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R-Spondin1 and tumor necrosis factor-alpha in infertile women with polycystic ovary syndrome: relationships with insulin resistance and other parameters

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ABSTRACT

Aim: To evaluate the relationship between R-spondin1 (RSPO1) and Tumor Necrosis Factor-Alpha (TNF-α) levels with insulin resistance (IR) and other parameters in infertile women with polycystic ovary syndrome (PCOS).

Material and Method: This case-control prospective observational study was carried out on 84 patients admitted to the University of Health Sciences Etlik Zübeyde Hanım Training and Research Hospital Gynecology and IVF Clinic and Medistate Hospital Gynecology and IVF Clinic between September 2020 and June 2021. Women aged 18-36 years diagnosed with infertility and PCOS constituted the PCOS group. Women who were diagnosed with infertility but not PCOS formed the control group. Cases were divided according to their body mass index (BMI) values into obese (BMI≥25) and non-obese (BMI<25) groups.

Results: The mean age of the study group was 26.9±5.37 years. RSPO1 and TNF-α levels were higher in PCOS patients ($p<0.001$). Hip circumference (HC) was found to be higher in those with a diagnosis of PCOS ($p<0.001$). The group with the highest waist-to-hip ratio (WHR) was the obese-PCOS group ($p<0.001$). Glucose, insulin, and homeostatic model assessment for IR (HOMA-IR) levels were the highest in the obese-PCOS group ($p<0.001$). A positive correlation was found between RSPO1 and TNF-α ($r=0.944$, $p<0.001$). There was a positive correlation between the hip circumference and RSPO1 ($r=0.255$, $p=0.0019$) and TNF-α ($r=0.\ 0.278$, $p=0.011$). There were positive correlations between blood glucose and RSPO1 ($r=0.343$, $p=0.001$) and TNF-α levels ($r=0.312$, $p=0.004$) and between insulin and RSPO1 (r value=0.577, $p<0.001$) and TNF-α levels ($r=0.569$, $p<0.001$). HOMA-IR values were also found to correlate with RSPO-1 and TNF-α levels (r= 0.619 and 0.608, $p<0.001$).

Conclusion: It can be concluded that RSPO1 and TNF-α levels may guide the assessment and management of IR and diabetes risk in infertile women with PCOS.

Keywords: Polycystic ovary syndrome, R-Spondin1, tumor necrosis factor-alpha, obesity, insulin resistance

INTRODUCTION

Infertility refers to failure to conceive after 12 months of regular unprotected sex, and almost 8-12% of couples worldwide suffer from infertility (1). Polycystic Ovary Syndrome (PCOS) is the most prevalent endocrine dysfunction in childbearing-age women, which is also the most common cause of anovulatory infertility (~%90) (2,3). PCOS is a multifaceted disease characterized by polycystic ovaries, clinical or biochemical hyperandrogenism, and chronic oligo/anovulation (3). When women with PCOS become pregnant, they have a higher risk of pregnancy-related diabetes mellitus (DM) and pregnancy complications than pregnant women without PCOS; the former creates a significant burden on healthcare (3).

Obesity is one of the common complications in patients with PCOS (4). It has been reported that obesity is associated with the exacerbation of metabolic and ovulatory dysfunction associated with PCOS, and weight loss restores ovulation and reduces hyperandrogenism (5). Increased waist-to-hip circumference (WHC) ratio and abdominal obesity decrease the menstrual frequency and fertility in relation to insulin resistance (IR) (3). One

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of the characteristics of PCOS is IR with compensatory hyperinsulinemia (6). Hyperinsulinemia causes an increase in ovarian androgen biosynthesis in vitro and in vivo, in addition to a decrease in sex hormone binding globulin (SHBG) protein synthesis in the liver, which causes an increase in the free androgens bioavailability (6). This production increase of local ovarian androgen, which is increased by hyperinsulinemia, leads to early follicular atresia and anovulation, which impairs the ability to conceive (3).

