Research Article ISSN 2639-9342

Gynecology & Reproductive Health

Vitamin D Supplementation İmproves Follicle Maturation By Regulating Oxidant/Antioxidant Balance İn Women With PCOS

Mehmet Ağar[1*] and Murat Önal[2]

[1]*Private Clinic, Sanlıurfa, Turkey.*

[2]*Gynolife IVF Clinic, Lefkosa, Cyprus.*

***Correspondence:**
Mehmet Ağar, MD, Özel Muayenehane, Sanlıurfa/ Turkey.

Received: 15 Apr 2022; **Accepted:** 18 May 2022; **Published:** 23 May 2022

Citation: Mehmet Ağar, Murat Önal. Vitamin D Supplementation İmproves Follicle Maturation By Regulating Oxidant/Antioxidant Balance İn Women With PCOS. Gynecol Reprod Health. 2022; 6(3): 1-4.

ABSTRACT

Objective: *To investigate the effect of vitamin D (VD) supplementation on follicular fluid total oxidant status (TOS) and total antioxidant status (TAS) in women undergoing IVF/ICSI due to PCOS.*

Materials and Methods: *Forty infertile women who were diagnosed with PCOS and decided on IVF/ICSI and whose serum VD levels were lower than 20 ng/mL were included in the study. The VD levels of the patients were determined according to the proposal of Endocrine Society. The patients were divided into two equal groups with 20 patients in each group. While the patients in Group 1 were given 400 IU/day of oral VD3 replacement, the patients in Group 2 were given 600 IU/day of oral VD3. VD treatment was started one month before the controlled ovarian stimulation cycle and continued throughout the following cycle. Fifteen patients with serum VD levels >30 ng/mL were taken as the control group. Oral VD replacement was not given to the patients in the control group. TAS and TOS values were measured in the follicle fluids taken from the patients in the VD replacement group and the patients in the control group on the day of ooist pick-up.*

Results: *When compared with the control group, the follicular fluid TAS levels of both groups with VD replacement were significantly higher, while TOS levels were found to be significantly lower. When 400 IU/day VD replacement and 600 IU/day VD replacement were compared within themselves, follicular fluid TAS and TOS levels were found to be similar. Increasing the VD replacement dose from 400 IU/day to 600 IU/day did not cause a significant change in TAS and TOS values. A positive and significant correlation was found between intrafollicular TAS levels and serum VD, MII oocyte, 2PN zygote and clinical pregnancy rates. A negative correlation was found between intrafollicular TOS levels and serum VD, 2PN zygote and clinical pregnancy rates.*

Conclusions: *VD replacement therapy contributes to follicle maturation by regulating intrafollicular oxidant/antioxidant balance in PCOS patients.*

Keywords

Vitamin D, PCOS TAS, TOS, Clinical pregnancy.

Introduction

Polycystic ovarian syndrome (PCOS) is a syndrome that causes inflammatory and oxidative stress as well as endocrine and metabolic pathologies. Ovarian-induced hyperandrogenism leads to a chronic clinical course of PCOS by disrupting the oxidant/antioxidant balance as well as insulin resistance [1-3]. Follicle development stages of a patient with PCOS show some differences compared to non-PCOS controls. High androgen and AMH levels as well as insulin resistance cause some pathologies in the regulation of folliculogenesis in PCOS patients. Defective follicular development results in anovulation. While chronic inflammation and high androgen increase the release of free oxygen radicals, antioxidant molecule synthesis decreases [4]. Increasing inflammatory molecules and ROS in the systemic circulation cause follicular maturation pathologies by accumulating in the follicle fluid [1-3].

Vitamin D is a steroid hormone that acts through the VD receptor (VDR). VDR is widely expressed in reproductive organs, especially in granulosa cells and endometrium [5]. The VD, which binds to the cytoplasmic VDR, goes to the nucleus and increases or decreases the expression of target genes [6]. Most PCOS patients have decreased serum VD levels. Increased inflammation and oxidative stress in PCOS may be regulated by the anti-inflammatory, antioxidant and immunomodulatory effects of VD, and may contribute to the normal conduct of follicle development [6,7]. To date, the effect of VD replacement on intrafollicular ROS production and follicle dynamics has not been investigated. We evaluated total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI), a method that has been used for a long time for the evaluation of intrafollicular oxidant/antioxidant balance [8-10]. These markers evaluate oxidative stress and antioxidant mechanisms in total [11]. This study was planned to determine how giving VD replacement to patients scheduled for IVF/ICSI due to PCOS affects follicular fluid TAS, TOS and OSI values and embryological parameters.

