

MULTİPL SKLEROZ HASTALARINDA VİTAMİN D, PARATHORMON, CİNSİYET, İNTERLÖKİN 10 VE TÜMÖR NEKROZ FAKTÖR ALFA İLİŞKİSİ

VITAMIN D, PARATHORMONE, GENDER, INTERLEUKIN 10 AND TUMOR NECROSIS ALPHA RELATIONSHIP IN MULTIPLE SCLEROSIS PATIENTS

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ÖZ

Giriş: Güneş ışığına maruziyetin fazla olduğu bölgelerde Multipl Skleroz'un daha az sıklıkla görülmesinde D vitamininin rolü olduğu düşünülmektedir. Vitamin D'nin klinik özellikler ile ilişkisi ve bu etkisinin kadın hastalarda daha belirgin olduğu görüşü tartışmalıdır.

Bu çalışmada relapsing-remitting multipl skleroz hastalarında vitamin D, sitokin ve cinsiyet arasındaki ilişki araştırıldı.

Gereç ve yöntem: Çalışmaya remisyon döneminde olan 74 Relapsing-remitting multiple skleroz hastası alındı. Kontrol grubu benzer yaş ve cinsiyette 50 sağlıklı bireyden oluşturuldu.

Hasta ve kontrol gruplarında serum vitamin D, interlökin-10, tümör nekroz faktör- α ve parathormon düzeyleri ölçüldü. Vitamin D'nin cinsiyet ve klinik özellikler ile ilişkisi araştırıldı.

Bulgular: Ortalama vitamin D düzeyi hasta grubunda 14.4 ng/ml, kontrol grubunda 16 ng/ml olarak saptandı. Hasta ve kontrol gruplarında vitamin D ($p:0.206$), Interlökin-10 ($p:0.081$), tümör nekroz faktör- α ($p: 0,340$) ve parathormon ($p:0.107$) düzeyleri benzer bulundu. Klinik ağırlaştıkça vitamin D düzeyinin düşme eğiliminde olduğu gözlemlendi ise de bu ilişki istatistiksel anlamlılığa ulaşmadı. Kadınlarda vitamin D düzeyi daha düşük saptandı.

Sonuç: Hem hasta hem de kontrol grubunda vitamin D düzeyi düşüktü ve vitamin D klinik özellikler ile ilişkili bulunmadı.

SUMMARY

Introduction: Vitamin D is thought to have a role on multiple sclerosis hence MS cases are less frequent in the areas where exposure to sunlight is high. The idea that vitamin D is related to clinical features and this effect is more prominent in female patients is controversial. In this study, the relationship between vitamin D levels, pro and anti-inflammatory cytokine levels and gender in relapsing-remitting multiple sclerosis patients were investigated.

Materials and Methods: 74 patients with relapsing-remitting multiple sclerosis who were in the remission period were included in the study. The control group consisted of 50 healthy individuals with similar age and gender. Serum vitamin D, parathormone, interleukin-10 and tumor necrosis factor- α levels were tested in the patient and control groups. The relationship of vitamin D with gender, clinical features and cytokine levels was also investigated.

Results: The mean vitamin D level was 14.4 ng/ml in the patient group and 16 ng/ml in the control group. The levels of vitamin D ($p: 0,206$), Interleukin-10 ($p: 0,081$), Tumor necrosis factor- α ($p: 0,340$) and parathormone ($p: 0,107$) were similar in the patient and control groups. Although vitamin D values tended to decrease as clinical severity was worsening, this relationship was not statistically significant. Vitamin D levels found to be lower in female.

Conclusion: Vitamin D levels were low in both the patient and control group. There was no correlation between vitamin D levels and clinical features.

INTRODUCTION

It is known that the incidence of multiple sclerosis (MS) is higher in areas with low exposure to sunlight. It is thought that the relationship between the risk of MS development and sunlight may be mediated by vitamin D hence the only hormone that requires sunlight for synthesis is vitamin D.