In some previous in-vivo studies, it has been reported that R-spondin1 (RSPO1) regulates food intake and increases insulin secretion (7-9). RSPO consists of four proteins (RSPO1–4), secreted as potent enhancers of Wnt/β-catenin signaling. The signaling system of wnt/β-catenin is essential in maintaining adult stem cells, self-renewal, and embryonic development. (9). It has been shown that increasing serum RSPO1 level is significantly related to infertility, obesity and IR, although the exact mechanisms are unknown (10,11). The potential link between PCOS and the increase in pro-inflammatory cytokine Tumor Necrosis Factor-Alpha (TNF-α) levels is particularly important because TNF-α has a multifaceted effect on the functions of ovarian, such as corpus luteum regression, ovulation, and follicular growth and ovaries contain TNF-α receptors and produce TNF-α (12). Therefore, TNF-α levels are variable in PCOS, contributing to the disease's short-term ovarian dysfunction and hyperandrogenic state, and may be associated with long-term effects on the ovaries and other organs.

This study evaluates the relationship between RSPO1 and TNF-α levels with IR and other parameters in infertile women with PCOS.

MATERIAL AND METHOD

This case-control prospective observational study was carried out on 84 patients admitted to the University of Health Sciences Etlik Zübeyde Hanım Training and Research Hospital Gynecology and IVF Clinic and Medistate Hospital Gynecology and IVF Clinic between September 2020 and June 2021.

The procedures were conducted according to the Clinical Research and Ethics Committee regulations and the Helsinki Declaration. The study was carried out with the permission of the Research Ethics Committee of University of Health Sciences Etlik Zübeyde Hanım Training and Research Hospital (Date: 09.09.2020, Decision No: 2020/128). All the patients were given signed informed consent.

The PCOS group consisted of women aged 18-36 with a diagnosis of infertility and PCOS who applied to the clinic within the study period. Women admitted to the infertility clinic who were not diagnosed with PCOS formed the control group. Women in the PCOS and control groups were divided into obese (BMI≥25) and non-obese (BMI<25) groups according to body mass index (BMI) values. The BMIs of the obese and non-obese groups in the study and control groups were not significantly different. Patients who participated in the study, had a diagnosis of PCOS, and had not been able to conceive for at least one year between the ages of 18-36, were included in the PCOS group. Patients excluded from the study had diabetes mellitus, thyroid dysfunction, hypertension, impaired glucose tolerance, hypercortisolism, hyperprolactinemia or any endocrinopathy, used oral contraceptives or any drugs altering hormone, insulin, or lipid metabolism three months before the study. Patients with vitamin B6 or B12 deficiency or those who used vitamin supplements within the last six months to treat previously diagnosed deficiency (because it may have affected homocysteine metabolism) and smokers were not included in the study group.

Patients were diagnosed with PCOS based on meeting at least two of the 2003 Rotterdam Consensus criteria (13).

A routine clinical procedure (history, physical examination, ultrasonography (US), blood analysis, etc.) was performed. Venous blood samples were collected from all subjects in the morning after overnight fasting. Serum samples were obtained by centrifuging the venous blood samples for 10 minutes at 4000 rpm after the 30-minute coagulation period. Serum samples collected for biochemical and hormonal evaluation were examined in the biochemistry and hormone laboratory of Medistate Hospital.

BMI values were calculated by dividing the weight (kg) by the square of the height (m). Waist circumference, hip circumference, and waist-hip ratio (WHR) were determined. Patients' systolic and diastolic blood pressure values were measured and recorded with standardized devices.

SBP (mm-Hg) was measured by using the tail-cuff technique. DBP (mm-Hg) , AMH (ng/mL), LH (IU/L), FSH (mIU/mL), Estradiol (pg/mL), Free T4(ng/dL), Prolactin (µg/L), TSH (uIU/mL), LDL (mg/dL), HDL (mg/dL), Triglyceride (mmol/L), and Insulin (IU/mL) were measured by commercial enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN, USA). Total cholesterol (mg/dl) was measured by the cholesterol-oxidase method and Glucose (mmol/L) was measured by a glucometer.

