High-normal thyroid stimulating hormone is a predictor of metabolic syndrome among young polycystic ovary syndrome women

Ahmed Saad-Aldeen Salama[a], Ragaa Abed-Elshaheed Matta[a], Sahar H. Elhini[b], Lamia Hamdi[c], Laila Adel[d], Hany Hassan[e]

[a]Departments of Internal Medicine, [b]Endocrinology and Diabetes Internal Medicine, [c]Clinical Pathology, [d]Radiology, [e]Gynecology and Obstetrics, Medical School, Minia University, Minia, Egypt

Correspondence to Ragaa Abed-Elshaheed Matta, MD of Internal Medicine, Department of Internal Medicine, Minia University Hospital, Minia, Egypt;
Tel: +20 100 879 2833;
e-mail: mattaragaa@yahoo.com

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Background and objectives
High-normal thyroid stimulating hormone (TSH) (2.6–4.5 μIU/ml) is associated with metabolic syndrome (MetS) in population studies. We hypothesized that euthyriod polycystic ovary syndrome (PCOS) with TSH of higher than 2.5 had altered anthropometric, metabolic, and endocrine parameters as well as higher percentage of MetS compared with those with lower TSH levels.
Patients and methods
The present study included 60 young euthyroid PCOS women without any thyroid risk factors and 60 age-matched and BMI-matched healthy, fertile women. Anthropometric measurements were obtained, biochemical and hormonal assay were evaluated, and the homeostatic model assessment-insulin resistance was calculated. PCOS women were divided into high-normal TSH (group 1) and low-normal TSH (group 2) groups. MetS was defined according to National Cholesterol Education Program/Adult Treatment Panel III.
Results
Group 1 had significantly higher waist circumference, systolic blood pressure and diastolic blood pressure, total cholesterol, triglycerides, low-density lipoprotein cholesterol, fasting glucose, fasting insulin, homeostatic model assessment-insulin resistance, and free androgenic index and significantly lower high-density lipoprotein cholesterol, free thyroxin, and sex hormone binding globulin compared with both group 2 and healthy controls. In addition, group 1 (compared with group 2) had significantly higher percentage and higher risk of MetS [46.7 vs. 16.7%, P=0.01; odds ratio (OR)=4.4] and some of its components such as fasting glucose of at least 100 mg/dl (26.7 vs. 6.7%, P=0.03; OR=4.3), high-density lipoprotein cholesterol of less than 50 mg/dl (50 vs. 23.3%, P=0.03; OR=3.3), TG of at least 150 mg/dl (50 vs. 20%, P=0.01; OR=4), and near-significant higher percentage of both waist circumference of 88 cm or more and systolic blood pressure of at least 130 (P=0.06 for both, OR=3.25, 5, respectively). TSH level of 2.85 was the best threshold to indicate MetS risk (sensitivity=68%, specificity=88%, Youden index=0.56, area under the curve=0.81).
Conclusion
High-normal TSH PCOS women had increased risk of MetS. The optimal cut-off point for diagnosis of MetS was 2.85 μIU/ml.

Keywords:
high-normal thyroid stimulating hormone, metabolic syndrome, polycystic ovary syndrome

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Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disease affecting 6–8% of reproductive age women [1]. It is characterized by hyperandrogenism, chronic anovulation, and polycystic ovaries after exclusion of related disorders [2]. The metabolic syndrome (MetS) and its individual components including abdominal obesity, dyslipidemia, and hypertension, along with increased risk for type 2 diabetes mellitus and cardiovascular disease, are common in PCOS, particularly among women with higher insulin levels and BMI. Insulin resistance (IR) is a likely common pathogenic factor for both PCOS and MetS [3,4].

Association of subclinical hypothyroidism (SCH) with IR and MetS is bidirectional [5,6]. PCOS women have a higher prevalence of autoimmune thyroiditis and SCH [7], whereas the association of thyroid stimulating hormone (TSH) with fasting insulin levels and IR among either SCH-PCOS women with MetS or IR-PCOS women has been reported in many studies; a few others have not proven this issue

[8–10]. SCH is defined as elevated serum TSH in association with normal thyroid hormones. TSH level is the most sensitive test for detecting minor degrees of primary thyroid dysfunction [11]. However, the diagnosis of SCH critically depends on the definition of the upper limit of 'normal' for TSH in serum. In 2003, the National Academy of Clinical Biochemistry (NACB) recommended lowering the upper reference limit of TSH to 2.5 μIU/ml in contrast to the conventionally used levels of 4–5 μIU/ml based on a large-scale epidemiological survey that revealed that more than 95% of normal individuals have TSH levels of less than 2.5 μIU/ml [12,13]. In accordance with this recommendation, high-normal TSH within the reference range (2.5–4.5 μIU/ml) is associated with higher blood pressure, less-favorable lipid profile, adiposity, and higher prevalence of MetS in recent population-based studies [14–17].

