

Low Vitamin D Levels can Effect the Balance of Immune Mediators in Fibromyalgia Syndrome

Objective: To investigate the relationship between vitamin D levels and inflammatory cytokine levels in patients with Fibromyalgia Syndrome (FMS).

Materials and Methods: 29 women with FMS who were diagnosed according to American College of Rheumatology (ACR) 2010 fibromyalgia diagnostic criteria and 25 healthy women as the control group was included in the study. Serum levels of vitamin D, Vitamin D Receptor (VDR) and Vitamin D Binding Protein (VDBP), and inflammatory cytokines (IL-2, IL-4, IL-6, IL-12, IFN- γ) were analyzed using by ELISA method. Widespread body pain, fatigue, morning stiffness, cognitive symptoms, somatic symptoms, Fibromyalgia Impact Questionnaire (FIQ) scores were evaluated in patients with FMS.

Results: Vitamin D, VDR, and VDBP levels were found to be higher in the healthy individuals compared to the patients with FMS ($p < 0.001$, $p < 0.002$, $p < 0.001$, respectively). Correspondingly, pro-inflammatory (IL-2, IL-12, IFN- γ), anti-inflammatory (IL-4), and both pro and anti-inflammatory (IL-6) cytokine levels of the control group were higher than the patients with FMS ($p < 0.001$, $p < 0.006$, $p < 0.004$, $p < 0.001$, $p < 0.049$, respectively).

Conclusion: Low vitamin D levels in FMS may negatively affect the release of inflammatory cytokines and this functional relationship may be in the etiology of this chronic pain disorder

Keywords: Cytokines • fibromyalgia • immune system • vitamin D

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Introduction

Fibromyalgia (FM) is a chronic pain disorder characterized by abnormal central sensory processing of pain signals which is thought to be related to interactions between different systems in the body [1]. Pain pathways associated with cortical, immunological, hormonal, and neuronal changes in chronic pain, are potentially also influenced by vitamin D levels [2]. Severe conditions such as fatigue, somnolence, hyperalgesia, cognitive dysfunctions, allodynia, anxiety, and depression are known symptoms of Fibromyalgia Syndrome (FMS) and recent studies have reported that the same symptoms are observed when vitamin D levels are low [3].

The main functional background for vitamin D deficiency and regulation of pain processing is based on the presence of Vitamin D Receptor (VDR) and Vitamin D Binding Protein (VDBP) in many areas of the Human Central

Nervous System (CNS) [4]. In the rat model, Vitamin D Binding Protein (VDBP) has been found in axonal projections in the lateral hypothalamus [5]. The presence of VDR and VDBP in the hypothalamus is suggested as the mechanism by which vitamin D deficiency is implicated in the pathophysiology of chronic pain in FM [6]. The etiology of FMS is still not clear but recent studies have highlighted the role of interactions between the central nervous system, the hypothalamic-pituitary-adrenal axis, and the immune system in the pathogenesis of FMS [7-10].

The expressions of immune mediators such as cytokines have been linked to the pathogenesis and traits of FMS. Cytokines are messengers of the immune system that are involved in many physiological and pathological processes. Pro-inflammatory cytokines such as interleukin (IL)-6, IL-8, IL-17, tumor necrosis factor-alpha (TNF- α), and interferon-gamma (IFN- γ) promote inflammation, while anti-inflammatory cytokines, such as IL-4, IL-10, and IL-

13, reduce inflammation and promote healing [11,12]. An imbalance or excessive production of these cytokines has been linked to different diseases and symptoms, ranging from fever to death and atherosclerosis to cancer [11-17]. Dysregulation of pro-inflammatory cytokines has also been linked to sickness behavior, such as pain perception, cognitive impairment, depression, and other neurologically related effects [11,12,18]. It has been further suggested to use serum levels of cytokines as potential biomarkers in diseases, such as in rheumatoid arthritis [19].

This study aims to determine serum vitamin D and inflammatory cytokine levels in patients with FMS and investigate the relationship between these factors. Studies on this subject are limited and our study is the first to be conducted. Additionally, the correlations between vitamin D, cytokine levels, and clinical findings of FMS were evaluated by Fibromyalgia Impact Questionnaire (FIQ).

