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Vitamin D Level in Patients with Chronic Lymphocytic Leukemia and Relationship Between Rai Stage

Kronik Lenfositik Lösemide Tanı Esnasında Vitamin D Seviyesi ve Rai Evresi Arasındaki İlişki

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ABSTRACT

Background: We aimed to compare the serum 25 hydroxy vitamin D [25(OH)D] levels at the time of diagnosis of patients with chronic lymphocytic leukemia (CLL) with the healthy control group. We also examined its association with disease prognosis using the Rai stage.

Materials and Methods: One hundred and twenty-six healthy control group and 126 CLL patients whose 25(OH)D levels were measured at the time of diagnosis were examined. Serum 25(OH)D levels of CLL patients and control groups were compared. In addition, CLL patients were divided into five groups as Rai stage 0, 1, 2, 3 and 4 and compared in terms of serum 25(OH)D levels among themselves.

Results: The mean age of CLL patients at diagnosis was 65.1 (±11.5) years. 59% (n=75) of the patients were male and 41% (n=51) were female. The mean 25(OH)D levels of the CLL patients and the control group were 18.4 ng/mL (±8.83) and 27.7 (±11.6) ng/mL, respectively (p<0.001). There was no statistically significant difference between the two groups in terms of age and sex. In terms of 25(OH)D levels, a statistically significant difference was found in the comparison of the Rai stage-0 group and Rai stage-2 group (p=0.002), Rai stage-0 group, and Rai stage-4 group (p=0.004).

Conclusion: Serum 25(OH)D deficiency may be an effective modifiable risk factor in the etiology and progression of CLL. More studies are needed to elucidate the relationship between CLL and vitamin D. We believe that our study will lead to more comprehensive studies to be carried out in the future.

Keywords: Vitamin D, chronic lymphocytic leukemia, prognosis

ÖZ

Amaç: Bu çalışmanın amacı kronik lenfositer lösemi (KLL) tanılı hastalarda tanı anındaki serum 25 hidroksi vitamin D [25(OH)D] düzeyini sağlıklı kontrol grubuyla karşılaştırmak ve hastalık prognozuyla ilişkini incelemektir.

Gereç ve Yöntemler: Tanı esnasında 25(OH)D düzeyi ölçülen 126 KLL hastası ve 126 sağlıklı kontrol grubu incelendi. KLL hastaları ile kontrol grubunun serum 25(OH)D değerleri karşılaştırıldı. Ayrıca KLL hastaları Rai evre 0, 1, 2, 3 ve 4 olarak beş gruba ayrılarak kendi aralarında ikişerli olarak serum 25(OH)D değerleri açısından karşılaştırıldı.

Bulgular: KLL hastalarının tanı anında mean yaşı 65,1 (±11,5) yıl idi. Hastaların %59'u (n=75) erkeklerden, %41'i (n=51) kadınlardan oluşmaktaydı. KLL hastalarının 25(OH)D değeri mean 18,4 ng/mL (±8,83) iken sağlıklı kontrol grubunun 27,7 (±11,6) ng/mL olarak saptandı (p<0,001). Her iki grup arasında yaş ve cinsiyet açısından istatistiksel olarak anlamlı fark saptanmadı. Hastaların tanı anında Rai evresine göre 25(OH)D karşılaştırıldığında gruplar arasında istatistiksel olarak anlamlı fark tespit edildi. Rai evre 0 grubunun mean serum 25(OH)D seviyesi Rai evre 2 grubundan (22 ng/mL vs 14.2 ng/mL, p=0.002) ve Rai evre 4 grubundan (22 ng/mL vs. 12.6 ng/mL, p=0.004) istatistiksel olarak anlamlı derecede yüksek saptandı.

Sonuç: Serum 25(OH)D düşüklüğü KLL etiyolojisinde ve progresyonunda etkili bir değiştirilebilir risk faktörü olabilir. KLL ve D vitamini arasındaki ilişkiyi aydınlatmak için daha fazla çalışmaya ihtiyaç vardır. Çalışmamızın gelecekte yapılacak daha kapsamlı çalışmalara ışık tutacağına inanıyoruz.

Anahtar Kelimeler: D vitamini, kronik lenfositik lösemi, prognoz

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Introduction

Chronic lymphocytic leukemia (CLL) is a neoplasia that occurs with a monoclonal increase of mature B lymphocytes. It is the verdienst prevalent adult leukemia and accounts for 25-35% of leukemias in the United States (1). The prevalence of the disease varies by race and geographical location. There is a higher incidence among white Americans compared to African Americans or Asian Pacific islanders (2,3). The presence of occupational or environmental risk factors predisposing to CLL is not known.

