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Insulin-like growth factor 1, liver enzymes, and insulin resistance in patients with PCOS and hirsutism

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Aim: Hyperinsulinemia and insulin resistance are commonly seen in patients with hirsutism and polycystic ovary syndrome (PCOS), and are associated with cardiovascular disease risk. However, it is not yet known whether insulin-like growth factor I (IGF-I) and alanine transaminase (ALT) produced by the liver play roles in hyperinsulinemia and subclinical atherosclerotic process in patients with PCOS and idiopathic hirsutism (IH).

Materials and methods: This was a prospective case-controlled study. The study population consisted of 25 reproductive-age PCOS women, 33 women with IH, and 25 control subjects.

Results: Mean IGF-I levels and median ALT levels were higher in patients with IH and PCOS than controls, but these differences were not statistically significant. The participants who had a homeostasis model assessment insulin resistance index (HOMA-IR) greater than 2.7 had significantly higher IGF-1 and ALT levels. ALT levels were positively correlated with body mass index, FG, insulin and HOMA-IR.

Conclusion: The study illustrated that IGF-1 and ALT levels were significantly higher in patients with increased insulin resistance. Due to short disease duration in younger participants, we did not observe any correlation between IGF-1 and hyperinsulinemia. These findings suggest that increased hepatic production of IGF-I and ALT might be an early indicator of insulin resistance in hirsutism.

Key words: Cardiovascular disease risk, carotid intima media thickness, insulin-like growth factor 1, insulin resistance, polycystic ovary syndrome

1. Introduction

Polycystic ovary syndrome (PCOS) consists of hyperandrogenism, menstrual irregularity, anovulation, infertility, and obesity (1), and is frequently seen in reproductive-age women (2). Cardiovascular disease (CVD) risk (3) and early atherosclerosis have been found to be associated with PCOS (4). Hyperinsulinemia and insulin resistance are commonly seen in PCOS patients (5) and are associated with low-grade chronic inflammation (6) and CVD (7,8).

Chronic elevation of insulin is associated with higher circulating insulin-like growth factor 1 (IGF-1) levels (9). Insulin and IGF-1 show their effect via protein receptors with intrinsic tyrosine kinase activity; they also activate mitogenic pathways (10).

The relationships between IGF-1, insulin sensitivity, and CVD risk have not yet been studied in patients with PCOS and idiopathic hirsutism (IH). Therefore, the aim of the present study was to evaluate IGF-1 levels in patients with PCOS and IH, and to assess its association with cardiometabolic factors including carotid intima media thickness (CIMT) and insulin resistance.

2. Materials and methods

2.1. Selection of participants

We studied 33 patients with IH, 25 patients with PCOS, and 25 healthy controls consisting of healthy women who had regular ovulatory cycles and normal androgen levels. All 3 groups were matched for age and body mass index (BMI). The protocol was approved by the local ethics committee. All patients gave written consent. All patients were female and nonsmokers. This was a prospective case-controlled study conducted in a training and research hospital between September 2011 and March 2012.

The revised diagnostic criteria of PCOS are given as below (11):

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1. Oligo and/or anovulation.

2. Clinical and/or biochemical signs of hyperandrogenism.

3. Polycystic ovarian appearance on ultrasound.

Patients with at least 2 of these criteria are defined as having PCOS.

Those participants who had a smoking history, diabetes mellitus, hyperprolactinemia, congenital adrenal hyperplasia, androgen-secreting tumors, thyroid disorders, Cushing's syndrome (as determined by a 1 mg dexamethasone suppression test), infectious disease, hypertension, or hepatic or renal dysfunction were not included in the study. Patients were excluded if they had used oral contraceptive agents, antilipidemic drugs, antihypertensive medications, or insulin-sensitizing drugs within the 3 months prior to enrollment.

The control group (n = 25) consisted of healthy patients who had a hirsutism score of <8 and regular menses without ultrasound appearance of polycystic ovaries.

A Ferriman–Gallwey (FG) score was used to obtain the degree of hirsutism (12). The BMI, WHR, and hirsutism scores were evaluated by a single investigator for all of the subjects.

