Anthropometric, hormonal and biochemical indices in patients with polycystic ovarian syndrome

Manal T Mutib[1], Farqad B Hamdan[2], Anam R. Al-Salihi[2]
1- High Institute of Infertility Diagnosis and Assisted Reproductive Technology, Al-Nahrain University. 2- Department of Physiology, College of Medicine, Al-Nahrain University. Corresponding Author E-mail: manaltaha2001@yahoo. com

Abstract:

Background:
Polycystic ovary syndrome is the most common, yet complex, endocrine disorder with features of anovulation, amenorrhea, ovulation-related infertility, polycystic ovaries, and obesity. Number of metabolic aberrations is now well recognized as a growing public health problem in PCOS as glucose intolerance, hyperinsulinemia and dyslipidemia.

Objective:
To investigate and analyze the anthropometric, hormonal profile and metabolic abnormalities of obese and non-obese women with this disorder.

Materials and Methods:
Sixty-five healthy Iraqi women serves as the control group and eighty-four infertile women divided into two subgroups depending on the body mass index (BMI < >30kg/m2) were studied. BMI, waist to hip ratio and waist to thigh ratio were measured. Serum levels of luteinizing hormone, follicle stimulating hormone, estradiol, testosterone, fasting blood glucose, and oral glucose tolerance test, and total lipid profile were measured.

Results:
BMI, waist/hip, waist/thigh ratio were significantly higher in PCOS than control women. Lower FSH level, higher LH and E2 levels, LH/FSH ratio, and lower E2/testosterone ratio was found in obese and non-obese PCOS. Higher blood sugar level, high cholesterol, triglyceride, LDL, VLDL and lower HDL levels in PCOS compared to control women.

Conclusion:
Obesity is a common finding of women with PCOS, but it is not part of the diagnostic criteria. Women with PCOS usually have the so-called central obesity (Visceral adiposity), and therefore tend to have an increased waist-hip ratio (WHR) and waist to thigh ratio, regardless of the weight factor (i. e. , in obese and non-obese). PCOS patients exhibited abnormal hormonal status and biochemical indices also regardless of obesity.

Keywords: PCOS, obesity, hormones, GTT, lipid profile

Introduction

Polycystic ovary syndrome (PCOS) is the most common, yet complex, endocrine disorder affecting women in their reproductive years. It affects between 6% and 8% of women worldwide(1), such that it can be considered one of the most common disorders of humans, and the single most common endocrine abnormality of women at reproductive age.

The exact etiology of PCOS is unknown; observation of an increased prevalence of PCOS among family members as compared to the general population favored the hypothesis that, at the basis of this syndrome, a genetic component may exist whose inheritance is still a matter of controversy. However, the heterogeneous clinical characteristics of this syndrome indicate that a more complex interaction between genetic and environmental factors may cause this disorder(2).

The principal features of PCOS are anovulation, resulting in irregular menstruation, amenorrhea, ovulation-related infertility, and polycystic ovaries; abnormal pituitary hormonal profile, excessive amounts or effects of androgenic (masculinizing) hormones, resulting in acne and hirsutism(3) ; reduced level of sex hormone binding globulin (SHBG) and often high level of prolactin(4).

Obesity is observed in up to 60% of patients with PCOS(5), mostly visceral or abdominal adiposity frequently noted, with an increase of the waist-to-hip ratio. It is often an inciting factor in the development of menstrual dysfunction and cutaneous signs of hyperandrogenism(6) ; also associated with extremity wasting, purple striae, easy bruisability, moon faces, and rubor. Most women with PCOS will relate a history of a sudden easily gain weight and lose it only with great effort(7). Number of metabolic aberrations is now well recognized as a growing public health problem in PCOS, there will be increase risk central obesity, hypertension, glucose intolerance, hyperinsulinemia(8,9), and dyslipidemia(10,11).

The metabolic abnormalities of PCOS imply an increased risk for infertility, dysfunctional bleeding, endometrial carcinoma(12), type 2 diabetes (T2DM) and cardiovascular disease. More evidences have shown that insulin resistance is an underlying pathophysiologic defect in PCOS(13).

