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Associations of plasma IL-8 levels with *Helicobacter pylori* seropositivity, gastric atrophy, and *IL-8* T-251A genotypes

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SUMMARY

There are few data on circulatory pro-inflammatory cytokine levels and cytokine gene polymorphisms in *H. pylori*-positive patients. A cross-sectional study was conducted to examine the effects of *H. pylori* infection, gastric atrophy, and the *IL-8* T-251A polymorphism on plasma IL-8 levels in 98 Japanese adults. Seventy-one subjects were positive for *H. pylori* infection. The geometric mean of plasma IL-8 concentration was significantly higher in subjects with *H. pylori* infection than in those without ($P=0{\cdot}001$). The development of atrophy was negatively associated with IL-8 levels in the *H. pylori*-positive subjects, although not significantly. Plasma IL-8 levels in the T/T genotype were associated with *H. pylori* infection and atrophy status ($P=0{\cdot}016$). Our findings suggested that circulating IL-8 levels were associated with *H. pylori* infection. The effect of *H. pylori* infection on plasma IL-8 levels was not clearly modified by the *IL-8* T-251A polymorphism.

Key words: *Helicobacter pylori*, interleukin-8, plasma, polymorphism.

INTRODUCTION

Helicobacter pylori (*H. pylori*) is the main cause of gastritis and peptic ulcer disease. There is now evidence that *H. pylori* may also play a role in various non-gastric diseases. *H. pylori* has also been shown to affect the vascular risks and complications in patients with diabetes mellitus [1, 2]. Prevalence of *H. pylori* infection is higher in patients with diabetes mellitus compared to healthy controls [1]. There are other studies showing a positive correlation between *H. pylori* infection and cardiovascular disease risk [2]. *H. pylori* colonization is associated with reduced circulating leptin levels, and fundic ghrelin and leptin levels are directly related, which suggests that *H. pylori* has an impact on human health and disease by its involvement in the regulation of leptin and ghrelin expression [3].

Epidemiological studies that have investigated circulating chemokine levels suggest that these processes are reflected at the systemic level [4–7]. Elevated levels of chemokines such as monocyte chemoattractant protein-1 (MCP-1), interleukin-8 (IL-8) and IL-10

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precede coronary events [4]. The circulating levels of MCP-1 and IL-8 may link obesity with obesity-related metabolic complications such as diabetes and atherosclerosis [5]. Other reports have elaborated on the involvement of chemokines in tumour growth, invasion and metastasis [6, 7], indicating that increased serum IL-8 in breast cancer patients is correlated with early dissemination and survival [7].

H. pylori plays an important role in the increased expression of various cytokines in gastric tissues, probably causing proliferation of gastric epithelial cells that enhances the development of gastric cancer [8]. High expression of IL-8 has been demonstrated in gastric mucosa infected with *H. pylori* [9], and IL-8 has been shown to cause chemotaxis and activation of inflammatory cells in gastric mucosa infected with *H. pylori* [10]. Moreover, significantly increased levels of mucosal IL-8 have been detected when *H. pylori* is associated with active gastritis and gastric cancer [11]. Although many studies have reported IL-8 production in the gastric mucosa of patients infected with *H. pylori*, few data are available on circulating IL-8 levels.

In addition, previous studies have reported association of the *IL-8* T-251A polymorphism within the IL-8 promoter with various diseases such as asthma, colorectal cancer and gastric diseases [12–14]. Individuals with both the *IL-8* –251T/T and *IL-10* –819T/T genotypes have a high probability of persistent *H. pylori* infection [12], and the *IL-8* –251T allele is significantly associated with either gastritis or duodenal ulcer in subjects infected with *H. pylori* [13]. The *IL-8* –251 A allele is associated with higher mucosal IL-8 production, more severe inflammation, mucosal atrophy, and intestinal metaplasia than *IL-8* –251T/T genotype in *H. pylori*-infected Koreans [14]. However, the association between the *IL-8* gene polymorphism and circulating IL-8 levels has not been investigated.

Therefore, we examined the associations of plasma IL-8 levels with *H. pylori* infection and gastric atrophy in Japanese adults. We also evaluated the effects of *H. pylori* infection and the severity of atrophy of the gastric mucosa on plasma IL-8 levels, stratified by the *IL-8* T-251A genotype.