The HOMA index is also used to evaluate insulin resistance. This index was calculated from the following formula using fasting serum glucose and insulin levels:

HOMA-IR = [Fasting Glucose (mg/dl) x Fasting Insulin (uU/ml)/22.5]

RSPO-1 and TNF-α measurements were performed with Human R-spondin-1 Enzyme-Linked Immunosorbent (ELISA) and TNF-α ELISA Kits (Reader Biotek ELx800). The ELISA quantitatively immunoassays human RSPO1 levels. The intra-assay coefficient of variation (CV) of the ELISA kit was 5.7% and the inter-assay CV of the ELISA Kit was 9.5% in our laboratory. Normal values for TNF-α were considered 75 +/- 15 pg/ml.

Statistical Analysis

All analyses were performed on SPSS v21 (SPSS Inc., Chicago, IL, USA). Shapiro-Wilk test determined the normal distribution. According to the normality of distribution, data are given as mean ± standard deviation or median. One-way variances (ANOVA) were used for normally distributed variables. Pairwise comparisons of these variables were performed with the Tukey or Tamhane test, depending on the homogeneity of variances. Kruskal Wallis test was used for non-normally distributed variables. The Bonferroni correction method was used for pairwise comparisons of these variables. Spearman correlation coefficients were calculated to evaluate relationships between variables. $p<0.05$ values accepted as statistically significant results.

RESULTS

The ages of the women in the study group ranged between 18-36, with a mean of 26.99±5.37 years. Waist circumference ($p<0.001$), BMI ($p<0.001$), and weight ($p<0.001$) were found to be higher in obese groups. Hip circumference was higher in those who were obese and those with a diagnosis of PCOS ($p<0.001$). The groups with the highest WHC ratio were obese women with PCOS and women in the obese control group, respectively ($p<0.001$). Systolic blood pressure was the lowest in the obese PCOS group ($p=0.013$).

As shown in **Figures 1-2** and **Table 1**, RSPO1 ($p<0.001$), TNF-α ($p<0.001$), and AMH ($p=0.006$) levels were significantly higher in the PCOS groups. LH level was significantly higher in obese women with PCOS than in other groups ($p<0.001$). LDL level was the lowest in the non-obese PCOS group ($p=0.022$). Glucose ($p<0.001$), insulin ($p<0.001$), and HOMA-IR ($p<0.001$) levels were highest in the obese PCOS group. The groups were similar in age, height, diastolic blood pressure, FSH, Estradiol, Free T4, TSH, Prolactin, total cholesterol, HDL, and triglyceride.

Table 1. Summary of the individuals characteristics and laboratory measurements with regard to groups

