

Assessment of Serum and Follicular Fluid Total Oxidant and Total Antioxidant Levels in In Vitro Fertilization (IVF) Cases

İn-Vitro Fertilizasyon Olgularında Serum ve Folliküler Sıvı Total Oksidan ve Antioksidan Seviyelerinin İncelenmesi

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ABSTRACT

Objective: To assess the effects of serum and follicular fluid total oxidant (TOC) levels, total antioxidant capacity (TAC), and oxidative stress index (OSI) on oocyte maturation, fertilization, embryogenesis, and clinical pregnancy outcomes in In Vitro Fertilization (IVF) cycles of infertile patients.

Material and Methods: One hundred patients having infertility and underwent ART enrolled the study group and blood samples were collected on gonadotropin starting, oocyte pick-up (OPU) and embryo transfer (ET) days. Additionally, follicular fluid specimen obtained during OPU was collected. TOC, TAC levels and OSI in serum samples and follicular fluid specimens between clinically pregnant and non-pregnant patients were compared.

Results: No significant difference was noted between clinically pregnant and nonpregnant patients in terms of the woman's age, duration of infertility, ovarian reserve or number of transferred embryos. There was also no significant difference in TAC, TOC and OSI levels in serum samples obtained during three phases of treatment cycle (basal gonadotropin starting day, OPU day and ET day) and in follicular fluid samples.

Conclusion: TAC, TOC and OSI seem to be ineffective to predict clinical pregnancy as an outcome in patients who underwent ART.

Keywords: infertility, total oxidant capacity, total antioxidant capacity, oxidative stress index

ÖZET

Amaç: İnfertilite teşhisi konmuş hastaların İn Vitro Fertilizasyon (IVF) uygulamalarında serum ve folliküler sıvılarında total oksidan kapasite (TOC), total antioksidan kapasite (TAC) ve oksidatif stres indeksi (OSI) değerlerinin oosit gelişimi, fertilizasyon, embriyogenezis ve klinik gebelik üzerine etkilerini değerlendirmek.

Gereç ve Yöntemler: Kliniğimizde IVF protokolüne alınan 100 hastanın çalışma grubunu oluşturdu ve gonadotropin başlandığı gün, embriyo transferi günü ve oosit toplandığı gün kan örnekleri alındı. İlave olarak oosit toplandığı sırada folliküler sıvı da toplandı. Serumda ve folliküler sıvıda TOC, TAC ve OSI değerleri klinik gebe olan ve olmayanlarda karşılaştırıldı.

Bulgular: Gebe olan ve olmayan hastaların yaş, infertilite süresi, ovarian rezerv ve transfer edilen embriyo sayıları arasında istatistiksel bir fark yok idi. Ayrıca serum TAC, TOC ve OSI değerleri arasında her üç fazda da (gonadotropin başlandığı gün, embriyo transferi günü ve oosit toplandığı gün) ve folliküler sıvıda anlamlı bir fark saptanmadı ($p < 0.05$).

Sonuç: Yardımcı üreme tekniklerine giden hastalarda TAC, TOC ve OSI değerleri klinik gebeliği öngörmeye etkisiz gözükmemektedir.

Anahtar Kelimeler: in vitro fertilizasyon, total oksidan kapasite, total antioksidan kapasite, oksidatif stres indeksi

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INTRODUCTION

Oxidative stress (OS) is a complicated process, which was defined as an imbalance between pro-oxidants and antioxidants in favor of oxidants. Generation and production of free radicals and antioxidant systems work together for providing a balance to achieve normal homeostasis [1]. Free radicals are known to have important functions including redox signalling and antibacterial defense. In normal biologic conditions, when increase in reactive oxygen species (ROS) exceeds the capacity of the mechanisms required to detoxify them, the system shifts toward OS and there may be oxidation in macromolecules like DNA, proteins, and lipids resulting changes in DNA configuration and may cause permanent damage [2].