METHODS

Subjects

Subjects were sampled from patients visiting the Daiko Medical Center of Nagoya University in 2004 who requested testing for *H. pylori* and subsequent eradication [15]. We analysed the first 103 participants (36 men and 67 women) of those aged 20–69 years, who gave written informed consent, provided a 7-ml blood sample, and completed a questionnaire on their lifestyle, medical histories and medication. Of the 103 participants, we had no information on *H. pylori* infection and atrophy in one subject, atrophy status was not determined in two subjects, and plasma IL-8 was not measured in two others, which left 98 eligible for the present analysis. No participant was taking antibiotics or steroid drugs, and none were current smokers. Two participants reported a history of gastric ulcer, and three a history of gastritis. This study was approved by the ethics committee of the Nagoya University School of Medicine on 16 June 2004 (approval number 155).

Blood samples

Plasma and the buffy coat fraction were separated from blood samples in a test tube that contained EDTA-2Na, and were kept at $-40\,^{\circ}C$ until analysis.

Tests for *H. pylori* infection

H. pylori infection was evaluated using a [^{13}C]urea breath test or serum anti-*H. pylori* antibody test. The [^{13}C]urea breath test was conducted at the Daiko Medical Center with the UBiT® (Otsuka Pharmaceutical, Japan). Anti-*H. pylori* IgG antibody was measured with Detaminor *H. pylori* antibody kits (Scimedx, USA) by a single laboratory (SRL, Japan). Those with $\Delta^{13}C > 0{\cdot}25\,\%$ or an ELISA value $\geqslant 2{\cdot}3$ were regarded as being infected.

Testing for pepsinogen (PG)

PGI and PGII in plasma were measured using a chemiluminescent enzyme immunoassay (CLEIA) by SRL. Those with PGI $\leqslant 70$ ng/ml and a PGI/PGII ratio $\leqslant 3$ were classified as atrophy-positive, and those with PGI $\leqslant 30$ ng/ml and a PGI/PGII ratio $\leqslant 2$ were classified as having severe atrophy. Those who were classified as atrophy-positive but did not satisfy the criteria of severe atrophy were defined as having mild atrophy.

Measuring plasma IL-8 level

Plasma IL-8 level was measured in 100 μl thawed plasma using a Biochip Array Technology Analyzer

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Table 1. *Subject characteristics by* H. pylori *infection and gastric atrophy*

		H. pylori-positive					
	H. pylori-negative	All subjects	No atrophy	Mild atrophy	Severe atrophy	*P*‡	*P* trend§
No. of subjects	27	71	42	24	5	—	—
Age, years [mean (S.D.)]	52·0 (9·2)	54·6 (8·1)	54·0 (7·9)	54·3 (8·2)	61·0 (8·0)	0·186	—
Gender (M/F)	8/19	25/46	16/26	7/17	2/3	0·601	—
IL-8 [mean* (pg/ml)]	13·2	19·1	20·6	17·6	15·3	0·001	0·138
IL-8 [adjusted mean† (pg/ml)]	13·6	18·9	20·3	17·5	15·0	0·005	0·154

S.D., Standard deviation.
* Geometric mean.
† Geometric mean adjusted for age, gender and comorbidities.
‡ *P* values between all subjects with *H. pylori* infection and those without *H. pylori* infection by *t* test.
§ *P* trend values for association between extent of gastric atrophy and plasma IL-8 in subjects with *H. pylori* infection.

Evidence Investigator, cytokines and growth factor array (Randox Laboratories, UK) at a single laboratory (Mitsubishi Kagaku Bio-clinical Laboratories, Japan). The principle of the array system has been described previously [16]. All assays were performed by staff that were blinded to the clinical and epidemiological data.

Genotyping of IL-8

DNA was extracted from the buffy coat fraction using a BioRobot EZ1 (Qiagen, Japan). The *IL-8* gene was genotyped at the polymorphic site T-251A using the polymerase chain reaction by confronting two-pair primers (PCR–CTPP) [17]. The PCR amplification of *IL-8* T-251A was conducted using the primers F1 (5′-CAT GAT AGC ATC TGT AAT TAA CTG) and R1 (5′-CAC AAT TTG GTG AAT TAT CAA A) for the T allele (a 169-bp fragment), and F2 (5′-GTT ATC TAG AAA TAA AAA AGC ATA CAA) and R2 (5′-CTC ATC TTT TCA TTA TGT CAG AG) for the A allele (a 228-bp fragment). The DNA amplified between F1 and R2 resulted in a 349-bp band common to both alleles.

