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Inflammatory Biomarkers and Subclinical Atherosclerosis in African-American Women with Systemic Lupus Erythematosus (SLE)

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Abstract

Women with lupus are at increased risk for developing cardiovascular disease (CVD). Previous studies of atherosclerosis in SLE have not been representative of the minority groups most affected by lupus and its complications. Therefore, a study of 41 lupus cases and 83 controls was conducted to investigate the relationship between carotid atherosclerosis and inflammation in African-American women. Participation consisted of a questionnaire, physical examination, fasting blood draw, and ultrasound of the carotid arteries. There were observed differences between cases and controls with regard to carotid intima media thickness (IMT) and traditional cardiovascular risk factors, although few reached statistical significance. Tumor Necrosis Factor-alpha was significantly related to carotid IMT, lupus, body mass index, and hypertension, indicating that it may be an important factor to consider in future studies of cardiovascular risk in African American women with lupus. This study adds to scientific literature by demonstrating that there may be other factors in the link between SLE and CVD.

Key Words: Carotid atherosclerosis, systemic lupus erythematosus, women

INTRODUCTION

In the United States, Systemic Lupus Erythematosus (SLE) disproportionately affects African American women. African Americans in the United States have three-fold higher incidence and prevalence rates of SLE, as well as cause-specific mortality rates, compared with Whites (Alarcon et al., 2005; Oates et al., 2003; Rus & Hochberg, 2002). Additionally, young women are most frequently affected by the disease, outnumbering male patients ten to one (Cooper, Dooley, Treadwell, St Clair, & Gilkeson, 2002).

A leading cause of death for persons with lupus is cardiovascular disease (CVD) (Office of Minority Health, 2004; Rus & Hochberg, 2002). Studies have shown that women with lupus are 5 to 8 times more likely to develop coronary heart disease (CHD) than women in the general population (Manzi, Meilahn, Rairie, & al, 1997; Petri, Perez-Gutthann, Spence, & al, 1992). Major cardiovascular risk factors for the general population (i.e., smoking, hypertension, obesity, sedentary lifestyle, and dyslipidemia) are also observed in SLE (Ho et al., 2005; Toloza et al., 2004). Very little is known about the risk factors in African Americans with SLE, although there is data to suggest that they may not be identical to those seen in Caucasian populations (Adeniyi et al., 2002; Henderson, Bretsky, Henderson, & Stram, 2001), indicating the critical nature of a study that explores the risk factors of CVD in African Americans with SLE.

The mechanism of the increased CVD rate in SLE is not well understood. Overall, literature indicates that SLE patients have multiple CVD risk factors present more often than individuals in the general population (Doria et al., 2003; Manzi & Wasko, 2000; Ramsey-Goldman & Manzi, 2001; Svenungsson et al., 2001; Van Doornum, McColl, & Wicks, 2002; Vlachoyiannopoulos et al., 2003). However, some studies that have used the Framingham risk factor assessment in SLE patients and controls, have shown that the 10-year risk of a CHD-related event is the same in patients and controls, indicating that other factors are contributing to the increased CVD rate in SLE (Bruce, Urowitz, & al, 2003; Chung, Oeser, Avalos, Raggi, & Stein, 2006; Manzi et al., 1997; Petri et al., 1992). In non-lupus patients, there is an association between inflammation markers and CVD (Kuller, Tracy, Shaten, Meilahn, & Group, 1996). This raises the possibility that the severe systemic inflammation seen in SLE may bring about high CVD mortality by causing accelerated atherosclerosis (Manzi & Wasko, 2000; Van Doornum et al., 2002). While it has been suggested that non-traditional mechanisms outside of traditional cardiovascular risk factors may modulate some of the increased risk for heart disease and increase in carotid IMT associated with lupus in women, this trend has not been explored specifically in African American women.