Very few nutritious foods contain vitamin D. The most important source of vitamins in humans is their synthesis in the skin. Vitamin D undergoes 25 and 1 hydroxylation in hepatocytes and kidney, respectively, and is eventually converted to its functional form, 1.25-dihydroxy vitamin D. The most accurate indicator of serum vitamin D concentration is the measurement of 25(OH)D. The serum vitamin D level in humans varies according to the geographical location of their residence and the average amount of time they are exposed to sunlight.

Several studies have focused on the effects of vitamin D deficiency on malignant diseases. Its benefit in cancer prevention and control has been reported in solid malignancies such as breast, colon, and prostate cancers (4,5,6). An inverse relationship was found between 25(OH)D and the development of colon cancer and adenoma (7,8). There are limited publications on the relationship between hematopoietic system malignancy and 25(OH)D. It has been shown that 25(OH)D deficiency is common in acute myeloid leukemia, and higher vitamin concentrations in these patients are associated with better outcomes in treatment (9). In addition, it has been reported that the survival of Hodgkin lymphoma (HL) patients with vitamin D deficiency at the time of diagnosis is poor (10).

In our study, we compared the 25(OH)D concentrations measured at the time of diagnosis in CLL patients with the control group (CG). We also aimed to examine whether serum 25(OH)D differs according to the Rai stage in CLL.

Material and Methods

Study Population

The information of patients diagnosed with CLL in the Hematology Clinic of University of Health Sciences Türkiye, Hamidiye Faculty of Medicine; İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital, between 2014 and 2022 was retrospectively analyzed from the hospital registry system and patient follow-up files. Among the patients whose 25(OH)D levels were measured at the time of CLL diagnosis, those who did not receive vitamin D replacement therapy were included in the study. Age, sex, serum 25(OH)D concentration, and Rai stage of the patients were recorded. A CG was determined to compare the 25(OH)D concentrations of CLL patients with the population without CLL. The CG was selected from patients who did not have any malignant disease and had not received vitamin D replacement in the last 6 months, and who applied to the internal medicine outpatient clinic for a control examination. In addition, the age, gender, and 25(OH)D concentrations of the CG were recorded.

Serum 25(OH)D levels of CLL patients and the CG were compared. Vitamin levels of CLL patients at different Rai stages were compared to research the relationship between vitamin and disease prognostic factors.

Serum 25(OH)D tests are routinely checked at the time of diagnosis of all patients with hematological malignancies, and vitamin D replacement therapy is applied to those with a deficiency in our center. Therefore, no extra blood was taken from the patients for the study.

Rai staging system was calculated using a complete blood count and physical examination findings (11). As a result of staging, the patients were categorized as Rai stages 0, 1, 2, 3, and 4. Serum 25(OH)D was measured in Roche Cobas e801 device with electrochemiluminescence immunoassay technique.

The study was approved by the University of Health Sciences Türkiye Hamidiye Faculty of Medicine (04.11.2022, decision number: 24/26). Since the study was retrospective, written consent was not obtained from the participants.

Statistical Analysis

We used the Kolmogorov-Smirnov test for the normal distribution of data. The results were reported as mean ± standard deviation for normally distributed continuous variables. Median and interquartile range were used for non-normally distributed variables, and frequency and percentage were used for categorical variables. We used the Student's t-test for the pairwise comparison of the data with normal distribution, and the ANOVA test (non-parametric) for continuous data with the abnormal distribution. A p-value of <0.05 was accepted as statistically significant. We used SPSS 20.0 statistical package for the analyses.

Results

The data of 214 patients diagnosed with CLL in our clinic between 2014-2022 were obtained. Sixty-one patients were excluded from the study because they had received vitamin D replacement at the time of diagnosis, and 27 patients were excluded because 25(OH)D tests were not performed. As a result, 126 newly diagnosed CLL patients were included in our study.

The mean age of CLL patients at diagnosis was 65.1 (±11.5) years. Fifty-nine percent (n=75) of CLL patients were male and 41% (n=51) were female. The Rai stage of CLL patients at the time of diagnosis is shown in Table 1. The mean 25(OH)D level of CLL patients was 18.4 ng/mL (±8.83), while it was 27.7 (±11.6) ng/mL in the CG (p<0.001) (Figure 1). Patients and CG were similar in terms of age and gender (Table 1).

