**Abstract**

Background: Host genetic factors of interleukin (IL)-1 polymorphisms influence *Helicobacter pylori* infection pathogenic activity. We examined whether *H. pylori*-infected patients with IL-1 polymorphisms are associated with myocardial infarction (MI).

Materials and methods: We recruited 594 consecutive coronary artery disease patients and excluded those who met exclusion criteria. After matching age and sex, 82 cases with MI and 82 controls were enrolled. Immunoglobulin G antibodies against *H. pylori* and IL-1 polymorphisms (IL-1 beta-511 base pairs and IL-1 receptor antagonist) were analyzed. We assessed high sensitivity C-reactive protein (hs-CRP) level and reactive hyperemia-peripheral arterial tonometry (RH-PAT) index (RHI) using the EndoPAT2000 system.

Results: The simultaneous prevalence of *H. pylori*-seropositivity and IL-1 polymorphisms was 45.1% and 19.5% in the cases and controls, respectively (P = 0.001). *H. pylori*-positive patients with IL-1 polymorphisms showed significantly higher serum levels of natural logarithm of hs-CRP in the cases and controls (−2.8 ± 1.0 vs. −3.4 ± 0.6, respectively; P = 0.005) and significantly lower levels of natural logarithm of RHI in the cases and controls (0.51 ± 0.13 vs. 0.61 ± 0.23, respectively; P = 0.039 and 0.47 ± 0.13 vs. 0.69 ± 0.23, respectively; P = 0.005). *H. pylori*-seropositivity with IL-1 polymorphisms was significantly associated with MI by logistic regression analysis (odds ratio, 4.83; 95% confidence interval, 1.99–11.7; P < 0.001).

Conclusions: *H. pylori*-positive patients with IL-1 polymorphisms showed higher levels of hs-CRP and lower levels of RHI, and were significantly correlated with the MI.

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1. Introduction

Chronic bacterial infections have been suggested to be associated with the risk of myocardial infarction (MI) [1,2]. *Helicobacter pylori* (*H. pylori*) is the most common chronic bacterial infection in humans, and it has been demonstrated worldwide and in individuals of all ages. Previous studies have shown an association between *H. pylori* and MI, [3] but other studies have not demonstrated such an association [4].

The pro-inflammatory cytokine, interleukin (IL)-1 beta, influences the clinical outcomes of an *H. pylori* infection [5]. The IL-1 beta gene has three diallelic polymorphisms at positions −511, −31, and +3954 base pairs (bp) from the transcriptional start site, [6] even though there are conflicting data regarding the functional effects of these polymorphisms on IL-1-beta production [7,8]. The gene for the IL-1 receptor antagonist (IL-1RN) has a variable number of identical tandem repeats of 86 bp in length in intron 2, and the less common allele 2 (IL-1RN^2) is associated with a wide range of chronic inflammatory and autoimmune conditions [6]. A previous study reported that carriage of the IL-1 beta-511 genotype or IL-1RN^2 allele was significantly associated with *H. pylori*-related gastric mucosal IL-1 beta levels and the incidence of gastric cancer [9].
The association between \textit{H. pylori} and MI may be explained by the IL-1 polymorphisms, and host genetic factors that affect inflammation may explain why some individuals infected with \textit{H. pylori} develop MI, while others do not. In this study, we examined the association of \textit{H. pylori} infection and IL-1 polymorphisms with MI.

2. Methods

2.1. Study population

Patients were recruited between January 2009 and December 2012. The case subjects were patients with MI who had been admitted to Kumamoto University Hospital. Kumamoto University Hospital is located in the Kumamoto Prefecture southwest of Tokyo and has a population of approximately 1.8 million people. MI included ST-elevation MI and non-ST-elevation MI, and we used the universal definition of MI\cite{10}. Cases subjects started with consecutive 594 coronary artery disease and patients with stable coronary artery disease (n = 344), serum sample deficiency (n = 93), unstable angina pectoris (n = 43), and patients with malignancy (n = 8), hypoxia (n = 2), other inflammatory diseases (n = 6), collagen disease (n = 2) and hemodialysis (n = 1) were excluded. As a result, 95 MI patients were analyzed. There are generally many elderly people in patients with coronary artery disease, so control subjects included individuals aged > 60 years undergoing medical examination or treatment at our hospital. We enrolled consecutive individuals who did not have a history of atherosclerotic diseases such as coronary artery disease, stroke, or peripheral arterial disease and as a result, 95 patients without atherosclerotic diseases agreed with participation in this study. After matching age and sex, 82 cases and 82 controls were enrolled (Fig. 1).

