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Insulin Resistance in Polycystic Ovarian Syndrome Women: Impact of Regularity and Hyperandrogenemia

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Abstract

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder and the main cause of infertility due to anovulation among reproductive-aged women. fetal programming is the beginning of the insulin resistance (IR) and hyperandrogenism which leads to a chain of health consequences. The disturbances in menstruation and fertility develop metabolic complications as age advances. An early and precise diagnosis is necessary for a satisfactory management of PCOS, especially at the extreme ends of the reproductive lifespan. In the present study, IR, hormones and irregularity was studied in PCOS and compared with the control groups. Sixty PCOS women and thirty apparently healthy women were participated in the present study. Serum Fasting serum glucose and sugar, prolactine, progesterone, Cortisol, LH, FSH, estradiol, total & Free testosterone were determined in all women's. In PCOS women (IR-PCOS) had more obesity comparing with (non IR-PCOS). HOMA%B and insulin were increased while HOMA%S decreased in IR-PCOS patients in comparing with (non-IR-PCOS). The IR-PCOS patients had higher LH/FSH, and BMI and lower FSH than Non IR-PCOS. Serum (Insulin, HOMA%B, LH, LH/FSH) significantly increased in IR-PCOS patients, while serum (FSH, HOMA S%) levels were decreased as compared with non IR-PCOS. Furthermore, IR-PCOS patients had higher PRG, Cortisol, E2, PRL and lower free Testosterone and total Testosterone. In Iraqi women with PCOS, IR state is increased as women having irregular menstruation or hyperandrogenism. Also, PCOS has many changes in hormones and other measured parameters duo to the metabolic bases of the syndrome.

Keywords: Insulin resistance, Polycystic Ovarian Syndrome, Regularity & Hyperandrogenemia.

INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is a hormonal disorder that affects between 5-10% women of reproductive age and remains the most enigmatic reproductive disorders. The most common symptoms of PCOS are insulin resistance (IR) irregular menstrual cycles obesity, acne, amenorrhea, hirsutism, and high cholesterol(1). Its hyperandrogenic manifestations include, dyslipidemia, IR, diabetes, cancer, infertility and coronary heart diseases (2). PCOS is a common endocrine disorder affecting females in reproductive age. It is a complex disorder whose pathogenesis is not well elucidated despite evidence of multiple interaction with genetic, behavioral and environmental factors that contribute to its occurrence (3). This syndrome is typically characterized by anovulatory cycles and infertility, altered gonadotropin levels, obesity, and bulky multi follicular ovaries on ultrasound, Hyperandrogenism and IR are hallmark features of its complex pathophysiology. Hyperandrogenemia is a salient feature of PCOS and a major contributor to cosmetic anomalies including hirsutism, acne, and male pattern alopecia in affected women. Increased androgen levels may be intrinsic or aggravated by preexisting IR in women with PCOS (4). Since IR and resulting compensatory hyperinsulinemia are closely related to its pathogenicity and comorbidities, obesity, which is prevalent in a half of PCOS women can be exacerbated (5). IR is a main pathophysiologic feature in PCOS women (6) since cells fail to respond normally to the insulin. The pancreas produces insulin when blood glucose starts to be elevated. Under normal conditions of insulin reactivity, this insulin response triggers glucose being taken into body cells, to be used for energy, and inhibits the body from using fat for energy, thereby causing the concentration of glucose in the blood to decrease as a result, staying within the normal range even when a large amount of carbohydrates is consumed. During IR, however, excess glucose is not sufficiently absorbed by cells even in the presence of insulin, thereby causing an increase in the level of blood sugar (7). IR and hyperandrogenism are considered to be a key factors contributing to PCOS, and the exact mechanisms behind the interactions between IR and hyperandrogenism in the female liver is not well understood. Hyperandrogenism itself or combined with IR contributes to liver damage in women with PCOS (8,9). In some PCOS models, elevated testosterone is reported as a marker of hyperandrogenemia (10). Hyperinsulinemia and hyperandrogenemia are play a prominent roles in PCOS. Insulin signaling in pituitary and the ovaries either directly or indirectly stimulates androgen production (11). Acne and alopecia are not commonly associated with hyperandrogenemia and therefore should not be regarded as evidence of hyperandrogenemia (12). Menstrual disturbances are common in PCOS, with oligomenorrhoea and amenorrhoea in different ratios (13, 14). Although the pathogenesis of PCOS is complex, it is typically associated with hyperandrogenism, hyperinsulinaemia and an elevated ratio of the gonadotropins luteinising hormone (LH) to follicle-stimulating hormone (FSH) (15). Many factors influence menstrual regularity, including physical activity, body size, alcohol intake, and smoking, in addition to pathologic conditions including PCOS (16, 17). Approximately 85-90% of women with oligomenorrhea have PCOS, usually defined as cycle length greater than 35 days (18). However, the diagnostic criteria for PCOS has evolved over time. Currently, there are three overlapping,

