Should Subclinical Hypothyroidism Be an Exclusion Criterion for the Diagnosis of Polycystic Ovary Syndrome?

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Abstract

Background: The purpose of the study was to examine whether patients with subclinical hypothyroidism (SCH) should be excluded before making a diagnosis of polycystic ovary syndrome (PCOS).

Methods: Seven hundred sixteen patients, 462 with true PCOS, 31 with PCOS-SCH, and 223 normal cycling women were enrolled. Clinical, metabolic, and hormonal parameters among the groups were investigated. Continuous variables were compared by one-way analysis of variance. Proportions were compared using Z test. Fisher test was used to compare categorical variables. Simple correlation was performed using Spearman’s coefficient. Correlation between thyroid stimulating hormone (TSH) and dependent variables were performed using backward multiple regression. The significance level was set at 0.05.

Results: True polycystic ovary and polycystic ovary with subclinical hypothyroidism patients presented similar anthropometrical parameters. C-peptide was higher in polycystic ovary patients than in the other groups (p=0.014). Prevalence of glucose intolerance (p=0.186) and insulin resistance (p=0.293) was not statistically different in polycystic ovary and polycystic ovary with subclinical hypothyroidism. TSH levels showed positive correlation with lean body mass (p=0.032), total cholesterol (p=0.046, insulin (p=0.048) and prolactin (p=0.047). Backward multiple regression model retained TC, insulin, and PRL as predictors of TSH levels (p=0.011).

Conclusion: Anthropometric parameters and ovary morphology were similar in both PCOS and PCOS-with-SCH patients. Regarding hormones, only C-peptide was higher in PCOS group. TSH correlated with total cholesterol, insulin, and prolactin. Before PCOS diagnosis, the exclusion criterion thyroid dysfunction should be standardized and subclinical hypothyroidism should not exclude a diagnosis of PCOS.

Keywords: Hyperandrogenism, Hypothyroidism, Polycystic ovary syndrome, Thyroid hormones.

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Introduction

In women of reproductive age, depending on the diagnostic criteria used, the prevalence of polycystic ovary syndrome (PCOS) ranges from 5 to 21% (1, 2). The condition is characterized by oligo/anovulation, hyperandrogenism, and polycystic ovary morphology (3). Dyslipidemia, dysglycemia, and insulin resistance are frequent metabolic abnormalities, increasing risks of type II diabetes mellitus (T2DM) and cardiovascular disease (CVD) (4). Primary hypothyroidism patients share many signs and symptoms with PCOS, such as menstrual disorders, infertility, hyperandrogenism, and weight gain (5, 6). Among the endocrine features, patients with primary overt hy-
Subclinical hypothyroidism (SCH) may have mild increase in total testosterone (T) and free testosterone (fT) total and free estradiol, prolactin (PRL) and luteinizing hormone (LH), and decreased sex hormone binding globulin (SHBG) levels (6, 7). Dyslipidemia and insulin resistance are frequent metabolic abnormalities with increased risk of type II diabetes melittus (T2DM) and cardiovascular diseases (CVD) (8). Moreover, ovaries with bilateral multicystic appearance are frequently found in these patients (9).

So, called subclinical hypothyroidism (SCH), found between 3–8% of women of reproductive age (10), has few signs or symptoms of thyroid dysfunction and often remains untreated; whether SCH leads to clinical, endocrine, or metabolic alterations that could be misdiagnosed as the early stages of PCOS remains to be demonstrated. Nearly 14% of SCH patients may present dyslipidemia, dysglycemia, insulin resistance, infertility, ovari- tory dysfunction, obesity, and abnormal menstrual cycle, mimicking PCOS (5, 7). High levels of total (T) and free testosterone (fT), LH, PRL, fasting and postprandial insulin, glycated hemoglobin (HbA1C), homeostasis model assessment of insulin resistance index (HOMA-IR), serum lipoprotein (a), triglyceride, total cholesterol (TC), unfavorable low-density lipoprotein (LDL) and high-density lipoprotein (HDL) and low levels of SHBG (8, 11), are independent risk factors for metabolic syndrome or myocardial infarction frequently described in SCH (11-13).