The study complied with the Declaration of Helsinki, and the human ethics committee of Kumamoto University approved it. The file number and year for the Ethics Committee approval is 64 and January 16 2003. Written informed consent was obtained from all the patients.

2.2. Laboratory methods

The cases and controls provided venous blood samples, which were centrifuged, and the serum was stored at $-80$ °C until analysis. Immunoglobulin G (IgG) antibodies against \textit{H. pylori} were measured using a direct enzyme-linked immunosorbent assay kit (E-Plate Eiken \textit{H. pylori} Antibody, Eiken Chemical Co., Ltd., Tokyo, Japan). Levels of IgG were categorized as seropositive and seronegative for \textit{H. pylori} according to a selective cutoff value (492 nm). Using the same kit, it was reported that the sensitivity and specificity of the kit with respect to cell culture and rapid urease test in 70 Japanese subjects were 100% and 80.0%, respectively\cite{11}. The measurements of high sensitivity C-reactive protein (hs-CRP) level were performed in the laboratory of our hospital using routine enzymatic methods. Since acute phase proteins such as hs-CRP are up-regulated in acute MI patients, we collected data of hs-CRP 6–9 months after admission for acute MI as far as possible, though medications such as statins subscribed on the admission might influence the CRP levels.

2.3. Genotyping

DNA was extracted from whole blood using the DNA extractor WB kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan) by following the modified protocol described by Richards et al.\cite{12}. A single bp polymorphism at $-511$ in the promoter region of the IL-1 beta (rs16944) was determined using a real-time TaqMan allelic discrimination assay (Step One Plus Real-Time PCR system, version 2.1; Applied Biosystems, Tokyo, Japan) according to the protocols provided by the manufacturer (assay no.: C_1839943_10). All the reagents were purchased from Applied Biosystems. It was reported that within the IL-1 beta gene, the T and C alleles at the $-511$ locus were in near total linkage disequilibrium with the C and T alleles at the $-31$ locus\cite{13}, and the frequency of another polymorphism at position +3954 is very rare in Japanese populations,\cite{14} so we restricted the analysis to the IL-1 beta-$511$ locus. The IL-1RN polymorphism was based on the number of an 86 bp repeat, and the analysis of the IL-1RN polymorphism was performed using polymerase chain reaction (PCR) as described previously.\cite{15} In this study, we defined IL-1 polymorphisms as the carriage of either IL-1 beta-$511$ T allele or IL-1RN *2 allele.

2.4. Assessment of endothelial function

A previous report suggested that inflammation and CRP might directly contribute to endothelial dysfunction\cite{16}. Peripheral

Fig. 1. Flow chart showing the protocol used for enrolling the patients. MI, myocardial infarction.
endothelial function was assessed by reactive hyperemia-peripheral arterial tonometry (RH-PAT) using the EndoPAT2000 system (Itamar Medical, Caesarea, Israel), as described previously [17]. Previous studies have demonstrated that RH-PAT has excellent reproducibility [18]. We measured RH-PAT index (RHI) in stable condition in MI patients. The time point of measurement was in stable condition before discharge of the MI admission or several months later. Because hs-CRP and RHI are not normally distributed and some previous study concerning endothelial function and cardiovascular diseases expressed these values as logarithmic transformation form, [19] we calculated the natural logarithmic (Ln)-hs-CRP and RHI values for statistical analyses.

2.5. Statistical analysis

Since RHI values are not normally distributed, we calculated the natural logarithmic (Ln)-RHI values for statistical analyses. We conducted a study on independent cases and controls with 1 control per case. Our pilot data indicated that the probability of the simultaneous presence of H. pylori infection and IL-1 polymorphisms among controls is 0.2. If the true probability of that among cases is 0.4, we needed to study at least 81 cases and 81 controls. Enrolled patients were matched by age and sex using a propensity score method. The Hardy–Weinberg equilibrium of alleles at individual loci was assessed using the chi-square test. The odds ratio (OR) and 95% confidence intervals (CI) were used to assess the risk of MI associated with the presence of H. pylori infection and IL-1 polymorphisms. Estimates of the C-statistic for the risk factors were calculated, and a comparison of C-statistics was estimated after the addition of the simultaneous presence of H. pylori and IL-1 polymorphisms to the risk factors of independent predictive values showed in the multivariate logistic regression analysis. The incremental effect of adding the presence of H. pylori and IL-1 polymorphisms to the conventional risk factors for predicting MI was evaluated using the net reclassification improvement (NRI), as previously described [20]. A P value < 0.05 was considered to denote the presence of a statistically significant difference. Statistical analyses were performed using SPSS, version 22 (IBM Corp., Armonk, NY, USA).

