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Comparison of the Paraoxonase and Arylesterase Activities in Follicle Fluid of Females with PCOS in ICSI Cycle

Polikistik Over Sendromlu Kadınların ICSI Sikluslarında Folikül Sıvıları İçinde Paraksonaz ve Arilesteraz Aktivitelerinin Karşılaştırılması

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ABSTRACT

Objective: The aim of our study was to compare the Paraoxonase (PON1) and arylesterase (ARES) activity in follicle fluids of infertile females with polycystic ovary syndrome (PCOS). **Material and Methods:** A total of 93 women who underwent assisted reproductive technologies (ART) using intra-cytoplasmic sperm injection (ICSI) were enrolled in this prospective study, of whom 46, with PCOS, comprised the study group, the remaining 47 women with MFI formed the control group. Women age, body mass index, (BMI) basal hormone levels, total follicle count, total oxidant status (TOS), total antioxidant status (TAS), PON1 and ARES activity, oxidative stress index (OSI), simulated paraoxonase (SPON) were compared between groups. **Results:** There were no significant differences for average age, BMI, Day3-FSH, Day3-LH, Day3-E2 and total follicle count among groups. Also there were no statistically significant differences among groups in terms of TOS, TAS, PON1, SPON, ARES and OSI ($p>0.05$). **Conclusion:** TOS, TAS, OSI, SPON, PON1 and ARES enzyme activities in follicular fluid of infertile females with PCOS are similar in those with male factor infertility.

Key Words: Paraoxonase; arylesterase; follicular fluid; polycystic ovary syndrome; infertility

ÖZET

Amaç: Çalışmamızdaki amacımız Poikistik Over Sendromu (PKOS) olan infertil kadınların folikül sıvılarındaki Paraksonaz (PON1) ve arilesteraz (ARES) aktivitelerinin karşılaştırılması. **Gereç ve Yöntemler:** Yardımcı üreme tekniği (YÜT) ile intra-sitoplazmik sperm enjeksiyonu (ICSI) uygulanan toplam 93 kadın, bu prospektif çalışmaya dahil edildi, bu kadınlardan PKOS'lu olan 46' sı çalışma grubunu ve sadece erkek faktörü bulunan geri kalan 47'si kontrol grubunu oluşturdu. Kadınalrın yaşı, vücut kitle indeksi (VKİ), bazal hormon seviyeleri, toplam folikül sayıları, total oksidan status (TOS), total antioksidan status (TAS), PON1 ve ARES aktiviteleri, oksidatif stres indeksi (OSİ), simüle edilmiş paraksonaz (SPON) gruplar arasında karşılaştırıldı. **Bulgular:** Gruplar arasında ortalama yaş, VKİ, 3. Gün FSH- LH- Estadiol seviyeleri ve total folikül sayıları açısından anlamlı bir fark yoktu. Ayrıca TOS, TAS, PON1, SPON, ARES ve OSI seviyeleri arasında anlamlı bir farklılık yoktu ($p>0.05$). **Sonuç:** Folikül sıvısı içindeki TOS, TAS, OSI, SPON, PON1 ve ARES'in enzim aktiviteleri PKOS'lu kadınlarda ve erkek faktörü nedeniyle YÜT uygulanan kadınlarda benzerdi.

Anahtar Kelimeler: Paraksonaz; arilesteraz; foliküler sıvı; polistik over sendromu; infertilite

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Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disease which affects to 10-15% of women at reproductive age; present with hyperandrogenism, chronic anovulation and/or polycystic ovaries.[1,2] It is associated with approximately 75% of women who have infertility problems due to anovulation.[3] Moreover, PCOS is associated with several metabolic changes; including glucose intolerance, dyslipidemia, obesity, insulin resistance, and these changes may worsen the metabolic abnormalities by developing local and systemic oxidative stress.[4-6]

Oxidants, tended to lose positive charge and gain electrons, are defined as chemical substances. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the products of normal cellular metabolism and oxidants include those substances. RNS derive from nitric oxide and ROS derive from molecular oxygen, oxygen ions, free radicals and peroxides. Both these systems have ability to support the normal cellular condition in both low and moderate concentrations; however in the high concentrations; those products may damage DNA, cellular lipids and proteins.[7] Glutathione peroxidase, catalase and superoxide dismutase support protective effect against ROS.[8] Paraoxonase-1 (PON-1) synthesized in the liver and has protective effect by preventing the generation of lipid peroxides, as well. PON-1 reduces the oxidation of low density- lipoprotein cholesterol (LDL-C) and also has anti-inflammatory properties.[9]

The effect of oxidative stress on etiology of PCOS and effect of PON1 in women with PCOS are still debate; there are a few studies with conflicting results about relationship between PON1 and PCOS.[10-13] Therefore we aimed to compare the follicular fluid level of PON and ARES activity in women with PCOS and those with Male factor infertility (MFI) who underwent assisted reproductive technique (ART) using intra-cytoplasmic sperm injection (ICSI).

