CLINICAL STUDY

High prevalence of polycystic ovary syndrome characteristics in girls with euthyroid chronic lymphocytic thyroiditis: a case–control study

Mohd Ashraf Ganie[1], Raman Kumar Marwaha, Rashmi Aggarwal and Satveer Singh

Department of Endocrinology and Thyroid Research Centre, Institute of Nuclear Medicine and Allied Sciences, Lucknow Road, Timarpur, New Delhi 110054, India and [1]Department of Endocrinology, Sheri-Kashmir Institute of Medical Sciences, Srinagar, Jammu and Kashmir 110014, India

(Correspondence should be addressed to R K Marwaha; Email: marwaha_ramank@hotmail.com)

Abstract

Objective: The aim was to find the prevalence of polycystic ovary syndrome (PCOS) phenotype in adolescent euthyroid girls with chronic lymphocytic thyroiditis (CLT).
Design: This was a prospective case–control study as part of an ongoing community-wide thyroid survey in Indian schools.
Methods: One hundred and seventy-five girls with euthyroid CLT and 46 age-matched non-CLT girls underwent clinical, biochemical, hormonal, and ultrasonographic evaluation for diagnosis of PCOS by Rotterdam 2003 criteria. All subjects underwent serum sampling for LH, FSH, testosterone, DHEAS, free thyroxine, TSH, and anti-thyroid peroxidase (TPO) antibodies. Oral glucose tolerance test (OGTT) was undertaken for plasma glucose and insulin.
Results: Significantly higher prevalence of PCOS was noted in girls with euthyroid CLT when compared to their control counterparts (46.8 vs 4.3%, $P=0.001$). The CLT girls had higher body mass index, waist circumference, and systolic blood pressure ($P=0.001$). Mean number of menstrual cycles/year was 8.4 ± 3.5 vs 10.1 ± 1.4, and mean Ferriman–Gallwey score was 11.9 ± 3.5 vs 3.0 ± 2.4 ($P=0.001$) in cases versus controls respectively. The fasting and postprandial glucose and serum cholesterol were also higher in the cases ($P=0.001$). Homeostasis model assessment-insulin resistance was 4.4 ± 4.2 vs 2.3 ± 2.7 in the cases versus controls ($P=0.001$).
Conclusion: Higher prevalence of PCOS characteristics in euthyroid CLT girls when compared to controls suggest possible role of autoimmune phenomenon in the etiopathogenesis of PCOS. Further studies are required to understand the pathogenic link between these two disorders.

European Journal of Endocrinology **162** 1117–1122

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age, affecting about 6.5–6.7% of all premenopausal women (1, 2). The disorder originally described as cystic disease of the ovaries by Stein & Levanthal (3) is now considered to be associated with a barrage of endocrine and metabolic disturbances such as hypertension, impaired glucose tolerance (IGT), type 2 diabetes mellitus, coronary artery disease, increased risk of endometrial and perhaps breast cancer (4). The main endocrine derangements responsible for the clinical manifestations are hyperandrogenemia and abnormal insulin response to glucose (5, 6). Insulin resistance (IR)/hyperinsulinemia in PCOS being the main pathogenic defect is also supported by clinical, biochemical, and hormonal response to insulin sensitizers (7, 8). With adipose tissue being one of the important sources of pro-inflammatory cytokines, IR in obese has been attributed to elevation of adipocytokines and other inflammatory markers. Women with PCOS have been demonstrated to have elevated cytokines like tumour necrosis factor-α (TNF-α) and IL-6 concentrations independent of obesity, and these alterations in serum inflammatory markers in PCOS are unrelated to PPAR-γ variants (9). It has been demonstrated that the therapy with metformin in PCOS leads to an increase in plasma adiponectin and TNF-α but not plasma IL-6 and C reactive protein (CRP) (10). Some authors demonstrated a high prevalence of autoimmune thyroiditis in PCOS women suggesting a potential pathogenic link with autoimmunity/sub-inflammatory cascade (11). Thus, the concept that inflammatory and immune markers may have a role in the pathogenesis of IR and hyperinsulinemia in PCOS has taken a front seat.

