Predictors of urinary albumin excretion in women with polycystic ovary syndrome

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Abstract

Objective—To evaluate UAE in normotensive and non-diabetic women with PCOS in relation to their clinical, endocrine and metabolic profiles. Polycystic ovary syndrome (PCOS) is associated with increased cardiovascular risks including evidence of altered endothelial function. Urinary albumin excretion (UAE) is closely related to endothelial function and closely correlates with nephropathy and adverse cardiovascular endpoints.

Design—Observational study.

Setting—University fertility center.

Patient(s)—Sixty-three women with PCOS were evaluated.

Intervention(s)—Clinical assessments and urine and blood testing.

Main Outcome Measure(s)—UAE, systolic and diastolic blood pressure, serum levels of LH, FSH, prolactin, testosterone, 17-hydroxyprogesterone, glucose, insulin, lipids, C-reactive protein and 24-hour urinary free cortisol.

Result(s)—In univariate and multivariate correlation analysis, UAE correlated with diastolic blood pressure, insulin area under the curve during glucose tolerance test, prolactin and 17-hydroxyprogesterone. Overt microalbuminuria was detected in a significant proportion of subjects.

Conclusion(s)—Urinary albumin excretion in women with PCOS correlates well with other cardiovascular risk factors. Since the relationship between UAE and adverse cardiovascular events is continuous, evaluation of UAE in the presence of PCOS may provide clinically relevant information and may aid in selecting appropriate patients for more aggressive treatment of likely aggravating factors, such as hyperinsulinemia or borderline hypertension.

Keywords

Polycystic ovary syndrome; urinary albumin; endothelial function
Introduction

Polycystic ovary syndrome (PCOS) is a complex and heterogeneous disorder affecting 5–10% of women in reproductive age (1,2). While definitions of PCOS focus on reproductive-endocrine dysfunction, a majority of women with this condition also display a broad range of metabolic derangements associated with increased risk of developing type 2 diabetes, atherosclerosis, hypertension and, ultimately, cardiovascular events (3–6).

Cardiovascular risk factors common in PCOS include dyslipidemia, insulin resistance, systemic inflammation and endothelial dysfunction (7–10). Growing evidence points at endothelial dysfunction as an important early finding predicting development of atherosclerosis.

Urinary excretion of albumin (UEA) reflects renal function and is thought to be directly related to endothelial function, or more specifically, endothelial “leakiness”. The concept of UEA as a marker of endothelial dysfunction was initially developed based on studies of patients with diabetes; in these patients, even modest increases in UAE have been shown to predict adverse cardiovascular outcomes (11). However, UEA also predicts cardiovascular events in non-diabetic subjects independently from traditional risk factors (12). Excessive UEA is considered to be a risk factor for all-cause mortality and cardiovascular morbidity (13,14). Even very low levels of microalbuminuria have been associated with coronary artery disease and death; this association was independent of renal function, hypertension or diabetes (15).

In view of the above considerations, this study was conducted to evaluate UEA in women with PCOS to determine whether it was increased above the reported normal range and to determine whether UEA correlates with clinical, endocrine and metabolic parameters relevant to PCOS.

Methods

Patients

Sixty-three women with PCOS (mean age, 24.7±7.9 years) were enrolled in the study; all subjects were evaluated at Fertility and Reproductive Endocrinology Center at Yale University School of Medicine. Fifty subjects were Caucasian (79%), nine African American (14%), two Asian (3%), one Hispanic and one Native Indian. The study protocol was approved by the Ethics Committee for Human Studies of Yale University in 2006. PCOS was defined as the presence of two of the following three features after the exclusion of other etiologies (16): (i) oligo- or anovulation (fewer than six menstrual periods in the preceding year); (ii) hyperandrogenism and/or biochemical signs of hyperandrogenism and/or (iii) polycystic ovaries. Exclusion criteria were: pregnancy, diabetes mellitus or blood pressure above 140 mmHg systolic or 90 mmHg diastolic; treated hypertension; hyperprolactinemia, adrenal dysfunction and thyroid dysfunction. Oligomenorrhea or amenorrhea was present in 56 patients (88.9%). All subjects had either clinical or biochemical evidence of hyperandrogenism/hyperandrogenemia; hirsutism was observed in 56 patients (88.9%) and acne was documented in 24 patients (38.1%). Polycystic ovarian morphology was detected (by transvaginal ultrasound) in 47 subjects (74.6%). Relevant clinical, hormonal and biochemical parameters evaluated in this study are summarized in Table 1.

Assays

Blood collections were carried out, following an overnight fast, in the morning, between cycle day three and nine of a spontaneous or medroxyprogesterone-induced menstruation. The serum concentrations of FSH, LH, prolactin (PRL), total testosterone and sex hormone-binding globulin were measured by chemiluminescent enzyme immunoassay (Chiron Diagnostics, Terry Town, New York, USA). Serum 17-hydroxyprogesterone was measured by RIA.