but not entirely consistent, clinical definitions of PCOS (19, 20). Menstrual cycle irregularity and length are features included in all three PCOS definitions. Late and irregular menstrual cycles have been associated with higher androgen and lower sex hormone binding globulin levels PCOS and this altered hormonal environment may increase the risk of specific histologic subtypes of ovarian cancer (21). In the present study, IR and irregularity was studied in PCOS and compared with the control groups. The measured parameters also were correlated with each other in order to estimate the factor affecting parameters levels in PCOS group.

MATERIALS AND METHODS

Patients

Sixty PCOS women patients participated in the study. Their age mean range was 20 to 32 year, All of them were diagnosed with PCOS by gynecology specialist according to the European Society of Human Reproduction and Embryology (ESRHE) consensus conference held in Rotterdam in 2003 (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004), American Society of Reproductive Medicine (ASRM) and ultrasonography examination in (Center of Fertility in Al-Sadr Teaching Medical City in Al-Najaf Al-Ashraf Governorate-Iraq). They were (6-8) hr overnight fasting and on treatment with metformin (Glucophage®). All the participated women were having polycysts in their ovaries by ultrasonography examination.BMI calculated from the following equation: BMI=Weight (Kg)/Height (m^2) (22), When BMI was larger than 30 Kg/m^2, the person was defined obese. All hormones were calculated by using Elisa Kit (The enzyme-linked immunosorbent assay). The estimate of serum Glucose, Total Protein provided by CYPRESS DIAGNOSTICS. The computer based HOMA2 calculator (available at www.dtu.ox.ac.uk/homa) (Figure 1) uses fasting insulin and glucose concentrations to calculate the index of IR, HOMA2IR, and the index of β cell function (HOMA-%B). In ideal normal-weight individual age <35 year had HOMA2IR of 1 mol.μU/L^2 and HOMA-% B cell function of 100% (23).

Exclusion Criteria

The present research excluded the patients with any obvious major systemic diseases including diabetes mellitus, hereditary diseases, or other endocrine disorders.

Controls

Thirty apparently healthy women were recruited as control group. Their age range was 28.73±7.40 year which was comparable to that of patients. None of these subjects were pregnant, irregular period, or have other endocrine disorders.

Statistical Analysis

Kolmogorov-Smirnov test has been used to examine the normality distribution of the results. The results of the normally distributed variables were expressed as (mean ± standard deviation). Pooled t-test was used for the comparison between the groups. Pearson's correlation coefficients (r) were calculated for the correlationstudy. For the nonparametric variables, that is not normally distributed, the results were expressed as medians, in addition to (mean±standard deviation). Mann-Whitney *U* test was used for the comparison and Spearman's correlation coefficients (ρ rho) were used to estimate the correlation between parameters. All statistical analyses were performed using SPSS Statistics version 25 program (2017), IBM-USA. While the figures constructed using EXELL program of Microsoft Office 2013.

RESULTS

The comparison between PCOS patients and controls in the measured parameters is presented in Table 1. There is a significant increase in BMI, WHR, insulin, HOMA2IR, HOMA%B, LH, E2, Prolactin, T.Testo and F.testo in PCOS patients group in comparing with the control group.

The comparisons between regular and irregular PCOS women in different parameters are presented in Table 2. Thirty-eight out of sixty PCOS women (63.4%) were irregular and 22 patients were regular (36.6%).

Table 1: Comparison between PCOS patients and controls.

