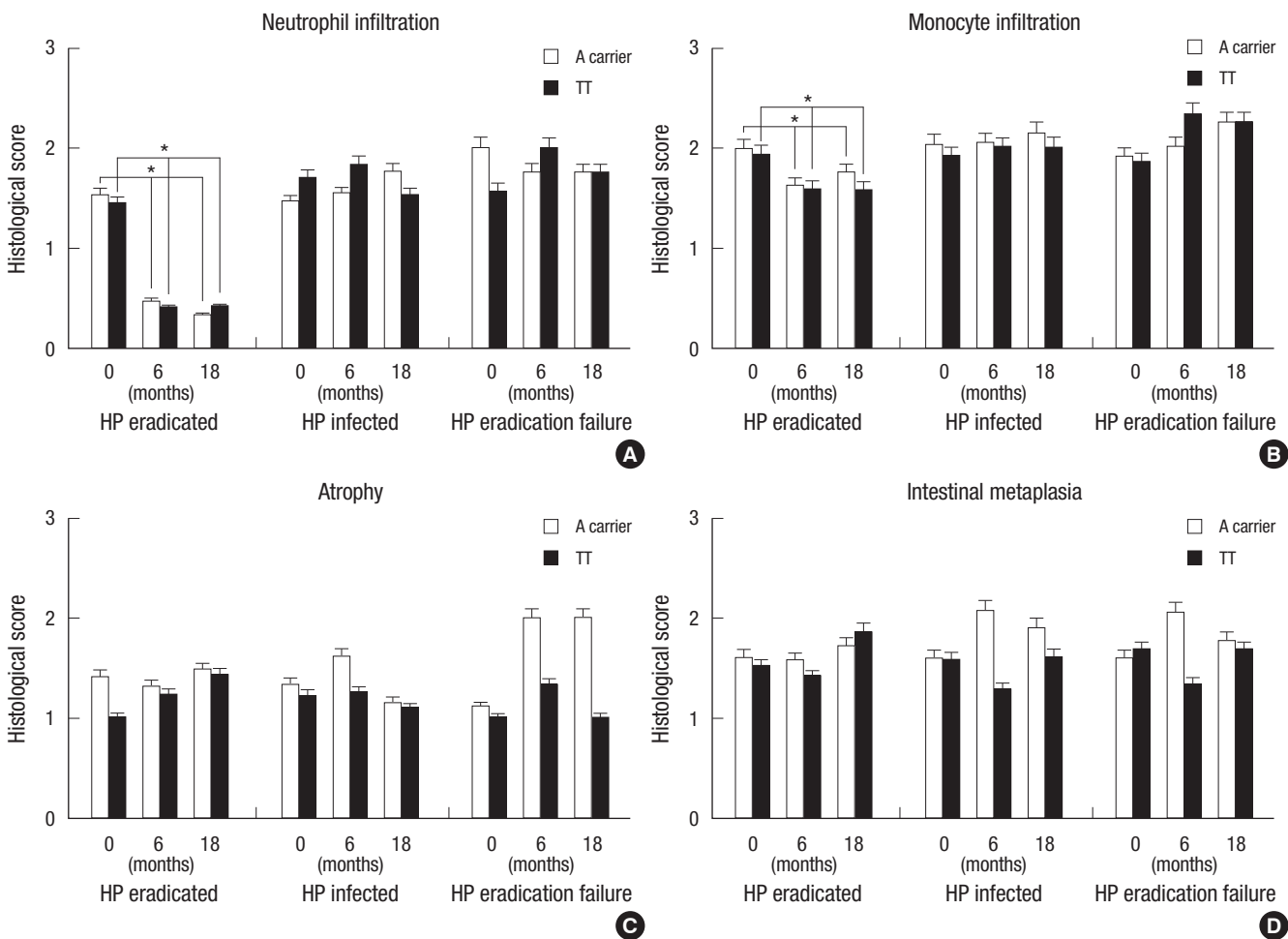


Fig. 1. Comparison of the *H. pylori*-eradicated, *H. pylori*-infected and *H. pylori*-eradication failure group in relation to histological grade of the antrum, according to the IL-8-251 A/T polymorphism. Scores of (A) neutrophil, (B) monocyte infiltration, (C) atrophy and (D) intestinal metaplasia. Results are shown as the mean ± S.E.M. * $P < 0.05$ when compared with baseline histology.

Effects of *H. pylori* eradication on histological scores according to the IL-8 polymorphism

Following endoscopic resection, changes in histological scores in the gastric antrum were compared in relation to IL-8-251 genotypes in the 3 groups, respectively (Fig. 1). Mean scores for neutrophil and monocyte infiltration, which represents acute and chronic inflammation, were significantly improved in the *H. pylori*-eradicated group, independent of IL-8-251 genotypes. There were no significant differences in mucosal atrophy and intestinal metaplasia in all 3 groups, independent of IL-8-251 genotypes.

