MEDICAL RECORDS-International Medical Journal

Research Article

Vitamin D, VDR, and VDBP Levels Correlate with Anti-inflammatory Cytokine Profile in FMS Patients

Pinar Ellergezen[1], Alev Alp[2], Sinan Cavun[1]

[1]Uludağ University Faculty of Medicine, Department of Medical Pharmacology, Bursa, Türkiye
[2]Uludağ University Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Bursa, Türkiye

Copyright@Author(s) - Available online at www.dergipark.org.tr/tr/pub/medr
Content of this journal is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International.

Abstract

Aim: The major target of this research is to examine whether there is any connection between the levels of vitamin D and anti-inflammatory mediators in patients with fibromyalgia syndrome (FMS).

Materials and Methods: The study contains 30 FMS diagnosed and 25 healthy female individuals and the determination of FMS was made according to the standards of 2010 American College of Rheumatology (ACR). Vitamin D, vitamin D receptor (VDR), vitamin D binding protein (VDBP) levels, and anti-inflammatory cytokine (IL-4, IL-10, TGF-β) levels in the serum of patients with FMS and healthy individuals were measured using enzyme-linked immunosorbent assay (ELISA).

Results: The concentrations of vitamin D, VDR, and VDBP were determined to be higher in healthy controls than in patients with FMS ($p<0.001$). Correlating with this, IL-4, IL-10, and TGF-β levels were measured remarkably higher in the healthy group than in the FMS patients ($p<0.001$).

Conclusion: Low vitamin D levels may cause a decrease in anti-inflammatory cytokine levels and their immunosuppressive effect in FMS.

Keywords: Anti-inflammatory cytokines, vitamin D, fibromyalgia syndrome

INTRODUCTION

The definition of “chronic widespread pain” is widely used for some pain-related diseases whose pathogenesis has not been fully resolved. One of the most well-known subgroups of these diseases is fibromyalgia syndrome (FMS). Fibromyalgia is defined as a pain processing disorder resulting from abnormal conditions in the pain signaling pathways opening to the central nervous system (1). The most prominent symptoms of FMS include chronic widespread pain, extreme fatigue, sleep disturbance, increased pain when touching the inflamed area, tingling in the skin, and prolonged muscle spasm (2). All these symptoms are actually related to inflammation, and studies suggest that inflammation has a noticable task in the pathogenesis of FMS. The most effective mediators involved in the formation of inflammatory responses are cytokines. These immune mediators can be divided two groups as pro-inflammatory and anti-inflammatory (3). Anti-inflammatory cytokines are regulatory molecules that work in coordination with pro-inflammatory cytokines and have immunosuppressive effects on various markers. In this study, the efficacy of anti-inflammatory mediators TGF-β, IL-4, IL-10, in FMS was discussed. IL-10 is an anti-inflammatory cytokine that controls the expression of several pro-inflammatory mediators (IL-1, IL-6, TNF-α etc.). In recent studies, IL-10 and IL-4 levels were found to be low in the blood of patients with chronic widespread pain, and it has been thought that these cytokines may have a prominent function in patients with chronic pain (4). TGF-β can be found in different regions such as peripheral ganglia, choroid plexus, and meninges, which are the basic elements of the nervous system (5). Its main function is to suppress cytokine release by inhibiting macrophage and T cell activity (6). It also participates in the regulation of nitric oxide (NO) production in macrophages. NO has an important action in the functioning of neuropathic pain pathways (7). In recent studies, it is thought that TGF-β with anti-inflammatory activity and other agents that stimulate this cytokine can be shown as therapeutic targets in cases related to neuropathic pain. On the other hand, there are many new perspectives on molecular markers that have critical assignments in the development and treatment of chronic pain. One of them is the immunoregulatory activity of vitamin D. The anti-inflammatory potential of vitamin

CITATION
Ellergezen P, Alp A, Cavun S. Vitamin D, VDR and VDBP Levels Correlate with Anti-inflammatory Cytokine Profile in FMS Patients. Med Records. 2023;5(1):24-8. DOI: 10.37990/medr.1131305

Received: 18.06.2022 **Accepted:** 31.08.2022 **Published:** 03.01.2023
Corresponding Author: Pınar Ellergezen, Uludag University Faculty of Medicine, Department of Medical Pharmacology, Bursa, Türkiye **E-mail**: pinarhiz@gmail.com

