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Endotrophin as a novel marker in PCOS and its relation with other adipokines and metabolic parameters: a pilot study

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Abstract

Background: Polycystic ovary syndrome is known to be the most common hormonal disorder in women of reproductive age. Current evidence shows that regulatory proteins secreted from the adipose tissue called adipokines may have a role in polycystic ovary syndrome. We planned to investigate the role of endotrophin that has never been researched in polycystic ovary syndrome before and its correlation with other metabolic parameters and adipokines such as adiponectin and ghrelin in patients with polycystic ovary syndrome.

Methods: Forty-three women (*n*: 43) with polycystic ovary syndrome and 43 (*n*: 43) women as a control group were enrolled in this cross-sectional study. Serum levels of endotrophin, adiponectin, and ghrelin levels were measured with the enzyme-linked immunosorbent assay method. High-density lipoprotein cholesterol, low-density lipoprotein cholesterol, total cholesterol levels, luteinizing hormone/follicle-stimulating hormone ratio, total testosterone, and triglyceride levels were measured. Homeostasis model assessment for insulin resistance index, body mass index, Ferriman Gallwey Score, and waist-to-hip ratio were also evaluated.

Results: Total testosterone, homeostasis model assessment for insulin resistance, C-reactive protein, luteinizing hormone/follicle-stimulating hormone ratio, and triglyceride levels were higher in patients with polycystic ovary syndrome ($p < 0.01$). No difference was detected between the groups in terms of body mass index, Ferriman Gallwey Score, waist-to-hip ratio, total cholesterol, low-density lipoprotein, and high-density lipoprotein levels ($p > 0.05$). We did not observe any significant difference in adiponectin and ghrelin levels between the groups ($p > 0.05$). Patients with polycystic ovary syndrome had significantly higher endotrophin levels ($p < 0.01$). According to our regression analyses [area under the curve: 0.973 (0.935–1.000), 95% confidence interval, 95.2% sensitivity, and 100% specificity], it was shown that endotrophin greater than 92 ng/ml and homeostasis model assessment for insulin resistance greater than 2.5 might be good predictors for polycystic ovary syndrome diagnosis.

Conclusion: We demonstrated that endotrophin level is higher in patients with polycystic ovary syndrome and may have predicted polycystic ovary syndrome with increased homeostasis model assessment for insulin resistance index. There was no significant difference in adiponectin and ghrelin levels in the polycystic ovary syndrome group. Endotrophin may have a role in polycystic ovary syndrome etiology rather than other adipokines.

Keywords: adiponectin, endotrophin, ghrelin, polycystic ovary syndrome

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Introduction

Polycystic ovary syndrome (PCOS) is described as oligoanovulation, polycystic ovarian morphology, and higher androgen levels and is one of the most common endocrinopathies in reproductive aged women.[1] As a main cause of anovulation in

infertile women, PCOS is still a disorder with multiple unknown causes and metabolical results.[2]

Adipose tissue secretes a wide variety of signaling molecules that regulate certain homeostatic systems, such as nutrient intake, energy expenditure, insulin secretion, and insulin functioning. Most of these factors secreted from adipose tissue are cytokines and adipokines such as tumor necrosis factor (TNF)-alpha, C-reactive protein (CRP), interleukin (IL)-6, adiponectin, and ghrelin, which regulate immune and inflammatory responses.[3] Studies showed that the expression profile of adipose tissue changed in patients with PCOS, and this change causes adipocytes to release an unbalanced amount of adipokines which adversely affects endocrine and reproductive systems. This unbalanced secretion with increased inflammatory responses and insulin resistance may be responsible for PCOS and its clinical results.[4]

