# A simple proof of vitamin D deficiency and inflammation relation: single center study, over two thousand patients

## Vitamin D eksikliği ve inflamasyon ilişkisinin basit bir kanıtı: tek merkezli çalışma, iki binin üzerinde hasta

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**Abstract**

**Aim:** Vitamin D deficiency is a very common condition in the world. Although vitamin D appears to be effective mainly on calcium and bone metabolism, it is an important molecule that also affects immune functions and inflammation. In our study, we tried to evaluate the contribution of vitamin D deficiency to inflammation by examining the platelet indices of patients with low and normal vitamin D levels.

**Methods:** Healthy patients who applied to internal medicine outpatient clinics of our hospital for routine control in 2017 were included in our study. Patients are included in the study by following needed criteria; creatinine <1.3 mg/dl, hemoglobine >12 gr/dl, vitamin B12 >150 pg/dl, red cell distribution width <14 fL, thyroid stimulating hormone (TSH) between 1-5 IU/L and C-reactive protein (CRP) <10 mg/dl. Patients were divided into two groups, those with vitamin D less than 10 ng/ml as the study group and those above 30 ng/ml as the control group.

**Results:** A total of 2179 patients, 644 male and 1535 female, were included in the study. 797 patients constituted the study group (27.1% male), and 1382 patients constituted the control group (31% male). When the two groups were compared in terms of platelet indices, we found that mean platelet volume (MPV) and plateletcrit (PCT) were significantly higher in the study group (p<0.001, p<0.001).

**Conclusion:** In our study, we found that MPV and PCT values increased significantly with vitamin D deficiency. We attributed this result to the absence of the anti-inflammatory activity of vitamin D. As a result, treating a patient's vitamin D deficiency can also protect patients from cardiovascular or autoimmune diseases through the anti-inflammatory effect of vitamin D.

**Keywords:** Inflammation; mean platelet volume; platelet; Vitamin D; vitamin D deficiency

**Öz**

**Amaç:** D vitamini eksikliği dünyada çok yaygın bir durumdur. D vitamini esas olarak kalsiyum ve kemik metabolizması üzerinde etkili gibi görünse de, bağışıklık fonksiyonlarını ve inflamasyonu da etkileyen önemli bir moleküldür. Çalışmamızda D vitamini düzeyi düşük ve normal olan hastaların platelet indekslerini inceleyerek D vitamini eksikliğinin inflamasyona etkisini değerlendirmeye çalıştık.

**Yöntemler:** Çalışmamıza 2017 yılında hastanemiz dahiliye polikliniklerine rutin kontrol için başvuran sağlıklı hastalar dahil edildi. Hastalardan tetkik sonuçları şu kriterleri sağlayanlar çalışmaya dahil edildi; kreatinin <1,3 mg/dl, hemoglobin >12 gr/dl, vitamin B12 >150 pg/dl, kırmızı hücre dağılım genişliği (RDW) <14 fL, tiroit uyarıcı hormon (TSH) 1-5 IU/L arası ve C-reaktif protein (CRP) <10 mg/dl. Hastalar D vitamini 10 ng/ml'nin altında olanlar çalışma grubu ve 30 ng/ml'nin üzerinde olanlar kontrol grubu olarak 2 gruba ayrıldı.

**Bulgular:** Çalışmaya 644 erkek, 1535 kadın olmak üzere toplam 2179 hasta dahil edildi. 797 hasta çalışma grubunu (%27,1 erkek), 1382 hasta kontrol grubunu (%31 erkek) oluşturdu. Platelet indeksleri açısından iki grup karşılaştırıldığında çalışma grubunda ortalama trombosit hacmi (MPV) ve plateletokrit (PCT) anlamlı olarak daha yüksek olduğu saptandı (p<0,001, p<0,001).

**Sonuç:** Çalışmamızda, MPV ve PCT değerlerinin d vitamini eksikliğinde önemli ölçüde arttığını tespit ettik. Sonuç olarak, bir hastanın d vitamini eksikliğinin tedavi edilmesi, d vitamininin anti-inflamatuar etkisi ile hastayı kardiyovasküler veya otoimmün hastalıklardan da koruyabilir.

