Original Article

Evaluation of Insulin and Estradiol in Women with Polycystic Ovary Syndrome and its Relationship With BMI

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Abstract

Background & Objective: Polycystic ovary syndrome (PCOS) is a complex endocrine and metabolic disorder, which is characterized by ovulatory dysfunction and hyperandrogenism. This study was done to evaluate insulin and estradiol (E2) in women with PCOS and its relationship with Body mass index (BMI).

Materials & Methods: This case-control study included, 104 women with polycystic ovary syndrome as a case group and 100 women without polycystic ovary syndrome as a control group. Concentrations of insulin, glucose and E2 were measured in fasting blood samples.

Results: Insulin level was 7.02 ± 3.29 in control group, 11.41 ± 3.84 in the case group. FBS level was 82.75 ± 7.18 in control group versus 84.03 ± 5.82 in case group. E2 level was 70.74 ± 53.03 in control group and 60.21 ± 40.58 in case group. The insulin resistance level was 1.45 ± 0.74 in the control group versus 2.37 ± 0.83 in case group. According to correlation analysis, the insulin variable had a significant positive association with BMI ($p < 0.0001$, $r=0.245$), although no significant correlation was seen between E2 and BMI (p-value = 0.245, $r=0.092$).

Conclusion: Present data showed that E2 levels were not different in PCOS and non-PCOS patients, but insulin levels in PCOS were significantly increased and PCOS women had significant insulin resistance which is dependent on BMI.

Keywords: Polycystic ovary syndrome, Insulin, estradiol, BMI

Introduction

Polycystic Ovary is a complex syndrome related to endocrine and metabolic disorders characterized by chronic ovarian failure, polycystic ovaries, and pathological symptoms like hyperandrogenism (1).

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Accordingly, it was shown that this syndrome has significant adverse effects on the body's physiology and metabolisms such as insulin resistance, hyperinsulinemia, abdominal obesity, and high blood pressure, which can also lead to type 2 diabetes, endometrial hyperplasia, and cardiovascular disease in long-term (2). Defects in the function of the hypothalamic-pituitary ovarian axis and insulin activity cause polycystic ovary syndrome (PCOS), which is associated with abnormal secretions of gonadotropins,

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the increased PCOS of steroids in the ovaries, and sometimes with insulin resistance (3, 4).

Since the early 1980s, a relationship has been found between increased insulin resistance and a lack of ovulation (5). Moreover, the link between the two complications and hyperandrogenism has also been confirmed in different races (6). Numerous studies have emphasized the important role of insulin resistance in the pathogenesis of this syndrome (7-9). They have also stated that insulin resistance and its resulting hyperinsulinemia are the main determinants of ovulation, infertility, and premature abortions in patients with this syndrome (8). Polycystic ovaries are diagnosed as type 2 diabetes, which is one of the manifestations of insulin resistance (10). Glucose intolerance (IGT) is a predisposing complication that was observed to be associated with insulin resistance, which is common among 20 to 40% of women with PCOS (11). Some studies have shown that metformin reduces the level of serum estradiol (E2) by reducing the level of insulin resistance and hyperandrogenism in people withPCOS (12). Concomitant treatment with FSH and IGF-I increases synergism in E2 production (13). The obtained data showed that relatively low doses of 17b.estradiol may have positive effects on glucose homeostasis, due to the protective effects of estrogen (14). Previous studies have also indicated that high doses of E2 increase insulin sensitivity and E2 therapy increase insulin sensitivity (13, 15). In recent years, the role of insulin resistance in the etiology of this syndrome has attracted much attention, because it was shown that insulin-lowering drugs can reduce the concentration of androgens in the blood and improve the changes in the metabolism of one gene (16).

The worldwide prevalence of PCOS is estimated at 2.2% to 26% in various countries (17). According to the NIH criteria, the prevalence of PCOS is about %12 in Iran (18). Therefore, due to the high relative prevalence of PCOS, this study aimed to evaluate the serum levels of insulin and E2 in women with PCOS and explore its association with body mass index.