Endotrophin, as a newly discovered adipokine, is a soluble proteolytic product of type VI collagen's A3 chain. It is secreted secondarily to a metabolic problem, especially in adipose tissue and plays a key role in many events such as inflammation, chemotaxis, apoptosis, angiogenesis, and regulation of myofibroblast accumulation. This accumulation eventually leads to fibrosis in the extracellular environment of both adipose tissue and other tissues in the body. Endotrophin increases the proinflammatory cytokines and causes insulin resistance and also associated with pathologies such as diabetes mellitus, atherosclerosis, metabolic syndrome, and some cancers such as breast and colon cancers.[5,6]

An increased number of studies conducted in recent years indicate that there is a chronic low level of inflammatory activity in PCOS. While some signal molecules secreted by leukocytes, oocyte, and follicular cells in the ovarian tissue regulate ovarian functions by paracrine and autocrine signals, molecules secreted from other tissues such as adipose tissue regulate ovarian functions by endocrine signals. Some of these molecules act as proinflammatory and some as anti-inflammatory. It is thought that this balance shifted to the proinflammatory direction in PCOS. This increased inflammatory state disrupts steroid synthesis and follicular maturation in the ovarian tissue, linking metabolic complications of PCOS such as obesity, cardiovascular disease, insulin resistance, and diabetes with each other.[7,8]

Adiponectin is one of these adipokines which has a role in adjusting energy balance, inhibiting vascular inflammation, improving lipid metabolism, and increasing insulin sensitivity.[9] Ghrelin is another adipokine and a gastric peptide that comprises 28 amino acids, which are mainly secreted from oxyntic cells of the stomach. Besides its effects on food intake, glucose metabolism, and energy balance, it regulates the reproductive axis from the hypothalamus to the ovary by decreasing the luteinizing hormone (LH) responsiveness to luteinizing hormone-releasing hormone (LH-RH).[10] Although publications are stating that adiponectin and ghrelin levels are low in PCOS, some researchers claimed a controversy about their levels.[11–13]

In the literature, several studies prove the relationship between metabolic alterations in adipose tissue and PCOS. In this study, we aimed to investigate the hypothesis that new adipokine endotrophin is associated with PCOS and its metabolic parameters. We also hypothesized that there is a relationship between endotrophin and other adipokines such as adiponectin and ghrelin.

Materials and methods

Participants and study design

In this cross-sectional study, we enrolled 43 patients with PCOS and compared them with healthy age-matched controls. We selected the study participants from our outpatient gynecology clinic between April and December 2019. Our research complies with the Helsinki Committee's requirements and was approved by the Ethics Committee of the Balıkesir University (number: 2019/069). Written informed consent was given by all patients before enrollment into the study. Based on the criteria of the European Society of Human Reproduction and Embryology and American Society for Reproductive Medicine (Rotterdam ESHRE/ASRM—Sponsored PCOS Consensus Workshop Group, 2004) if two of the following three criteria are present, a diagnosis of PCOS is accepted: the polycystic appearance of ovaries on the ultrasound image (the presence of 12 follicles or more in the range of 2–9 mm in diameter and the ovarian volume above 10 cm^3),

anovulation and oligo-ovulation, and biochemical and clinical signs of hyperandrogenism.[1] The definition of oligo-anovulation was made according to the existence of oligomenorrhea (menstrual cycle longer than 35 days) or amenorrhea (failure to menstruate for 6 months or longer). We chose 43 regularly menstruating healthy women for our control group. Patients were consecutively referred and written informed consent was obtained from all individuals who participated in our research. Blood was drawn in the early follicular period of the menstrual cycle or the early follicular period in progesterone-affected patients with amenorrhea. Transvaginal ultrasound was performed on the same day using a 7.5-MHz vaginal transducer (Voluson 730, GE Healthcare, Austria). The same physician examined all patients. The study's exclusion criteria were the presence of a history of taking oral contraceptive agents, glucocorticoids, anticoagulants or anti-platelet agents, insulin sensitizers, and anti-androgens, anti-lipidemic, and antihypertensive drugs, at least 4 months before the beginning of our research. We did not include patients with body mass index (BMI) over 30 in our study.

