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Vitamin D supplementation during pregnancy inhibits the activation of fetal membrane NF-κB pathway

N. GURKAN

Department of Obstetrics and Gynecology, Medical Park Hospital, Samsun, Turkey

Abstract. – OBJECTIVE: Nuclear Factor-κB (NF-κB) is an important member of the basic cellular inflammatory pathway that regulates inflammation and apoptosis in fetal membranes. Vitamin D (VD) exerts its anti-inflammatory and immunomodulatory effects via the NF-κB pathway. This study was designed to investigate amniotic fluid NF-κB levels in pregnant women undergoing VD replacement therapy.

PATIENTS AND METHODS: Sixty patients who received antenatal vitamin D supplementation from the 14th week of pregnancy until delivery were included in the study. Participants were selected among those whose serum vitamin D levels were compatible with insufficiency (20-30 ng/mL), according to the Endocrine Society proposal. Participants were divided into three groups with 20 patients in each group and one of the cholecalciferol or placebo treatments was given. Patients in Group 1 were given 500 IU/day of cholecalciferol, while patients in Group 2 were given 1000 IU/day of cholecalciferol. Patients in group 3 were not given cholecalciferol treatment (placebo). Patients in all groups underwent elective cesarean section. Amniotic fluid samples were collected after the fetal membranes were cut and before the fetal parts were manually removed.

RESULTS: The amniotic fluid NF-κB level of the control group who did not receive VD replacement was 9.33±2.02 ng/mL. The amniotic fluid NF-κB level of the 500 IU/day VD replacement group was found to be 6.12±1.23 ng/mL. Compared to the control group, NF-κB levels of pregnant women given 500 IU/day VD replacement were significantly lower (9.33±2.02 ng/mL *vs.* 6.12±1.23 ng/mL, $p<0.03$). The amniotic fluid NF-κB level of the 1000 IU/day VD replacement group was found to be 3.09±0.44 ng/mL. Compared to the control group, amniotic fluid NF-κB levels of pregnant women given 1000 IU/day VD replacement were significantly lower (9.33±2.02 ng/mL *vs.* 3.09±0.44 ng/mL, $p<0.01$). When the VD replacement groups were compared among themselves, the amniotic fluid NF-κB level decreased approximately twice as much in the 1000 IU/day replacement group compared to the 500 IU/day replacement group (3.09±0.44 ng/mL *vs.* 6.12±1.23 ng/mL, $p<0.01$). A negative correlation was found between amniotic fluid NF-κB level and VD dose (r=-0.789, $p<0.04$).

CONCLUSIONS: The present study showed for the first time that amniotic fluid NF-κB levels decreased in pregnant women who underwent VD replacement dose dependent manner.

Key Words:
Pregnancy, Vitamin D, Amniotic fluid, NF-κB.

Introduction

Although the changes caused by pregnancy in vitamin D (VD) metabolism are not known exactly, biochemically low VD levels are frequently encountered during pregnancy[1,2]. Deficiency in dietary intake of ergocalciferol or cholecalciferol or increased skin pigmentation due to pregnancy may lead to a decrease in VD levels[3]. On the other hand, the increased need for calcium and phosphate during pregnancy due to the fetus and its appendages may also lead to a decrease in VD levels. As it is known, the fetus is completely dependent on the mother in terms of VD. Therefore, maternal and umbilical cord VD levels are highly correlated[4]. The VD passes through the placenta and reaches the fetus and its appendages[5]. While maternal serum ionised calcium levels are kept stable during pregnancy, active 1,25(OH)2 D levels are increased[5]. Trying to keep calcium and phosphate metabolism stable and increasing 1,25(OH)2 D during pregnancy are important in providing immune tolerance to the fetus[6].