Materials and Methods

Patients and clinical assessment

In this study, 29 female FMS patients were recruited from the University outpatient clinic. All of the patients enrolled in the study were screened and identified based on the 2010 American College of Rheumatology Criteria (ACR) for the diagnosis of FMS [20]. ACR 2010 criteria include 2 items; the Widespread Pain Index (WPI), a 0-19 count of the number of body regions reported as painful by the patient, a 0-3 severity Scale of Symptoms (SS) 0-31 points that are characteristic of fibromyalgia: fatigue, non-refreshed sleep and cognitive problems [20].

The Fibromyalgia Impact Questionnaire (FIQ) is an instrument developed to assess the current health status of women with fibromyalgia syndrome in research settings. It gives information about the functional and clinical status of the patient; work, depression, anxiety, morning tiredness, pain, stiffness, fatigue, and well-being over the past week. The FIQ appears to be a relatively useful measure of disease impact in patients with fibromyalgia. Overall, the FIQ has demonstrated good responsiveness to change in clinical studies and a good correlation with similar questionnaires, including the Health Assessment Questionnaire (HAQ), Arthritis Impact Measurement Scale (AIMS), and 36-item short-form survey (SF-36) [21].

The healthy control group consisted of 25 female volunteers. Patients who had infectious or autoimmune diseases were excluded from the study. The healthy controls had no clinical findings suggestive of FMS or any other inflammatory disease. The study protocol was approved by the Ethical Committee of the University and was supported by the Scientific Investigation Unit of the Faculty with the project number KUAP (T)-2020/2.

Blood Sampling

Venous blood samples were collected into Vacutainer tubes

and allowed to clot at room temperature for 30 minutes. The coagulated blood was centrifuged for 10 minutes at $3.000 \times g$; the serum was aliquoted into sterile tubes and stored frozen (-80°C) till the date of analysis.

Enzyme-linked immunosorbent assay (ELISA)

The concentrations of vitamin D, VDR, VDBP, and inflammatory cytokines (IL-2, IL-4, IL-6, IL-12, IFN- γ) were measured using a commercial ELISA kit (BT-LAB, Shanghai, China) following the manufacturer's instructions. Tests were performed in duplicate for each sample and the vitamin D, VDR, VDBP, and cytokine concentrations were calculated using standard curves. The sensitivity of the cytokine detection system of the assays was 0.23 ng/ml for vitamin D, 2,51 pmol/L for VDR, 5.41 $\mu\text{g/ml}$ for VDBP, 2.51 ng/L for IL-2, 2.53 ng/L for IL-4, 1.03 ng/L for IL-6, 0.13 ng/L for IL-12 and 0.49 ng/ml for IFN- γ , respectively).

Statistical Analysis

A priori power analysis was performed based upon findings of the study conducted by Behm et al. [22]. The sample size calculation was based on the mean (\pm sd) "IL-6" value. "IL-6" values were found to be 2799 (\pm 4182) in the control group (n=91) and 276 (\pm 437) in the patient's group (n=110) for a total of 201 participants. Using the large effect size ($d=0.85$) total of 46 participants were estimated for a power of 0.80 and alpha of 0.05. Power analysis was performed under GPower 3.1 (<http://www.gpower.hhu.de/>). Shapiro Wilk test was used for assessing whether the variables follow normal distribution or not. Continuous variables were presented as median (IQR) and mean \pm standard deviation values. According to the normality test results, Mann Whitney U test was used in comparison between two groups. Correlations between continuous variables were examined by correlation analysis and Spearman correlation coefficients were calculated. Multiple linear regression analysis was performed to estimate vitamin-D. Variables are included in the multiple linear regression model by using enter method. The variables found to be significant in the model were determined as independent variables. Multiple linear regression model was found to be significant ($p<0.001$). SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY: IBM Corp.) was used for statistical analysis, and a p-value <0.05 was considered statistically significant.