The 25(OH)D distribution of the patients according to the Rai stage at the time of diagnosis is shown in Table 2. Benforoni correction was applied in the pairwise comparison between the groups. In terms of 25(OH)D levels, a statistically significant difference was found in the comparison of the Rai stage-0 group and Rai stage-2 group (p=0.002), Rai stage-0 group and Rai stage-4 group (p=0.004) (Table 3).

Of the entire study population, 142 were male and 110 were female. The mean serum 25(OH)D concentrations were 22.5 ng/mL (±10.8) in men and 23.8 ng/mL (±11.8) in women. When the whole group was evaluated, there was no difference between the genders in terms of 25(OH)D (p=0.362).

Discussion

At present, cytogenetic and molecular markers are used to determine the prognosis and to guide the treatment of CLL. The main ones are TP53 mutation and IGHV gene mutation status (23,24). In many parts of the world, especially in low-income countries, there are difficulties in accessing cytogenetic and molecular analyzes due to their high cost. For this reason, low-cost and easily accessible markers may be attractive to some researchers.

In addition to serum calcium and skeletal homeostasis, vitamin D has functions in many places, such as regulation of cell proliferation, apoptosis, immune system, tumor metastasis, and angiogenesis (12,13). It has been previously reported that vitamin D deficiency elevates the risk of solid and hematological malignancies (4,5,6,9). Vitamin D

Table 1. Characteristics of patients and control group

	CLL patients, n=126	Control group, n=126	p
Age, y, mean (SD), 95% CI	65.1 (±11.5)	64.1 (±12.9)	0.115
Sex, n (%) Male Female	 75 (59%) 51 (41%)	 67 (53%) 59 (47%)	0.31
Rai stage at diagnosis, n (%) 0 I II III IV	 39 (31.0%) 28 (22.2%) 37 (29.4%) 6 (4.8%) 16 (12.7%)		
25(OH)D levels, ng/mL Mean (SD)	18.4 (±8.83)	27.7 (±11.6)	<0.001

SD: Standard deviation, CI: Confidence interval, CLL: Chronic lymphocytic leukemia

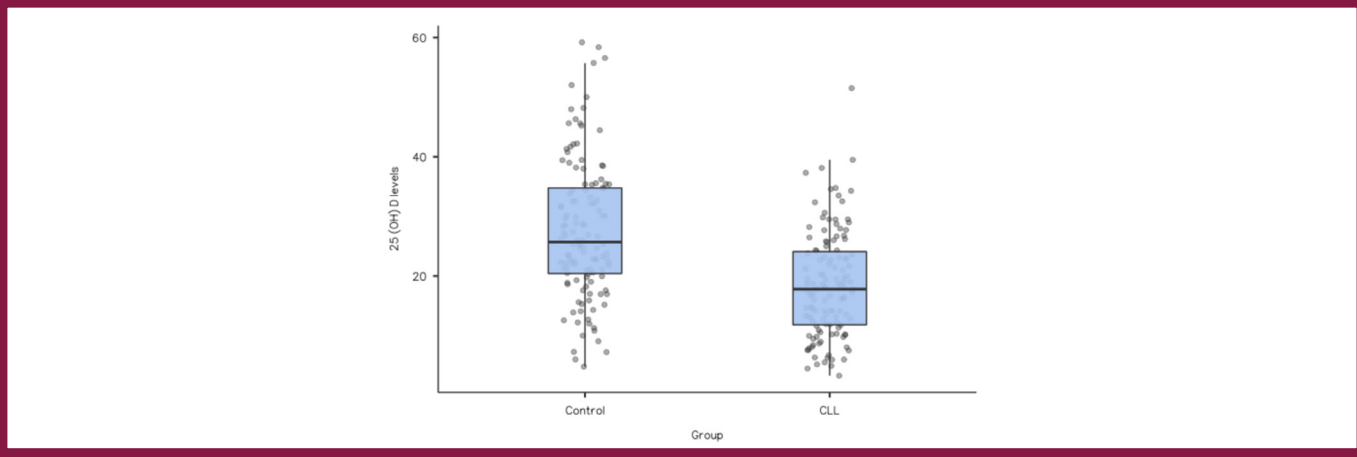

Figure 1. Mean 25(OH)D comparison of CLL and control group
CLL: Chronic lymphocytic leukemia

deficiency not only affects tumor formation but may also affect tumor growth and progression (14). *In vitro* studies have demonstrated that vitamin D has a direct antitumor effect against leukemia and lymphoma cells. These studies demonstrated antiproliferative activity in non-HL, HL, and multiple myeloma, and induction of apoptosis in B-cell CLL (10,15,16,17).