While many studies have focused on clinically overt heart disease as a cardiovascular endpoint, an emerging trend has been the use of ultrasound to study thickening of the interior lining of the arteries or intima media thickness (IMT) as a measure of subclinical atherosclerosis. The carotid arteries are easily accessible to noninvasive study using ultrasound techniques, and these

techniques provide accurate and reliable measurement of atherosclerosis in its subclinical stages (Espeland et al., 1996; Li et al., 1996; Persson, Formgren, Israelsson, & Berglund, 1994; Sutton-Tyrrell, Wolfson, Thompson, & Kelsey, 1992). However, few studies have used ultrasound to measure subclinical atherosclerosis in women with SLE (Manzi & Wasko, 2000; Ramsey-Goldman & Manzi, 2001; Van Doornum et al., 2002; Vlachoyiannopoulos et al., 2003; Wolak, Todosoui, & al, 2004). In those that have, the majority observed increased IMT compared with control subjects (Manzi & Wasko, 2000; Ramsey-Goldman & Manzi, 2001; Van Doornum et al., 2002; Vlachoyiannopoulos et al., 2003). While such studies have documented modest rates of plaque lesions, this trend has not been explored specifically in African American women and estimates may be substantially higher in this population.

In order to examine the role of inflammation in the relationship between SLE and subclinical atherosclerosis in African American women with SLE, the extent of subclinical carotid atherosclerosis measured as carotid IMT in 41 SLE cases identified on the east side of Buffalo, New York and 83 individually matched controls were measured, and its relationship to inflammation markers in the blood examined.

PATIENTS AND METHODS

Study Population

The Breakfast with a Buddy Biomarkers of Lupus Study was a case-control investigation of pre-clinical heart disease, inflammation, and traditional cardiovascular risk factors in the largely African American cohort of women with Systemic Lupus Erythematosus (SLE) from the Buffalo Lupus Project. Forty-one African American women with SLE were recruited to participate from the Buffalo Lupus Project, a survey and registry component of a five year community-based participatory research project investigating lupus and other autoimmune diseases on the east side of Buffalo, NY. Eighty-three friend controls were individually matched to cases on the basis of sex, age (within five years), and race. Cases identified themselves for participation in the Buffalo Lupus Project and released their medical records to be validated against the 1997 American College of Rheumatology revised criteria for the classification of SLE (Hochberg, 1997) by an independent rheumatologist. For the purposes of the present study, cases were asked to invite two or more friends to participate as controls. The present study desired controls representing the same population as cases, and friends may be likely to use the medical system in similar ways. Also biases due to social class are usually reduced since the case and friend control will be of similar socioeconomic background (Wacholder, McLaughlin, Silverman, & Mandel, 1992; Wacholder, Silverman, McLaughlin, & Mandel, 1992a, 1992b). Controls were required to be free of SLE and/or other autoimmune diseases and could not be a relative of a case since autoimmune diseases often run in families and controls may be unaware of existing disease. All eligible women 18 years of age or older were invited to participate

in this study, which was approved by the Health Sciences Institutional Review Board at the State University of New York at Buffalo. All participants provided their written informed consent. Of the 63 participants in the Buffalo Lupus Project survey, 33 cases participated in the Breakfast with a Buddy Study, corresponding to a 52 percent participation rate. Seven additional cases expressed interest and participated in the study without having done a survey, after learning of the study from other participants or joining the registry but not meeting residential inclusion criteria of the Buffalo Lupus Project Survey. Of the 124 controls invited to participate in the Breakfast with a Buddy Study, 99 participated, corresponding to an 80 percent participation rate.

Variable Measurements

All research was conducted in the Center for Preventive Medicine (CPM) at the State University of New York at Buffalo. Participation in the Breakfast with a Buddy Biomarkers of Lupus Study consisted of blood collection, physical measurements, an interviewer-administered questionnaire, and an ultrasound scan of the carotid arteries, all of which took place during the same two-hour clinic visit. All participants received a light breakfast following blood collection, a custom-designed gift basket, and culturally appropriate heart-health materials developed by the National Heart, Lung and Blood Institute of the National Institutes of Health for use in African American populations. Several risk factors were assessed.

Traditional cardiovascular risk factors. Information was obtained on age, race, education level, household income, smoking habits (current and past use), family history of CVD (MI, stroke, or sudden death in a first-degree relative before the age of 60 years), diabetes (previous diagnosis and/or current use of oral hypoglycemic agents or insulin) and fasting glucose levels, physical activity, and body mass index (calculated from height and weight). Current hypertensive status was determined using an average of three consecutive sitting blood pressure readings of SBP > 140 mmHg or higher or DBP > 90 mmHg or higher. Total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels were measured in fasting blood samples using standardized laboratory tests. Information on current use of anti-hypertensive agents and lipid-lowering agents was also obtained.