The intention of our study is to assess the anthropometric, hormonal and biochemical (GTT, lipid profile) between PCOS and control women and between obese and non-obese groups.

Subjects and methods

Sixty-five healthy Iraqi women serves as the control group and eighty-four infertile women with PCOS were selected from those who attend the Higher Institute of Infertility Diagnosis and Assisted Reproductive Technologies. The study was approved by the Local Medical Ethical Committee of the College of Medicine, Al-Nahrain University, and written consent was obtained from patients or their surrogates to participate in the study.

PCOS fulfilling at least two of the following three criteria based on the Rotterdam ESHRE/ASRMS sponsored PCOS consensus workshop group(13):

1. Clinical or biologic Hyperandrogenism.
2. Chronic anovulation or oligovulation.
3. Polycystic ovaries on ultrasound.

The PCOS patients and control women were divided into two subgroups according to their BMI:

- Obese PCOS: includes 34 Obese PCOS (BMI ≥30 kg/m2) with an age range between 22 years and 39 years (mean ± SD = 29.67 ± 4.23).
- Non-obese PCOS: comprised of 50 non- obese PCOS (BMI <30 kg/m2) with an age range between 22 years and 40 years (mean ± SD = 28.83 ± 4.43).
- Obese control women: included 22 obese women (BMI ≥30 kg/m2) with an age range between 23 years and 36 years (mean ± SD = 33.85 ± 2.83).
- Non-obese control women: consist of 43 non obese women (BMI <30 kg/m2) with an age range between 23 years and 39 years (mean ± SD = 25.53 ± 1.9).

Body mass index (BMI):

The BMI was measured by dividing the weight in kilograms by the height in squared meters (kg/m2).

Waist to hip ratio

This ratio was determined by using measuring tape to determine the circumference of hips at the widest part of the buttocks. Waist circumference was measured at the level midway between the lowest rib margin and the iliac crest. The ratio calculated by dividing the waist measurement by hip measurement.

Waist to thigh ratio

Waist-to-thigh ratio (WTR) was calculated by dividing the waist circumference by the thigh circumference that is measured directly below the gluteal fold.

Biochemical tests

-Hormonal assay: Hormonal analysis was performed by using Addendum-Mini VIDAS apparatus (VIDAS), through an enzyme linked fluorescent assay (ELFA) technique.

-Glucose tolerance test (GTT): This test was performed using spectrophotometer (Cecil CE 1011, UK) by measuring blood sugar following 14 hours fasting; then the patient take 75 gm of glucose and repeats the blood glucose measurement half hour, one hour and two hours then after.

Lipid profile

The cholesterol, triglyceride, and high density lipoprotein were measured by spectrophotometer at 500 nm absorbance (using cholesterol kit, Biomaghreb Company-Tunisia, and triglyceride and HDL kits, Linear chemical Company-Spain). The low density lipoprotein is estimated by using the following equation:

LDL = Cholesterol – (VLDL + HDL)

The very low density lipoprotein is estimated by using the following equation: VLDL = Triglyceride /5.

Statistical analysis

Statistical analysis were done using student T-test with p value less than 0.05 considered to be significant and those less than 0.01 was highly significant.

Results

PCOS patients versus Control subjects

Table 1 illustrates the demographic data of the PCOS patients and control women. No significant difference in the age was observed between the two groups. The BMI, waist to hip ratio and waist to thigh ratio of PCOS were significantly higher when compared the values of the control group ($P < 0.05$; $P < 0.05$; $P < 0.01$, respectively).

Concerning the hormonal status, significantly lower FSH level, higher LH level and LH/FSH ratio, higher testosterone level and lower E2/testosterone ratio ($P<0.01$) were noticed in PCOS patients in comparison to that of the control group, in addition, higher E2 level ($P<0.05$) was demonstrated between the two groups.

With regard to the glucose tolerance test (GTT), it was demonstrated that fasting blood sugar (FBS) level and blood sugar measured after half, one and two hours were significantly higher in PCOS patients with respect to that of the control group {$P < 0.05$ (for FBS) ; $P < 0.01$}.

