

HELICOBACTER PYLORI INFECTION AND CYTOKINE GENE POLYMORPHISMS IN UZBEKS

SHAVKAT ABDIEV¹, KYN SOU AHN², ABDUKHAKIM KHADJIBAEV²,
YUSUF MALIKOV², SAIDKARIM BAHRAMOV², BAKHODIR RAKHIMOV³,
JUNICHI SAKAMOTO⁴, YASUHIRO KODERA¹, AKIMASA NAKAO¹
and NOBUYUKI HAMAJIMA⁵

¹Department of Surgery II, Nagoya University Graduate School of Medicine, Nagoya, Japan

²Department of Emergency Surgery, Republic Research Center of Emergency Medicine, Tashkent, Uzbekistan

³UNICEF CO, Tashkent, Uzbekistan

⁴Young Leaders Program, Medical Administration, Nagoya University Graduate School of Medicine, Nagoya, Japan

⁵Department of Preventive Medicine, Nagoya University Graduate School of Medicine, Nagoya, Japan

ABSTRACT

Recent studies have reported that *Helicobacter pylori* (*H. pylori*) persistent infection and gastric atrophy development were associated with genetic polymorphisms of cytokines. This study aimed to determine possible associations of host genotypes with the seropositivity of anti-*H. pylori* IgG and anti-CagA IgG, as well as gastric atrophy measured with serum pepsinogens (PG) among an Uzbek population. Subjects were 84 patients with peptic ulcer disease, 35 with other miscellaneous diseases, and 48 healthy persons, for a total of 167 participants. Using a polymerase chain reaction with confronting two-pair primers, their DNA was genotyped for polymorphisms of interleukins (*IL*) (*IL-1B* C-31T, *IL-2* T-330G, *IL-4* C-33T, *IL-8* T-251A, *IL-10* T-819C, and *IL-13* C-1111T) and tumor necrosis factor A (*TNF-A*) (C-857T and T-1031C). Among 167 participants, 124 (74.9%) were anti-*H. pylori* IgG seropositive, 142 (85.6%) were anti-CagA IgG seropositive, and 44 (26.3%) exhibited gastric atrophy (PG1<70 ng/ml and PG1/PG2<3). The adjusted odds ratio (OR) of *IL-4* -33CT for anti-*H. pylori* IgG seropositivity was significant; OR=2.33 (95% confidence interval (CI), 1.04–5.19), relative to -33CC. In addition, those with *TNF-A*-1031TC had a significantly increased risk for anti-*H. pylori* IgG seropositivity; OR=2.82 (95%CI, 1.05–7.57), relative to -1031TT. No alleles were associated with the risk of anti-CagA IgG seropositivity or gastric atrophy. The significant associations with cytokine polymorphisms indicated that genetic traits might play a role in the persistent infection of *H. pylori* among Uzbeks. In addition to confirming the above associations, lifestyle interactions with the genotypes also remain to be elucidated.

Key Words: Single nucleotide polymorphisms, *Helicobacter pylori*, Interleukins, Peptic ulcer disease, Uzbekistan

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection plays a key role in the pathogenesis of gastric diseases, including stomach cancer.¹⁾ Two thirds of world populations are reportedly infected,²⁾ but only some of them actually develop *H. pylori*-associated diseases. CagA-positive strains are

Corresponding author: Shavkat Abdiev, MD

Department of Surgery II, Nagoya University Graduate School of Medicine,
65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

TEL: +81-52-744-2444, FAX: +81-52-744-2444, E-mail: abdiev_shavkat@yahoo.com

associated with a higher degree of gastric mucosal damage, inducing an immune response.³⁻⁵⁾ It seems reasonable to assume that the degree of immunological response corresponds to the levels of mucosal inflammation, which could influence the conditions conducive to persistent *H. pylori* infection and subsequent gastric atrophy. The inflammatory process is regulated by biological mediators including cytokines produced by epithelial cells. *H. pylori* secretes several factors that contribute to the inflammatory process by stimulating the release of cytokines such as interleukins (IL) and tumor necrosis factor (TNF)- α , with the difference in the amount of cytokines produced possibly accounting for the varying clinical presentations of *H. pylori* infection.⁶⁻¹⁷⁾

Since cytokine production is regulated partly at the transcriptional level,^{18,19)} functional polymorphisms in the promoter region could act as potential determinants of *H. pylori* infection susceptibility and its outcomes.^{20,21)} In the present study, polymorphisms of relevant cytokines were evaluated in an Uzbek population along with their associations with anti-*H. pylori* and anti-CagA seropositivity, as well as with gastric atrophy (GA) measured with serum pepsinogen (PG), were examined.