To evaluate hirsutism, the Ferriman Gallwey Score (FGS) was used and a score greater than 8 was accepted as clinical hyperandrogenism.[14] The serum level of total testosterone greater than 0.80 ng/ml was accepted as biochemical hyperandrogenism.[15] Waist-to-hip ratio (WHR) shows the visceral fat accumulation. BMI was calculated by dividing a person's weight in kilograms by the square of their height in meters. The same physician evaluated the hirsutism scores, WHR, and BMI.

Biochemical analysis

Between 9.00 a.m. and 10.00 a.m. following an overnight fast, each venous blood samples from all patients was sent to the laboratory for hormonal and biochemical analysis. All samples were centrifuged at 1300*g* for 10 min and stored at −40°C until analyzed. The enzyme-linked immunosorbent assay (ELISA) kits, which were commercially available, were used to determine serum ghrelin (300–4800 pg/ml), adiponectin (2–32 ng/ml), and endotrophin levels(12.5–200 ng/ml) by the manufacturer's instructions (Sunred Biological Technology Co., Ltd, Shangai, China). The dilution method was applied to the high adipokine values during biochemical analysis. Commercially available kits (Cobas Integra 800; Roche Diagnostics GmbH, Germany) were used on a chemistry autoanalyzer to measure low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol, and triglycerides. In a hormone autoanalyzer (Beckman Coulter; Unicel DXI 600; Access Immunoassay, Brea, CA), we measured the fasting insulin levels with Access kits. Serum testosterone levels were measured by using commercially available kits (eBioscience, Vienna, Austria) with an ELISA technique on a diagnostic instrument (BioTek, ELx 800, Winooski, VT). Insulin resistance was calculated using the homeostasis model [homeostasis model assessment for insulin resistance (HOMA-IR)], as fasting glucose (mmol/l) × fasting insulin (mU/l)/22.5[13]

Data analysis

We used Statistical Package for Social Sciences (SPSS 25.0; SPSS Inc., Chicago, IL) for statistical analyses. The following descriptive statistics were used to describe the study data: mean, median, standard deviation, and frequency. The Mann–Whitney *U* test and Spearman's correlation coefficients were used to analyze the data. A p-value <0.05 was considered statistically significant. A logistic regression method was used to evaluate the predictors of PCOS. To evaluate the multivariable model, any variables whose univariable test result had a p-value <0.05 was accepted as a candidate. Adjusted odds ratios for each variable were calculated at a 95% confidence interval. Two-sided p values were considered statistically significant at $p < 0.05$.

Results

The demographic characteristics—clinical, biochemical, hormonal parameters, endotrophin, adiponectin, and ghrelin levels—are shown in Table 1. We did not observe any difference for BMI, FGS, WHR, total cholesterol, LDL, and HDL levels between PCOS and control group ($p > 0.05$). The mean ages were similar between the groups ($p > 0.05$). Adiponectin and ghrelin levels also showed no difference between the groups ($p > 0.05$). The patients with PCOS had significantly higher endotrophin levels than the control group (188.03 ± 82.88, 32.99 ± 11.31, respectively) ($p < 0.01$) (Table 1).

Table 1. Clinical characteristics, hormonal, and biochemical results of the groups.