3. Results

Table 1 shows the subjects’ clinical characteristics. The cases had a higher prevalence of diabetes (P = 0.025), dyslipidemia (P < 0.001), history of smoking (P < 0.001), medical history of previous MI (P = 0.014) and peripheral arterial disease (P = 0.028). We found no significant differences between the cases and controls in the levels of natural logarithm of hs-CRP (Ln-hs-CRP) (−3.1 ± 0.9 vs. −3.1 ± 0.7, respectively; P = 0.87). The Ln-RHI was significantly lower in the cases than in the controls (0.57 ± 0.2 vs. 0.65 ± 0.23, respectively; P = 0.024).

All 164 subjects were successfully genotyped for the 2 loci (the IL-1 beta-511 genotype and IL-1RN alleles). Fig. 2 shows the results of H. pylori seropositivity, IL-1 beta-511 genotype and IL-1RN alleles, respectively. The prevalence of seropositivity to H. pylori IgG antibody in the case and control groups was 47.6% and 31.7%, respectively (P = 0.055) (Fig. 2A). The genotype frequencies of the IL-1 beta-511 C/C, C/T, and T/T were 26.8%, 46.3%, and 26.8%, respectively, in the cases and were 32.9%, 45.1%, and 22%, respectively, in the controls (P = 0.63) (Fig. 2B). The alleles of the IL-1RN were in Hardy–Weinberg equilibrium in the cases and controls (P = 0.54 and P = 0.41, respectively). The distribution of the IL-1RN L/L, *2/L, *2/2 genotype were 87.8%, 12.2%, and 0%, respectively, in the cases and were 91.5%, 8.5%, and 0%, respectively, in the controls (P = 1.0) (Fig. 2C). The IL-1RN alleles were also in Hardy–Weinberg equilibrium in the cases and controls (P = 0.52 and P = 0.68, respectively).

The rate of the simultaneous presence of H. pylori seropositivity and IL-1 polymorphisms was 45.1% and 19.5% in the cases and controls (P = 0.001) (Fig. 3). Subjects with H. pylori seropositivity and IL-1 polymorphisms showed significantly higher serum levels of Ln-hs-CRP in the cases and controls (−2.8 ± 1.0 vs. −3.4 ± 0.6, respectively; P = 0.003 and −2.8 ± 0.9 vs. −3.2 ± 0.6, respectively; P = 0.02). The Ln-RHI was significantly lower in patients with H. pylori seropositivity and IL-1 polymorphisms than in other patients of the case and control groups (0.51 ± 0.13 vs. 0.61 ± 0.23, respectively; P = 0.039 and 0.47 ± 0.13 vs. 0.69 ± 0.23, respectively; P = 0.005) (Fig. 4).

Logistic regression analysis for MI revealed that the carriage of H. pylori seropositivity and IL-1 polymorphisms was associated with a significantly high increased risk of MI (OR, 4.83; 95% CI: 1.99–11.7; P = 0.001) after adjusting for diabetes, hypertension, dyslipidemia, obesity, smoking, and chronic kidney disease (Table 2). The P value of the Hosmer and Lemeshow test was 0.8. We estimated the C-statistic of the risk factors for MI (C-statistic, 0.78; 95% CI: 0.71–0.84). The addition of H. pylori seropositivity with IL-1 polymorphisms to the risk factors resulted in a significant increase in the C-statistics from 0.78 to 0.82 (P = 0.028) (Fig. 5). We reclassified the risk of the risk factors for MI, and the net reclassification improvement (NRI) was significant with the inclusion of H. pylori seropositivity and IL-1 polymorphisms (P < 0.001) (Table 3).