	Controls (BMI<25)	Controls (BMI≥25)	PCOS (BMI<25)	PCOS (BMI≥25)	P
N	21	21	21	21	N/A
Age (year)	25 (23-28)	27 (23-35)	26 (21-29)	27 (24-32)	0.55
Height (cm)	163 (158-165)	165 (160-168)	163 (159-167)	163 (158-170)	0.76
Weight (kg)	53 (52-58)[a]	75 (70-78)[b]	56 (53-61)[a]	81 (69-92)[b]	0.001
BMI (kg/m2)	20.31 (19.5-22)[a]	27.5 (25.8-30.1)[b]	20.96 (20.0-22.5)[a]	29.5 (27.8-31.3)[b]	0.001
WC (cm)	74.19±9.35[a]	88.19±13.06[b]	74.76±5.25[a]	97.43±13.43[b]	0.001
HC(cm)	94 (92-100)[a]	104 (98-112)[bc]	99 (93-102)[ab]	111 (107-119)[c]	0.001
WHR	0.78±0.07[ab]	0.83±0.07[bc]	0.76±0.05[a]	0.87±0.10[c]	0.001
SBP (mm-Hg)	110 (100-110)[ab]	120 (110-120)[b]	110 (110-120)[ab]	100 (100-110)[a]	0.013
DBP (mm-Hg)	60 (60-80)	65 (60-80)	60 (60-80)	60 (60-60)	0.131
RSPO1 (µg)	434.0 (386.8-550.4)[a]	691.7 (418.9-1079.1)[a]	2814.1 (1702.7-3762.0)[b]	2694 (2051-3371)[b]	0.001
TNF-α(ng/ml)	61.59 (54.3-71.91)[a]	87.71 (57.15-139.67)[a]	352.51 (227.44-487.7)[b]	287 (236.4-391.7)[b]	0.001
AMH (ng/mL)	1.96 (1.78-2.31)[ab]	1.69 (1.45-2.11)[a]	2.54 (1.94-2.85)[b]	2.19 (1.67-3.2)[b]	0.006
FSH (mIU/mL)	6.11±1.84	5.58±1.63	6.36±1.66	5.91±1.18	0.451
LH (IU/L)	4.56 (3.26-5.62)[a]	4.9 (4.1-5.25)[a]	4.5 (4.19-6.2)[a]	9.64 (6.93-12.2)[b]	0.001
Estradiol (pg/mL)	44 (28-51)	41 (32-52)	32.5 (29-55.8)	37 (33-48.4)	0.947
Free T4(ng/dL)	1.03 (0.98-1.11)	1.07 (0.96-1.37)	1.08 (1.05-1.13)	1.07 (0.96-1.13)	0.610
TSH (uIU/mL)	1.99 (1.46-2.39)	1.61 (1.28-2.13)	1.75 (1.26-2.36)	1.72 (1.26-2.36)	0.755
Prolactin (µg/L)	15.83±5.69	17.10±4.97	14.75±6.74	15.31±5.26	0.582
Total cholesterol (mg/dl)	201.29±29.41	222.57±67.01	192.43±51.80	189.19±41.08	0.263
LDL (mg/dL)	124 (92-142)[b]	102 (72-135)[ab]	89 (69-123)[a]	115 (105-151)[b]	0.022
HDL (mg/dL)	69.46±11.78	61.43±14.78	59.90±15.20	59.18±13.38	0.069
Triglyceride (mmol/L)	106 (98-125)	96 (68-132)	92 (84-108)	102 (68-126)	0.617
Glucose (mmol/L)	88 (85-92)[ab]	86 (82-92)[a]	95 (90-104)[bc]	96 (91-101)[c]	0.001
Insulin (IU/mL)	6.14 (5.4-8)[a]	8.1 (6.6-11.2)[ab]	10.3 (7.8-12.1)[bc]	12.1 (10.9-16.3)[c]	0.001
HOMA-IR	1.35 (1.22-1.63)[a]	1.65 (1.42-2.54)[ab]	2.35 (1.61-2.68)[bc]	3.15 (2.59-3.9)[c]	0.001

Data are given as mean ± standard deviation or median (1st quartile - 3rd quartile) according to the normality of distribution
The same letters and format denote the lack of statistically significant difference between groups
BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; AMH, anti mullerian hormone; FSH, folicle stimulating hormone; LH, luteinizing hormone; TSH, thyroid stimulating hormone; LDL; low dansity lipoprotein; HDL, high dansity lipoprotein