Although thyroid function as reflected by TSH levels was associated with IR in PCOS women [18], data regarding the association of TSH within normal range with IR, metabolic, and endocrine parameters in PCOS are limited and inconsistent [19–21]. Most of these studies had many limitations as they were performed in obese women; thyroid peroxides (TPO) autoantibody assessment was neglected, and not compared with non-PCOS women. To the best of our knowledge, no study has reported the existence of an association between high-normal TSH and MetS among PCOS women. The present study was conducted in young PCOS women with BMI of 28 kg/m^2 or less. They were also euthyroid, with negative TPO autoantibodies, and had normal thyroid morphology on ultrasound to exclude any risk factor for thyroid disease and to allow better evaluation of the role TSH levels per se.

This study aimed to assess the clinical implications of using a TSH level of 2.5 μIU/ml, as the upper limit of normal, to detect changes in clinical, metabolic, and hormonal parameters in euthyroid PCOS women, as well as to evaluate the relationships between TSH and previously mentioned parameters. Second, we aimed to examine the associations of high-normal TSH levels with IR indices, MetS, and its components. Third, the intention was to identify a possible cut-off point of TSH for the diagnosis of MetS.

Patients and methods

The present, observational, case–control study was conducted from March 2013 to February 2015. Women with PCOS were recruited from the Endocrinology Out-patient Clinic at the Internal Medicine Department, El-Minia University Hospital. The present study involved 60 euthyroid PCOS women between the ages of 18 and 35 years and 60 age-matched and BMI-matched healthy, fertile females who served as a control group. Our study was carried out on euthyroid PCOS women without any risk of thyroid disorder. They had normal TSH levels that ranged from 0.3 to 4.5 μIU/ml. On the basis of this new upper limit of TSH according to the NACB, we divided PCOS women into high-normal TSH with TSH values of 2.6–4.5 μIU/ml and low-normal TSH with TSH values of 0.3–2.5 μIU/ml groups. The protocol was approved by the local ethics committee of the Faculty of Medicine, El-Minia University. All the patients gave their written informed consent.

Patients met the revised Rotterdam diagnostic criteria after exclusion of other causes of hyperandogenism and menstrual irregularity as well as thyroid disorders [2]. PCOS women had at least two of the following criteria: (i) amenorrhea (absence of menstrual cycle for three cycles) or oligoamenorrhea (menstrual cycle for more than 35 days); (ii) clinical and/or biochemical hyperandogenism [hirsutism with a Ferriman–Galleway score of more than 7 and acne; total testosterone of at least 2.5 nmol/l, or free androgen index (FAI) of more than 4] [22,23]; or (iii) polycystic ovaries on ultrasound scan (at least one ovary more than 10 ml in volume and/or with at least 12 follicles of 2–9 mm in diameter). Diagnosis of MetS was based on the National Cholesterol Education Program/Adult Treatment Panel III. MetS was defined by presence of three or more of the following criteria: abdominal obesity [waist circumference (WC) ≥88 cm], elevated fasting serum triglycerides (TG) of at least 150 mg/dl, low levels of high-density lipoprotein cholesterol (HDL-c) of less than 50 mg/dl, elevated blood pressure of at least 130/85 mmHg, and elevated fasting glucose of at least 100 mg/dl [24].

All women in control group had regular menstrual cycles every 21–35 days; none of them had hyperandogenism, polycystic ovaries on ultrasound, or family history of PCOS. Normal ovulation was assessed by measuring serum progesterone on days 22–23 of the menstrual cycle.

Exclusion criteria

Women who had any one of the following disorders were excluded: hyperprolactinemia, Cushing's syndrome, nonclassic congenital adrenal hyperplasia, androgen-secreting tumors, thyroid disorders involving subclinical and manifest hypothyroidism or

hyper thyroidism, on thyroxin or antithyroid therapy, radioactive iodine intake or previous thyroid surgery, abnormal thyroid morphology on ultrasound scan (goiter, nodule, or altered echogenicity), abnormal thyroid hormones levels [TSH less than 0.3 or more than 4.5 μIU/ml, free thyroxin (FT4) less than 0.8 or more than 2.8 ng/dl, or FT3 less than 2.3 or more than 4.2 pg/ml] or TPO antibody titer of more than 5.6 IU/ml, and any system or chronic illness. Women were also excluded if they were under 18 years of age and over 35 years of age, smokers, pregnant or lactating, or had BMI of more than 28 kg/m^2. None of participants were diabetic or used confound medications (hormonal therapy, steroids, and lipid-lowering drugs or insulin sensitizers) for at least 6 months before enrollment into the study.

All patients answered a standardized questionnaire that collected information on age, marital state, menstrual cycle, hirsutism, acne, family history, and medications. Complete physical examination was performed with special emphasis to signs of hyperandogenism. Anthropometric measurements were taken in a standardized manner. Height and weight were mea- sured to calculate BMI by dividing body weight (kg) by square of height (m^2). Patients were positioned with their feet apart by 25–30 cm; waist circumference was measured directly on skin with a flexible tape placed horizontally midway between the last rib and the highest point of the iliac crest. Systemic arterial blood pressure was measured using a mercury sphygmomanometer with the patients seated for 15 min before measurement. The thyroid gland was examined according to standard procedures with combined inspection and palpation.

