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Oxidative Stress, Inflammation, and Atherosclerotic Changes in Retinal Arteries in the Japanese Population; Results from the Mima Study

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Abstract. Oxidative stress and inflammation are known to play roles in the pathogenesis of vascular events. The aim of this study was to investigate the relationship between oxidative stress, inflammation, and atherosclerosis in the general population. A population-based, cross-sectional study was made of 282 people (126 men and 156 women, mean age; 65 ± 13, mean BMI; 25.4 ± 2.7 kg/m^2) recruited from the Mima study in Tokushima Prefecture. Risk factors included age, sex, body mass index (BMI), cigarette smoking, systolic and diastolic pressure, fasting blood glucose, serum lipids, and high-sensitive C-reactive protein (hs-CRP). Oxidative stress in blood samples was measured by the diacron reactive oxygen metabolites (ROMs) test. The degree of sclerotic change was determined from fundus photographs according to Scheie's classification. After adjustment for age and sex, ROM levels positively correlated with hs-CRP levels, but not with ghrelin, leptin and adiponectin levels. Furthermore, ROM and hs-CRP levels positively and individually correlated with the grade of sclerotic change in the fundus oculi independent of age in a multiple regression analysis. These results suggest that oxidative stress and chronic inflammation promote atherosclerosis in the retinal arteries in the general population.

Key words: Oxidative stress, C-reactive proteins, Atherosclerosis, General population
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OXIDATIVE stress, through the production of reactive oxygen species (ROS), has been proposed as the root cause underlying the development of insulin resistance, β-cell dysfunction, impaired glucose tolerance and type 2 diabetes mellitus [1–3]. Furthermore, this mechanism has been implicated as the underlying cause of both the macrovascular and microvascular complications of diabetes [3, 4]. Recently, the Framingham study suggested that smoking, diabetes, and body mass index were highly associated with systemic oxidative stress [5]. However, it has yet to be clear that oxidative stress is associated with atherosclerosis in the general population.

Recently, Tedschi-Reiner *et al*. indicated that the extent and severity of atherosclerosis in the retinal arteries correlated strongly with the extent and severity of coronary artery disease (CAD) [6]. Thus, atherosclerotic changes in the retinal arteries may be a predictor for the extent of CAD. The aim of this study was to investigate the relationship between oxidative stress, inflammation, and atherosclerotic changes in the retinal arteries in the general population.

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Materials and Methods

A total of 282 Japanese subjects (126 men and 156 women, mean age; 65 ± 13 years, range; 24-88 years) were enrolled in a population-based, cross-sectional study performed in an area of the Mima city in Tokushima Prefecture. The study was approved by the Ethics Committee of Kyoto Medical Center. All subjects gave their written informed consent. Subjects were recruited from a general community-dwelling population. Eligible subjects were not taking medication known to influence lipid/glucose metabolism or were without a history of diabetes and CAD. Blood pressure was measured in the upper arm of seated subjects. Overnight fasting blood glucose, total cholesterol and triglyceride levels were enzymatically determined, and high-density (HDL) lipoprotein cholesterol levels were determined by a homogeneous method. Serum leptin levels were measured using an enzyme immunoassay kit (Cayman Chemical Co., Ann Arbor, MI, USA). Serum total ghrelin levels were measured with an EIA kit (Peninsula Laboratories Inc., San Carlos, CA). Serum adiponectin levels were measured by enzyme-linked innmusorbent assay. Serum high-sensitive C-reactive protein (hs-CRP) levels were measured with a commercial enzyme-linked immunosorbent assay kit (Assaypro, St. Charles, MO, USA). As a marker of reactive oxygen species, the levels of reactive oxygen metabolites (ROM) were measured using a FRAS4 system (Health & Diagnostics Ltd. Co., Parma, Italy) [7–10]. A current cigarette smoking was defined as smoker.

Photographs of the ocular fundus were taken with an automatic-focus fundus camera under amydriatic conditions. The degree of atherosclerosis in the retinal vessels (stage 1–4 according to Scheie) was determined by examination [6, 11].

Statistical analyses were conducted using SPSS version 12. All data are shown as means ± SD. Data were analyzed by the nonparametric Mann-Whitney U test, and a multiple regression analysis controlling for age, sex, BMI, cigarette smoking, and blood pressure (explanatory variables) was performed to evaluate the impact of ROM or hs-CRP levels (explanatory variables) on atherosclerotic changes in the retinal arteries (response variables). Simple and age-sex matched correlation analyses among hs-CRP, ROM levels, and vascular risk factors (including age, blood pressure, and serum lipids) were conducted. $P<0.05$ was used as the determinant of statistical significance for all analyses.