With reference to the lipid profile, in PCOS patients, the cholesterol and LDL; the triglyceride and VLDL levels were significantly higher ($P < 0.01$; $P < 0.05$, respectively), whereas the HDL was significantly lower ($P < 0.01$) in comparison to the control group.

Table 1. Comparison of demographic parameters between PCOS and control women

Parameters	Control Women N = 65 (mean ± SD	Control Women N = 65 (mean ± SD)
Age (yrs)	29.02 ± 4.50	30.31 ± 3.71
BMI (kg/m2)	29.9 ± 5.13	28.10 ± 4.51*
Waist/ Hip Ratio	0.82 ± 0.07	0.80 ± 0.06*
Waist/thigh ratio	1.42 ± 0.18	1.33 ± 0.11**
FSH (IU/ml)	5.35 ± 1.55	7.22 ± 2.32**
LH (IU/ml)	6.24 ± 3.00	3.97 ± 1.55**
LH/FSH	1.20 ± 0.52	0.56 ± 0.15**
E2 (pg/ml)	60.27 ± 19.68	50.28 ± 18.90*
Testosterone (ng/ml)	0.79 ± 0.44	0.26 ± 0.15**
E2/Testosterone	103.22 ± 58.34	230.62 ± 132.57**
FBS (mg/dl)	94.81 ± 15.30	87.07 ± 12.78*
BS (after 12/ hr.) (mg/dl)	148.53 ± 36.84	133.08 ± 18.77**
BS (after 1 hr) (mg/dl)	142.79 ± 33.16	124.63 ± 17.39**
BS (after 2 hr) (mg/dl)	112.71 ± 24.32	99.12 ± 11.97**
Cholesterol (mg/dl)	163.70 ± 30.72	140.92 ± 17.87**
Triglyceride (mg/dl)	124.68 ± 33.51	111.25 ± 23.09*
VLDL (mg/dl)	24.85 ± 6.76	22.25 ± 4.62*
LDL (mg/dl)	98.90 ± 29.36	77.98 ± 17.36**
HDL (mg/dl)	39.11 ± 2.49	40.73 ± 2.30**

* = P< 0.05, ** = < 0.01, PCOS = polycystic ovary syndrome, FBS = fasting blood sugar, BS = blood sugar, VLDL = very low density lipoprotein, LDL = low density lipoprotein, HDL = high density lipoprotein

Obese PCOS patients versus obese control women

As demonstrated in table 2, no significant difference in the age and BMI of obese PCOS patients and obese control women was noticed, whereas the waist to hip and waist to thigh ratio were significantly higher in the obese PCOS patients when compared to the obese control women ($P < 0.01$).

Significantly higher LH level and LH/FSH ratio ($P<0.01$) ; E2 and testosterone levels ($P<0.05$), while lower E2/testosterone ratio ($P<0.05$) was identified in obese PCOS patients in comparison to the control subjects. Moreover, the FSH level was decreased but to insignificant level. With regard to the blood sugar measured after half, one hour and two hours, they were increased significantly ($P < 0.01$) while the FBS level was not different between obese PCOS

patients and obese control women. Furthermore, the cholesterol and LDL; the triglyceride and VLDL levels were significantly elevated ($P < 0.01$; $P < 0.05$, respectively) whereas the HDL level was lower in obese PCOS patients when compared to obese control women ($P < 0.01$).