Genomic DNA (30–100 ng) was used in a 25-μl reaction mixture that contained 0·2 mM dNTPs, 12·5 pmol of each primer, 0·5 U polymerase (AmpliTaq Gold; Applied Biosystems, USA), and 2·5 μl 10 × PCR buffer including 15 mM $MgCl_2$. The amplification conditions were an initial 10 min at 95 °C, followed by 30 cycles of 1 min each at 95 °C, 58 °C and 72 °C, and then 5 min at 72 °C for final extension. The DNA products were visualized on 2% agarose gels with ethidium bromide staining.

Statistical analysis

Distribution of circulating IL-8 levels was skewed, and the geometric means of plasma IL-8 were calculated to compare groups. Differences in the demographic characteristics were analysed using the χ^2 test, *t* test and one-way analysis of variance (ANOVA). The linear trend in the geometric means of IL-8 across atrophy status was assessed in subjects with *H. pylori* infection. The geometric mean levels for subjects with *IL-8* –251 T/T and A allele carrier in respect to *H. pylori* infection and severity of atrophy were compared using the general linear model, with age, gender and comorbidities such as hyperuricaemia and bronchiectasis, which may affect plasma IL-8 as covariates. The frequency of the polymorphism was tested against Hardy–Weinberg equilibrium using the χ^2 test.

All statistical analyses were performed using the Statistical Package for the Social Sciences version 14.0 for Windows (SPSS Inc., USA), and $P < 0{\cdot}05$ was considered statistically significant for all analyses.

RESULTS

Table 1 summarizes the characteristics of the 98 study subjects, of whom 71 were positive for *H. pylori* infection. Seven subjects (9·9%) with *H. pylori* infection were negative in the [^{13}C]urea breath test and positive for serum anti-*H. pylori* antibody, while 64 (90·1%) with *H. pylori* infection were positive in both the [^{13}C]urea breath test and for serum anti-*H. pylori* antibody. Of the 71 participants with *H. pylori* infection, 42 did not show gastric atrophy, while 29 had

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gastric atrophy, based on PG tests. Only one subject with gastric atrophy was negative for *H. pylori* infection.

Plasma IL-8 levels in the subjects with *H. pylori* infection were significantly higher than in those without *H. pylori* infection (geometric mean: 13·2 and 19·1 pg/ml, respectively; $P=0{\cdot}001$). Adjustments for age, gender and comorbidities did not change this result ($P=0{\cdot}005$). Of the 71 participants with *H. pylori* infection, individuals who were positive for the [^{13}C]urea breath test showed higher IL-8 levels than those who were [^{13}C]urea breath test negative/serum anti-*H. pylori* antibody-positive (geometric mean: 19·5 and 16·1 pg/ml, respectively; $P=0{\cdot}536$).

The four groups, *H. pylori*(−), *H. pylori*(+)/atrophy(−), *H. pylori*(+)/mild atrophy(+), and *H. pylori*(+)/severe atrophy(+), had statistically different IL-8 levels ($P=0{\cdot}005$, by ANOVA), whereas no differences were observed according to age or gender. The *H. pylori*(+)/atrophy(−) group had the highest mean IL-8 level of all the groups. There was a statistically significant difference in the mean IL-8 level between *H. pylori*(−) and *H. pylori*(+)/atrophy(−) groups ($P=0{\cdot}001$). Development of atrophy was negatively associated with the geometric means of IL-8 in the 71 participants with *H. pylori* infection, although not significantly so. There was no statistically significant difference of mean IL-8 level between *H. pylori*(−) and *H. pylori*(+)/severe atrophy(+) groups ($P=0{\cdot}61$).

The distribution of the *IL-8* T-251A genotype was not significantly different from that expected by Hardy–Weinberg equilibrium ($P=0{\cdot}13$). IL-8 levels in A allele carriers of the *IL-8* T-251A genotypes were slightly higher than those in subjects with T/T genotype, but the difference was not statistically significant (geometric mean: 17·5 and 17·0 pg/ml, respectively).

For both the T/T genotype and A allele carriers, plasma IL-8 levels were elevated in individuals with *H. pylori*(+)/atrophy(−), and decreased with the development of atrophy. Development of atrophy was negatively associated with the geometric means of IL-8 levels in those with the T/T genotype in *H. pylori*-positive individuals (P trend $=0{\cdot}088$).

