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Complement protein C3 and coronary artery calcium in middle-aged women with polycystic ovary syndrome and controls

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Abstract

Circulating complement protein C3 (C3) levels have been associated with coronary artery calcification (CAC) in women with systemic lupus erythematosus, but have yet to be evaluated in women with polycystic ovary syndrome (PCOS). We aimed to determine whether C3 levels were elevated in women with PCOS compared to controls, and to quantify the association of C3 with cardiovascular disease (CVD) risk factors and CAC, and if PCOS modified this association. This cross-sectional analysis included 132 women with PCOS and 155 controls 35-62 years old from the third visit of a case-control study. CAC was measured during the study visit and circulating C3 was measured in stored sera. The presence of CAC and CAC categories (Agatston score 0, 1-9.9, and ≥10) were used for logistic and ordinal regression analysis, respectively. C3 levels were not significantly different between women with PCOS and controls. Among all women, C3 was associated with the presence of CAC and increasing CAC groups after adjusting for age, PCOS status, and insulin or BMI, all $p<0.05$. In addition, C3 was associated with the presence of CAC after adjusting for age, PCOS status, BMI, insulin and African American race, $p=0.049$. PCOS status did not modify these associations. In conclusion, circulating C3 levels may prove beneficial in identifying women at risk of CVD in women with PCOS and the general population.

Keywords

Polycystic ovary syndrome; subclinical cardiovascular disease; coronary artery calcium; inflammation; complement protein C3

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Introduction

Polycystic ovary syndrome (PCOS) is a common reproductive endocrine disorder affecting 6-8% of women in the United States [1]. Women with PCOS have a high burden of cardiovascular disease (CVD) risk factors including insulin resistance [2], abdominal adiposity [3], type 2 diabetes [4] and inflammation [5]. Women with PCOS have increased subclinical CVD as measured by carotid intima-media thickness [6], flow mediated dilation [3, 6], and coronary artery calcification (CAC) [7] compared with controls. There is limited evidence of an increased risk in CVD events, but a meta-analysis of five studies showed women with PCOS had a 1.55 fold increase risk of coronary heart disease or stroke compared with controls [8].

PCOS related factors involved in CVD are inadequately defined, but recent studies suggest that PCOS is a low-grade inflammatory state [5]. A part of the innate immune system involving circulating complement proteins may play a role in systemic inflammation. In particular, complement protein C3 (C3) can be elevated in adults with CVD [9] and is associated with tissue damage at the site of myocardial infarctions [10]. Circulating C3 has also been associated with subclinical CVD as measured by CAC [11] and vascular stiffness [12] in women with systemic lupus erythematosus (SLE), which is another population with systemic inflammation and a high CVD risk.

No investigation has evaluated the association between C3, CVD risk factors and subclinical CVD measured by CAC in women with PCOS and controls. CAC is a non-invasive indicator of coronary artery disease and predicts cardiovascular events and mortality [13, 14]. The aims of this study were the following: 1) to determine whether C3 levels are elevated in women with PCOS relative to controls, and 2) to quantify the association between C3 with traditional CVD risk factors and CAC in women with PCOS and controls, and if PCOS modified the association between C3 and CAC.

Methods

Subjects and study design

This present analysis includes 132 women with PCOS and 155 controls 35-62 years old from the third visit of the Cardiovascular Health and Risk Measurement Study (CHARM III; 2001-2003). Recruitment and methodology for CHARM has previously been described [2]. Briefly, investigators identified women diagnosed with PCOS through medical records in the Division of Reproductive Endocrinology at Magee-Women's Hospital dating 1970-1993 (Pittsburgh, PA). Investigators used NIH criteria to define PCOS [15], and matched cases to neighborhood controls by age (±5 years) and race using voter's registration tapes and the 1993 Cole's Cross Reference Directory of Household. The University of Pittsburgh institutional review board approved the protocols and all participants gave consent before enrolling. Women with a body mass index (BMI) ≥50 kg/m^2 (6 women with PCOS) were excluded in the present analysis to reduce the discrepancy in BMI between women with PCOS and controls since the women were not matched for BMI. There were no other exclusions.

Data collection

The study visit has been previously described [2, 16]. In brief, investigators collected medical, menstrual and reproductive history, medication use, lifestyle, anthropometric measurements, blood pressure and serum concentrations of total cholesterol, high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), triglycerides, and fasting glucose and insulin. The quantitative insulin sensitivity check index (QUICKI) was also calculated [17].