VD replacement in pregnant women increases umbilical cord VD levels as well as neonatal serum VD levels[7]. In addition to providing bone mineral density and muscle development of the fetus, VD also plays an important role in the continuation of the strong immunomodulatory effect mediated by T and B cells and the prevention of

Corresponding Author: Naziye Gurkan, MD; e-mail: nazeyg@yahoo.com

inflammatory reactions[8]. Chorioamnionitis with antenatal inflammation, which is thought to develop in VD deficiency, may lead to the emergence of different pathophysiological processes[9,10]. In VD deficiency, the fetus becomes more prone to many acute and chronic inflammatory diseases especially respiratory system and fetal membranes[11,12]. It has been reported that low VD induces premature rupture of membranes and uterine contractions through chorioamnionitis and may lead to preterm delivery[13,14]. However, the researchers reached this conclusion with observational studies and did not investigate the inflammatory changes that are claimed to occur in the fetal membranes.

Nuclear Factor-κB (NF-κB) is an important member of the basic cellular inflammatory pathway that regulates inflammation and apoptosis[15]. NF-κB is inactive in the cytosol by complexing with inhibitor kB[16,17]. VD regulates its anti-inflammatory and immunomodulatory effects via T and B cells as well as NF-κB pathway. Thinning and tearing of fetal membranes is a multifactorial regulated process. In addition to hormonal factors and uterine contractions, inflammation of the chorioamniotic membranes also plays an important role in the initiation of labor[17]. Due to its anti-inflammatory and immunomodulatory effects, VD may play a role in the initiation of labor. Decreased tensile strength and rupture of fetal membranes in VD deficiency may be regulated via the NF-κB pathway. The reason for preterm rupture of membranes in VD deficiency may be the activation of the NF-κB-IkB complex and the triggering of inflammatory reaction[13,18]. This study was designed to determine amniotic fluid NF-κB levels in pregnant women undergoing VD replacement. If the amniotic fluid NF-κB levels are found to be higher in pregnant women who did not undergo VD replacement, we can say that the increased frequency of preterm labor in VD deficiency may be NF-κB-dependent.

Patients and Methods

Patient Selection and Grouping

Sixty patients who received antenatal vitamin D supplementation from the 14th week of pregnancy until delivery were included in the study. Participants were selected among those whose serum vitamin D levels were compatible with insufficiency (20-30 ng/mL), according to the Endocrine Society propsal[19]. Sixty pregnant women were divided into three groups with 20 patients in each group and one of the cholecalciferol or placebo treatments was given. Patients in Group 1 were given 500 IU/day of cholecalciferol, while patients in Group 2 were given 1000 IU/day of cholecalciferol. Cholecalciferol doses were determined according to the Maternal Vitamin D Osteoporosis Study (MAVIDOS)[20]. The patients in group 3 were not given cholecalciferol treatment (placebo). All the patients in group 1 and 2 remained in the study until delivery. Missed dose was re-administered up to 7 days. Preparations containing calcium, folic acid and iron were also provided to each group during vitamin D treatment[21].

Inclusion criteria were determined as follows. Women aged 20 years and older with spontaneous pregnancy and 14 completed weeks of gestation according to USG or last menstrual period (LMP). For those with more than one USG record, the first record was considered. Accordingly, we considered 14 weeks + 0 days to be included in the study. Estimated date of birth was determined according to LMP. If there was a difference of more than ten days between USG and LMP, the estimated date of birth was determined according to second trimester USG. Those with a history of hypersensitivity to VD, hypercalcemia, or kidney stones, as well as those with active tuberculosis, parathyroid pathology, liver or kidney disease, hypertension or proteinuria were excluded from the study. Multiple gestations, IVF/ICSI pregnancies, oligohydramnios and epilepsy drug users were also excluded from the study. Patients who planned for normal vaginal delivery were not included in the study because it was not suitable for the study design. Those with a history of PPROM, patients with placenta previa or ablatio placenta, patients with a history of diabetes mellitus or gestational diabetes mellitus, and those with a C/S decision due to preeclampsia and eclampsia were not included in the study. Since the cervical softening mechanism is different in breech presentations, these patients were excluded from the evaluation.