Inflammation Markers. Levels of tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), Fibrinogen, and C-reactive protein (CRP) were assessed from fasting blood samples. TNF-α was analyzed using Luminex technology, wherein multiplex assay kits measure multiple analytes simultaneously in a single reaction well. The Human Serum Adipokine Panel B LINCOplex Kit (Linco Research, Inc.; St. Charles, MO): Panel B was used to measure TNF-α and IL-6. IL-6 was measured by ultra-sensitive ELISA (R&D Systems, Minneapolis, MN) and CRP was measured using the BNII nephelometer from Dade Behring utilizing a particle enhanced immuno-nepholometric assay (Harris et al., 1999). Fibrinogen analysis was conducted using the Stago-Compact method

with a Claus Assay and Automated Coag Analyzer kit. Women were included regardless of their history of cardiovascular events. We obtained information about previous MI, angina, coronary revascularization, stroke, or other blood vessel diseases. The prevalence of a CVD event was similar between cases and controls (13 cases and 10 controls), systematically controlling for history of an event potentially impacting multivariate findings.

Vascular Disease Measurements. Ultrasound technicians were trained and certified to scan and read vascular ultrasounds at the Center for Medical Ultrasound at Wake Forest University School of Medicine and Wake Forest University Baptist Medical Center in Winston-Salem, North Carolina. B-mode ultrasonographic examinations were performed using a Biosound EASOTE AU5 duplex scanner (Italy) equipped with a 7.5-10 MHz linear array imaging probe/transducer (LA13A, Florence, Italy) on subjects in the supine position. Images of near and far wall IMTs were recorded on the right and left internal, common, and bifurcation of the carotid arteries according to previously developed protocols as for carotid vessels in the Buffalo Cardiometabolic Occupational Police Stress study (BCOPS) (Violanti, 2004). For each side of the neck, ultrasound technicians first identified both the internal and external vessels of the carotid artery by Doppler to make sure the internal vessel was the focus. They then identified the optimal angle of interrogation at long view, depending on the transverse view of the artery coming up the neck. From the first angle, the ultrasound technician moved circumferentially around the neck, producing images of the vessel from 30 to 45 degrees anterior and posterior from the optimal angle of interrogation, scanning from the distal common carotid artery to the internal carotid artery at each angle.

All scans were recorded on super-VHS videotape for offline analysis. Measurement of IMT was performed using an image analysis work station consisting of an Intel P4 3.0 GHz computer (PC800FSB), a DT 3152 scientific frame grabber, an optical disk drive, a Sony super-VHS video cassette recorder with a Sony 18.1" active matrix LCD monitor and DVI input speakers, and the ImagePro measuring software (Media Cybernetics, 2007). To measure the average IMT of a segment, digitized B-mode images were displayed on the monitor and two lines were electronically drawn, one along the lumen-intima interface and one along the media-adventitia interface. Interfaces were determined across part or all of a 1-cm segment depending on clarity of the image. The computer then generated one measurement for each pixel over this area, for a total of approximately 140 measurements. The average distance in pixels was converted to mm using the equation: 1 pixel = 0.109 mm.

In the present study, multiple carotid sites were used to measure IMT. This approach consists of measuring IMT in the near and far walls of the three main segments of extracranial carotid arteries (common carotid, bifurcation, internal carotid) on both sides of the neck. The mean common carotid artery (CCA) IMT was measured in 12 CCA segments approximately 10mm in length: the near and far wall, from three interrogation angles, on both the right and left side (2 x 3 x 2=12). The mean value of these 12 mean CCA IMT values is

the MMCCA. In the rare event (2-3%) one of the segments was not adequately visualized, the mean of all the visualized segments was used. The maximum IMT was measured in the CCA, bulb and internal carotid artery (ICA) from three interrogation angles on the right and left side. After selection of the single maximum IMT from the three angles of the near and far wall in each anatomical segment (1 x 2 x 3=6 on the right and the left), the mean values of these 12 single maximum IMT values were computed as the MMXIMT (Byington et al., 1997; The ACAPS Group, 1992).