Since it has been shown that hypovitaminosis D has a positive correlation with increased risk of MS, rapid progression, and high disease activity in brain MRI and patients receiving vitamin D were shown to have a slower progression and less frequently relapsing and developing new brain lesions the relationship between MS and vitamin D has gained importance (1-5).

In addition to the role of Vitamin D in calcium metabolism, it also has antiinflammatory and immunomodulatory effects. Immunological effects of vitamin D include the inhibition of the conversion of monocytes to antigen-presenting dendritic cells, the inhibition of T-helper1, T-helper 17 and B cell proliferation, inhibiting the expression of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF- α) and increasing the expression of anti-inflammatory cytokines such as T-helper 2 phenotyped T-regulator activity and interleukin (IL) -10 (3-5). IL-10 and TNF- α that mediates the immunomodulatory effect of Vitamin D, are the key regulatory factors of immun system and have an important role on MS pathophysiology. In recent years, it has been reported that vitamin D deficiency plays a role in increased incidence MS in women (6). But this relationship is still not clarified.

In this study vitamin D, IL-10, TNF- α and parathormone (PTH) levels were measured in

Relapsing Remitting MS patients and the relationship between vitamin D, cytokine and clinical features were investigated.

MATERIALS AND METHODS

Patients and Design

Seventy-four patients with definite RRMS diagnosis according to McDonald criterias were included in this study. Patients were in remission period.

Patients with an autoimmune disease other than MS, active infection, psychiatric disorders and patients using corticosteroids, nonsteroidal anti-inflammatory drugs, antidepressants or drugs effecting calcium and/or vitamin D metabolism in the last 3 months were not included in the study. Patients neurological examinations were performed and Expanded Disability Status scale (EDSS) scores were calculated. Disease duration was recorded. Hemogram, sedimentation biochemical tests were done.

The control group consisted of 50 healthy individuals with similar age and gender.

Biochemical analysis

Serum samples of the patient and control groups were taken in June and July and stored at -85 celsius after centrifugation at 5000 rpm for 5 minutes until the measurement day for vitamin D, IL-10 and TNF- α levels. PTH levels were measured immediately after the serum sample was taken.

Vitamin D level was measured with LIAISON brand 25(OH) vitamin D total Assay (310600) kit. After the measurements <20 ng/mL vitamin D level accepted as deficiency, 21-29 ng/mL

accepted as insufficiency and ≥ 30 ng/mL accepted as normal.

IL-10 level was measured using the Solid Phase Enzyme Amplified Sensitivity Immunoassay (EASIA) method using the Biosource IL-10-EASIA kit (KAP1321) on a DIASORIN brand, fully automated Elisa instrument. The lowest value that the device could measure was $0.2 \mu\text{mol/L}$ and the normal value was $<3.3 \mu\text{mol/L}$.

TNF- α was measured via EAISA method in a Siemens brand ELISA device. Normal values of TNF- α ranged from 0 to 8.1 pg/mL .

Normal PTH level was in between $10\text{-}69 \text{ pg/mL}$.

Vitamin D, IL-10, TNF- α and PTH levels were compared in between patient and control groups. The relationship of vitamin D with clinical features and cytokine levels was investigated.

Statistical analysis

Statistical evaluations are performed with SPSS program, version 15,0. The demographic and clinical characteristics and laboratory findings of the patient and control groups were determined by descriptive analyzes and frequency analyzes. Fisher's exact test for IL-10, Mann-Whitney U test for TNF- α , UniAnova test for vitamin D were used; correlation analysis performed with Pearson and Spearman tests. A value of $p < 0,05$ accepted as significant.

RESULTS

Characteristics of patient and control groups

The mean age of the patients was 36.60 ± 9.01 (20-56) and the mean age of the control group was 35.04 ± 8.55 (21-58). 64.9% of the patient

group and 64.5% of the control group were female. Patient and control group were similar in terms of gender ($p: 0.210$) and age ($p: 0.970$) distribution.