Polycystic ovary syndrome patients with SCH patients (PCOS-with-SCH) may be just like those with true PCOS and the SCH is incidental. It has been proposed that exclusion of hypo- or hyperthyroidism is not mandatory to make a diagnosis of PCOS in the absence of other symptoms or signs of thyroid dysfunction (14). However, according to the current recommendation, definitive PCOS diagnosis should be made after exclusion of thyroid dysfunction (3, 14). This has not been universally accepted however (7, 14, 15) and, in many publications, TSH levels are sometimes not measured or are dismissed in brief statements excluding thyroid function (16). Criteria frequently used to exclude thyroid disorders nowadays include overt hypothyroidism (14), clinical suspicion of hypothyroidism (17), and different levels of TSH (18-21). Given the lack of a clear guideline on this matter and the required standardiza- tion among researchers, the present study aimed to examine whether patients with subclinical hy-
weighed on an electronic scale, and their height was measured using a Harpenden stadiometer (Holtain Limited, Crymych, Dyfed, UK). The waist was measured at the midpoint between the lower rib margin and the iliac crest, and the hip was measured from the widest circumference over the great trochanters. The body mass index (BMI) was calculated as body weight (kg)/height (meter) squared. Obesity was defined as a body mass index (BMI) ≥30 kg/m². Lean body mass (LBMI) was calculated according to James’s formula: (1.07 × weight kg) – 148 (weight²/100 × height m²) (23). Fat mass (FM) was calculated as body weight minus LBMI. Abdominal adiposity was estimated by the conicity index (CI), using the following equation: [(WC (m))/√BW (kg) • height(m)) (24).

Blood samples were obtained between 7:00 and 9:00 am by venupuncture after a 10-12 hr fast. All patients with regular cycles were tested in the early follicular phase of the menstrual cycle (days 3-5 of the cycle). Patients with oligomenorrhea or amenorrhea had their blood collected at any time provided the progesterone was less than 2 ng/ml (6.4 nmol/L). Triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and total cholesterol (TC) levels were measured using an enzymatic assay (Wiener Laboratories, Rosário, Argentina). Low-density lipoprotein cholesterol (LDL-C) was calculated as TC-(HDL-C+TG/5) (25). Glucose concentration was analyzed using the glucose oxidase technique (Beckman Glucose Analyses, Fullerton, CA, USA). Subjects were diagnosed with impaired fasting glucose (IFG) when fasting plasma glucose (FPG) level was between 100 mg/dL (5.5 mmol/L) and 126 mg/dL (6.99 mmol/L) (26), and in 2 hr oral glucose tolerance test (GT), glucose level was between 140 mg/dL (7.8 mmol/L) and 199 mg/dL (11.0 mmol/l) (26). Implied insulin resistance (IR) was defined using fasting insulin levels >12.2 µU/ml (84.7 pmol/L) (27) and/or HOMA-IR ≥2.8 (28). HOMA%β was estimated using the following equation: HOMA% B=[(20×fasting insulin µU/ml)/fasting glucose (nmol/L) – 3.5] (28). For the GT, blood samples were collected at 0, 30, 60, 90, 120, and 180 min after ingestion of 75 g of dextrose for the measurement of plasma glucose and insulin levels (29).

Hormone levels were measured using previously validated methods and their imprecisions in the local laboratory were extensively presented in previous publications (29). High-performance liquid chromatography (HPLC)/radioimmunoassay (RIA) was performed for compound S (11-desoxy cortisol) measurement following extraction using a method developed in-house by the Alvaro Center of Analysis and Clinical Research in Paraná, Brazil. 17-hydroxyprogrenolone level was measured with an HPLC/RIA using titrated steroids (NEN Life Science Products, Boston, MA, USA) and antiserum from ICN Biochemical Inc (Costa Mesa, CA, USA). The free androgen index (FAI) was estimated as the total T (nmol/L)/sex hormone-binding globulin (SHBG), nmol/L×100. Taking into account the limited agreement with respect to the Ferriman–Gallwey score, a specific value to define hirsutism was not used and clinical hyperandrogenism was defined as a binomial variable by the single presence or absence of hirsutism (30). TSH and free thyroxine were measured using electrochemiluminescence assays (Elecsys 1010, Roche Diagnostics GMBH, Mannheim, Germany). Ovary transvaginal ultrasonography was performed using a Voluson machine (Voluson® E8, GE Health Care, England) and PCO morphology was defined according to previous recommendations (31).