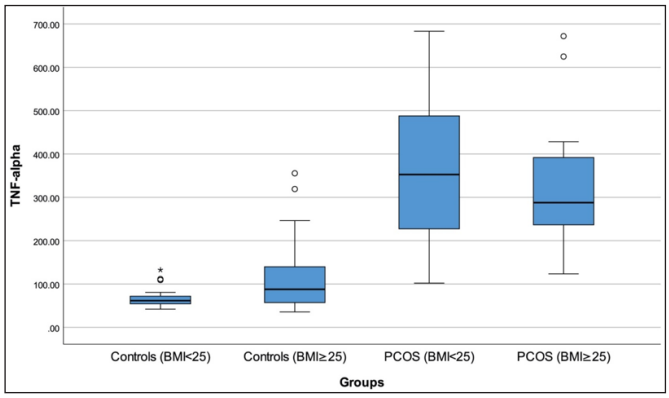

Figure 1: TNF-α levels in groups

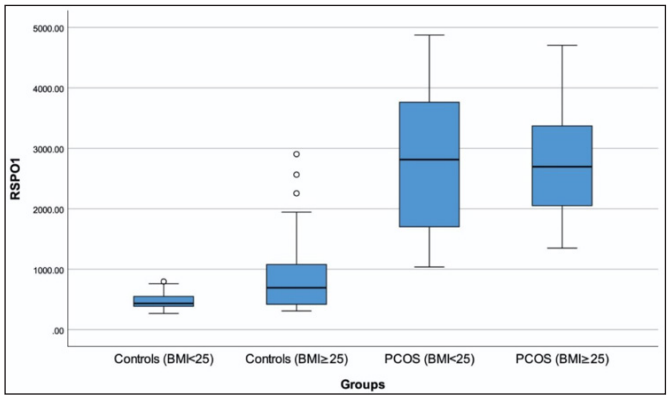

Figure 2. RSPO1 levels in groups

Table 2 shows a high positive correlation between RSPO1 and TNF-α levels (r=0.944, p<0.001). Positive correlations were found between the hip circumference and the concentrations of RSPO1 (r=0.255, p=0.019) and TNF-α (r=0.278, p=0.011). There were weak positive correlations between LH levels and the concentrations of RSPO1 (r=0.221, p=0.043) and TNF-α (r=0.242, p=0.026). There was a weak negative correlation between prolactin and TNF-α levels (r=-0.269, p=0.013). Weak positive correlations were found between blood glucose levels and the concentrations of RSPO1 (r=0.343, p=0.001) and TNF-α (r=0.312, p=0.004). Moderate positive correlations were found between insulin levels and the concentrations of RSPO1 (r=0.577, p<0.001) and TNF-α (r=0.569, p<0.001). Again, moderate positive correlations were found between HOMA-IR values and the concentrations of RSPO-1 (r=0.619, p<0.001) and TNF-α (r=0.608, p<0.001). No statistically significant correlation was found between other parameters and the levels of RSPO1 and TNF-α.

DISCUSSION

PCOS is one of the most common endocrine disorders in women of reproductive age. In addition to disorders related to the reproductive system, PCOS patients are relatively likely to have several systemic problems, such as functional disorders in adipose tissue, metabolic syndrome, Type 2 DM, and an increased risk of cardiovascular disease (14). This study investigated the association between RSPO1 and TNF-α level, IR, and other parameters in infertile women with PCOS.

Table 2. Correlations between RSPO1, TNF- alpha and other variables of participants

		RSPO1	TNF- α			RSPO1	TNF- α
RSOP1	r	-	0.944*	Estradiol	r	0.038	0.067
	p	-	<0.001		p	0.731	0.545
Age	r	0.004	-0.049	Free T4	r	-0.007	-0.007
	p	0.973	0.660		p	0.951	0.952
Height	r	0.046	0.101	TSH	r	0.029	-0.069
	p	0.676	0.362		p	0.795	0.530
Weight	r	0.151	0.161	Prolactin	r	-0.196	-0.269*
	p	0.171	0.142		p	0.074	0.013
Body mass index	r	0.152	0.147	Total cholesterol	r	-0.177	-0.188
	p	0.166	0.182		p	0.107	0.086
Waist circumference	r	0.131	0.144	LDL	r	-0.026	0.040
	p	0.236	0.192		p	0.816	0.720
Hip circumference	r	0.255*	0.278*	HDL	r	-0.169	-0.142
	p	0.019	0.011		p	0.124	0.196
Waist / Hip ratio	r	-0.046	-0.039	Triglyceride	r	-0.163	-0.098
	p	0.680	0.722		p	0.139	0.374
Systolic blood pressure	r	-0.002	0.016	Glucose	r	0.343*	0.312*
	p	0.989	0.883		p	0.001	0.004
DBP	r	-0.072	-0.073	Insulin	r	0.577*	0.569*
	p	0.515	0.508		p	<0.001	<0.001
AMH	r	0.147	0.163	HOMA-IR	r	0.619*	0.608*
	p	0.182	0.139		p	<0.001	<0.001
FSH	r	0.081	0.111				
	p	0.462	0.314				
LH	r	0.221*	0.242*				
	p	0.043	0.026				

r: Spearman correlation coefficient, * Correlation is significant at the 0.05 level (2-tailed).

Depending on the demographic characteristics of the studied populations and the definition of PCOS, 30 to 75% of women with PCOS have obesity, and 50-70% of those with normal BMIs have abdominal obesity (increased central fat) (15). The result we found in the present research supports this information. We found that hip circumference was higher in women diagnosed with PCOS, and the WHC ratio was higher in obese women with PCOS. Since obesity is more common in women with PCOS, these women are at greater risk of increased insulin resistance, Type 2 DM, metabolic syndrome, and impaired glucose tolerance (15,16). It has been reported that IR can be an integral part of the syndrome in 65-80% of PCOS patients and may have a role in its etiology (6). The current study found that glucose, insulin, and IR levels were highest in the obese-PCOS group. We believe it is necessary to repeat that PCOS patients must be monitored more closely regarding the risk of developing obesity and insulin resistance.

