**Evaluation of Vitamin D-levels and Bone Mineral Density in Lymphoma and Solid Tumors of Childhood after Treatment**

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**Abstract:** As the survival rate of childhood cancers increases, late effects of the treatment are becoming significantly more critical. The aim of this study is to evaluate serum 25 Hydroxy Vitamin D levels and bone mineral density (BMD) of patients with lymphoma and solid tumors. In this study, we included patients diagnosed with cancer in our pediatric oncology department between 2004 and 2013. These patients were all in remission and completed their treatment. Patient data were collected from corresponding medical files. Vitamin D levels, BMD measurements and other laboratory tests were performed prospectively. The study group consisted of 105 patients who completed treatment at least one year ago. Overall, 59.6 % of the subjects were found to have vitamin D levels less than 20 ng/mL. When the factors affecting vitamin D deficiency were evaluated, there were not any factor related to vitamin D deficiency. However, a positive correlation was observed in the blood sampling time, namely test results taken in summer months (April-September) were higher. In our study, bone mineralization disorder (low BMD) was found in 44.4% of the patients. In addition, we observed that bone health was significantly affected in children who recovered from cancer. 59.6 % of the vitamin D deficiency rate was found to be higher than the similar age group in our country. To our knowledge, this is also the lowest vitamin D levels in children with cancer compared to previously published studies. The rate of low BMD was determined as 44.4%, similar to earlier studies.

**KeyWords:** 25 hydroxy vitamin D, bone mineral density, lymphoma, solid tumor.

1. **INTRODUCTION**

 Survival rate in childhood cancers has significantly increased with use of chemotherapy (CT) and radiotherapy (RT) and intensive care. As the survival rate increases, the late effects of treatment are becoming more critical. They may occur months or years after the treatment. As children's growth and development process continue, they become more susceptible to these late effects. These effects may vary depending on the tumor itself, the treatment regimen (surgery, CT and RT), the age at diagnosis and treatment, and the time elapsed in remission. Several earlier studies have noted that survivors of pediatric cancers have a higher risk of vitamin D insufficiency or deficiency (Andıran *et al.*, 2012; Choudhary *et al.*, 2013; Sinha *et al.*, 2011; Simmons *et al.*, 2011; Rossen *et al.*, 2013). This risk depends on the tumor itself, treatment regimens, poor nutrition.

 On the other hand, in patients undergoing cancer treatment, bone mineral density typically decreases. Osteopenia may also occur during diagnosis due to factors such as increase in metabolism, release of tumor cytokines and change in bone mineralization density. During the treatment period, corticosteroids therapy, low levels of physical activity and poor nutrition significantly increase osteoporosis and fracture risk in patients who are already osteopenic. In this study, we aimed to evaluate 25-Hydroxy Vitamin D (vitamin D) levels and bone mineral densities (BMD) of children with lymphoma and solid tumors after completion of chemotherapy.

1. **MATERIALS AND METHOD**

 Patients diagnosed with lymphoma and solid tumor in our hospital's pediatric oncology department between 2004 and 2013, were included in the study. They were all in remission and completed the treatment at least one year ago. Patient data on age, gender, primary diagnosis, treatment details were collected from corresponding medical files. Vitamin D levels, BMD measurements and other laboratory tests were performed prospectively. Before starting the study, approval was obtained from Ankara Childrens’ Hematology and Oncology hospital ethics committee (Date: 06.06.2013, Decision No: 158). The subjects were included in the study after verbal consent was obtained from those who accepted to be included in the study and from their families when they were invited to participate in the study.

 In the data collection process; sex, age, diagnosis, stage, diagnosis age, treatment, chemotherapy and radiotherapy information of all patients included in the study were recorded. Chemotherapeutics and their total doses were calculated during the treatment period. Radiotherapy doses and regions were recorded in patients receiving radiotherapy. Bone mineral density (BMD) was measured from the lumbar region (L2-L4) by Dual Energy X-Ray Absorptiometry (DEXA) using the Hologic QDR-4500A (S / N 45780) ® computerized densitometry. Results were reported as BMD (g / cm2). Deviations according to normal BMD values ​​were expressed as Z-score calculated according to healthy control group of the same age and sex in children. Bone mineral density Z-score lower than -2.5 standard deviation (SD) is considered as osteoporosis and Z-score was between -1-2.5 SD is considered as osteopenia .