There is growing evidence on the effects of oxidative stress in the field of gynecology and obstetrics. Polycystic ovary syndrome, endometriosis, infertility and pregnancy complications such as preeclampsia, and recurrent miscarriage, may arise in response to OS [3, 4]. Reproduction system is another area where OS and ROS have an important contribution. The role of OS on male infertility has been extensively studied [5-7]. In female infertile patients, there is growing evidence on the effects of OS on the female infertility including embryo culture medium, the endometrial implantation phase, continuity of corpus luteum [3, 8]. To the best of our knowledge, OS on follicular fluid in In Vitro Fertilization (IVF) cycles were evaluated in limited number of studies and requires further examination with the evaluation of clinical pregnancy outcomes [8-11]. Therefore, aimed to assess the effects of serum and follicular fluid total oxidant (TOS) levels, total antioxidant capacity (TAC), and oxidative stress index (OSI) on oocyte maturation, fertilization, embryogenesis, and clinical pregnancy outcomes in In Vitro Fertilization (IVF) cycles of infertile patients.

MATERIAL AND METHOD

This prospective cohort study was carried out between January 2015 and July 2015 at Eskisehir Osmangazi University Department of Obstetrics and Gynecology. The study was approved by the Ethical Review Board of the hospital and informed consent was obtained from all individual participants included in the study.

One hundred patients having infertility with unknown etiology and PCOS cases with male factor, tubal factor, advanced stage endometriosis, low ovarian reserve and 2 or 3 times being applied unsuccessful IUI attempts who applied to the hospital due to nonpregnancy even though they did not use any pregnancy prevention method and had regular sexual relationship were included in the study. The patients with over 5 millions of motile sperms, unilateral or bilateral tubal patent ones with infertility with unknown etiology, PCOS patients without any trial of IUI, early-stage endometriosis patients, and patients with sufficient ovarian reserve were excluded from the study.

Patients included in the study were applied antagonist protocol for ovulation induction, E2 antagonist protocol, the long Lucrin and hypogonadotropic hypogonadism protocols.

Venous blood samples were taken from the forearm at least after 8 hours of fasting. All venous blood samples were centrifuged at 3000 rpm for 10 min. Samples were stored at -80° C until analysis. Blood serum samples were analysed using 3-5 ml of the routine blood taken on the 3rd day of the follicular phase, oocyte retrieval and embryo transfer days. They were centrifuged for 10 minutes at 3000 rpm and the serum were stored at -80° C.

The fluid sample taken during oocyte retrieval was centrifuged for 5 minutes and 600xg to remove debris and granulosa cells. The supernatant was stored at -80 ° C until the day of analysis. Total Oxidant Capacity (TOC), total antioxidant capacity (TAC) and oxidative stress index (OSI) were studied on serum and follicular fluid.

Serum total oxidant status (TOS); (Rel Assay Diagnostics ®, Gaziantep, Turkey) was measured using a new automated colorimetric measurement method found by Erel. According to this method, oxidants present in the sample oxidize ferrous-o-dianisidine complex into the ferric ions [12]. The results are expressed in micromoles hydrogen peroxide equivalent per liter (H₂O₂eq micromol / L).

Serum total antioxidant status (TAS); (Rel Assay Diagnostics ®, Gaziantep, Turkey) was measured using a new automated colorimetric measurement method found by Erel. According to this method, the hydroxy radical as the product of Fenton reaction, reacts with colorless o-dianisidine to form a radical bright yellowish brown dianisyl substrate. The measurement results were expressed in Trolox equivalent of millimoles per liter (TroloxEq mmol/L) [13].

The samples were analysed in 680 Beckman Coulter (Beckman Coulter Inc. ®, CA, USA) device after reaching the room temperature. The intraassay %CV values for the TAS measurement were 4.12% for the 0.50 (0.35-0.65) mmol Trolox equiv / L and 1.53% for the 2.0 (1.7-2.3) mmol Trolox equiv / L. The intraassay CV% values for TOS measurements were 3.57% for 5.5 (3.0-8.0) µmol/L and 5.17% for 19.5 (16-23) µmol/L. Oxidative Stress Index (OSI) was calculated with the formula: Total Oxidant Status (TOS) / Total Antioxidant Level (TAS).