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Dehydroepiandrosterone sulfate (DHEAS) was measured by RIA (Diagnostic Products Corporation, Los Angeles, CA). All patients underwent a 2-hour glucose tolerance test (2h GTT) using a 75-g glucose load with determinations of both glucose and insulin at baseline and then at 30, 60, 90, and 120 minutes after glucose load. Serum glucose was measured by hexokinase calorimetric method using a Bayer Dax-48 system analyzer (Bayer Diagnostics Corporation, West Haven, CT, USA). Plasma insulin levels were measured by chemiluminescent enzyme (a solid-phase, two-site sequential chemiluminescent immunometric assay) using commercial kits from Bayer Diagnostic Corporation (West Haven, CT, USA). Lipid analysis in fasting serum was performed for all patients. The lipid profile included measurement of the levels of total cholesterol; triglyceride, high- and low-density lipoprotein (HDL and LDL). Cholesterol and triglyceride were measured by enzymatic colorimetric method in the Quest laboratory (Wallingford, CT, USA) using Olympus AU 600 auto analyzer and reagents from Olympus Diagnostics (New York, USA). High-density lipoprotein was separated by precipitating apolipoprotein-B (Boehringer Mannheim, Germany). Low-density lipoprotein was calculated using the Friedwald formula (17). Plasma hsCRP concentrations were measured by immunoturbidimetry with an image analyzer (Hitachi 917; Quest Laboratories, Wallingford, CT, USA) with intra-assay CV of 8.7% and sensitivity of 0.03 mg/L. Twenty-four hour urinary free cortisol was measured by radioimmunoassay (DPT Coat-A-Count Diagnostic Product Corporation, Los Angeles, CA, USA) in the Quest laboratory (Wallingford, CT, USA). Urinary albumin was measured by spectrophotometry (Perk&Elmer Corporation, Oak Brook, IL, USA); intra-assay and inter-assay CV was 4%. Creatinine was assayed using an Ectachem 400 Analyzer (Eastman Kodak Co, Rochester, New York, USA).

Statistical analysis—Statistical analysis was performed using JMP program version 7 (SAS Institute, Cary, NC, USA). Associations between variables were analyzed by Pearson correlation and multiple linear regression modeling. Normality of distribution was assessed by Shapiro-Wilk W test. In the absence of a normal distribution, variables were logarithmically transformed. Results are expressed as means ± S.D.

Results

Characteristics of the studied population of women with PCOS are summarized in Table 1. Women with PCOS excreted albumin in urine over a wide range of concentrations from 2 to 97 μg/mL with a median of 10 μg/mL. The ratio of urinary excretion of albumin per mg of creatinine ranged from 2 to 145.9 μg/mg with a median of 5.7 μg/mg. Microalbuminuria, defined in women as excretion of >25 μg of albumin per mg of creatinine (18), was observed in 16% of subjects. When applying another commonly used criterion of microalbuminuria (>20 μg of albumin per liter of urine) (19); 24% of subjects would be classified as displaying microalbuminuria.

Since the distribution of excretion of albumin was not normal, subsequent correlations were carried out on logarithmically transformed data. Correlation of urinary albumin to creatinine ratio was determined for each of the variables listed in Table 1. These variables were selected for analysis in view of their likely or postulated relationship to urinary albumin excretion and/or their prominent role in the pathophysiology of PCOS. Variables with Pearson correlation at P<0.1 are listed in Table 2.

Multiple linear regression modeling was then carried out to characterize the relationship between several predictors and the primary studied measure of UAE: the ratio of urinary albumin excretion to creatinine. In addition to variables identified as predictors in univariate analysis (Table 2), all other variables (listed in Table 1) were also tested but were excluded...
from the final model when their respective P-values in the model exceeded 0.1. As shown in the final model (Table 3 and Fig. 1), four independent predictors were: diastolic blood pressure, 17-hydroxyprogesterone, prolactin and insulin area under the curve (during 2 h GTT). The $R^2$ of the final model was 0.43 indicating that it explained forty-three percent of the variation in the response variable.

Discussion

This report presents new findings describing UAE in normotensive and non-diabetic women with PCOS. First, UAE appears to be independently related to several clinical and endocrine parameters. Second, a high rate of overt microalbuminuria has been detected.

Among the four independent predictors of UAE identified in this study, diastolic blood pressure is the best, established parameter, which can mechanistically explain albumin excretion as a function of increased intraglomerular pressure. As in the present study, a close correlation of blood pressure and UAE has been described previously in various populations of both hypertensive and normotensive subjects (20,21). The likely contribution of blood pressure to UAE in women with PCOS is highly relevant, since these patients are at increased risk of developing hypertension (22). However, importantly, in this study, a correlation of blood pressure with UAE was detected among normotensive subjects.