Materials and Methods

Forty infertile women who were diagnosed with PCOS and decided on IVF/ICSI and whose serum VD levels were lower than 20 ng/mL were included in the study. Women were diagnosed as PCOS based on the revised Rotterdam criteria, which require two of the following three manifestations: (1) oligo and/or anovulation, (2) clinical and/or biochemical hyperandrogenism, and (3) polycystic ovaries determined by ultrasonography. Participants in the control group were selected from infertile cases without clinical and laboratory findings of PCOS. The causes of infertility of the patients in the control group consisted of non-ovulatory causes such as tubal, male factor, endometriosis or endometrioma. The VD levels of the all participants were determined according to the proposal of Endocrine Society. Women with PCOS were divided into two equal groups with 20 patients in each group. While the patients in Group 1 were given 400 IU/day of oral VD3 replacement, the patients in Group 2 were given 600 IU/day of oral VD3. VD treatment was started one month before the controlled ovarian stimulation cycle and continued throughout the following cycle. Fifteen patients with serum VD levels >30 ng/mL were taken as the control group. Oral VD replacement was not given to the patients in the control group.

Blood was drawn from the patients in the PCOS group for basal hormone evaluation on the third day of progesterone withdrawal bleeding. The blood samples in the control group were collected on the 3rd day of the spontaneous menstrual cycle. Serum follicular stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and insulin, total testosterone, and dehydroepiandrosterone sulfate were measured. Insulin resistance was evaluated by calculating homeostatic model assessment of insulin resistance (HOMA-IR = fasting blood glucose (mg/dL) x fasting insulin (mIU/L)/405). Total plasma 25-OH vitamin D were measured with chemiluminescent enzyme immunoassay and results were given as ng/mL. Inclusion criteria were primary infertile cases older than 20 years of age, younger than 35 years of age, diagnosed with PCOS and decided on IVF/ICSI. Those who had metabolic and endocrine diseases such as diabetes other than PCOS, those who used hormonal medication in the last six months, patients with poor ovarian response and those who used antioxidant therapy were excluded from the study. Antagonist protocol was applied to the patients in all groups.

COS with antagonist protocol

rFSH was started to the patients in all VD replacement and control groups on the third day of the cycle. GnRH was initiated daily when the leading follicle reached a diameter of 14 mm. When the mean diameter of two or three leading follicles reached 16-17 mm or more ovulation was trigerred with agonist. The oocyte pick-up was carried out either 35 hours after ovulation trigger. TAS and TOS values were measured in the follicle fluids taken from the patients in the VD replacement group and the patients in the control group on the day of oocyte pick-up. A single top quality embryo on day 5 was transferred to each patient under ultrasound guidance. Luteal phase support with progesterone orally and intramuscularly until the day of the pregnancy test. The primary outcome of the study is to investigate the effects of vitamin D supplementation on follicular fluid TAS, TOS and TOS/TAS ratio. The secondary outcome was to determine the relationship between TOS, TAS, OSI and MII oocyte, 2 PN zygotes, clinical pregnanc and other parameters. Clinical pregnancy was defined as evidence of a gestational sac, confirmed by ultrasound examination at the 4th week of embryo transfer.

Follicular fluid TAS and TOS Analysis

Amniotic fluid samples taken during oocyte collection were centrifuged to remove blood and debris. Samples containing blood still after centrifugation were not evaluated. The centrifugation of follicular fluid samples was carried out at 400 rpm for 10 minutes, and aliquoted follicular fluid samples were stored at -20 °C until analyses. TAS and TOS levels were studied in an autoanalyzer by using TAS or TOS kits. While TAS results were presented as mmol Trolox Equivalent/L the TOS results were presented as μmol H2O2 Equivalent/L. OSI value was obtained by dividing TOS data by TAS data (TOS/TAS=OSI). OSI results are presented as percentages [9].

Statistical Analysis

Analyses of data were performed on SPSS 21 (SPSS Inc., Chicago, IL, USA). For the normality check, the Shapiro-Wilk test was used. Normally distributed variables were analyzed with the independent samples t-test. To compare the mean values of three independent groups one way ANOVA parametric test was used. The correlations between TAS and TOS levels and demographic and embryological parameters were assessed by calculating Spearman's correlation coefficient. Data are presented as mean ± SD. Differences were considered statistically significant if the p-value <0.05.