Chronic lymphocytic thyroiditis (CLT) is the most prevalent cause of hypothyroidism in areas with sufficient iodine intake, and is characterized by high levels of thyroid autoantibodies, lymphocytic infiltration

of the thyroid gland, and a typical hypoechoic pattern on thyroid ultrasound. High prevalence of CLT in Down's, and Turner's syndromes (12), and its association with the HLA DR3, DR5, and HLA DRB1*1404 genes (13, 14) suggests that CLT may have a genetic contribution. Similarly, the evidence of a strong familial association in the first-degree relatives of PCOS subjects suggests a role of genetics in its etiopathogenesis, and several genes have been identified to date (15–17). Since both these disorders have a similar pathogenic mechanism comprising of familial aggregation, subinflammation/inflammation, and elevated adipocytokines, we speculate that there may be a potential cross-link between the two conditions.

Since no systematic analysis of PCOS phenotype in women with CLT has been undertaken to date, we therefore planned this prospective case–control study to understand the association between these two genetically predisposed inflammatory disorders.

Subjects and methods

Study participants

The study was conducted in 13–18-year-old girls identified during a country-wide survey of thyroid diseases in schools and in those who reported to the outpatient department of our institutes. Informed consent was taken from the parents of all the children studied. The study protocol was approved by the ethics committee of the Institute of Nuclear Medicine and Allied Sciences, New Delhi.

Two hundred consecutive girls with fine-needle aspiration cytology (FNAC) and antibody-proven CLT (cases) were enrolled for the study. These girls were then evaluated for any evidence of PCOS on the basis of Rotterdam 2003 criteria as follows: i) clinical and/or biochemical hyperandrogenism, ii) oligo-anovulation, and iii) polycystic ovaries on ultrasonography (18), after excluding girls with suggestion of nonclassical congenital adrenal hyperplasia, Cushing's syndrome, hyperprolactinemia, androgen-secreting tumors or androgen intake. Forty-six age-matched apparently healthy euthyroid non-CLT girls from the same school survey group were taken as controls. These girls had normal cytology or colloid goiter on FNAC, negative anti-TPO antibody, and normal thyroid echogenicity on ultrasonography (USG). Subjects suffering from any systemic illness and those with a history of receiving any drugs known to interfere with thyroid function, hypothalamo-pituitary axis, insulin sensitivity, or glucose tolerance were excluded from the study.

Methods

The study girls were subjected to detailed history, clinical examination, biochemical, hormonal, cyto-pathological, and sonographic evaluation. FNAC was performed under aseptic conditions by using a 23-G needle, and USG thyroid was done by a single operator who was blinded to the clinical and biochemical status of the subjects. The echogenicity of the thyroid gland was assessed in relation to the surrounding neck muscles, and the brightness gain was adjusted for optimum visualization of the thyroid parenchyma. USG abdomen was done by the same observer to study polycystic ovarian morphology and to rule out any adrenal or ovarian mass lesion. The diagnosis of PCOS on transabdominal USG was based on the presence of >12 peripheral follicles each 2–9 mm in diameter in one or both ovaries, increased ovarian volume (>10 cm) on one or both sides and thecal hyperechogenicity in the mid-follicular phase.

For making a positive diagnosis of PCOS, all these subjects were interviewed to furnish details of menstrual history including age of menarche, regularity, and duration of cycles, number of cycles per year or intermenstrual interval. Oligomenorrhea was defined as intermenstrual interval of ≥ 35 days or a total of ≤ 8 menses per year. Amenorrhea was defined as the absence of menstruation for the last 6 or more months. Quantification of hirsutism was done using modified Ferriman–Gallwey (F–G) score by counting of nine body areas by a single observer with good reproducibility (19). A score of ≥ 8 of 36 was taken as significant.