Statistical analyses: Data were missing in some cases because of unavailable vital signs, inadequate specimens, or subjects not providing specimens. Anthropometrical, biochemical, and endocrinological continuous data are presented as mean and standard deviation (SD) and compared using one-way analysis of variance and the Tukey post hoc test. The Z test was used to compare two categorical variables. Fisher exact test was used to compare more than two categorical variables. Correlations between TSH and other variables were performed using Spearman’s rho correlation coefficient. Further, using TSH as the criterion variable, backward regression analysis including variables that presented a significant simple correlation coefficient with TSH was performed to determine the best model that could be used to examine the weight of different predictors in the criterion variable. Durbin-Watson test was used to verify correlation between residuals. All tests were two-sided, and the significance level was set at 0.05. All analyses were performed using SPSS for Windows, version 17 (SPSS Inc., Chicago, IL, USA).

Results

Patients with true PCOS were younger than those in the control group (p=0.010), but im-
importantly, as the primary outcome of the study, PCOS and PCOS-with-SCH subjects presented similar ages (p=0.088). The number of patients with acne was higher in PCOS with SCH than in PCOS patients (42.08% vs. 38.7, p=0.032). On the other hand, hirsutism (50.54% vs. 38.7%) was higher in PCOS than in PCOS-with-SCH patients (p=0.001). Acanthosis nigricans and PCOS morphology were of similar prevalences in PCOS and PCOS-with-SCH (p=0.468 and p=0.48, respectively) but these conditions were more frequent in PCOS and PCOS-with-SCH than in controls (p<0.001 and <0.001, respectively). Clinical and anthropometrical characteristics among the three groups of patients are compared in table 1. All anthropometric variables were equal in PCOS and PCOS-with-SCH and higher in control subjects (p<0.001). Weight, waist circumference, W:H ratio, CI, and LBM were higher in PCOS-with-SCH than in controls (p<0.001). As depicted in table 2, all metabolic markers were significantly different when PCOS patients were compared with controls. PCOS-with-SCH patients had higher fasting glucose, glucose 120 and total cholesterol levels and HOMA% than controls as well as lower HDL-C. After comparing PCOS with PCOS-with-SCH, only C-peptide levels showed to be higher in PCOS.

Using IFG as a marker, glucose intolerance was more prevalent in PCOS (13.9%) and PCOS-with-SCH groups (16.1%) than in control group (1.24%) (p=0.001) and not statistically different between PCOS patients than controls as well as lower HDL-C. Using a cutoff HOMA-IR of 2.8, 15.83% of PCOS patients and 9.7% of PCOS-with-SCH had higher HOMA-IR than controls (0.8%) (p<0.001). HOMA-IR was not statistically different in PCOS and PCOS-with-SCH patients.

Regarding endocrine parameters, only C-peptide levels were of similar prevalences in PCOS and PCOS-with-SCH patients. Interestingly, PRL was lower in PCOS than in controls (p<0.001). Sex steroids, LH and FAI were equally higher in PCOS and PCOS-with-SCH than in controls. TSH and FT4 concentrations were similar between PCOS and controls. FT4 concentrations were lower in PCOS-with-SCH patients compared with PCOS and control patients but yet in the normal range. TSH levels were associated with fasting glucose (rho=0.170; p=0.016) but were not associated with any anthropometric, or endocrine parameters in the control group. In PCOS patients, TSH levels had a weak positive correlation with LBM (rho=0.10; p=0.032), TC (rho=0.105; p=0.046), insulin (rho=

**Table 1.** Comparison of clinical and anthropometrical characteristics among PCOS and PCOS-with-SCH patients, and normal controls

<table>
<thead>
<tr>
<th>Variable **</th>
<th>PCOS TSH &lt;4.2 µUI/ml</th>
<th>PCOS-with-SCH TSH ≥4.2 µUI/ml</th>
<th>Control TSH &lt;4.2 µUI/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>460 26.72±5.38</td>
<td>30 28.73±5.21</td>
<td>223 30.34±4.74</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>407 118.82±12.43</td>
<td>27 119.63±15.80</td>
<td>206 115.11±7.87</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>407 77.13±9.37</td>
<td>27 75.97±8.36</td>
<td>206 73.82±8.11</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>443 74.34±17.51</td>
<td>31 75.17±19.73</td>
<td>215 63.95±10.84</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>412 29.11±6.74</td>
<td>26 26.75±6.18</td>
<td>206 24.47±4.03</td>
</tr>
<tr>
<td><strong>Waist (cm)</strong></td>
<td>385 86.02±15.29</td>
<td>25 83.72±15.17</td>
<td>204 73.93±9.39</td>
</tr>
<tr>
<td><strong>Hip (cm)</strong></td>
<td>384 106.68±12.36</td>
<td>25 105.68±12.57</td>
<td>204 100.12±8.54</td>
</tr>
<tr>
<td><strong>W:H ratio</strong></td>
<td>384 0.80±0.08</td>
<td>25 0.79±0.07</td>
<td>204 0.73±0.06</td>
</tr>
<tr>
<td><strong>CI</strong></td>
<td>378 1.15±0.10</td>
<td>23 1.14±0.09</td>
<td>203 1.07±0.07</td>
</tr>
<tr>
<td><strong>LBM (kg)</strong></td>
<td>412 45.61±6.37</td>
<td>25 44.85±5.91</td>
<td>216 20.67±7.17</td>
</tr>
<tr>
<td><strong>FM (kg)</strong></td>
<td>412 28.76±11.99</td>
<td>26 25.21±11.45</td>
<td>201 20.66±6.86</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; CI, conicity index; LBM, lean body mass; FM, fat mass

