The Association of C-Reactive Protein Levels With Carotid Intima-Media Complex Thickness and Plaque Formation in the General Population

Shinji Makita, MD; Motoyuki Nakamura, MD; Katsuhiko Hiramori, MD

Background and Purpose—An inflammatory response has been associated with the development of atherosclerosis. Our aim was to clarify which atherosclerotic changes (intima-media complex thickness [IMT] increase, plaque formation, and arterial dilatation) are associated with C-reactive protein (CRP) levels and to determine whether there are any gender differences.

Methods—Carotid ultrasound and measurement of high-sensitivity CRP (hs-CRP) levels were performed in 2056 subjects selected from a general population (mean age 58.3 years; 1290 men).

Results—In both genders, IMT significantly increased with increasing hs-CRP quartile ($P<0.001$), but this relationship disappeared after adjustment for age and other traditional cardiovascular risk factors. In men, but not women, carotid luminal diameter significantly increased with increasing hs-CRP levels ($P<0.05$), but again, this relationship disappeared with adjustment for age and other risk factors. However, in men, but not women, plaque score increased significantly with increasing hs-CRP quartile ($P<0.01$), even after adjustment for age and other traditional risk factors.

Conclusions—CRP level was closely associated with early atherosclerotic changes represented by carotid plaque formation. However, the IMT increase was strongly associated with aging and other traditional cardiovascular risk factors rather than CRP level. In the general population, CRP may serve as a complementary and quantitative marker for atherosclerotic plaque formation in men but not women. (Stroke. 2005;36:2138-2142.)

Key Words: atherosclerosis ▪ inflammation ▪ intima-media thickness ▪ ultrasonography

Many epidemiological studies have indicated that inflammation is associated with the process of atherosclerosis. It is also known that detectable but still relatively low levels of C-reactive protein (CRP) reflect chronic low-grade inflammation and are related to some of the traditional cardiovascular risk factors. Furthermore, CRP per se has been thought to be a risk factor for various cardiovascular events.1–3

Currently, ultrasonography is widely used for the noninvasive detection of atherosclerotic changes in the arterial wall. Many previous studies have demonstrated that specific findings on carotid ultrasound examination can accurately reflect the many different cardiovascular diseases and atherosclerotic risk factors that can occur.4,5

Various evaluations, such as the measurement of intima-media complex thickness (IMT), observation of plaque formation, estimation of lumen diameter, and wall elasticity are performed to assess carotid atherosclerosis. However, it is likely that each of these indices has a different role to play in the evaluation of atherosclerosis.6 Furthermore, gender differences in the relationship between inflammation and atherosclerosis have not been fully clarified. Thus, to identify which atherosclerotic changes are associated with the inflammatory response and to examine whether any association is common to both genders, we investigated the relationship of CRP levels with early carotid atherosclerotic changes and with traditional cardiovascular risk factors in subjects drawn from the general population.

Methods

Subjects
The study subjects were 2056 consecutive individuals (mean age 58.3 ± 9.8 years; range 25 to 86 years; 1290 men and 766 women) who visited the Iwate Health Service Association for a health checkup. The subjects consisted of inhabitants of a mixed urban/rural area. The majority of the subjects participated periodically in annual mass health screening programs in the community or physical checkups in the workplace. In the present study, all participants underwent a routine clinical examination, which included a medical history, physical examination, high-sensitivity CRP (hs-CRP) measurement, and carotid ultrasound examination. Subjects with an autoimmune disease or a recent infectious disease were excluded. As well, subjects who were using cholesterol-lowering therapy such as statin medications were excluded from this study because it has been suggested that these drugs may reduce CRP levels. In the study population, there were no patients with cardiovascular diseases.
including myocardial infarction and stroke, within the previous year. The study protocol was approved by the institutional ethics committee of the Health Service Association.

**Measurements**

Venous blood samples from the antecubital vein of subjects resting in the supine position were collected after an overnight fast. The serum CRP level was measured by high-sensitivity nephelometric immunoassay using N Latex CRP II (Dade Behring) with a lower limit of detection of 0.1 mg/L. Body fat was estimated by bioelectrical impedance analysis.