Serum hCG levels were measured in all patients on the 14th day following ET. Patients with >50 IU/L hCG level were evaluated as pregnancy positive. In patients with a positive initial hCG measurement, a twofold increase was evaluated after 48 h. Transvaginal ultrasonography was performed 3 weeks later in patients who showed an increase, and those with a fetal structure and fetal cardiac activity were considered as clinical pregnancy positive. Statistical analyses were performed using the program Statistical Package for the Social Sciences (SPSS), v.11.5 for Windows.

For normally distributed variables, paired-sample t-tests and independent-sample t-tests were applied, and the mean \pm standard deviation values were presented. Wilcoxon's signed rank test and the Mann-Whitney U test were applied for non-normally distributed variables, and median (25 %-75 %) percentiles were presented. P-values < 0.05 were taken to indicate statistically significant differences between group mean values.

RESULTS

Among 100 patients mean age, BMI, and duration of infertility were 30.3 ± 0.50 , 24.93 ± 0.43 and 5.71 ± 0.40 respectively. When patients were divided into two study groups according to their clinical pregnancy achievement there was no significant difference in our study between clinical pregnancies (n=27) and nonpregnants (n=73) on the variables of age, BMI, duration of infertility, sperm volume, total forward progressive motile sperm count, basal FSH and AMH level, total gonadotropin dose, antral follicle count, number of retrieved oocyte, fertilization rate and number of transferred embryos ($p > 0.05$) (Table 1). There was no significant difference on the TAS, TOS and OSI values analysed in the serum sample obtained on the second day, obtained during OPU, analysed in the follicular fluid and during embryo transfer between those with and without clinical pregnancy (Table 2-5).

Table 1: Characteristics of the patients who achieved pregnancy compared to those who did not.

Mean \pm sd	Pregnant (n=27)	Non-pregnant (n=73)	P value	
	Mean \pm sd			
Age (Years)	30,07 \pm 0,54	30,30 \pm 0,48	0,726	
BMI (kg/m ²)	23,97 \pm 0,39	25,37 \pm 0,52	0,256	
Duration of infertility (years)	6,20 \pm 0,48	5,51 \pm 0,37	0,778	
Basal FSH	6,49 \pm 0,21	7,89 \pm 0,71	0,962	
AMH	2,76 \pm 0,19	2,75 \pm 0,25	0,627	
Total gonadotrophin dose	2014,35 \pm 0,85	2113,3 \pm 0,87	0,463	
Number of retrieved oocytes	12 \pm 1,8	9,7 \pm 0,6	0,183	
	Median(Q1-Q3)	Median(Q1-Q3)	P value	
Fertilization rate (%)	82(57-100)	83(61-100)	0,509	
Antral follicle count	n(%)	n(%)	P value	
	< 5	3 (11,1)		13(17,8)
	5-12	9 (33,3)		30(41,1)
	>12	15(55,6)		30(41,1)
Number of transferred embryos	1	18	54	0,587
	2	9	18	
	3	0	1	

Table 2: Day 2 levels of serum total oxidant (TOS), total antioxidant capacity (TAS), and oxidative stress index (OSI).

	Pregnant	Non-pregnant	P value
TAS	1,46 \pm 0,35	1,460 \pm 0,34	0,745
TOS	24,2 \pm 0,36	29,9 \pm 0,32	0,588
OSI	15,61 \pm 0,20	20,53 \pm 0,24	0,647

Serum total anti-oxidant (TAS) in mmol Trolox Eq/L unit and serum total oxidant (TOS) in $\mu\text{mol H}_2\text{O}_2$ Eq/L unit. P-value < 0.05 was considered statistically significant.