* One way analysis of variance followed by post hoc Tukey test; ** PCOS=patients with PCOS and TSH <4.2 µUI/ml. PCOS-with-SCH=patients with PCOS and TSH >4.2 µUI/ml. Control=normal women with TSH <4.2 µUI/ml.

a: p<0.001 PCOS vs. control; b: p=0.001 PCOS-with-SCH vs. control.
only 2.8% (R²=0.028) and this model is a good fit for the variability of TSH accounted for by all predictors was 0.031. The importance of these associations was further tested using a backward multiple regression. Considering the model (Table 4), the variability of TSH accounted for by all predictors was only 2.8% (R²=0.028) and this model is a good fit of the data (F=3.771, p=0.011). The exclusion of LBM from the model decreased the TSH variability in 1.6% (0.044-0.028). The contribution, given by standardized coefficients, of each predictor variable was as follows: TC (B=0.113, t=1.919, p=0.056), insulin (B=0.136, t=2.291, p=0.023), and PRL (B=0.107, t=1.799, p=0.073).

**Discussion**

All current diagnostic criteria of PCOS emphasize the importance of excluding thyroid dysfunc-
tion for accepting a definitive diagnosis, suggesting that a high TSH level in the presence of normal thyroxin concentration (so-called subclinical hypothyroidism) should be an exclusion criterion from a precise diagnosis of PCOS. The present study was designed to examine this group of patients and to compare them with definitive PCOS and definite control subjects.

In summary, the current study demonstrated higher blood pressure in PCOS patients than in controls. Anthropometrical features were similar in PCOS and PCOS-like-SCH in most of the patients. Even though PCOS patients presented similar metabolic profile than controls, PCOS-like-SCH and controls had similar concentrations of fasting insulin, HbA1C, HOMA-IR, C-peptide, LDL-C and TG. These two groups presented similar BMI, hip circumference, W-H ratio, CI, and BMI were higher in PCOS-like-SCH than in controls. Free thyroxin was lower in PCOS-like-SCH than in PCOS and control patients. Estradiol levels were higher in PCOS and PCOS-like-SCH patients than in control group. PCOS and PCOS-like-SCH patients presented similar concentrations of LH, androgens and FAI. TSH levels presented weak positive correlation with LBM, TC, insulin, and PRL, in PCOS patients. In the PCOS-like-SCH group, TSH was positively correlated only with TC and HDL-C concentrations.

A few possible limitations need to be considered when interpreting the present findings. The normal upper limit of the TSH level is not standardized yet. The adopted cutoff for TSH level of 4.2 µU/ml in the current study was higher than that proposed by several authors (7, 18-20, 32, 33) and lower than those used in other studies (21, 32). The small sample size in PCOS-like-SCH group may have reduced the power of the comparisons. On the other hand, the current findings have scientific strength. One of the strengths of the study is the large number of patients examined prospectively with comprehensive anthropometric, metabolic and endocrine investigations. Additional insights into the relevance of establishing a single cutoff for TSH level in PCOS patients’ diagnosis are provided, and the results of the present study should be considered for the exclusion of thyroid disorders before the diagnosis of true PCOS.

The finding of an equal prevalence of many signs of hyperandrogenism and PCOS morphology, in PCOS and PCOS-like-SCH patients demonstrated the high clinical similarity between these two conditions and confirms previous observations (7, 19, 32-35). Although a clear diagnosis of PCOS-like-SCH had been established in 43% of patients from a group of patients presenting with PCOS and autoimmune thyroiditis in a previous study, the entire group also had equal prevalence of acne, hirsutism, androgenic alopecia, and PCO morphology (35). Another study found the same degree of hirsutism, as evaluated by Ferriman-Gallwey score, in PCOS and PCOS-like-SCH patients (32). Even some studies had shown that clinical hyperandrogenism is not common in patients with hypothyroidism (14, 17), biochemical increase in androgen levels, mainly in total, free testosterone and FAI in hypothyroidism are frequently found (18, 34, 36).