Statistical Analysis

All analyses were conducted using the SPSS 15.0 computer program (SPSS Inc., 2007). Descriptive data were expressed as mean + standard deviation for continuous variables and percentage of cases and controls for categorical variables. To define the relationship between lupus and cardiovascular risk factors, independent samples t-tests were used to assess differences in mean values of continuous variables and chi square analysis conducted to determine the statistical significance of independence of categorical explanatory variables from SLE disease status. The bivariate relationships between IMT and each cardiovascular risk factor and the bivariate relationship between SLE and IMT were assessed in separate simple linear regression models. Traditional cardiovascular risk factors that reached statistical significance based on a bivariate relation with IMT of $p < 0.25$ and were also significantly correlated with inflammation were added to linear models with SLE and selected inflammation marker(s), one at a time. This was done to assess their individual effects on each other and their individual/combined effects on the relationship between SLE and inflammation. For multivariate linear regression analyses, all variables with a bivariate relation with IMT of $p<0.25$ were evaluated for inclusion into statistical models to ensure that important variables from a biological standpoint were not excluded.

RESULTS

Table 1 compares traditional cardiovascular, inflammatory, and SLE-specific variables between SLE cases and controls. All SLE cases and controls were African American females. The mean age of the entire sample was 49.5 + 13.3 (mean + SD) years. Annual household income was similar between groups, while education level was different between cases and controls ($p=0.07$). Compared with cases, more controls reported not completing high school (13.2% of controls compared with 6.7% of cases), while more controls reported graduating from college (38.2% of controls compared with 16.7% of cases).

Table 1: Comparison of traditional cardiovascular, inflammatory, and SLE-specific variables in SLE cases and controls

Explanatory Variable	Cases (n = 41)	Controls (n = 83)	P
Age, years	50.8 ± 11.2	49.6 ± 14.7	0.70
Annual household income, %			0.69
Under $10,000	26.7	11.8	
$10-29,999	36.6	40.8	
$30-49,999	16.7	19.7	
Over $50,000	16.7	23.7	
Level of education, %			0.07
< 12 years	6.7	13.2	
High school graduate or GED	76.7	48.7	
College graduate or more	16.7	38.2	
Hypertension (SBP ≥ 140 mmHg, DBP ≥ 90 mmHg, or self-report and medication use), %	66.7	36.8	0.01
Total cholesterol, mg/dL	189.7 ± 36.5	192.3 ± 28.5	0.70
LDL cholesterol, mg/dL	115.8 ± 31.0	123.7 ± 25.5	0.18
HDL cholesterol, mg/dL	56.2 ± 22.0	51.1 ± 13.2	0.15
Triglycerides, mg/dL	88.6 ± 38.2	86.9 ± 64.2	0.90
Lipid lowering medication use, %	20.0	14.5	0.56
Antimalarial use, %	46.7	1.3	<0.01
Steroid use, %	33.3	9.2	0.01
Family history of cardiovascular disease, %	33.3	16.0	0.06
Smoking status, Never compared with Past/Current			0.03
Never, %	40.0	65.3	
Past smoker, %	50.0	21.4	
Current smoker, %	10.0	13.3	
BMI (kg/m^2)	31.12 ± 7.5	31.23 ± 6.2	0.94
Physical activity, Inactivity compared with any activity			0.37
None, %	16.7	7.9	
Once in a while, %	26.7	34.2	
At least 3 times per week, %	56.7	57.9	
Mean max IMT, mm	0.87 ± 0.18	0.84 ± 0.18	0.64
Mean CCA IMT, mm	0.68 ± 0.12	0.67 ± 0.13	0.87
C-reactive protein, ug/ml	4.1 ± 3.6	3.4 ± 3.9	0.43
Tumor necrosis factor-alpha, pg/ml	6.3 ± 2.8	5.1 ± 2.4	0.03
Interleukin-6, pg/ml	4.0 ± 3.4	3.2 ± 2.8	0.17
Fibrinogen, mg/dL	357.2 ± 64.0	365.7 ± 61.7	0.54

Except where otherwise indicated, values are the mean ± standard deviation. P-values derived from independent samples t-tests for equality of means for continuous measures and chi-square calculations for independence of categorical variables (equal variances assumed). SBP-Systolic blood pressure, DBP-Diastolic blood pressure, IMT-intima media thickness, LDL-Low density lipoprotein, HDL-High density lipoprotein