Laboratory analyses

Blood samples were collected from females during the early follicular phase on days 2–5 of menstrual the cycle by sterile venipuncture at 8:00 a.m. after 12 h of fasting. Among women with amenorrhea, a random blood sample was acceptable. Blood was processed within 30 min of collection for measurement of plasma glucose, lipid profile, TPO antibody, and hormonal assays; 1 ml of plasma samples were aliquoted and frozen at −70°C until assay for fasting insulin. Among women with PCOS, serum prolactin and 17-hydroxyprogesterone were measured to exclude hyperprolactinemia and nonclassical congenital adrenal hyperplasia, respectively; 24-h urine free cortisol and 17-hydroxyprogesterone stimulation tests were performed if there was suspicion of Cushing's syndrome and nonclassic congenital adrenal hyperplasia, respectively [25–27]. Serum dehydroepian-drosterone sulfate was also measured. Fasting plasma glucose was measured by enzymatic colorimetry using kits from Elitech Diagnostic (ELtech group, Inc., North America). Quantitative determination of fasting plasma insulin was performed using an insulin enzyme immunoassay kit provided by IBL International GMBH (Hamburg, Germany) company. Homeostatic model assessment-insulin resistance (HOMA-IR) was calculated as [fasting plasma glucose (mg/dl)×fasting plasma insulin (μIU/ml)]/405 [28]. Serum total cholesterol (TC), HDL-c, and TG were assessed by colorimetry using kits supplied by Human Germany. Low-density lipoprotein cholesterol (LDL-c) was calculated according to the Friedewald equation [29]. Assays for luteinizing hormone (LH), follicle stimulating hormone, progesterone, total testosterone (TT), prolactin, TSH, and FT4 were performed by automated quantitative enzyme linked fluorescent immune assays using Mini-VIDAS with available kits from Biomerieux, Marcyl L'Etoile, France. Serum dehydroepian-drosterone sulfate in human serum was analysed by Elecsys (Roch Diagnostic, Mannheim, Germany) using Elecsys kits. Sex hormone binding globulin (SHBG) was assessed, and FAI was calculated as the quotient of 100×TT/SHBG [30]. TPO antibody levels were assessed by immunoenzymatic assay using Accubind ELISA microwells (Monobind Inc., USA); normal values ranged up to 5.6 IU/ml. Seven hydroxyprogesterone was assessed by ELISA (Human reader Germany Company; Human Diagnostic, Madeburg, Germany).

Ultrasonographic evaluation

Transvaginal ultrasound was performed using a 7.5-mHz transducer (TOSHIBA SSA 340 machine, Tokyo, Japan) for all women. The antral follicle count and ovarian volume of both ovaries were assessed. Thyroid ultrasonography was performed by the same equipment using a high-frequency linear probe of 7.5 MHz. Patients were examined in the supine position with a small pillow under their shoulder for better exposure of the thyroid gland. Scanning with longitudinal and transverse planes allows measurements of length, width, and depth of each lobe as well as the isthmus separately. Echogenicity was assessed as compared with echogenicity of adjacent muscles.

Statistical analysis

Statistical analyses were performed using SPSS (version 22). Categorical data were expressed as percentages and compared by χ^2 or fisher's extract tests. Quantitative data are expressed as mean±SD. The Kolmogorov–Smironov test was used to examine normal distribution. Non-normally distributed variables were log transformed before analysis and back transformed to original units for presentation in tables. Comparison between groups

were carried out using the student *t*-test for normally distributed data and the Mann–Whitney test for non-normally distributed data. Pearson's correlation coefficient was calculated to determine the strength of association between each continuous variable. Simple logistic regression analysis test assessed the odds ratio (OR) of the value. To determine the optimal thresholds, the point on the receiver operating characteristics curve with the maximum Youden index [sensitivity–(1–specificity)] was calculated. Statistical significance and highly statistical significance were defined as * and ** with probability levels of $P<0.05$ and $P<0.01$ respectively.

Results

The baseline characteristics of the study participants are given in Table 1. Total PCOS women had significantly higher WC, systolic blood pressure (SBP), diastolic blood pressure (DBP) ($P<0.001$, 0.04, 0.02, respectively), total cholesterol, TG, and LDL-c ($P=0.04$, <0.001, 0.01, respectively) compared with healthy controls. Fasting plasma glucose, fasting plasma insulin, HOMA-IR, and TSH were significantly higher in women with total PCOS than in healthy controls ($P=0.02$, 0.001, 0.001, 0.01, respectively). Total PCOS women had significantly higher LH, TT, and FAI ($P<0.001$ for all) and significantly lower HDL-c, FT4, and SHBG ($P<0.001$, 0.04, 0.01, respectively) compared with healthy controls.