D suggests a remarkable relationship with the immune system (8). Vitamin D acts through intracellular receptors and works with its associated connectors. Especially vitamin D receptor (VDR), and vitamin D binding protein (VDBP) are major contributors to vitamin D activation. VDR is expressed in various tissues in the body and involved in events such as regulation of gene transcription and calcium transport (9). Many immune cell types can express VDRs on their surfaces. In this way, the VDR has a significant function in the orchestration of immune reactions (10). VDBP is the main transport molecule for all vitamin D metabolites, and is involved in immune regulation by binding to the surface of leukocytes and activating the complement system (11). Recent studies on FMS have been suggested that vitamin D concentrations are generally lower in patients than in healthy individuals, and this may be associated with neuropathic pain (12,13). On the other hand, studies on chronic pain reveal that one of the most important factors underlying FMS and similar diseases is immunological parameters (14). The fundamental goal of this study is to evaluate whether there is any connection between vitamin D and anti-inflammatory cytokine profile in FMS patients and to research the therapeutic use of this condition in a case of chronic widespread pain.

MATERIAL AND METHOD

Study Groups

This study was accepted by the Uludağ University Faculty of Medicine Ethics Committee and an informed permission form was gotten from whole individuals. A total of 55 female people, including 25 healthy controls and 30 individuals with FMS, took part in this research. The patients were known to have FMS in the last 1 year and were determined according to American College of Rheumatology (ACR) diagnostic standards. All FMS patients were included in the study without using any FDA-approved fibromyalgia medication for at least 2 weeks. In addition, patients with autoimmune disease, inflammatory disease, infectious disease, cancer and those receiving anti-inflammatory drug therapy were not included in the research. In the healthy group, people who did not have any chronic diseases and did not use any medication were included.

Serum samples of patients

Blood samples were taken into sterile tubes of 20 ml and was centrifuged at 3000 x g for 10 minutes to get serum of patients. Collected samples were transferred to eppendorf tubes and stored at -80°C until the date of use.

Quantification of vitamin D and cytokine levels

Vitamin D, VDR, VDBP, and cytokine (IL-4, IL-10, TGF-β) levels in samples were obtained by ELISA method. The ELISA kit procedure used in the study was applied (BT-LAB, Shangai, China). Each sample was measured twice and the results were calculated according to the standard curve. Validated detection limits were 0.23ng/ml for vitamin D, 2,51pmol/L for VDR, 5.41µg/ml for VDBP, 2,53 ng/L for IL-4, 2,59 pg/ml for IL-10 and 5,11 ng/L for TGF-β, respectively.

Statistical Analysis

The Shapiro Wilk test was used to examine how the variables were distributed. Continuous variables were presented as median and mean±standard deviation values and Mann Whitney U test was performed for comparison of FMS patients and healthy controls. Correlations between variables were evaluated by Spearman correlation test. ROC analysis method was applied to measure the range of values that can be predicted in diagnosing the disease. Statistical analysis was evaluated using SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY: IBM Corp.) and p value <0.05 was considered to indicate a statistically significant difference.

RESULTS

There was no notable difference in terms of mean age values in 25 healthy individuals (48.60±10.59 years old) and 30 FMS patients (50.93±9.92 years old) who participated in the study. Details showing vitamin D, VDR, VDBP, and cytokine concentrations are displayed in Table 1. Vitamin D concentration was obtained to be higher in the healthy group (18.06±8.41) than in the FMS patients (15.67±1.83) ($p<0.001$). It was observed that there was an important difference between the patient (120.83±22.06) and healthy (142.02±67.61) groups in terms of VDR values ($p=0.002$) and VDBP values were also different from each other in both groups; patients (310.77±33.68) and healthy individuals (368.03±188.92) ($p<0.001$). VDR and VDBP concentrations of the patients with FMS were lower than healthy group. All the levels of IL-4 (130.47±56.70), IL-10 (182.84±57.02) and TGF-β (547.28±252.27) were also reported to be higher in the healthy group compared to FMS patients (104.03±14.63, 156.83±20.97, 440.71±54.39, respectively) and there was a remarkable difference among the groups in terms of cytokine concentrations ($p=0.001$, $p<0.001$, $p<0.001$, respectively). In addition, a noticable correlation was detected between the values of vitamin D, VDR, VDBP, and IL-4, IL-10, TGF-β ($p<0.05$). As vitamin D, VDR and VDBP concentrations decrease, a decline is observed in IL-4, IL-10, TGF-β values (Table 2). According to the ROC analysis results, vitamin D, VDR, VDBP, and cytokine values were determind to be significant ($p<0.001$). In table 3, cut-off values for vitamin D, VDR, VDBP, and anti-inflammatory cytokines that can be predicted in patient diagnosis are demonstrated. It has been shown that people with or below these values can be diagnosed with FMS (Table 3).