Results

The study included 25 healthy controls (47.74 ± 10.89 years old) and 29 patients with FMS (51.52 ± 9.55 years old). No statistically significant difference was found between the groups in terms of mean age ($p=0.183$).

Table 1 contains laboratory variables; vitamin-D, VDR, VDBP, IFN- γ , IL-2, IL-4, IL-6, IL-12 in FMS and control groups. Vitamin-D, VDR and VDBP levels of healthy controls were higher than patients with FMS ($p<0.05$). Furthermore, pro-inflammatory cytokine levels (IL-2, IL-12, IFN- γ) in the healthy individuals were also significantly higher than the patients with FMS. Likewise,

anti-inflammatory cytokine IL-4 level ($p < 0.001$) and both pro-/anti-inflammatory cytokine IL-6 level ($p < 0.049$) were higher in the control group (Figure 1).

In Table 2, serum levels of vitamin D, VDR, VDBP, and pro-and anti-inflammatory cytokines with respect to self-reported symptoms such as Widespread Intensity (WSI), tiredness, waking unrefreshed, cognitive/ somatic symptoms, symptom severity total score, and FIQ total score. There was a significant relationship between the age and WSI total score ($p = 0.037$). WSI total score was positively correlated with increasing age. There was no significant correlation between the serum levels of vitamin D, VDR, VDBP, pro- (IFN- γ , IL-2, IL-12), anti- (IL-4), and both pro/anti (IL-6) inflammatory cytokines and reported symptoms.

The relationship between serum vitamin D, VDR, VDBP and IL-2, IL-4, IL-6, IL-12, IFN- γ levels are presented in Table 3. There was a positive correlation between vitamin D, VDR, VDBP and IL-2, IL-4, IL-6, IL-12, IFN- γ concentrations ($p < 0.05$). As the vitamin D, VDR, VDBP levels increased, an increase in IL-2, IL-4, IL-6, IL-12, and IFN- γ values was also observed.

For the Vitamin-D value, the cut-off point value that can be predicted for the diagnosis of the patient was ≤ 17.13 and the area under the Receiver-Operating Characteristic (ROC) curve containing this cut-off point was 0.799 ($p < 0.001$). It has been shown that women with vitamin D ≤ 17.13 , VDR ≤ 120.83 , VDBP ≤ 319.07 , IFN- $\gamma \leq 42.28$, IL-2 ≤ 9.76 , IL-4 ≤ 113.13 , IL-6 ≤ 77.32 , and IL-12 ≤ 218.58 values can get sick (Table 4).