Materials & Methods

This study was a Case/Control study performed in the research laboratory of the Department of Clinical Biochemistry of Hamadan University of Medical Sciences, the Fatemieh Medical Center of Hamadan in 2020. The present research was conducted in women aged between 18 – 40 years old on serum samples of 104 women with PCOS as the case group and 100women without ovarian syndrome as the control group. The subjects did not have any acute or chronic inflammatory disease and regarding the role of metabolic factors in the development of the disease, the two groups were matched in terms of age and BMI. This study was accepted by the Ethics Committee of Hamadan University of Medical Sciences with the ethics code A-10-2903-1 of IR.UMSHA.REC.1399.040. Informed consent was obtained from all the subjects before participating in the project.

Inclusion and exclusion criterial

Persons who had been referred to medical centers for other reasons were classified as the control groups. In this study, the inclusion criteria were as follows: having PCOS for patients in which the disease was confirmed by a gynecologist through performing clinical and laboratory diagnoses. According to the Rotterdam Protocol Indicators, the PCOS diagnostic criterion is subjected to having at least 2 out of the 3 features of this syndrome were necessary: hyperandrogenism, chronic ovulation failure, and polycystic ovary view on ultrasound (19).

The selected individuals lacked etiologies such as Cushing's syndrome and androgenic secretory tumors. Notably, the control group was matched to each identified PCOS patient based on age, sex and BMI. The control group had a regular menstrual period without hirsutism and acne. In addition, all people were diagnosed as clinically healthy at the discretion of the gynecologist and they had no known chronic or acute diseases. Moreover, the exclusion criteria were the following: cardiovascular diseases, thyroid disease, underlying diseases such as acute or chronic inflammatory disease, liver disease, cancer, diabetes, hypertension above 140/90 mm Hg,

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renal failure registered, and smoking. Demographic information including age, height, weight, BMI, blood pressure, and test results was recorded in a pre-prepared form.

Measurement

Body weight was tested to the closest 0.1kg while wearing light clothing by a balanced-beam scale. Besides, height was computed using a stadiometer to the nearest 0.5 cm. Afterward, BMI was computed based on the weight/ (height) 2 equation. Waist circumferences among the least rib and the iliac crest in umbilicus level were handled in the duplicate to the nearest millimeter by utilizing an adaptable tape.

Preparation of serum samples

10 ml of venous blood sample was taken from all women included in this study after fasting at night (8-12 hours). Subsequently, serum was collected by centrifugation for 15 minutes at 3000 g. The serums were elicited and then stored in a (-80) degree freezer until use. The study has collected information on the age, weight, BMI, waist circumference, and blood pressure of the participants by completing a questionnaire at the time the participants were registered by filling a questioner at the start of the study.

Measurement of glucose in serum samples

Serum glucose levels were measured using Pars Azmoun kit Obtained from a local company and enzymatic method was performed using a BS-800 autoanalyzer.

Measurement of cholesterol, triglyceride, LDL and HDL

Serum cholesterol, triglyceride, LDL and HDL levels were measured using a Pars test kit and the enzymatic method was then performed using a BS-800 autoanalyzer.

Measurement of serum E2

Serum E2 levels were measured using the ZellBio kit by ELISA. The sensitivity of this method was 0.024 ng / mL and the measurement range was between 0.1 and 0.8 ng / mL.

Insulin measurement in serum samples

The serum insulin levels were measured using a quantitative method of luminescence (CLIA) and Liason.

Measurement of insulin resistance

Insulin resistance was measured using the following formula: HOMA-IR (fasting plasma insulin (mU/l) ×fasting plasma glucose (mmol/L)/22.5) for insulin resistance were calculated. Besides, insulin resistance was considered as HOMA index of more than 2.1 (20, 21).

Statistical analysis

The obtained data were represented as mean±SD. Statistical analyses were done using SPSS version 20. The group was contrasted using the students'-test, Mann-Whitney and ANOVA test. The Spearman correlation measurements were also utilized to survey correlations among variables. Multiple logistic regression analyses were used to assess the independent effect of variables on the odds for PCOS. The statistical significance level was set at $P<0.05$.