Effects of *H. pylori* eradication on angiogenic factor levels according to the IL-8 polymorphism

During the follow-up period, changes in IL-8 concentrations and angiogenic factors were compared in relation to IL-8-251 genotypes in the 3 groups, respectively (Fig. 2). IL-8 and MMP-9 concentrations showed a significant reduction for 18 months follow-up period in the eradicated group, irrespective of IL-8-

251 genotypes. However, IL-8 and MMP-9 levels in the *H. pylori*-infected group and *H. pylori*-eradication failure group showed no significant changes during the follow-up period. In contrast, VEGF levels showed a significant reduction in the *H. pylori*-infected group. Ang-1 levels showed no significant changes during the follow-up period in all 3 groups.

Angiogenic factor levels at 6 months and 18 months after endoscopic resection were compared between IL-8-251 A carriers and IL-8-251 TT genotypes. No significant difference was observed in all angiogenic factor levels between IL-8-251 A carrier and TT genotypes, irrespective of *H. pylori* eradication.

DISCUSSION

In Korea, gastric adenoma and EGC without concomitant lymph node metastasis are often treated with endoscopic resection. Because endoscopic resection removes only the tumor and surrounding mucosa, a metachronous gastric tumor can develop in the remaining gastric mucosa (18, 19). Previous studies to

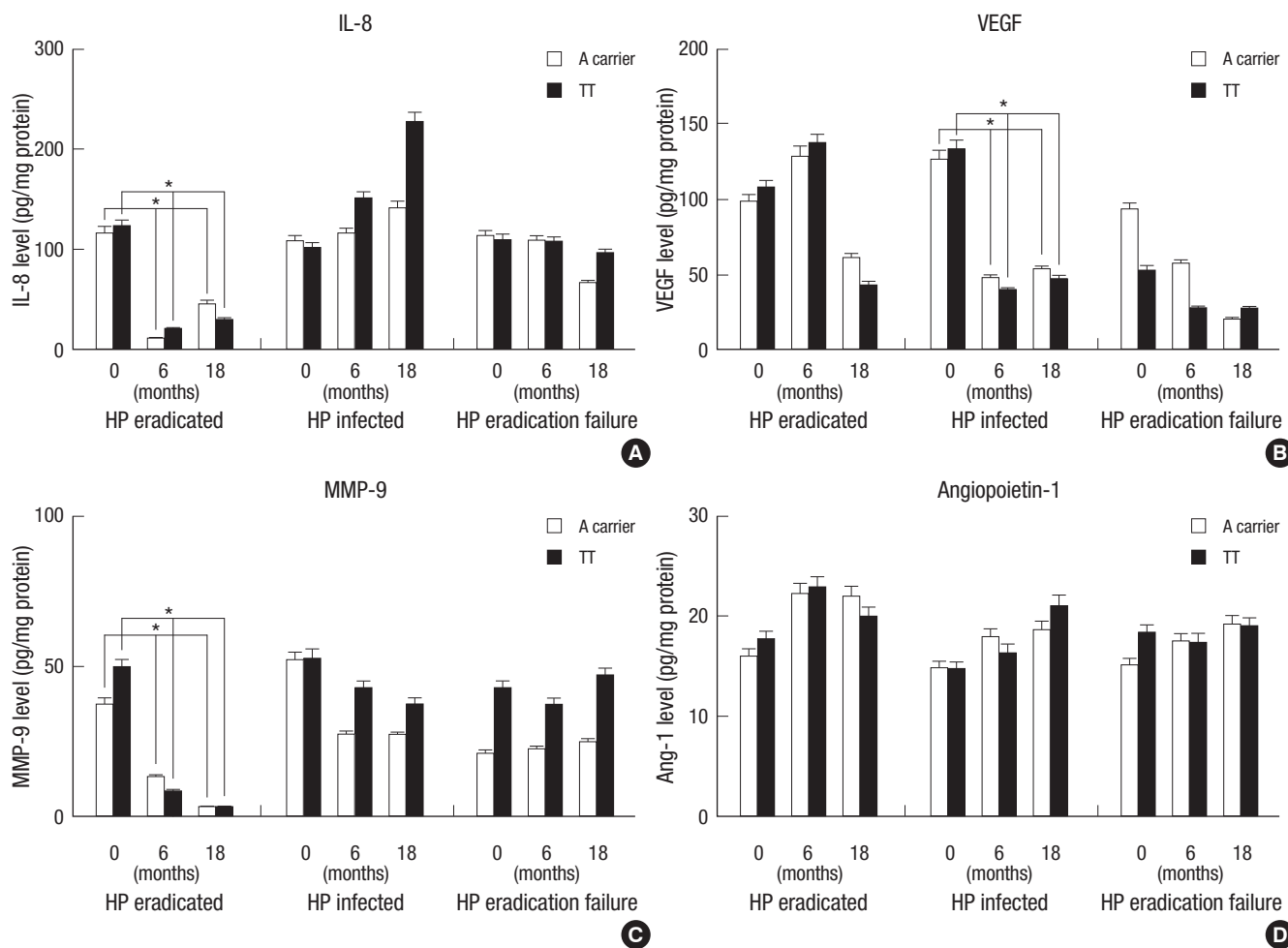


Fig. 2. Comparison of the *H. pylori*-eradicated, *H. pylori*-infected and *H. pylori*-eradication failure group in relation to concentration of IL-8, VEGF, MMP-9, and Ang-1, according to the IL-8-251 A/T polymorphism. Concentrations of (A) IL-8, (B) VEGF, (C) MMP-9 and (D) Ang-1. Results are shown as the mean \pm S.E.M. * $P < 0.05$ when compared with baseline concentrations.