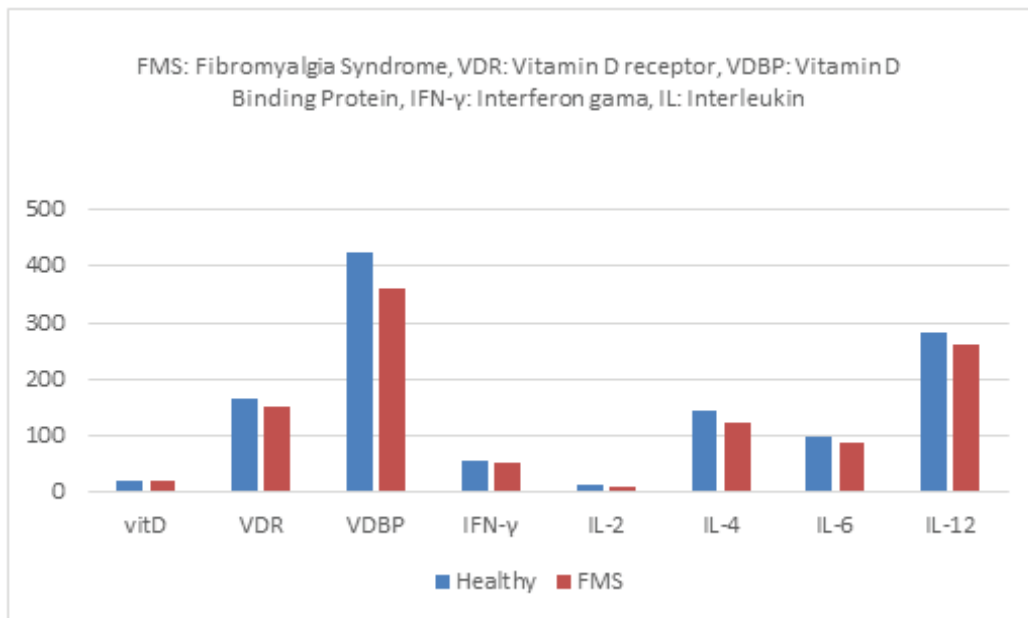


Figure 1: Comparison of healthy controls and FMS patients.

	Healthy Controls (n=25)	FMS Patients (n=29)	p-value ^a
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
IFN- γ	47,07(20,43)	43,06(7,85)	0,004
IL-2	10,17(4,76)	8,90(1,25)	0,001
IL-4	130,47(56,70)	104,03(14,63)	0,001
IL-6	82,39(39,67)	74,84(13,95)	0,049
IL-12	250,57(118,72)	218,58(32,98)	0,006

FMS: Fibromyalgia Syndrome, VDR: Vitamin D receptor, VDBP: Vitamin D Binding Protein, IFN- γ : Interferon gama, IL: Interleukin a: Mann-Whitney U Test $p < 0,05$

Table 2. Comparison of clinical findings and age, vitamin D, VDR, VDBP and inflammatory cytokines.

(n=29)	WPI		Fatigue		Waking Unfreshed		Cognitive Symptoms		Somatic Symptoms		Sypmtom Severity		FIQ	
	r _s	p	r _s	p	r _s	p	r _s	p	r _s	p	r _s	p	r _s	p
Age	0,39	0,037	-0,11	0,574	0,10	0,608	0,06	0,774	0,08	0,694	0,00	0,997	0,29	0,133
Vitamin-D	-0,06	0,776	0,14	0,467	0,28	0,140	0,02	0,929	-0,14	0,483	0,11	0,561	-0,07	0,702
VDR	0,07	0,719	0,03	0,866	0,20	0,294	0,02	0,911	0,07	0,701	0,13	0,487	-0,34	0,070
VDBP	0,05	0,791	0,09	0,651	0,32	0,086	-0,08	0,695	-0,06	0,756	0,10	0,606	-0,22	0,251
IFN-γ	0,07	0,712	0,13	0,515	0,31	0,098	-0,07	0,712	0,17	0,370	0,17	0,379	-0,25	0,185
IL-2	0,09	0,637	-0,12	0,537	-0,03	0,882	0,15	0,451	-0,26	0,175	-0,04	0,851	0,05	0,809
IL-4	0,03	0,862	0,04	0,859	0,19	0,321	-0,04	0,820	-0,01	0,950	0,10	0,622	-0,10	0,606
IL-6	0,01	0,950	-0,13	0,501	0,09	0,656	0,09	0,629	-0,07	0,731	0,04	0,845	-0,23	0,230
IL-12	0,07	0,732	0,04	0,838	0,21	0,277	0,03	0,896	0,07	0,724	0,14	0,474	-0,34	0,071

FMS: Fibromyalgia Syndrome, VDR: Vitamin D receptor, VDBP: Vitamin D Binding Protein, IFN-γ: Interferon gama, IL: Interleukin
rs: Spearman correlation coefficient p<0,05

Table 3. Comparison of vitamin D, VDR, VDBP and inflammatory cytokine levels between the healthy controls and FMS patients.