DISCUSSION

In the recent study, we reported that the levels of vitamin D, VDR, and VDBP were correlate with anti-inflammatory cytokine profile (IL-4, IL-10, TGF-β) and all markers were higher in the healthy group than in the FMS patients ($p<0.05$).

Table 1. Values of the FMS patients and healthy controls

	Healthy Controls (n=25)	FMS Patients (n=30)	p-valuea
Vitamin-D	18.06(8.41)	15.67(1.83)	<0.001
VDR	142.02(67.61)	120.83(22.06)	0.002
VDBP	368.03(188.92)	310.77(33.68)	<0.001
IL-4	130.47(56.70)	104.03(14.63)	0.001
IL-10	182.84(57.02)	156.83(20.97)	<0.001
TGF-β	547.28(252.27)	440.71(54.39)	<0.001

FMS: Fibromyalgia Syndrome, VDR: Vitamin D receptor, VDBP. Vitamin D Binding Protein, TGF-β:transforming growth factor beta, IL: Interleukin
Data was presented as median(IQR). a:Mann-Whitney U Test p<0.05

Table 2. Comparison of vitamin D, VDR, VDBP and cytokine levels between the healthy controls and FMS patients

	Healthy Controls (n=25)						FMS Patients (n=30)						Total (n=55)					
	Vitamin-D		VDR		VDBP		Vitamin-D		VDR		VDBP		Vitamin-D		VDR		VDBP	
	rs	p	rs	p	rs	p	rs	p	rs	p	rs	p	rs	p	rs	p	rs	p
IL-4	0.90	**<0.001**	0.92	**<0.001**	0.82	**<0.001**	0.70	**<0.001**	0.82	**<0.001**	0.89	**<0.001**	0.86	**<0.001**	0.91	**<0.001**	0.87	**<0.001**
IL-10	0.87	**<0.001**	0.73	**<0.001**	0.71	**<0.001**	0.55	**<0.001**	0.71	**<0.001**	0.66	**<0.001**	0.82	**<0.001**	0.79	**<0.001**	0.77	**<0.001**
TGF-β		**<0.001**	0.94	**<0.001**	0.83	**<0.001**	0.89	**<0.001**	0.81	**<0.001**	0.85	**<0.001**	0.91	**<0.001**	0.90	**<0.001**	0.89	**<0.001**

FMS: Fibromyalgia Syndrome, VDR: Vitamin D receptor, VDBP. Vitamin D Binding Protein, TGF-β: transforming growth factor beta, IL: Interleukin
rs: Spearman correlation coefficient p<0.05

Table 3. ROC Analysis results

	AUC	p-value	Cut-off Value	Sensitivity	Specificity	PPV	NPV
Vitamin-D	0.799	**<0.001**	≤17.13	86,21	80	83,3	83,3
VDR	0.741	**<0.001**	≤120.83	51.72	96	93,7	63,2
VDBP	0.784	**<0.001**	≤319.07	68.97	88	87	71
TGF-β	0.821	**<0.001**	≤478.84	82.76	88	88.9	81.5
IL-10	0.786	**<0.001**	≤169.75	79.31	80	82.1	76.9
IL-4	0.763	**<0.001**	≤113.13	79.31	80	82.1	76.9

AUC: Area under the ROC curve, PPV:Positive predictive value, NPV:Negative predictive value

Current approaches to FMS have determined that the underlying causes of this disease are more than one. It is emphasized that one of these reasons may be immunological factors and attention should be paid to this issue. Although the mechanism of FMS has not been fully resolved, there are limited studies on the connection of the disease with the immune system. In current study, our main purpose was to obtain the concentrations of vitamin D and anti-inflammatory mediators in FMS patients and to research the link between them. In recent years, it has been discovered that there is a much wider area where vitamin D benefits beyond its known functions such as bone development, regulation of Ca level, and contribution of muscle functions. Additionally, there are several studies showing that vitamin D has a remarkable activity in the pathogenesis of FMS and pain related disorders (15,16). New approaches suggest that vitamin D has an active role in supporting neurological pathways, reducing inflammation and preventing the risk of many chronic diseases (17,18). Vitamin D shows its effectiveness by binding to VDR and modulates the immune system through vitamin D responsive elements (19). Besides, in current studies, it has been determined that the level of VDBP in the serum decreases in liver diseases, kidney-related disorders, and various trauma situations (20). In a study on vitamin D function pulmonary tuberculosis patients, it was shown that vitamin D increased IL-10 production by down-regulating Th1 and Th17 cytokines (21). In addition, it inhibits immune functions by suppressing the transcription factor nuclear factor kappa B (NF-κB) (22). Another possible mechanism is that vitamin D down-regulates toll-like receptor (TLR) expression by reducing pro-inflammatory cytokine release

