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Vitamin D supplementation inhibits NF-kß signaling pathway in lean and obese women with PCOS

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Abstract. – OBJECTIVE: This study aims at investigating the effect of vitamin D (VD) replacement therapy on serum nuclear factor-kappa β (NF-kβ) levels in both lean and obese women with Polycystic Ovary Syndrome (PCOS).

PATIENTS AND METHODS: 50 women with PCOS with VD levels lower than 20 ng/mL were included in the study. Participants were equally divided into two groups, as lean and overweight/obese PCOS, according to their body mass index (BMI) values. Patients in both groups received 2000 IU/day oral VD replacement for two months. Serum NF-kβ, VD, demographic and hormonal values of the patients were recorded before and after VD replacement therapy.

RESULTS: Serum insulin and homeostatic model assessment (HOMA-IR) values of overweight/obese women with PCOS were significantly higher than lean women with PCOS. Pre-replacement NF-kβ levels were found to be significantly higher in the overweight/obese PCOS group (3.22 ± 1.09 ng/mL) than in the lean PCOS group (1.22 ± 0.43 ng/mL) ($p < 0.03$). Serum NF-kβ levels of the patients in the overweight/obese group (1.10 ± 0.30 ng/mL) and the lean group (0.83 ± 0.10 ng/mL) decreased significantly after VD replacement. No significant difference was found between the groups in terms of HOMA-IR, insulin, and total testosterone levels at the end of VD replacement therapy.

CONCLUSIONS: VD replacement therapy contributes to the improvement of subfertility and metabolic imbalance by reducing serum NF-kβ levels in both lean and obese women with PCOS.

Key Words:
MiRNA-106, Pediatric osteosarcoma, PI3K/AKT sigVitamin D, NF-kβ pathway, Lean PCOS, Obese PCOS.

Introduction

In addition to the roles of vitamin D in regulating bone mineralization and calcium hemostasis, the immunological effect of VD is also needed for the follicle development and implantation[1,2]. VD exerts its immunomodulating effect *via* T cells, B cells, and antigen-presenting cells[3]. VD exerts its immunomodulatory effect through the balance between Th1, Th2, Th17 and Treg cells[4]. Since the immunomodulating effects of VD dose does not occur in populations with deficient VD levels, many autoimmune and inflammatory diseases may occur. Such associations have been suggested by clinical studies not only in inflammatory and metabolic diseases but also in PCOS[5,6]. Possible association between low vitamin D status and increased risk of PCOS has now been generally accepted. In line with this, VD replacement improves follicle developmental capacity as well as its estrogen and progesterone synthesis capacity in women suffering from PCOS[5]. It has also been reported that VD replacement reduces serum androgen levels in PCOS and improves follicle morphology[6].

Inflammation is causally related to the occurrence of endocrine and metabolic pathologies. A chronic inflammatory process is accepted as an important factor in the pathogenesis of PCOS[7]. By inhibiting inflammatory molecules and immune cells, VD replacement regulates endocrine and ovulatory dysfunctions in PCOS[8]. Many studies[9,10] showed that VD administration has inhibitory effects on the release of COX2, PGs, MAP kinase and nuclear factor kappa β (NF-kβ), which are the main markers of inflammation. Clinical

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and experimental studies have reported that VD replacement therapy in women with PCOS leads to improvement in clinical and laboratory findings of the disease[5,11]. However, there are limited studies investigating the reason for the improvement provided by VD in the clinical and laboratory findings of PCOS. The reason for the improvement in the clinical findings may be the regulation of inflammatory pathways by the VD[7]. One of the main targets of the VD replacement therapy is the NF-kβ signaling pathway. The NF-kβ pathway is active in many immune cells in the reproductive tract. It generally regulates the immune response and inflammation *via* T cells[12,13].

There is no study on whether VD replacement affects the functions of the NF-kβ pathway. The aim of this study is to investigate the effect of VD replacement on serum NF-kβ levels in women with PCOS. We will try to find the answer whether NF-kβ mediates a causal relationship between VD replacement and improvement in PCOS findings.

Patients and Methods

50 infertile patients diagnosed with PCOS, according to the revised Rotterdam criteria, were included. Participants were selected from those with a VD < 20 ng/mL. Patients with normal VD levels were excluded. BMI values of the participants were recorded as normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2), and obese (≥ 30 kg/m^2). Participants were divided into two groups (n=25), lean and overweight/obese PCOS. Patients in Group 1 were selected from women with a BMI of 18.5-24.9 kg/m^2. The patients in Group 2 consisted of overweight and obese women with PCOS. Serum VD levels of both groups were measured before VD replacement therapy. According to the Endocrine Society proposal, patients with VD levels lower than 20 ng/mL were considered to have VD deficiency and were included in the study. Patients in both groups received 2000 IU/day oral VD replacement for 2 months. Serum NF-kβ levels were measured before and 2 months after VD replacement. Demographic and hormonal values of both groups were recorded before and after VD replacement therapy.