Another predictor of UAE identified in this report is the insulin area under the curve during a 2h GTT. A large proportion of women with PCOS are insulin resistant and have compensatory hyperinsulinemia (8). As in the present study, a correlation of insulin resistance and hyperinsulinemia with UAE has been described in various other populations (23,24). Effects of insulin on UAE may be related to both acute and long-term actions. An acute injection of insulin has been shown to immediately increase UAE (25). In the long-term, hyperinsulinemia may act by inducing mesangial hyperplasia (26). Postulated mechanisms of actions of insulin on UAE include increased glomerular filtration (27) and increased vascular permeability (28). However, the role of insulin is still not fully understood and in some studies an association between UAE and insulin was not observed (29,30).

A third factor found to be associated with UAE in women with PCOS is prolactin. This observation correlates well with a report that hyperprolactinemic pre-menopausal women had impaired endothelial function, as detected by flow-mediated dilatation of the brachial artery and decreased insulin sensitivity (31). Furthermore, treatment of these patients with bromocriptine resulted in improvement of endothelial function and insulin. Notably, in the present study of women with PCOS, UAE correlated with prolactin in the absence of hyperprolactinemia and, as determined by multiple regression analysis (Table 3), independently of insulin level. While the mechanistic link between prolactin and UAE is not readily apparent, it is interesting to note that endothelial cells express prolactin mRNA and there is evidence for autocrine actions of prolactin on endothelium (32,33). There is also evidence that prolactin may modulate angiogenesis and affect endothelial cell proliferation, apoptosis and migration (34).

The final and unexpected predictor of UAE in this study is serum level of 17-hydroxyprogesterone. The association with UAE was observed at levels of 17-hydroxyprogesterone well below those found in subjects with 21-hydroxylase deficiency. At this time no plausible explanation of the present observation is offered and it is possible that levels of 17-hydroxyprogesterone may co-vary with other not yet elucidated factors which may be linked to endothelial function and albumin excretion. PCOS is associated with excessive 17-hydroxyprogesterone responses to GnRH agonist and a recent study has found that women with an exaggerated 17-hydroxyprogesterone response to a GnRH analog also had
greater insulin resistance and β-cell insulin secretion (35). Hence, one may propose that the level of 17-hydroxyprogesterone may be a surrogate marker for metabolic derangements such as excessive insulin secretion, which in turn may affect UAE. However, such an explanation is highly speculative and in the present study, the multivariate model of UAE excretion indicates that 17-hydroxyprogesterone was an independent predictor of UAE when accounting for insulin levels.

Surprisingly, UAE did not correlate with some parameters, which in view of their association with cardiovascular risks would be expected to predict UAE. Thus, in this study neither BMI, nor hsCRP had any significant correlation with urinary albumin concentration or with urinary albumin to creatinine ratio. Previous studies evaluating the relationship of microalbuminuria and BMI led to conflicting results. In some reports obesity was associated with microalbuminuria (36); however, surprisingly, in other studies an unexpected decrease of microalbuminuria among obese individuals was observed (37). Similarly, the correlation of microalbuminuria and hsCRP has been previously evaluated and unexpectedly has been found to be quite weak (38).

While this study was not designed to compare the level of UAE among women with PCOS to general population, a high frequency of overt microalbuminuria was observed. In a large study evaluating a large sample from the general population, microalbuminuria (defined as UAE from 20 to 200 mg/L) was found in 7.2% of the subjects; among diabetics it was 16.4%, among hypertensives in 11.5% and among non-diabetics and non-hypertensives in 6.6% (19) of the subjects. When the same criteria of microalbuminuria are applied to the present study, then 24% of women with PCOS had overt microalbuminuria. Thus, it may appear that excessive UAE may be even more common in PCOS than in subjects with overt diabetes and/or hypertension. However, comparisons of findings from different reports need to be interpreted with caution and verification of the present observations should be obtained from further studies on larger populations of women with PCOS.

The present understanding of the mechanisms underlying UAE is still incomplete. While traditionally UAE has been thought to be primarily related to blood pressure and glomerular endothelial integrity, several recent studies indicate that this process may be also affected by other factors such as tubular re-absorption of filtered albumin (39,40).

Overall, the present findings indicate that normotensive and nondiabetic women with PCOS have significant UAE correlating well with other established cardiovascular risk factors. Notably, even high “normal” levels of UAE (<25 μg of albumin per mg of creatinine) in subjects without diabetes or hypertension predicts increased risk of subsequent development of hypertension (41). Since the boundary between normal albumin excretion and microalbuminuria is artificial, and the relationship between UAE and cardiovascular risks is continuous (42), evaluation of UAE in subjects with PCOS may provide new and clinically relevant information. Furthermore, since interventions lowering UAE have been shown to significantly decrease cardiovascular risks (43,44), consideration may be given to more aggressive treatment of likely aggravating factors, such as hyperinsulinemia or borderline hypertension.