(23). In line with these studies, it is thought that vitamin D has a suppressive effect on the activity of immune system cells through various inhibitory mechanisms (24). Clinical research on the link between chronic pain and vitamin D is limited. However, there are strong evidences to suggest that vitamin D has a potential role in pain-related conditions. In studies conducted, vitamin D levels were found to be related with symptoms such as headache, muscle-joint, chest and back pain, and were found to be low in patients with FMS (25). Besides, it has been determined that long-term vitamin D deficiency is associated with a weakened immune system and chronic inflammation. Inflammatory responses have a prominent effect in the formation of various pain pathways in the peripheral and central nervous systems. The major molecules involved in the occurance of these responses are cytokines. In addition to being effective in the regulation of immune responses, their physiological and pathological roles in inflammation are becoming increasingly important (26). The anti-inflammatory cytokines discussed in this study are generally those that have an inhibitory effect and act in the direction of suppressing the inflammatory response. Recent studies have shown that low blood concentrations of IL-10 and another anti-inflammatory cytokine, IL-4, are detected in patients with chronic widespread pain, which is a remarkable finding (27). In addition, studies have shown that IL-10 suppresses spinal-mediated pain facilitation in acutely administered animal models. It has also been reported that neuropathic pain can be prevented when spinal IL-10 is blocked (28). TGF-β inhibits macrophage and T cell cell activation thus suppresses cytokine release. It also antagonizes nitric oxide (NO) release in macrophages. NO has a prominent task in neuropathic pain pathways. This anti-feature of TGF-β, which affects cytokine release, suggests that it can be used in neuropathic pain therapy (29). In a study performed on microglia and astrocytes, vitamin D was found to upregulate TGF-β and IL-4 (30). Mahon et al. reported that vitamin D inhibited CD4+ Th2 cell proliferation in animal models by promoting IL-4 production and reducing pro-inflammatory cytokine expression (31). Additionally, it is known that very low doses of circulating vitamin D cause a decrease in Treg cells. However, the function of vitamin D in immune responses is quite complex and depends on the involvement of various factors such as innate immunity, brain pathways, gut microbiome. It has been observed that enriched Treg cells in patients with SLE increase the Th2 type immune response following long-term vitamin D supplementation (32). In this study, vitamin D, VDR and VDBP concentrations were detected to be lower in FMS patients than in healthy individuals, in correlation with the anti-inflammatory mediators IL-4, IL-10 and TGF-β. There are some limitations in this study. It was a single center research prevents the interpretation of the results throughout the country. In addition, the fact that it was studied only in women does not give an idea about the course of the disease in men. Some of the anti-inflammatory cytokines have been studied so further extended studies with other groups of anti-inflammatory cytokines may be done.

CONCLUSION

In conclusion, we would like to emphasize that this is the first study to correlate vitamin D, VDR, and VDBP levels with anti-inflammatory cytokine profile in patients with fibromyalgia. The results we obtained from the study suggested that there was a remarkable link between vitamin D and anti-inflammatory cytokine profile. This finding may help to understand the role of vitamin D and cytokines in pathological process of fibromyalgia and can be a guide for similar disease states.

Financial disclosures: *The authors received no support from any financial institution or organization for this study.*

Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *This study was accepted by the Uludağ University Faculty of Medicine Ethics Committee and an informed permission form was gotten from whole individuals.*

REFERENCES

1. Clauw DJ, Arnold LM, McCarberg BH. The science of fibromyalgia. Mayo Clin Proc. 2011;86:907–11.
2. Clauw DJ. Fibromyalgia: a clinical review. JAMA. 2014;311:1547–55.
3. Zhang JM, An J. Cytokines, Inflammation and Pain. Int Anesthesiol Clin. 2007;45:27–37.
4. Uceyler N, Valenza R, Stock M, et al. Reduced levels of antiinflammatory cytokines in patients with chronic widespread pain. Arthritis Rheum. 2006;54:2656–64.
5. Unsicker K, Flanders KC, Cissel DS, et al. Transforming growth factor beta isoforms in the adult rat central and peripheral nervous system. Neuroscience. 1991;44:613-25.
6. Roberts AB, Sporn MB. Physiological actions and clinical applications of transforming growth factor-beta (TGF-beta). Growth Factors. 1993;8:1–9.
7. Ding A, Nathan CF, Graycar J, et al. Macrophage deactivating factor and transforming growth factors-beta 1 -beta 2 and -beta 3 inhibit induction of macrophage nitrogen oxide synthesis by IFN-gamma. J Immunol. 1990;145:940–4.
8. Wang TT, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol. 2004;173:2909–12.
9. Fleet JC, Schoch RD. Molecular mechanisms for regulation of intestinal calcium absorption by vitamin d and other factors. Crit Rev Clin Lab Sci. 2010;47:181–95.
10. Provvedini DM, Tsoukas CD, Deftos LJ, Manolagas SC. 1,25-dihydroxyvitamin D3 receptors in human leukocytes. Science. 1983;221:1181-3.
11. Abdella NA, Mojiminiyi OA. Vitamin D-Binding protein clearance ratio is significantly associated with glycemic status and diabetes complications ina predominantly vitamin D-deficient population. J Diabetes Res. 2018, Article ID:6239158.