There were no statistically significant differences between SLE cases and controls with respect to total cholesterol, and triglycerides. However, controls displayed higher levels of LDL-cholesterol than cases, and cases displayed higher levels of HDL-cholesterol than controls (p=0.18 and p=0.15 respectively). More cases than controls reported lipid lowering medication use (20.0 percent compared with 14.5 percent) (p=0.56). Additionally, significantly more cases compared with controls used steroids and antimalarials (p=0.01 and p<0.01 respectively). High blood pressure, defined by both SBP/DBP measurements and blood pressure lowering medication use, was significantly more prevalent in cases compared with controls (p=0.01). Cases also reported family history of premature cardiovascular disease more often than controls (p=0.06). The distribution of SLE cases and controls according to smoking status was significantly different (p=0.03). More cases were past or current smokers than controls (60 percent compared with 35 percent). Although average BMI was not significantly different across SLE cases and controls (p=0.94), more cases than controls were of normal weight (BMI=18.5-24.9 kg/m2) (21 percent compared with 13 percent) and more controls than cases were obese (BMI > 30 kg/m2) (49 percent compared with 41 percent). There was no difference in overall physical activity (p=0.37) between cases and controls. However, more controls than cases reported engaging in physical activity once in a while and at least three times per week (92 percent compared with 84 percent), while more cases than controls reported not engaging in physical activity at all (17 percent compared with 8 percent). While levels of CRP and Fibrinogen were not different across cases and controls (p=0.43 and p=0.54 respectively), levels of TNF-alpha and IL-6 were higher in cases compared to controls (p=0.03 and p=0.17 respectively). In both cases and controls, distributions of IMT (both mean max and mean CCA) were skewed toward high values. Cases displayed a mean of 0.87 (+ 0.18) mm for mean max IMT and a mean of 0.68 (+ 0.12) mm for mean CCA IMT. Controls were almost identical with a mean of 0.84 (+ 0.18) mm for mean max IMT (p=0.64) and a mean of 0.67 (+ 0.13) mm for mean CCA IMT (p=0.87).

Variables independently associated (p<0.05) with increased mean max IMT, using linear regression, included hypertension, past or current smoking, TNF-α, and lower BMI (Table 2). Although not statistically significant, there was a 0.10 mm increase in mean max IMT observed in SLE cases compared with controls (p=0.33), and a 0.02 mm increase in mean CCA IMT observed in SLE cases compared with controls (p=0.87). BMI displayed an inverse relationship with carotid IMT. There was a reduction of 0.29 mm in mean max IMT and a 0.19 mm reduction in mean CCA IMT per kg/m2 of BMI.

Table 2: Variables associated with increased intima-media wall thickness in 124 SLE cases and controls

	Mean max IMT (mm)	Mean CCA IMT (mm)	
Explanatory variable	Parameter estimate	Parameter estimate	*P-value*
Hypertension (SBP > 140 mmHg or DBP > 90 mmHg or self-report and medication use)	0.01, 0.08	0.01, 0.08	**0.01**, 0.08
Smoking status (Never and Past/Current Smoker)	0.03, 0.31	0.03, 0.31	**0.03**, 0.31
BMI (per kg/m2)	<0.01,0.05	<0.01,0.05	**<0.01,0.05**
TNF-α (pg/ml)	0.05, 0.17	0.05, 0.17	**0.05**, 0.17

Table 3 shows that after controlling for TNF-alpha, BMI, and hypertension, SLE disease status remained unrelated to mean max IMT and declined further in significance with adjustment for other covariates (p=0.33 to p=0.81 after adjustment for TNF-alpha, BMI, and hypertension). However, when only adjusted for TNF-alpha, the relationship between SLE and mean max IMT diminished in strength from a 0.10 mm increase in mean max IMT in SLE cases compared with controls to a 0.04 mm increase in mean max IMT for SLE cases, suggesting that levels of TNF-alpha partially explain the relationship between SLE and mean max IMT. When BMI was added to the model, the relationship between TNF-alpha and mean max IMT diminished in strength from a 0.18 mm increase to a 0.16 mm increase in mean max IMT per pg/ml increase in TNF-alpha, and TNF-alpha declined further in significance (p=0.08 to p=0.10). This trend continued with the addition of hypertension to the model, which resulted in the relationship between TNF-alpha and mean max IMT diminishing even more in strength to only a 0.09 mm increase in mean max IMT per pg/ml increase in TNF-alpha and losing more significance (p=0.35). When BMI was added to multivariate models with hypertension status, its relationship with mean max IMT became more protective, moving from a 0.29 mm reduction to a 0.35 mm reduction in mean max IMT per kg/m2 increase in BMI. Similarly, when hypertension was added to multivariate models with BMI, its relationship with mean max IMT increased, from a 0.26 mm increase to a 0.32 mm increase in mean max IMT for hypertensive subjects compared with normotensive participants, and it gained significance (p=0.01 to p<0.01). This trend suggests that BMI and hypertension are more important correlates of mean max IMT in this population than are SLE or TNF-alpha.