MATERIALS AND METHODS

Study subjects

Subjects were participants in a case-control study of digestive ulcers among 167 ethnic Uzbeks (89 males and 78 females), including 84 with a definitive diagnosis of peptic ulcer disease (PUD) (77 with duodenal ulcer and 7 with gastric ulcer), 35 with other miscellaneous diseases (14 with acute appendicitis, 17 with acute cholecystitis, and 4 with hernia) and 48 healthy subjects (students and staff), with subjects divided into three groups according to recruitment sources.²²⁾ Blood samples were obtained between January and March 2007 in the Republic Research Center of Emergency Medicine, Tashkent, Uzbekistan, after informed consent was obtained. The samples were immediately stored at -20°C until analysis. None of the participants enrolled in the study had been treated for PUD before enrollment, and none were under medication with non-steroid anti-inflammatory drugs.

Laboratory tests

Anti-*H. pylori* and anti-CagA IgG antibodies were measured by enzyme-linked immunosorbent assay (ELISA). E-plate Eiken *H. pylori* antibody (Eiken Chemical Co., Ltd., Tokyo, Japan) and CagA IgG EIA WELL (Radim SpA, Rome, Italy), respectively, were used. Serum PG1 and PG2 were measured by chemiluminescent enzyme immunoassay (CLEIA). Gastric mucosal atrophy was categorized as "none" ($\text{PG1} \geq 70$ ng/ml or $\text{PG1}/\text{PG2} \geq 3$), or "positive" ($\text{PG1} < 70$ ng/ml and $\text{PG1}/\text{PG2} < 3$).

Genotyping

DNA was extracted from a heparinized buffy coat with a BioRobot M48 Workstation (QIAGEN Group, Tokyo). All cytokine polymorphisms (*IL-1B* C-31T, *IL-2* T-330G, *IL-4* C-33T, *IL-8* T-251A, *IL-10* T-819C, *IL-13* C-1111T, *TNF-A* C-857T, and *TNF-A* T-1031C) were genotyped using a polymerase chain reaction with confronting two-pair primers (PCR-CTPP), as described in the previous studies.²³⁻²⁶⁾

Statistical analysis

Statistical analysis was conducted using SPSS 11.0. Logistic regression analysis was applied for the estimation of odds ratios (OR) and 95% confidence intervals (CI). The Hardy-Weinberg

equilibrium was examined with a chi-square test. Age, sex, and recruitment source (three subject groups) were adjusted to exclude any potential confounding.

The study was approved by the local medical authority in Uzbekistan and the Ethics Committee of Nagoya University, Graduate School of Medicine (approval number 459).

RESULTS

Table 1 shows the seropositivity for anti-*H. pylori* antibody and anti-CagA antibody, as well as those with GA. The mean age for all participants was 37.9 years, ranging from 15 to 86 years. One-hundred-twenty-five samples (74.9%) were positive for anti-*H. pylori* antibody and 143 (85.6%) for anti-CagA antibody. GA was found in 44 cases (26.3%). Anti-*H. pylori* IgG seropositivity was higher among women (80.8%) than men (69.7%), though roughly the same prevalence of anti-CagA IgG seropositivity and GA were found in both sexes. Anti-*H. pylori*

Table 1 Seropositive subjects for anti-*H. pylori* (*Hp*) and anti-CagA, and those with gastric atrophy* (GA), according to sex and subject recruitment source

Characteristics	N	Mean age (range)	Hp (%)	CagA (%)	GA(%)
Sex					
Males	89	37.3 (16–75)	62 (69.7)	78 (87.6)	24 (27.0)
Females	78	38.7 (15–86)	63 (80.8)	65 (83.3)	20 (25.6)
Recruitment source					
PUD	84	42.9 (16–86)	59 (70.2)	77 (91.7)	19 (22.6)
Non-PUD	35	36.7 (17–75)	27 (77.1)	26 (74.3)	9 (25.7)
Healthy	48	30.3 (15–58)	39 (81.3)	40 (83.3)	16 (33.3)

* Gastric atrophy was defined with PG1<70 ng/ml and PG1/PG2<3.

