Is PCOS an inflammatory process?

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Abstract

PRO-- PCOS is associated with low-grade systemic inflammation as evidenced by elevation of multiple markers of inflammation such as C-reactive protein, interleukin-18, monocyte chemoattractant protein-1 and white blood count as well as endothelial dysfunction and increased oxidative stress.

CON-- The evidence in support of the presence of chronic inflammatory state in the majority of women with PCOS is incontrovertible. It is apparent that PCOS is associated with a significant elevation of multiple markers of inflammation including CRP, IL-18, MCP-1, and white blood count. Furthermore, PCOS is associated with other derangements associated with inflammation such as increased oxidative stress and endothelial dysfunction. While the etiology of systemic inflammation in PCOS remains unclear, recent data raise the intriguing possibility of a link between PCOS, inflammation and chronic low grade infectious agents such as *Chlamydia pneumoniae*, *Helicobacter pylori* and pathogens inducing periodontal inflammation.

Introduction

While the etiology of PCOS remains a mystery, the evidence in support of the presence of chronic low-grade inflammation in women with this syndrome is emerging. Inflammation is also likely to be associated with other prominent aspects of PCOS including insulin resistance and cardiovascular disease (CVD) risk factors. Indeed, inflammation is considered to be the key feature of endothelial dysfunction and atherosclerosis (1). Women with PCOS are predisposed to increased visceral adiposity and this appears to be across all categories of BMI. Using dual x-ray absorptiometry (DEXA) it has been shown that subjects with PCOS had similar percentage of total and trunk fat but higher percentage of central abdominal fat compared with weight-matched controls (2). The presence of increased visceral adipose tissue is associated with insulin resistance, hyperglycemia and dyslipidemia which as mentioned above are co-morbidities associated with PCOS. The visceral adipocytes exert these effects in a paracrine and endocrine manner via the secretion of a number of molecules some of which are markers of inflammation. In the Women's Health Initiative Observational Study it has been shown that overweight/obese women without clustering of cardiometabolic risk factors still possess abnormal levels of inflammatory

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markers (3). Furthermore, recent studies raise the possibility of an intriguing association of PCOS with low-grade infections. We will review the current evidence in support of and against low grade chronic inflammation in women with PCOS with special emphasis on the associated influence of obesity. Manifestations of chronic inflammation as evidenced by increases of C-reactive protein (CRP), pro-inflammatory cytokines and chemokines, white blood count (WBC), oxidative stress and various markers of endothelial inflammation will be discussed.

Views in favor of inflammation-- Antoni J. Duleba M.D.

CRP

One of the most important markers of inflammation is CRP. CRP is an acute-phase reactant produced by hepatocytes under the stimulatory control of pro-inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor α (TNFα) (4). Growing evidence supports the concept that CRP may be, not only a marker, but also a mediator of inflammatory processes (5,6). For example, CRP induces endothelial dysfunction and promotes monocyte chemoattractant protein-1-mediated chemotaxis (5). Elevation of high sensitivity CRP (hs-CRP) is considered to be one of the most important predictors of the risk of cardiovascular events; the predictive power of hs-CRP is independent of and complementary to lipid profile (7, 8).

The first study demonstrating elevation of CRP in women with PCOS was carried out by Kelly and associates who compared 17 women with PCOS (defined according to NIH criteria) and 15 control subjects (9). The increased CRP levels in women with PCOS remained significant when age and BMI were accounted for. These findings were confirmed by a large number of diverse studies evaluating various populations of women with PCOS defined according to NIH (10) as well as Rotterdam (11) criteria. Recent meta-analysis evaluated 31 clinical trials and included 2,359 women with PCOS and 1,289 controls (12). It concluded that CRP in women with PCOS is on average 96% (95% CI: 71-122%) greater than in healthy subject. Since BMI has a significant impact on CRP, an additional analysis was performed using data from 26 studies properly matched for BMI; in this analysis, CRP in women with PCOS was increased by 102% (95% CI: 73-131%). Funnel Plot assessment of the above meta-analysis revealed no evidence of publication bias. The above findings are of importance since even modest elevations of CRP are associated with a significant increase of vascular risks. For example, the age-adjusted relative risk of adverse cardiovascular events among subjects with blood levels of hs-CRP at 1-3 mg/L, and >3 mg/L were progressively and significantly increased above the relative risk of subjects with hs-CRP <1 mg/L (13).