Patients in all three groups underwent elective cesarean section. Amniotic fluid samples were collected after the fetal membranes were cut and before the fetal parts were manually removed. Sterile 10 cc injectors were used for amniotic fluid collection. Care was taken not to contaminate the samples with blood. Amniotic fluid samples containing dense blood and vernix caseosa were not included in the study. Samples containing mild to moderate blood or vernix were included in the study after centrifugation. The primary

outcome was to determine the amniotic fluid NF-κB levels of pregnant women who received and did not receive VD. The secondary outcome was to correlate the amniotic fluid NF-κB with the dose of vitamin D administered and other laboratory, demographic parameters. All procedures performed in this study were in accordance with the Ethical Standards of International Research Committee and local approval was obtained from the OMU. All patients recruited to the study were fully counseled and written informed consent was obtained.

NF-κB Analysis in Amniotic Fluid

NF-κB levels were measured using ELISA after thawing the amniotic fluid samples of all groups that were taken during cesarean section and frozen in RNA later. The immunological kit used can measure NF-κB levels in biological fluids with great sensitivity (CusabioBiotechCo., Ltd., WUHAN, CHINA). The kit can make *in vitro* and quantitative measurements in humans. Thawed amniotic fluids were treated with phosphate buffer and centrifuged at 2500 rpm for 5 minutes. Thus, blood and vernix were removed. NF-κB was analyzed according to the method specified by the manufacturer in the kit. Results are given in ng/mL. The detection range of the kit was 0.3 to 20 ng/mL and the minimum measurable level was 0.078 ng/mL. The intra- and inter-assay coefficients of variation of the kit were <8% and <10%, respectively. Test results are expressed as ng/mL.

Statistical Analysis

The data obtained following VD replacement was analyzed with the use of the Statistical Package for Social Sciences software 21.0 for windows package software (SPSS Inc., Armonk, NY, USA). Normality of data was examined by nonparametric Kolmogorov–Smirnov test. Continuous variables were analyzed using parametric One-Way ANOVA test. Pearson's correlation analysis was used to determine the correlation between amniotic fluid NF-κB levels and other parameters. Data are presented as mean ± SD. A p-value <0.05 was considered statistically significant.

Results

Demographic, laboratory, maternal and perinatal characteristics of patients with and without VD replacement are presented in Table I. There was no significant difference between the groups in terms of serum VD, age, gravida, parity, and gestational age. The VD levels of all experimental and placebo groups were determined as 20-30 ng/mL, consistent with insufficiency. Post-replacement VD levels were not evaluated. There was no difference between the groups in terms of fetal birth weights. Elective cesarean delivery was performed without complications in all groups.

The amniotic fluid NF-κB level of the control group who did not receive VD replacement was 9.33±2.02 ng/mL. The amniotic fluid NF-κB level of the 500 IU/day VD replacement group was found to be 6.12±1.23 ng/mL. Compared to the control group, NF-κB levels of pregnant women given 500 IU/day VD replacement were significantly lower (9.33±2.02 ng/mL *vs.* 6.12±1.23 ng/mL, p<0.03). The amniotic fluid NF-κB level of the 1000 IU/day VD replacement group was found to be 3.09±0.44 ng/mL. Compared to the control group, amniotic fluid NF-κB levels of pregnant women given 1000 IU/day VD replace-

Table I. Demographic and laboratory characteristics of vitamin D replacement and placebo groups.