In each ANCOVA, IL-8 levels in those with the T/T genotype were associated with *H. pylori* infection and atrophy status ($P=0{\cdot}016$) for three groups, while the differences for those carrying the A allele were not significant ($P=0{\cdot}326$) (Fig. 1). There was a statistically significant difference in the mean IL-8 level in those with the T/T genotype between *H. pylori*(−) and *H. pylori*(+)/atrophy(−) groups ($P=0{\cdot}021$). The differences in the IL-8 concentration between those with the T/T and A allele carriers in each group in relation to *H. pylori* infection and atrophy status were not statistically significant.

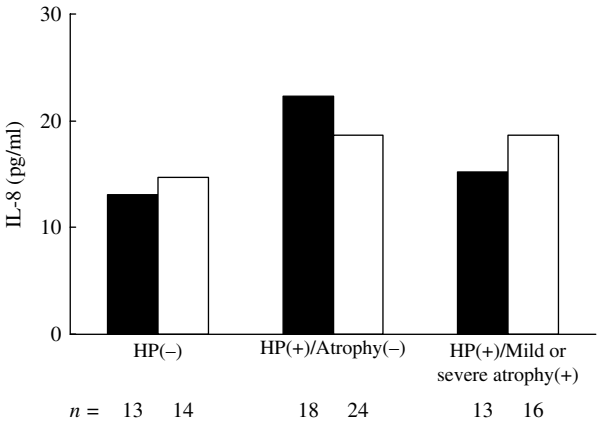

Fig. 1. Geometric mean of plasma IL-8 levels in subjects with *IL-8*–251T/T (■) and A allele carriers (□) in respect of *H. pylori* infection and severity of gastric atrophy. IL-8 levels were adjusted for age, gender and comorbidities. In each analysis of covariance, IL-8 levels in those with the T/T genotype were associated with *H. pylori* infection and atrophy status ($P=0{\cdot}016$) for three groups, while the differences for those carrying the A allele were not significant ($P=0{\cdot}326$). There was a statistically significant difference in the mean IL-8 level in those with the T/T genotype between *H. pylori*(−) and *H. pylori*(+)/atrophy(−) groups ($P=0{\cdot}021$).

DISCUSSION

This is believed to be the first study to examine the effects of *H. pylori* infection, gastric atrophy and *IL-8* T-251A polymorphism on circulating IL-8 levels. We found plasma IL-8 levels were significantly associated with *H. pylori* infection. Higher plasma IL-8 levels were particularly observed in *H. pylori*(+)/atrophy(−) participants. The differences in plasma IL-8 levels were clearer in those with the *IL-8* –251T/T genotype than those carrying the A allele.

There is a paradigm according to which a host–microbial interaction occurs in some cases that may promote pathological conditions, whereas in other cases it may protect against pathology. Current studies showed that eradication of *H. pylori* reduced the risk of gastric cancer [18]. Meanwhile, it has now become clear that it is inversely associated with the

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development of oesophageal diseases, and the more interactive CagA-positive strains are associated with the strongest inverse effects [19]. Inverse associations of *H. pylori* and childhood asthma, allergic rhinitis and atopy have also been found [20]. Our findings revealed the possibility that *H. pylori* has an impact on human health by its involvement in the regulation of IL-8 expression.

Previous reports have described conflicting results concerning the association between *H. pylori* infection and circulating IL-8 levels. We found a positive association, which supports the results of Cichoz-Lach *et al.* [21] and Mehmet *et al.* [22], but not those of Russo *et al.* [23], Bayraktaroğlu *et al.* [24], and Di Bonaventura *et al.* [25]. Bayraktaroğlu *et al.* [24] examined circulating cytokine levels in 42 patients with dyspeptic symptoms and found that 30 were infected with *H. pylori*, while 12 were not. They observed elevated circulatory levels of IL-8 in those who were *H. pylori*-positive, but the difference was not statistically significant ($P = 0{\cdot}079$). In part, the sample sizes might account for the discrepancy in the results. Infections with specific *H. pylori* strains that possess the CagA pathogenicity island induce significantly higher levels of chemokines than CagA-negative strains [26]. The distribution of *H. pylori* strains possessing CagA might also have affected the results.

Antigens released by *H. pylori* can stimulate endothelial cells, macrophages and epithelial cells to make large amounts of chemokines, such as IL-8 and growth-regulated oncogene-alpha, which produces a chemotactic gradient for the migration of neutrophils into the gastric mucosa [27–29]. The stomach has a large surface area, with locally produced cytokines which might spill over into the bloodstream continuously, and *H. pylori* infection and the severity of gastric atrophy in those infected may alter the expression levels of IL-8.