 The venous blood sample taken in the routine follow-up examinations was used in laboratory investigations; Serum Ca, P, ALP, PTH-intact tests were studied in the central laboratory to evaluate the metabolism of vitamin D. Ca, P, ALP were studied by colorimetric method (Beckman Coulter) ® AU-680 autoanalysers. Serum PTH chemiluminescence was measured by means of immunometric method (Beckman Coulter) ® UniCel DxI 800 with autoanalysers. Plasma Vitamin D levels were studied using MS / MS (Agilent Technologies) ® methods. The effects of all these data on vitamin D and bone mineralization levels were investigated.

 Statistical analysis of the data was done by SPSS for Windows 11.5. In the two-category comparisons, student t-test was used for the variables with normal distribution. For the categorical data, Chi-Square test was applied in the relations. In addition, Pearson and Spearman Rank correlation analysis were used to evaluate the relationships between the variables. The statistical significance limit of the study was accepted as 0.05.

1. **RESULTS**

 In this study, there were a total of 105 patients with lymphoma and solid tumors who were in remission after cancer treatment.. The mean age of the patients at the time of diagnosis was 6.77 ± 4.51 years (6 months-19 years). The mean follow-up period was 4.76 ± 3.00 years (1.5-13 years). The follow-up period after treatment was 3.56 ± 2.76 years (1-12.5 years). Distribution of patients in terms of diagnosis is as follows; 45 patients were diagnosed with lymphoma and 60 patients were diagnosed with solid tumor. There were Hodgkin lymphoma in 28 patients, Non-Hodgkin Lymphoma in 19 patients, Wilms tumor in 19 patients, central nervous system tumors in 12 patients, rhabdomyosarcoma in 8 patients, neuroblastoma in 6 patients, and 13 patients with other tumor types. There were 67 female and 38 male patients. At the time of diagnosis, the patients were divided into two groups. Patients with stage 1, 2 and non-metastatic stages are considered as in the early stage; stage 3, 4 and metastatic patients were evaluated as advanced stage. 47.6% (n = 39) of the patients were in the early stage group and 52.4% (n = 43) were in the advanced stage group. When the time of blood sampling was evaluated, it was observed that in 53.8% of the patients (n = 56) blood sample was taken in winter months (October-March) and in 46.2% of the patients (n = 49) was taken in summer months (April-September). Demographic data of patients are summarized in Table 1.

**Table 1**. General features of study population (n=105).

|  |  |
| --- | --- |
| Male | 67 |
| Female | 38 |
| Age [year, mean±SD, (range)]At the time of diagnosisAt the time of study | 6.77 ± 4.51 (6 months-19 years)11.82 ± 5.10 (3-28 years) |
| Follow up period after treatment [years, mean±SD, (range)] | 4.76 ± 3.00 (1.5-13 years) |
| Stage1 and 2 (early stages) 3 and 4 (advanced stages) Stage Not specified | 394323 |
| Tumor typesHodgkin LymphomaNon Hodgkin LymphomaWilms TumorCNS TumorsRhabdomyosarcomaNeuroblastomaOthers | 281919128613 |

**Vitamin D Results:** The mean of vitamin D results was 18.40 ± 38.99 ng/ml (4-56 ng/ml). Vitamin D values were found to be low (<15 ng/ml) in 31 (28.4%) patients. Vitamin D values ​​were found to be insufficient (15-20 ng/ml) in 34 (31.2%) patients. Vitamin D values ​​were normal (> 20 ng/ml) in 40 (36.7%) patients as shown in Table 2. The mean Ca levels ​​of all patients were 9.88 ± 0.47 mg/dl (8.70-10.80 mg/dl), the mean of P values were ​​4.52 ± 0.81 mg/dl (2.20-6.60 mg/dl), the mean ALP values ​were ​229.07 ± 99.26 U / L (90-528 U/L).