determine whether or not *H. pylori* eradication reduces preneoplastic lesions and the prevalence of gastric cancer have been inconclusive (20-22). Polymorphism of the IL-8 gene has recently been associated with important host genetic factors for gastric carcinogenesis (4, 13). Findings from a recent study indicated that *H. pylori* infection causally promoted host angiogenesis, which has been attributed to either augmented inflammation or enhanced carcinogenesis, and proton pump inhibitor could potentially be used for inhibition of *H. pylori*-provoked angiogenesis (23).

Previous studies suggested that the IL-8-251 A allele is associated with severe inflammation and angiogenesis in *H. pylori*-infected Koreans (13, 14). However, there has been no data to see the association of IL-8-251 polymorphism and *H. pylori* eradication in gastric neoplasm. Therefore we aimed to evaluate whether IL-8-251 polymorphism affects *H. pylori* eradication rate in the first place. There was no significant difference in the *H. pylori* eradication rate depending on the IL-8-251 A/T polymorphism. This result suggested that IL-8-251 polymorphism did not affect *H. pylori* eradication rate.

As expected, *H. pylori* eradication induced significant improvement in acute and chronic inflammation characterized by neutrophil and monocyte infiltration. However, these improvements in the inflammatory process did not lead to recovery of preexisting atrophy and intestinal metaplasia. In consideration of genetic polymorphisms, there was no significant difference in gastritis scores between the IL-8-251 A allele and the TT genotypes. Results from previous studies have suggested that the IL-8-251 A allele is associated with severe inflammation, atrophy, and intestinal metaplasia of gastric body mucosa in patients aged 49 or younger; however, these phenomena were not observed in the older age group (13). Because the mean age of patients was 60.5 yr in our study, this could explain the lack of an association and would be consistent with the previous results. Furthermore, results concerning the effects of *H. pylori* eradication on premalignant conditions such as atrophic gastritis and intestinal metaplasia, are conflicted (24-26). In this study, the duration of follow-up after endoscopic resection was not long enough to evaluate the effect of *H. pylori* eradication on atrophy and intestinal metaplasia.

H. pylori infection was recently reported to induce IL-8 and MMP-9 expression in gastric mucosa, and increment of these factors contributes to tissue damage in *H. pylori*-associated gastritis (11, 27). In this study, IL-8 and MMP-9 concentrations were significantly decreased in gastric mucosa following *H. pylori* eradication, and low concentrations were maintained during the follow-up period. This result could indicate that down-regulation of IL-8 and MMP-9 by eradication of *H. pylori* might inhibit gastric mucosal inflammation and angiogenesis which is essential to gastric carcinogenesis. In the present study, VEGF concentrations in the *H. pylori*-infected group were significantly

decreased unlike IL-8 and MMP-9 concentrations. VEGF showed varied results in relation to *H. pylori* infection in previous studies. One study reported that *H. pylori* down-regulated VEGF receptors in vascular endothelial cells and this could explain our results (28). Findings from a recent study revealed significant correlation between mucosal concentrations of angiogenic factors and gastric disease progression in *H. pylori*-infected Koreans, particularly in the IL-8-251 AA genotype (14). However, several studies conducted in Western countries did not find any significant association between IL-8-251 polymorphism and risk of gastric cancer (29, 30). In this study, significant difference in angiogenic factor levels was caused by *H. pylori* eradication, not by the IL-8 polymorphism. These results suggest that the IL-8-251 polymorphism had relatively little influence on angiogenic factor levels compared with the effect of *H. pylori*.

This study had some limitations. Data were retrospectively reviewed and patients were not equally randomized either to receive *H. pylori* eradication therapy or not after endoscopic resection of gastric neoplasm. Furthermore, *H. pylori*-eradication failure group was too small to analyze the effect of the IL-8-251 polymorphism. Most of the studies suggesting the association between IL-8-251 polymorphism and gastric carcinogenesis included a lot more cases (3, 4, 13). More well-designed studies based on larger subjects are needed to clarify our results.

In conclusion, it is suggested that elevated IL-8 and MMP-9 concentrations in *H. pylori*-infected gastric mucosa are altered significantly after successful eradication of *H. pylori* and these conditions continue for 18 months follow-up period. However, IL-8-251 polymorphism does not affect *H. pylori* eradication rate and the sequential changes of related angiogenic factors after *H. pylori* eradication in Koreans.

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