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Complement proteins and arterial calcification in middle aged women: Cross-sectional effect of cardiovascular fat. The SWAN Cardiovascular Fat Ancillary Study

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ABSTRACT

Background: CVD risk increases in women after menopause. Recent data suggest higher levels of complement protein C3 and cardiovascular fat (CF) in postmenopausal women. Whether complement proteins are associated with early markers of atherosclerosis in healthy midlife women has never been evaluated. Additionally, the potential impact of the local CF on these associations has never been assessed.

Methods: Participants (n = 100, age (mean(SD)):50.48(2.63), 50% premenopausal) were from the Study of Women's Health Across the Nation (SWAN). Arterial calcification (aortic-AC and coronary-CAC) and CF volumes around the heart and aorta (total heart-TAT and aortic perivascular adipose tissue-PVAT) were quantified using EBCT scans. AC and CAC were each evaluated as presence (Agatston scores >0) and extent of calcification (log (Agatston scores+1)). Logistic and linear regression models were used for statistical analysis.

Results: Adjusting for age, race, menopausal status and lipids, C3 was significantly associated with both presence and extent of AC and CAC, all P values <0.05. Associations between C3 and presence and extent of AC and CAC were explained by additional adjustment for log TAT and log PVAT, respectively. Association between C3 and log(AC+1) was more pronounced at higher volumes of log TAT (interaction-P = 0.013) adjusting for study variables. No associations were found with C4.

Conclusions: Higher C3 was significantly associated with presence and greater extent of arterial calcification in midlife women. These associations were explained by higher volumes of CF, suggesting CF as a potential source of C3. C3 could be a potential non-invasive biomarker of early diagnosis of atherosclerosis. These findings need to be replicated in larger studies.

1. Introduction

The risk of cardiovascular diseases (CVD) in women increases significantly after the fifth decade of life [1], a time period coincident with the menopausal transition. During this period, women are subjected to several biological alterations including adverse changes in sex hormones, lipid/lipoprotein profile and body fat composition [2,3]. Interestingly, women over 50 years of age significantly had higher level of circulating complement protein C3 [4], which was found to be associated with postmenopausal status [5]. Several lines of evidence suggest a potential role of complement proteins in the atherosclerotic process. Various studies have reported significant associations between complement proteins (e.g. C3, C4) and subclinical measures of atherosclerosis [6,7] and demonstrated the activation of complement

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proteins within plaque in the arterial wall [8,9]. In light of the recent findings that complement protein C3 is higher in postmenopausal women [5], it is plausible to hypothesize that levels of complement proteins may play a potential role in explaining the higher risk of CVD after menopause [1]. No previous study has assessed the association between complement proteins and subclinical measures of atherosclerosis in healthy midlife women.

Adipose tissue could be a potential source of complement proteins. C3 and C4 proteins are strongly associated with abdominal adiposity and visceral adipose tissue in human related studies [10]. In mouse models, increased C3 and C4 deposition have been found connected to the adventitial and medial fibers including collagen and elastin at early time points prior to luminal lesion development [8]. The detection of C3 and C4 depositions on the outside layers of the vasculature, which are usually surrounded by a local fat depot known as perivascular fat [11], suggested this fat depot as a potential local source of these proteins. Interestingly, postmenopausal women have higher volumes of cardiovascular fat [3]. Therefore, it is possible that cardiovascular fat could be a potential local source of complement proteins [10,12] and could contribute to any associations between complement proteins and subclinical atherosclerosis in midlife women. No previous study has assessed the role of cardiovascular fat in explaining or modifying associations between complement proteins and subclinical atherosclerosis in midlife women.

For the present study, we aim first to evaluate the association between circulating complement proteins (C3 and C4) and arterial calcification in the coronary arteries and aorta (measures of subclinical atherosclerosis, CAC and AC, respectively) in healthy midlife women, and then to evaluate whether this association could be explained or modified by the volume and location of the cardiovascular fat.

2. Methods

The Study of Women's Health Across the Nation (SWAN) is an ongoing, multi-site, longitudinal study of women examining the physiological and psychological changes during their transition through the middle years. The study design has been previously published [13]. Between 1996 and 1997, 3302 participants were recruited from 7 different sites across the US (Boston, MA; Oakland, CA; Los Angeles, CA; Detroit, MI; Chicago, IL; Pittsburgh, PA & Newark, NJ). The baseline eligibility criteria for the SWAN study were An intact uterus and at least 1 ovary; Not pregnant or breast feeding; At least 1 menstrual period within the past 3 months; No hormone therapy use within the past 3 months.