**Carotid Ultrasonography**

Carotid ultrasound examination was performed using an SSD-5500 (Aloka Co. Ltd) and Power Vision 6000 (Toshiba Medical Systems) scanner equipped with a 7.5-MHz linear array imaging probe. The IMT was measured in the common carotid artery (CCA) at sites 1.0 and 3.0 cm proximal from the beginning of the carotid bulb and at the bottom of the bulb. Each of the measurements done in these 3 sites was done at the far wall. If plaque lay at an IMT measurement point an appropriate adjacent portion was chosen. The IMT was defined as the distance between the leading edge of the lumen–intima and the leading edge of the media– adventitia echo. The average value of the 3 points was calculated for each side, and the largest value (maximum IMT) was used for the analysis. Plaque was defined as a regional intimal thickening, >1.4 mm in height or double the adjacent IMT height. The plaque score was defined as the sum of the maximum height of each plaque located in the whole extracranial portion of both carotid arteries. End-diastolic luminal diameters (Dd) were carefully measured on M-mode imaging with longitudinal guide. The average value of the 3 points was calculated for each side, and the largest value (maximum Dd) was used for the analysis. If a plaque lay at the Dd measurement site, a site at a proximal point was chosen to avoid the plaque.16 Average values derived from data of both sides were used for the analysis.

**Risk Factor Definitions**

Hypertension was assumed to be present in patients with a history of hypertension who were taking antihypertensive drugs. Hyperlipidemia was diagnosed in patients with serum cholesterol levels >200 mg/dL. Diabetes mellitus was diagnosed in patients with a blood glucose level >126 mg/dL, a hemoglobin A1c level >6.2%, or in patients with a history of antidiabetic therapy. In addition to these, a history of a smoking habit and the presence of obesity (body mass index >25 kg/m²) were regarded as other traditional cardiovascular risk factors.

**Statistical Analysis**

To determine the effect of the various factors on the hs-CRP level, simple or multiple regression analysis was performed. Natural log-transformed hs-CRP levels were used for regression analysis because of the skewed distribution. ANOVA with the Bonferroni post hoc test was used to compare the hs-CRP levels and the number of cardiovascular risk factors that were present, as well as to compare the carotid ultrasound indices and the hs-CRP quartiles. Because carotid atherosclerotic changes depend largely on age and other cardiovascular risk factors, an ANCOVA was also used to adjust for these factors. All these analyses were performed separately in men and women using SYSTAT ver.10.2 (SYSTAT Software Inc). Demographic data are expressed as mean±SD, and the other results are expressed as mean±SE.

**Results**

The demographic data of this study population are shown in Table 1. On univariate analysis, age, history of hypertension, diabetes mellitus, high-density lipoprotein level, smoking index (pack-years), body mass index, and body fat scale were significantly correlated with serum CRP level in both genders. Total cholesterol level and history of hyperlipidemia did not show a significant correlation with the CRP level (data not shown). On multivariate analysis, age, presence of diabetes mellitus, high-density lipoprotein level, smoking index, and body fat scale were significantly correlated with CRP levels in both genders (Table 2).

Comparisons of the carotid ultrasound indices with hs-CRP quartiles are shown in Figures 1 and 2. Carotid maximum IMT significantly increased with increasing hs-CRP levels in both genders (mean value in men: 0.71±0.01 first, 0.76±0.01 second, 0.78±0.01 third, and 0.80±0.01 mm fourth quartile, P<0.001; mean value in women: 0.69±0.02 first, 0.71±0.01 second, 0.74±0.01 third, and 0.78±0.01 mm fourth quartile, P<0.001; Figure 1). As shown in Figure 2, the plaque score increased significantly with an increasing hs-CRP level in men (0.49±0.07 first, 0.75±0.08 second, 0.86±0.09 third, and 1.01±0.13 mm fourth quartile; P<0.01). However, there

**TABLE 1. Demographic Data of the Study Population**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men (n=1290)</th>
<th>Women (n=766)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>57.8±10.3</td>
<td>59.1±8.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>122±16</td>
<td>119±18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>71±9</td>
<td>68±10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>205±34</td>
<td>219±35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>131±102</td>
<td>100±50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>56±14</td>
<td>63±15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>121±30</td>
<td>130±30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m2</td>
<td>24.3±2.8</td>
<td>23.9±3.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Body fat scale, %</td>
<td>23.4±5.0</td>
<td>29.6±6.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.2±0.7</td>
<td>5.3±0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking index, pack years</td>
<td>382±411</td>
<td>15±78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>21.2</td>
<td>23.6</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>5.4</td>
<td>3.9</td>
<td>NS</td>
</tr>
<tr>
<td>Hyperlipidemia, %</td>
<td>56.7</td>
<td>71.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Obesity, % (BMI &gt;25)</td>
<td>26.9</td>
<td>23.9</td>
<td>NS</td>
</tr>
<tr>
<td>Cigarette smoking, %</td>
<td>66.1</td>
<td>6.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cardiovascular disease, %</td>
<td>3.1</td>
<td>2.7</td>
<td>NS</td>
</tr>
<tr>
<td>Cerebrovascular disease, %</td>
<td>1.8</td>
<td>2.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, hemoglobin A1c; BMI, body mass index.