12. Makrani AH, Afshari M, Ghajar M et al. Vitamin D and fibromyalgia: a meta-analysis. Korean J Pain. 2017;30:250–7.

13. Baygutalp NK, Baygutalp F, Şeferoğlu B, Bakan E. The relation between serum vitamin D levels and clinical findings of fibromyalgia syndrome. Dicle Med J. 2014;41:446-50.

14. Marchand F, Peretti M, McMahon SB. Role of the Immune system in chronic pain. Nat Rev Neurosci. 2005;6:521–32.

15. Okyay R, Koçyigit B, Gürsoy S. Vitamin D levels in women with fibromyalgia and relationship between pain, tender point count and disease activity. Acta Med Mediterr. 2016;32:243–7.

16. Labeeb AA, Al-Sharaki DR. Detection of serum 25(OH)-vitamin D level in the serum of women with fibromyalgia syndrome and its relation to pain severity. Egypt Rheumatol Rehabil. 2015;42:196–200.

17. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357:266-81.

18. Brannon PM. Key questions in Vitamin D research. Scand J Clin Lab Invest. 2012;243:154–62.

19. Boonstra A, Barrat FJ, Crain C et al. 1alpha,25-Dihydroxyvitamin d3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. J Immunol. 2001;167:4974.

20. Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. Am J Clin Nutr. 2008;88:491–9.

21. Kumar NP, Gopinath V, Sridhar R et al. IL10 dependent suppression of type 1, type 2 and type 17 cytokines in active pulmonary tuberculosis. PLoS One. 2013;8:e59572.

22. Song Y, Hong J, Liu D et al. 1,25-dihydroxyvitamin D3 inhibits nuclear factor kappa B activation by stabilizing inhibitor IkappaBalpha via mRNA stability and reduced phosphorylation in passively sensitized human airway smooth muscle cells. Scand J Immunol. 2013;77:109.

23. Khoo AL, Chai LY, Koenen HJ et al. Vitamin D(3) down-regulates proinflammatory cytokine response to Mycobacterium tuberculosis through pattern recognition receptors while inducing protective cathelicidin production. Cytokine. 2011;55:294.

24. Harishankar M, Afsal K, Banurekha VV et al. 1,25-Dihydroxy vitamin D3 downregulates pro-inflammatory cytokine response in pulmonary tuberculosis. Int Immunopharmacol. 2014; 23:148.

25. Plotnikoff GA, Quigley JM. Prevalence of severe hypovitaminosis D in patients with persistent, nonspecific musculoskeletal pain. Mayo Clin Proc. 2003;78:1463–70.

26. Watkins LR, Milligan ED, Maier SF. Glial proinflammatory cytokines mediate exaggerated pain states: implications for clinical pain. Adv Exp Med Biol. 2003;521:1–21.

27. Uceyler N, Valenza R, Stock M, et al. Reduced levels of antiinflammatory cytokines in patients with chronic widespread pain. Arthritis Rheum. 2006;54:2656–64.

28. Milligan ED, Sloane EM, Langer SJ, et al. Controlling neuropathic pain by adeno-associated virus driven production of the anti-inflammatory cytokine, interleukin-10. Mol Pain. 2005;1:9.

29. Meller ST, Gebhart GF. Nitric oxide (NO) and nociceptive processing in the spinal cord. Pain. 1993;52:127–36.

30. Garcion E, Wion-Barbot N, Montero-Menei CN et al. New clues about vitamin D functions in the nervous system. Trends Endocrinol Metab. 2002;13:100–5.

31. Mahon BD, Wittke A, Weaver V, Cantorna MT. The targets of vitamin D depend on the differentiation and activation status of CD4 positive T cells. J Cell Biochem. 2003;89:922–32.

32. Piantoni S, Andreoli L, Scarsi M, et al. Phenotype modifications of T-cells and their shift toward a Th2 response in patients with systemic lupus erythematosus supplemented with different monthly regimens of vitamin D. Lupus. 2015;24:490–8.