EDSS score of the patients was in between 0-3.5, the mean EDSS of patients was $1.45 \pm 0,74$ and the median was 1,00. The mean duration of disease was $9.92 \pm 6,2$ (min-max 1-27) years. The mean number of relapses were 4.63 ± 2.6 (min-max: 1-17).

Vitamin D, IL-10, TNF- α and PTH serum levels

The vitamin D, IL-10, TNF- α and PTH results of the patient and control groups are given in Table 1

The mean level of vitamin D was 14.4 ± 6.5 (2.0-28.9) ng/ml in patients and 16.0 ± 7.4 (2.0-31.9) ng/ml in the control group and there was no statistically significant difference in between two groups ($p: 0,206$).

Vitamin D levels were <20 ng/mL in 60 patients (81.1%), 20-29 ng/mL in 14 patients (18.9%) and in the control group; <20 ng/mL in 32 people (64.0%), 20-29 ng / mL in 17 people (34.0%) and ≥ 30 ng / mL in 1 person (2.0%).

IL-10 levels of all individuals both in the patient and control groups were below the upper limit of the normal range which was $3.3 \mu\text{mol/L}$, and no difference was found between the two groups. However, IL-10 levels were not further evaluated because IL-10 levels of all the individuals in the control group were below the device threshold. The mean TNF- α levels were 17.9 ± 11.3 (7.4-54.0) pg/ml in the patient group, 15.8 ± 34.7 (6.7-120.0) in the control group. Although it was higher in the patient group, this difference was not statistically significant ($p: 0.340$).

Table 1. The level of vitamin D, IL-10, TNF- α and PTH in the patients and control groups

	Patients		Control		p
Vitamin D mean \pm SD (min-max) ng/ml	$14,4 \pm 6,5$ (2,0-28,9)		$16,0 \pm 7,4$ (2,0-31,9)		0,206
Interlökin-10	$<0,02 \mu\text{mol/L}$ n (%)	$\geq 0,02 \mu\text{mol/L}$ n (%)	$<0,02 \mu\text{mol/L}$ n(%)	$\geq 0,02 \mu\text{mol/L}$ n(%)	0,081
	69(93,2)	5(06,8)	50(100)	0(0)	
TNF- α mean \pm SD (min-max) pg/ml	$17,9 \pm 11,3$ (7,4-54,0)		$15,8 \pm 34,7$ (6,7-120,0)		0,340
Parathormon mean \pm SD (min-max) pg/ml	$54,0 \pm 27,8$ (4,9-132,0)		$61,8 \pm 28,3$ (18,6-176,0)		0,107

Mean PTH levels were 54.0 ± 27.8 (4.9-132.0) pg/ml in patient group and 61.8 ± 28.3 (18.6-176.0) pg/ml in control group and between the two groups there was no statistically significant difference was found (p: 0.107).

The relationship of Vitamin D level with gender, clinical and biochemical parameters

Mean vitamin D levels were 13.5 ± 7.0 (2.0-31.9) ng/mL in women and 17.9 ± 5.7 (7.9-28.7) ng/mL in men (Table 2). Vitamin D levels were not different between male and female in both patient and control groups. But when all the study group evaluated, it was found to be lower in females (p: 0.001).

Although there was a tendency to a negative relationship between the duration of the disease with Vitamin D (r: - 0.174, p: 0.138) and TNF- α levels (r: - 0.075, p: 0.528) this result did not reach to statistically significant value. Although Vitamin D levels were lower in the patients with high EDSS score, this difference was not found to be statistically significant (r: -0.110, p: 0.350).

TNF- α level (p: 0.784) was not associated with EDSS score. While there was no statistically significant correlation between vitamin D levels and TNF- α levels (r: 0.193, p: 0.100), there was a negative correlation between vitamin D and PTH levels (r: - 0.540, p<0.001).