In our study, we showed that serum 25(OH)D concentration in CLL patients was lower than in CG. In addition, we found that the 25(OH)D concentration in Rai stage 2 and stage 4 groups was statistically significantly lower than stage 0 in CLL patients.

There are very few studies in the literature on the relationship between CLL and vitamin D. Dehghani et al. (18) reported that CLL patients (n=86) had lower 25(OH) D than the CG (28.666±17.528 vs 47.77±25.69 ng/mL, p<0.001). They could not show a significant relationship between age, sex, Rai stages, and 25(OH)D concentrations in the CLL group (18). Molica et al. (19) reported that the time to the first treatment was shorter in patients with low 25(OH)D in early-stage CLL patients. They suggested that a study should be conducted to determine whether normalizing vitamin D with replacement could delay disease progression in early-stage CLL. Pepper et al. (17) showed that pharmacological doses of a vitamin D analog (EB1089) induced preferentially *in vitro* cell death in CLL cells via a p53-independent mechanism. Shanafelt et al. (20) reported that vitamin D below 25 ng/mL was an indicator of poor prognosis in 390 patients with newly diagnosed CLL/SL. In multivariate analysis, vitamin D deficiency was associated with a shorter time to treatment onset [hazard ratio (HR) 1.47; 95% confidence interval (CI) 1.11-1.96] and a trend toward shorter survival (HR 1.47; 95% CI 0.97-2.23).

Table 2. 25(OH)D distribution according to Rai stage

Rai stage in diagnosis	25(OH)D levels in diagnosis, ng/mL, median (min-max)
0	22 (10.1-51.5)
I	19.5 (4.90-37.3)
II	14.2 (3.30-34.8)
III	9.85 (4.50-27.7)
IV	12.6 (5.50-32.4)

Table 3. Pairwise comparison of Rai groups

Rai stage in diagnosis	I	II	III	IV
0	p=0.464	0.002	0.053	0.004
I		0.556	0.613	0.570
II			0.704	0.946
III				0.804

We did not encounter any other publication in the literature showing that 25(OH)D is lower in advanced Rai stages. We are the first to demonstrate and publish this finding.

Many publications are showing the poor prognostic effect of low vitamin D concentration on malignancy. Despite this, uncertainties remain about whether pharmacological dose vitamin supplementation can turn a poor prognosis into a good one. There have been studies investigating the impact of vitamin D on carcinogenesis. Vitamin D has been shown to induce apoptosis in cancer cells through both downregulations of the anti-apoptotic proteins B-cell lymphoma 2 (Bcl-2) and Bcl-XL and upregulation of proapoptotic proteins (21). Furthermore, stimulation of apoptosis has been demonstrated by upregulation of other proapoptotic proteins such as G0-G1 switch 2, death-associated protein, and caspases (22,23). It is also claimed that vitamin D inhibits the anti-apoptotic signaling pathway. It has been claimed that it does this through protein kinase B by increasing the expression of phosphatase and tensin homolog (24). Vitamin D also can promote apoptotic events by activating calcium-dependent apoptotic effectors like calcium-dependent μ-calpain and calcium/calpain-dependent caspase-12 (25).

The etiology of CLL remains an enigma. Publications are claiming an increased risk of CLL in agricultural and asbestos workers (26,27). Exposure to radiation or leukemogenic drugs, which play a role in the etiology of many other hematological malignancies, has not been proven to be an etiological factor in CLL (28). Based on our study results and due to the effects of vitamin D on apoptosis mentioned above, we can claim that vitamin D deficiency may be a factor contributing to the development and progression of CLL.

Study Limitations

Factors such as latitude and exposure to sunlight, age, gender, and season of the year may affect the measured serum vitamin D concentration (29,30). We ignored the season when vitamin D was measured in the study population. There are also publications in the literature that vitamin D is lower in patients with diabetes mellitus (31). We did not take into account chronic diseases such as diabetes mellitus in our study population when evaluation. These may have affected our study results.

Conclusion

CLL patients had low vitamin D levels at the time of diagnosis, and this was more evident in the Rai stage 2 and 4 groups. These findings support the suspicion that vitamin D may play a role in the etiology and progression of CLL. Further studies involving more patients are needed to elucidate this suspicion.

Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Türkiye Hamidiye Faculty of Medicine (04.11.2022, decision number: 24/26).

Informed Consent: Since the study was retrospective, written consent was not obtained from the participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.K., M.K.K., Concept: E.K., M.K.K., Design: E.K., M.K.K., Data Collection or Processing: E.K., Analysis or Interpretation: E.K., M.K.K., Literature Search: E.K., Writing: E.K., M.K.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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