Investigation

Both case and control subjects underwent oral glucose tolerance test between 0800 and 0900 h with 75-g glucose load after an overnight (10–12 h) fast and a normal diet for at least 3 days prior to testing. The fasting sample was taken in the follicular phase of spontaneous or medroxyprogesterone-induced cycle for baseline biochemistry, anti-TPO antibodies, glucose, lipids, insulin, hormones, and thyroid profile. Samples for plasma glucose and insulin were also collected 2 h after the glucose load. Plasma glucose was assayed by enzymatic calorimetric method (GOD-POD, Roche). Hemogram, renal, and liver function tests were done for both controls and CLT subjects. Hormonal evaluation included LH, FSH, prolactin, testosterone, DHEAS, serum cortisol (0800/1600 h or after overnight dexamethasone suppression test, in cases diurnal rhythm was reversed or serum cortisol levels were raised), and 17-hydroxyprogesterone (17-OHP) estimations. Serum 17-OHP levels <4.8 ng/ml were taken as normal, and ACTH stimulation was done if the levels were from 4.8 to 10 ng/ml. Free thyroxine (FT_4), TSH, anti-TPO antibody, and insulin were estimated by electrochemiluminescence assay (Elecsys Roche Diagnostics) and LH, FSH by IRMA (Radium, Italy). Serum cortisol, serum testosterone, DHEAS, 17-OHP, and prolactin were assayed by RIA (Radium, Italy). The analytical sensitivity, intra-assay and inter-assay

coefficients of variation were within the manufacturer's prescribed limits. Transabdominal ultrasound was performed using a 3.5 MHz transducer (HDI-5000 Phillips, Bothel, WA, USA) and thyroid USG using portable equipment with 7.5 MHz transducer (Aloka SSD-500, Tokyo, Japan). Thyroid gland was considered hypoechogenic when its signal was equal or below that of the surrounding neck muscles.

Calculations

Glucose intolerance was graded according to the ADA guidelines, and homeostasis model assessment-IR (HOMA-IR) was calculated as follows: fasting insulin (mU/l)×fasting glucose (mmol/l)/22.5 (20). Anti-TPO antibodies were considered positive when the levels were above 34 U/ml. Biochemical hyperandrogenism was defined as a serum testosterone >0.65 ng/ml.

Statistical analysis

The statistical analysis was performed with SPSS version 11.5 (Chicago, IL, USA). The quantitative variables have been described as mean±s.d. To compare quantitative variables between two groups, unpaired *t*-test was used. The χ^2 test/Fischer's exact test was used to compare the qualitative variables. Comparison between controls, PCOS, and non-PCOS subgroups of CLT girls was done by ANOVA. FT_4 quartiles were correlated with the presence of PCOS components using Pearson's correlation. $P < 0.05$ was considered significant.

Results

Of the 200 euthyroid CLT girls recruited for the study, only 175 girls, whose data were complete, were analyzed. The clinical, biochemical, and hormonal parameters of the study and control subjects are presented in Tables 1 and 2. Diagnosis of PCOS by Rotterdam 2003 criteria was established in 82/175 girls (46.85%) with proven CLT and 2/46 (4.32%) in controls. Since the diagnosis of PCOS in adolescent girls is considered to be relatively difficult (21), we therefore adopted more stringent criteria recommended by Sultan & Paris (22) and found a similar prevalence of PCOS (45.9%) in girls with CLT. Mean anti-TPO antibody levels as expected were significantly higher in subjects than in controls (321.4±189.6 vs 22.5±7.2, $P = 0.001$). The average number of menstrual cycles/year was significantly lower in cases as compared to controls (8.4±3.5 vs 10.1±1.4, $P = 0.001$). Among the CLT group, ten patients had spells of amenorrhea, and four others had more than eight cycles/year but their cycles were grossly irregular. Mean F–G score was 11.9±3.5 vs 3.0±2.4 ($P = 0.001$). F–G score of more than eight was present in 111/175 (63.42%) of cases as compared to 3/46 (6.52%) controls. Acne vulgaris affected 24/175 (13.71%) cases with grade I in 9.7% and grade II in 4% of cases. Hypertension, defined as BP≥135/85 mmHg, was present in 22/175 (12.57%) of cases and none of the controls.

The girls with CLT had significantly higher systolic blood pressures, higher body mass index (BMI), and wider waist circumference ($P = 0.001$, 0.001, and 0.02 respectively). The fasting and postprandial plasma glucose and serum cholesterol were also higher in the CLT girls than in controls ($P = 0.001$, 0.001, and 0.001 respectively). Fourteen cases (8%) had impaired fasting plasma glucose, 13 (7.4%) had IGT, and only one patient had diabetes mellitus. The serum LH/FSH ratio, serum testosterone, and fasting insulin were also significantly higher in the cases than in the controls ($P = 0.003$, 0.001, and 0.005 respectively). More than 18.85% (33/175) cases and 4.34% (2/46) controls had an elevated (≥2) LH/FSH ratio. Serum total testosterone >0.65 ng/ml was observed in 48.57% (85/175) cases and 10.86% (5/46) controls. However, when the serum testosterone cut-off was taken as 0.7 ng/ml, the respective observations were 45.7% in cases and 9.98%

Table 1 Comparison of clinical characteristics of cases versus control girls.