Variables	PCOS ($n=43$)	Control ($n=43$)	p value
Age (years)	31.0 ± 8.38	28.42 ± 5.22	0.262
BMI (kg/m^2)	28.05 ± 5.77	27.13 ± 4.44	0.674
WHR	0.78 ± 0.18	0.73 ± 0.16	0.194
Ferriman Galleway Score (FGS)	13.19 ± 3.94	11.69 ± 3.43	0.099
HOMA-IR	3.85 ± 1.70	1.62 ± 0.68	<0.001*
CRP	10.90 ± 3.49	6.43 ± 3.12	<0.001*
LH/FSH ratio	1.55 ± 1.08	0.71 ± 0.39	<0.001*
Total testosterone (ng/ml)	0.82 ± 0.39	0.52 ± 0.21	<0.001*
Triglyceride (mg/dl)	123.80 ± 53.76	107 ± 76.07	0.024*
Total cholesterol (mg/dl)	185.71 ± 38.33	175 ± 31.24	0.163
LDL (mg/dl)	110.84 ± 38.60	100.74 ± 27.38	0.171
HDL (mg/dl)	49.52 ± 9.79	54.23 ± 12.20	0.081
Endotrophin (ng/ml)	188.03 ± 82.88	32.99 ± 11.31	<0.001*
Adiponectin (ng/ml)	31.04 ± 28.25	48.49 ± 42.78	0.17
Ghrelin(pg/ml)	4162 ± 2468	5071.21 ± 3260	0.21

BMI, body mass index; CRP, C-reactive protein; FGS, Ferriman Galleway Score; FSH, follicle-stimulating hormone; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; LDL, low-density lipoprotein; LH, luteinizing hormone; PCOS, polycystic ovary syndrome; WHR, waist-to-hip ratio.
Data presented as mean ± SD.
*$p < 0.05$ accepted as statistically significant.

HOMA-IR and CRP levels were higher in patients with PCOS than in the control group ($p < 0.01$). Luteinizing hormone (LH)/follicle-stimulating hormone (FSH) ratio, total testosterone, and triglyceride levels were also higher in the PCOS group when compared with the control group ($p < 0.01$) (Table 1).

In our correlation analysis, as shown in Table 2, there was a positive correlation between endotrophin and HOMA-IR, CRP, LH/FSH ratio, and total testosterone levels in all patients. The optimal cut-off point of the endotrophin that discriminated groups from each other was evaluated using a receiver-operating characteristic (ROC) curve analysis [area under the curve (AUC): 0.973 (0.935–1.000), 95% confidence interval (CI), 95.2% sensitivity, and 100% specificity]. According to this multivariate analysis, we detected that endotrophins greater than 92 ng/ml and HOMA IR greater than 2.5 predicted the PCOS best (Table 3).

Discussion

In the current study, we found that endotrophin levels were significantly higher in patients with PCOS. There were also higher CRP, HOMA-IR, total testosterone, LH/FSH ratio, and triglyceride levels in patients with PCOS. No difference was detected in terms of adiponectin and ghrelin levels between the patients with PCOS and healthy controls. We also detected that higher endotrophin and HOMA-IR index predicted the PCOS best.

Literature suggests that the vascular bed's inability to fufil the metabolic requirements of adipose tissue expansion might cause hypoxia at the tissue level. After hypoxia, the excess collagen secreted at the tissue level turns into fibrosis and limits the adipose

Table 2. Associations of different clinical, hormonal, and metabolic parameters with serum endotrophin, adiponectin, and ghrelin levels in all patients (correlation analysis).

	Endotrophin		Adiponectin		Ghrelin	
BMI (kg/m^2)	*r*: 0.033	*p*: 0.769	*r*: –0.019	*p*: 0.862	*r*: 0.076	*p*: 0.491
WHR (waist-to-hip ratio)	*r*: 0.143	*p*: 0.194	*r*: 0.026	*p*: 0.815	*r*: –0.044	*p*: 0.691
Ferriman Galleway Score (FGS)	*r*: 0.128	*p*: 0.247	*r*: –0.078	*p*: 0.480	*r*: 0.089	*p*: 0.421
HOMA-IR	*r*: 0.623**	$p < 0.001$	*r*: –0.097	*p*: 0.378	*r*: –0.044	*p*: 0.691
CRP	*r*: 0.508**	$p < 0.001$	*r*: –0.131	*p*: 0.235	*r*: 0.016	*p*: 0.888
LH/FSH ratio	*r*: 0.402**	$p < 0.001$	*r*: –0.158	*p*: 0.152	*r*: –0.193	*p*: 0.079
Total testosterone (ng/dl)	*r*: 0.306**	*p*: 0.005	*r*: –0.237*	*p*: 0.030	*r*: –0.107	*p*: 0.331
Triglyceride (mg/dl)	*r*: 0.170	*p*: 0.121	*r*: –0.031	*p*: 0.777	*r*: 0.016	*p*: 0.883
Total cholesterol (mg/dl)	*r*: 0.124	*p*: 0.262	*r*: –0.137	*p*: 0.212	*r*: –0.047	*p*: 0.674
LDL (mg/dl)	*r*: 0.123	*p*: 0.261	*r*: –0.102	*p*: 0.356	*r*: –0.028	*p*: 0.799
HDL (mg/dl)	*r*: –0.86	*p*: 0.436	*r*: –0.139	*p*: 0.209	*r*: –0.111	*p*: 0.314

