

***Helicobacter pylori* Infection Enhances Gastric Mucosal Inflammation in Individuals Carrying the 260-T Allele of the CD14 Gene**

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Background/Aims: We aim to evaluate the association between promoter polymorphism of the clusters of differentiation 14 (CD14) gene and *Helicobacter pylori*-induced gastric mucosal inflammation in a healthy Korean population. **Methods:** The study population consisted of 267 healthy subjects who visited our hospital for free nationwide gastric cancer screening. Promoter polymorphism at -260 C/T of the CD14 gene was determined by polymerase chain reaction and restriction fragment length polymorphism analysis. The severity of gastric mucosal inflammation was estimated by a gastritis score based on the sum of the values of the grade and activity of the gastritis. Expression of soluble CD14 (sCD14) was assessed by quantitative sandwich ELISA. **Results:** CD14 polymorphism was not associated with *H. pylori* infection. There were no significant differences in gastritis scores among the genotype subgroups, but subjects carrying the CD14 -260 CT/TT genotype had significantly higher sCD14 levels than those carrying the CC genotype. Subjects with the 260-T allele of the CD14 gene and *H. pylori* infection had significantly higher sCD14 levels than those with the same genotype but without infection. **Conclusions:** In individuals with the T allele at the -260 site of the promoter region of the CD14 gene, *H. pylori* infection accentuates gastric mucosal inflammation. (**Gut Liver 2013;7:317-322**)

Key Words: *Helicobacter pylori*; CD14; Genetic polymorphism; Gastritis

INTRODUCTION

Helicobacter pylori infection is strongly associated with the development of gastric cancer, particularly for intestinal type

gastric cancer. With regard to the association between *H. pylori* induced gastritis and gastric cancer, it has been proposed that gastritis with severe mucosal damage may lead to the pre-malignant stage of atrophic gastritis/intestinal metaplasia, and subsequently the development of gastric cancer.¹ Epidemiological evidence has accumulated that chronic inflammation of the gastric mucosa contributes to the pathogenesis of gastric cancer. The hyperproliferation of gastric epithelium caused by *H. pylori* infection is the starting point of a sequence of events that leads to gastric cancer.² Moreover, immunogenetic factors play important roles in the progression and severity of gastric mucosal inflammation, thereby influencing the clinical outcome of *H. pylori* infection.

The clusters of differentiation 14 (CD14) are stimulated by lipopolysaccharide (LPS) generated by gram-negative bacteria.³ LPS binds to LPS-binding protein, and is transported to CD14 on the surfaces of monocytes.^{4,5} *H. pylori* is a gram negative rod and LPS of *H. pylori* activates monocytes to express cytokines. This results in various cellular responses and inflammatory cell recruitment in *H. pylori* induced gastritis.^{6,7} There is single nucleotide polymorphism in the CD14 promoter sequence, located in the locus 5q23-31; this polymorphism involves a transition from cytosine (C) to thymine (T) in the position -260 from the translation starting site of the gene.⁸ This polymorphism is found near the recognition site for transcription factor Sp1 and seems to have a significant role in CD14 regulation.⁹ Recent studies have reported on the association between CD14 gene polymorphism and gastric cancer, but there have been no consistent results.¹⁰⁻¹² The reason for the inconsistent results may be due to the heterogeneity of the races investigated, as gastric cancer is a multifactorial disease that results from a complex interaction of genetic and environmental factors.

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Herein we focused on the acute and chronic inflammation of gastric mucosa in a healthy population without specific gastrointestinal disease and symptom. Furthermore, it is not known whether CD14 gene polymorphism is associated with *H. pylori* induced gastric inflammation in the Korean population. In this study, we aim to evaluate the association between CD14 promoter polymorphism and *H. pylori* infection. Also, we plan to elucidate the association between CD14 promoter polymorphism and gastric mucosal inflammation.