High-normal TSH PCOS women showed significantly higher WC, SBP, DBP, total cholesterol, TG, LDL-c, fasting plasma glucose, fasting insulin, HOMA-IR, TSH, LH, TT, and FAI and significantly lower HDL-c, FT4, and SHBG ($P<0.001$ for all except $P=0.005$ for glucose and $P=0.002$ for SHBG) compared with healthy controls (Table 1).

High-normal TSH PCOS women as compared with low-normal TSH PCOS had significantly higher BMI, WC, SBP, DBP ($P=0.03$, 0.04, 0.0007, 0.01, respectively), total cholesterol, TG, LDL-c ($P=0.004$, 0.003, 0.007, respectively), fasting plasma glucose ($P=0.04$), fasting plasma insulin, HOMA-IR ($P<0.001$ for both), TSH, LH ($P=0.001$ for both), and FAI ($P=0.01$) and significantly lower HDL-c, FT4, and SHBG ($P<0.001$, <0.001, 0.04, respectively) (Table 1).

As compared with controls, low-normal TSH PCOS women showed significant increases in WC ($P=0.01$), LH, TT, and FAI ($P\leq0.001$ for all) and significant decreases in HDL-c ($P=0.003$) as shown in Table 1.

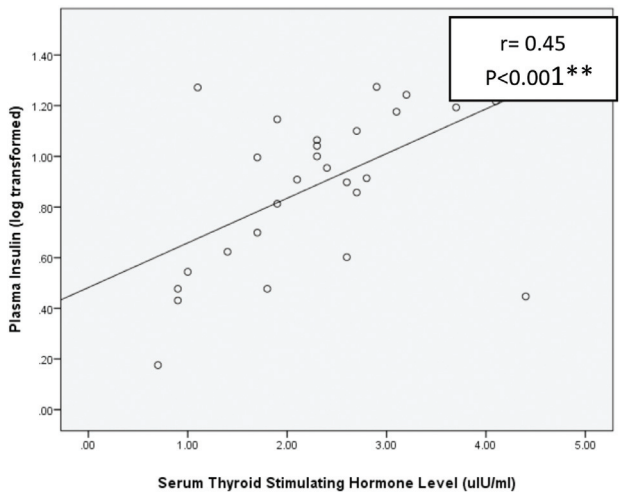
Among PCOS women, TSH showed significant positive correlations with BMI ($r=0.32$, $P=0.01$), WC

Table 1 Comparison of anthropometric, metabolic, and hormonal characteristic of polycystic ovary syndrome women and healthy control group

Variables	Healthy control (n=60)	Total PCOS (n=60)	Low-normal TSH PCOS (n=30)	High-normal TSH PCOS (n=30)
Age(years)	25.13±3.55	24.4±3.4	24.43±2.9	24.36±3.9
BMI (kg/m^2)	25.21±1.28	25.35±1.9	24.98±2.14^{c,*}	25.9±1.6
WC (cm)	80.9±3.76^{a,c,**,b,*}	84.68±4.7	83.3±5.21^{c,*}	85.9±3.9
SBP (mmHg)	113.91±7.25^{a,*,c,**}	117.2±9.5	113.96±8.6^{c,**}	120.46±9.3
DBP (mmHg)	72.81±5.33^{a,*,c,**}	75.4±6.1	73.43±5.78^{c,*}	77.43±5.78
Total cholesterol (mg/dl)	162.36±15.92^{a,*,c,**}	168.4±19	162.67±17.1^{c,**}	174.23±12.3
Triglyceride (mg/dl)	93.7±21.04^{a,c,**}	115.6±31	104.1±28.8^{c,**}	127.1±29.45
HDL-c (mg/dl)	57.3±4.36^{a,b,c,**}	52.1±4.41	54.33±4.41^{c,**}	49.86±4.2
LDL-c (mg/dl)	88.01±14.1^{a,*,c,**}	94.38±14.66	89.36±15.18^{c,**}	99.43±12.04
Fasting glucose (mg/dl)	82.5±7.65^{a,*,c,**}	86.22±9.6	83.7±8.5^{c,*}	88.73±10.1
Fasting insulin (μU/ml)	7.19±2.07^{a,c,**}	10.02±3.9	8.1±3.1^{c,**}	11.93±3.75
HOMA-IR	1.48±0.42^{a,c,**}	2.14±0.95	1.69±0.76^{c,**}	2.57±0.95
FSH (mIU/ml)	4.32±1.49	4.69±1.24	4.72±0.99	4.66±1.46
LH (mIU/ml)	3.8±1.6^{a,b,c,**}	9.5±1.92	9.85±1.79^{c,**}	8.25±1.74
Total testosterone (nmol/l)	1.16±0.6^{a,b,c,**}	2.8±2.7	2.47±1.77	2.98±2.3
SHBG (nmol/l)	49.5±26.18^{a,*,c,**}	36.2±19.4	42.67±20.3^{c,*}	29.73±16.4
Free androgen index	2.1±1.1^{a,b,c,**}	8.9±4.9	7.33±4.85^{c,*}	10.53±4.48
TSH (μIU/ml)	2±0.81^{a,*,c,**}	2.43±0.97	1.8±0.61^{c,*}	3.06±0.85
Free T3 (pg/ml)	2.8±0.8	2.72±0.69	2.59±0.67	2.9±1.34
Free T4 (ng/dl)	1.34±0.26^{a,*,c,**}	1.27±0.63	1.51±0.76^{c,**}	1.02±0.32

