Serum Interleukin-6 Levels, Not Genotype, Correlate With Coronary Plaque Complexity

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SUMMARY
An increased serum interleukin-6 (IL-6) level is associated with an increased risk of cardiovascular events in healthy subjects. However, it is unknown whether the level of serum IL-6 or genetic IL-6 polymorphism is correlated with the complexity of coronary plaque in patients with stable coronary artery disease (CAD).

Patients with stable CAD (n = 135) were divided into 3 groups: insignificant coronary plaque (n = 77), simple coronary plaque (n = 15), and complex coronary plaque (n = 43). IL-6-174G > C polymorphism and serum levels of IL-6 and C-reactive protein (CRP) were investigated.

No significant difference in the distribution of IL-6 genotypes was found among the groups. The presence of complex coronary plaque was associated with higher serum concentrations of IL-6 (P = 0.026) and CRP (P < 0.0001). To predict the presence of complex lesions, IL-6 > 5.8 ng/L and CRP > 2.6 mg/L had sensitivities of 86% and 74%, and specificities of 61% and 62%, respectively. By multivariate analysis, IL-6 > 5.8 ng/L and CRP > 2.6 mg/L were independently related to the presence of complex coronary plaque (P = 0.0002 and 0.004, respectively). IL-6 > 5.8 ng/L and CRP > 2.6 mg/L were associated with a 4.5-fold increase in the odds of having complex coronary plaque (P < 0.005).

A simple measurement of the serum IL-6 level in patients with CAD can potentially identify subjects with complex coronary lesions and provide the option of aggressive medical strategies in a clinical setting. (Int Heart J 2008; 49: 391-402)

Key words: Interleukin-6, C-reactive protein, Coronary artery disease

ATHEROSCLEROSIS can be described as a chronic inflammatory process.1) Circulating levels of several inflammatory markers rise in individuals at risk for atherosclerotic events and have strong predictive values for cardiovascular events. Appreciation of the role of inflammation in atherosclerosis provides a
mechanistic framework for understanding the clinical benefits of a variety of anti-atherosclerotic therapies. Cytokines, such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α, are soluble polypeptides which act as important humoral regulators in inflammatory cascades. IL-6 expression has been observed on macrophages and vascular smooth muscle cells in stable coronary plaque from patients with ischemic heart disease undergoing heart transplantation. IL-6 is involved in the function of a variety of cells, including B-cell immunoglobulin production, T-cell cytotoxic activity, platelet reactivity, vascular smooth muscle proliferation, and endothelial cell activation, which may lead to plaque growth or instability.

Epidemiological data have demonstrated that IL-6 is associated with clinical and subclinical cardiovascular diseases. Importantly, elevation of the circulating IL-6 level is associated with an increased risk of myocardial infarction among healthy men, increased late cardiovascular events among patients undergoing coronary artery bypass grafting, and an increased mortality rate among patients with acute coronary syndromes. However, the mechanistic role of IL-6 in mediating this poor cardiovascular outcome is still not fully understood. We hypothesized that the morphological complexity of coronary atherosclerotic plaque may fundamentally represent the status of inflammation and may be correlated with the level of serum IL-6 or other inflammatory cytokines in patients with stable coronary artery disease (CAD). Furthermore, the value of genetic polymorphism investigations was also estimated by evaluating whether any relationship exists between the genetic polymorphisms of IL-6 and IL-6 serum levels or coronary plaque morphology.