Pro-inflammatory cytokines and chemokines

Chronic inflammatory processes are associated with elevations of a host of proinflammatory cytokines and chemokines including IL-18, monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1□ (MIP-1□). Interleukin-18 appears to be closely related to insulin resistance, metabolic syndrome and has emerged as an important predictor of long-term cardiovascular mortality (14,15). MCP-1, one of the most studied chemokines, plays a major role in the development of atherosclerosis (16). Another chemokine, MIP-1□, also known as chemokine (C-C motif) ligand3 (CCL3) is involved in recruitment and activation of leukocytes and predicts future cardiovascular events (17). PCOS is associated with elevation of these agents. In particular, increased levels of IL-18 have been demonstrated in several studies evaluating this cytokine in women from different ethnic groups with PCOS defined according to NIH as well as Rotterdam criteria (18,19,20,21) While IL-18 correlated also with obesity, the association with PCOS was observed when

accounting for age and BMI of the subjects. Furthermore, serum level of IL-18 correlated with total testosterone level and inversely with insulin sensitivity index (18,21) .Several studies have also revealed that women with PCOS have elevated MCP-1 (22, 23, 24). Most importantly, this observation was verified in PCOS and control subjects matched for age and BMI (24). Increased levels of MCP-1 and MIP-1□ were also noted among women with isolated hirsutism (23). Furthermore, serum from women with PCOS, when compared to serum from controls, induced significantly increased expression of MCP-1 in THP-1 human monocyte cell line(24).

WBC

Another marker of inflammation is white blood count. Even modest elevations of WBC are associated with multiple cardiovascular risk factors including increased BMI, adverse lipid profile and periodontal disease (25). In a large national cohort study, WBC was a predictor of coronary heart disease mortality independent of smoking and other traditional risk factors (26). The first study demonstrating an increase of WBC among women with PCOS was published by Orio and associates in 2005 (27). This study evaluated 150 women with PCOS (defined according to NIH criteria) and 150 controls matched for age and BMI. Median WBC in the PCOS and control groups was, respectively, 7,260 and 5,220 cells/mm^3 (P<0.0001). Significant elevation of lymphocytes and monocytes were found. WBC in women with PCOS correlated with insulin resistance determined by the homeostasis model (HOMA). Elevation of WBC in women with PCOS was subsequently confirmed in several other studies (28, 29, 30).

Oxidative stress

Oxidative stress and chronic inflammation are closely inter-related; indeed, extensive evidence supports the concept of a vicious cycle, whereby inflammation induces generation of reactive oxygen species (ROS), while oxidative stress promotes and aggravates inflammation (31). Such a vicious cycle has been particularly well documented in endothelium and in adipose tissues. Increased oxidative stress is associated with atherosclerosis, diabetes, obesity and metabolic syndrome. The first report documenting increased oxidative stress in women with PCOS was published a decade ago (32). Twenty-seven women with PCO were matched for age and BMI with 18 healthy controls. Oxidative stress was determined by evaluation of lipid peroxidation using an erythrocyte malondialdehyde assay. Lipid peroxidation was significantly increased in PCOS and this increase correlated positively with BMI, insulin level and blood pressure. These findings were confirmed by subsequent studies evaluating various markers of oxidative stress including lipid peroxidation and protein carbonyl content (33,34). Complementary to the increase of oxidative stress, women with PCOS also have a reduced total antioxidant status (34), reduced glutathione (35), as well as decreased level of haptoglobin, a protein with anti-oxidant properties (36). Women with PCOS have also increased susceptibility of DNA to damage induced by oxidative stress; this susceptibility correlates with free testosterone level (35). An interesting concept linking oxidative stress to insulin resistance has been proposed by Gonzalez and associates who have demonstrated that ROS generation by mononuclear cells in response to hyperglycemia is greater among women with PCOS than in control subjects (37).

Advanced glycation end-products

Advanced glycation end-products (AGEs) are generated by non-enzymatic reactions between reducing sugars and amino groups of proteins forming reversible Schiff bases, Amadori products and, ultimately, reactive cross-linked derivative molecules. AGEs act on signal-transducing receptors (RAGE) leading to induction of oxidative stress. AGEs, by acting both directly and through RAGE promote development and progression of

cardiovascular disease (38). While elevation of AGEs is usually detected in the presence of diabetes, recent studies have demonstrated an elevation of AGEs in women with PCOS (39,40). In a carefully designed study, several sub-groups of women with PCOS were compared to control subjects, and to women with isolated hyperandrogenism, polycystic ovaries, and non-hyperandrogenic anovulation (39). The greatest elevations of AGEs were found among women with NIH-defined PCOS irrespective of normal or polycystic ovarian morphology. Intermediate levels of AGEs were detected in hyperandrogenic ovulatory women with normal ovaries and anovulatory normoandrogenic women. In contrast, levels of AGEs were normal in women with isolated hyperandrogenism (ovulatory with normal ovarian morphology) as well as isolated polycystic ovarian morphology (normoandrogenic and ovulatory) and isolated anovulation (normoandrogenic and with normal ovarian morphology). More recently, the same group of investigators has shown that AGEs correlate with the level of anti-mullerian hormone (AMH), presence of PCOS and anovulation (40).