Table 2. Comparison of demographic parameters between obese PCOS and obese control women

Parameters	Control Women N = 65 (mean ± SD	Control Women N = 65 (mean ± SD)
Age (yrs)	29.67 ± 4.23	30.64 ± 3.74
BMI (kg/m2)	35.22 ± 3.46	33.85 ± 2.83
Waist/ Hip Ratio	0.87 ± 0.06	0.8 ± 0.06**
Waist/thigh ratio	1.55 ± 0.14	1.36 ± 0.11**
FSH (IU/ml)	5.08 ± 0.98	6.62 ± 3
LH (IU/ml)	6.51 ± 2.96	3.88 ± 1.44**
LH/FSH	1.38 ± 0.55	0.6 ± 0.15**
E2 (pg/ml)	68.71 ± 21.09	52.54 ± 21.25*
Testosterone (ng/ml)	0.85 ± 0.44	0.28 ± 0.08**
E2/Testosterone	101.69 ± 54.96	183.9 ± 69.1**
FBS (mg/dl)	99.44 ± 15.74	90.45 ± 12.22
BS (after 12/ hr.) (mg/dl)	160.63 ± 23.92	134.45 ± 22.93**
BS (after 1 hr) (mg/dl)	153.81 ± 25.47	129.38 ± 18.05**
BS (after 2 hr) (mg/dl)	124.26 ± 23.21	102.33 ± 12.4**
Cholesterol (mg/dl)	181.31 ± 31.59	145.69 ± 12.11**
Triglyceride (mg/dl)	141.86 ± 32.02	120.47 ± 24.63*
VLDL (mg/dl)	28.36 ± 6.39	24.17 ± 4.81*
LDL (mg/dl)	114.29 ± 30.09	83.05 ± 13.25**
HDL (mg/dl)	38.29 ± 2.24	40.23 ± 1.3**

* = P< 0.05, ** =< 0.01, PCOS = polycystic ovary syndrome, FBS = fasting blood sugar, BS = blood sugar, VLDL = very low density lipoprotein, LDL = low density lipoprotein, HDL = high density lipoprotein

Non-obese PCOS patients versus non-obese control women

Table 3 exemplify that no significant difference was declared in the age and waist to thigh ratio between non-obese PCOS patients and non-obese control women while the BMI and waist to hip significantly higher in non-obese PCOS ($P < 0.05$). Siignificant increment in the LH and testosterone levels, LH/FSH and E2/testosterone ratios and significant decrement in the FSH level was observed in the non-obese PCOS patients as compared to the non-obese control group ($P<0.01$) ; whereas no significant difference in E2 level was noticed between the two groups. With regard to the GTT, the FBS level and blood sugar measured after half, one hour and two hours were significantly different between non-obese PCOS patients and non-obese control women ($P < 0.05$). Lipid profile show no significant difference in the level of cholesterol, triglyceride, VLDL and LDL levels while the HDL level was significantly lower in non-obese PCOS patients compared to non-obese control women.

Table 3. Comparison of demographic parameters between non-obese PCOS and non-obese control women

Parameters	Control Women N = 65 (mean ± SD	Control Women N = 65 (mean ± SD)
Age (yrs)	28.83 ± 4.43	30.33 ± 3.85
BMI (kg/m2)	26.45 ± 2.18	25.53 ± 1.9*
Waist/ Hip Ratio	0.83± 0.06	0.8± 0.05*
Waist/thigh ratio	1.34 ± 0.16	1.32 ± 0.15
FSH (IU/ml)	5.13 ± 1.64	7.46 ± 1.99**
LH (IU/ml)	6.39 ± 2.89	4 ± 1.61**
LH/FSH	1.3 ± 0.52	0.54 ± 0.16**
E2 (pg/ml)	55.84 ± 18.54	49.11 ± 17.91
Testosterone (ng/ml)	0.69 ± 0.33	0.25 ± 0.17**
E2/Testosterone	107.42 ± 59.39	249.31 ± 147.77**
FBS (mg/dl)	93.31 ± 13	85.76 ± 10.39*
BS (after 12/ hr.) (mg/dl)	148.98 ± 36.15	133.29 ± 10.6*
BS (after 1 hr) (mg/dl)	140.52 ± 33.83	126.37 ± 15.24*
BS (after 2 hr) (mg/dl)	113.33 ± 25.14	103.82 ± 8.07*
Cholesterol (mg/dl)	152.91 ± 24.52	144.37 ± 15.48
Triglyceride (mg/dl)	112.57 ± 25.15	107.06 ± 21.44
VLDL (mg/dl)	22.01 ± 5.3	21.38 ± 4.32
LDL (mg/dl)	90.24 ± 25.03	81.4 ± 14.28
HDL (mg/dl)	39.7 ± 2.51	40.91 ± 2.57*