Table 2 Genotype distribution, allele frequency, and p value of Hardy-Weinberg equilibrium for selected cytokine polymorphisms

Polymorphism	Genotype distribution (%)			Allele frequency		χ^2	p
	<i>TT</i>	<i>CT</i>	<i>CC</i>	<i>T</i>	<i>C</i>		
<i>IL-1B</i> C-31T	49 (29.5)	78 (47.0)	39 (23.5)	0.530	0.470	0.536	0.464
	<i>TT</i>	<i>TG</i>	<i>GG</i>	<i>T</i>	<i>G</i>		
<i>IL-2</i> T-330G	57 (34.3)	82 (49.4)	27 (16.3)	0.590	0.410	0.075	0.784
	<i>CC</i>	<i>CT</i>	<i>TT</i>	<i>C</i>	<i>T</i>		
<i>IL-4</i> C-33T	57 (34.3)	88 (53.0)	21 (12.7)	0.608	0.392	2.103	0.147
	<i>TT</i>	<i>TA</i>	<i>AA</i>	<i>T</i>	<i>A</i>		
<i>IL-8</i> T-251A	56 (33.7)	69 (41.6)	41 (24.7)	0.545	0.455	4.347	0.037
	<i>CC</i>	<i>CT</i>	<i>TT</i>	<i>C</i>	<i>T</i>		
<i>IL-10</i> T-819C	64 (38.6)	79 (47.5)	23 (13.9)	0.623	0.377	0.031	0.860
	<i>CC</i>	<i>CT</i>	<i>TT</i>	<i>C</i>	<i>T</i>		
<i>IL-13</i> C-1111T	90 (54.2)	70 (42.2)	6 (3.6)	0.753	0.247	2.966	0.085
	<i>CC</i>	<i>CT</i>	<i>TT</i>	<i>C</i>	<i>T</i>		
<i>TNF-A</i> C-857T	110 (66.4)	53 (31.9)	3 (1.8)	0.822	0.178	1.419	0.234
	<i>TT</i>	<i>TC</i>	<i>CC</i>	<i>T</i>	<i>C</i>		
<i>TNF-A</i> T-1031C	115 (69.3)	47 (28.3)	4 (2.4)	0.834	0.166	0.097	0.755

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Table 3 Age, sex and subject group adjusted odds ratio (OR) and 95% confidence interval (CI) of genotypes for anti-*H. pylori* (Hp) antibody and anti-CagA antibody, and gastric atrophy