Result

In the present study, 104 patients with PCOS (average age: 25.82 ± 4.16 years old; average BMI: 26.84 ± 3.48) and 100 healthy persons (average age: 26.05 ± 4.85 years old; average BMI: 26.43 ± 3.82) were evaluated. According to Table 1, in the study, the included women were matched based on age ($p = 0.775$). Also, due to the effect of weight interference on the disease, the groups were similar in terms of body mass index (BMI), and serum lipid concentrations were higher in patients with PCOS compared to the controls. Some significant differences were observed in serum concentrations of LH and FSH between the case and control groups ($P LH< 0.001$, $P FSH=0.013$). The mean of the studied parameters in the two studied groups was estimated by Mann-Whitney analysis, which is given in Table 1. relationship between serum insulin levels of the two groups of PCOS patients and healthy individuals was examined as well,

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there was a significant difference between PCOS patients and healthy individuals with ($p < 0.001$.) In addition, the relationship between E2 levels of PCOS patients and healthy individuals was examined. There was no significant difference between E2 levels in the two groups with (P-value = 0.245). In Figure 1(1a,1b), after dividing the subjects based on their body mass index, regardless of the case and control groups,the association between average insulin and E2 concentration in these individuals with the body mass index is shown. Based on the results of this figure, insulin levels have a direct linear relationship with weight gain and index. It has shown that with BMI and increasing weight, insulin levels and insulin resistance increase. While no correlation was found between estradiol and BMI. Table 2 showed the serum insulin level in women with PCOS was significantly higher in overweight and obese individuals than in normal-weight individuals ($p< 0.001$) and the serum level of E2 between the two groups is not statistically significant.

Results of logistic regression analysis of variables indicated that each unit insulin increasing, the risk of PCOS increases 1.15 times (P= 0.031) and by increasing one unit of BMI, 0.63 times Increased Chance of PCOS (P< 0.001). While the variable E2 is not significant. (P-value = 0.031)

Table1. Biochemical indexes and clinical characteristics of the women with PCOS and their control group (mean ± SD)

Variable	Control group (n=100)	PCOS group (n=104)	*p-value*
Age	26.05±4.85	25.82±4.16	0.775
BMI[b]	±3.82 26.43	26.84±3.48	0.420
Waist	86.44±10.95	±11.12 91.57	0.003
B.Psystolic	112± 11.9	115± 12.3	0.153
B.Pdiastolic	73.7± 8.1	75.3± 9.4	0.192
TC	162.99±34.26	172.63±33.85	0.044
TG	124.18±22.96	±58.34 151.21	0.002
LDL	92.76±22.53	±22.04 101.34	0.007
HDL	48.77±9.36	47.33±9.34	0.261
FBS	82.82±8.26	84.45±8.87	0.163
E2	70.34± 52.91	60.21±40.57	0.245
Insulin	7.20± 3.33	11.68±5.80	0.000
HOMA-IR	1.48± 0.74	2.44± 1.25	0.000

*BMI: Body Mass Index; FBS: Fasting Blood Sugar; TC: Total Cholesterol,TG: Triglyceride; LDL : Low Density Lipoprotein, HDL: High Density Lipoprotein; FSH: Follicle-Stimulating, Hormone; LH: Luteinizing Hormone, B.P= blood pressure, Statistical significance level was set at P<0.05

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Table2. Differences in Waist, Insulin and HOMA-IR, E2 in the subjects with and without PCOS based on their BMI

Parameter	Control group (n = 100)			Case group (n = 104)		
	Normal Weight(39)	Overweight (60)	p	Normal Weight(28)	Overweight (76)	p
WC(cm)	79.92± 7.79	90.68 ±10.6	0.000	80.00± 6.08	95.62 ± 9.52	0.000
E2	70.34±52.91	66.38± 49.1	0.313	57.89± 29.6	61.02± 43.90	0.732
Insulin(µU/ml)	7.20 ± 3.33	7.54 ± 3.64	0.037	9.06 ± 3.24	12.23 ± 3.72	0.000
HOMA-IR	1.48± 0.74	1.58 ± 0.81	0.019	1.82 ± 0.66	2.57 ± 0.79	0.000