**METHODS**

**Patients:** Initially, 306 patients were consecutively enrolled from May to October 2002 if they had undergone coronary angiography because of chest pain suspected of being of coronary origin. Patients were excluded if they had acute coronary syndromes (n = 54), elevated cardiac troponin I (n = 67) or other cardiac isoenzymes (n = 14), or any chronic or acute conditions associated with inflammation (n = 28). The racially diverse aboriginals of Taiwan were also excluded (n = 8). Finally, 135 subjects were included in the analysis of this study. All of the enrolled subjects were unrelated Han Chinese living in Taiwan. Angiograms were independently assessed by two experienced cardiologists. Patients with normal coronary angiograms or lesions with < 50% stenosis were defined as the group with insignificant plaque. Patients with lesions with ≥ 50% stenosis in any major coronary artery were separated into 2 groups: a simple stenotic plaque group and a complex stenotic plaque group. The simple stenotic plaque group was defined
as patients with type A plaque according to the classification proposed by Ambrose, *et al* [9] and the American Heart Association/American College of Cardiology classification [10]. The complex stenotic plaque group was defined as patients with type B or C plaque (Figure 1). Other coronary risk factors, including hypertension, diabetes mellitus, hypercholesterolemia, a high body mass index, and smoking, were determined by history taking, previous medical records, or examining the patients during hospitalization. “Smoking” was defined as smoking more than one pack a day within the last 5 years. This study was performed in
In accordance with the principles of the Helsinki Declaration and Ethics Review Board of Chang Gung Memorial Hospital. Written informed consent was obtained from all patients after explanation of the purpose, nature, and potential risks of the interventions to the subjects.

**Genotyping of IL-6:** Genomic DNA was extracted with a QIAmp blood kit (QIAGEN, Chatsworth, CA, USA) from peripheral blood leukocytes. Genotyping of the IL-6 polymorphism, -174G > C, was performed according to a previously described method\(^{11}\) with minor modifications. The sense primer was 5’CA-GAAGAAACTCAGATGACTGG3’, and the antisense primer was 5’GCTGGG-CTCCTGGAGGG3’. Amplification was performed with 5 minutes of denaturation at 94°C, followed by 37 cycles of 1 minute at 94°C, 1 minute at 63°C for annealing, 2 minutes of DNA synthesis at 72°C, and then 10 minutes at 72°C. PCR products were digested with Sfa NI, resulting in a single nondigested 614-bp fragment corresponding to the C allele and digested 377- and 237-bp fragments corresponding to the G allele. Genotyping of the IL-6 polymorphism was classified as GG, GC, and CC for further analysis.

**Inflammatory markers:** Arterial blood samples were collected in anticoagulant-free tubes before the coronary angiography and centrifuged at 2500 g and 20°C for 10 minutes. Serum was frozen at -80°C until being assayed. IL-6, TNF-α, the IL-1 receptor antagonist (IL-1Ra), and high sensitivity C-reactive protein (CRP) were measured with quantitative enzyme-linked immunoassay (ELISA) techniques. The IL-6, TNF-α, and IL-1Ra kits were purchased from R&D Systems (Minneapolis, MN, USA). All serum levels of biochemical markers were measured according to the manufacturer’s protocols. The minimal detection limits were 1.6 ng/L for IL-6 (DuoSet DY206 kit), 2.2 ng/L for TNF-α (DY210 kit), and 119 ng/L for IL-1Ra (DRA00 Quantikine kit). High-sensitivity C-reactive protein concentrations were measured using a particle-enhanced immunoturbidimetric method (Roche Diagnostics) with a Hitachi 912 analyzer (Roche Diagnostics) and Tina-quant C-reactive protein [latex] ultrasensitive assay reagents (Roche Diagnostics). The minimal detection limit for the CRP assay was 0.1 mg/L.

**Statistical analysis:** Categorical variables are presented as the frequency (percentage). Differences in the categorical variables and frequencies of the genotypes and alleles between the groups (insignificant, simple, and complex plaque groups) were evaluated using the chi-squared test. For continuous variables, results are presented as the mean ± SD, and differences among the 3 groups were evaluated by analysis of variance (ANOVA) with Tukey’s test. For variables analyzed by nonparametric methods, the Mann-Whitney U test or Kruskal-Wallis test was used. A multivariate logistic regression model was used to analyze the relationship between the presence of complex coronary plaque and serum variables or other coronary risk factors. Variables included in the analysis were age,
hypertension, diabetes mellitus, statin therapy, smoking, body mass index, and the serum levels of cytokines. The odds ratio (OR) and 95% confidence intervals (CIs) were calculated. A two-tailed probability value of < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS 10.0 for Windows (SPSS, Chicago, IL).