4. Discussion

This is the first study to reveal the association among H. pylori infection, IL-1 polymorphisms, and the incidence of MI. The present study has three important findings. First, H. pylori infection and IL-1 polymorphisms were found more significantly in patients with MI than in those without atherosclerotic diseases. Second, patients with H. pylori infection and IL-1 polymorphisms showed significantly higher levels of hs-CRP and significantly lower levels of Ln-RHI. Third, the addition of H. pylori infection with IL-1 polymorphisms to the previously described risk factors improved the risk stratification of the incidence of MI, resulting in a substantial increase in the C-statistics and a significant NRI.

H. pylori infection is associated with clinical outcomes that range from simple gastritis to more serious conditions such as peptic ulcer and gastric neoplasia; however, the evidence of a relationship between H. pylori infection and MI is controversial. [3,4] and H. pylori is not considered to have any detrimental effect on the classic coronary risk factors. [20] In contrast, some reports have shown that H. pylori infection was significantly associated with the incidence of MI in younger patients [21,22]. Therefore, H. pylori infection would be associated with MI in patients with specified clinical characteristics. Detail mechanisms...
of the association between H. pylori infection and MI are unclear, but previous studies have demonstrated that H. pylori infection is associated with endothelial dysfunction [23] and that it might cause vulnerable plaques. [24] Some of H. pylori strains possess cytotoxin-associated gene-A (CagA), which is one of the major virulence factors of H. pylori, and high prevalence of CagA-positive H. pylori strain has been reported in Japan [25]. The anti-CagA antibodies react with antigens localized within coronary atherosclerotic plaques leading to destabilization, [26] and a past study showed a significant association between CagA sero-positivity and risk of cardiovascular diseases [24].

Previous reports have suggested the importance of IL-1 in H. pylori-associated diseases and that IL-1 genetic polymorphisms are related to gastric inflammation, atrophy, and gastric carcinogenesis [9]. Among various genetic polymorphisms, C–T base transitions at position –511 bp from the transcriptional start site and IL-1 RN *2 were reportedly strongly associated with gastric inflammation and carcinogenesis, and these genetic polymorphisms showed higher local levels of IL-1 beta than non-carriers. [9] Thus, the IL-1 polymorphisms could be important determinants of the inflammatory response in patients with H. pylori infection, because cytokines such as IL-1 beta is important in the inhibition of gastric acid secretion, [27] and the inhibition of gastric acid enables larger colonization of H. pylori, [5] leading to continuous chronic infection. To the best of our knowledge, there have not been any reports that evaluate both the H. pylori infection and IL-1 polymorphisms in the increased risk of MI. At the present study, we hypothesized that H. pylori infection and IL-1 polymorphisms are important in chronic inflammation, pathogenesis of atherosclerosis, and incidence of acute coronary events, and we investigated the H. pylori infection and the genetic factors of IL-1 in cases and controls. As a result, H. pylori-infected patients with IL-1 polymorphisms showed a significantly higher incidence of MI than the other subjects.

Atherosclerosis is an inflammatory disease, and inflammation has a fundamental role in all the stages of atherosclerosis from initiation through progression and, finally, in the thrombotic complications of atherosclerosis. H. pylori is reported to be associated with increased concentrations of systemic markers of inflammation and inflammatory cytokines [28]. Although there is some controversy regarding the effect of cytokine polymorphism on the increased levels of serum cytokines, [8,9, 29] previous studies have stressed the role of cytokine polymorphisms in determining the levels of inflammation associated with chronic low-grade infections such as periodontal infections and Chlamydia pneumoniae, leading to an increased risk of atherosclerosis, [30,31] so the association between atherosclerosis and pathogen burden may be modulated by cytokine polymorphisms. In the present study, patients...
with H. pylori infection and IL-1 polymorphisms showed significantly higher levels of hs-CRP and significantly lower levels of Ln-RHI both in MI and control groups. Therefore, there is a possibility that host cytokine polymorphisms could enhance the inflammation from H. pylori infection and cause the development of vascular endothelial dysfunction and atherosclerosis, resulting in the progression of coronary plaque and acute coronary event. This low-grade systemic inflammation may influence the initiation and progression of atherosclerosis and the incidence of acute coronary event. Our study suggests that there will be treatment options for eradicating the H. pylori pathogen or anti-inflammatory agents to prevent adverse cardiovascular events; however, further studies are needed to confirm our findings.