2.2. Measurement of carotid intima media thickness

Carotid intima media thickness (CIMT) was evaluated by a high-resolution ultrasound machine (Sonoline G 40, Siemens) with 7.5-MHz mechanical sector transducer and a single investigator in our outpatient clinic. The intima media thickness was measured as the distance between the blood-intima and media-adventitia on B-mode imaging.

2.3. Biochemical evaluation

Venous blood was sampled from each patient after a 12-h overnight fast in the follicular phase of a spontaneous or progesterone-induced menstrual cycle for the determination of hormone, liver enzyme, lipid profile, high-sensitive C-reactive protein (hs-CRP), insulin, and glucose levels.

The glucose oxidase/peroxidase method (Gordion Diagnostic, Ankara, Turkey) was used to obtain plasma glucose levels. The serum levels of follicle-stimulating hormone (FSH) (normal range: 3–10.9 mIU/mL), luteinizing hormone (LH) (normal range: 2.1–12.8 mIU/mL), prolactin (normal range: 3.1–29.8 ng/mL), estradiol (normal range: 18.9–246.7 pg/mL), dehydroepiandrosterone sulfate (DHEAS) (normal range: 35–430 μq/dL), total testosterone (T) (normal range: 14.2–73.1 ng/dL), insulin (normal range: 3–25 mU/L), and thyroid stimulating hormone (TSH) (normal range: 0.5– 4.78 mIU/L) were measured with specific electrochemiluminescence immunoassays (Elecsys 2010 Cobas, Roche Diagnostics, Mannheim, Germany). The levels of alanine transaminase (ALT), total cholesterol, high density lipoprotein cholesterol (HDL-C), and triglyceride (TG) were determined with enzymatic colorimetric assays by spectrophotometry (BioSystems S.A., Barcelona, Spain). The Friedewald formula was used to calculate the low density lipoprotein cholesterol (LDL-C).

IGF-1 was evaluated by enzyme-labeled chemiluminescent immunometric assay (Immulite 2000 XP). Serum hs-CRP (normal range: 0–3 mg/L) was measured by high-sensitive CRP immunonephelometry (BN, Dade-Behring; Marburg, Germany).

The homeostasis model assessment insulin resistance index (HOMA-IR) (13), including the formula [fasting plasma glucose (mmol/L) × fasting serum insulin (mU/mL) / 22.5] was used to calculate insulin resistance. The cut-off value was taken as 2.7 for HOMA-IR (14).

2.4. Statistical analyses

Collected data were entered into SPSS version 17. Continuous data were shown as means ± SD. Chi-squared tests were used to compare differences in rates. Normally distributed variables were compared using the one-way ANOVA. Data that were not normally distributed, as determined using the Kolmogorov–Smirnov test, were compared by K independent sample analyses. The data are expressed as means ± SD. The degree of association between variables was calculated using the Pearson correlation coefficient. Multiple linear regression using the enter method was used to determine the independent predictors. Univariate analyses were used to adjust the IGF-1 with respect to age and BMI.

A P value lower than 0.05 was regarded as statistically significant, and all computed P values were 2-tailed.

3. Results

Clinical and endocrinological parameters were screened in the patients with PCOS and IH and in the healthy control subjects (Table 1). We studied 33 patients with IH (mean age 23.24 ± 5.65 years, range 18–34 years; BMI, 24.86 ± 6.06 kg/m^2), 25 patients with PCOS (mean age 22.51 ± 4.07 years, range 18–31 years; BMI, 24.58 ± 4.62 kg/m^2), and 25 age- and BMI-matched healthy controls (mean age 22.36 ± 4.44 years, range 18–32 years; BMI, 22.30 ± 3.66 kg/m^2).

3.1. Comparing parameters between groups and the linear regression analyses

Mean age, BMI, and waist-to-hip ratios were similar between the groups (P > 0.05). The mean fasting insulin, T, DHEAS, and CIMT levels were significantly higher and the estradiol levels were significantly lower in the patients with IH and PCOS (P < 0.05) (Tables 1 and 2).