Results

Intrafollicular TAS, TOS and OSI values of the patient group given

400 IU/day VD were found to be 0.77 ± 0.02, 24.10 ± 4.32, 31.29 ± 5.33, respectively. Intrafollicular TAS, TOS and OSI values of the patient group given 600 IU/day VD were found to be 0.81 ± 0.20, 23.40 ± 6.40, 28.81 ± 3.04, respectively. Intrafollicular TAS, TOS and OSI values of the patient group in the control group were 0.64 ± 0.32, 26.71 ± 4.67, 41.71 ± 8.40, respectively. When compared with the control group, the follicular fluid TAS levels of both groups with VD replacement therapy were significantly higher ($p<0.01$, and $P<0.02$). Follicular fluid TOS levels of both groups given VD replacement were significantly lower than the control group ($p<0.02$ and $p<0.03$). When 400 IU/day VD replacement and 600 IU/day VD replacement were compared within themselves, follicular fluid TAS and TOS levels were found to be similar ($p<0.54$, $p<0.44$). Increasing the VD replacement dose from 400 IU/day to 600 IU/day did not cause a significant change in TAS and TOS values. A positive and significant correlation was found between intrafollicular TAS levels and serum VD, MII oocyte, 2PN zygote and clinical pregnancy rates. A negative correlation was found between intrafollicular TOS levels and serum VD, 2PN zygote and clinical pregnancy rates.

Discussion

PCOS, which is characterized by chronic inflammation due to hyperandrogenemia, insulin resistance, and metabolic syndrome, leads to delayed fertility in many women of reproductive age. In addition to primary metabolic pathologies related to PCOS, increased oxidative stress on the background of chronic inflammation negatively affects folliculogenesis. Abnormal follicle development and anovulation increase the IVF/ICSI rates as well as prolong the duration of pregnancy in PCOS patients. Testicular and ovarian germ cells have a different developmental stage than other somatic cells. The testicular blood barrier does not allow circulating immune cells and oxidative radicals to pass through this barrier during spermatogenesis. Thus, mature sperm formation takes place in a more protected microenvironment. However, follicle development from the ovaries does not occur in a sheltered environment, but in an environment open to oxidative and inflammatory stress. Since there is no immunological barrier in the ovaries, the follicle grows open to the effects of harmful molecules in the circulation [12-14].

Oxidative stress is a process that is balanced by antioxidant mechanisms by a healthy cell under physiological conditions and in physiological amounts. If the oxidant stress exceeds the antioxidant capacity, damage to the cell membrane and organelles begins. The increase in the amount of ROS during germ cell development leads to the development of both genomically and morphologically unhealthy oocytes [3]. PCOS is an oxidative and inflammatory disease [12-14]. Antioxidant defense systems originating from Thiol or sulphydryl (–SH) are involved in the neutralization of the harmful effects of ROS during folliculogenesis in PCOS [8,9]. Since there is no immunological barrier in the ovaries, the follicles of PCOS patients are exposed to oxidative stress. The follicle needs exogenous antioxidant support to recover from this stress. Vitamin D is a secosteroid hormone synthesized in the skin by the effect of UV light and activated in the liver and kidney. Thanks to its anti-inflammatory and antioxidant properties, it exhibits a ROS-scavenging effect. In this study, we compared the effects of VD replacement on oxidant/antioxidant balance in follicular fluids of PCOS patients who underwent IVF/ICSI for the first time. We compared the effects of two different VD doses on intrafollicular TAS and TOS. While both 400 IU/day and 600 IU/day VD increased TAS levels, they decreased TOS levels. In the control group that did not receive VD, TAS levels were low while TOS levels were high.

A minimum of 400 IU/day VD replacement provided intrafollicular TAS and TOS balance. A positive correlation was found between MII oocyte, 2PN zygotes and clinical pregnancy rates and TAS levels of patients who underwent VD replacement, and a negative correlation was found between TOS levels. The oxidant/antioxidant balance provided by VD replacement significantly improved the follicular development of PCOS patients and led to an increase in clinical pregnancy rates. Similar effects were not found in patients who did not undergo replacement. Increasing the VD dose to 600 IU/day did not provide any additional gain. It has been reported that VD replacement decreases MDA levels in the circulation of PCOS patients while increasing GSH and TAS [15,16]. However, there is no data on the effect of VD replacement on direct follicle development. Gungor et al. reported for the first time the link between VD levels and sperm DNA damage [17]. In another study by the same team, it was stated that estrogen replacement had a restorative effect on in vitro maturation cycles. Since estrogen is an antioxidant steroid hormone, it is not surprising that VD also exhibits similar effects. The progesterone-like effect of VD may also be responsible for the improvement in follicle parameters.

Despite the small number of cases, this study is the first to demonstrate that VD replacement regulates follicular development and maturation in PCOS patients. Obtaining more MII oocytes and more 2 PN zygotes with VD replacement before IVF/ICSI will contribute to clinical pregnancy rates. It is obvious that there is a need for studies comparing different VD doses in order to reach a clear conclusion.

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