**TABLE 2. Association of Cardiovascular Risk Factors With Log-Transformed hs-CRP Levels on Multivariate Analysis**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>0.097</td>
<td>0.091</td>
</tr>
<tr>
<td>Hypertension (yes/no)</td>
<td>−0.012</td>
<td>−0.013</td>
</tr>
<tr>
<td>Diabetes mellitus (yes/no)</td>
<td>0.139</td>
<td>0.069</td>
</tr>
<tr>
<td>HDL level, mg/dL</td>
<td>−0.184</td>
<td>−0.129</td>
</tr>
<tr>
<td>Smoking index, pack-years</td>
<td>0.168</td>
<td>0.092</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>0.038</td>
<td>0.067</td>
</tr>
<tr>
<td>Body fat scale, %</td>
<td>0.148</td>
<td>0.292</td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein.
was no significant relationship between the plaque score and the hs-CRP level in women (0.42 ± 0.10 first, 0.48 ± 0.12 second, 0.39 ± 0.07 third, and 0.57 ± 0.08 mm fourth quartile; NS). As shown in Table 3, other than with adjustment for diabetes mellitus in men, the statistical associations between hs-CRP levels and the maximum IMT disappeared in men and women when adjusted for age or cardiovascular risk factors that statistically affected the IMT increase. However, in men, the significant association of hs-CRP levels with the plaque score was maintained even after adjustment for age and the relevant risk factors. In contrast, the significant association between hs-CRP levels and the plaque score disappeared in women when adjusted for age only or for the presence of hypertension in addition to the age adjustment.

Corrected Dd (Dd/square of body surface area) significantly increased with increasing hs-CRP levels in men (4.54 ± 0.03 first, 4.63 ± 0.02 second, 4.63 ± 0.03 third, and 4.66 ± 0.03 mm fourth quartile; \( P \) trend < 0.05), and the relationship was maintained after adjustment for age (\( P < 0.05 \)). However, this association in men disappeared when adjusted for cardiovascular risk factors and age. In women, no significant association of hs-CRP levels and Dd was found even on the crude analysis (4.59 ± 0.05 first, 4.61 ± 0.03 second, 4.66 ± 0.04 third, and 4.66 ± 0.03 mm fourth quartile; NS).

### Discussion

The present cross-sectional study revealed that plaque formation in men was strongly associated with elevated hs-CRP levels, and this was independent of age and other traditional cardiovascular risk factors. However, the relationship between IMT and CRP level appeared to be largely dependent on age or the presence of other cardiovascular risk factors. On the other hand, in women, there were no obvious relationships between carotid atherosclerosis and CRP levels.

Although elevated CRP levels have been associated with a higher IMT,8,9 this has not been confirmed by all studies.10,11 One study reported that an increased IMT occurred in an early phase of the atherosclerotic process.12 However, data from elderly individuals may mask the effects of the risk factors associated with early atherosclerosis because age is the primary contributor to carotid IMT increases (\( \beta = 0.479; t = 24.7; P < 0.001 \), based on a preliminary analysis of the data for our study). Such an increased IMT has been thought to represent arterial remodeling that occurs as an adaptive response to the tensile stress and hypertensive pressure occurring with lower degrees of IMT.13,14 Thus, it is possible that the association between IMT and CRP level is not strong enough to surpass aging or traditional risk factors. Unexpectedly, the statistical association of IMT with CRP level was maintained on covariate analysis for diabetes mellitus in men. This could be explained by the fact that diabetes mellitus may be synergistically associated with an increased IMT, or, alternatively, diabetes mellitus by itself may have a relatively small IMT-increasing effect.

