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Helicobacter pylori-seropositivity along with pro-inflammatory interleukin-1 polymorphisms correlated with myocardial infarction☆

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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; $P = 0.003$ and -2.8 ± 0.9 vs. -3.2 ± 0.6, respectively; $P = 0.02$) 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.

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].

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The association between *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 *H. pylori* develop MI, while others do not. In this study, we examined the association of *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 [10]. Case 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 *H. pylori* were measured using a direct enzyme-linked immunosorbent assay kit (E Plate Eiken *H. pylori* Antibody, Eiken Chemical Co., Ltd., Tokyo, Japan). Levels of IgG were categorized as seropositive and seronegative for *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 [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. [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 [13], and the frequency of another polymorphism at position +3954 is very rare in Japanese populations, [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. [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 [16]. Peripheral

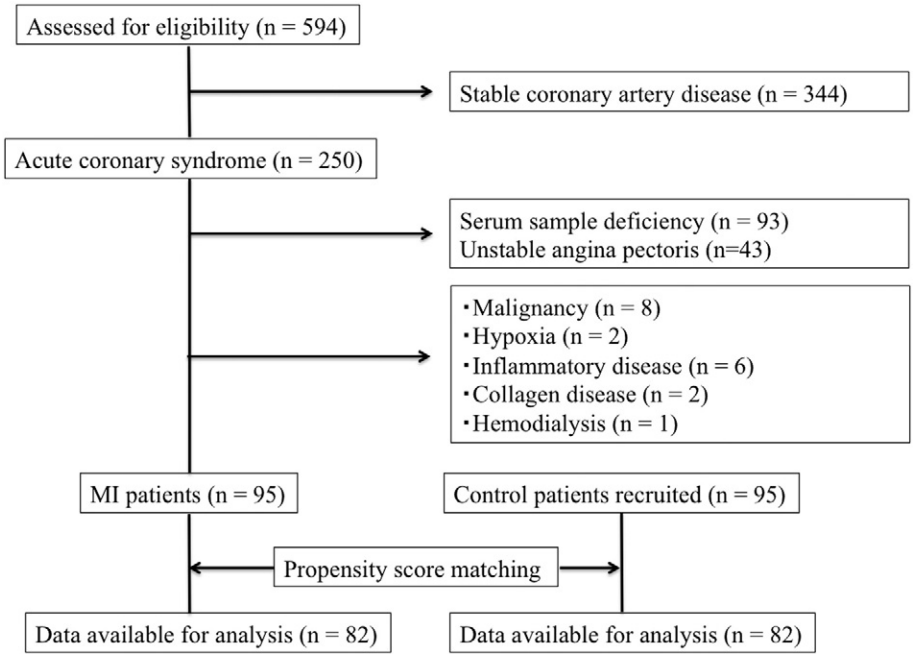

Fig. 1. Flow chart showing the protocol used for enrolling the patients. MI, myocardial infarction.