Parameter	PCOS n=30	Controls n=60	Significance
Age(year)	28.28 ± 6.48	27.98 ± 7.10	N.S
BMI (kg/m^2)	28.72±4.66	26.23±3.48	0.012
WHRo	0.91±0.13	0.81±0.86	0.024
FBS (mg/ml)	92.13±19.26	89.88±21.23	N.S
Insulin (μlU/ml)	17.22	8.10	0.008
HOMA2IR	2.41	1.92	0.011
HOMA%B	94.45	86.98	0.033
HOMA%S	88.77	92.08	N.S
FSH (mIU/ml)	11.89	11.10	N.S
LH (mIU/ml)	14.12	11.13	0.017
LH/FSH	1.39	1.57	N.S
E2 (pg/ml)	67.58	38.69	<0.001
Prolactin(ng/ml)	13.20	11.97	N.S
PRG(ng/ml)	0.97±0.04	0.94±0.04	N.S
F.Testo. (pg/ml)	4.18	3.58	0.041
T.Testo. (ng/ml)	0.9±0.23	0.71±0.32	0.021

Table 2: Comparison between PCOS patients with regular and irregular Cycle.

Parameter	PCOS with Irregular Cycle n=38	PCOS with Regular Cycle n=22	Significance
Age(year)	26.13±6.67	29.45±8.765	N.S
BMI(kg/m^2)	28.67±7.60	26.11±8.03	N.S
WHR	0.7803±0.20	0.8266±0.04	N.S
FBS	83.75±13.78	87.70±42.63	N.S
Insulin(µIU/ml)	16.56	7.21	0.006
I/G	3.65±2.71	3.69±3.82	N.S
HOMA2IR	2.68	1.64	0.031
HOMA%B	94.65	83.59	0.027
HOMA%S	88.52	91.22	N.S
FSH (mIU/ml)	12.62±5.23	10.99±4.76	N.S
LH(mIU/ml)	12.48	11.26	N.S
LH/FSH	1.32±1.03	1.18±0.70	N.S
Cortisol(µg/dI)	13.25	7.85	0.008
E2(pg/ml)	46.08±19.74	42.68±14.39	N.S
PRL.(ng/ml)	17.33±8.38	19.38±7.36	N.S
Prog.(ng/ml)	0.93±0.03	0.94±0.04	N.S
F.Testo. (pg/ml)	4.38	2.14	0.024
T.Testo. (ng/ml)	0.72±0.15	0.61±0.11	0.043

Table 3: Comparison between IR patients (HOMA2IR≥3) and non-IR patients.

Parameters	IR-PCOS (HOMA2IR≥3) (n=15)	Non-IR-PCOS (HOMA2IR<3) (n=45)	Significance
Age (year)	28.45±7.54	26.80±7.76	N.S
BMI (kg/m^2)	30.48±5.32	26.75±8.46	0.046
WHR	0.819±0.04	0.79±0.18	N.S
FBS (mg/ml)	88.67±13.04	85.39±30.78	N.S
Insulin (µlU/ml)	38.52±8.73	11.07±5.59	<0.0001
I/G	8.04±2.49	2.48±1.41	<0.001
HOMA%B	343.16±131.17	169.75±91.16	<0.001
HOMA%S	22.72±5.29	98.65±57.46	<0.001
FSH (mIU/ml)	9.80±1.69	12.65±5.67	0.006
LH (mIU/ml)	13.84	11.45	0.035
LH/FSH	2.17±1.30	1.45±0.78	0.021
Cortisol (µg/dI)	15.28±22.64	11.83±11.90	N.S
E2 (pg/ml)	49.35±21.49	44.43±17.02	N.S
PRL.(ng/ml)	17.93±9.52	17.54±7.78	N.S
PRG (ng/ml)	0.95±0.05	0.93±0.03	N.S
F.Testo. (pg/ml)	3.15	3.46	N.S
T.Testo. (ng/ml)	0.67±0.09	0.69±0.11	N.S

The results of serum Insulin, HOMA2IR, HOMA%B, Cortisol, F.Testosterone, and T.Testosterone appeared significantly higher ($p<0.05$) in PCOS women with irregular cycle as compared with PCOS women with regular cycle, while serum HOMA%S decreased.

