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ORIGINAL ARTICLE

Does Serum R-Spondin-1 Play a Role in Pathophysiology of Polycystic Ovary Syndrome?

PCOS Patofizyolojisinde Serum R-Spondin-1'in Bir Rolü Var mıdır?

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ABSTRACT

Objective: It has been thought that many novel metabolic and inflammatory markers are involved in the etiology of Polycystic ovary syndrome (PCOS). R-spondin-1 (Rspo1) is a member of the roof plate-specific spondin protein family. Rspo1 levels have been associated to obesity and insulin resistance. In this study, it was aimed to investigate whether the Rspo1 plays role in the PCOS pathophysiology.
Materials and methods: This cross-sectional study included newly diagnosed, treatment-naïve PCOS patients and healthy controls. The Rspo1 levels were compared between PCOS patients and healthy controls. In addition, the Rspo1 levels were compared within PCOSS group according to body mass index (BMI) and Ferriman Gallwey (FG) score.
Results: verall, 83 subjects (47 patients with PCOS and 36 healthy controls) were included in the study. TheO PCOS and control groups were comparable regarding age and BMI. However, FG score, homeostasis model assessment of insulin resistance score, Rspo1 and total testosterone levels were significantly higher in patients with PCOS when compared to controls (p<0.001, p=0.01, p=0.02, p=0.001 respectively). In the PCOS group, there was no significant difference in the Rspo1 levels among BMI and FG scores subgroups It was also found that the Rspo1 had a significant positive correlation with total testosterone and dehydroepiandrosterone sulfate levels (p=0.03, r=0.23; p=0.08, r=0.30, respectively).
Conclusion: The Rspo-1 may be associated with PCOS pathophysiology through total testosterone and dehydroepiandrosterone sulfate. Further molecular and genetic studies are needed to support this hypothesis.

Keywords: R-spondin-1, Polycystic ovary syndrome, Ferriman Gallwey scores

ÖZ

Amaç: Pek çok yeni inflamatuar ve metabolik belirtecin Polikistik Over Sendromu (PCOS) etiyolojisinde rol oynadığı düşünülmektedir. R-spondin-1 (Rspo1), tepe tabakaya özgü spondin protein ailesinin bir üyesidir. Rspo1 seviyeleri obezite ve insülin direnci ile ilişkilendirilmiştir. Rspo1'in PCOS patofizyolojisinde rolü olup olmadığını araştırmayı amaçladık.
Gereç ve Yöntem: Bu kesitsel çalışma, yeni tanı konmuş ve tedavi edilmemiş PCOS olguları ve PCOS'u olmayan bir kontrol grubu ile planlandı. PCOS'lu hastalar ve sağlıklı katılımcılar, Rspo1 seviyeleri için karşılaştırıldı. Ayrıca PCOS grubu vücut kitle indeksi (VKİ) ve Ferriman Gallwey skorlarına (FGS) göre de gruplandırılarak Rsop1 düzeyleri açısından karşılaştırıldı.
Bulgular: PCOS grubunda 47, kontrol grubunda 36 olmak üzere toplam 83 katılımcı çalışmaya dahil edildi. PCOS ve kontrol grupları benzer yaş ve VKİ'ye sahipti. Kontrol grubu ile karşılaştırıldığında, PCOS hastalarının FGS, insülin direnci skorlarının homeostaz modeli değerlendirmesi, Rspo1 ve toplam testosteron düzeyleri anlamlı olarak daha yüksekti (sırasıyla p<0,001, p=0.01, p=0.02, p=0.001). PCOS hastaları BMI ve FGS değerlerine göre alt sınıflara ayrıldığında, hem BMI hem de FGS değerleri açısından Rspo1 seviyeleri arasında istatistiksel olarak anlamlı bir fark yoktu. Ayrıca, Rspo1, toplam testosteron ve dehidroepiandrosteron sülfat seviyeleri ile anlamlı bir pozitif korelasyon gösterdi (sırasıyla p=0.03, r=0.23; p=0.08, r=0.30).
Sonuç: Rspo-1, toplam testosteron ve dehidroepiandrosteron sülfat yoluyla PCOS patofizyolojisi ile ilişkilendirilebilir. Bu hipotezi desteklemek için daha fazla moleküler ve genetik çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: R-spondin-1, Polikistik over sendromu, Ferriman Gallwey skorları