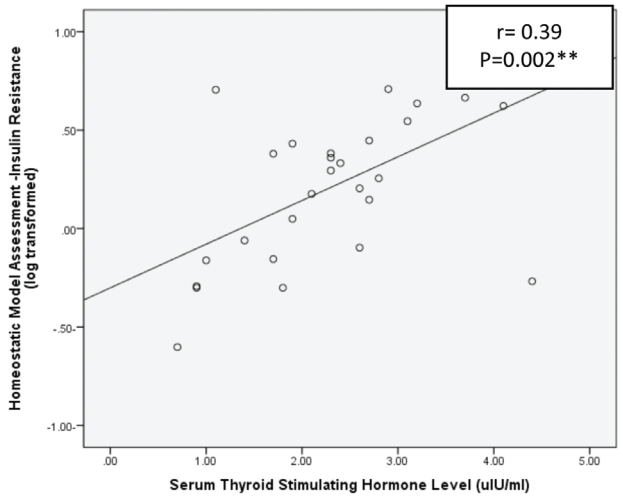
Data are expressed as mean±SD. DBP, diastolic blood pressure; FSH, follicle stimulating hormone; HDL-c, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-c, low-density lipoprotein cholesterol; LH, luteinizing hormone; PCOS, polycystic ovary syndrome; SBP, systolic blood pressure; SHBG, sex hormone binding globulin; TSH, thyroid stimulating hormone; WC, waist circumference. [a]Versus total PCOS. [b]Versus low-normal TSH PCOS. [c]Versus high-normal TSH PCOS. *$P<0.05$. **$P<0.01$.

Figure 1



Correlation between serum thyroid stimulating hormone and fasting insulin among total polycystic ovary syndrome group.

Figure 2



Correlation between serum thyroid stimulating hormone and homeostatic model assessment-insulin resistance among total polycystic ovary syndrome group.

(r=0.29, P=0.02), SBP (r=0.27, P=0.03), TG (r=0.45, P<0.001), total cholesterol (r=0.31, P=0.01), LDL-c (r=0.31, P=0.01), fasting glucose (r=0.34, P=0.008), fasting insulin (r=0.45, P<0.001, Fig. 1, HOMA-IR (r=0.39, P=0.002) (Fig. 2), and FAI (r=0.625, P<0.001) and significant negative correlations with HDL-c (r=–0.44,P<0.001), FT4 (r=37, P=0.003), and SHBG (r=–0.54, P<0.001) (Table 2).

Among total PCOS women, the percentage of MetS was 31.7% (19 out of 60), of central adiposity 23.3% (14/60), of high blood pressure 27.7% (16/60), of high fasting glucose 17% (10/60), of high TG level 35% (21/60), and of low HDL-c it was 36.7% (22/60).

Table 2 Correlation of thyroid stimulating hormone level with clinical, metabolic, and hormonal parameters among polycystic ovary syndrome women

Variables	Thyroid stimulating hormone	
	R	*P*
Age (years)	0.04	0.72
BMI (kg/m^2)	0.32	0.01*
Waist circumference (cm)	0.29	0.02*
Systolic blood pressure (mmHg)	0.27	0.03*
Diastolic blood pressure (mmHg)	0.24	0.06
Total cholesterol (mg/dl)	0.31	0.01*
Triglyceride (mg/dl)	0.45	<0.001**
High-density lipoprotein-cholesterol (mg/dl)	–0.44	<0.001**
Low-density lipoprotein-cholesterol (mg/dl)	0.31	0.01*
Fasting glucose (mg/dl)	0.34	0.008**
Fasting insulin (μU/ml)	0.45	<0.001**
Homeostatic model assessment of insulin resistance	0.39	0.002**
Follicle stimulating hormone (mIU/ml)	0.07	0.553
Luteinizing hormone (mIU/ml)	–0.08	0.53
Total testosterone (nmol/l)	0.13	0.32
Sex hormone binding globulin (nmol/l)	–0.453	<0.001**
Free androgen index	0.625	<0.001**
Free thyroxine (ng/dl)	–0.37	0.003**

High-normal TSH PCOS women compared with low-normal TSH PCOS women had significantly higher percentage of MetS (46.7 vs. 16.7%, P=0.01) and its components such as elevated fasting glucose level (26.7 vs. 6.7%, P=0.03), high TG levels (50 vs. 20%, P=0.01), and low HDL-c (50 vs. 23.3%, P=0.03). They had also near-significant higher percentage of central adiposity and high SBP (P=0.06 for both) than low-normal TSH PCOS women (Table 3).