The mean IGF-1 levels and median ALT levels were higher in the patients with IH and PCOS compared with the controls, but these differences were not statistically significant (P = 0.120, P = 0.266, respectively) (Table 1). The mean IGF-1 levels are shown as a bar graph in Figure 1. After adjusting

Table 1. The clinical and biochemical data in women with idiopathic hirsutism (IH) and polycystic ovary syndrome (PCOS), and healthy controls.

	PCOS	IH	Control	P
	(n = 25)	(n = 33)	(n = 25)	
Age, years	22.51 ± 4.07	23.24 ± 5.65	22.36 ± 4.44	0.943
BMI, kg/m^2	24.58 ± 4.62	24.86 ± 6.06	22.30 ± 3.66	0.164
Waist-hip ratio	0.84 ± 0.05	0.82 ± 0.06	0.81 ± 0.07	0.138
Ferriman–Gallwey score	9.81 ± 4.98	11.28 ± 3.61	0.20 ± 0.70	**< 0.001**
Fasting glucose, mg/dL	83.45 ± 8.10	87.60 ± 10.04	88.84 ± 10.05	0.108
Fasting insulin, µIU/mL	15.90 ± 9.71	15.09 ± 7.61	10.63 ± 6.07	**0.036**
HOMA-IR	3.24 ± 1.90	3.26 ± 1.74	2.36 ± 1.42	0.116
Total cholesterol, mg/dL	165.48 ± 21.89	167.47 ± 44.24	159.32 ± 27.86	0.637
Triglyceride, mg/dL	97.36 ± 66.12	111.86 ± 59.52	87.52 ± 52.45	0.226
HDL-C, mg/dL	52.96 ± 13.05	47.95 ± 9.40	52.52 ± 12.49	0.364
LDL-C, mg/dL	92.84 ± 20.83	96.82 ± 37.30	88.92 ± 23.13	0.603
AST, IU/L	21.15 ± 6.96	19.27 ± 6.10	19.50 ± 2.12	0.837
ALT, IU/L	18.44 ± 13.55	17.17 ± 7.05	14.14 ± 4.56	0.266
IGF1, ng/mL	258.96 ± 112.46	275.84 ± 92.49	220.20 ± 77.34	0.120
hsCRP, mg/L	1.60 ± 1.28	1.65 ± 1.52	0.87 ± 1.10	**0.020**
Carotid intima media thickness, mm	4.32 ± 0.64	4.47 ± 0.61	3.83 ± 0.49	**0.002**

HOMA-IR: homeostasis model assessment insulin resistance index; HDL-C: high density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; hs-CRP: high-sensitive C- reactive protein.

Table 2. The hormonal value and thyroid volume levels in women with polycystic ovary syndrome (PCOS) and idiopathic hirsutism (IH), and healthy controls.

	PCOS	IH	Control	P
	(n = 25)	(n = 33)	(n = 25)	
TSH, µIU/mL	1.93 ± 0.91	1.85 ± 0.63	1.89 ± 0.98	0.944
FSH, m IU/mL	5.19 ± 1.86	6.04 ± 1.88	5.94 ± 2.33	0.229
LH, m IU/mL	5.73 ± 2.71	5.65 ± 2.81	4.80 ± 2.27	0.383
Estradiol, pg/mL	57.97 ± 29.30	50.80 ± 19.34	86.54 ± 54.55	**0.007**
Total testosterone, ng/dL	60.06 ± 23.31	53.04 ± 20.82	42.50 ± 13.65	**0.008**
PRL, ng/mL	12.76 ± 8.96	12.42 ± 5.98	9.99 ± 5.19	0.384
DHEAS, µq/dL	249.69 ± 130.07	264.65 ± 115.62	172.64 ± 80.43	**0.011**

fT4: free thyroxine; TSH: thyroid stimulating hormone; FSH: follicule stimulating hormone; LH: luteinizing hormone; PRL: prolactin; DHEAS: dehydroepiandrosterone sulfate.

for age and BMI, the IGF-1 levels were significantly higher in patients with IH and PCOS (P < 0.001) (Figure 2). In linear regression analyses, FG was found to be an independent risk factor for adjusted IGF-1 (P = 0.002).