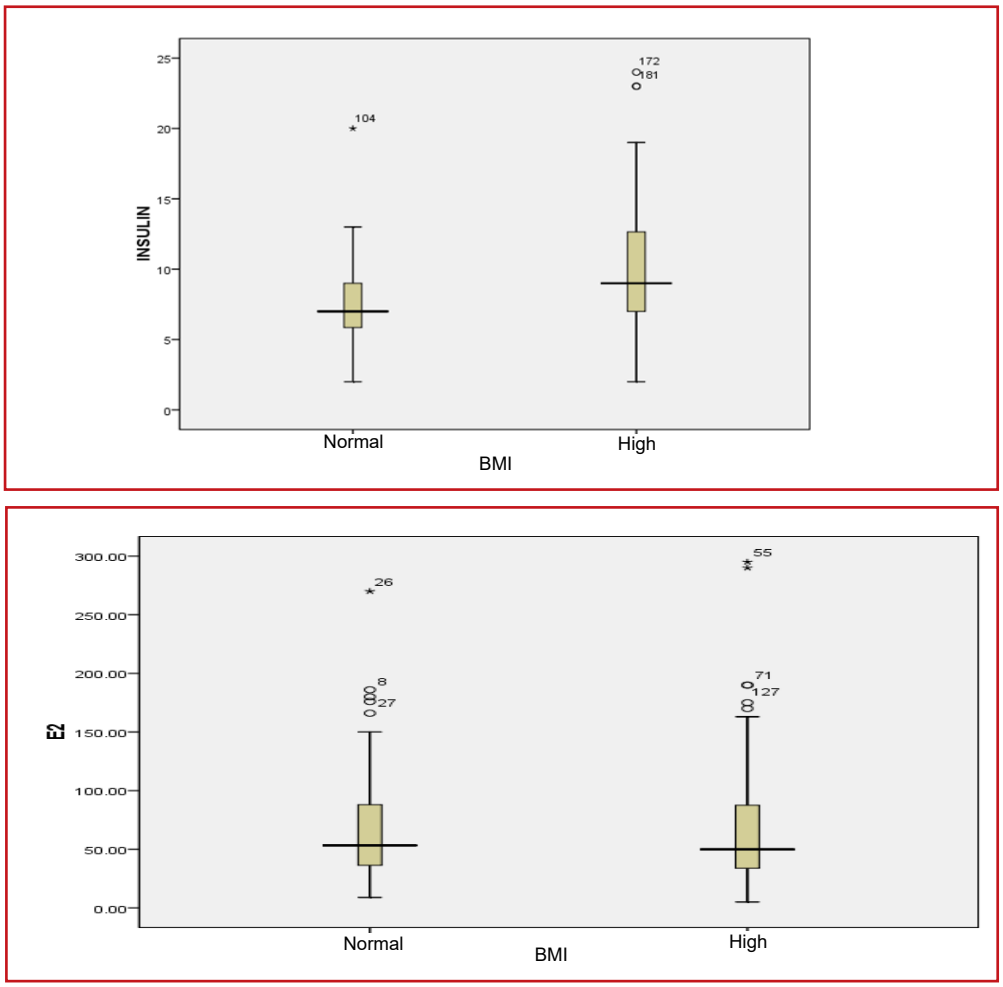

Figure 1. (a) The relationship between insulin and body mass index (b) The relationship between E2 and body mass index

*Insulin level has a direct linear relationship BMI, but there is no correlation between E2 and BMI.