Characteristics	Cases $n=175$ Mean±s.d. (range)	Controls $n=46$ Mean±s.d. (range)	P value
Age (years)	14.70±1.49 (13–18)	14.75±1.08 (13–18)	0.83
Age of menarche (years)	12.4±1.0 (9–16)	11.8±0.7 (11–13)	0.002
No. of menstrual cycles/year	8.4±3.5 (4–13)	10.1±1.4 (8–12)	0.001
Ferriman–Gallwey score	11.9±3.5 (4–24)	3.0±2.4 (0–7)	0.001
Height (cm)	151.7±4.4 (137–165)	150.0±4.1 (140–159)	0.02
Weight (kg)	64.7±12.3 (38–96)	57.9±8.4 (38–76)	0.01
BMI (kg/m²)	28.1±5.1 (16.2–39.3)	25.8±3.7 (17.2–33.3)	0.001
Waist circumference (cm)	75.1±7.9 (60–88)	71.4±13.6 (42–101)	0.02
Hip circumference (cm)	85.7±8.0 (71–98)	80.8±14.1 (49–110)	0.08
WH ratio	0.88±0.06 (0.72–1.08)	0.88±0.12 (0.63–1.23)	0.09
SBP (mmHg)	118.8±9.8 (90–148)	110.5±5.9 (100–120)	0.001
DBP (mmHg)	73.8±8.4 (56–110)	73.9±8.0 (61–86)	0.41

Table 2 Comparison of biochemical and hormonal characteristics of cases versus control girls.

Characteristics (normal range)	Cases $n=175$ Mean ± s.d. (range)	Controls $n=46$ Mean ± s.d. (range)	*P* value
BGF (<100 mg/dl)	81.4 ± 13.7 (53–127)	69.1 ± 5.9 (56–84)	0.01
BGPP (<140 mg/dl)	107.1 ± 119.4 (78–187)	91.5 ± 7.1 (76–105)	0.001
Cholesterol (150–200 mg/dl)	197.4 ± 30.1 (78–283)	148.8 ± 24.8 (89–198)	0.001
Free T_4 (nmol/dl)	15.7 ± 2.8 (10.2–25.5)	15.9 ± 3.0 (11.9–22.8)	0.44
TSH (0.5–5.5 IU/ml)	2.4 ± 1.4 (0.03–7.0)	3.0 ± 1.0 (1.3–5.3)	0.06
Anti-TPO (0–35 IU/l)	321.4 ± 189.6 (5–600)	22.5 ± 7.2 (8–33)	0.001
LH (7–22 IU/l)	11.5 ± 4.6 (2.5–33.4)	8.2 ± 3.0 (4.11–21.3)	0.001
FSH (7–22 IU/l)	6.2 ± 2.9 (1.9–17.2)	7.2 ± 4.3 (2.01–21.36)	0.128
LH/FSH (<2)	2.4 ± 1.6 (0.18–10.2)	1.6 ± 1.2 (0.12–5.17)	0.003
Testosterone (0.12–70 ng/ml)	0.8 ± 0.5 (0.23–1.99)	0.5 ± 0.2 (0.21–0.82)	0.001
DHEAS (1.2–3.6 ng/ml)	4.2 ± 4.7 (0.91–22)	1.6 ± 0.5 (0.4–3.55)	0.001
Cortisol (μg/dl) (morning)	14.1 ± 3.9 (4.17–27.7)	8.1 ± 5.6 (0.51–18.5)	0.001
Prolactin (μg/dl)	10.9 ± 4.9 (3.32–31.7)	9.2 ± 4.9 (1.92–19.6)	0.06
Insulin fasting (μIU/l)	21.4 ± 19.6 (3.4–132)	13.0 ± 14.3 (1.2–63.7)	0.005
HOMA (<2)	4.4 ± 4.2 (0.71–27.4)	2.3 ± 2.7 (0.23–13.2)	0.001

in controls, which were statistically insignificant ($P=0.29$ among cases and 0.50 among controls).