The OR for the presence of MetS among high-normal TSH PCOS women compared with low-normal TSH PCOS women was 4.4. In addition, the ORs for central adiposity, high SBP, high DBP, elevated fasting glucose level, high TG level, and low HDL-c were 3.25, 5, 3.5, 4.3, 4, and 3.3, respectively (Table 3).

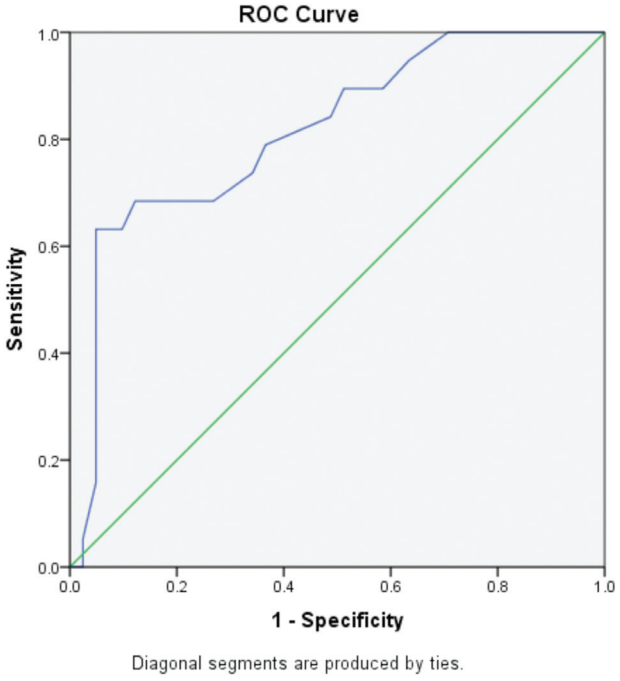
Figure 3 shows the point on the receiver operating characteristics curve with the maximum Youden index that was used to distinguish women at risk of MetS from those who were not. The area under the curve was 0.81. Among the total PCOS women, the cut-off value of 2.85 μIU/ml for TSH was the best threshold to indicate MetS risk according to National Cholesterol Education Program/Adult Treatment Panel III definition, maximizing Yuoden index (sensitivity=68%, specificity=88%, Youden index=0.56).

Table 3 Percentage and odds ratio of metabolic syndrome and its components among low-normal and high-normal thyroid stimulating hormone polycystic ovary syndrome women

Variables	Low-normal TSH PCOS (*n*=30)	High-normal TSH PCOS (*n*=30)	*P*
Metabolic syndrome [*n* (%)]	5 (16.7)	14 (46.7)	0.01*
Odds ratio		4.4	
Waist circumference ≥88 cm [*n* (%)]	4 (13.3)	10 (33.3)	0.06
Odd Ratio		3.25	
Systolic blood pressure ≥130 mmHg [*n* (%)]	2 (6.7)	7 (23.3)	0.06
Odd ratio		5	
Diastolic blood pressure ≥85 (mmHg) [*n* (%)]	2 (6.7)	6 (20)	0.12
Odd ratio		3.5	
Fasting glucose≥100 mg/dl [*n* (%)]	2 (6.7)	8 (26.7)	0.03*
Odd ratio		4.3	
Triglyceride ≥150 mg/dl [*n* (%)]	6 (20)	15 (50)	0.01*
Odd ratio		4	
High-density lipoprotein cholesterol <50 mg/dl [*n* (%)]	7 (23.3)	15 (50)	0.03*
Odds ratio		3.3	

PCOS, polycystic ovary syndrome; TSH, thyroid stimulating hormone.

Figure 3



Receiver operating characteristics (ROC) curve analysis of thyroid stimulating hormone (TSH) cut-off for metabolic syndrome in polycystic ovary syndrome women .The point on the ROC curve with the maximum Youden index was calculated to determine the optimal threshold. A cut-off value of 2.85 for TSH was the best threshold to indicate MetS risk according to National Cholesterol Education Program/Adult Treatment Panel III definition, maximizing the Yuoden index (sensitivity=68%, specificity=88%, Youden index=0.56). The area under the curve=0.81.

Discussion

To the best of our knowledge, this is the first study to report that high-normal TSH is associated with higher percentage and increased risk of MetS and many of its components among euthyroid PCOS women. The optimal TSH cut-off point for MetS risk among PCOS women in our study was 2.85 μIU/ml, an aspect that has not been previously evaluated.

PCOS women have elevated serum TSH levels and higher prevalence of autoimmune thyroiditis and thyroid nodules [5]. There are conflicting data about the association of these above-mentioned thyroid risk factors with IR and MetS [31–33]. To explore the sole role of TSH levels per se in the present study, PCOS women who were nonobese (BMI<28 kg/m^2) and euthyroid without any thyroid risk factors were selected. They had negative TPO antibody and normal thyroid morphology on ultrasound. These selection criteria were neglected among PCOS women in most previous studies, which also did not compare PCOS women with a healthy control group [18,19,34].