Comparison between IR-patients (HOMA2IR≥3) and non-IR patients HOMA2IR<3 PCOS patients group is subdivided into IR patients (IR-PCOS) which have HOMA2IR≥3 and noninsulin resistant patients (Non IR-PCOS which have HOMA2IR<3 (23). The results of the measured parameters in IR-PCOS and NIR-PCOS groups are presented in Table 3.

In our research fifteen patients out of 60 (25%) are IR (IR-PCOS) and 45 out of 60 patients (75%) are noninsulin resistant (non IR-PCOS). The result in the table showed (IR-PCOS) had significantly higher BMI than (non IR-PCOS). Serum (Insulin, I/G, HOMA B%, LH, LH/FSH) significantly increased in IR-PCOS patients, while serum (FSH, HOMA S%) levels were significantly decreased as compared with non IR-PCOS. Furthermore, IR-PCOS patients had higher PRG, Cortisol, E2, PRL, and lower free Testosterone and total Testosterone. The facial hair is present in 28 out of 60 (47%) of PCOS women under study. The comparisons between PCOS patients with and without facial hair groups revealed a significant difference in the following parameters (Cortisol (11.65 *vs.* 9.41µg/dI), T.Test. (0.94 *vs.* 0.71 ng/ml), and PRG (0.92 *vs.* 0.74 ng/ml) in patients group with /without facial hair, respectively. In the present research, 25 out of 60 patients (42%) of the patients were having acne. The results showed that the PCOS patients with acne have higher age (28.69±8.42 *vs.* 25.48±5.97 yrs, p=0.031); BMI

(29.79±6.29 *vs.* 24.73±8.85 kg/m^2, p=0.021); F.Teso (2.85 *vs.* 5.12 pg/ml, p=0.08) than the corresponding patients without acne (p=0.021). The following IR parameters also showed an increase in patients with acne in comparing with patients without acne (Insulin=17.3 *vs.* 11.54 µlU/ml, p=0.014; I/G= 3.12 *vs.* 4.68, p=0.23; HOMA%B= 91.86 *vs.* 88.42, p=0.021).

DISCUSSION

The results in Table 1 showed the typical results of the PCOS as a syndrome related to IR, and hormonal disturbances (24-25). In the present research, only fifteen patients out of 60 (25%) are insulin resistant (IR-PCOS) and 45 out of 60 patients (75%) are non-IR (non IR-PCOS) and they had significantly higher BMI (obese) than (nonIR-PCOS) This result has been recorded with *Miriam et al* (26) found ratio of IR diagnoses was higher in obese women than in normal BMI women. they observed a significant association between IR and BMI. Even though obesity is not regarded as the cause in the development of PCOS (27), but it was observer in the other studies (28) that reduction in body weight brings many benefits and is the best method of treatment for women with PCOS. The prevalence of obesity in PCOS ranges from 38% to 87%. It has been reported in previous studies that the prevalence of IR is higher in obese PCOS women than obesity without PCOS *(29).*Body mass reduction is so beneficial that it is even achieved in patients through bariatric surgeries. It contributes to improvement in parameters indicative of metabolic disorder such as IR and affects the regulation of menstrual cycles. However, in case of bariatric procedure the patient is not taught new rational eating habits and healthy lifestyle, and her self-assessment decreases despite body mass reduction (30-31). Other studies (32) found Infertile women with PCOS had higher BMI and serum leptin levels. Some studies appears that cinnamon significantly decreased serum fasting blood glucose, insulin, homeostatic model assessment for IR, and weight and increased high-density lipoprotein cholesterol compared with placebo (all p<0.05). Serum body mass index significantly decreased in the cinnamon group (33) ,Cinnamon reduce IR by increasing phosphatidyl, inositol, 3-kinase activity in the insulin signaling pathway and thus potentiating insulin action (34). However, *Sahin et al., (2017)* (35) results are in accordance with the results of the present study and they found that the Insulin, LH, and E2 were higher in HOMA-IR-PCOS. Hyperandrogenism is wide spectrum of clinical features in PCOS women (29, 36). We obtained in our results the serum Insulin, HOMA2IR, HOMA%B, significantly higher (p<0.05) in PCOS women with irregular cycle as compared with PCOS women with regular cycle, while serum HOMA%S decreased. New studies (37) found the prevalence of IR was 57% in women with BMI ≥ 25. However The median HOMA-IR values were the highest in overweight women in both IR and nonIR groups. However, we find that Cortisol, F. Testo, T. Testo significantly higher (p<0.05) in PCOS women with irregular cycle as compared with PCOS women with regular cycle, while serum HOMA%S decreased. Positive correlations were found between BMI, free testosterone and HOMA-IR (37). Farthermore, F.Testo. level is more sensitive than the measurement of total T.testo. for establishing the existence of androgen excess and should be ideally determined through equilibrium dialysis techniques. Value of measuring levels of androgens other than T in patients with PCOS is relatively low (38). Daghestani *et al (2018)* (39) showed in their results that patients in the PCOS group exhibited increases in testosterone (202.3%), fasting glucose (9.2%), fasting insulin (49.4%), LH/FSH ratio (205.3%), and decreases in progesterone (-7.4%). While Zhang *et al 2018* (40) found The levels of LH, LH/FSH, T.testo, fasting insulin and homeostasis model assessment of IR (HOMA2IR) index in PCOS group were higher than those in normal control group. A significant difference was found in basal LH, fasting glucose, insulin, HOMA-IR, F.Testo levels, WHR ratios between the PCOS and non-PCOS patients in the lean and overweight groups (p<0.05) (41).