**Anahtar Sözcükler:** İnflamasyon; platelet; ortalama trombosit hacmi; Vitamin D; Vitamin D eksikliği

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## INTRODUCTION

Vitamin D is a hormone with two forms; vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Foods or supplements are sources of vitamin D2. Vitamin D3 is produced by sun exposure on the skin from pre-vitamin D3. Vitamin D deficiency is a very common condition worldwide (1-3). Although the main role of this vitamin in our body is calcium homeostasis and bone mineralization (4), also has important effects on cell differentiation, immunity, anti-coagulation, and prevention of inflammation (5,6,8).

Vitamin D deficient patients with hypertension can have benefits from supplementation of this vitamin in the regulation of hypertension (9). This antihypertensive effect of vitamin D is associated with renoprotective effects, suppression of renin–angiotensin–aldosterone system and direct effects on vascular cells. Patients with vitamin D deficiency with heart failure have a shorter life expectancy. Vitamin D replacement improves the duration of life in these patients by the same mechanisms that provide its antihypertensive effect (10). Insufficient level of vitamin D is also associated with cognitive impairment, especially in the elderly population (11).

The conditions pointed out above can be related to the immunomodulatory effects of vitamin D (12). In addition, proinflammatory cytokines (ie. IL6, TNF-α) increase by deficiency of vitamin D. After supplementation, cytokine levels decrease back (12).

At this point, we can mention about platelets and platelet indices (mean platelet volume [MPV] – plateletcrit [PCT] – platelet distribution width [PDW]), which is an indicator of inflammation. Platelets are anucleated small cells that originate from megakaryocytes. Although the main role of these cells is in hemostasis, they also affect the atherosclerotic process, immune functions, and inflammation (13, 14, 15, 16). Inflammatory conditions increase the size of platelets. MPV shows us the size and function of platelets (17).

MPV also can be considered as an indicator of platelet activation. Large platelets with high MPV measurements are more reactive and thrombogenic, they can easily aggregate into the tissue, carry and express more cytokines (18). For that reason, higher MPV levels are associated with ischemic cardiovascular diseases, hypertension, and diabetes (19, 20, 21).

The other two platelet indices are PDW and PCT. Inflammation affects platelet size and MPV, platelets are produced in different sizes and PDW is affected by platelet size variation. The PCT value is calculated from platelet count and MPV (22). In the same way, a higher level of MPV affects PCT value. As a result, all three platelet indices are affected by inflammation.

Many studies examined the relation between vitamin D deficiency and MPV levels. In this study, we aimed to investigate this relationship with a huge population. We also evaluated other platelet indices such as PCT and PDW to increase the evidence level of the study.

## MATERIAL AND METHODS

This is a retrospective study that includes internal medicine outpatient clinic patients. All of the study participants were healthy routine control subjects and were screened from the hospital information management system. The present study includes the exact one-year population (1.1.2017-31.12.2017) of outpatient clinics to avoid seasonal changes in vitamin D. This study was approved by Clinical Research Ethics Committee of Şişli Hamidiye Etfal Training and Research Hospital (date: 28.08.2018, decision no: 2093).

Patients between the age of 18-80 without any chronic illnesses are included in the study by following needed criteria; creatinine <1.3 mg/dl, hemoglobin>12 gr/dl, B12 >150 pg/dl, RDW <14 fL, TSH between 1-5 IU/L and CRP <10 mg/dl. These criteria are used to exclude pathologies that can affect platelet indices.

Patients are divided into two groups according to vitamin D levels. Those with vitamin D levels <10 ng/ml constituted the vitamin D deficient group and those with >30 ng/ml constituted the control group. International guidelines agree that a vitamin D level of less than 10 ng/ml is severe vitamin D deficiency, while a level of more than 25 ng/ml is optimal. (23). In this study we enrolled subjects with a vitamin D level lower than 10 ng/ml and higher than 30 ng/ml just because of to see the exact effect of vitamin D deficiency on platelet indices.