Anthropometrical parameters were equal in PCOS and PCOS-like-SCH patients in the current study. Similar BMI and WHR in PCOS and PCOS-like-SCH were already reported in several studies (19-21, 32, 34, 37). Increased BMI was found in a study when PCOS patients with TSH >2 µU/ml and PCOS patients with lower TSH levels were compared (7). Regarding carbohydrate metabolism, only C-peptide was higher in PCOS than in PCOS-like-SCH. Supporting the current study, the PCOS-with-SCH subjects were equal to those of PCOS with respect to fasting glucose, glucose after GTT 120, and HOMA-IR was also found in other studies (9, 21, 32, 34, 37). Higher or equal fasting insulin between PCOS and PCOS-like-SCH patients were also previously reported (18, 19, 32). Notably, increased HOMA-IR in PCOS-like-SCH patients was reported only in one study (7).

### Table 4. Backward multiple regression between TSH and significant predictors found after simple linear correlation

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R square</th>
<th>Adjusted R square</th>
<th>Std. error of the estimate</th>
<th>F</th>
<th>Durbin-Watson</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.211&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.044</td>
<td>0.031</td>
<td>0.85480</td>
<td>3.289</td>
<td>--</td>
<td>0.012&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>0.196&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.038</td>
<td>0.028</td>
<td>0.85602</td>
<td>3.771</td>
<td>1.971</td>
<td>0.011&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

a: Criterion variable: TSH mU/ml; b: Predictors (constant), PRL, LBM, TC, insulin; c: Predictors (constant), PRL, TC, insulin
In general, lipid levels are similar between PCOS and PCOS-like-SCH (18, 37). TG levels have been found to be lower (37), higher (18, 21) or similar (34) in PCOS-like-SCH patients when compared with PCOS patients. Although HDL-C levels had been equal in PCOS and PCOS-like-SCH patients in the current study, other studies have shown lower levels of this lipid in PCOS-like-SCH patients (18, 34). Higher TC levels were also seen in PCOS-like-SCH patients in other studies (18, 34) LDL-C level was reported to be higher in PCOS-like-SCH patients, in addition to showing a positive correlation with TSH levels (21, 34). No difference in lipid levels between SCH patients and controls was reported in non-PCOS populations (38), but in the current study, HDL-C levels were lower in PCOS-with-SCH than in controls.

With the exception of C-peptides, in the present study, PCOS and PCOS-like-SCH patients showed to be endocrinologically equal. Results of other studies comparing hormonal parameters between PCOS and PCOS-like-SCH have not been consistent. Higher levels of T, I1T, and FAI in PCOS than in PCOS-like-SCH were found in a few reports (7, 19). On the other hand, other studies reported equal concentrations of these hormones in both groups (32, 34). Higher levels of DHEAS, PRL, and LH in PCOS-like-SCH compared with PCOS were also reported (32, 39), but equal concentrations of LH, FSH, T, PRL, DHEAS and E2 were also found by others (7, 19, 21, 34). Lower levels of SHBG in PCOS-like-SCH patients than in PCOS patients were reported in only one study (18). Free thyroxine was lower in PCOS-with-SCH than in PCOS and control patients indicating mild thyroid dysfunction at a statistical but not clinical level. Regarding endocrine profiles between PCOS and PCOS-with-SCH, the discrepant results suggest heterogeneity among studies precluding unbiased comparisons.

**Conclusion**

The current study demonstrated that anthropometrical parameters and ovarian morphology were similar in PCOS and PCOS-with-SCH patients. Signs of hyperandrogenism were present in both groups. Regarding hormones, only C-peptide was higher in PCOS group. TSH correlated with total cholesterol, insulin, and prolactin. Collectively, current data indicated that the exclusion criterion "thyroid dysfunction" should be standardized before PCOS diagnosis. Subclinical hypothyroidism as manifested by a raised TSH with normal free T4, should not be used as an exclusion criterion for a diagnosis of PCOS. Studies designed to treat PCOS-with-SCH with thyroxin before excluding them from true PCOS group are still needed.

**Conflict of Interest**

Authors declare no conflict of interest.

**References**


33. Pei YJ, Wang AM, Zhao Y, Yan L, Li M, White RE, et al. Studies of cardiovascular risk factors in polycystic ovary syndrome patients combined with...
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