Anovulatory women with PCOS were subjected to withdrawal bleeding with progesterone. Serum samples were taken on the third day of the follicular phase for complete hormonal assays. Serum levels of NF-kβ, VD, luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, and insulin were measured. Homeostatic model assessment (HOMA-IR) formula was used for calculating insulin resistance. Serum total 25-hydroxyvitamin D levels were measured before and after VD replacement using an enzyme chemiluminescence immunoassay (ECLIA, Elecsys Vitamin D total II, Roche Cobas E602, Japan). The intra- and inter-assay coefficients of variations were 10% and 15%, respectively. Serum NF-kβ (p105) levels of lean and overweight/obese women with PCOS were measured by enzyme-linked immunosorbent assay (USCN Life Sci Inc, Wuhan China). The detection range of the kit was 0.156-10 ng/mL. The minimum detection rate of the kit was lower than 0.056 ng/mL. The intra- and inter-assay coefficients of variation were smaller than 10% and 12%, respectively.

The primary outcome of the study were (1) to compare serum NF-kβ and VD levels between lean and overweight/obese women with PCOS and (2) to determine the effects of VD replacement on serum NF-kß and other metabolic parameters.

Statistical Analysis

The data obtained before and after VD replacement was analyzed with the use of the Statistical Package for Social Sciences software 21.0 for Windows package software (IBM Corp., Armonk, NY, USA). The normality of data was examined by the use of the Shapiro-Wilk test. Pearson's Chi-square and Fischer's exact tests were used to compare categorical variables. Continuous variables were analyzed using the Mann-Whitney U-test. Data are presented as mean ± SD. Categorical data are described either by the number of cases or percentages. A p-value < 0.05 was considered as statistically significant.

Results

Demographic and laboratory findings of both groups are shown in Table I. The age and infertility duration of lean and overweight/obese groups were similar. There was no significant difference between the groups in terms of serum FSH, LH, and estradiol values. Serum insulin and HOMA-IR values of overweight/obese women with PCOS were significantly higher than

Table I. Pre- and post-replacement laboratory findings of both groups.

	Lean PCOS (n = 25)			Overweight/obese PCOS (n = 25)		
	Before VD	After VD	*p*	Before VD	After VD	*p*
Total VD	16.3 ± 4.01	27.3 ± 4.01	0.04	15.4 ± 6.02	26.9 ± 6.22	0.03
NF-kß	1.22 ± 0.43	0.83 ± 0.10	0.03	3.22 ± 1.09	1.10 ± 0.30	0.01
FSH	5.05 ± 1.22	5.30 ± 2.40	0.56	5.66 ± 2.20	5.41 ± 1.30	0.40
LH	8.60 ± 2.10	7.44 ± 1.02	0.33	9.01 ± 3.43	8.54 ± 2.09	0.08
Estradiol	41.2 ± 8.70	35.9 ± 6.33	0.20	39.1 ± 8.66	34.3 ± 5.33	0.11
TT	0.40 ± 0.11	0.33 ± 0.03	0.50	0.55 ± 0.30	0.51 ± 0.20	0.46
Insulin	11.9 ± 4.02	8.43 ± 1.20	0.04	13.5 ± 5.04	9.03 ± 3.66	0.02
HOMA-IR	3.01 ± 1.20	2.23 ± 0.40	0.02	3.99 ± 1.55	2.87 ± 0.77	0.01

FSH: Follicle stimulating hormone; HOMA-IR: Homeostatic model assessment; LH: Luteinizing hormone, NF-kß: Nuclear factor kappa ß; TT: Total testosterone.

lean women's with PCOS. A non-significant increase in total testosterone level was noted in the obese group compared to the lean group. Serum total VD levels of both groups were < 20 ng/mL (16.3 ± 4.01 ng/mL *vs.* 15.4 ± 6.02 ng/mL respectively). There was no significant difference between the groups in terms of VD levels. Serum NF-kß levels measured before VD replacement were found to be significantly higher in the overweight/obese PCOS group (3.22 ± 1.09 ng/mL) than in the lean PCOS group (1.22 ± 0.43 ng/mL) ($p < 0.03$).

Following VD replacement, an increase in VD levels was observed in both groups. Post-replacement VD levels of both groups were similar (27.3 ± 4.01 ng/mL *vs.* 26.9 ± 6.22 ng/mL, $p < 0.30$). After VD replacement, no difference was found between the groups in terms of HOMA-IR, insulin, FSH, LH, estradiol, and total testosterone levels. VD replacement improved the HOMA-IR and insulin levels of patients both in the obese and the lean group. The decline in serum insulin and HOMA-IR values after VD replacement was significant in both groups. After VD replacement, the serum NF-kß levels of the patients in the overweight/obese group were 1.10 ± 0.30 ng/mL, while the NF-kß level of the lean group was 0.83 ± 0.10 ng/mL. There was no significant difference between the groups in terms of serum NF-kß levels after VD replacement ($p < 0.01$). Receiving VD supplementation decreased serum NF-kß levels in both obese and lean women with PCOS. Although the decline in NF-kß levels was more pronounced in the obese group, the decline in both groups was found to be significant. Serum NF-kß, VD and other hormonal values of both groups before and after VD replacement are shown in Table I.