DISCUSSION

The mean vitamin D level in our study was 14.4 ng/ml in the patient group and 16 ng/ml in the control group. Vitamin D level was found to be deficient in 81.1% of patients and insufficient in 18.9% of patients. In the control group, 64% had deficiency, 34% had insufficiency, vitamin D level was normal in only 1 patient (2%). Studies conducted in many countries have reported low vitamin D levels in most of the MS patients (7-

12). It is noteworthy that in the majority of these studies vitamin D deficiency was also frequently found in the control group. In studies, vitamin D deficiency (<20 ng/ml) was reported to be as high as 77% in the patient group and 71% in the control group (8-10). In the study by Karampaar et al. vitamin D levels were normal (> 30 ng/ml) in only 3.4% of the patients and 24.2% of the control group (9). In the study of Eskandari et al., Vitamin D levels were not normal (> 30 nmol/L) in any of the patients as in our study and vitamin D levels were normal in only 2.7% of the control group (7).

This result is supported by the fact that the studies in many countries have reported that vitamin D levels are very low in the normal population as well (7,13,14).

Possible causes of low vitamin D levels in both the patient and control groups are thought to be low exposure to sunlight, poor intake, air pollution, clothing habits, and vitamin D receptor gene polymorphism (7,10).

The effect of vitamin D on the disability of MS patients is contentious and not clear. In some studies, a negative correlation between vitamin D levels and EDSS score and disease activity was reported (12,15), but no correlation was found in between the others (7,9,16-18). In some studies, negative correlation was found between serum vitamin D level and EDSS score only in female patients (15, 19). Although vitamin D levels decreased as the EDSS score increased in our patients, this association did not reach statistical significance level. This result was thought to be due to the low disability scores of our patients, but in one study vitamin D levels were found to be associated with disability grade only in fully ambulatory patients (20). In addition, our study showed a tendency to decrease in vitamin D

Table 2. The level of vitamin D in the patients and control groups

	Female		Male		P
	n	Vitamin D (ng/ml) mean \pm SD (min-max)	n	Vitamin D (ng/ml) mean \pm SD (min-max) ng/ml)	
Patients	48	12,9 \pm 6,6 (2,0-28,9)	26	17,2 \pm 5,4 (9,2-27,1)	0,201
Controls	32	14,3 \pm 7,6 (2,0-31,9)	18	19,0 \pm 6,1 (7,9-28,7)	0,201
Total	80	13,5 \pm 7,0 (2,0-31,9)	44	17,9 \pm 5,7 (7,9-28,7)	0,001

levels as the duration of the disease was prolonged. But this data was not found to be statistically significant either. In many studies, vitamin D levels were not associated with the duration of the disease (7,16,21) while negative correlations were found in others (12).

The relationship between vitamin D level and gender was also evaluated in our study. Although vitamin D levels were lower in our female patients, this finding did not reach statistically significant level. However, when we evaluate the whole group together, vitamin D levels were lower in women.

It is reported that probably due to the synergistic effect between beta-estradiol and vitamin D the immunomodulator effect of vitamin D is more pronounced in women (22). However, there is no consensus on the relationship between vitamin D and gender in the literature, and studies have reported lower (7,18), similar (23) even higher (24) vitamin D levels in female MS patients compared to male patients .

IL-10 and TNF- α , which are known to play important roles in MS pathophysiology, are thought to be the most important cytokines that

mediate the role of vitamin D on the immune system (21). In our study, IL-10 and TNF- α levels were not different from the control group and there was no relationship between TNF- α level and EDSS and duration of illness .

In our study, vitamin D levels were negatively associated with PTH levels as expected (25).

The relationship between vitamin D level and TNF- α level is not clear. In some studies, it is reported that TNF- α levels were decreasing in MS patients who were also on having vitamin D supplementation (26,27) , on the contrary in the other studies TNF- α and vitamin D levels were not associated (28). The reason we did not find a relationship between vitamin D level and TNF- α level in our study may be due to the low level of vitamin D in most of our group. Experimental studies have also reported that the immunomodulator effect of vitamin D occurs only in the hyperphysiological concentration, which makes hypercalcemia in human (29).

In conclusion, this study concluded that vitamin D levels are low in MS patients and vitamin D is not related to clinical features

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