**RESULTS**

**Clinical and angiographic characteristics:** Throughout the study period, 77 patients were found to have normal coronary arteries or insignificant coronary plaque. There were 58 patients with significant coronary stenosis, including 15 with simple coronary plaque and 43 with complex coronary plaque. The baseline clinical and angiographic characteristics of these 3 groups are shown in Table I.

<table>
<thead>
<tr>
<th>Demographic, Clinical, and Angiographic Characteristics of Subjects With Different Coronary Plaque Morphologies</th>
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<tbody>
<tr>
<td><strong>Insignificant plaque</strong></td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Male (%)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
</tr>
<tr>
<td>Diabetes (%)</td>
</tr>
<tr>
<td>Statin therapy (%)</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
</tr>
<tr>
<td>Smoking (%)</td>
</tr>
<tr>
<td>Angiographic parameters</td>
</tr>
<tr>
<td>Diseased vessels</td>
</tr>
<tr>
<td>LVEF (%)</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
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* $P < 0.05$, ** $P < 0.001$, compared to the insignificant plaque group. LVEF indicates left ventricular ejection fraction and LVEDP, left ventricular end-diastolic pressure. Values are presented as the number (%) or mean ± SD.

<table>
<thead>
<tr>
<th>Interleukin (IL)-6 Genotype and Coronary Plaque Complexity</th>
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<tbody>
<tr>
<td><strong>Insignificant plaque</strong></td>
</tr>
<tr>
<td>IL-6 GG homozygote (%)</td>
</tr>
<tr>
<td>IL-6 GC heterozygote (%)</td>
</tr>
<tr>
<td>IL-6 CC homozygote (%)</td>
</tr>
</tbody>
</table>

Values are presented as the number (%).
No significant differences were noted among the groups in terms of age, gender, body mass index, or the prevalence of hypertension and statin therapy. The incidence of diabetes mellitus and left ventricular end diastolic pressure were significantly higher in the simple plaque group compared to the insignificant plaque group. The prevalence of smoking was significantly higher in both the simple and complex coronary plaque groups compared to the insignificant plaque group. The left ventricular ejection fraction was significantly lower in the complex plaque group compared to the insignificant plaque group. Furthermore, the left ventricular end diastolic pressure was correlated to the presence of diabetes mellitus ($r = 0.22, P = 0.017$).

**Relationships of genotypes of IL-6 with plaque complexity and serum IL-6 levels:** The frequency of the genotype of the IL-6 -174G > C variant in patients of the simple and complex coronary plaque groups did not significantly differ from that of the insignificant plaque group (Table II). The frequencies of the CC homozygote in the simple and complex coronary plaque groups did not significantly differ from that of the insignificant plaque group either ($P = 0.153$ and $0.956$, respectively). Furthermore, the relationship between the genotypes of IL-6 and serum IL-6 levels was investigated. There was no significant relationship between the genotypes of IL-6 and serum IL-6 levels either in the insignificant plaque group or the entire study population (Figure 2).

![Figure 2](image-url). Relationship between genotypes of interleukin (IL)-6 and serum IL-6 levels.
Inflammatory markers and plaque complexity: Compared to the insignificant plaque group, complex coronary plaques were associated with higher serum concentrations of IL-6 ($P = 0.026$, Figure 3A) and CRP ($P < 0.0001$, Figure 3B), and tended to be associated with higher serum TNF-α concentrations ($P = 0.79$, Figure 3C). Serum IL-1Ra levels were not associated with coronary plaque complexity ($P = 0.926$, Figure 3D). CRP levels were not significantly related to IL-6 ($P = 0.45$). ROC curves were drawn to compare the predictive value of the IL-6 level on the presence of complex coronary plaque (Figure 4). The best-balanced sensitivity and specificity values for IL-6 and CRP were 5.8 ng/L and 2.6 mg/L, respectively, and were used as cutoff values for further analysis in the logistic regression model. To predict the presence of complex coronary lesions, IL-6 > 5.8 ng/L and CRP > 2.6 mg/L had sensitivities of 86% and 74%, and specificities of 61% and 62%, respectively. Multivariate analysis showed that IL-6 > 5.8 ng/L