3.2. The correlations between parameters

IGF-1 levels were found to be negatively correlated with age (r: –0.471, P < 0.001) and estradiol (r: –0.240, P = 0.03), whereas they were weakly positively correlated with FG score (r: 0.232, P = 0.03). ALT levels were found to be positively correlated with BMI (r: 0.466, P < 0.0001), HOMA-IR (r: 0.394, P < 0.05), and insulin (r: 0.469, P < 0.001), and weakly correlated with FG score (r: 0.248, p <0.05), total testosterone levels (r: 0.280, P < 0.05), and CIMT (r: 0.272, P = 0.037). CIMT measurements were

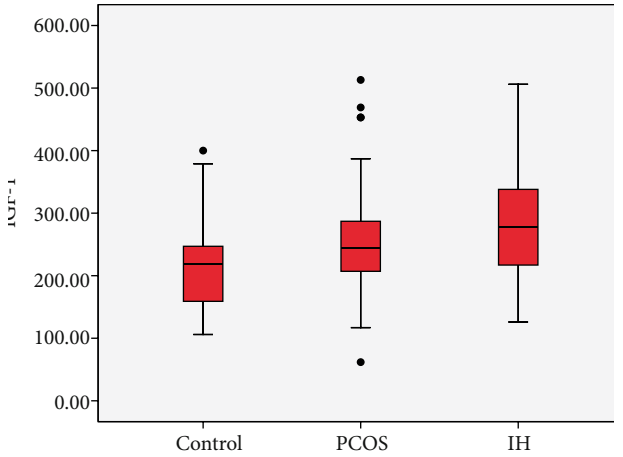

Figure 1. Comparison of IGF-1 levels between the groups.

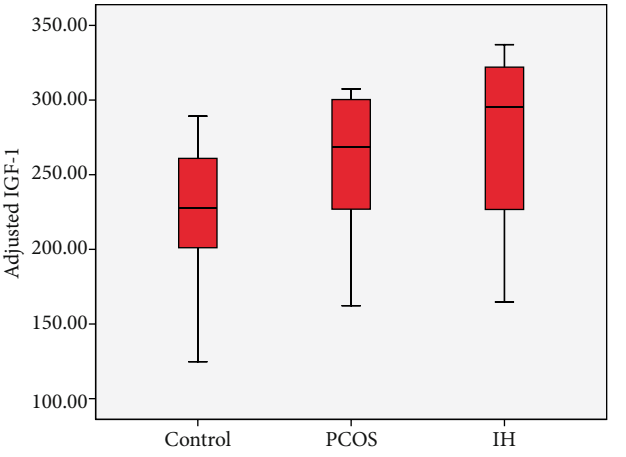

Figure 2. Comparison of adjusted IGF-1 levels between the groups.

found to be positively correlated with BMI (r: 0.459, P < 0.0001), TG (r: 0.544, P < 0.0001), insulin (r: 0.405, P < 0.0001), hsCRP (r: 0.464, P < 0.0001), FG score (r: 0.329, P = 0.005), HOMA-IR (r: 0.385, P = 0.001), and ALT (r: 0.272, P = 0.037).

3.3. Comparison of IGF-1 and ALT levels in groups classified according to HOMA-IR and FG score

The participants were divided into 2 groups according to HOMA-IR levels. The cut-off point was 2.7 (14). The participants who had a HOMA-IR greater than 2.7 are as follows: 15 (45.5%) patients in the PCOS group, 16 (64%) in the IH group, and 8 subjects (32%) in the control group (P = 0.075). Although the number of patients with HOMA-IR greater than 2.7 was higher in the PCOS and IH groups compared with the control, the difference was not statistically significant.

Those participants who had a HOMA-IR greater than 2.7 had significantly higher IGF-1 and ALT levels. Additionally, ALT and IGF-1 levels were higher in the groups with a higher FG score (FG ≥ 8) (Table 3).

4. Discussion

The results of the present study demonstrate that the mean IGF-I, ALT, hsCRP, and CIMT levels were higher in patients with IH and PCOS. Furthermore, IGF-1 showed an independent association with FG scores. FG scores also showed a significant correlation with ALT, which reflects hepatic insulin resistance, according to the study by Bonnet et al. (15). Additionally, IGF-1 and ALT levels were significantly higher in patients with a HOMA-IR greater than 2.7. However, there was no relationship between IGF-1, HOMA-IR, and CIMT. This is thought to be due to the younger age of participants.