	Healthy Controls (n=25)						FMS Patients (n=29)						Total (n=54)					
	Vitamin-D		VDR		VDBP		Vitamin-D		VDR		VDBP		Vitamin-D		VDR		VDBP	
	r _s	p	r _s	p	r _s	p	r _s	p	r _s	p	r _s	p	r _s	p	r _s	p	r _s	p
IFN-γ	0,85	<0,001	0,87	<0,001	0,83	<0,001	0,67	<0,001	0,79	<0,001	0,84	<0,001	0,79	<0,001	0,85	<0,001	0,85	<0,001
IL-2	0,77	<0,001	0,78	<0,001	0,78	<0,001	0,71	<0,001	0,63	<0,001	0,66	<0,001	0,83	<0,001	0,83	<0,001	0,81	<0,001
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-6	0,90	<0,001	0,82	<0,001	0,81	<0,001	0,62	<0,001	0,81	<0,001	0,82	<0,001	0,76	<0,001	0,82	<0,001	0,82	<0,001
IL-12	0,80	<0,001	0,96	<0,001	0,74	<0,001	0,61	<0,001	1,00	<0,001	0,87	<0,001	0,76	<0,001	0,97	<0,001	0,79	<0,001

FMS: Fibromyalgia Syndrome, VDR: Vitamin D Receptor, VDBP: Vitamin D Binding Protein, IFN-γ: Interferon gama, IL: Interleukin
rs: Spearman correlation coefficient
p<0,05

Table 4. Results of ROC analyse.

	AUC	p-value	Cut-off Value	Sensitivity	Specificity	PPV	NPV
Vitamin-D	0,8	<0,001	≤17.13	86,21	80	83,3	83,3
VDR	0,74	<0,001	≤120.83	51.72	96	93,7	63,2
VDBP	0,78	<0,001	≤319.07	68.97	88	87	71
IFN-γ	0,73	<0,001	≤42.28	48.28	100	100	62.5
IL-2	0,75	<0,001	≤9.76	82.76	72	77.4	78.3
IL-4	0,76	<0,001	≤113.13	79.31	80	82.1	76.9
IL-6	0,66	0,039	≤77.32	72.41	60	67.7	65.2
IL-12	0,72	0,003	≤218.58	51.72	92	88.2	62.2

VDR: Vitamin D receptor, VDBP: Vitamin D Binding Protein, IFN-γ: Interferon gama, IL: Interleukin
AUC: Area Under the ROC Curve, PPV:Positive Predictive Value, NPV:Negative Predictive Value
p<0,05

Discussion

In the present study, we demonstrated that the levels of vitamin D, VDR, and VDBP were low in FMS patients and there was a significant difference between the healthy and patient groups (p<0.001, p<0.002, p<0.001, respectively).

The inflammatory cytokines (IL-2, IL-6, IL-12, IFN-γ) and anti-inflammatory cytokine IL-4 were also low in patients with FMS (p<0.001, p<0.001, 0.049, p<0.006, p<0.004, respectively) and we suggested that there may be a relationship between low vitamin D levels and impaired

immune response None of the laboratory variables were correlated with clinical variables.

FMS is a chronic syndrome with an increasing prevalence and characterized by widespread musculoskeletal pain in combination with different symptoms. Pain pathways involve interactions between different systems in the body. The immune system is one of them and cytokines are thought to play an important role in FMS. Recent studies revealed that patients with FMS have low levels of vitamin D [23,25]. Furthermore, it has been reported that vitamin D acts as an immune system regulator in FMS and is also associated with musculoskeletal pain [24].