MATERIAL AND METHODS

THE STUDY POPULATION

A total of 93 women who underwent ART using ICSI were enrolled into this prospective study, of whom 46, with PCOS, comprised the study group, the remaining 47 women with MFI formed the control group. The present study was approved by the Ethical Committee and Institutional Review Board of the hospital and written informed consent was obtained from each participant.

A complete history, laboratory evaluation including basal hormonal assays (Day 3 of spontaneous or progesterone-induced menstruation): luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), prolactin, serum estradiol (E2), progesterone (P), total testosterone, free testosterone, and DHEAS levels, physical examination and transvaginal sonography was performed on all patients to detect any uterine abnormalities or adnexal pathologies, and patients with abnormal hormonal levels and uterine or adnexal pathologies were excluded from the study. Patients between 20 and 35 years old, with normal basal hormone levels (FSH <12 IU/ml, PRL, TSH normal) and normal HSG (tubal patency and adequate uterine cavity) were included in the study. Diagnosis of PCOS was based on the revised 2003 Consensus on diagnosis criteria.[14] Transvaginal ultrasound (TVUSG) was performed for antral follicle count, ovarian volume, and endometrial thickness in all patients using 5 MHz TVUSG (ALOKA Co. Ltd. SD1000, Tokyo, Japan). The women with abnormal semen analysis for their partners according to the modified criteria of the World Health Organization (1999) were included to the MFI group (control group).

STIMULATION REGIMEN AND ICSI PROCEDURE

The cases in both groups received daily gonadotropin injections. Regular ultrasound monitoring was performed for follicle growth and endometrial thickness inspection. Also serum E2 levels were monitored. When a dominant follicle reached 17-20 mm diameter and with E2 levels that are greater than 500 pg/ml, ovulation is triggered by 10000 units of hCG. After 34 to 36 hours from hCG injection; oocyte pick up (OPU) is performed and the oocytes were subjected to ICSI.

EMBRYO TRANSFER

Embryo transfer was performed using a Wallace catheter (Edwards- Wallace catheter; Marlow Technologies, Willoughby, OH) by the same clinical team. It was determined based on the American Society for Reproductive Medicine (ASRM) guidelines and gametes were prepped in GMOPS medium (Vitrolife, Frolunda, Sweeden) plus 0.3% human serum albumin.[15]

Clinical pregnancy was defined as the presence of gestational sac containing fetal hearts on ultrasound scan. Clinical pregnancy rates and cancellation rates were calculated per the number of ART cycles.

BIOCHEMICAL PARAMETERS

Fluid samples were obtained through the ovarian follicles by OPU process. PON, PON stimulated, ARES activities were investigated in follicular fluid of both group. Reagents were commercially obtained from Rel Assay Diagnostics® (Gaziantep, Turkey). Studies were performed in Cobas C501 automated analyzer (Roche Diagnostics®, Berlin, Germany).

Paraoxon was hydrolyzed and the rate of formation of p-nitrophenol was measured according to the Eckerson method.[16] The activity was expressed as U/L based on the molar absorptivity (18 290) of p-nitrophenol at 405 nm, at pH 10.5. Stimulated PON was measured by using same method in PON measurement, except additional 1 mol/L NaCl was used in this procedure.

Arylesterase uses phenyl acetate as substrate, and the measurement of the arylesterase activity was based on this function.[17] The activity, expressed as kU/L, was based on the molar absorptivity (1310) of phenol at 270 nm, at pH 8.0.

The TAS levels were determined by a method described by.[18] The assay results are expressed in millimole Trolox equivalent units per liter, and the precision of this assay is excellent – less than 3%.