	Group 1 (n=20) 500IU/day Vitamin D	Group 2 (n=20) 1000 IU/day Vitamin D	Group 3 (n=20) Placebo
Age (years)	27.9±6.10	29.1±5.30	28.3±7.20
VD level (ng/mL)	24.4±4.51	25.9±6.03	26.5±8.22
Gravidity	3.20±1.02	2.90±1.44	2.87±0.50
Parity	2.11±0.33	1.87±0.40	1.90±0.21
Gestational age (weeks)*	37.2±8.33	36.6±5.31	37.3±7.10
Fetal birthweight (gr)	2895.1±149.2	2945.4±202.1	2830.4±104.5
Amniotic fluid NF-kB (ng/mL)**	6.12±1.23	3.09±0.44	9.33±2.02

*Estimated date of birth was determined according to LMP or USG. **Amnotic fluid NF-kB levels of both groups who received 500 or 1000 IU/day VD replacement were found to be significantly lower than the controls.

ment were significantly lower (9.33±2.02 ng/mL *vs.* 3.09±0.44 ng/mL, $p<0.01$). When the VD replacement groups were compared among themselves, the amniotic fluid NF-κB level decreased approximately twice as much in the 1000 IU/day replacement group compared to the 500 IU/day replacement group (3.09±0.44 ng/mL *vs.* 6.12±1.23 ng/mL, $p<0.01$). A negative correlation was found between amniotic fluid NF-κB level and VD dose (r=-0.789, $p<0.04$). No significant correlation was found between other maternal and perinatal parameters and NF-κB levels.

Discussion

There is no consensus on both the definition of VD deficiency in pregnancy and the dose of VD to be used for supplementation. The threshold values used for the definition of deficiency vary from country to country. In this study, we selected the patient groups among pregnant women with VD insufficiency (25-35 ng/mL). In general, the recommended dose for VD support during pregnancy varies between 400-600 IU/day. However, the World Health Organization has not determined a specific dose for VD support during pregnancy[22]. On the other hand, while the Endocrine Society determines the upper limit of the maximum safe VD dose as 10,000 IU/day in pregnant women with VD deficiency, the dose is 600 IU/day in pregnant women who do not have VD deficiency[19]. Due to all these debates, we administered 500 IU/day to one group of the participants and 1000 IU/day of VD to the other group in order not to cause any maternal or fetal complications. Since these doses were far below the safe upper limits recommended by the Endocrine Society, we did not encounter any maternal and fetal complications related to VD throughout the study.

The mechanisms that initiate labor are not fully known, loacal and systemic hormones, inflammator cytokines whose synthesis and release in fetal membranes are increased as a result of the synchronized activation of the fetal adrenal cortex, maternal brain and placental compartment. While cascading local and systemic reactions cause inflammation of the membranes, matrix metalloproteinases cause the inflamed membranes to rupture and stimulate the onset of labor. NF-κB may play a role in this reaction cascade as the main regulator of inflammation. The release of adhesion molecules such as E-selectin, intercellular adhesion molecule 1, and vascular cell adhesion molecule 1 is regulated by the NF-κB pathway[23]. VD may contrbute to the regulation of inflamatuar pathways in fetal membranes with its immunomodulatory and anti-inflammatory effects. In good agreement with this in the LPS-induced placental inflammation model, Vit D administration has been shown to inhibit both placental NF-kB signaling and VD receptor expression. In the same study, it was emphasized that VD exerts its anti-inflammatory effect in placental tissue via NF-kB and VD receptors[24]. On the other hand, since the main target of VD in the inflammatory pathway is NF-κB[25,26], the onset of labor may be altered in case of VD deficiency[13,18]. Our study is the first clinical study to present data on the effect of VD replacement on amniotic fluid NF-κB levels. The amniotic fluid NF-κB levels of both groups in which we underwent VD replacement were significantly lower than the group that did not receive VD replacement therapy (placebo). We found a negative correlation between the given VD dose and the reduction in amniotic fluid NF-κB. Compared to the group given 500 IU VD daily, the amnotic fluid NF-κB levels of the patients given 1000 IU/day VD showed a two-fold decrease.