IR as calculated by HOMA-IR was 4.4 ± 4.2 and 2.3 ± 2.7 in cases and controls respectively ($P=0.001$), and consequently, HOMA-IR ≥ 2 was observed in a significantly higher percentage of cases than of controls (91/175 (52%) vs 9/46 (19.56%) ($P=0.001$)). Though fasting serum insulin ≥ 14 μIU/ml was present in 94/175 (53.71%) cases when the fasting insulin cutoff was brought down to 10 μIU/ml, a significantly higher number of subjects (146/175 (83.42%)) qualified for the presence of IR. Number of menstrual cycles, hirsutism score, serum testosterone, fasting and postprandial glucose correlated positively with fasting serum insulin and HOMA-IR. Even after adjusting for BMI, the number of menstrual cycles per year (8.64 ± 3.4 vs 9.94 ± 1.5; $P=0.03$), F–G score (10.71 ± 2.8

vs 3.0 ± 2.4; $P=0.001$), serum total testosterone (0.7 ± 0.38 vs 0.48 ± 0.18 ng/ml; $P=0.002$), fasting serum insulin (21.01 ± 18.3 vs 13.02 ± 13.8 μIu/ml; $P=0.02$) and HOMA-IR values (4.12 ± 3.89 vs 2.26 ± 2.51; $P=0.009$) were significantly different in cases when compared to controls.

On categorization of CLT girls based on Rotterdam 2003 criteria, two distinct subgroups (PCOS versus non-PCOS) emerged whose clinical, biochemical, and endocrine parameters differed both within themselves and from the control group (Table 3). On correlating the serum FT_4 levels with various components of PCOS, FT_4 quartiles showed a significant inverse correlation with PCOS components ($r=-0.46$, $P=0.05$). Thirteen PCOS girls (7.4%) had their serum FT_4 in the highest quartile, whereas 27 (15.42%) non-PCOS girls had the serum FT_4 in this quartile (Fig. 1).

Table 3 Comparison of clinical, biochemical, and hormonal parameters of polycystic ovary syndrome (PCOS) and non-PCOS subsets of chronic lymphocytic thyroiditis (CLT) girls with controls.

Characteristics	PCOS $n=82$ Mean ± s.d. (range)	Non-PCOS $n=93$ Mean ± s.d. (range)	Controls $n=46$ Mean ± s.d. (range)
Age (years)	15.7 ± 2.8 (10.2–25.5)	14.72 ± 1.57 (11–18)[d]	15.9 ± 3.0 (11.9–22.8)[e,c]
Ferriman–Gallwey score	13.42 ± 2.5 (8–21)	10.21 ± 3.53 (4–24)[a]	03.0 ± 2.4 (0–7)[b,c]
BMI (kg/m^2)	29.89 ± 4.04 (21–39)	27.03 ± 4.92 (16–39)[a]	25.8 ± 3.7 (17.2–33.3)[b,c]
Waist circumference (cm)	74.21 ± 12.29 (52–98)	68.13 ± 12.22 (42–99)[a]	75.1 ± 7.9 (60–88)[d,c]
Free T_4 (nmol/dl)	15.17 ± 2.67 (10–22)	16.20 ± 3.0 (11–25)[c]	15.9 ± 3.0 (11.9–22.8)[d,e]
BGF (<100 mg/dl)	85.38 ± 12.67 (64–112)	77.69 ± 13.99 (53–127)[a]	69.1 ± 5.9 (56–84)[b,c]
Cholesterol (150–200 mg/dl)	209.26 ± 27.17 (142–276)	190.87 ± 26.26 (141–283)[a]	148.8 ± 24.8 (89–198)[b,c]
Triglycerides (70–150 mg/dl)	138.94 ± 28.56 (78–198)	128.71 ± 26.80 (86–190)[a]	108.23 ± 20.18 (80–171)[b,c]
HDL (<50 mg/dl)	39.07 ± 4.74 (30–51)	38.58 ± 4.67 (26–48)[d]	41.58 ± 6.46 (30–53)[e,c]
LDL (70–100 mg/dl)	112.04 ± 17.16 (74–162)	104.47 ± 14.51 (82–164)[a]	99.15 ± 12.12 (80–151)[b,c]
Testosterone (0.12–0.7 ng/ml)	0.84 ± 0.52 (0.22–1.93)	0.69 ± 0.38 (0.23–1.80)[a]	0.5 ± 0.2 (0.21–0.82)[b,c]
DHEAS (1.2–3.6 ng/ml)	4.70 ± 0.62 (0.54–4.88)	3.66 ± 0.48 (0.39–2.92)[d]	1.6 ± 0.5 (0.4–3.55)[e,f]
Cortisol (μg/dl) (morning)	13.97 ± 3.62 (5.78–21.97)	14.74 ± 4.28 (4.17–27.73)[d]	8.1 ± 5.6 (0.51–18.5)[b,c]
Insulin fasting (μIU/l)	24.21 ± 23.20 (3.50–113.9)	20.29 ± 19.23 (4.3–132.4)[d]	13.0 ± 14.3 (1.2–63.7)[b,c]
HOMA (<2)	5.28 ± 5.54 (0.71–27.4)	3.84 ± 3.36 (0.91–22.23)[a]	2.3 ± 2.7 (0.23–13.2)[b,c]