TOS can be measured spectrophotometrically by using colour intensity, which, is related to the total amount of oxidant molecules present in the sample. The total peroxide levels of the samples were calculated as the differences between the absorbance readings related to the H_2O_2 standard curve as described by.[19]

OSI, an indicator of the degree of oxidative stress, is the percent ratio TOS to TAC To perform the calculation, we changed the resulting unit of TAC, millimoles of Trolox per litre, to micromoles per litre, and the OSI value was calculated from the formula: OSI = [TOS μmol/l / TAC μmol of Trolox X 100]

STATISTICAL ANALYSIS

The sample size G * Power (G * Power Ver. 3.1.7, Franz Faul, Universität Kiel, Germany) were calculated with the statistic package. 88 patients were required for completion with %90 force, $\alpha = 0.05$ type 1 error, $\beta = 0.10$ type 2 error and d=0.70 of study group. Therefore; we formed the study with a total of 93 women to support a powerful analyze.

The mean and standard deviation were calculated for continuous variables. The normality of the variables was analyzed by the Kolmogorov-Smirnov test. In the abnormal distribution of variables, median and interquartile range was given. The Student's t -test and the Mann-Whitney U test were used to evaluate associations between the categorical and continuous variables. Chi-square or Fisher's exact tests were used to compare differences between groups for categorical variables. Statistical analyzes were performed using of Statistical Packages for the Social Sciences (SPSS) 17.0 for Windows (SPSS Inc., Chicago, IL, USA). A p value less than 0.05 was considered statistically significant.

RESULTS

There were no significant group differences for average age, body mass index (BMI), Day 3-FSH, Day 3-LH, Day 3-E2; total follicle count and male average age (Table 1).

There were no statistically significant differences among groups in terms of TAS, TOS, PON1, SPON, ARES, OSI ($p>0.05$) (Table 2).

When we compared the pregnant and non-pregnant women in study group; we found no dif-

TABLE 1: Descriptive characteristics and basal hormone levels of the patients.

Variables	Women with PCOS	Women with MFI	P value
Women age (years)	29.9±3.1	30.0±5.4	0.562
BMI (kg/m^2)	25.14±4.36	25.68±4.40	0.375
Day3-FSH (IU/L)	5.56±0.76	7.26±3.49	0.102
Day3-LH (IU/L)	7.30 (6.59)	5.73 (4.90)	0.201
Day3-E2 (pq/ml)	39.56±9.67	33.96±18.21	0.205
Total Follicle Count*	20.0 (12.0)	12.0 (6.0)	0.123

Data were given as mean ± standard deviation and *:median, (interquartile range)

TABLE 2: Comparison of oxidative markers in follicular fluid in women with PCOS and controls with male factor infertility.

Variables	Women with PCOS, serum median, (IQR)	Women with MFI, serum median, (IQR)	P value
PON (U/L)	64.50 (96.03)	66.00 (81.20)	0.994
SPON (U/L)	113.00 (162.55)	101.50 (127.40)	0.884
ARES (kU/L)	139.46±45.44*	143.98±39.97	0.611
TAS (µmol/l)	0.89 (1.43)	1.38 (1.08)	0.070
TOS (µmol/l)	6.89 (8.79)	6.36 (7.03)	0.607
OSI	0.89 (78.50)	0.59 (2.89)	0.117

TAS: Total antioxidant status, TOS: Total oxidant status, PON: Paraoxonase, SPON: Stimulated paraoxonase, ARES: Arylesterase, OSI: Oxidative stress index, OSI = [TOS µmol/l/TAC µmol of Trolox X 100], IQR: Interquartile range, *= mean ± standard deviation.

TABLE 3: Comparison of oxidative markers in follicular fluid of pregnant and non-pregnant women in PCOS group.

Variables	Pregnant, serum median, (IQR)	Non-pregnant, serum median, (IQR)	P value
PON (U/L)	96.70 (117.00)	60.60 (72.65)	0.849
SPON (U/L)	148.80 (169.00)	104.00 (137.65)	0.505
ARES (kU/L)	137.60±45.84*	134.43±40.05	0.827
TAS (µmol/l)	0.30 (1.22)	0.86 (1.24)	0.409
TOS (µmol/l)	10.18 (9.05)	6.04 (3.73)	0.150
OSI	3.50 (143.68)	0.75 (19.52)	0.125

TAS: Total antioxidant status, TOS: Total oxidant status, PON: Paraoxonase, SPON: Stimulated paraoxonase, ARES: Arylesterase, OSI: Oxidative stress index, OSI = [TOS µmol/l/TAC µmol of Trolox X 100], IQR: Interquartile range, *= mean ± standard deviation.

ferences in terms of TAS, TOS, PON, SPON, ARES, OSI ($p>0.05$) (Table 3).