We do not clearly know the rationale for the decrease in NF-κB levels in parallel with the increase in VD dose. Since this is not a dose-response study, we cannot clearly state what the minimum VD dose is that reduces amniotic fluid NF-κB levels. By choosing the doses, we chose the VD that have close to the minimum feto-maternal side effects according to the Endocrine Society criteria. We showed that VD at a dose of 500 IU/day significantly reduced amniotic fluid NF-κB levels. The inhibitory effect of VD on the fetal membrane NF-κB pathway may also be dose-dependent, since the dose of VD required for the improvement of clinical and laboratory findings in paratroid dysfunction is higher than the pharmacological VD dose[27]. However, more detailed dose-response studies are needed to determine the minimum VD dose required to meet the needs of both mother and fetus during pregnancy.

VD exerts its inhibitory effect on fetal membrane NF-κB pathway via the VD receptor (VDR). VDR is intensely expressed in the placenta and decidua as well as in all reproductive organs[24,28]. In addition to those in the systemic circulation, 1,25(OH)2D3 production also takes place locally at the maternal-fetal interface. However, we do not have data on whether VD affects the VDR in fetal membranes in a genomic or nongenomic way. Since VD exerts its anti-inflammatory effect on fetal membranes through uterine natural killer cells, macrophages, and T lymphocytes, it may also reduce NF-κB in fetal membranes by

a similar mechanism[24,28,29]. The inhibition of the decidual cells differentiation by 1,25(OH)2D3[30,31] may explain the reduction of NF-κB synthesis in fetal membranes by VD. The increased synthesis of pro-inflammatory cytokines in VDR knockout mice[32] is an important proof that the NF-κB-blocking effect of VD is mediated by VD receptors. Moreover, in line with our results, another strong evidence is that 1,25(OH)2D3 blocks cytokine release in uNK cells[33].

VD inhibits the NF-κB pathway through different mechanisms in different cells. While it blocks the binding of NF-κB to DNA in some cells, it decreases RelB and VD receptor expression in others and sometimes prevents the nuclear translocation of p65[34-36]. On the other hand, it is not known exactly how VD blocks NF-κB in fetal membranes. VD replacement may increase the synthesis of many local and systemic factors that block NF-κB translocation to the nucleus[37-39]. Since the patients were sent to elective cesarean section we could not establish a correlation between the delivery times of the VD repecement groups and NF-κB levels. Since active labor can affect NF-κB levels by stimulating inflammation in fetal membranes, we had to design the study in this way.

Conclusions

We showed for the first time that amniotic fluid NF-κB levels decreased in pregnant women who underwent VD replacement. Our results are clinically important in terms of demonstrating the role of the NF-κB pathway in the initiation of labor. In addition, this study may open new horizons in the treatment of obstetric emergencies such as preterm labor and premature rupture of fetal membranes due to the inhibitory effect of VD on the placental NF-κB signaling pathway[24].

Conflicts of Interest

The authors declare no conflicts of interest.

Ethical Committee

Ethical Standards of International Research Committee and local approval was obtained from the OMU.

Informed Consent

All patients recruited to the study were fully counseled and written informed consent was obtained.