The current study was a pilot study conducted among participants from the SWAN Heart ancillary study at the Pittsburgh site. SWAN Heart ancillary study was a study of changes in subclinical measures of atherosclerosis during the menopausal transition [3]. The sample size for this pilot study was determined based on several factors including availability of blood specimens, CT scans, subclinical atherosclerosis measures, and menopausal status at SWAN Heart baseline visit. Baseline blood specimens (stored locally at the Pittsburgh site) and CT scans were available for 100 SWAN Heart participants (50 pre/early peri-, and 50 late peri-/postmenopausal) who were all included in this pilot study.

The participants provided written informed consent prior to enrollment and research protocols were approved by the University of Pittsburgh institutional review board (IRB).

2.1. Study measures

2.1.1. Arterial calcification

Coronary artery calcification (CAC) and aortic calcification (AC) were measured using electron beam computed tomography (EBCT) scans in 3 passes. The first pass marked the anatomical landmarks for the coronary and aortic scans. The second pass showed the coronary arteries and was captured at maximal breath hold using electrocardiographic triggering to obtain the 100-ms exposure in the same phase of cardiac cycle of R–R interval (60%). The third pass captured the aortic artery from the aortic arch to the aortic bifurcation. The scans were saved on optical discs. To assess CAC, 30–40 contiguous scans of 3-mm were obtained from the level of root of aorta to the apex of the heart at maximal breath holding. To assess aortic calcification, 6-mm images were obtained picturing the arch of the aorta to bifurcation of the iliac vessels with a 300-ms exposure. AC and CAC were assessed using a DICOM workstation equipped with AcuImage, Inc software (South San Francisco, CA) at the University of Pittsburgh using the Agatston scoring technique [14]. Calcification was determined as present if three contiguous pixels showed >130 Hounsfield units (HU). CAC was determined as the sum of Agatston scores of the 4 major coronary arteries [15].

2.1.2. Blood assay

Blood samples were collected in the morning after fasting overnight (8–12 h). The blood collection was scheduled on days 2–5 of a regular menstrual cycle. In cases when a scheduled sample could not be obtained (due to less regular menstrual cycles), a random fasting sample was collected within 3 months of the scheduled annual visit. After sample collection, the blood was maintained at 4 °C and separated and frozen at −80 °C. The sample was then transported to the medical research laboratories on dry ice for analysis. High density lipoprotein cholesterol (HDL-C) was measured using heparin-2M manganese chloride while triglycerides and total cholesterol were measured by enzymatic methods using a Hitachi 747 analyzer [16–18]. Low density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation [19], after excluding triglyceride value ≥400 mg/dl. Insulin resistance was measured as homeostasis model assessment insulin resistance index (HOMA-IR) using fasting insulin and glucose levels [20]. C3 and C4 were assessed from frozen serum samples using commercial immunoturbidimetric assay kits (Tina-quant C3 cassette and a Tina-quant C4 cassette) that were run on the Integra 800.

2.1.3. Cardiovascular fat (CF) volumes

Volumes of total heart adipose tissue (TAT) and perivascular adipose tissue (PVAT) of the descending thoracic aorta were quantified using existing EBCT scans previously obtained to measure arterial calcification. TAT was defined as the fat around the heart and PVAT was defined as the fat around the descending thoracic aorta. TAT volume was quantified at the Biomedical lab at the Harbor UCLA Medical center. For TAT volume (cm^3), slices within 15 mm above and 30 mm below the superior extent of the left main coronary artery were included. The anterior border was defined by the chest wall and the posterior border by aorta and bronchus. This region was chosen as it also consists of the epicardial fat around the proximal coronary arteries including left anterior descending, left main coronary artery, right coronary artery and circumflex arteries. The protocol to quantify TAT showed excellent reproducibility with a between-readers Spearman correlation coefficient of 0.99 and within-reader Spearman coefficients of at least 0.97 [21]. PVAT volume was quantified as previously described [22] using the existing scans originally obtained to quantify AC. The scans were re-read at the University of Pittsburgh using the software Slice-O-matic (Tomovision, Montreal, Canada) by one local reader. The posterior border for PVAT was defined as the anterior portion of the spinal foramen while the anterior and lateral borders were defined by the left bronchus, esophagus and crus of