DISCUSSION

The probable relationship between PCOS and increased oxidative stress may speculate the cause of infertility in those women with this increased oxidative condition. However; in current study, we found similar metabolic conditions in FF of women with PCOS and those controls with MFI who underwent ICSI.

In the literature, to our knowledge, there are three studies investigated the relation between PON activity in human FF and female infertility. The findings were reported as (i): PON1 and arylesterase activity was positively associated with embryo cell cleavage rate 13, (ii): PON1-arylesterase activity in FF was a positive predictor for Day 3 embryo cell number 10, and (iii): PON3 activity in FF of women with female factor infertility was significantly lower in comparison with male factor infertility 12. And contrary to others; this current study has reported similar PON1-arylesterase activity in FF of women with PCOS and controls with MFI.

Oxidative stress is defined as an imbalance derived from excessive formation of oxidants in the presence of limited antioxidant defenses.[20] In several studies; excessive oxidant formation of subtracts were claimed as the main etiological factors on PCOS, and it was associated with dyslipidemia, elevated metabolic syndrome-type 2 diabetes and increased oxidative stress risks.[21-24] In addition, it has been reported that oxidized low-density lipoprotein (LDL) which starts the atherogenesis, through foam cell formation and inflammatory responses, were found to be higher in women with PCOS than in controls.[25,26] Contrary to this process; high-density lipoprotein (HDL) shows anti-inflammatory and antioxidant effects.[27,28] Due to its small size (lower than 300 kDa); the permanent of HDL is possible to the FF. HDL is only the lipoprotein that may exist in FF. Therefore; HDL is only the source of cholesterol in FF.

PCOS is considered to be a part of metabolic syndrome, and is a disease presented with insulin resistance, obesity, broken lipid profile, and endothelial dysfunction.[11] In recent years, the decreased antioxidant and increased oxidant activity have been reported in women having PCOS.[29,30] PON1, which support a protection from oxidative and peroxidative transformation has been identified in FF by proteomic analysis.[31-33] In a recently published systematic review and meta-analysis, it

has been reported that the concentrations of several promoters and by-products of oxidative stress such as homocysteine and malondialdeyde were significantly increased, and some circulating antioxidant markers, such as PON1 activity and glutathione levels, were decreased in patients with PCOS compared with those controls.[34]

In genomic variation study of San Milla et al., it has been reported that the homozygosity of PON1 gene, for (-108T) variants, has been found to be higher in PCOS group than control group.[35] PON1 activity decreases with -108T allele homozygosity and oxidative stress rises; as a result of these, insulin resistance occurs in patients having PCOS.[35] And in the study of Dursun et al., the serum paraoxanase level of PCOS patients has been found to be lower than that of control group.[11] Ovarian follicular fluid represents the biological window of ovary, and indicates the oocyte quality and the capacity to create embryo. The phagocytic macrophages in ovary, the endothelium cells, and stromal-steroid-producing cells produce the oxidative molecules.[36] It is thought that oxidative stress increases the proliferation of ovarian mesenchymal cells, and contributes on PCOS development as a result of that, but this thought should be supported with further studies.[37] Mohamadin et al. and Dursun et al. have evaluated the PON activity in serum, and serum PON activity in PCOS patients has been evaluated to be low.[11,38]

To our knowledge, the first study investigated PON activity in FF was conducted by Browne et al. and they have found that PON1-arylesterase activity in FF was a positive predictor for Day 3 embryo cell number.[10] Contrary to that study, we found similar FF levels of PON1 and arylesterase activity in women with PCOS and in controls. It is thought that proven conditions including increased oxidative stress in women with PCOS is not the cause of the infertility in those women, however; in our study, differently from other studies, the oxidative molecules have been investigated in follicle fluid representing the biological window of ovary. Follicle fluid has been taken from ICSI-planned PCOS patients and non-PCOS patients during OPU process after ovulation induction, and PON and ARES activities have been investigated. The PON and ARES enzyme activities in PCOS patients have been found to be similar with non- PCOS patients. As a result; it is seen in conducted studies that oxidative stress plays important role in PCOS etiology and broader prospective studies should be carried out in this topic. The previous studies have focused on serum oxidative activity or genomic variation. We have found PON and ARES enzyme activities in follicle fluids of PCOS patients to be similar with those of non-PCOS patients. Our results should be corroborated with further studies.

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Conflict of interest

No conflict of interest was declared by the authors.

Disclosure

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