Endothelial inflammation

Chronic low-grade inflammation is closely linked to endothelial inflammation and consequent endothelial dysfunction. Most importantly, endothelial dysfunction plays a key role in cardiovascular disease and in particular in the development of atherosclerotic plaques and hypertension (1,41). PCOS is associated with abnormal endothelial function and with elevation of various markers of endothelial inflammation. In an early study on this subject, 12 obese women with PCOS were matched for age and weight with 13 healthy control subjects and endothelial function was evaluated by determination of leg blood flow following infusion of the endothelium-dependent vasodilator methacholine chloride and insulin (42). PCOS was characterized by endothelial dysfunction and resistance to insulin-induced vasodilation. These findings are concordant with subsequent studies evaluating endothelial function (43,44,45,46). Furthermore, PCOS is associated with elevation of various markers of endothelial dysfunction and inflammation including: endothelin-1, soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1) asymmetric dimethylarginine and plasminogen activator inhibitor-1 (43,45,47,48,49). Abnormal endothelial function in PCOS correlates with insulin resistance.

Infections

Probably the most controversial and least studied aspect of the relationship between PCOS and inflammation is the concept that PCOS may be associated with low-grade chronic infection or infections. The concept is appealing since various pathogens such as *Chlamydia pneumoniae* and *Helicobacter pylori* are associated with chronic inflammation and cardiovascular disease (50). In particular, *Chlamydia pneumonia* seropositivity correlates with the presence of atherosclerosis and even acute myocardial infarction (51). In a similar fashion, seropositivity for *Helicobacter pylori* is associated with arterial stiffness and unstable angina (52,53). Other sources of chronic infectious processes linked to inflammation and cardiovascular risks include pathogens involved in periodontal disease. Indeed, a recent large, prospective study evaluating over 10,000 participants verified the relationship between periodontal disease and cardiovascular mortality (54). Literature evaluating the role of pathogens in PCOS is still minimal but suggests a possible association of this syndrome with *Chlamydia* species, *Helicobacter pylori* and periodontal disease (55,56,57). Specifically, seropositivity to *Chlamydia pneumonia* and *Chlamydia trachomatis* was significantly greater among women with self-reported oligomenorrhea and hirsutism than in control subjects (55). The simultaneous presence of elevated hs-CRP levels strengthened this association. In another study, seropositivity to *Helicobacter pylori* was significantly more common among women with PCOS than in an age-matched control group (56). Most recently, a study evaluating markers of periodontal disease compared 25 non-obese women with PCOS with 27 age and weight-matched control women (57).

Women with PCOS had multiple elevated clinical periodontal parameters consistent with gingivitis. On a cautionary note, the above studies are preliminary and demonstrate only an association of PCOS with some infections; the cause and effect relationship of these conditions has yet to be determined. Furthermore, larger studies on diverse populations of subjects are needed.

Views not supporting chronic inflammation-- Anuja Dokras, MD., PhD

Circulating Cytokines

In addition to the above mentioned biomarkers, there are other proinflammatory cytokines, chemokines and adipokines that are not elevated in the peripheral circulation in women with PCOS compared to matched controls. A recent meta-analysis by Escobar-Morreale et al (12) showed no difference in the serum levels of IL6 between women with PCOS (defined per NICHD and Rotterdam criteria) and controls (15% relative difference, confidence interval CI −15-45% p=0.33). This was a rigorous meta-analysis which included cross-sectional studies with a minimum of 25 subjects in each group and all studies used high sensitivity assays. In the 10 studies included in the meta-analysis examining IL6 levels the total number of subjects in the PCOS group was 523 compared to 330 controls. The results were similar after excluding one study that had a mismatch in BMI. TNFα is another cytokine primarily secreted by the visceral adipocytes. In the same publication as above Escobar-Morreale et al (12) performed a meta-analysis of 9 studies and found TNFα levels were not significantly different in women with PCOS compared to controls (5% relative difference CI −12-23%, p=0.56). A few of these studies have shown an association between markers of hyperinsulinemia and insulin resistance (HOMA) and the above mentioned circulatory markers of chronic inflammation (58,59,60). The lack of significant association with androgens in most studies suggests that PCOS is not independently associated with these markers of inflammation.