The Wnt signaling pathway significantly regulates cells' survival, proliferation, polarity, and fate during embryonic development and tissue homeostasis. Abnormal regulation of Wnt signaling often results in pathological conditions in humans, including congenital disabilities, cancer, and other diseases (17). RSPO1 contributes to ovarian differentiation by activating the standard Wnt signaling pathway (18). It is stated that RSPO1 has a critical role in inhibiting testis formation during early ovarian development since it stimulates the downstream catenin pathway (19). This has been evidenced by testis formation and the development of androgen-related features in female mice in an experimental study where RSPO1 deletion was performed (20). However, it has been reported that RSPO1 level is increased in 8% of ovarian cancers (18). Liu et al. (21) reported that RSPO1 promotes growth, survival, and migration in ovarian cancer cells and protects cancer cells against chemotherapy. No study evaluating the relationship between PCOS and RSPO1 could be found in the literature. It is noteworthy that the RSPO1 level is significantly higher in women with PCOS in our study group. Although we could not evaluate the mechanism and cause of this elevation, our finding is valuable in that it raises important questions about the role of RSPO1 in PCOS.

The RSPO family can bind to the LGR4–6 receptors and leucine-rich repeating domains containing G protein, thereby amplifying Wnt signaling (9). Wang et al. (22) reported that the ablation of LGR4 (which encodes the specific receptor for RSPO1) in mice caused a transition from white to brown adipose tissue with increased energy consumption and reduced fat. It has also been reported that LGR4 expression is significantly increased in obese patients' subcutaneous and intraabdominal fat tissue compared to normal individuals. Kang et al. (10) reported that RSPO1 levels were higher in obese patients than in non-obese patients, and RSPO1 serum levels showed a significant correlation with BMI. Our study found no significant difference between the RSPO1 levels of obese and non-obese patients. While there was no correlation between BMI and RSPO1 level, a positive correlation was found between the hip circumference and RSPO1 level.

Previous publications report that RSPO1 can be detected in murine islets and that RSPO1 could stimulate insulin secretion (regardless of glucose level) (23). In addition, it was reported that serum RSPO1 levels were higher in IR subjects compared to insulin-sensitive subjects, and the levels were correlated with fasting C-peptide level and the degree of IR (10). Furthermore, in some previous in-vivo studies, it has been reported that RSPO1 increases insulin secretion (7-9). Similarly, in the current study, a positive correlation was found between glucose, insulin, IR, and the concentration of RSPO1. The increase in RSPO1 level in infertile women with PCOS could be important in monitoring the risk of developing DM.

Low-grade chronic inflammation of PCOS patients plays a role in the disease pathogenesis, and PCOS is widely accepted as a pro-inflammatory condition (24). TNF-α is an inflammation marker that plays a role in many immunological functions as well as acute bacterial infection, viral replication, septic shock, and fever (15). In our study group, TNF-α level was significantly higher in women diagnosed with PCOS. Sayın et al. (25) reported similar results. Some studies reported no difference in serum TNF-α levels between obese women with and without PCOS (26). In the metanalysis performed by Toulis et al. (27), TNF-α levels were reported to be higher in women with PCOS compared to controls. Despite such supporting evidence, another meta-analysis study found no difference between PCOS and control groups in TNF-α levels (28), indicating a need for future studies with better design and patient selection to determine the TNF-α role in PCOS. The current study reports remarkably elevated TNF-α levels in the presence of PCOS, providing support to the former group of studies. However, it must be noted that the possible existence of many clinical situations related to TNF-α level may have caused the differences; however, this is true for all studies focusing on this topic and further highlights the necessity of collaborative attempts and prospective studies to elucidate TNF-α and its role in PCOS.