ORCID

N. Gurkan 0000-0003-1088-018X.

References

1) Blomberg Jensen M. Vitamin D and male reproduction. Nat Rev Endocrinology 2014; 10: 175-186.
2) Zhang JY, Lucey AJ, Horgan R, Kenny LC, Kiely M. Impact of pregnancy on vitamin D status: a longitudinal study. Br J Nutr 2014: 1-7.
3) McAree T, Jacobs B, Manickavasagar T, Sivalokanathan S, Brennan L, Bassett P, Rainbow S, Blair M. Vitamin D deficiency in pregnancy - still a public health issue. Matern Child Nutr 2013; 9: 23-30.
4) Maghbooli Z, Hossein-Nezhad A, Shafaei AR, Karimi F, Madani FS, Larijani B. Vitamin D status in mothers and their newborns in Iran. BMC Pregnancy Childbirth 2007; 7: 1.
5) Curtis EM, Moon RJ, Harvey NC, Cooper C. Maternal vitamin D supplementation during pregnancy. Br Med Bull 2018; 126: 57-77.
6) Tamblyn JA, Hewison M, Wagner CL, Bulmer JN, Kilby MD. Immunological role of vitamin D at the maternal-fetal interface. J Endocrinol 2015; 224: R107-21.
7) Brooke OG, Brown IR, Bone CD, Carter ND, Cleeve HJ, Maxwell JD, Robinson VP, Winder SM. Vitamin D supplements in pregnant Asian women: effects on calcium status and fetal growth. Br Med J 1980; 280: 751-754.
8) Guillot X, Semerano L, Saidenberg-Kermanac'h N, Falgarone G, Boissier MC. Vitamin D and inflammation. Joint Bone Spine 2010; 77: 552-557.
9) Adams JS, Hewison M. Update in vitamin D. J Clin Endocrinol Metab 2010; 95: 471-478.
10) Bikle D. Nonclassic actions of vitamin D. J Clin Endocrinol Metab 2009; 94: 26-34.
11) Brehm JM, Schuemann B, Fuhlbrigge AL, Hollis BW, Strunk RC, Zeiger RS. Childhood Asthma Management Program Research G Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. J Allergy Clin Immunol 2010; 126: 52-58; e55.
12) Clifford RL, Knox AJ. Vitamin D – a new treatment for airway remodelling in asthma? Br J Pharmacol 2009; 158: 1426-1428.
13) Urrutia RP, Thorp JM. Vitamin D in Pregnancy: Current Concepts. Curr Opin Obstet Gynecol 2012; 24: 57-64.
14) Palacios C, Kostiuk LK, Peña-Rosas JP. Vitamin D supplementation for women during pregnancy. Cochrane Database Syst Rev 2019; 7: CD008873.
15) Hu W, Zhou PH, Rao T. Adrenomedullin attenuates interleukin-1beta-induced inflammation and apoptosis in rat Leydig cells via inhibition of NF-kappaB signaling pathway. Exp Cell Res 2015; 339: 220-230.

16) Celik O, Celik E, Turkcuoglu I, Yilmaz E, Ulas M, Simsek Y, Karaer A, Celik N, Aydin NE, Ozerol I, Unlu C. Surgical removal of endometrioma decreases the NF-kB1 (p50/105) and NF-kB p65 (Rel A) expression in the eutopic endometrium during the implantation window. Reprod Sci 2013; 20: 762-770.

17) Fischer C, Page S, Weber M, Eisele T, Neumeier D, Brand K. Differential effects of lipopolysaccharide and tumor necrosis factor on monocytic IkappaB kinase signalsome activation and IkappaB proteolysis. J Biol Chem 1999; 274: 24625-24632.

18) Bodnar LM, Klebanoff MA, Gernand AD, Platt RW, Parks WT, Catov JM, Simhan HN. Maternal vitamin D status and spontaneous preterm birth by placental histology in the US Collaborative Perinatal Project. Am J Epidemiol 2014; 179: 168-176.

19) Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011; 96: 1911-1930.

20) Harvey NC, Javaid K, Bishop N, Kennedy S, Papageorghiou AT, Fraser R, Gandhi SV, Schoenmakers I, Prentice A, Cooper C. MAVIDOS Maternal Vitamin D Osteoporosis Study: study protocol for a randomized controlled trial. The MAVIDOS Study Group. Trials 2012; 13: 13.

21) Roth DE, Gernand AD, Morris SK, Pezzack B, Islam MM, Dimitris MC, Shanta SS, Zlotkin SH, Willan AR, Ahmed T, Shah PS, Murphy KE, Weksberg R, Choufani S, Shah R, Al Mahmud A. Maternal vitamin D supplementation during pregnancy and lactation to promote infant growth in Dhaka, Bangladesh (MDIG trial): study protocol for a randomized controlled trial. Trials 2015; 16: 300.

22) World Health Organisation. Guideline: Vitamin D supplementation in pregnant women. Geneva: 2012.

23) Zhong L, Simard MJ, Huot J. Endothelial microRNAs regulating the NF-κB pathway and cell adhesion molecules during inflammation. FASEB J 2018; 32: 4070-4084.