H. pylori has been well documented to upregulate the Th1 cytokines. Goll *et al.* [30] have reported that the cytokine profile of the *H. pylori*-infected gastric mucosa shows a mixed Th1–Th2 profile. Both the Th1 and Th2 mediator genes are upregulated in the gastric mucosa of *H. pylori*-positive subjects. Our finding of decreased IL-8 levels in the plasma of *H. pylori*-infected subjects with atrophy, compared to those without, is consistent with switching of the immune response from an innate to an adaptive one mediated by Th1 cells [31]. *H. pylori* gradually decreases during the development of glandular atrophy [32], which also supported our findings.

Our results did not clearly show the modification of effects of *H. pylori* infection on plasma IL-8 levels by the *IL-8* T-251A polymorphism, which were significantly elevated in individuals with *H. pylori*(+)/atrophy(−) for the T/T genotype. The association between the *IL-8* gene polymorphism and disease in *H. pylori*-infected patients has not been well documented. Shirai *et al.* [33] have revealed that gastric carcinoma with a high frequency of microsatellite instability occurs in *H. pylori*-infected patients, and is associated with the *IL-8* –251T/T genotype (low-expression genotype). The *IL-8* –251T/T genotype is also significantly associated with an increased risk of non-cardia gastric carcinoma in *H. pylori*-positive individuals [34]. The low inflammatory cytokine expression genotype may lead to carcinogenesis via the mutator pathway, presumably due to a higher rate of *H. pylori* colonization [33]. Meanwhile, there are contradictory reports. Ye *et al.* [14] found a positive association between the *IL-8* –251 A allele and the degree of atrophy and intestinal metaplasia. The *IL-8* –251 A allele carriers showed a higher risk of gastric adenocarcinoma in that study. We could not sufficiently analyse the associations of IL-8 with severity of gastric atrophy and the *IL-8* T-251A polymorphism in *H. pylori*-positive subjects because of the small sample size. Further study in a larger sample size is needed to examine the modifying effect of *IL-8* gene polymorphism on circulating IL-8 levels and disease progression over time in *H. pylori*-infected patients.

Our study found that IL-8 levels were elevated in *H. pylori*(+)/atrophy(−) individuals. Circulating IL-8 was significantly higher in patients with coronary artery disease with *H. pylori* infection than in control subjects [35]. *H. pylori* infection may be a trigger factor in the pathophysiology of ischaemic heart disease, through induction of an inflammatory cascade concentrated on the gastric mucosa [25]. Similarly, the associations between *H. pylori* infection, vascular diseases and diabetes mellitus could be mediated by increasing cytokine levels [36]. Follow-up data for *H. pylori*(+)/atrophy(−) individuals may provide a clue concerning the potential role of circulating IL-8 as a risk marker for gastric diseases and other systemic illnesses, such as cardiovascular diseases.

The current study has some limitations. Circulating IL-8 levels can be affected by factors other than *H. pylori* infection. Most of our subjects were apparently healthy, and the analysis included adjustment for potential confounding factors. Information on the use of antibiotics or steroid drugs and smoking status

was all self-reported, which may have led to misclassification. Study subjects were outpatients who requested testing for *H. pylori* and subsequent eradication, although only 5% were found to suffer from gastric disease. Since the subjects did not know their PG level, IL-8 level, and IL-8 genotype, the selection bias seemed to be limited. The cross-sectional study design and small sample size were also limitations of this study.

Another limitation involves the diagnosis of gastric atrophy. This was done entirely on the basis of serum PG levels and not through histological assessment. However, the PG method is well established as a surrogate marker of gastric atrophy [37–39]. The validation criterion is PGI $\leqslant$70 ng/ml and PGI/PGII ratio of $\leqslant$3. A relatively small proportion of our participants had severe gastric atrophy (5·1%), which prevented analysis stratified by the level of atrophy. Although cagA antibody was not tested in the plasma of our subjects, nearly 100% of *H. pylori* strains in Japan possess CagA [40].

In conclusion, we found that plasma IL-8 levels were significantly higher in those with *H. pylori* infection, and inversely correlated with the level of gastric atrophy in *H. pylori*-infected Japanese individuals. Our findings did not indicate that the effects of *H. pylori* infection on plasma IL-8 levels were modified by the *IL-8* T-251A polymorphism.

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DECLARATION OF INTEREST

None.

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