Complement protein C3

Blood sera were frozen and stored at the University of Pittsburgh. In 2010, Quest Diagnostics (Pittsburgh, PA) measured C3 levels on stored sera using an immunoturbidimetric assay (C3c Serum Complement Assay, ID 44859W). Quality control measures include a monthly audit comparing data bias and precision against other Quest laboratories operating the same platform and a guarantee through regulated College of American Pathologists and New York State Proficiency.

Coronary artery calcium

CAC was quantified using electron beam computed tomography scans of the heart and aorta at the University of Pittsburgh Medical Center Cardiovascular Institute Preventive Heart Care Center. Blinded to PCOS status, radiology and computer tomography technicians (RT/CT) performed one scan per participant using the Imatron C-150 Ultrafast CT scanner (Imatron, South San Francisco, CA) [16]. The RT/CT technician scanned 30-40 contiguous images 3 mm from the aortic root to the apex of the heart with a 100 millisecond exposure time. Images were taken at the same phase of cardiac cycle, approximately 60% of the R-R interval.

Coronary calcium scores were computed using the base value region of interest computer software program (AccuImage, Diagnostics Corp., San Francisco, CA). Calcification was defined as pixels greater than 130 Hounsfield units and 1 mm^2 within an operator-defined region of interest (ROI) in each 3 mm thick image. The Agatston method was used to calculate the calcium score for each ROI by multiplying the area of significant pixels by a grade number (1-4) indicative of the peak computerized tomography number (Hounsfield unit) [18]. The individual ROI's were summed for a total coronary calcium score.

The director of the Institute (Dr. Daniel Edmundowicz) adjudicated questionable scans and artifacts. This center has been reported to have excellent reproducibility. Blinded replicate readings were not significantly different and the intraclass correlation for a non-zero CAC score was 0.99 [19].

Statistical analysis

Participant characteristics were calculated using descriptive statistics for women with PCOS and controls and were compared using Chi-square tests or Fisher's exact tests for categorical data, independent t-tests for normally distributed continuous variables, and Mann-Whitney U tests for non-parametric variables. Spearman correlations were used to determine associations between C3 and CVD risk factors in women PCOS and controls. Since few

subjects had a CAC score >100, we created three CAC groups (0, 1-9.9 and ≥10), which were modified from guidelines [20]. We used independent t-tests and analysis of variance (ANOVA) to evaluate C3 levels by the presence of CAC and across CAC groups, respectively.

The association between C3 and CAC was evaluated by logistic regression analysis of the presence of CAC and by ordinal regression analysis of CAC groups. Covariates with $p<0.20$ in univariate analysis of CAC were evaluated in a forward stepwise method. Covariates included anthropometric measurements, systolic blood pressure (SBP), fasting insulin and glucose, lipids, postmenopausal status, race, current smoking, oral contraceptive use, and hormone replacement therapy use. For regression analyses, insulin and glucose levels were categorized into quartiles and C3 was expressed as a unit of 10 mg/dL. We forced PCOS status and age into each regression model and tested first order interactions with C3 and PCOS status. Analyses were performed with PASW (version 18; IBM SPSS Inc., Chicago, IL) with a 2-sided $p<0.05$ for statistical significance.

Results

Women with PCOS were younger and had higher BMI, waist circumference, fasting insulin, QUICKI, triglycerides, and HDL-C compared with controls, all $p<0.05$ (Table I). There were fewer African Americans and fewer postmenopausal women with PCOS compared with controls. In addition, a significantly greater number of women with PCOS had any CAC and higher levels of CAC compared with controls (Table I).

Complement C3

Although not significant, mean levels of C3 were greater in women with PCOS (173.5 mg/dL) versus controls (169.0 mg/dL), $p=0.31$ (Table I). Levels of C3 were positively correlated with BMI, waist circumference, SBP, fasting insulin, fasting glucose, triglycerides, total cholesterol, HDL-C and LDL-C in women with PCOS and controls, all $p<0.05$ (Supplemental Table SI). Additionally, mean levels of C3 were positively associated with increasing CAC in women with PCOS and controls (Figure 1).

Regression analysis

We did not find significant interactions with PCOS status, thus the regressions were not stratified. C3, PCOS status, age, BMI, fasting insulin quartiles, and African American race were associated with CAC in univariate logistic and ordinal regression analysis. A 10 mg/dL increase in C3 was associated with the presence of CAC after adjusting for age and PCOS status (odds ratio [95% confidence interval]: 1.39 [1.27-1.52]). This association remained significant after separately adjusting for insulin quartiles (1.26 [1.14-1.39]) or BMI (1.14 [1.03-1.27]). C3 was associated with the presence of CAC (1.12 [1.00-1.25]) in a fully adjusted model including age, PCOS status, BMI, insulin quartiles and African American race (Table II). After adjusting for age and PCOS status, a 10 mg/dL unit increase in C3 was associated with a higher CAC category (1.91 [1.15-1.27]). This association remained significant after separately adjusting for insulin quartiles (1.14 [1.08-1.20]) or BMI (1.09 [1.03-1.15]). The association between C3 and CAC was attenuated in a fully adjusted model

including age, PCOS status, BMI, insulin quartiles and African American race (1.06 [0.99-1.12]; Table II).