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Table 3. logistic regression analyses of BMI and other confounding variables to predict PCOS

Dependent Variables	independent Variables	Odds Ratio (Exp (β))	CI for Exp (β)	P-value
Patient	BMI	0.63	0.51 - 0.79	< 0.001
	INSULIN	1.15	1.03 - 1.30	0.031
	E2	0.994	0.98 – 1.00	0.245

* Statistical significance level was set at P<0.05

Discussion

In this study, we have examined insulin and E2 levels as well as their relationship with BMI. According to the results of this study, the mean serum insulin level and insulin resistance were higher in PCOS patients compared to the control group, which were statistically significant. The relationship between both parameters and BMI was also investigated. Correspondingly, there was a significant relationship between serum insulin concentration and BMI (P< 0.001). However, no significant relationship was found between E2 and BMI (P = 0.245). It was shown that in PCOS, insulin resistance and compensatory hyperinsulinemia could lead to fertility and metabolic disorders (22). Some studies have shown that insulin resistance increases along with BMI increasing (23). Notably, insulin resistance is accompanied by a wide range of manifestations including cardiovascular disease and hypertension, and it is also associated with type 2 diabetes and lipid disorders (24). A study has proved a 31–35% prevalence of impaired glucose tolerance and 7.5–10.0% prevalence of type 2 diabetes mellitus in women with PCOS (25, 26).

Several previous studies have shown that insulin resistance is a risk factor in PCOS (27-29). In recent studies, the rate of insulin resistance in women with polycystic ovaries has been significantly increased (30, 31). Our findings, inconsistent with the findings of a study by Doddappa et al., showed that insulin resistance was significantly higher in overweight women with PCOS compared to normal-weight women with PCOS (32). Serpeterman et al; conducted a study to examine glucose tolerance and insulin resistance in parents of patients with PCOS compared with parents of healthy women in the University of Chile in Santiago in 2002; Accordingly, 200 mothers with evidence of PCOS and 120 parents of healthy women were included; Afterward, the samples were tested for glucose tolerance with 75 g of glucose. As a result, insulin resistance was significantly higher in the parent group of patients compared to the control group (33).

Kanafchian et al, who performed a study in 2017 in Iran, showed that the rate of insulin resistance was significantly higher in women with PCOS compared to the control group (34).

Moreover, the results of a study by Kulhan et al. in 2017 in Turkey showed that the rate of insulin resistance was significantly higher in women with PCOS compared to the control group (35). Correspondingly, these results are consistent with the results of our study. It was observed that LH levels increase in women with PCOS. In this regard, Zafari et al; (2010) in their study showed that chamomile extract is involved in the reduction of LH / FSH and E2 levels in polycystic ovaries (36). Doldi et al. also showed that progesterone and E2 production of granulosa cells is not normal in women with PCOS (20). This indicates that patients with PCOS show different responses to gonadotropins (20, 37). Furthermore, they stated that serum concentrations of E2 and progesterone showed a significant decrease and increase in the samples, respectively (20). A study by Bartolone et al., Conducted in Italy in 2000, showed that, high levels of E2 in the case of polycystic ovary syndrome (38). In our study, there was no statistically significant difference in terms of serum E2 levels between the two groups, which is contrary to the results of previous studies (21, 39, 40). In this regard, the reason for this difference could be the smaller number of samples in the study. Also, in the study by Dolhi et al., the studied groups were not matched in terms of BMI (20). According to these differences in the design of the two studies, the difference in the results can be explained.

People who are even predisposed to PCOS genetically can prevent or control the disease by having a healthy lifestyle (21). The results show that short-term exercise could reduce serum levels of testosterone and beta-E2 in a model of PCOS (21). Therefore, it should be considered as an essential element of the treatment of this disease. Also, further studies with larger sample sizes are required for comparing the two groups (21).

Conclusion

The results of the present study showed that high insulin and glucose levels can indicate insulin resistance in PCOS. A significant correlation was observed between serum concentration of insulin with BMI. Women with PCOS have normal estrogen levels. This may be because high levels of insulin and testosterone in women with PCOS are sometimes converted to estrogen.

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This study was approved by the Ethics Committee of Hamadan University of Medical Sciences with the ethics code of IR.UMSHA.REC.1399.040. Informed consent was obtained from all the subjects before participating in the project

Conflicts of Interest

The authors have no conflicts of interest relevant to this paper.

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