MATERIALS AND METHODS

1. Study population

Healthy adults were enrolled from St. Vincent's Hospital, The Catholic University of Korea College of Medicine from February 2009 to March 2010 and they visited the health care center for free nationwide gastric cancer screening in a Korean adult population. None of them had a history of *H. pylori* eradication or previous gastric surgery. They were asymptomatic examinees of regular health screening with a simple symptom questionnaire at the Health Promotion Center of the same hospital. This study was conducted on 267 healthy adults (169 males and 98 females) without hypertension, diabetes, cardiac disease, or any past history of chronic illness. Individuals with grossly severe gastric atrophy or ulceration, or who were taking specific gastrointestinal medication, nonsteroidal anti-inflammatory drugs, or any other drugs were excluded. Patients with conditions that might have substantial effects on our study results (e.g., serum creatinine >2.5 mg/dL, total bilirubin >3.0 mg/dL), or who were pregnant, had a psychiatric disorder or did not sign a consent form were also excluded. Alcohol drinking was defined as consumption of at least 20 g alcohol/day and up to 3 times per week. Smoking was defined as current smoker.

2. Specimen collection

Two biopsy specimens were taken during upper gastrointestinal endoscopy from greater curvature side of the mid-truncum and corpus for histology. About 2 mL of peripheral blood and 3 mL of serum samples were collected for analysis of CD14 polymorphism and sCD14 level.

3. Pathologic evaluation

During the endoscopic examinations, two biopsy specimens were taken from the antrum (the greater curvature of the mid-truncum) and the corpus (the greater curvature of the midbody) for histological assessment. The diagnosis of *H. pylori* infection was made by showing histologic evidence of *H. pylori* on a Warthin-Starry silver stain in any of two specimens from the antrum and corpus. A gastritis score was calculated by the summation of the grade and activity of the gastritis. The grading of gastritis was based on the degree of infiltration by lymphocytes and plasma cells. Gastritis activity was based on the degree

of infiltration by neutrophilic granulocytes. Both values were scored using the updated Sydney system and scores ranged from 0 (none) to 3 (severe).¹³ Intestinal metaplasia was noted as absent, complete, or incomplete.

4. Polymorphism analysis

Genomic DNA was obtained from the peripheral blood lymphocytes of study subjects using the Genomic DNA Extraction Kit (Bioneer Corp., Daejeon, Korea). Genotypes of the CD14 promoter were determined by the polymerase chain reaction (PCR)-restriction fragment length polymorphism method. The CD14 promoter region containing -260C/T site was determined by using the PCR primer pairs 260 F5'-TGAGGAT-CATCCTTTTCCCAAAC-3'/260 R5'-CAGGCTTCACACTTGT-GAACTCTTC-3'.

PCR reactions were set up using i-Star Taq DNA polymerase (iNtRON Biotechnology, Seoul, Korea). In a total reaction volume of 20 μ L, 2 μ L 10 \times buffer, 10 pmol primer, 2 μ L genomic DNA, and 2 μ L dNTP mixture were combined. Reactions were run on Bio-Rad MyCycler thermal cyclers (BIO-RAD, Philadelphia, PA, USA). The PCR conditions were as follows: an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturing at 94°C for 30 seconds, annealing at 64°C for 30 seconds, extension at 72°C for 30 seconds and final incubation at 72°C for 10 minutes and cooling to 4°C. The resultant PCR products (3 μ L) were digested overnight at 37°C with Hae III, the appropriate restriction enzyme (New England BioLabs, Beverly, MA, USA), and the digests were electrophoresed on 1.5% agarose gel. The CD14 C allele was cut into two fragments of 155 and 263 base pairs, whereas the T allele remained uncut, with a length of 418 base pairs (Fig. 1).

5. Estimation of sCD14 level

By a quantitative sandwich ELISA according to the manufacturer's recommendations (catalog no. DC140; R&D Systems Europe, Abingdon, UK), the soluble CD14 (sCD14) level was determined.¹⁴ Samples (diluted 1:200) were added in duplicate to a microplate precoated with monoclonal anti-sCD14 antibodies, followed by incubation with, in succession, enzyme-linked polyclonal antibody specific for sCD14, substrate solution, and stop solution. Optical density was measured at 450 nm in a

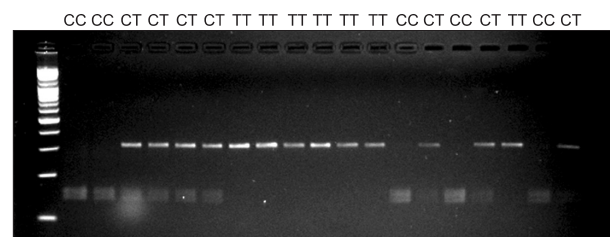


Fig. 1. Digestion of polymerase chain reaction products yielded bands of 418 bp in TT homozygotes, 263 and 155 bp in CC homozygotes, and all three bands in heterozygotes.