TNF-α is mainly expressed in monocytes, macrophages, and adipose tissue, and levels of TNF-α are elevated in obesity and Type 2 DM (29). Studies show that increased serum TNF-α levels in women with PCOS correlate positively with BMI (30). The present study found a

positive correlation between hip circumference and TNF-α level. Also, no significant correlation was found between BMI and TNF-α. No difference was found in TNF-α between obese and non-obese women in the PCOS and control groups. Sayın et al. (25) reported similar results. In the meta-analysis performed by Gao et al. (32), there was no relationship between TNF-α and BMI in women with PCOS. However, since TNF-α expression is implicated in the development of IR (29), it is evident that the lack of difference concerning obesity presence/absence deserves a more detailed analysis. Araya et al. (30) reported that increased serum TNF-α levels in women with PCOS were inversely correlated with insulin sensitivity. Some studies also report no relationship between TNF-α and glucose, insulin, and IR (25,26). As a result of a previously reported meta-analysis, the increase in TNF-α level is directly related to the increase in IR in PCOS (31). This study found a positive correlation between TNF-α and glucose, insulin, and IR, even though a difference was not observed concerning obesity status in either group of patients. The results may suggest that such elevations in TNF-α levels warrant investigation for the level of IR in PCOS patients. These findings also imply that, rather than the presence of obesity, the metabolic outcome of obesity or the progression towards clinically-relevant disease states are the triggering factors for the elevation of TNF-α.

Although the information on RSPO1 and TNF-α has increased, relatively little is known regarding the association between RSPO1 and TNF-α in women with PCOS. In the study conducted by Krönke et al. (32) on TNF-α -transgenic mice, they reported that RSPO1 was highly effective in protecting joint structural integrity in arthritis by preventing inflammation-related injury to the bone and cartilage. As a result, it was concluded that RSPO1 had therapeutic potential as an anabolic agent in arthritis. Another study reported significantly lower serum RSPO1 concentrations in rheumatoid arthritis patients compared to matched controls, and anti-TNF-α therapy significantly increased serum RSPO1 levels (33). The current study found a positive correlation between RSPO1 levels and TNF-α levels. The correlation between the two values is not surprising, as we found that both TNF-α and RSPO1 levels were significantly higher in the presence of PCOS. However, the dearth of evidence about RSPO1 and its effects on PCOS indicates a need for in-vivo and in-vitro studies evaluating the relationship between these two parameters in the presence of PCOS.

Apart from those mentioned previously, one of the study's limitations is that the research group consisted of a relatively small number of patients for a disease as prevalent and variable as PCOS. More robust evidence could be obtained with population-based and larger-scale studies to confirm the role of RSPO1 and TNF-α among PCOS patients (and possibly those with infertility due to PCOS). Another limitation is that the primary source of RSPO1 and TNF-α evaluated in the study cannot be determined since measurements were performed from serum samples. Finally, data were obtained from a single point in time, and since variations are possible –especially concerning period characteristics, there may be a need to assess patients longitudinally and possibly, concerning their management and treatment processes. On the other hand, we believe that this study, which compared infertile women with and without PCOS, is valuable for evaluating the relationship between obesity, insulin resistance, RSPO1, and TNF-α.

CONCLUSION

As a result of the analyses carried out in this study, it was found that RSPO1 and TNF-α levels were higher in women with PCOS than in controls. A very strong positive correlation was found between RSPO1 and TNF-α levels. IR was highest in the obese-PCOS group. While there was no correlation between BMI and the concentrations of RSPO1 and TNF-α, a notable positive correlation was between the levels of RSPO1 and TNF-α and the following parameters: hip circumference, insulin level, HOMA-IR, and glucose. Considering the lack of data on RSPO1 levels in PCOS or obesity and the conflicts in the literature concerning the role of TNF-α in these conditions, drawing direct conclusions about the results is difficult. Also, it may be feasible to suggest that RSPO1 and TNF-α levels may guide the assessment and management of IR and diabetes risk in infertile women with PCOS. More comprehensive studies are required to investigate the relationship of RSPO1 and TNF-α with IR and other parameters in PCOS patients in the presence/absence of obesity and infertility.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Etlik Zübeyde Hanım Training and Research Hospital Clinical Researches Ethics Committee (Date: 09.09.2020, Decision No: 2020/128).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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