In the present study, high-normal TSH PCOS women had significant higher markers of IR – namely, fasting insulin and HOMA-IR – as compared with low-normal TSH PCOS women. This was in accordance with previous studies among PCOS women [19,34]. Furthermore, we found significant, positive correlations between these markers and TSH levels among total PCOS women. In one study, TSH level was not connected to insulin resistant indices among PCOS women who were grouped on the basis of TSH levels [21]. However, other studies were in line with our results. IR assessed by either euglycemic hyperinsulinemic clamp or insulin resistant indices was significantly correlated with TSH levels among euthyroid, overweight, and obese PCOS women [20,35]. In the present study, high-normal TSH PCOS but not low-normal TSH PCOS women had significant higher markers of IR compared with

healthy women. This means an association of TSH and IR regardless of PCOS. In support of our observation, El-Hafez *et al.* [32] found that euthyroid, IR-PCOS women had higher TSH levels compared with euthyroid, non-IR-PCOS women. PCOS women have intrinsic IR as a cause or a consequence of the syndrome [3]. In addition, whether IR is a cause or a consequence of high-normal or high TSH levels is still unclear. Among IR-PCOS patients, 6 months of treatment with insulin sensitizers significantly reduces TSH levels [8]. However, SCH PCOS women with MetS treated with low-dose eltroxin (25–50 μg) displayed significantly decreased fasting insulin levels and enhanced insulin sensitivity [9].

The presence of MetS among PCOS women was previously linked to higher markers of IR [36]. In the present study, higher percentage and risk of both MetS and many of its components among high-normal TSH PCOS may be partially attributed to higher IR and altered metabolic parameters. Muller *et al.* [18] reported that TSH level of 2 μIU/ml was associated with IR among PCOS women. Moreover, we have identified a TSH level of 2.85 μIU/ml as the optimal cut-off point for MetS risk among PCOS women – a novel finding. Association of high-normal TSH level with increased risk and prevalence of MetS has recently been reported among euthyroid population study groups [17,37,38]. In these studies, high-normal TSH was associated with up to two-fold versus 4.4-fold increased risk of MetS in our study. Moreover, a gradual increase in the prevalence MetS according to TSH tertiles was reported in one study, but the presence of counter studies in obese euthyroid Turkish women created discrepancy [38,39]. Data on PCOS women are sparse. Only Anaforoglu *et al.* [31] showed that PCOS alone was not associated with thyroid disease, but the MetS and some of its components appear to be related to thyroid volume, function, and antithyroid antibody levels.

In our study, TSH level was significantly positively related to BMI and WC among total PCOS women. Novel findings were presence of both near-significant higher percentage and 3.25 times higher risk of central obesity among high-normal TSH PCOS women compared with low-normal TSH PCOS women. In addition, the former had significantly higher WC and BMI compared with the latter. Central obesity is more sensitive than BMI in the assessment of obesity [40]. Previous euthyroid population studies show similar associations of BMI with TSH levels. They also reported that the prevalence and risk of obesity showed gradual increase according to TSH tertiles. The highest tertile of TSH was associated with 1.872-fold increases in the OR for obesity after adjusting for age and HOMA-IR [38,41]. Higher serum TSH levels among obese than in lean euothyroid patients suggested setting up a TSH reference range according to BMI in previous studies [16]. However, there were conflicting results [39]. Previous studies among PCOS women showed that TSH correlated with BMI and waist-to-hip ratio. In addition, high-normal TSH was associated with higher BMI [19,32,34,35].

The question remains as to whether the changes in the levels of TSH are the cause or result of adiposity status. Binding of TSH to its receptor in fat tissue induces differentiation of preadipocytes into adipocytes, adipogenesis, and leptin release [42,43]. In addition, administration of low-dose thyroxine to SCH PCOS women significantly reduced BMI and WC [9]. The most favored hypothesis attributes the elevated TSH levels in obesity to increased leptin-mediated production of prothyroid releasing hormone [44].

In the present study, a linear increase in TC, LDL-c, and TG and a linear decrease in HDL-c have been observed with increasing TSH levels among total PCOS women. Therefore, increased TSH level within the normal range was associated with a less-favorable lipid profile. These positive associations with TG, TC, and LDL-c were in line with recent different euthyroid population-based studies among adolescents, adults, and postmenopausal women [16,37,45], whereas others did not find these associations with TG and LDL-c [15]. Takamura *et al.* [46] found a significant correlation between HDL-c and TSH level.

In our study, PCOS women with high-normal TSH compared with those with low-normal levels had significantly higher TC, TG, and LDL-c and lower HDL-c. Previous data among PCOS women are little and conflicting. Studies have reported that TSH PCOS women with TSH of more than 2 or 2.5 mIU/l had only significantly higher TG and lower HDL-c, but not LDL-c [18,34]. In contrast, euthyroid PCOS women subgrouped according to TSH levels had no change in TG levels among different groups. With elevation in TSH concentration, a trend toward higher TC and LDL-c and lower HDL-c was in fact observed, but only the differences in TC and LDL-c level were significant in those with TSH of more than 2.5 compared with a range of 0.02–1 μIU/ml. Optimal TSH of4.07 μIU/ml was identified for elevated LDL-c risk [21].