In the study by Akanji et al., 3099 subjects were evaluated and it was demonstrated that lower IGF-1 levels were related with increasing numbers of MetS abnormalities (16). Moreover, in a recent review, authors have suggested that IGF-1 levels decrease consistently in subjects with metabolic syndrome and its components, and in those with ischemic CVD (17).

Although several studies showed that lower IGF-1 levels were associated with increased metabolic syndrome risk, in the present study we observed higher IGF-1 levels in insulin-resistant patients. This might be due to the population of the study, which included PCOS and IH patients. In relation to this, in a previous study, follicular fluid concentration of IGF-1 levels were found to be higher in patients with PCOS, which is consistent with the present study (18).

Insulin resistance and compensatory hyperinsulinemia are commonly seen in PCOS. Insulin stimulates hepatic IGF-1 production and suppresses insulin-like growth factor binding protein (IGF-BP) production and increases IGF-1 levels (19–21). Additionally, hyperinsulinemia stimulates theca-cell receptors via IGF-1 and causes

Table 3. Comparison of the IGF-1 and ALT levels in the HOMA-IR and FG groups.

Groups Variables	HOMA-IR <2.7	HOMA-IR ≥2.7	P	FG score <8	FG score ≥8	P
IGF-1 Mean ± standard deviation	231.94 ± 89.38	280.61 ± 101.72	0.035	220.78 ± 87.89	272.19 ± 100.32	0.020
ALT Mean ± standard deviation	14.84 ± 6.29	16.88 ± 5.39	0.031	14.51 ± 4.68	18.30 ± 12.17	0.160

ovarian hyperandrogenemia (22). Furthermore, in a recent study the IGF-1 receptor was found to play a critical negative regulator role in insulin sensitivity (23). Likewise, in the present study, IGF-1 levels were found to be higher in patients with insulin resistance.

Additionally, women with PCOS have biological or clinical hyperandrogenemia and it has been shown that maternal prenatal androgenization causes upregulation of fetal IGF-1 mRNA expression in adult sheep. Although circulating testosterone concentrations were not altered in these sheep, liver androgen receptor was upregulated after maternal androgen exposure; therefore, these livers may be more responsive to androgens. The human IGF-1 gene is known to contain androgen-responsive elements in the promoter region that bind androgen receptor and stimulate IGF-1 transcription (24).

In the present study, the FG score, which reflects clinical hyperandrogenemia, was obtained as an independent risk factor for increased IGF-1 levels.

There are conflicting data about the relationship between IGF-1 and increased risk of atherosclerosis and ischemic heart disease (25–29). In the present study we did not find any correlation between IGF-1 and CIMT, which reflects the subclinical atherosclerotic process. This is thought to be due to the younger age of participants and short disease duration.

IGF-1 has been found to be a potent mitogen and antiapoptotic factor. Additionally, in several epidemiologic in vitro studies and metaanalyses, IGF-1 has been found to be associated with cancer risk, particularly of breast cancer (30–34). Hyperinsulinemia and increased IGF-1 levels modulate cellular proliferation, differentiation and apoptosis mechanisms, and tumor development (35–37). Genetic variation and polymorphisms also contribute to IGF-1–mediated cancer risk (30).

Therefore, PCOS and IH patients should be followed for increased malignancy risk and scanned for breast cancer at an early age.

The study illustrated that the IGF-1 and ALT levels were significantly higher in patients with a HOMA-IR score greater than 2.7. Additionally, increased ALT levels were found to be associated with hyperinsulinemia. Due to the short disease duration of younger participants, we did not observe any correlation between hyperinsulinemia and IGF-1. These findings suggest that increased hepatic production of IGF-I and ALT might be an early indicator of insulin resistance in hirsutism. An increased FG score was discovered to be an independent risk factor for elevated IGF-1 levels. Further studies are needed to evaluate the relationship between IGF-1, insulin resistance, and atherosclerotic CVD.

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