FMS is more prevalent on women than men and it can develop at any age, especially more common between the ages of 30-55 [20,23]. Although the cause of FMS is not known exactly, it has been proposed that the FMS studies have reported that low vitamin D levels are associated with FMS [24] and literature findings on vitamin D levels in FMS patients show varying outcomes. In some of the studies, vitamin D levels were reported to be lower in FMS patients compared to healthy individuals [24-28]. On the contrary, there are studies that have not reported differences in serum levels of vitamin D between FMS patients and healthy individuals [29]. In this study, we found that vitamin D levels of healthy controls were higher than patients with FMS ($p < 0,001$). Likewise, VDR and VDBP levels were reported to be higher in healthy subjects ($p < 0,002$, $p < 0,001$, respectively). In addition, among the clinical findings, there was a significant difference only between WPI and age ($p < 0,037$). Findings about VDR and VDBP levels in patients with fibromyalgia are very limited, so our study is the first to associate VDR and VDBP with FMS as a consequence of the subclinical impairment of immunoregulation. On the other hand, in normal conditions, vitamin D level may be sufficient (>30 ng/mL), insufficient (20-30 ng/mL), deficient (<20 ng/L) or seriously deficient (<10 ng/mL) [20,30-32]. However, it may differ according to the genetic characteristics of the races, their eating habits, and the geographical features of their region. In our study, blood samples were collected from female adult individuals living in Bursa-Turkey. In a current study, vitamin D levels were analyzed retrospectively from the records of 11,734 adult subjects (9142 women, 2592 men) admitted to 24 family health centers located in different districts of Bursa to evaluate the vitamin D status and its seasonal variation in the adult population [33]. Vitamin D levels <20 ng/mL are more prominent in women, and decline in spring, in the adult population of Bursa [33]. Depending on these results, the low vitamin D levels in our study are actually consistent within itself, therefore, the cut-off level for vitamin D was accepted as 17 ng/ml for deficiency. In addition, significant differences were observed between the healthy individuals and the patients with FMS in serum levels of VDR and VDBP in our patient group also.

Although there are so many speculations about the etiology of FMS, one of the main theories is that cytokines may play a role both in the etiology of the disease and the intensity of the main symptoms [34,36]. The cytokines are important

mediators of the immune system that affect the formation and progression of the inflammation cascade. In this study, the relationship of pro-and anti-inflammatory cytokines with serum vitamin D levels was investigated in patients with FMS. The importance of vitamin D on the regulation of cells of the immune system has gained increased appreciation over the past decade with the discovery of the VDR and key vitamin D metabolizing enzymes expressed by cells of the immune system. Animal studies have supported a potential role for vitamin D in maintaining immune system balance. Pro-inflammatory cytokines are mediators of inflammatory pain, while anti-inflammatory tend to block it [36]. Different results have been obtained in studies on cytokines. IL-2 is a pro-inflammatory cytokine that has essential roles in key functions of the immune system. Wallace et al. found no significant differences between control and patient groups for IL-2 levels in FMS [37]. On the other hand, it was observed by Kapoor et al. that IL-2 levels were lower in FMS patients compared to healthy controls [38]. Similarly, FMS patients had less IL-2 level than healthy controls in our study. Another important cytokine IL-6 which has both pro and anti-inflammatory properties is an important acute phase reactant. According to Wallace et al. IL-6 may be associated with hyperalgesia, depression, stress, fatigue, and sympathetic nervous system activation [37]. On the other hand, most published studies on the role of IL-6, show no differences in plasma concentration of IL-6 from FMS patients compared to healthy women [37,39]. Conversely, recent studies have reported high levels of IL-6 in FMS patients [40-42]. IL-12 is a pro-inflammatory cytokine that induces the production of interferon- γ (IFN- γ) and forms a link between innate and adaptive immunity [43]. There are very limited studies of IL-12 in FMS patients. However, the association of IL-12 with FMS has not been described yet. In our study, IL-12 levels were lower in FMS patients than in healthy subjects. IFN- γ ; another pro-inflammatory cytokine in our study was found to be non significantly higher in the patients with FMS compared to healthy individuals [39]. In another study, a significant decrease was observed in IFN- γ levels in patients with FMS when compared to controls [38]. Likewise, IFN- γ levels of FMS patients were lower than healthy controls in our study. Furthermore, the last cytokine IL-4 has anti-inflammatory properties. Stürigill et al. detected a decrease in the concentration of IL-4 in the serum of patients with FMS compared to the healthy controls [44] and Kapoor et al. obtained low levels of IL-4 in FMS patients [38]. We also report similar results regarding the recent study with comparatively low serum IL-4 levels in the intervention group.

On the other hand, clinical research in the area of chronic pain and Vitamin D deficiency remains limited. Persistent pain is associated with Vitamin D-related bone demineralization, myopathy, and musculoskeletal pain. Pain pathways associated with cortical, immunological, hormonal, and neuronal changes are potentially also influenced by vitamin D levels. Additionally, long-term vitamin D deficiencies have been linked to a weakened immune system and chronic inflammation [45].