Results

Compared with the men, the women had significantly higher total cholesterol, HDL-cholesterol, leptin, and adiponectin levels, lower triglyceride and fasting blood glucose levels, and fewer smokers. There was no difference in hs-CRP and ROM levels between men and women (Table 1). In the age-sex matched correlation analyses, hs-CRP correlated positively with BMI and leptin levels (Table 2), although there was no significant correlation between hs-CRP and other clinical parameters. After adjustments for age and sex, ROM levels positively correlated with hs-CRP levels ($\gamma = 0.148$; $p = 0.013$), but not with ghrelin, leptin, or adiponectin levels. According to the Scheie classification, the numbers of subjects in stages 1, 2, 3 and 4 were 198, 15, 21 and 4, respectively. Forty-six subjects had no sclerotic change in the retinal arteries. Hs-CRP levels and ROM levels are positively correlated with the grade of sclerotic changes in the fundus oculi. Further-

Table 1. Demographic Characteristics of the subjects

	Men (n = 126)	Women (n = 156)	P value
Age, y (range)	63 ± 15	66 ± 11	0.059
BMI (kg/m^2)	23.7 ± 2.8	24.3 ± 3.3	0.132
Systolic blood pressure (mmHg)	136 ± 20	140 ± 19	0.106
Diastolic blood pressure (mmHg)	76 ± 11	78 ± 12	0.193
Total cholesterol (mg/dL)	179 ± 38	195 ± 32	<0.001
HDL-cholesterol (mg/dL)	48 ± 16	57 ± 15	<0.001
Triglycerides (mg/dL)	109 ± 63	95 ± 39	<0.001
Fasting blood glucose (mg/dL)	105 ± 35	97 ± 28	0.036
Ghrelin (pg/ml)	57.8 ± 32.9	59.5 ± 26.2	0.639
Leptin (ng/ml)	2.2 ± 2.5	6.4 ± 5.7	<0.001
Adiponectin (μg/ml)	6.5 ± 5.3	9.7 ± 5.7	<0.001
hs-CRP (μg/ml)	0.43 ± 1.39	0.48 ± 1.34	0.753
ROM (IU.CARR)	311 ± 126	320 ± 57	0.218
Smoking (%)	32.5	1.3	<0.001

BMI, body mass indx; HDL, high-density lipoprotein; hs-CRP, high-sensitive C-reactive protein.Data are the means ± SD or percentages.

Table 2. Age-sex matched correlation

Parameters	hs-CRP	ROM
BMI (kg/m^2)	0.148*[1]	–0.065
Systolic blood pressure (mmHg)	0.047	–0.001
Diastolic blood pressure (mmHg)	0.043	–0.027
Total cholesterol (mg/dL)	–0.078	0.066
HDL-cholesterol (mg/dL)	–0.107	–0.096
Triglycerides (mg/dL)	–0.010	0.040
Fasting glucose (mg/dL)	0.037	–0.022
Ghrelin (pg/ml)	–0.078	0.040
Leptin (ng/ml)	0.147*[1]	0.013
Adiponectin (μg/ml)	–0.073	–0.011
Sclerotic changes*[2]	0.153*[1]	0.165*[1]

*[1]$p<0.05$.
*[2]The grade of sclerotic changes was scored as follows; no sclerotic changes = 0, stage 1 = 1, stage 2 = 2, stage 3 = 3, and stage 4 = 4.

Table 3. Multiple regression analysis for sclerotic changes in retinal arteries with clinical parameters

Parameters	Regression coefficient	P value
Age	0.163	0.012
Sex	0.086	0.215
Body mass index	–0.053	0.374
Smoking	–0.034	0.641
Systolic blood pressure	0.060	0.322
hs-CRP	0.135	0.022
ROM	0.141	0.017

more, ROM levels positively associated with the grade of sclerotic change in the fundus oculi independent of age and hs-CRP in a multiple regression analysis (Table 3).

Discussion

This study shows that inflammation (represented as in hs-CRP) and oxidative stress (represented as in ROM) promote atherosclerosis in the retinal arteries. The systemic and chronic inflammatory process has been recognized to play a role in the pathogenesis of atherosclerosis, and CRP has been considered a marker during the progression of cardiovasucular/cerebrovascular disease and metabolic disease. Recent studies emphasize that hs-CRP is a good marker of cardiovascular risk in the general population [12]. CRP levels are higher in obese subjects almost certainly because of increased insulin resistance [13]. CRP levels correlated positively with BMI in this study. However, we did not check insulin resistance indexes such as HOMA-IR. Interventions that alter insulin resistance, such as weight loss and exercise, alter CRP levels. Further examination of insulin levels and interventions is needed to clarify these issues.

In this study, ROS correlated positively with sclerotic changes in the retinal arteries. Carotid artery intima-media thickness (IMT) is a widely accepted index for assessing atherosclerosis, and known to be a risk indicator for cardiovascular/cerebrovascular disease. Kanaya *et al*. and Watanabe *et al*. reported that carotid artery IMT may be affected by increased levels of ROS produced by mononuclear cells (MNCs), and that increased ROS production by MNCs may be related to the development of atherosclerosis [14, 15]. Their data have shown the coexistence of increased oxidative stress and an inflammatory state in long-term renal graft recipients. The mechanism by which increased oxidative stress and an inflammatory state worsen the grade of sclerotic changes is unknown. Oxidative stress is considered to be involved in endothelial changes [16]. Further examinations including of endothelial function are needed to clarify these points.

Tedeschi-Reiner *et al*. reported that changes in lipoproteins and apoproteins associated with arthrosclerosis of the retinal arteries correspond well to those associated with at sclerosis of the coronary artery [6]. Pulse wave velocity (PWV) has been used as a marker for arterial stiffness and reflective damage [17]. Further examination including other methods such as IMT and PWV is needed to clarify these issues.

In conclusion, the present study suggests that an increased oxidative stress together with an inflammatory state promote atherosclerosis in the retinal arteries in the general population.

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