Introduction

Polycystic ovary syndrome (PCOS) is a clinical syndrome associated with menstrual irregularity and androgen excess, which is seen in 6-20% of women reproductive age (1). It is characterized by chronic anovulation, infertility, hyperandrogenaemia, obesity, dyslipidemia, and chronic low-intensity inflammation (2). The PCOS has a complex, multifactorial etiology including genetic, environmental, and inflammatory factor or their combination (3,4). Some inflammatory markers such as serum C-reactive protein, tumor necrosis factor-a and interleukin-6 have been linked to hyperandrogenism and PCOS (5,6). The serum markers for inflammation have become increasingly important as markers of atherosclerosis and cardiovascular disorders. In many studies, it was found that increased inflammatory marker concentrations are associated with traditional cardiovascular risk factors such as obesity, dyslipidemia, glucose intolerance, type 2 diabetes and hypertension. In fact, it has been suggested that chronic low-grade inflammation is involved in the pathogenesis of insulin resistance and later in the development of cardiovascular disease (7-9). Given that the frequencies of insulin resistance, cardiovascular events, type II diabetes mellitus and hypertension in

patients with PCOS, it was shown that there is a close relationship between chronic inflammation and PCOS etiology (10).

The R-spondin is a member of the specific spondin protein family of the roof plate involving four members. It is structurally related to the cysteine-rich furin-like and thrombospondin domains. R-spondin-1 (Rspo1) is an intestinal growth factor known to exert its effects through activation of the Wnt signaling pathway, which, in turn, results in expression of Wnt target genes (11). The Wnt signaling has been proven to play important roles in the development and pathogenesis of various diseases including diabetes mellitus (12). In addition, the R-spondin-1 levels have been shown to be associated with obesity and insulin resistance, and are significantly increased compared to the healthy population in both clinical conditions (13).

Given that the insulin resistance and obesity are common comorbid conditions in PCOS, it was aimed to investigate whether the Rspo1 has a role in the pathophysiology of PCOS in this study.

Materials and Methods

The study included 83 subjects (47 patients with PCOS and 36 healthy controls) among patients presented to endocrinology and metabolism disorders outpatient clinic of Kayseri City Hospital between 2019 and 2021. The inclusion criteria were: subjects aged >18 years without clinical signs of infection or inflammation, alcohol or drug use, history of chronic diseases such as diabetes mellitus, chronic kidney disease, and heart disease, or pregnancy. The exclusion criteria included: smoking, pregnancy, breastfeeding and presence of an endocrine disorders including congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome, hyperprolactinemia, thyroid dysfunction and adrenal disorders. The diagnosis of PCOS was made based on the European Society of Human Reproduction and Embryology / American Society of Reproductive Medicine (ESHRE / ASRM) criteria: the presence of two of the three features of hyperandrogenism (hirsutism, high testosterone or free androgen index, oligo or amenorrhea and the presence of polycystic ovary on sonography) (14). All patients had newly diagnosed PCOS and were treatment naive. The height and body weight measurements were made after an overnight fasting and BMI was calculated according to these measurements. Based on the World Health Organization (WHO) classification, the patients with BMI≥30.0 kg/m^2 were defined as obese (15). In the study, all patients were evaluated for hirsutism by a trained staff member using modified Ferriman-Gallwey (FG) score (16). All pelvic sonography studies were performed by same radiologist.

The blood samples were drawn following 12-hours fasting after 20 minutes resting in sitting position. The sera were stored -80°C until assays. The serum Rspo1 concentration was evaluated by Enzyme-Linked Immunosorbent Assay (ELISA) using a commercial kit (Limit of Quantification: 3.62 pg/mL, Assay Range: 10-1000 pg/mL, YL Biont, Shanghai, China). The Rspo1 level was analyzed according to the manufacturer's instructions and expressed as pg/mL. The concentrations of the samples were calculated through calibration curves obtained from study standards with known levels. The regression coefficient of our calibration curve was 0.991. The intra-assay and inter-assay coefficient of variation (CV) is <8% and <10%, respectively.