FMS is a chronic pain syndrome and progresses with chronic inflammation observed in the musculoskeletal system. Furthermore, spinal sensitization is one of the most important symptoms of FMS and involves interactions between several neuronal and glial cells. The glial cells in the spinal cord play a significant role by responding to various cytokines and neurochemicals released from infiltrating macrophages, neutrophils, and from the damaged peripheral nerve fibers. This response can be considered as part of protective measures, akin to acute pain, and vitamin D is thought to have a role in regulating the synthesis of cytokines [46]. For example, vitamin D is known to affect a number of inflammatory pathways associated with the development and persistence of chronic pain and vitamin D upregulates transforming growth factor-beta 1 (TGF- β 1) and IL-4 found in astrocytes and microglia [47-55]. Indirectly, TGF- β 1 has the ability to suppress the activity of various cytokines, namely, IFN- γ , TNF- α , and IL-2.

This study has several limitations; such as the small patient size and the absence of male patients. Anyway, there is no study in the literature conducted on the association between vitamin D, VDR, VDBP, and inflammatory cytokines in FMS. In our study, differently, a significant decrease in all cytokines was observed in parallel with vitamin D, VDR, and VDBP levels in patients with FMS. Normally, pro-inflammatory cytokines work in opposition to anti-inflammatory cytokines but in our study, a conflicting outcome is obtained. The levels of both pro-and anti-inflammatory cytokines were low in patients with FMS. Therefore, we suggest that low vitamin D levels may lead to an insensitivity by elevating immune stimulus threshold or impairment in the balance of inflammation cascade.

Conclusion

In conclusion, FMS is a disease that can be diagnosed with clinical symptoms with no diagnostic laboratory findings. Corroborating this fact, clinical symptoms were not correlated with low vitamin D or cytokine levels in our study. Our study supports the hypothesis that vitamin D levels are lower in FMS patients. Low serum vitamin D levels and their relation to impaired immune response may help us to understand etiopathogenesis. In addition to this, the positive correlation of VDR and VDBP levels with vitamin D can provide a versatile perspective for studies on this subject. We focused on vitamin D and cytokine levels in adult women who appeared to be healthy or having FMS. It would be of great interest to do similar studies on patients with other chronic diseases and inflammatory conditions. The study further requires detailed multicenter investigations with large sample sizes and understand the underlying mechanism for the development of various symptoms in patients with FMS.

Authors Contributions

P.E. wrote the manuscript. A.A. and S.Ç. revised the manuscript.

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Declaration of Competing Interest

The authors declare no conflict of interest regarding the publication of this paper.

Executive summary

Objective: To investigate the relationship between vitamin D levels and inflammatory cytokine levels in patients with Fibromyalgia Syndrome (FMS).

Materials and Methods: 29 women with FMS who were diagnosed according to American College of Rheumatology (ACR) 2010 fibromyalgia diagnostic criteria and 25 healthy women as the control group was included in the study. Serum levels of vitamin D, Vitamin D Receptor (VDR) and Vitamin D Binding Protein (VDBP), and inflammatory cytokines (IL-2, IL-4, IL-6, IL-12, IFN- γ) were analyzed using by ELISA method. Widespread body pain, fatigue, morning stiffness, cognitive symptoms, somatic symptoms, Fibromyalgia Impact Questionnaire (FIQ) scores were evaluated in patients with FMS.

Results: Vitamin D, VDR, and VDBP levels were found to be higher in the healthy individuals compared to the patients with FMS ($p < 0.001$, $p < 0.002$, $p < 0.001$, respectively). Correspondingly, pro-inflammatory (IL-2, IL-12, IFN- γ), anti-inflammatory (IL-4), and both pro and anti-inflammatory (IL-6) cytokine levels of the control group were higher than the patients with FMS ($p < 0.001$, $p < 0.006$, $p < 0.004$, $p < 0.001$, $p < 0.049$, respectively).

Conclusion: Low vitamin D levels in FMS may negatively affect the release of inflammatory cytokines and this functional relationship may be in the etiology of this chronic pain disorder.

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