MRX Microplate Reader connected with Revelation Software (Dynateck Laboratories, Chantilly, VA, USA). A soluble CD14 standard (250 to 8,000 pg/mL) was included on each plate for standard curve production. A control sample was also included on each plate.

6. Statistics

Statistical analysis was conducted using IBM SPSS version 18.0 software (IBM, Armonk, NY, USA). Values were expressed as means±SD. Allele and genotype frequencies between the sexes were compared via chi-squared tests. The normality in the distributions was assessed using normal probability plots. Analysis of variance (ANOVA) or Student's t-test was used to assess normally distributed variables. Differences at the level of $p < 0.05$ were regarded as statistically significant.

7. Ethics statement

Informed consent was obtained from all patients and the study was approved by the Institutional Review Board of The Catholic University of Korea College of Medicine (VC08TI-SI0082).

RESULTS

1. Basal characteristics of enrolled patients and CD14 polymorphism

Characteristics of the study population included in this study are presented in Table 1. The distributions of age and sex among

subjects were not significantly different. For the CD14 -260 C/T allele, 144 heterozygotes (53.9%) and 123 homozygotes (46.1%) were identified and T allele frequency was 0.63. There were no differences between genotype subgroups; the frequencies of the CD14 -260 CC, -CT, and -TT genotypes were 10.4%, 53.9%, and 35.6%, respectively.

2. *H. pylori* status and gastritis score according to CD14 polymorphism

We next examined *H. pylori* infection and inflammatory activity of the gastric mucosa according to CD14 polymorphisms. The frequencies of the CC, CT, and TT genotypes were 10.4%, 53.9%, and 35.6%, respectively. There were no differences in age, sex, and the ratio of smokers and alcohol drinkers between the three genotypes. The frequencies of the CC, CT, and TT genotype with *H. pylori* infection were 71.4% (20/28), 48.6% (70/144), and 47.4% (45/95) and T allele frequency in subjects with *H. pylori* infection was 0.59. No association between CD14 genotypes and *H. pylori* infection status or intestinal metaplasia was found.

Inflammation of the gastric mucosa was expressed by the gastritis score described above. It was calculated by the summation of the grading and activity of gastritis based on the pathologic data. The gastritis scores for each genotype were 4.31 ± 1.59 , 3.93 ± 2.22 , and 4.00 ± 2.40 in CC, CT, and TT genotype subgroups, respectively, and no relevant associations were observed. Among subjects with *H. pylori* infection, the gastritis scores were 4.95 ± 1.19 , 5.31 ± 2.10 , and 5.26 ± 2.19 in subjects

Table 1. Demographic Characteristics, Gastritis Score, and Soluble CD14 Levels for Each Genotype of the CD14 -260 C/T Polymorphism

Characteristic	Genotype			p-value
	CC	CT	TT	
Total	28 (10.4)	144 (53.9)	95 (35.6)	
Sex				
Male	18	89	62	0.86
Female	10	55	33	
Age, yr	43.22±7.51	48.26±12.07	48.48±9.47	0.44
Smoker	14 (50.0)	50 (34.7)	35 (36.8)	0.31
Alcohol drinking	15 (53.6)	64 (44.4)	42 (44.2)	0.65
Intestinal metaplasia	14 (50.0)	55 (38.2)	32 (33.7)	0.17
Complete	2	15	5	0.34
Incomplete	12	40	27	
<i>H. pylori</i> infection	20 (71.4)	70 (48.6)	45 (47.4)	0.06
Gastritis score of all patients	4.31±1.59	3.93±2.22	4.00±2.40	0.54
Gastritis score of patients with <i>H. pylori</i> (+)	4.95±1.19	5.31±2.10	5.26±2.19	0.72
sCD 14 level of all patients, ng/mL	1,265.12±313.05	1,515.40±566.51	1,506.24±391.90	0.82
sCD 14 level of patients with <i>H. pylori</i> (+), ng/mL	1,278.63±342.69	1,607.16±659.28	1,546.00±398.39	0.04*

Data are presented as number (%) or mean±SD.

H. pylori, *Helicobacter pylori*; sCD14, soluble CD14.

*Statistically significant.