Genotype	Anti-Hp antibody		Anti-CagA antibody		Gastric atrophy	
	N (%)	OR (95% CI)	N (%)	OR (95% CI)	N (%)	OR (95% CI)
<i>IL-1B</i> C-31T						
<i>TT</i>	39 (31.5)	1 (Reference)	44 (31.0)	1 (Reference)	11 (25.0)	1 (Reference)
<i>CT</i>	55 (44.4)	1.01 (0.35-2.95)	64 (45.1)	0.52 (0.17-1.59)	22 (50.0)	1.39 (0.58-3.34)
<i>CC</i>	30 (24.1)	0.59 (0.25-1.39)	34 (23.9)	0.66 (0.16-2.76)	11 (25.0)	1.49 (0.52-4.27)
<i>CT+CC</i>	85 (68.5)	0.69 (0.31-1.57)	98 (69.0)	0.58 (0.19-1.69)	33 (75.0)	1.41 (0.62-3.19)
<i>IL-2</i> T-330G						
<i>TT</i>	40 (32.3)	1 (Reference)	45 (31.7)	1 (Reference)	13 (29.5)	1 (Reference)
<i>TG</i>	61 (49.2)	1.34 (0.62-2.91)	73 (51.4)	2.43 (0.90-6.56)	24 (54.5)	1.57 (0.69-3.54)
<i>GG</i>	23 (18.5)	2.29 (0.65-8.19)	24 (16.9)	2.18 (0.51-9.36)	7 (15.9)	1.86 (0.49-6.91)
<i>TG+GG</i>	84 (67.7)	1.44 (0.69-2.99)	97 (68.3)	2.41 (0.97-5.99)	31 (70.4)	1.50 (0.69-3.29)
<i>IL-4</i> C-33T						
<i>CC</i>	39 (31.4)	1 (Reference)	51 (35.9)	1 (Reference)	16 (36.4)	1 (Reference)
<i>CT</i>	72 (58.1)	<u>2.33 (1.04-5.19)</u>	75 (52.8)	0.62 (0.21-1.83)	21 (47.7)	0.71 (0.31-1.59)
<i>TT</i>	13 (10.5)	0.92 (0.29-2.92)	16 (11.3)	0.37 (0.09-1.53)	7 (15.9)	0.86 (0.26-2.87)
<i>TT+CT</i>	85 (68.6)	1.81 (0.86-3.78)	91 (64.1)	0.56 (0.20-1.54)	28 (63.6)	0.81 (0.37-1.73)
<i>IL-8</i> T-251A						
<i>TT</i>	42 (33.9)	1 (Reference)	48 (33.8)	1 (Reference)	12 (27.3)	1 (Reference)
<i>TA</i>	55 (44.3)	1.27 (0.53-3.02)	61 (43.0)	1.22 (0.41-3.69)	22 (50.0)	1.64 (0.68-3.96)
<i>AA</i>	27 (21.8)	0.59 (0.23-1.48)	33 (23.2)	0.69 (0.23-2.17)	10 (22.7)	1.13 (0.41-3.13)
<i>TA+AA</i>	82 (66.1)	0.97 (0.46-2.06)	94 (66.2)	1.00 (0.39-2.57)	32 (72.7)	1.44 (0.65-3.19)
<i>IL-10</i> T-819C						
<i>CC</i>	47 (37.9)	1 (Reference)	53 (37.3)	1 (Reference)	13 (29.5)	1 (Reference)
<i>CT</i>	62 (50.0)	1.38 (0.62-3.07)	71 (50.0)	1.67 (0.61-4.59)	23 (52.3)	1.75 (0.77-3.99)
<i>TT</i>	15 (12.1)	0.54 (0.18-1.61)	18 (12.7)	0.61 (0.17-2.18)	8 (18.2)	1.78 (0.58-5.48)
<i>CT+TT</i>	77 (62.1)	1.13 (0.55-2.35)	89 (62.7)	1.32 (0.54-3.23)	31 (70.5)	1.67 (0.77-3.62)
<i>IL-13</i> C-1111T						
<i>CC</i>	66 (53.3)	1 (Reference)	78 (54.9)	1 (Reference)	22 (50.0)	1 (Reference)
<i>CT</i>	52 (41.9)	0.96 (0.46-2.00)	58 (40.9)	0.71 (0.28-1.78)	20 (45.5)	0.97 (0.46-2.06)
<i>TT</i>	6 (4.8)	not calculated	6 (4.2)	not calculated	2 (4.5)	2.95 (0.36-24.35)
<i>CT+TT</i>	58 (46.7)	1.09 (0.52-2.25)	64 (45.1)	0.81 (0.33-2.02)	22 (50.0)	0.99 (0.48-2.08)
<i>TNF-A</i> C-857T						
<i>CC</i>	79 (63.7)	1 (Reference)	90 (63.4)	1 (Reference)	29 (65.9)	1 (Reference)
<i>CT</i>	44 (35.5)	2.06 (0.88-4.79)	50 (35.2)	3.22 (0.89-11.58)	15 (34.1)	0.95 (0.43-2.07)
<i>TT</i>	1 (0.8)	0.34 (0.03-4.22)	2 (1.4)	0.42 (0.03-5.70)	0 (0)	not calculated
<i>CT+TT</i>	45 (36.3)	1.75 (0.79-3.87)	52 (36.6)	2.57 (0.81-8.06)	15 (34.1)	0.89 (0.41-1.94)
<i>TNF-A</i> T-1031C						
<i>TT</i>	82 (66.1)	1 (Reference)	97 (68.3)	1 (Reference)	29 (65.9)	1 (Reference)
<i>TC</i>	41 (33.1)	<u>2.82 (1.05-7.57)</u>	44 (31.0)	3.22 (0.85-12.19)	15 (34.1)	1.17 (0.52-2.62)
<i>CC</i>	1 (0.8)	0.12 (0.01-1.25)	1 (0.7)	0.10 (0.01-1.16)	0 (0)	not calculated
<i>TC+CC</i>	41 (33.9)	1.93 (0.82-4.55)	45 (31.7)	1.72 (0.59-4.91)	15 (34.1)	1.01 (0.46-2.26)

Underlined ORs are statistically significant (p < 0.05)

antibody-positive and GA were the most common among the healthy volunteers, although they were the youngest as a group. The anti-CagA antibody-positive was found to be the most prevalent in the PUD group. The difference in seropositivity between anti-*H. pylori* antibody and anti-CagA antibody in the PUD group was 21.5%. Table 2 shows genotype distributions and allele frequencies, as well as p values for a Hardy-Weinberg equilibrium test. All genotype distributions except for *IL-8* T-251A ($p < 0.05$) were in a Hardy-Weinberg equilibrium.