Discussion

This is the first study to investigate circulating C3 levels, CVD risk factors and CAC in women with PCOS. Circulating C3 levels did not differ by PCOS status. C3 significantly correlated with traditional CVD risk factors in women with PCOS and controls. Additionally, increased C3 levels were associated with the presence of CAC and more severe CAC in women with PCOS and controls, and this association was not modified by PCOS status, suggesting that C3 may be a CVD inflammatory marker.

One of the most important observations is that C3 was not different between women with PCOS and controls. Similar to our results, *Wu et al.* found that C3 levels were higher but not significantly different between premenopausal women with PCOS (2.1 g/L) and controls (1.8 g/L) [21]. It may be that elevated C3 levels are partially mediated by adiposity and insulin resistance rather than PCOS *per se*. Our findings are consistent with reports showing strong associations of C3 with BMI and features of insulin resistance in women with [21, 22] and without [23] PCOS.

C3 is not traditionally evaluated in PCOS, but is a circulating inflammatory marker routinely measured in the diagnosis and treatment of SLE and considered an SLE-specific risk factor for CVD. Significant positive relationships between C3 and subclinical CVD measures including vascular stiffness [12] and CAC [11] have been confirmed in SLE patients and are consistent with these findings in women with PCOS. Additional epidemiological studies report that C3 is an inflammatory risk factor that predicts initial and recurrent CVD events [9, 24, 25], indicating a potential benefit of monitoring C3 levels in women with a high CVD risk.

C3 is a part of the innate immune system and the convergence point of the classical, alternative and mannose-binding lectin pathways which elicit an inflammatory response. Elevated circulating C3 levels can be linked to insulin resistance [26] and obesity [27]. In addition, C3 has been found in atherosclerotic lesions [28] and may play a role in compromising mechanical integrity of the vascular wall leading to increased vascular stiffness as demonstrated in mouse models [29].

The cross-sectional design of this study limits our ability to determine causality; however as mentioned previously, prospective studies have shown C3 to be a significant predictor of CVD events. There was some concern for the length of serum sample storage of approximately 8.5 years; however, Muscari *et al.* successfully evaluated C3 levels in samples stored for 7 years [30]. To minimize assay error, we had all sera samples evaluated by an independent, local laboratory (Quest Diagnostics) with approved quality control measures in place at the initiation of this study. Any future analyses should include a more comprehensive panel of inflammatory factors including complement protein C4 and high sensitivity C-reactive protein (hsCRP). Additional investigations are needed to prospectively measure C3 among women at high risk for CVD.

In conclusion, C3 was not significantly elevated in middle-aged women with PCOS when compared to controls. C3 was significantly associated with CVD risk factors and CAC in women with PCOS and controls. Our findings suggest C3 may be a CVD risk marker among women with PCOS and the general population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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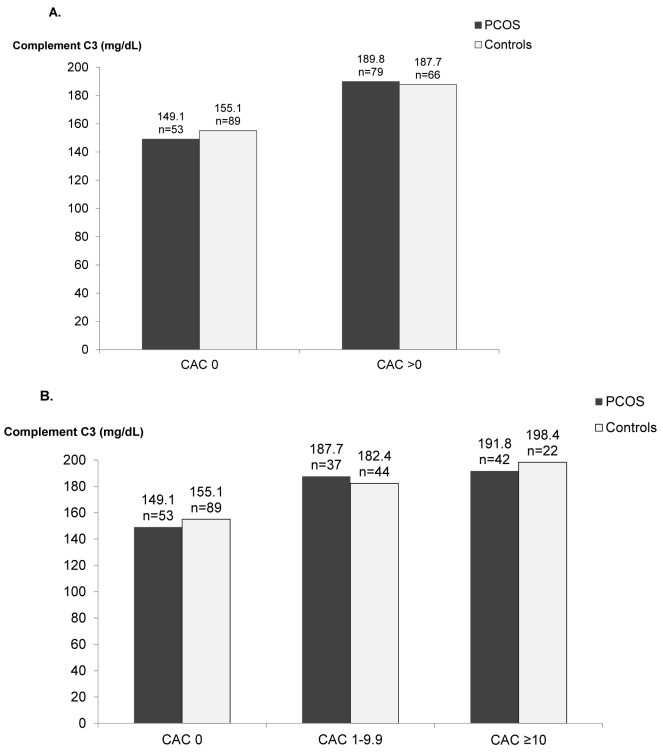

Figure 1.