In the laboratory, hemogram is studied in Mindray BC6800 (Shenzhen Mindray Bio-Medical Electronics Co. China) device, TSH, 25OH vitamin D, and B12 are

**Table 1.** Sex, age and vitamin D distribution of the groups

| | | Vitamin D deficient group | | Control group | | |
|---|---|---|---|---|---|---|
| | | n (797) | % | n (1382) | % | p |
| Gender | Male | 216 | 27.1% | 428 | 31% | ***0.000**** |
| | Female | 581 | 72.9% | 954 | 69% | ***0.000**** |
| | | mean±SD | min-max | mean±SD | min-max | p |
| Age | | 44.2±15.4 | 18-84 | 53.4±15.8 | 18-85 | ***0.000***** |
| Vitamin D (ng/ml) | | 7.49±1.69 | 0-9.9 | 42.2±15 | 30-162.4 | ***0.000***** |

max: Maximum, min: Minimum, n: Number, SD: Standard deviation
*Chi square test, **Independent t test

**Table 2.** Comparison of groups in terms of laboratory results

| | Vitamin D deficient group | | | Control group | | | |
|---|---|---|---|---|---|---|---|
| | mean±SD | min-max | median | mean±SD | min-max | median | p |
| Hgb* (gr/dl) | 13.37±0.82 | 12.1-17.5 | 13.2 | 13.49±0.78 | 12.1-16.5 | 13.4 | 0.209 |
| RDW* (fL) | 12.97±0.49 | 11.09-13.9 | 13.0 | 13.03±0.52 | 10.85-13.9 | 13.1 | ***0.000*** |
| MPV* (fL) | 10.56±0.96 | 7.8-13.4 | 10.5 | 10.28±1.07 | 7.3-13.8 | 10.3 | ***0.000*** |
| PCT* (fL) | 0.28±0.06 | 0.07-0.47 | 0.3 | 0.27±0.06 | 0.09-0.5 | 0.3 | ***0.000*** |
| PDW* (fL) | 13.56±2.06 | 9-21.9 | 13.2 | 14.16±2.09 | 9.1-22.7 | 14.3 | ***0.000*** |
| CRP** (mg/dl) | 3.68±1.9 | 0.33-9.9 | 3.0 | 3.24±1.89 | 0-9.96 | 3 | ***0.000*** |
| TSH** (IU/L) | 2.21±0.93 | 1.01-4.88 | 2.0 | 2.28±0.91 | 1.01-4.91 | 2.1 | 0.225 |
| Cre.* (mg/dl) | 0.68±0.12 | 0.37-1.11 | 0.7 | 0.72±0.13 | 0.34-1.29 | 0.7 | ***0.000*** |
| B12** (pg/dl) | 336.5±156.9 | 150.3-1752 | 304.6 | 424.3±212.7 | 152-1708 | 371.6 | ***0.000*** |

Cre: creatinin, CRP: C reactive protein, Hgb: Hemoglobin, max: Maximum, min: Minimum, n: Number, MPV: Mean platelet volüme, PCT: plateletcrit, PDW: Platelet distibution width, RDW: Reticulocyte disribution width, SD: Standard deviation, TSH: Thyriod stimulating hormone.
* Independent t test, **Mann Whitney-U testi

studied in Beckman Coulter DXI800 (Beckman Coulter, Inc. California, USA) device, and CRP, creatinine are studied in Roche Cobas 8000 (601 series) device.

## Statistical Analyses

In the power analysis performed for the study, the minimum number of patients to be included was calculated as 262, based on an effect size of 0.2 in the t-test, by assuming the first type error at the 0.05 level and the second type error at the 0.95 level. Statistical Package for the Social Sciences package program, version 15.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Descriptive statistics were; numbers and percentages for categorical variables, mean, standard deviation, minimum, and maximum for numerical variables. Comparisons of numerical variables in two independent groups were made using the Mann-Whitney U test since numerical variables

did not meet the normal distribution condition. The ratios in the groups were compared by Chi-Square Analysis. Relationships between numerical variables were analyzed using Spearman Correlation Analysis since parametric test conditions were not met. Determining factors were determined by Linear Regression Analysis. The statistical significance level of alpha was accepted as $p < 0.05$.