Figure 3. Serum levels of inflammatory markers and coronary plaque complexity. (A) IL-6 indicates Interleukin-6; (B) CRP, C-reactive protein; (C) TNF-α, Tumor necrosis factor-α; and (D) IL-1Ra, IL-1 receptor antagonist.
and CRP > 2.6 mg/L were independently related to the presence of complex coronary plaque even after adjusting for such variables as hypertension, diabetes mellitus, smoking status, age > 65 years, statin therapy, and a body mass index > 25 (kg/m²).

Table III. Multivariate Logistic Regression Analysis for Interleukin-6 (IL-6) and C-Reactive Protein (CRP) in Predicting the Presence of Complex Coronary Plaque

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>95% interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 &gt; 5.8 ng/L</td>
<td>4.5</td>
<td>1.9-10.2</td>
<td>0.0002</td>
</tr>
<tr>
<td>CRP &gt; 2.6 mg/L</td>
<td>4.5</td>
<td>1.6-12.6</td>
<td>0.004</td>
</tr>
</tbody>
</table>

After adjusting for the variables of hypertension, diabetes mellitus, smoking status, age > 65 years, statin therapy, and a body mass index > 25 (kg/m²).

DISCUSSION

This study showed that, in patients suspected of having stable coronary artery disease, an elevated serum IL-6 level was significantly associated with the presence of complex coronary plaque (type B and C lesions). The predictive value of IL-6 for the presence of complex coronary plaque was even better than that of serum CRP levels. However, IL-6 genotypes were not related to either
serum levels of IL-6 or the coronary plaque complexity. This discrepancy might be related to other unknown environmental or genetic factors, or the inflammatory status. Our study suggests that a simple measurement of serum IL-6 levels in patients suspected of having stable CAD may identify subjects with complex coronary lesions and provide the option of early coronary intervention or aggressive medical strategies in a clinical setting.

**Genotypes of IL-6 and coronary plaque complexity:** Although the genotype is thought to be related to gene expression and protein production, our study, however, could not demonstrate a relationship between the IL-6 gene promoter polymorphism of -174G > C and serum IL-6 levels or its possible impact on the morphology of coronary atherosclerosis in a clinically stable population. In the literature, the -174G > C polymorphism is the most important and functional IL-6 gene promoter polymorphism. The genotype of the -174C allele has been found to be associated with thickened carotid artery intima-media, and an increased risk of developing CAD or hypertension in generally healthy subjects. However, not all reports are consistent with this theory. Rauramaa, et al reported a protective role of the -174C allele genotype on the development of carotid intima-media thickening. On the other hand, Brull, et al performed an elegant study demonstrating the existence of a conditional discrepancy between genotypes and serum levels of IL-6. They showed that although IL-6 levels were similarly low in subjects with a stable status, their serum IL-6 levels increased quite differently in response to a variety of strong stimuli such as coronary bypass surgery. Moreover, Georges, et al also reported the viral and bacterial pathogen burden to be associated with IL-6 expression in patients with CAD and the IL-6 -174 polymorphism. Our report lends further support to a simple investigation of IL-6 genotypes not benefiting the application of inflammatory markers to risk stratification in a clinical setting. IL-6 expression seems to depend on an unknown environmental or inflammatory status, or the status of vascular plaque complexity, but not simply on genetic factors. However, whether the presence of complex coronary plaque plays an active or passive role in increases in serum IL-6 levels still needs to be elucidated.

**Serum IL-6 levels and coronary plaque complexity:** Increased blood levels of inflammatory markers are a consistent finding in patients with CAD, especially in those with acute coronary syndromes. Activation of local and systemic inflammation, however, may interfere with the interpretation of the relationship between inflammatory markers and coronary plaque characteristics. The current study excluded all subjects with acute coronary syndromes, evidence of myocardial injury, or any grossly identifiable acute inflammatory status in an attempt to clarify this issue. Our results showed that an increased serum IL-6 level correlated with a high possibility of the presence of complex coronary
plaque in patients suspected of having clinically stable CAD.