Table 3. Variables associated with increased mean max IMT in 124 SLE cases and controls, using multivariate linear regression

		Adjusted parameter (p)		
Independent Variable	Crude parameter (p)	1	2	3
SLE status (case/control)	0.10 (0.33)	0.04(0.53)	0.05 (0.60)	-.02 (0.82)
TNF-alpha (pg/ml)	0.19 (0.05)	0.18 (0.08)	0.16 (0.10)	0.09 (0.35)
BMI (kg/m2)	-.29 (<.01)		-.28 (<.01)	-.35 (<.01)
Hypertension (yes/no)	0.26 (0.01)			0.32 (<.01)

1-adjusted for TNF-alpha

2-adjusted for TNF-alpha and BMI

3-adjusted for TNF-alpha, BMI, and Hypertension

Sample previously matched on age

DISCUSSION

In this study, we sought to determine the role of inflammatory biomarkers in the increased risk of heart disease in SLE, with a focus on African American women who are at highest risk for both conditions. In doing so, both traditional cardiovascular risk factors and more novel inflammatory parameters were investigated in a sample of African American women with (cases) and without (controls) SLE. We found that SLE cases and controls were very similar with respect to risk factor levels, but differed in the traditional CVD risk factor variables of hypertension, family history of CVD, and smoking status. Previous studies have observed cardiovascular risk profile similarities between SLE cases and controls (Bruce et al., 2003; Chung et al., 2006; Manzi et al., 1997; Petri et al., 1992), suggesting that the 10-year risk of a CHD-related event would be the same in SLE cases and controls and that other factors are contributing to the documented increased CVD rate in SLE. The most surprising finding of the current study was that we did not find a statistically significant association between SLE and carotid IMT, especially when the existing literature consistently suggests that the development of atherosclerosis is accelerated in SLE. The mean values of carotid IMT for SLE cases and controls in the present study were not significantly different from each other [mean max IMT (cases)=0.86 mm, mean max IMT (controls)=0.84 mm, p=0.64 and mean CCA (cases)=0.68 mm, mean CCA IMT (controls)=0.67 mm, p=0.87]. A

0.03 mm difference in mean max IMT observed between cases and controls has clinical significance, however. Population data shows that a 0.03 mm difference in IMT is equivalent to three years of vascular age and a difference of 0.04 mm separates stroke patients from those without stroke (Burke et al., 1995). A significant number of cases compared with controls reported use of antimalarials and steroids since that is the usual regimen for secondary prevention of SLE, which may explain the slight increase in IMT observed in cases compared with controls (Bruce et al., 2003). Other studies have linked increased dosage and duration of use of Prednisone to carotid abnormalities (Doria et al., 2003; Manzi et al., 1999), although in the present study neither steroid nor antimalarial use were associated with carotid IMT. In general, our study population (both SLE cases and controls) displayed higher IMT values than have been observed in previous studies (Doria et al., 2003; Selzer et al., 2004; van der Meer et al., 2002). In these studies, conducted in predominantly White populations, the average IMT values (both mean max and common carotid) of both cases and controls range between 0.50-0.70 mm (Doria et al., 2003; Manzi et al., 1999; Selzer et al., 2004; van der Meer et al., 2002). The Atherosclerosis Risk in Communities (ARIC) study was a prospective investigation of cardiovascular risk factors and outcomes in sub-samples across the country. In investigations of IMT, study samples were between 25 and 37 percent African American and African American men and women displayed higher IMT than White participants. For example, Burke and associates (1995) observed average IMT of 0.73 mm in normotensive African American women (Ranjit et al., 2006). High IMT values observed in the current study may reflect the fact that Western New York has one of the highest rates of CHD in the US (National Center for Chronic Disease Prevention and Health Promotion; Centers for Disease Control and Prevention; Office for Social Environment and Health Research, 2000). Thus, in a population already demonstrating a high propensity for CHD, IMT measurement may not be too discriminatory.