## RESULTS

A total of 2179 patients were included in the study. 797 patients constituted the vitamin D deficient group and the remaining 1382 patients constituted the control group with adequate vitamin D levels. The distribution of both groups according to gender, age, and vitamin D levels were given in Table 1. When two groups were compared in terms of platelet indices, in the vitamin D deficient group MPV levels were significantly higher than the control group ($p < 0.001$). Also PCT levels were significantly high in the Vitamin D deficient group than the control group ($p < 0.001$). PDW which is the last platelet index that we evaluated was significantly low in the Vitamin D deficient group ($p < 0.001$). These comparisons are also given in **Table 2.**

## DISCUSSION AND CONCLUSION

The present study aimed to evaluate the relationship between vitamin D deficiency and inflammation over platelet indices. Results showed that vitamin D deficiency affect platelet indices. The absence of the anti-inflammatory effect of vitamin D leads to the formation of platelets that are larger and more prone to aggregation, resulting in increased MPV and other platelet indices.

In a similar study, Park et al. evaluated the inverse relation between vitamin D levels and platelet indices in Korean adults (24). In their study, they showed that platelet count and also MPV levels were higher in the Vitamin D deficient group. They linked this inverse relationship to the anti-thrombogenic and anti-inflammatory effects of vitamin D.

Sivritepe et al. showed that in their study decreasing vitamin D levels increases cardiovascular risk (25). They showed that cardiovascular risk as assessed by the Framingham's scale increases with decreasing vitamin D levels. They attributed this effect to the adverse effects of myocyte hypertrophy and interstitial fibrosis that occur in vitamin D deficiency on cardiac remodeling.

In another study, Kebapçılar et al. evaluated primary ovarian insufficiency (POI) patients with vitamin D deficiency (26). They found that vitamin D deficiency has an important role in POI patients and is also associated with coagulation which is an independent effect of age and BMI. They accepted elevated levels of D-dimer, white blood cell, MPV, and prothrombin time as indicators of risk factors for thrombosis. Eventually, this study also confirms the same association between vitamin D deficiency and inflammation.

In a study that included pregnant subjects, Gür et al. investigated the relationship between MPV and vitamin D deficiency in gestational diabetes mellitus (GDM) (27). They suggested that a low serum vitamin D level leads to a decrease in β-cell function and insulin sensitivity in pancreatic β-cells. On the other hand, the author stated that, due to the decreased anti-inflammatory effect vitamin D deficiency in pregnant women with GDM can increase cardiovascular and thrombotic risks.

Our study has shown that the vitamin D deficient group is younger than the adequate vitamin D group. We think that older people giving more care about their health and because of that, they apply to medical centers for routine tests and as a result of this vitamin D deficiency is determined and treated. The limitation here is that it is not known whether the patients included in the study had received vitamin D treatment before. The other limitation that may affect the results is the sex difference in the vitamin D-deficient group. In the results of our study, we saw that most of the vitamin D-deficient group members were female. We believe that as a result of traditional and/or religious wearing styles, Turkish women are more prone to vitamin D deficiency because more body surface area is covered.

When the results are evaluated, it is seen that there is a significant difference between the two groups in terms of B12, creatinine, RDW, and CRP. However, since all of the patients were selected from patients without B12 deficiency, renal failure, normal CRP

values, and anemia, it is thought that this did not affect the results of the study, but we believe that a study with matched groups in terms of these parameters will eliminate the doubts.

Another striking negative aspect of the study is that the number of the control group was higher than the vitamin D-deficient group. We believe that this was due to the addition of B12, hemoglobin, and RDW levels to the inclusion criteria. Because we think that many patients with vitamin D deficiency have anemia or B12 deficiency.

The present study showed that vitamin D deficiency increased inflammation. This study evaluated three different platelet indices in demonstrating this relationship and two of them showed a significant increase. Vitamin D replacement may protect patients from conditions that may occur secondary to increased inflammation caused by deficiency. Future studies with groups without the limitations mentioned above will provide clearer results.

## Conflict-of-interest and financial disclosure

The authors declare that they have no conflict of interest to disclose. The authors also declare that they did not receive any financial support for the study.