Statistical Analysis

Statistical analyses were performed using the 22.0 SPSS statistical program. Parametric variables are presented as mean ±standard deviations while non-parametric variables are presented as median (min.-max). Shapiro-Wilks test and histograms were used to determine whether continuous variables were normally distributed. Two independent groups of parametric variables were compared using Student's t test. Mann Whitney U test was administered to non-parametric variables The relationship correlation among non-parametric variables were analyzed using Spearman correlation tests. Pearson's correlation tests were used for parametric variables. Qualitative data was defined as %. A Chi-square test was applied to look for differences between groups. A *p* value of < 0.05 was considered to indicate statistically significant differences.

Ethical Consideration

The study was conducted in accordance to tenets of the Declaration of Helsinki. The was approved by Kayseri City Hospital Ethics Committee (approval number: 2019/391, date: 22.05.2019). With the principle of voluntarism, written and verbal information was provided, and the data was collected by filling out the forms by the participants who gave informed consent. The authors expressed no conflict of interest.

Results

Overall, 83 subjects (47 patients with PCOS and 36 healthy controls) were included in the study. The mean age was 24.7±5.7 years in the PCOS group and 25.3±5.1 years in the control group, indicating no significant difference (p=0.26). Again, there was no significant difference in BMI between PCOS and control groups (29.3±4.9 kg/m2 vs. 27.0±6.0 kg/m2, respectively). It was found that the FG score, homeostasis model assessment of insulin resistance score, Rspo1 and total testosterone levels were significantly higher in patients with PCOS when compared to controls (p<0.001, p=0.01, p=0.02, p=0.001 respectively). However, there was no significant difference in fasting and postprandial plasma glucose, thyroid stimulating hormone, free thyroxin, white blood cells, leukocyte, lymphocyte, dehydroepiandrosterone sulfate, low-density lipoprotein, high-density lipoprotein values between groups (Table 1). The Rspo1 had a significant positive correlation with total testosterone and

dehydroepiandrosterone sulfate levels (p=0.03, r=0.23; p=0.08, r=0.30, respectively).

The patients with PCOS were classified into 3 subgroups according to FG scores: group 1, patients with FG score<8; group 2, patients with FG score of 8-13; and group 3, patients with FG score>13. No significant difference was found in the Rspo1 levels among 3 groups (p=0.31). Again, the patients with PCOS were classified into 3 subgroups according to BMI: obese patients, BMI≥30 kg/m2 and non-obese patients, BMI<30 kg/m2. When the Rspo1 levels were compared obese and non-obese patients, no significant difference was found (422.7±287.4 vs. 413.0±283.7, respectively; p=0.48)

Discussion

The Rspo1 is a new marker shown to play a role in many events in the body such as gut epithelium regeneration, diabetes mellitus in recent studies (12,17). In PCOS and diabetes mellitus, there are many common metabolic abnormalities including obesity and insulin resistance (18). The Rspo1 was also detected in the human pancreas, but its role is not known clearly (19). In a cellular study, it was shown that the Rspo1 activates Wnt signaling in MIN6 β-cells cells and that Rspo1 not only promotes cell growth and survival, but is also an insulin secretagogue (19). Interestingly, in the cell study, the highest dose of Rspo1 failed to induce proliferation in MIN6 β-cells and it was though that this cell line is desensitized by a recombinant protein (20).