4.1. Limitations

As a limitation of this study, it was performed at a single center, and the number of this study subjects was relatively small, though sample power analysis was done. We cannot deny that this background might make our findings less convincing, and multicenter studies in much larger subjects are needed in the future to confirm our findings.

H. pylori infection is associated with multiple cofactors such as hygienic environment and smoking habits, and H. pylori-associated diseases might be partly explained by confounders. Also, the frequencies of IL-1 beta-511 genotype and IL-1RN *2 allele are different among ethnic populations, and our results may not be applicable all over the world. Moreover, we did not measure the serum levels of inflammatory cytokines such as IL-1 beta, IL-6, and tumor necrosis factor; thus, we did not demonstrate that the systemic inflammation was induced by the elevation of inflammatory cytokine levels.

5. Conclusions

The simultaneous presence of H. pylori infection and IL-1 polymorphisms showed higher levels of hs-CRP and lower levels of RHI, and were significantly correlated with the incidence of MI.

<table>
<thead>
<tr>
<th>Variable</th>
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<th>Multivariate regression</th>
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<td></td>
<td>OR 95% CI P value</td>
<td>OR 95% CI P value</td>
<td></td>
<td></td>
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<tr>
<td>H. pylori with IL-1 polymorphisms</td>
<td>3.39 1.69–6.82 0.001</td>
<td>4.83 1.99–11.7 &lt;0.001</td>
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<td>Diabetes</td>
<td>2.32 1.16–4.66 0.018</td>
<td>1.22 0.5–3.0 0.66</td>
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<tr>
<td>Hypertension</td>
<td>0.73 0.36–1.47 0.38</td>
<td>0.51 0.21–1.25 0.14</td>
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<tr>
<td>Dyslipidemia</td>
<td>4.45 2.27–8.74 &lt;0.001</td>
<td>5.82 2.46–13.8 &lt;0.001</td>
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<tr>
<td>Obesity</td>
<td>1.4 0.73–2.72 0.32</td>
<td>1.7 0.72–3.98 0.23</td>
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<tr>
<td>Smoking</td>
<td>19.6 4.48–86 &lt;0.001</td>
<td>14.2 2.89–69.9 0.001</td>
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<tr>
<td>CKD</td>
<td>1.96 0.54–6.07 0.071</td>
<td>2.56 1.04–6.33 0.041</td>
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Abbreviations: OR, odds ratio; CI, confidence interval; H. pylori, Helicobacter pylori; IL-1, interleukin-1; CKD, chronic kidney disease.

Fig. 4. Natural logarithm of reactive hyperemia index (Ln-RHI) between patients with Helicobacter pylori infection and interleukin (IL)-1 polymorphisms and the other patients. The bars represent the average Ln-RHI. The T-bars indicate the standard deviations. MI, myocardial infarction.

Fig. 5. ROC curves for the model of conventional risk factors (age, male sex, obesity, diabetes, hypertension, dyslipidemia, smoking, and chronic kidney disease) (A) and the model after the addition of the simultaneous presence of Helicobacter pylori and interleukin-1 polymorphisms (B).
Table 3
Reclassification of risk factors for MI after the addition of H. pylori infection and IL-1 polymorphisms.

<table>
<thead>
<tr>
<th>Original risk category</th>
<th>Reclassification</th>
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<td>RF + H. pylori + IL-1</td>
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<tr>
<td></td>
<td>Polymorphisms low risk</td>
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<tr>
<td>Patients without cardiovascular events</td>
<td>RF low risk 0</td>
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<tr>
<td></td>
<td>RF Intermediate risk 29</td>
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<tr>
<td></td>
<td>RF High risk 0</td>
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<tr>
<td>Patients with cardiovascular events</td>
<td>RF low risk 0</td>
</tr>
<tr>
<td></td>
<td>RF intermediate risk 2</td>
</tr>
<tr>
<td></td>
<td>RF high risk 0</td>
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Abbreviations: MI, myocardial infarction; H. pylori, Helicobacter pylori; IL-1, interleukin-1; RF, risk factors (dyslipidemia, current smoking, and chronic kidney disease).

Acknowledgments and disclosures

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[11] Fujioka T, Tokieda M. Validity of serum anti-14 work was supported in part by the Japan Heart Foundation, Tokyo.