Acknowledgments

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References


40. Clavant SP, Greive KA, Nikolovski J, Reeve S, Smith AI, Comper WD. Albumin fragments in normal rat urine are derived from rapidly degraded filtered albumin. Nephrology (Carlton) 2003;8:72–9. [PubMed: 15012737]


Figure 1.
Multiple regression model as presented in Table 3: actual by predicted plot of the logarithm of urinary albumin to creatinine ratio; RSq represents the square of the multiple correlation coefficient; RMSE represents the root mean square error.
Table 1
Clinical, endocrine and biochemical parameters evaluated in women with PCOS (n=63).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>Median (10th to 90th percentile)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>24.7±7.9</td>
<td>24.0 (16–35)</td>
</tr>
<tr>
<td>BMI</td>
<td>32.9±7.4</td>
<td>32.7 (22.5–42.9)</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>115.3±12.4</td>
<td>117 (98–130)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>68.7±9.8</td>
<td>70 (58–80)</td>
</tr>
<tr>
<td>Total testosterone (ng/dL)</td>
<td>61.9 ± 22.5</td>
<td>61.5 (34.0–88.4)</td>
</tr>
<tr>
<td>DHEAS (g/dL)</td>
<td>244.9 ± 127.1</td>
<td>236.0 (99–401)</td>
</tr>
<tr>
<td>17-OH Prog (ng/dl)</td>
<td>56.4 ± 36.4</td>
<td>47.0 (26.2–104)</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>40.8 ± 38.6</td>
<td>25.0 (10.4–89.6)</td>
</tr>
<tr>
<td>Urinary 24-hour free cortisol (μg)</td>
<td>31.2 ± 16.5</td>
<td>29.8 (12.2–56.5)</td>
</tr>
<tr>
<td>LH (mIU/mL)</td>
<td>5.7 ± 3.4</td>
<td>5.3 (2.3–11.1)</td>
</tr>
<tr>
<td>FSH (mIU/mL)</td>
<td>4.4 ± 1.7</td>
<td>4.3 (2.2–6.6)</td>
</tr>
<tr>
<td>PRL (ng/mL)</td>
<td>12.7 ± 5.3</td>
<td>11.2 (7.2–105)</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>91.3 ± 10.0</td>
<td>89 (82–105)</td>
</tr>
<tr>
<td>Fasting insulin (μIU/mL)</td>
<td>15.2 ± 9.5</td>
<td>13.0 (4.2–28.6)</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>113 ± 30.8</td>
<td>112 (70–155)</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>52.7 ± 15.2</td>
<td>49.0 (38.6–76.8)</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>103 ± 57.8</td>
<td>87 (50.3–181)</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>5.6 ± 7.7</td>
<td>3.2 (0.4–13.3)</td>
</tr>
<tr>
<td>Urinary albumin (μg/mL)</td>
<td>17.2 ± 20.1</td>
<td>10.0 (3.7–51.9)</td>
</tr>
<tr>
<td>Urinary albumin to creatine ratio (μg/mg)</td>
<td>13± (20.1)</td>
<td>5.7 (3.2–32.5)</td>
</tr>
</tbody>
</table>
Table 2

Univariate analysis evaluating correlation of logarithm of urinary albumin (μg/mg urinary creatinine) with other variables. * Only variables correlating at P<0.1 are presented.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>0.05</td>
<td>0.07</td>
</tr>
<tr>
<td>17-Hydroxyprogesterone (ng/dL)</td>
<td>0.07</td>
<td>0.03</td>
</tr>
<tr>
<td>Prolactin (ng/mL)</td>
<td>0.09</td>
<td>0.03</td>
</tr>
<tr>
<td>LH (mU/mL)</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td>Insulin AUC (μU/mL/min)</td>
<td>0.11</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* Outlier with urinary albumin of 146 μg per mg of creatinine was excluded from this analysis
Table 3

Multiple regression model for logarithm of urinary albumin (μg/mg urinary creatinine). The $R^2=0.43$

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate ± standard error</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>0.0112 ± 0.0047</td>
<td>0.02</td>
</tr>
<tr>
<td>17-Hydroxyprogesterone (ng/dL)</td>
<td>0.0035 ± 0.0011</td>
<td>0.003</td>
</tr>
<tr>
<td>Prolactin (ng/mL)</td>
<td>0.0197 ± 0.0073</td>
<td>0.01</td>
</tr>
<tr>
<td>Insulin AUC (μU/mL/min)</td>
<td>1.32e-5 ± 6.69e-6</td>
<td>0.056</td>
</tr>
</tbody>
</table>

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