[a,d]Comparison between PCOS versus non-PCOS. [b,e]Comparison between PCOS versus controls. [c,f]Comparison between non-PCOS versus controls. Significance (*t*-test for independence). [a,b,c]$P \leq 0.05$, [d,e,f]$P \geq 0.05$.
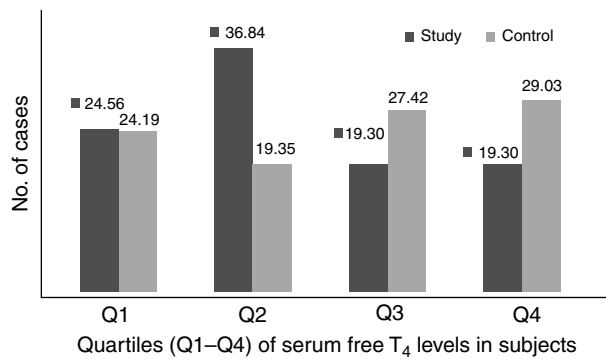
Figure 1 Case distribution of CLT girls versus controls among various quartiles of serum FT_4.

Discussion

PCOS and IR are reported to be on the rise in the Asian subcontinent, even though systemic studies on this issue are awaited. We have previously reported high prevalence of juvenile autoimmune thyroiditis in young and adolescent girls from India (23). Based on our preliminary observation of high prevalence of PCOS phenotype in these CLT girls during nationwide thyroid surveys and reported high prevalence of thyroid autoimmunity in subjects with established PCOS earlier (11), we undertook this first prospective study to evaluate the association of PCOS with thyroid autoimmunity.

Significantly higher prevalence of PCOS phenotype in CLT girls as compared to controls was noted in the present study for the first time to the best of our knowledge by both Rotterdam 2003 and Sultan & Paris criteria (46.85 vs 4.2%, $P = 0.001$). This observation lends indirect support to the findings of Janssen *et al.* (11) who conversely reported elevated thyroperoxidase or thyroglobulin antibodies in 26.9% of PCOS subjects in contrast to 8.3% in controls. Besides, they also observed that 42.3% of PCOS subjects had hypoechoic tissue pattern typical of autoimmune thyroiditis on USG in comparison to 6.5% in controls ($P < 0.001$). Familial aggregation and genetic predisposition of these two apparently diverse disorders along with a recent study demonstrating high prevalence of serologic parameters of autoimmunity (anti-histone and anti-dsDNA antibodies) in women with PCOS may suggest the role of autoimmunity in the pathogenesis of PCOS (13–17, 24). Similar findings supporting a potential link between PCOS and autoimmunity have been reported by two other groups (25, 26).