Previous reports revealed that an elevated serum IL-6 level is associated with an increased risk of a future myocardial infarction among healthy men and that early intervention benefits patients with acute coronary syndromes and elevated IL-6 levels.7,8) Our findings provide a mechanistic link between laboratory data and cardiovascular outcomes in patients with a stable status. Previously, we showed that CRP actively promotes a proatherosclerotic and proinflammatory phenotype, and these effects are mediated, in part, via the production of IL-6 by activated endothelial cells.18) The data in the present study showed that both serum IL-6 and CRP concentrations correlated with the complexity of coronary lesions, in line with the central theory of inflammatory processes in atherosclerotic development. Furthermore, the value of IL-6 levels in predicting the presence of complex coronary plaque was better than CRP. However, the predictive value of IL-6 may have been overestimated by the major limitation of measuring the IL-6 level, which could be detected in only 24% of our patients. More information would be gained if a more sensitive kit could be designed to measure IL-6 in the future. On the other hand, serum IL-6 levels did not correlate well with CRP levels, suggesting that IL-6 may provide information about different components of risk for complex coronary lesions, not accounted for per se by CRP levels. In a clinical setting, the high specificity of IL-6 and the high sensitivity of CRP can be used to provide different values in predicting the presence of complex coronary artery lesions.

Other inflammation markers and coronary plaque severity: CRP, one of the most extensively studied inflammatory markers, is not only a downstream inflammation indicator but also actively participates in the processes of atherosclerosis formation.19) Suzuki, et al20,21) previously identified a relationship between CRP concentrations and the coronary plaque burden quantified by an intravascular ultrasound technique in a population with acute coronary syndrome. Our data further demonstrated that the serum CRP level is correlated with the complexity of coronary plaque and explains in part why CRP has prognostic value among healthy men. Recent progress in understanding the role of CRP has shown that the most powerful form of CRP in terms of endothelial dysfunction is the monomeric form.22) The traditional measurement of high sensitivity CRP only identifies the pentameric form, and this might explain its modest association with the presence of complex coronary plaque. IL-1Ra is an acute reactive protein and has been found to be prominently expressed at an early stage of acute myocardial infarction.23) However, our data revealed that serum IL-1Ra levels were not correlated to either the CRP levels or the morphology of coronary plaque in patients with stable CAD.

Compared to the complex plaque group, our study revealed that the inci-
dence of diabetes mellitus and the left ventricular end diastolic pressure were higher only in the simple plaque group. However, the patient number in the simple plaque group is quite small with a high probability of a type II statistical error. More patients need to be recruited to reach a solid conclusion. On the other hand, our data demonstrated that the left ventricular end diastolic pressure was correlated to the presence of diabetes mellitus. The elevated left ventricular end diastolic pressure may be attributed to the presence of microvascular disorders with left ventricular diastolic dysfunction in diabetic patients.

**Limitations:** The size of our study population was not large enough to reach definitive conclusions; however, the distribution of IL-6 polymorphism was compatible with Hardy-Weinberg equilibrium. The evaluation of coronary lesions was obtained by coronary angiography rather than the standard methodology by coronary intravascular ultrasound. However, the main theme of this study was to estimate the morphology of coronary plaque instead of the anatomy. Finally, the cross-sectional study design did not allow a direct assessment of the association between serum IL-6 levels and changes in plaque morphology or the development of future cardiovascular events. Long-term follow-up may clarify the prognostic value of serum IL-6 levels in a population with initially stable CAD.

**Conclusions:** An elevated serum IL-6 level is associated with an increased incidence of complex coronary lesions in patients with stable CAD independent of genetic polymorphisms. Our data suggest that a simple measurement of serum IL-6 levels in patients suspected of having stable CAD may identify subjects with complex coronary lesions and provide the option of early coronary intervention or aggressive medical strategies in a clinical setting.

**References**