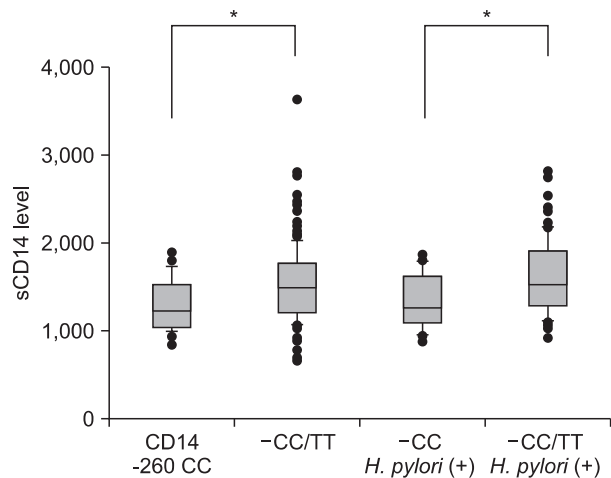


Fig. 2. Soluble CD14 (sCD14) levels in different CD14 -260 C/T genotypes and in subjects with *Helicobacter pylori* infection.

* $p < 0.05$.

with CC, CT, and TT genotype, respectively, and there was also no statistical difference ($p = 0.47$) (Table 1).

3. sCD14 level according to CD14 polymorphism

We also analyzed the relationships between CD14 genotypes, *H. pylori* infection and serum sCD14 levels. Although serum sCD14 levels (ng/mL) varied greatly among individuals, subjects carrying the CD14 -260 CT or TT genotype had significantly increased sCD14 levels compared with those carrying the CC genotype ($p < 0.01$). The carriers of the T allele at the -260 site of the promoter region of the CD14 gene had the higher sCD14 levels than subjects with the non-T genotype (Fig. 2). Analyses with combined CD14 genotypes and *H. pylori* infection showed that subjects having the CD14 -260 CT or TT genotype with *H. pylori* infection had significantly higher sCD14 levels than those with the same genotype but without infection (Fig. 3).

DISCUSSION

In innate immunity, the germ line encoded pattern-recognition receptors play a central role in pathogen recognition and immune responses.¹⁵ CD14 is one of the pattern-recognition receptors and is an important mediator of the inflammatory response as the first line of host defense due to its ability to recognize LPS.¹⁶ Although *H. pylori* is minimally invasive and LPS of *H. pylori* has relatively low potency, it is important in CD14-mediated monocyte activation in gastric mucosal inflammation.^{6,7} CD14 exists in two forms: a membrane-bound form of CD14 anchored by glycosyl phosphatidylinositol, and a sCD14 without anchors, which is present in the plasma.^{3,5,16} In modulating LPS-induced apoptosis of endothelial cells, sCD14 may be an important molecule and is usually elevated in many chronic infectious and inflammatory conditions. As for gastric mucosal inflammation, LPS of *H. pylori* has the ability to bind

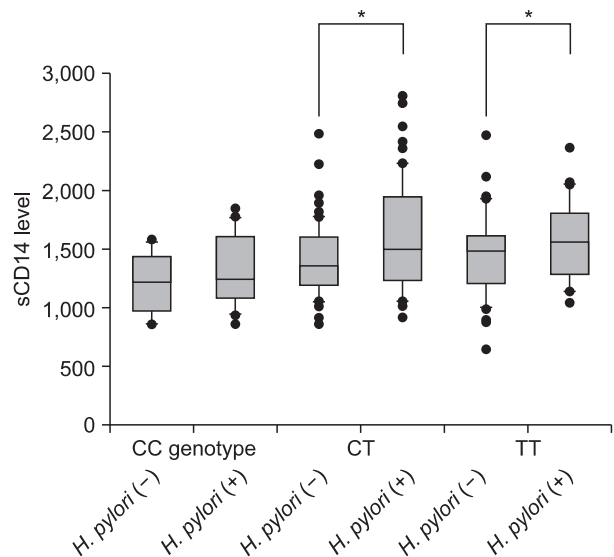


Fig. 3. Soluble CD14 (sCD14) levels in each CD14 -260 C/T genotype with or without *Helicobacter pylori* infection.

* $p < 0.05$.