In our study, it was found that the PCOS patients had higher Rspo1 and HOMA-IR levels than healthy controls. As expected, insulin resistance was higher in PCOS patients compared to the healthy controls in our study. It was considered that the significant Rspo1 elevation in PCOS patients might be related to the finding of higher insulin resistance in PCOS patients in our study. Studies have shown that the Wnt pathway and Rspo1 may also affect the glucagon-like peptide-1 (GLP-1) pathway (21,22). The anti-obesity effect of GLP-1 is well- known and is used in the treatment (23). Again it was thought that the reason underlying higher frequency of obesity among PCOS patients may be abnormalities in Wnt pathway and their effects on GLP-1, although it was not statistically significant in our study.

The Rspo1 also has significant effects on the reproductive system. In a previous study on Rspo1 knockout female mice, it was found that there was masculinization in the reproductive system. Therefore, the Rspo1 may be required for activation of the Wnt signaling pathway in female gonadal differentiation (24). Many mutations have been identified in the Rspogen family. For example, Parma and colleagues describe a recessive mutation in the gene encoding Rspo1; by addition of a single nucleotide, this recessive mutation resulted in a frameshift and a new stop codon, leading removal of Rspo1 (25). Although the Rspo-1 values were higher in PCOS patients in our study, the presence of masculinizing findings such as increased FG score and hair loss suggests that there may be a mutation in the Rspo-1 gene in these patients.

The Rspo1 is required for female sex development in pregnancy. It activates the WNT/β catenin pathway to inhibit development towards male sex development. During the critical gonadal stages, including 6 to 9 weeks after fertilization, their production decreases in the testes and increases in the ovaries (26). Decreasing of Rspo1 function can cause female-to-male gender reversal (27). The Rspo1 is one of the most important factors for ovarian differentiation in XX gonads (28). Due to difference in R-spondin levels between PCOS and control groups in our study, it may be suggested that there may be mutations in R-spondin genes in patients with PCOS.

The study has some limitations. Firstly, the study should be evaluated with more different markers and genetic parameters. Secondly, further studies are needed in groups with higher patient numbers.

Conclusion

We found that the Rspo-1 levels were significantly higher in PCOS patients compared to the healthy controls. In addition, the HOMA-IR values and FG scores were higher in the PCOS group than the healthy controls. The Rspo1 had a significant positive correlation with total testosterone and dehydroepiandrosterone sulfate levels

Table 1. Clinical and laboratory data of the patients

	PCOS Group (n=47)	Control Group (n=40)	p
Age (year)	26 (18-38)	24 (18-37)	0.260
BMI (kg/m^2)	30.1 (17.9-43.2)	28.5 (18.0-38.5)	0.060
White blood cells (10^3/μL)	7.8 (5.6-11.4)	7.8 (4.7-13.4)	0.830
Neutrophil (10^3/μL)	4.7 (2.6-6.6)	4.8 (1.9-9.6)	0.500
Lymphocyte (10^3/μL)	2.5 (1.6-3.5)	2.3 (1.3-3.4)	0.160
Fasting plasma glucose (mg/dL)	89.4±8.2	87.9±6.7	0.370
Postprandial plasma glucose (mg/dL)	109.9±7.8	103.8±15.0	0.640
HOMA-IR	5.4±2.4	2.9±1.4	**0.010**
Low-density lipoprotein (mg/dL)	101.3±27.9	92.6±27.5	0.170
High-density lipoprotein (mg/dL)	52.2±14.6	53.9±12.3	0.590
Thyroid stimulating hormone (mU/L)	2.2±0.84	2.1±0.9	0.720
Free thyroxine (ng/L)	12.2 (9.6-15.3)	12.2 (9.7-16.4)	0.650
Total Testosteron (μg/L)	0.5 (0.1-0.8)	0.3 (0.09-0.7)	**0.001**
Dehydroepiandrosterone sulphate (μg/L)	1554.4±229.1	982.6±173.7	0.350
Ferriman Gallwey scores	12 (7-21)	8 (2-12)	**<0.001**
Rspo-1 (pg/mL)	342.9 (55-904)	147.8 (70-564)	**0.002**

BMI: Body mass index, HOMA-IR: Homeostasis model assessment of insulin resistance, Rspo-1: R-spondin-1.

Data were expressed as mean±standard deviations or median (min.-max.) according to parametric or non-parametric distribution.

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