BMI, body mass index; CRP, C-reactive protein; FGS, Ferriman Galleway Score; FSH, follicle-stimulating hormone; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; LDL, low-density lipoprotein; LH, luteinizing hormone; WHR, waist-to-hip ratio. r, correlation coefficient.
**Correlation is significant at $p < 0.01$.
*Correlation is significant at $p < 0.05$.

tissue by disrupting the remodeling of the extracellular tissue that must be flexible.[16] Supporting this relation, various studies have shown that the extracellular matrix balance is disturbed by fibrotic factors, resulting in an increase in the production of ovarian stromal elements, which may have caused PCOS. During this unhealthy remodeling, Li *et al.*[17] detected that the collagen degradation product, endotrophin, was released into the circulation and caused both local and systemic inflammation. Confirming this relationship, they tried to block endotrophin using a neutralizing antibody and saw that the metabolic side effects first gradually disappeared. After that, metabolic dysfunction reversed. To explain endotrophin's mechanism, Park *et al.* showed that abnormal expression of transforming growth factor beta 1 (TGF-β1) signaling a pathway has a role in its fibrosis. Besides its fibrotic effects, increased expression of TGF-β1 stimulates the theca-interstitial cells, which causes increased androgen production seen in PCOS.[18] Despite the relationship between ovarian theca cells and androgens, studies have also indicated that there may be a mutual interaction between androgens and adipose tissue.[19] In one study, Klöting and Blüher[20] observed that androgens could be overexpressed both from disorganized adipose tissue and a rise secondary to conditions, such as PCOS, which may disrupt the adipose tissue structure.

Molecular studies to elucidate the mechanism of this reciprocal vicious circle have not yet provided a clear reason; however, we think that endotrophin plays a key role in this relationship, as we observed a positive correlation between endotrophin and total testosterone levels.[21] Although the results between adipokines other than endotrophin and androgens are controversial in the literature, we think our result is important, as we have not encountered the relationship between endotrophin and testosterone now in the literature.[22–25] Endotrophin levels have been revealed to play a role in regulating energy balance and insulin sensitivity.[18] In a study by Sun *et al.*,[16] suppression of endotrophin decreases adipose tissue inflammation and increases insulin sensitivity in animals. In another study, mice were exposed chronically to endotrophin, and impaired glucose and insulin tolerance were observed as a result.[26]

Table 3. Predictors of PCOS in logistic regression analysis.