24) Chen YH, Yu Z, Fu L, Wang H, Chen X, Zhang C, Lv ZM, Xu DX. Vitamin D3 inhibits lipopolysaccharide-induced placental inflammation through reinforcing interaction between vitamin D receptor and nuclear factor kappa B p65 subunit. Sci Rep 2015; 5: 10871.

25) Griffin MD, Xing N, Kumar R. Vitamin D and its analogs as regulators of immune activation and antigen presentation. Annu Rev Nutr 2003; 23: 117-145.

26) van EE, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. J Steroid Biochem Mol Biol 2005; 97: 93-101.

27) Bouillon R. Vitamin D: from photosynthesis, metabolism, and action to clinical applications, endocrinology: adult and pediatric: vitamin D, 7th ed. Philadephia, PA: Elsevier, 2015; pp. 1018-1037.

28) Arck PC, Hecher K. Fetomaternal immune cross-talk and its consequences for maternal offspring 's health. Nat Med 2013; 19: 548-556.

29) Tamblyn JA, Hewison M, Wagner CL, Bulmer JN, Kilby MD. Immunological role of vitamin D at the maternal-fetal interface. J Endocrinol 2015; 224: R107-121.

30) Kachkache M, Rebut-Bonneton C, Demignon J, Cynober E, Garabedian M. Uterine cells other than stromal decidual cells are required for 1,25-dihydroxyvitamin D3 production during early human pregnancy. FEBS Lett 1993; 333: 83-88.

31) Griffin MD, Lutz W, Phan VA, Bachman LA, McKean DJ, Kumar R. Dendritic cell modulation by 1,α-25 dihydroxyvitamin D3 and its analogs: a vitamin D receptor-dependent pathway that promotes a persistent state of immaturity in vitro and in vivo. Proc Natl Acad Sci 2001; 98: 6800-6805.

32) Liu NQ, Kaplan AT, Lagishetty V, Ouyang YB, Ouyang Y, Simmons CF. Vitamin D and the regulation of placental inflammation. J Immunol 2011; 186: 5968-5974.

33) Evans KN, Nguyen L, Chan J, Innes BA, Bulmer JN, Kilby MH. Effects of 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 on cytokine production by human decidual cells. Biol Reprod 2006; 75: 816-822.

34) Berry DM, Clark CS, Meckling-Gill KA. 1alpha,25-dihydroxyvitamin D3 stimulates phosphorylation of IkappaBalpha and synergizes with TPA to induce nuclear translocation of NFkappaB during monocytic differentiation of NB4 leukemia cells. Exp Cell Res 2002; 272: 176-184.

35) Adams LS, Teegarden D. 1,25-dihydroxycholecalciferol inhibits apoptosis in C3H10T1/2 murine fibroblast cells through activation of nuclear factor kappaB. J Nutr 2004; 134: 2948-2952.

36) Ribeiro MC, Moore SM, Kishi N, Macklis JD, MacDonald JL. Vitamin D Supplementation Rescues Aberrant NF-κB Pathway Activation and Partially Ameliorates Rett Syndrome Phenotypes in Mecp2 Mutant Mice. eNeuro 2020; 7: ENEURO.0167-20.

37) Harant H, Wolff B, Lindley IJ. 1a,25-dihydroxyvitamin D3 decreases DNA binding of nuclear factor-kB in human fibroblasts. FEBS Lett 1998; 436: 329-334.

38) Sadeghi K, Wessner B, Laggner U. Vitamin D3 down-regulates monocyte TLR expression and triggers hyporesponsiveness to pathogen-associated molecular patterns. Eur J Immunol 2006; 36: 361-370.

39) Celik O, Ersahin A, Acet M, Celik N, Baykus Y, Deniz R. Disulfiram, as a candidate NF-κB and proteasome inhibitor, prevents endometriotic implant growing in a rat model of endometriosis. Eur Rev Med Pharmacol Sci 2016; 20: 4380-4389.