A novel finding of our study, high-normal TSH, was associated with higher percentage and risk of both hypertriglyceridemia and low HDL-c levels (two dyslipidemic components of MetS), with ORs of ~4 and 3.3, respectively. There are little previous data regarding HDL-c; Zhang *et al.* [45] reported that decreased TSH level could reduce the risk of low HDL-c levels. An association between hypertriglyceridemia and high-normal TSH levels has been previously reported [17].

Unclear mechanisms may explain the association between TSH within the normal range and lipid profile. TSH might bind to its receptor expressed on hepatocytes to upregulate the expression of hydroxy methyl glutaryl coenzyme A reductase, resulting in increased TC [47]. In addition, TSH has a direct enhancing effect on apoB-lipoproteins that greatly affect plasma cholesterol ester transfer rates and are able to accept cholesteryl esters from HDL [48,49]. TSH levels correlate linearly with circulating proprotein convertase subtilisin/kexin type 9. This protein binds to hepatic LDL receptors and targets them for lysosomal degradation, resulting in impaired clearance of LDL-c. In addition, proprotein convertase subtilisin/kexin type 9 level showed correlations with TC, non-HDL-c, LDL-c, ApoB, and TG levels [50,51].

For the first time, we described the significant positive association of TSH level with SBP and near-significant association with DBP among euthyroid PCOS women; high-normal TSH PCOS women had significant higher SBP and DBP and increased risk of having systolic and diastolic hypertension with ORs of 5 and 3.5, respectively. However, they did not have significant higher percentage of systolic or diastolic hypertension. The relationship between arterial blood pressure and TSH levels within normal range is not clear. Previous studies described positive correlations with either SBP, DBP, or both [17,35,45,52], whereas others did not find any association [53]. Oh *et al.* [17] found that the prevalence of hypertension did not differ between high-normal and low-normal TSH groups. In support to our findings, a study found that high-normal TSH is an independent risk factor for hypertension in middle-aged and elderly Chinese women [14]. The underlying mechanisms for the relationship between high-normal TSH and hypertension may be a common genetic variant, increased arterial stiffness, and endothelial dysfunction. Binding of TSH to its receptors on microvascular endothelial cells attenuates endothelial nitric oxide and prostacyclin expressions [54–56].

Our study showed for the first time, a positive correlation between TSH and fasting plasma glucose (FPG). In addition, high-normal TSH PCOS had significant higher FPG with higher percentage and increased risk (OR=4.3) of impaired FPG. This association can be explained by its stimulatory effect on hepatic glucose production *in vivo* and *in vitro*, possibly by enhancing hepatic gluconeogenesis and inhibiting glycogen synthesis by binding to its receptor [57].

In our study, a significant association between level of TSH with SHBG and androgen levels exist. Women with high-normal TSH had significantly higher FAI and lower SHBG. Our data are in line with Ditrich *et al.* [19] but in contrast with Muller *et al.* 2009 [18]. Our study found that TSH had a significant positive correlation with FAI and a significant negative correlation with SHBG. These results are in line with Sumarac-Dumanovic *et al.* [20]. Ditrich and colleagues reported a correlation among only high-normal TSH PCOS group, whereas El-Hafez *et al.* [32] found a correlation with total testosterone. In contrast, others did not find any correlations ([35]. IR is a common contributing factor for hyperandogenemia and high TSH levels in PCOS women. IR-PCOS women had higher FAI and lower SHBG. Testosterone regulates TSH levels and its receptors [58].

Conclusion

Results of our study are meaningful in clinical practice. We found that high-normal TSH PCOS women had altered clinical, metabolic, and hormonal parameters. They have higher WC, BP, FBS, and marker of IR as well as a less-favorable lipid profile. They have higher percentage and risk of MetS and many of its individual components. The risk and presence of MetS are related to TSH levels rather than PCOS alone. We recommend lowering the upper limit of normal TSH level to 2.5 μIU/ml instead of 4.6 μIU/ml among PCOS women. PCOS women with TSH levels more than 2.85 μIU/ml should be assessed for the presence of the MetS. We did not evaluate IR by the hyperinsulinemic–euglycemic clamp method, which is the gold standard. We used HOMA-IR, which is a less sensitive marker for insulin sensitivity. This was a cross-sectional study which did not address any cause–effect relationships. The small number of participants in our study was another limitation.

Recommendations

We recommended further population-based studies among PCOS women of different age groups as well as among adolescent and postmenopausal women to verify the accuracy of using TSH 2.5 μIU/ml as the upper normal level and TSH of at least 2.85 μIU/ml to detect MetS.

Further longitudinal studies to establish a cause–effect relationship between TSH levels and MetS are required.

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Conflicts of interest
There are no conflicts of interest.

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