* = P< 0.05, ** =< 0.01, PCOS = polycystic ovary syndrome, FBS = fasting blood sugar, BS = blood sugar, VLDL = very low density lipoprotein, LDL = low density lipoprotein, HDL = high density lipoprotein

Discussion

The BMI of our PCOS patients was higher when compared to the control women; and even within the subgroups. Women with PCOS usually have the so-called central obesity (Visceral adiposity), and therefore tend to have an increased waist-hip ratio (WHR) and waist to thigh ratio(14). This was also noticed in this study. It is worthy to state that even among subjects of normal weight; it is higher than control women with adjusted BMI.

A likely explanation for the mechanisms underlying the development of obesity in women with PCOS is the combined effect of a genetic factor in which certain single-nucleotide polymorphisms associated with obesity contribute to elevated BMI in PCOS(15), in the context to other factors like obesogenic environmental factors where women with PCOS appear to have a significantly lower basal metabolic rate than do age- and BMI-controls (1446 kcal/day versus 1841 kcal/day)(16).

Hormonal factors also play role in PCOS obesity, including the insulin in which insulin resistance that is predominant in PCOS patients are associated with a decrease in mitochondrial function (mitochondrial oxidative activity and mitochondrial ATP synthesis) that contributes to the ectopic fat accumulation(17).

The results of this study showed that regardless of the weight factor (i. e. in obese and non-obese), PCOS patients had higher levels of LH

and LH/FSH ratio, while FSH was found to be low; a findings that was also reported by Saxena *et al*. ,(18).

An increased LH-pulse frequency in PCOS women, independent of BMI is well established. The cause is linked to an accelerated gonadotrophin releasing hormone (GnRH) pulse generator activity and heightened pituitary response to GnRH (22). LH and FSH synthesis and secretion are highly dependent on the pattern of the GnRH stimulus. Obesity seems to lower the LH-pulse amplitude and the peak increment of LH in response to GnRH stimulation, but the LH-pulse frequency in PCOS women is not influenced by BMI(18).

The mechanism (s) underlying the abnormal regulation of GnRH in PCOS women has remained unclear. It has been postulated that altered inputs to the GnRH neuronal system by insulin and/or sex steroids may induce a dysregulation of GnRH pulse generator activities. LH dysregulation is not primary but secondary to the peripheral events within the ovary. However, the mechanism of neuroendocrine dysfunction may be due to chronically elevated levels of estrone, a weak estrogen aromatized peripherally from andostenedione, which is not counteracted by progesterone so an uncoupling of hypothalamic estradiol inhibition by elevated ovarian androstenedione(19).

This abnormal secretion of ovarian androstenedione seems to be an intrinsic property of PCOS theca/granulosa cells. It can augment pituitary sensitivity to GnRH both by a direct action on gonadotrophin synthesis and by enhancing GnRH-induced GnRH receptors. At a particular threshold (determined by estradiol levels), this uncoupling is associated with an estradiol-related sensitization of pituitary LH release and hence an increase in LH secretion(20).

Regardless of their body weight, PCOS patients of the current study have an elevated testosterone and estrogen levels, yet, E2/testosterone ratio was lower than that of control women. Several studies have indicated that polycystic ovaries usually produce excess androgen(21). A key step in androgen formation is the regulation of P450c17 enzyme. Activation of this enzyme in ovary and/or adrenal cortex is regulated by a number of hormones or growth factors including LH, insulin and IGFs. Hyperactivity of the P450c17 enzyme represents one of mechanisms leading to ovarian hyperandrogenism(22).

Chronic LH stimulation in PCOS induces sustained hypersecretion of androgens by the theca compartment(23). Insulin in supraphysiological level and IGFS also augments ovarian androgen production, it increase activity of the human P450c17 mRNA expression(23).