The association of IR and hyperinsulinemia of PCOS with elevation of various cytokines such as IL-4, IL-6, TNF-α, and their alteration after treatment with insulin sensitizers is well established (9, 10). Elevated fasting insulin and HOMA-IR, indicative of significantly higher IR in both of our PCOS and non-PCOS sub-groups of CLT girls when compared to controls, may indicate

some pathogenic link between autoimmunity and IR (12, 13). Similarly, higher levels of fasting blood glucose, serum cholesterol and testosterone in non-PCOS subgroup of CLT girls as compared to controls also suggest some commonality in the etiopathogenesis of CLT and PCOS. The non-PCOS subgroup of CLT may be a mild pathophysiologic equivalent of PCOS, and may eventually evolve into fully-fledged PCOS.

Obesity is known to be responsible for IR and associated hyperinsulinemia in women with PCOS, and obese PCOS women have more severe hyperandrogenism and related clinical features (such as hirsutism, menstrual abnormalities, and anovulation) than normal-weight PCOS women (27). Even after BMI adjustment, oligomenorrhea, hirsutism score, serum testosterone, serum insulin, and HOMA values remained significantly high in the cases compared to controls, suggesting that the PCOS pathophysiology in these cases is not due to obesity alone. However, obesity may account for some exaggeration of their clinical phenotype.

Previous data on the interaction of sex steroids and immune regulation have shown that androgens and progesterone can have inhibitory effects and estrogens, a facilitatory effect on humoral immunity. There is evidence that activating immune/inflammatory response is exerted through nuclear factor (NF)κB pathway (28). Though normal to high estrogen coupled with low progesterone milieu in PCOS girls may be considered conducive for the development of autoimmunity, it is difficult to explain the role of hyperandrogenemia. The apparent paradox of positive correlation between anti-TPO antibodies and serum total testosterone observed in our study suggests that the mild hyperandrogenemia typical of PCOS may not be dominant enough to ameliorate autoimmunity. Whether this peculiar hormonal milieu of PCOS is responsible for autoimmunity of CLT (11) or vice versa needs to be pursued in future research.

Hypothyroidism is known to induce a phenotype similar to PCOS or produce features suggestive of metabolic syndrome. The interesting observation of inverse correlation of serum FT_4 quartiles with various components of PCOS phenotype in euthyroid CLT girls also lends indirect support to association between PCOS and CLT.

We conclude that the CLT girls have high prevalence of clinical and metabolic derangements suggestive of PCOS as compared to normal age-matched girls. Whether autoimmune thyroiditis predisposes subjects to develop PCOS characteristics or if PCOS is a forerunner of autoimmune thyroiditis, remains an unanswered speculation and mandates follow-up longitudinal studies.

Declaration of interest

Funding

Acknowledgements

References

1 Diamanti-Kandarakis E, Kouli CR, Bergiele AT, Filandra FA, Tsianateli C, Spina GG, Zapanti ED & Bartzis MI. A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 4006–4011.

2 Asuncion M, Calvo RM, San Millan JL, Sancho J, Avila S & Escobar-Morreale HF. A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 2434–2438.

3 Stein IF & Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. *American Journal of Obstetrics and Gynecology* 1935 **29** 181–191.

4 Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. *Endocrine Reviews* 1997 **18** 774–800.

5 Goodarzi MO & Korenman SG. The importance of insulin resistance in polycystic ovary syndrome. *Fertility and Sterility* 2003 **80** 255–258.

6 Kulshreshtha B, Ganie MA, Praveen EP, Gupta N, Khurana ML, Seith A, Dwivedi SN, Kumar G & Ammini AC. Insulin response to oral glucose in healthy, lean young women and patients with polycystic ovary syndrome. *Gynecological Endocrinology* 2008 **24** 637–643.

7 Nieuwenhuis-Ruifrok AE, Kuchenbecker WK, Hoek A, Middleton P & Norman RJ. Insulin sensitizing drugs for weight loss in women of reproductive age who are overweight or obese: systematic review and meta-analysis. *Human Reproduction Update* 2009 **15** 57–68.

8 Ganie MA, Khurana ML, Eunice M, Gupta N, Gulati M, Dwivedi SN & Ammini AC. Comparison of efficacy of spironolactone with metformin in the management of polycystic ovary syndrome: an open-labeled study. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 2756–2762.