Since no statistically significant associations were observed between SLE and carotid IMT (both mean max and mean CCA) in the analytic sample, conclusions could not be drawn regarding the role of inflammation in the pathway between SLE and carotid IMT with any confidence. However, within the limits of sample size, inflammation appears to at least partially explain the relationship between SLE and carotid IMT. A study with a larger sample size would be necessary to better understand the relationship between SLE and IMT when selected inflammation markers were introduced to statistical models. Additionally, the current study demonstrated that levels of proinflammatory cytokines (limited to levels of TNF-alpha and IL-6) were significantly increased in SLE cases compared with controls ($p<0.01$). Additionally, TNF-alpha was related to both mean max and mean CCA IMT, although these relationships were diminished or disappeared after adjustment for BMI and hypertension. These findings were consistent with previous study findings that have linked TNF-alpha to functional changes of the endothelium and hypertension (Manabe, Okura, Watanabe, & Higaki, 2005; Sebestjen, Zegura, Videcnik, & Ke-

ber, 2005). The observed relationships of TNF-alpha with carotid IMT, as well as with BMI, in a predominantly African American sample with high values of IMT and BMI, may indicate that TNF-alpha is an important factor to consider in future investigations of cardiovascular risk in African Americans.

We found that BMI and hypertension were more important correlates of carotid IMT (both mean max and mean CCA) than SLE and the investigated inflammation markers. When added to multivariate models, relationships between SLE disease status and IMT didn't change much, but the observed changes (although non-significant), indicate that hypertension may be in the pathway. Such findings are in agreement with other literature in both the general population and SLE-specific groups that have also demonstrated the importance of high blood pressure in the development of heart disease (Davis, Dawson, Riley, & Lauer, 2001; Doria et al., 2003; Iglseder, Cip, Malaimare, Ladurner, & Paulweber, 2005; Paul et al., 2005; Selzer et al., 2004). However, the observed negative relationship between BMI and IMT was contradictory, since most studies have observed an increase in cardiovascular risk as BMI increases (Diehr, Newman, Jackson, Kuller, & Powe, 2002; Ranjit et al., 2006). In the current study, BMI was negatively correlated to TNF-alpha in both SLE cases and controls, and TNF-alpha in turn was positively associated with carotid IMT, which may explain BMI appearing protective if heavier participants had lower levels of a factor (TNF-alpha) that corresponded with increased carotid IMT. There is also the possibility that the observed effects, or lack thereof, were a function of a BMI threshold. Approximately 83 percent of the study sample fell within overweight or obese BMI categories, making it possible that the observed trends are specific to obese African American women.

Findings may have been a function of the homogeneity of the current study population with regard to race, since the current study population shares gender and age characteristics of previous study samples (Manzi et al., 1999), which have generally been middle-aged or older and predominantly if not completely female. Since African Americans overall have higher risk of cardiovascular disease and are more likely to have characteristics that promote the atherosclerotic process (high blood pressure, dyslipidemia, smoking, physical inactivity, obesity, diabetes) compared with other racial ethnic groups (Adeniyi et al., 2002; Adeyemo et al., 2005; Alderman, Cohen, & Madhavan, 2000; Crespo, Smit, Andersen, Carter-Pokras, & Ainsworth, 2000; Finkelstein, Khavjou, Mobley, Haney, & Will, 2004; Freedman et al., 2005; Ranjit et al., 2006), this trend may have masked any disease-specific trends in the current study that are apparent in other less diverse study populations. Although not statistically significant, the apparent increase in carotid IMT in SLE cases compared with controls may have been due to differences between the two groups with respect to medication use. Significantly more SLE cases than controls reported steroid use, and other researchers have found that increased prednisone dosage and duration of use were associated with carotid plaque in women with SLE (Cooper et al., 2002; Doria et al., 2003; Manzi et al., 1999).

Strengths and Limitations

Our results must be interpreted cautiously since the study sample was small and highly select. The most compromising shortcoming of the current study was small sample size (N=124). Such a small sample increases the risk of Type I error, or inability to detect statistically significant differences that may in fact have been present. This is particularly true in the case of multivariate analyses, wherein effective analytical sample size was further reduced for each level of analysis. Small sample size may have also contributed to an increase in the likelihood of Type II error, and negative or aberrant associations observed may in fact be false.