Table 3: Serum levels of total oxidant (TOS), total antioxidant capacity (TAS), and oxidative stress index (OSI) on the day of oocyte pick-up.

	Pregnant	Non-pregnant	P value
TAS	1,51 \pm 0,53	1,26 \pm 0,25	0,762
TOS	36,5 \pm 0,46	24,9 \pm 0,30	0,720
OSI	23,1 \pm 0,28	18,3 \pm 0,24	0,723

Serum total anti-oxidant (TAS) in mmol Trolox Eq/L unit and serum total oxidant (TOS) in $\mu\text{mol H}_2\text{O}_2$ Eq/L unit. P-value < 0.05 was considered statistically significant.

Table 4: Levels of total oxidant (TOS), total antioxidant capacity (TAS), and oxidative stress index (OSI) in follicles on the day of oocyte pick-up.

	Pregnant	Non-pregnant	P value
TAS	1,25 \pm 0,39	1,22 \pm 0,32	0,966
TOS	11,17 \pm 0,26	16,5 \pm 0,24	0,535
OSI	12,7 \pm 0,14	13,5 \pm 0,17	0,689

Serum total anti-oxidant (TAS) in mmol Trolox Eq/L unit and serum total oxidant (TOS) in $\mu\text{mol H}_2\text{O}_2$ Eq/L unit. P-value < 0.05 was considered statistically significant.

Table 5: Serum levels of total oxidant (TOS), total antioxidant capacity (TAS), and oxidative stress index (OSI) on the day of embryo transfer.

	Pregnant	Non-pregnant	P value
TAS	1,41 \pm 0,38	1,34 \pm 0,30	0,839
TOS	20,55 \pm 0,30	29,11 \pm 0,30	0,580
OSI	13,8 \pm 0,17	22,08 \pm 0,25	0,666

Serum total anti-oxidant (TAS) in mmol Trolox Eq/L unit and serum total oxidant (TOS) in $\mu\text{mol H}_2\text{O}_2$ Eq/L unit. P-value < 0.05 was considered statistically significant.

DISCUSSION

TAS, TOS and OSI values on cycles beginning, obtained during OPU and ET and in the follicular fluids obtained during OPU were found to be insignificant in terms of pregnancy prediction in patients undergoing ART treatment.

Although the negative effect of the oxidative stress starting from folliculogenesis and going on with embryo development and implantation steps are clear, the major portion of the work is in vitro or animal studies [4, 14]. In this sense, the studies on the oxidative stress effect in ART cycles will be quite guiding to us.

In a clinical study, Das et al., found that ROS levels in follicular fluid measured by the chemiluminescence method have an impact on the development and quality of embryos; additionally, the oocyte fertilization capacity diminished as ROS increased in the follicular fluid of 78 couples who were admitted to IVF treatment because of tubal infertility [9]. In another clinical study conducted by Oyawoye et al., it is demonstrated that the total antioxidant capacity is related proportionally to the oocytes fertilization and embryo viability [15]. The serum total antioxidant level during OPU and ET in the couples in which only the male factor infertility exists has been shown to be significantly correlated with the clinical pregnancy rate in a study in terms of the clinical pregnancy, which is one of the major outcomes of the ART treatment [16].

One of limitations of the study is the heterogeneity of our patient groups. Although there was no significant difference in terms of infertility factor in both of the pregnant and the group of being not able to become pregnant, all female and male factors are

taken into consideration. It would be better to analyze subgroups of patients who underwent ART to define the effect of TAS, TOS and OSI on endometriosis, PCOS and others however our study population was inadequate to perform this analysis. Moreover, it would be better to compare the results with normal female follicle TAS, TOS and OSI levels however it was not possible for ethical reasons but our study still may give an idea for future research. In conclusion when all of the ART group is totally examined TAS, TOS and OSI seem to be ineffective to predict clinical pregnancy as an outcome, however more accurate results can be obtained through examining of isolated groups with different infertility factors.

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