Mean C3 level by women with PCOS (black bars) and controls (light gray bars) and A) presence of coronary artery calcium (CAC), $p<0.0001$ by independent t-tests, and B) CAC groups (0, 1-9.9, ≥10), both $p<0.001$ by analysis of variance.

Table I

Characteristics of women with PCOS and controls (N=287)

	PCOS (n=132)	Controls (n=155)	*p* value
Age (yr)	46.9 ± 5.3	49.3 ± 5.7	<0.0001
BMI (kg/m^2)	31.5 ± 7.6	28.6 ± 6.1	<0.0001
Waist circumference (cm)	93.4 ± 17.4	86.0 ± 14.0	<0.0001
SBP (mm Hg)	118.1 ± 11.5	117.3 ± 14.3	0.6
DBP (mm Hg)	76.1 ± 8.4	75.5 ± 8.4	0.5
Fasting insulin (μU/mL)	16.1 (9.8, 25.2)	11.8 (8.5, 16.2)	<0.0001[b]
Fasting glucose (mg/dL)	96.3 (86.0, 100.9)	93.0 (87.0, 98.0)	0.5[b]
QUICKI	0.3 ± 0.03	0.3 ± 0.03	<0.0001
Triglycerides (mg/dL)	132.1 (82.3, 210.5)	110.9 (78.0, 150.0)	0.03[b]
Total cholesterol (mg/dL)	208.9 ± 44.1	209.4 ± 35.3	0.9
HDLc (mg/dL)	52.2 ± 15.7	56.8 ± 14.3	0.01
LDLc (mg/dL)[a]	124.8 ± 40.2	127.4 ± 32.1	0.5
African American (n, %)	13 (9.9)	31 (20.0)	0.02[c]
Current smoker (n, %)	23 (17.4)	22 (14.2)	0.5[c]
Current OC user (n, %)	10 (7.6)	10 (6.5)	0.5[c]
Current HRT user (n, %)	17 (12.9)	29 (18.7)	0.2[c]
Postmenopausal (n, %)	33 (25.0)	61 (39.4)	0.01[c]
Complement C3 (mg/dL)	173.5 ± 38.9	169.0 ± 36.3	0.3
CAC any (n, %)	79 (59.9)	66 (42.6)	0.004[c]
CAC groups (n, %)			
0	53 (40.2)	89 (57.4)	0.0008[c]
1-9.9	37 (28.0)	44 (28.4)	
≥10	42 (31.8)	22 (14.2)	

Values are mean ± SD, median (25%-75% inter-quartile range), or number (percent); OC= oral contraceptive; HRT= hormone replacement therapy; postmenopausal was defined as no menstrual period in the last 12 months; CAC= coronary artery calcium expressed as Agatston score.

[a] n=130 women with PCOS.

[b] *p* value by Mann-Whitney U test, otherwise by unpaired t-test.

[c] *p* value between women with and without PCOS by chi-square test.

Table II

Logistic and ordinal regression analysis of coronary artery calcium (CAC)

	Logistic regression of presence of CAC		Ordinal regression of CAC groups	
Variable	**OR [95% CI]**	***p* value**	**OR [95% CI]**	***p* value**
Age (yrs)	1.10 [1.04-1.17]	0.001	1.04 [1.01-1.07]	0.015
PCOS[a]	1.79 [0.91-3.53]	0.092	1.68 [1.13-2.48]	0.01
Complement C3 (per 10 mg/dL)	1.12 [1.00-1.25]	0.049	1.06 [0.99-1.12]	0.082
BMI (kg/m^2)	1.22 [1.13-1.32]	<0.0001	1.13 [1.09-1.17]	<0.0001
Insulin quartiles	1.30 [0.90-1.87]	0.166	1.29 [1.05-1.60]	0.017
African American[b]	2.83 [1.09-7.34]	0.033	1.73 [1.09-2.75]	0.021

The presence of coronary artery calcium (CAC) was defined as an Agatston score >0; the CAC groups was defined as Agatston score 0, 1-9.9, ≥10; the Cox & Snell R Square was 0.40 and the Nagelkerke R Square was 0.53 for the logistic regression, and Cox & Snell R Square was 0.44 and the Nagelkerke R Square was 0.51 for the ordinal regression.

[a] Women without PCOS are the referent group.

[b] Caucasians are the referent group.