CD14 and activate monocytes and induce an autoimmune response. It results in gastric mucosal injury, and consequently, the sCD14 level may be a marker of *H. pylori* induced gastric inflammation.^{17,18} It has been known that CD14 polymorphism is associated with circulating sCD14 levels and it modifies the environmental effect in many other disease conditions.^{19,20} However, there is a limited data on the functional effects of this polymorphism on gastric mucosal immunity and inflammation.

CD14 -260 polymorphism has been studied in various inflammatory conditions, including cardiovascular disease, infectious disease, chronic hepatitis, and inflammatory bowel disease, and it is known to be associated with enhanced inflammatory reaction.²¹⁻²⁸ With regard to gastrointestinal conditions, individuals carrying the T-allele or TT genotype have an increased risk for ulcerative colitis and Crohn's disease.^{27,28} In this study, taking the gastritis score as a marker for gastric mucosal inflammation, we could not find an association between CD14 -260 polymorphism and gastritis score. Although the differences were not statistically significant, the subjects who had the T allele and *H. pylori* infection tended to have higher gastritis scores, compared with the CD14 -260 CC genotype. In this study, two pathologists independently reviewed for histological assessment. To some extent, subjective differences of interpretation between pathologists would exist and we measured sCD14 level as supplementary marker or objective evidence.

A previous study showed that increased concentrations of serum sCD14 have been found in *H. pylori*-infected patients and in patients with pronounced gastric inflammation.¹⁸ In this study, we found that the promoter polymorphism at -260 C/T of the CD14 gene had an effect on circulating sCD14 levels. Although none of the specific genotypes of CD14 polymorphism was susceptible to *H. pylori* infection, it was found that the T

allele was associated with increased CD14 expression. Moreover, the subjects with the T allele had a much more prominent response to *H. pylori*. However, a limitation of this study was that we did not consider other risk factors, such as diet, age and duration of *H. pylori* infection. In our results, no significant difference of age was noted between the three groups. However, in post hoc analysis, mean age with CD14 -260 CT or TT genotype was significantly high than CC genotype (The data was not shown in the result.). By exclusion of macroscopically severe atrophy and intestinal metaplasia, age factor and duration of *H. pylori* infection would have little effect on gastric mucosal inflammation. Salt consumption is the single most important modifiable and preventable risk factor for gastric mucosal inflammation and gastric carcinogenesis. To overcome this limitation, a fairly large population study is necessitated.

As for CD14 polymorphism and gastric disease, it has been reported that there is no apparent association between this polymorphism and *H. pylori*-related gastric carcinoma in the Caucasian population, whereas this polymorphism was associated with a greater risk of *H. pylori*-related gastric carcinoma in the Han Chinese population.^{10,11} Our opposite results to those of the Caucasian population study may arise from the differences in the distribution of CD14 -260 C/T genotypes between the Korean and Caucasian populations, demonstrating the existence of ethnic genetic differences. There were very similar patterns in the distribution of CD14 -260 C/T genotypes between the Korean and Chinese populations, and the development of gastric cancer depends on ethnic-specific host susceptibility. However, in Japanese study, it was shown that the CD14 -159 TT and T carrier, considered as the high producing CD14 genotype, were associated with the reduced risk of intestinal type gastric cancer.^{29,30} Although the T allele seems to favor the inflammatory process, it was not associated with gastric cancer. As a reason for this inconsistent result, it is also possible that CD14 polymorphism is a risk factor only in the early stages of gastric mucosal inflammation, and is not involved in subsequent carcinogenic progression. In Japanese, it may confer protection against gastric mucosal atrophy caused by severe inflammation and thus may reduce the risk of intestinal type gastric cancer.³⁰

The disease pattern is determined through the host immune responses affected by many other environmental factors. In view of this point, we provided new information regarding the association between the promoter polymorphism of the CD14 gene and *H. pylori*-induced gastric mucosal inflammation in healthy population without specific gastrointestinal symptom. To our knowledge, there were a few papers describing association of CD14 polymorphism with gastric inflammation in patients with gastric cancer but not in patients with just gastritis. In conclusion, CD14 polymorphism was associated with circulating sCD14 levels. Although CD14 polymorphism was not associated with *H. pylori* infection, there was a close relationship between promoter polymorphism of CD14 -260 C/T and *H. py-*

lori induced gastric mucosal inflammation in a Korean population. In individuals with T allele at the -260 site of the promoter region of the CD14 gene, *H. pylori* infection accentuates gastric mucosal inflammation.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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