Discussion

Although the results of previous studies[14,15] on serum VD levels in women with PCOS are heterogeneous, most studies have reported reduced VD levels. There are no comparative data on how obesity affects serum VD levels in women with PCOS. Obesity-related hemodilution and VD accumulation in adipose tissues in PCOS patients cause low serum VD levels[16]. However, since the VD deficiencies of both lean and obese groups are similar, it can be thought that the decline in VD levels is independent from body fat accumulation.

In population-based studies[5,11,15,17], it has been reported that VD positively contributes to fertility by improving the clinical and metabolic findings of PCOS. However, the mechanism by which VD replacement increases fertility outcomes is not known exactly. It has been suggested that the fertility-enhancing effect of VD is mediated by both the ovary and the endometrium[18]. Consistent with this, increased serum Antimüllerian hormone (AMH) levels have been reported after VD replacement[15,17]. The VD receptor is expressed in the endometrium and granulosa cells. The increase in serum AMH levels after VD replacement therapy may be due to increased VD receptor expression in granulosa cells. The addition of VD to granulosa cell culture increases VD receptor expression and production of genes responsible for steroid synthesis[19]. Makieva et al[20] reported that oral VD replacement therapy altered gene expression in luteinized granulosa cells of infertile women. However, no correlation was found between VD replacement and serum AMH levels[15]. The second target tissue of the VD may be the endometrium. VD3 supplementation increases estrogen and progesterone synthesis by stimulating the 3β-hyd-

roxysteroid dehydrogenase enzyme. Increasing estrogen and progesterone may contribute to the fertility outcome by stimulating the genes responsible for endometrial receptivity[21]. However, until now there has been no study investigating receptivity genes and VD receptor expression after VD replacement. All these findings have brought to the fore the idea that VD replacement can show its effects on fertility through other mechanisms besides the endometrium and ovary. Since PCOS is a chronic inflammatory disease and VD replacement affects major cellular components responsible for innate and adaptive immunity[12,13].

One of the main targets of VD is NF-kß, which is responsible for inflammation and immune response[12,13]. In a recent study[22], increased expression of endometrial NF-kß p65 has been reported in both lean and obese women with PCOS. The present study represents the first clinical evidence that VD replacement reduces serum NF-kβ levels in lean and obese women with PCOS. In order to clearly demonstrate the effect of VD replacement, participants were selected among women with PCOS suffering from VD-deficiency (< 20 ng/mL). In pre-replacement evaluation, we found that the serum NF-kβ levels of overweight/obese women with PCOS were significantly lower than those of lean women with PCOS. The high NF-kβ levels in the obese group may be due to the increased inflammatory process secondary to excess body fat accumulation. Indeed, by increasing the release of many bioactive molecules known as adipokines, adiposity induces inflammation[23-25]. Increased fat accumulation in women with obesity may increase the inflammatory events and cause NF-kβ levels to be higher than lean women with PCOS. However, a recent study[24] reporting that lean and obese women with PCOS have similar endometrial NF-kβ levels has weakened our idea.

After VD replacement therapy, serum NF-kβ levels were significantly decreased in both lean and obese women with PCOS. The decrease in NF-kβ levels in the obese group was more pronounced than in the lean group. This may be due to the fact that the inflammatory events, due to the increase in adipose tissue, are more pronounced in the obese group than in the lean group[25]. Since PCOS is a systemic, inflammatory, and autoimmune disease, it is not surprising that serum NF-kβ levels are increased. Similarly, since obesity stimulates inflammation more, it is usual to detect a more pronounced NF-kβ increase compared to the lean group. Since VD is a secosteroid with immunomodulatory effect[12,13], it inhibits the synthesis of inflammatory cytokines and prostaglandins *via* T cells, B cells, and antigen-presenting cells[4,9]. Another important target of VD is the NF-kβ pathway[12]. VD both prevents the binding of NF-kβ to DNA and inhibits the expression of the NF-kβ reporter gene[10]. The significant decrease in serum NF-kβ levels in patients given VD replacement may be due to the NF-kβ inhibitory effect of VD. On the other hand, previous reports[26] affirming that VD stimulates the NF-kβ pathway in immune cell types have weakened our opinion. However, the normalization of serum insulin and HOMA-IR values after the VD replacement is important evidence that VD inhibits inflammation in immune cells and adipocytes.

Conclusions

Regardless of the underlying mechanism, VD replacement therapy improves subfertility and hormonal imbalance by reducing serum NF-kβ levels in both lean and obese women with PCOS. Although the small number of cases is a limitation, this study is important as it is the first clinical investigation showing that VD replacement improves chronic inflammation *via* NF-kβ pathway in women with PCOS. Nevertheless, it is clear that there is a need for large-scale randomized studies investigating the relationship between VD administration, inflammatory pathway, and fertility outcome in women with PCOS.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethical Approval

The study was performed according to the guidelines of the Helsinki Declaration on human experimentation and was approved by the Ethics Committee.

Informed Consent

Informed consent was obtained from participants at the time of enrollment.

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