Hyperandrogenism remains as one of the key features in PCOS and can be assessed clinically or determined by biochemical assays. Hirsutism is the most common clinical manifestation of hyperandrogenism. The clinical assessment is subjected to wide variability due to poor inter observer agreement and multiple population factors such as ethnic variation, cosmetic procedures and genetic trait. The difficulty in resolving the androgen excess biochemically is due to a lack of consensus as to which serum androgen should be measured for the diagnosis of PCOS (42). However new research showed that hyperandrogenic disorders have been associated with various psychological distress and disorders (43). Other study (44) showed that, in patients with PCOS, some treatment regimns can effectively improve biochemical and clinical parameters of hyperandrogenism (45) showed that long and irregular menstrual cycles, a hallmark of PCOS, have been associated with higher androgen and lower sex hormone binding globulin levels and this altered hormonal environment may increase the risk of specific histologic subtypes of ovarian cancer, while Panidis *et al.,* 2015 (46) showed that The proportion of women with regular menstrual cycles did not differ between normal weight and obese women but was higher in overweight women. However, Harris *et al.,* 2018 (47) suggest that menstrual cycle characteristics influence ovarian cancer risk differentially based on histotype. Impact**:** These results highlight the importance of examining ovarian cancer risk factors associations by histologic subtype. It could be concluded that PCOS treatment has to treat the irregular menstrual cycle and to ameliorate the related underlying metabolic dysfunctions. The recommended herbs in ancient medicine, which have the most scientific proof for their related actions, can be studied further in experimental analyses (48).

In our study we find that thirty-eight out of sixty PCOS women (63.4%) were irregular and 22 patients were regular (36.6%). However Ybarra *et al.,* 2018 (49) found Irregular menstrual cycles were found in 65.3% of patients. Clinical hyperandrogenism was observed in 16.3% of girls and 18.4% had elevated serum androgen values.

In our study we find that PCOS patients with and without facial hair groups revealed a significant difference in the following parameters (Cortisol (11.65 *vs.* 9.41µg/dI), T.Test. (0.94 *vs.* 0.71 ng/ml), and Prog (0.92 *vs.* 0.74 ng/ml) in patients group with /without facial hair, respectively. In the present research, 25 out of 60 patients (42%) of the patients were having acne. The results showed that the PCOS patients with acne have higher age (28.69±8.42 *vs.* 25.48±5.97 yrs, p=0.031); BMI (29.79±6.29 *vs.* 24.73±8.85 kg/m^2, p=0.021); F.Teso (2.85 *vs.* 5.12 pg/ml, p=0.08) than the corresponding patients without acne (p=0.021). However a recent research (50) conclude that Bariatric surgery improves key diagnostic features seen in women with PCOS and ovarian volume, and free testosterone may have utility in predicting likelihood of metabolic benefit from surgery.

CONCLUSION

In Iraqi women with PCOS, IR state is increased as women having irregular menstruation or hyperandrogenism. Also, PCOS has many changes in hormones and other measured parameters duo to the metabolic bases of the syndrome.

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