Materials and Methods

Subjects

The Dallas Heart Study is a multiethnic (~50% African American), probability based sample of the Dallas County population. The details on the Dallas Heart Study design and population sample have been previously published.[1-3] The current study included 3509 subjects with complete biomarker data consisting of IgG and IgM autoantibodies to malondialdehyde (MDA)-LDL and IgG and IgM ApoB-Immune complexes (ApoB-IC) and of those 2,740 subjects had coronary artery calcium (CAC) data and 2475 with abdominal aortic MRI data.

Determination of IgG and IgM Autoantibodies to Malondialdehyde-LDL and ApoB-Immune complexes

Plasma titers of IgG and IgM MDA-LDL (1:200 plasma dilution) autoantibodies and IgG and IgM ApoB- -IC were measured as previously described.[4,5] Briefly, MDA-LDL (5 µg/ml) was coated on microtiter well plates, plasma was added, and IgG or IgM antibodies binding to MDA-LDL was detected with alkaline phosphatase labeled goat anti-human IgG or IgM (Sigma). ApoB-ICs were detected by plating murine monoclonal antibody MB47 to bind a saturating amount of human apoB. Plasma was added and IgG or IgM antibodies binding to the captured apoB were detected with alkaline phosphatase labeled goat anti-human IgG or IgM as above.

Determination of laboratory variables

Total cholesterol, LDL cholesterol, high-density lipoprotein cholesterol, triglycerides, and high-sensitivity C-reactive protein were measured as previously described.[2]

Electron beam computed tomography protocol and CAC classification

Electron beam computed tomography image acquisition was performed using an Imatron C-150XP EBCT scanner (Imatron Inc., San Bruno, California), as previously described.[6] Results were expressed in Agatston units and the mean of the two scores was used as the final CAC score. Individuals with a mean EBCT score >10 Agatston U were classified as CAC+.

Abdominal aortic plaque quantitation with magnetic resonance imaging (MRI)

Abdominal magnetic resonance imaging was performed using a 1.5-T whole-body system (Intera, Philips Medical Systems, Best, the Netherlands) as previously described.[7] Six transverse slices of the infrarenal abdominal aorta were obtained using a free breathing, electrocardiography-gated, T2-weighted turbo spin-echo (black-blood) sequence. Slice thickness was 5 mm and interslice gap was 10 mm. Image acquisition utilized a commercial 4-element abdominal phased-array receiver coil. Images were analyzed by trained observers using the Magnetic Resonance Analytical Software Systems (MASS) cardiac analysis software package (Version 4.2 beta, Medis Medical Imaging Systems, Inc.). Adventitial and luminal borders were drawn for each slice using a free-hand manual contour drawing tool. Atherosclerotic plaque was identified as hyper-intense signal volume that protruded ≥1 mm from the endoluminal surface of the aortic wall as previously defined. Plaque was manually contoured in each image, and voxel summation was used to calculate the following endpoints: total vascular area (TVA) = ∑vessel area in each slice for all slices; total plaque area (TPA) = ∑plaque area in each slice for all slices; and aortic plaque burden (APB) = 100 × (TPA/TVA). Aortic wall thickness (AWT) was calculated by dividing the total vessel wall area by the aortic circumference in each slice. The mean AWT for each participant was determined adding the values of AWT for each slice, and dividing the result by the number of slices

Determination of Major Adverse Cardiovascular Events

The subjects were followed from January 2001 until December 31, 2011 for a median of 10.5 years of follow-up. Event adjudication beyond 2011 is not currently available for the DHS. Major adverse cardiovascular events (MACE) was defined as cardiac death, non-fatal MI, stroke/TIA, unstable angina requiring hospitalization and arterial vascularization that included coronary artery bypass surgery, percutaneous coronary intervention, carotid endarterectomy, carotid stenting and peripheral artery revascularization. Of the 3509 individuals, all had complete biomarker data. 2914 had the MACE endpoint adjudicated until the latest follow-up of Dec 31, 2011, therefore the MACE analyses were performed on these subjects. There were no significant baseline differences in the individuals with or without adjudicated MACE events.

If a patient had more than one MACE event, only one event was counted. Additional analyses were performed by removing arterial revascularization from the MACE endpoint.

Statistical Analyses

Analyses were performed with SPSS 23.0 software package. Continuous variables were presented as means ± standard deviations (SD) or medians and interquartile range (IQR) and dichotomous variables as percentages. Differences in baseline attributes between subjects were analyzed with ANOVA and X^2-test. Correlations between variables were determined with the Spearman test. The base-10 logarithms of triglycerides and the 4 biomarkers of oxidized lipoproteins were used in the logistic and Cox regression models to account for skewness in the distributions. Multivariate-adjusted (variables in Table 1) binary logistic regression models were used to estimate the associations between of biomarkers oxidized lipoproteins and CAC or plaque burden by MRI. The odds ratios (OR) are presented as age per decile and as per 1 standard deviation (SD) for LDL-C and HDL-C, and as per 1 SD for the log transformed triglycerides and the 4 biomarkers of oxidized lipoproteins. Multivariable-adjusted Cox regression analysis was used to estimate the associations between the 4 biomarkers of oxidized lipoproteins and time to MACE, with adjustment for sex, age in deciles, ethnicity, smoking status, the presence or absence of hypertension and diabetes, body mass index and levels of LDL-C per SD increase, HDL-C per SD increase, and log triglycerides per SD increase. Further analyses were performed according to ethnicity additionally using interaction terms. For ethnicity, analyses were performed for Blacks (n=1814) versus other ethnicities (n=1695) to make groups of relatively equal size. There were 1814 subjects who self-identified as Black, 1031 as White, 589 as Hispanic and 75 with no ethnic self-identification.

References

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