	Univarite analysis		Multivariate analysis	
	Odds ratio (95% CI)	***p* value**	**Odds ratio (95% CI)**	***p* value**
Obesity (>30)	2.772 (0.994–7.766)	0.047	0.634(0.094–48.577)	0.634
Waist-to-hip ratio (>0.86)	1.250 (0.495–3.159)	0.637	–	
Biochemical hiperandrogenism (T.Tes > 0.80)	8.140 (2.675–24.774)	<0.001	0.009–11.702	0.544
Clinical hyperandrogenism (FGS > 8)	1.900 (0.512–7.052)	0.332	–	
Dyslipidemia	3.692 (1.273–10.714)	0.013	0.149 (0.003–8.077)	0.350
Insulin resistance (HOMA-IR > 2.5)	90.25 (21.022–387.457)	<0.001	0.487 (0.307–0.773)	0.036
Endotrophin > 92	260.0 (41.179–1641.619)	<0.001	1.847 (1.216–2.805)	<0.001
LH/FSH > 2	12.813 (1.558–105.361)	0.004	1.200 (0.020–72.680)	0.931
Inflammation (CRP > 10)	4.130 (1.499–11.383)	0.005	0.172 (0.005–5.408)	0.317

AUC, area under the curve; CI, confidence interval; CRP, C-reactive protein; FGS, Ferriman Galleway Score; FSH, follicle-stimulating hormone; HOMA-IR, homeostasis model assessment for insulin resistance; LH, luteinizing hormone; PCOS, polycystic ovary syndrome.
$p < 0.05$ accepted as statistically significant; AUC: 0.973 (0.935–1.000), 95% CI, 95.2% sensitivity, and 100% specificity.

Our study found a significant positive correlation between endotrophin and HOMA-IR index similar to previous animal studies.

Karsdal *et al.*[27] showed a correlation between BMI and endotrophin levels. Our study did not find any correlation between serum endotrophin levels and BMI, which is associated with unhealthy fat mass, such as white adipose tissue located near visceral organs. As BMI does not always reflect the unhealthy fat ratio in the body, we think that our correlation may be accepted.[28] So, due to our results, we believe that unhealthy fat tissue may have a role in PCOS etiology and other confounding factors such as increased insulin resistance and inflammatory response, together with the higher endotrophin levels in the PCOS group.

Despite the current evidence of adipose tissue's role in PCOS, several studies show contradictory results in the level of both adiponectin and ghrelin in PCOS.[29] We did not observe any significant difference about both of their levels in PCOS group. It was shown that adiponectin levels are inversely related to the degree of adiposity, but we did not find any correlation between adiponectin with WHR and BMI.[30] However, it was observed that ghrelin might have a negative effect on fertility by modifying the insulin resistance and androgen levels; likewise, we did not see any correlations between these parameters and ghrelin.[31] So, we conclude that endotrophin is an additional and potentially useful biomarker for PCOS.

Many studies have shown that systemic inflammation is increased in patients with PCOS, and CRP may indicate this.[32] We found that CRP was higher in our patients with PCOS. We also detected a positive correlation between endotrophin and CRP, as we thought that endotrophin might have a role in this inflammation. Indeed, supporting our opinion, Aydin Yoldemir *et al.*[33] and Khan *et al.*[34] also stated in their studies that there was a relationship between CRP and endotrophin.

In this study, we did not separate patients based on their BMI, which can be considered as a limitation, because we did not investigate the possible correlation between endotrophin and BMI. Another limitation of this study is our relatively small number sample size. However, we believe that comparing the adiponectin and ghrelin levels with endotrophin constitutes a strength of our

research, as both of their levels are related to the adipose tissue amount, to which we did not show any correlation.[35]

Conclusion

In the current study, we report the association between endotrophin and PCOS, for the first time. Our results demonstrate that higher endotrophin and HOMA-IR index might be good predictors for PCOS diagnosis. Endotrophin was positively correlated with HOMA-IR, CRP, total testosterone, and LH/FSH ratio. Although we evaluated two adipokines, such as adiponectin and ghrelin levels, we did not observe any significant differences in their levels between the groups.

Author contributions

GG contributed to conceptualization; formal analysis; methodology; writing—original draft; and writing—review and editing.MIT contributed to methodology; supervision; and writing—review and editing. OB contributed to methodology. EA contributed to supervision. SG and SS contributed to investigation. CK contributed to formal analysis and software.ET contributed to writing—review and editing.

Conflict of interest statement

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