Chemokines and adiponectins

A number of chemokines can be also detected in the circulation; however, few studies have examined the levels of these biomarkers in women with PCOS. In a large population-based study, elevated IL-18 levels associated with risk factors for atherosclerosis and with metabolic syndrome. However the association between IL-18 and atherosclerosis diminished after accounting for traditional cardiovascular risk factors indicating that IL-18 is not independently predictive of atherosclerosis in asymptomatic individuals (14). IL18 levels have been shown to be higher in women with PCOS compared to controls (18, 19) and in both these studies IL18 levels correlated with indices of insulin sensitivity. In another recent study where obese PCOS and obese control groups were compared, there was no difference in serum IL18 levels indicating that when obesity was controlled, the PCOS effect completely disappeared (20). Future studies should stratify subjects by BMI classes to better identify the independent association between IL18 and PCOS. Adiponectin levels have also been measured in women with PCOS. A recent meta-analysis (61) (number of studies =16) showed that adiponectin levels are significantly lower in women with PCOS compared to controls (weighted mean difference −1.71 CI −2.82 − 0.6, p<0.01). However, there was significant heterogeneity between studies and both insulin resistance and study size attributed to some of these findings. Lower adiponectin levels were not associated with total testosterone levels.

CRP - Limitations of studies in PCOS

CRP is a circulatory marker of chronic inflammation that has been used for risk stratification of patients with known coronary artery disease (CAD) and for primary prevention of cardiovascular disease (CVD). CRP has also been incorporated in screening strategies for

CAD such as the Reynolds risk score. Both IL6 and TNFα which are not significantly elevated in women with PCOS (as described above) stimulate the hepatic production of CRP. As mentioned above in a recent meta-analysis, mean CRP levels were two-fold higher in PCOS subjects even after including only BMI matched studies (12). All studies included in the meta-analysis compared mean hsCRP levels between the 2 groups. Further examination of these individual studies shows that the mean hsCRP levels were in the "high risk" category (>3mg/L) in only a few studies (62) indicating that at the time of testing these differences may not be clinically relevant. There is currently controversy regarding the utility of hsCRP in a "screen everyone" versus "selective screening" in intermediate risk patients approach (63). Most reproductive age women with PCOS are "low risk" for CVD using the Framingham risk score assessment making it more meaningful to stratify the data based on the hsCRP values (64). Although hsCRP has been shown to be an independent predictor of CVD, given the overall low levels detected in the young PCOS population, inclusion of multiple risk factors such as dyslipidemia, hypertension, and diabetes in conjunction with hsCRP levels in an individual subject maybe more useful. An overall risk profile in this young population would provide a better assessment of long term risk.

Atherosclerosis and inflammation in PCOS

Another consideration is that the presence of individual biomarkers alone in reproductive age women with PCOS may not be predictive of CVD risk. Long term cardiovascular outcomes data is limited in women with PCOS and measures of endothelial function, carotid intima media thickness and coronary artery calcium (CAC) scores (65) have been used to assess subclinical atherosclerosis in this population. The section on Endothelial Inflammation has reviewed the evidence for endothelial dysfunction in women with PCOS and its association with adhesion molecules. Presence of calcium deposits within the coronary arteries provides direct evidence of atherosclerosis and may provide better assessment for the link between inflammation and CAD. A few studies have examined the prevalence of coronary artery calcifications in women with PCOS (NICHD definition) and compared that with controls. In a case controlled BMI matched study of 48 subjects the prevalence of CAC was significantly higher in women with PCOS (33%) compared to controls (8%, p<0.03) (66). Interestingly there was no difference in serum IL6, TNFα, hsCRP and adiponectin levels between the 2 groups. In another study with a larger number of subjects prevalence of CAC (defined as Agatston unit >10) was similar between women with PCOS (Rotterdam definition) and controls (67). Also, in this population there was no significant difference between serum hsCRP and monocyte chemoattractant protein 1 (MCP-1) levels in women with PCOS and controls. Currently the data is limited to support a direct link between chronic inflammation and atherosclerosis in women with PCOS.

Limitations of published data on low grade inflammation in PCOS

There are several limitations to the individual studies discussed above and those included in the meta-analysis. Most studies had small numbers and included women with the Rotterdam definition of PCOS which introduces marked heterogeneity in the studied population due to the inclusion of different phenotypes. The impact of obesity (especially visceral adiposity) on the serum levels of biomarkers has not been adequately addressed in all studies. Although BMI was controlled statistically in some studies, it has been shown that stratification of the subjects based on BMI classes is useful to assess the independent effects of PCOS (20,68). Most studies are cross sectional and thereby prohibit determination of causality between adiposity/metabolic risk and chronic inflammation in PCOS. For some of the biomarkers discussed above (AGES, markers of oxidative stress, adhesion molecules) there are only a few small studies describing the findings and verification in larger studies will be needed to confirm these findings.

Conclusions

The above studies collectively suggest that women with PCOS have altered circulatory levels of some markers of inflammation, which may reflect a state of chronic low grade inflammation. The data however are limited with respect to the association of chronic inflammation and PCOS independent of adiposity and the long term implications of these findings remain to be confirmed. Future studies in this field should define the Rotterdam phenotype of subjects, stratify subjects based on BMI, use clinically relevant cut offs rather than comparison of means and ultimately provide longitudinal data on changes in biomarkers and their correlation with atherosclerosis.

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