We considered 2 reasons to explain why an increased CRP level was linked so strongly to carotid plaque formation in men. First, the actual sites of plaque formation are likely sources of measurable systemic inflammation. In fact, various inflammatory cells, such as T cells and macrophages, are abundant in atherosclerotic plaques.15,16 Second, the development of atherosclerosis in patients with the metabolic syndrome, which includes insulin resistance and a visceral fat increase, could have an increased formation of carotid plaque. Thus, the elevated systemic inflammation seen with the metabolic syndrome could be the result of an increased plaque score. Indeed, a significant association of diabetes with the plaques score was found in the present study (1.33 ± 0.27 in diabetes versus 0.78 ± 0.05 in nondiabetes; \( P < 0.01 \)). However, because a significant relationship between the plaque score and the hs-CRP quartiles was maintained on covariate analysis for diabetes, as well as in individuals with neither diabetes nor obesity (\( P < 0.01; F = 5.37; n = 858 \) in men), the first hypothesis that the actual sites of plaque formation are sources of measurable systemic

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**Figure 1.** Unadjusted comparison of carotid maximum IMT with hs-CRP quartiles in men and women. Quartile box plots depict 25th, 50th, and 75th percentiles as horizontal lines, and error bars depict the range of the 10th to the 90th percentiles. *\( P < 0.01 \) vs first quartile; **\( P < 0.01 \) vs first, second, and third quartiles of hs-CRP level.

**Figure 2.** Unadjusted comparison of the carotid plaque score with hs-CRP quartiles in men and women. Bar graph shows mean value, and error bars indicate SE. *\( P < 0.05 \) vs first quartile; **\( P < 0.05 \) vs first, second, and third quartiles of hs-CRP level.
inflammation may be regarded as the main mechanism that explains the linkage of elevated CRP level and carotid plaque formation.

Interestingly, previous reports have suggested that some circulating proinflammatory factors may indicate that the process of abdominal aortic dilatation is occurring even within aortas that have a diameter in the normal range.17 Our results show that although carotid arterial dilatation was associated with the CRP level when adjusted for age in men, no associations strong enough to surpass the association with known cardiovascular risk factors was found. Pathoanatomic studies have suggested that adaptive and compensatory increases in artery size, as manifested by an increased IMT, are found in the early stages of the atherosclerotic process.13,14,18 Bots et al and Kazmierski et al revealed that a graded positive linear association was found between lumen diameter and IMT at lower degrees of IMT (<1.1 or 1.2 mm).14,19 Such a result could be expected on the basis of postulating carotid arterial remodeling attributable to tangential and perpendicular tensile stress. Taking this into account, the weaker correlation between Dd and CRP level in our study is not unexpected because the association between IMT and CRP level was weak.

Many studies have revealed a close association between smoking and the presence of systemic inflammation,10,20 Tracy et al, in a detailed analysis of smoking status, found that CRP is primarily related to pack years and not to years since cessation of smoking. This suggests that there may be a persistent long-term effect that results in the association between smoking and inflammation. Although the mechanism of the inflammatory effect of smoking is unknown, it could be linked to plaque formation at the site of endothelial damage in the arterial wall, if plaque is the source of the inflammation.10

To date, the gender difference with respect to the association of inflammation with atherosclerosis has been not fully clarified. We could not find a strong relationship between CRP level and carotid atherosclerotic indices in women. Although in the present study, several background factors were found to be different between men and women, in women, there also are several other factors relating to inflammation, such as cyclical hormonal changes associated with the menstrual cycle and subclinical autoimmune reactions, that may modify the relationships between inflammation and carotid atherosclerosis. Overall, there are conflicting data on gender differences in the relationship between inflammation and atherosclerosis. Blackburn et al showed that in dyslipidemic patients, a significant association between CRP level and advanced carotid plaques was found only in male subjects.9 On the other hand, a report that analyzed data obtained from participants in the Framingham Study showed that there was an association between CRP and carotid atherosclerosis as assessed by IMT measurement of the internal carotid artery in women but not in men after adjustment for other possible factors.9 These disagreements may be partly explained by methodological variables, such as differences in the measurement site within the carotid artery21 and differences in the study populations. In the report using data from the Framingham Study, IMT measurement was performed at the internal carotid/bulb portion, and the average IMT was thus much larger than that observed in our study population.

Study limitations include the determination of CRP by a single measurement, which could result in imprecision attributable to measurement error. Moreover, CRP has a poor specificity in the presence of a coexisting inflammatory condition such as rheumatoid arthritis, chronic pulmonary diseases, and other infections. Although subjects with known inflammatory diseases or recent infection were excluded, our data were obtained in a manner that may not have allowed for the exclusion of all patients with subclinical inflammatory diseases. Recently, Chapman et al reported that the monocyte count is a better predictor of carotid atherosclerosis than CRP and other inflammatory factors.22 Our study did not examine such other inflammatory markers. Further research into the many inflammatory markers, including CRP, is needed.
In conclusion, this study showed that serum CRP level was closely associated with early atherosclerotic alterations, as measured by carotid plaque formation, in apparently healthy men.

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References
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