As Table 3 shows, the *IL-4* -33CT genotype was significantly associated with anti-*H. pylori* seropositivity; OR=2.33 (95% CI, 1.04–5.19) relative to -33CC, although the OR of -33CT/TT relative to -33CC was not significant. *TNF-A* -1031TC heterozygotes posed a significantly high risk for anti-*H. pylori* seropositivity relative to -1031TT; OR=2.82 (95% CI, 1.05–7.57). Similarly, the OR of -1031TC/CC relative to -1031TT was not significant. No other genotypes were significantly associated with the risks of anti-*H. pylori*- seropositive, anti-CagA-seropositive, or gastric atrophy.

DISCUSSION

The present study revealed that the enrolled Uzbek subjects were highly infected with CagA-positive *H. pylori* strains. Among the eight polymorphisms, *IL-4* C-33T and *TNF-A* T-1031C were significantly associated with the anti-*H. pylori* antibody. Such significant associations were observed only for the heterozygotes of both polymorphisms relative to the major homozygotes; when the minor homozygotes were included the OR became insignificant. No polymorphisms were associated with anti-CagA antibody or GA. All genotypes except *IL-8* T-251A were in a Hardy-Weinberg disequilibrium. When the p value for *IL-8* T-251A (0.037) was corrected for a multiple comparison with the Bonferroni method, it was not found to be significant ($0.037 \times 8 = 0.296 > 0.05$).

IL-4 has been considered to inhibit *H. pylori* infection and gastric atrophy by decreasing the serum level of IFN- γ , which plays an important role in Th1 immune responses.¹⁶⁾ Nakashima *et al.* found a high expression level of *IL-4* protein among the population with a TT genotype of *IL-4* C-33T.²⁷⁾ A significant association between *IL-4* -33CT and *H. pylori*-induced atrophic gastritis was reported.²⁴⁾ Our study found that *H. pylori* seropositivity was associated with that same genotype. These data clearly indicate that *IL-4*-33CT genotype contributes to infection persistence and a further development of *H. pylori*-induced mucosal atrophy.

Our data also revealed that *TNF-A* -1031CT genotype carriers pose about a three-fold higher risk for *H. pylori* seropositive prevalence. These findings agreed with those in other research groups, who have noted a decreased OR for anti-*H. pylori* seroprevalence among *TNF-A* -1031CC genotype carriers²⁶⁾ and lower seroprevalence of *H. pylori* in males with -1031 C allele than with T allele.²⁸⁾ Contradictory data on *TNF-A* T-1031C polymorphism and the level of TNF- α production were published. Lu *et al.* reported that the severity of *H. pylori*-induced inflammation was determined by *TNF-A*-1031C and/or -863A carriers.²⁹⁾ High levels of TNF- α were found to be determined by -857T or -1031C alleles in an *in vitro* study.³⁰⁾

Among Brazilian subjects³¹⁾ the *IL-1B* C-31T genotype was not associated with *H. pylori* seropositivity, however in the Japanese population it showed an association.³²⁾ The G allele of *IL-2* T-330G was related to a lower risk of *H. pylori* infection in Brazilians and gastric atrophy in Japanese.^{24,31)} Those with *IL-8* -251TT and *IL-10* -819TT were reported to be at a low risk of *H. pylori* seropositivity.²⁵⁾ *IL-13* C-1111T was not associated with *H. pylori* infection, but was associated with the presence of gastric atrophy.²⁴⁾ Although *TNF-A* C-857T was associated with *H. pylori* seropositivity,³²⁾ however other studies showed no association.^{26,28)} In our study,

loci of the cytokines *IL-1B* C-31T, *IL-2* T-330G, *IL-8* T-251A, *IL-10* T-819C, *IL-13* C-1111T, and *TNF-A* C-857T were not individually associated with persistent *H. pylori* infection itself, nor with CagA carrying strains nor gastric atrophy in Uzbeks.

There were several limitations in this study. First, it was conducted using data from a case-control study on digestive ulcers, in which about half of the study subjects were patients with PUD. Although three subject groups (PUD patients, those with other miscellaneous diseases, and healthy volunteers) were adjusted, the associations reflected mainly those among PUD patients. A second limitation was the number of subjects. Since large-scale studies in Uzbekistan have proved difficult, the current study size was most feasible. Finally, the abnormally high infection rate reduced the study's statistical power.

In summary, *IL-4* C-33T and *TNF-A* 1031 might be ones of the host factors determining the persistence of *H. pylori* infection in Uzbeks. The suboptimal secretions of IL-4 and TNF- α that do not cause an acute response or elimination probably promote the long-term chronic inflammation favorable for infection. To confirm these associations, further studies with a large sample should be conducted, as well as biological studies of the gene expressions and roles of molecules in the regulatory mechanisms for the underlying persistent infection.

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