Familial aggregation of reproductive endocrine biochemical abnormalities in PCOS relatives suggests that these traits have a genetic basis. The genetic susceptibility for a poor function of the aromatase enzyme amplifies the androgen elevation by a slow conversion of androgens to estrogens. Aromatase activity might be decreased in follicles from patients with PCOS, and that the possible androgen excess resulting from this decreased activity might contribute to abnormal follicle development(24).

Granulosa cells in the arrested follicles of PCOS women are few in number and are virtually devoid of aromatase activity. At baseline, there is a tendency toward a mild E2 excess as a result of a response to FSH and premature responsiveness to LH(25). Also partly the consequence of excess androgen substrate for E2 secretion, aromatase activity in granulosa cells in the PCOS follicle is very low which results in a higher androgen to estrogen ratio and follicular arrest.

Most women with PCOS have insulin resistance to a significantly greater extent than in age and BMI-matched control women. Saxena *et al* (18) found that irrespective of weight, PCOS patients (lean as well as overweight or obese), are inherently insulin resistant. There will be compensatory hyperinsulinemia and this plays a central role in the pathogenesis of PCOS (28). The results of our study were compatible to the aforementioned studies where there was higher GTT level in PCOS patients than control women.

Since hyperandrogenemia is present in both obese and non-obese PCOS without significant difference, and androgens may induce the insulin-resistance state; then additional factors are necessary to explain the insulin resistance because suppressing androgen levels does not completely restore normal insulin sensitivity(26). It acts both through the activation of the lipolytic cascade, and the modification of the muscle histological structure, by decrease in red, oxidative, insulin-sensitive, type I muscle fibers, an increase in white, glycolytic type II

less insulin-sensitive fibers, a reduction of capillary density and an inhibition of glycogen synthase system (27).

The causes of insulin resistance appear to be complex and multi-factorial. As insulin resistance affects both obese and non-obese PCOS and there are familial aggregation consistent with a genetic trait may predispose an individual to insulin resistance(28). At a cellular level, there are a number of factors which affect the way an insulin receptor functions. The major abnormality in insulin action in PCOS is abnormalities of insulin receptor binding(29) and a post binding defect in insulin signaling, excessive serine phosphorylation of the insulin receptor, or a defect in the autophosphorylation of tyrosine residues in the insulin receptor-which reduces receptor signal transduction(29).

High levels of the cholesterol, triglyceride LDL, VLDL and low HDL were observed in women with PCOS when compared with healthy women. A finding that is in harmony with other studies(30).

The causes of dyslipidaemia in PCOS are again multifactorial. Insulin resistance appears to have a pivotal role mediated in part by stimulation of lipolysis and altered expression of lipoprotein lipase and hepatic lipase(31). Insulin resistance cause increasing hepatic gluconeogenesis and inhibiting glucose uptake and oxidation in skeletal muscle, glucose in the liver converted to free fatty acids and cholesterol(32).

High androgen levels additionally worsen the disturbances in the lipid metabolism, it may lead to the abnormalities in lipoprotein profile by working directly at the liver through the induction of hepatic lipase activity and it decreases lipoprotein lipase activity in abdominal fat cells(33).

The presence of obesity usually leads to a more atherogenic lipoprotein pattern suggesting a reduced capacity for cholesterol removal from tissues with diminished antiatherogenic potential. In the present study, the data shows that non-obese PCOS patients have higher but not significant lipid profile than non-obese control women. They suggest that presence of obesity makes these patients susceptible to deranged lipid profile(34).

The lipolytic sensitivity varies in different regions of adipose tissue, with high sensitivity of adipocytes in central depots, particularly in the visceral region(35).

In conclusion, obesity is a common finding of women with PCOS, but it is not part of the diagnostic criteria. The BMI of our PCOS patients was higher when compared to the control women; and even within the subgroups, the BMI was higher. Women with PCOS usually have the so-called central obesity (Visceral adiposity), and therefore tend to have an increased waist-hip ratio and waist to thigh ratio, regardless of the weight factor. PCOS patients had abnormal biochemical indices and disturbed hormonal status.

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