## REFERENCES

1. Adams JS, Hewison M. Update in vitamin D. J Clin Endocrinol Metab. 2010;95:471-8.
2. Holick MF. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc. 2006;81:353-73.
3. Heaney RP. Vitamin D in health and disease. Clin J Am Soc Nephrol . 2008;3:1535-41.
4. Anderson PH, Turner AG, Morris HA. Vitamin D actions to regulate calcium and skeletal homeostasis. Clin Biochem. 2012;45(12):880-6.
5. Bikle DD. Vitamin D regulation of immune function. Vitam Horm. 2011;86:1-21.
6. Samuel S, Sitrin MD. Vitamin D's role in cell proliferation and differentiation. Nutr Rev. 2008;66(10 Suppl 2):S116-S124.
7. Koyama T, Hirosawa S. Anticoagulant effects of synthetic retinoids and activated vitamin D3. Semin Thromb Hemost. 1998;24(3):217-26.
8. Krishnan AV, Feldman D. Mechanisms of the anti-cancer and anti-inflammatory actions of vitamin D. Annu Rev Pharmacol Toxicol. 2011;51:311-36.
9. Pilz S, Tomaschitz A, Ritz E, Pieber TR. Vitamin D status and arterial hypertension: a systematic review. Nat Rev Cardiol. 2009;6(10):621-30.
10. Gotsman I, Shauer A, Zwas DR, et al. Vitamin D deficiency is a predictor of reduced survival in patients with heart failure; vitamin D supplementation improves outcome. Eur J Heart Fail. 2012;14(4):357-66.
11. Llewellyn DJ, Lang IA, Langa KM, Melzer D. Vitamin D and cognitive impairment in the elderly U.S. population. J Gerontol A Biol Sci Med Sci. 2011;66(1):59-65.
12. Di Rosa M, Malaguarnera M, Nicoletti F, Malaguarnera L. Vitamin D3: a helpful immuno-modulator. Immunology. 2011;134(2):123-39.
13. Wang Y, Andrews M, Yang Y, et al. Platelets in thrombosis and hemostasis: old topic with new mechanisms. Cardiovasc Hematol Disord Drug Targets. 2012;12(2):126-32.
14. Li N. CD4+ T cells in atherosclerosis: regulation by platelets. Thromb Haemost. 2013;109(6):980-90.
15. Semple JW, Freedman J. Platelets and innate immunity. Cell Mol Life Sci . 2010;67(4):499-511.
16. Rondina MT, Weyrich AS, Zimmerman GA. Platelets as cellular effectors of inflammation in vascular diseases. Circ Res. 2013;112(11):1506-19.
17. Davì G, Patrono C. Platelet activation and atherothrombosis. N Engl J Med. 2007;357(24):2482-94.
18. Park Y, Schoene N, Harris W. Mean platelet volume as an indicator of platelet activation: methodological issues. Platelets. 2002;13(5-6):301-6.
19. Khode V, Sindhur J, Kanbur D, Ruikar K, Nallulwar S. Mean platelet volume and other platelet volume indices in patients with stable coronary artery disease and acute myocardial infarction: A case control study. J Cardiovasc Dis Res. 2012;3(4):272-5.
20. Bulur S, Önder HI, Aslantas Y, et al. Relation between indices of end-organ damage and mean platelet volume in hypertensive patients. Blood Coagul Fibrinolysis. 2012;23(5):367-9.
21. Kodiatte TA, Manikyam UK, Rao SB, et al. Mean platelet volume in Type 2 diabetes mellitus. J Lab Physicians. 2012;4(1):5-9.
22. Lombarts AJ, Koevoet AL, Leijnse B. Basic principles and problems of haemocytometry. Ann Clin Biochem. 1986;23 ( Pt 4):390-404.
23. Kennel KA, Drake MT, Hurley DL. Vitamin D deficiency in adults: when to test and how to treat. Mayo Clin Proc. 2010;85(8):752-8.
24. Park YC, Kim J, Seo MS, Hong SW, Cho ES, Kim JK. Inverse relationship between vitamin D levels and platelet

indices in Korean adults. Hematology. 2017;22(10):623-9.
25. Sivritepe R, Basat S, Ortaboz D. Association of vitamin D status and the risk of cardiovascular disease as assessed by various cardiovascular risk scoring systems in patients with type 2 diabetes mellitus. Aging Male. 2019;22(2):156-62.
26. Kebapcilar AG, Kulaksizoglu M, Ipekci SH, et al. Relationship between mean platelet volume and low-grade systemic coagulation with vitamin D deficiency in primary ovarian insufficiency. Arch Gynecol Obstet. 2013;288(1):207-12.
27. Gur EB, Karadeniz M, Genc M, et al. Relationship between mean platelet volume and vitamin D deficiency in gestational diabetes mellitus. Arch Endocrinol Metab. 2015;59(5):448-54.