9 Tarkun I, Cetinarslan B, Türemen E, Cantürk Z & Biyikli M. Association between circulating tumor necrosis factor-alpha, interleukin-6, and insulin resistance in low grade inflammatory serum markers in patients with polycystic ovary syndrome (PCOS) and their relationship to PPAR gamma gene variants. *Experimental and Clinical Endocrinology & Diabetes* 2008 **116** 481–486.

10 Jakubowska J, Bohdanowicz-Pawlak A, Milewicz A, Szymczak J, Bednarek-Tupikowska G & Demissie M. Plasma cytokines in obese women with polycystic ovary syndrome, before and after metformin treatment. *Gynecological Endocrinology* 2008 **24** 378–384.

11 Janssen OE, Mehlmauer N, Hahn S, Offner AH & Gärtner R. High prevalence of autoimmune thyroiditis in patients with polycystic ovary syndrome. *European Journal of Endocrinology* 2004 **150** 363–369.

12 Idris I & O'Malley BP. Thyrotoxicosis in Down's and Turner's syndromes: the likelihood of Hashimoto's thyroiditis as the underlying etiology. *International Journal of Clinical Practice* 2000 **54** 272–273.

13 Marwaha RK, Sen S, Tandon N, Sahoo M, Walia RP, Singh S, Ganguly SK & Jain SK. Familial aggregation of autoimmune thyroiditis in first degree relatives of patients with juvenile autoimmune thyroid disease. *Thyroid* 2003 **13** 299–302.

14 Kanga U, Tandon N, Marwaha RK, Goswami D, Gupta N, Ray D & Mehra NK. Immunogenetic association and thyroid auto-antibodies in juvenile autoimmune thyroiditis in North India. *Clinical Endocrinology* 2006 **64** 573–579.

15 Crosignani PG & Nicolosi AE. Polycystic ovarian disease: heritability and heterogeneity. *Human Reproduction Update* 2001 **7** 3–7.

16 Franks S, Gharani N & McCarthy M. Candidate genes in polycystic ovary syndrome. *Human Reproduction Update* 2001 **7** 405–410.

17 Urbanek M, Legro RS, Driscoll DA, Azziz R, Ehrmann DA & Norman RJ. Thirty-seven candidate genes for polycystic ovary syndrome: strongest evidence for linkage is with follistatin. *PNAS* 1999 **96** 8573–8578.

18 The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Human Reproduction* 2004 **19** 41–47.

19 Ferriman D & Gallwey JD. Clinical assessment of body hair growth in women. *Journal of Clinical Endocrinology and Metabolism* 1961 **21** 1440–1447.

20 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF & Turner RC. Homeostasis model assessment (HOMA): insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985 **28** 412–419.

21 Diamanti-Kandarakis E. PCOS in adolescents. *Best Practice & Research. Clinical Obstetrics & Gynaecology* 2010 **24** 173–183.

22 Sultan C & Paris F. Clinical expression of polycystic ovary syndrome in adolescent girls. *Fertility and Sterility* 2006 **86** S6.

23 Marwaha RK, Tandon N, Karak AK, Gupta N, Verma K & Kochupillai N. Hashimoto's thyroiditis: countrywide screening of Goitrous healthy young girls in post-iodization phase in India. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 3798–3802.

24 Hefler-Frischmuth K, Walch K, Huebl W, Baumuehlner K, Tempfer C & Hefler L. Serologic markers of autoimmunity in women with the polycystic ovary syndrome. *Fertility and Sterility* 2009 **92** e59–e60.

25 Van Gelderen CJ & Gomes dos Santos ML. Polycystic ovarian syndrome. Evidence for an autoimmune mechanism in some cases. *Journal of Reproductive Medicine* 1993 **38** 381–386.

26 Fenichel P, Gobert B, Carre Y, Barbarino-Monnier P & Hieronimus S. Polycystic ovary syndrome in autoimmune disease. *Lancet* 1999 **353** 2210.

27 Gambineri A, Pelusi C, Vicennati V, Pagotto U & Pasquali R. Obesity and the polycystic ovary syndrome. *International Journal of Obesity and Related Metabolic Disorders* 2002 **26** 883–896.

28 Cutolo M, Sulli A, Capellino S, Villaggio B, Montagna P, Seriolo B & Straub RH. Sex hormones influence on the immune system: basic and clinical aspects in autoimmunity. *Lupus* 2004 **13** 635–638.