Overall, participants were highly educated and of higher socioeconomic status than middle-aged African American women in the general population. Additionally, cases may have appeared healthier than controls with respect to traditional cardiovascular risk factors due to increased medical surveillance. An additional limitation of the current study is that the SLE cases included were not incident cases of the disease, which could affect their risk for CVD (i.e., risk factors, lifestyle habits, etc.) and other comorbid disorders. Other studies have observed that cardiovascular risk is associated with disease duration (Alarcon et al., 2005; Cooper et al., 2002), suggesting that our group of participants with SLE were inherently at increased risk for CVD compared with controls, regardless of other factors. However, SLE is a disease that often goes misdiagnosed or undiagnosed in patients for many years (Calvo-Alen et al., 2005; Office of Minority Health, 2004; Rus & Hochberg, 2002), making it difficult to truly classify cases as incident.

Another limitation of the present study was reliance on a previous diagnosis of SLE to confirm SLE case status and self-report of SLE control status. The current study grew out of a community-based participatory research project. Therefore, the study design and implementation incorporated community-based participatory research methods to recruit participants and develop goodwill in the community with available resources. While we reviewed their medical charts, there was not a clinician on-site to assess symptoms of cases and perform laboratory analyses to confirm a diagnosis of lupus. Due to financial constraints, the extent of disease (organ systems involved), its severity and impact (measured as damage accrual), and activity (assessed with high-sensitivity CRP levels, urinalysis, and complete blood cell count) were not defined and more novel markers of inflammation such as MMP-9 were not investigated. Patients were not examined to determine the presence of rash, mucosal lesions, or other markers of active disease, although self-reported arthritis (both rheumatoid and osteoarthritis) was assessed. However, medical records containing a positive diagnosis of lupus from at least one rheumatologist were obtained for all 30 cases included in analysis. Since it is usual clinical practice to ascertain a diagnosis of lupus using current American College of Rheumatology (ACR) criteria, it is assumed that these criteria were used to diagnose cases in the current study. The Connective Tissue Disease Screening Questionnaire (CSQ) was used in cases to further validate self-reported lupus

diagnoses and previous clinical diagnoses reflected in medical records. CSQ review, performed by Brigham and Women's Hospital, revealed that 94 percent of cases in the present study had probable SLE. The CSQ, however, was not used to screen out controls who may have a connective tissue disease. Thus, there is a possibility that some degree of misclassification occurred, which may partially explain some of the unexpected results found. However, questionnaire responses available from both cases and controls regarding other comorbid disorders, including asthma, COPD, Emphysema, cancer, and arthritis (both rheumatoid and osteo), revealed that no controls reported previously being diagnosed with lupus, COPD, Emphysema or rheumatoid arthritis, which increases certainty that controls are indeed controls free of these inflammatory mediated conditions. There is also a reasonable level of confidence in self-reported disease status since SLE is a hard diagnosis to ascertain for cases, who identified themselves in order to join the Buffalo Lupus Project registry and participate in corresponding support activities for lupus patients. Additionally, SLE is a serious enough condition to confidently assume that controls should remember experiencing it or being told they had it. Also, studies relying solely on the CSQ to identify controls may not be able to identify participants with mild or atypical SLE.

In addition to strengths of individual matching and a cost-efficient study design, the present study was the first of its kind to examine the relationship between SLE and carotid IMT in the context of inflammation, in an African American sample.

CONCLUSION

Although this study was not able to conclude that SLE was related to carotid IMT, it did demonstrate the importance of TNF-alpha, BMI, and hypertension as correlates of both mean max and mean CCA IMT in an African American study population. The clinical consequences of the increased risk of atherosclerosis in SLE could be considerable. Other studies have documented modest rates of plaque lesions, but this trend has not been explored specifically in African American women and estimates may be substantially higher in this population. As other complications and disease manifestations associated with lupus are better controlled with current therapeutic modalities, the relative importance of atherosclerotic disease may increase further. The implication that African Americans share a certain level of CVD risk, regardless of SLE disease status, makes it possible that the cardiovascular risk profile of African American women with SLE may be different from other racial/ethnic groups. Clinicians providing care for African American patients with SLE and other diseases characterized by chronic systemic inflammation should be aware of the propensity to atherosclerosis that these patients share. More meticulous screening for cardiovascular risk factors (both traditional and non-traditional) and more aggressive management of the identified risk factors may need to be considered for African American SLE patients.

There is a need for larger studies focusing on African American women with SLE to determine if the same or different factors are associated with CVD risk in this group. This study's role may primarily be to act as a sentinel that more research is needed to make sure that the most relevant factors are the focus of disease prevention on the primary and secondary level in this high-risk population.

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