 PTH values ​​were 45.85 ± 20.84 pg/ml (13.20-133 pg/ml). PTH elevation was not observed in patients whose Vitamin D level were lower than 20. When the factors affecting vitamin D deficiency were evaluated; age, gender, use of radiotherapy and chemotherapy, tumor type were not correlated with the level of vitamin D. While vitamin D measurements were low in winter months (October-March), it was observed that tests in summer were higher (p<0.05). A detailed presentation of vitamin D status for 105 patients are summarized in Table 2.

**Table 2.** Detailed analysis of Vitamin D status for 105 patients.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Deficiency****(Vit. D < 15 ng/ml)****No. of pts (%)** | **Insufficiency****(Vit. D :15-20 ng/ml)****No. of pts (%)** | **Sufficiency****(Vit. D > 20 ng/ml)****No. of pts (%)** |
| **Age**<5 years 5-10 years >10 years | 2 (40)12 (28.6)17 (29.3) | 0 (0)16 (38.1)18 (31.0) | 3 (60)14 (33.3)23 (39.7) |
| **Gender** male female | 16 (24.2)15 (35.9) | 24 (36.4)10 (25.6) | 26 (39.4)14 (35.9) |
| **Radiotherapy**Patients treated with RT Patients not treated with RT | 18 (36.0)13 (23.6) | 14 (28.0)20 (36.4) | 18 (36.0)22 (40.8) |
| **CT regimen**Steroid Cisplatin Other | 8 (30.8)10 (29.6)13 (30.2) | 11 (42.3)12 (34.3)11 (25.6) | 7 (26.9)15 (37.1)19 (44.2) |
| **Season** Winter Summer | 21 (37.5)10 (22.2) | 18 (32.1)13 (28.9) | 17 (30.4)22 (48.9) |
| **Diagnosis** Lymphoma Solid Tumors | 15 (32.6)16 (27.1) | 16 (34.8)18 (30.5) | 15 (32.6)25 (42.4) |
| **Stage** Early Advanced | 9 (23.7)15 (36.6) | 13 (34.2)13 (31.7) | 16 (42.1)13 (31.7) |

**Bone Mineral Density Results:** The bone mineral density of the patients was evaluated with Dexa in 99 children. Z-scores of the patients according to age were corrected using the oxology 2014 package program. The mean of the DEXA z-score was (-0.59) ± 1.25 [(-3.80) -3.08], and the mean BMD (g / cm2) was 0.641 ± 0.168 (0.399-1.053). Out of 99 patients, 55 patients (55.6%) are diagnosed with a normal BMD z-score, 41 (41.4%) patients had a low BMD z-score [(-1.00) - (- 2.5)] (osteopenia), and 3 patients (3.0%) were found to be severe [-2.5 and under] (osteoporosis). The results are summarized in Table 3.

**Table 3.** BMD z-score analysis on our patient set.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Normal (>-1) | Osteopenia (-1, - 2.5) | Osteoporosis (<-2.5) |
| No of Patients / Percentage | 55 / 55.6 | 41 / 41.4 | 3 / 3 |
| Mean BMD (g / cm2) level | 0.641 ± 0.168 (0.399-1.053) |
| Mean DEXA z-score level | (-0.59) ± 1.25 [(-3.80) -3.08] |

1. **DISCUSSION**

 Children are more sensitive to late effects of cancer treatment as they are in the process of growth and development. These effects include endocrine system problems and metabolic problems, pulmonary and cardiovascular problems, learning difficulties, psychological problems and secondary cancer development.

 Late side effects of the endocrine system are more common (Bhatia *et al.*, 2006; Cohen, 2003; Bircan, 2003; Nandagopal *et al.*, 2008). Changes in bone mineral density, growth retardation, malnutrition and obesity are common problems in children undergoing cancer treatment. 25-OH-vitamin D is the major circulating form of vitamin D and its analysis is recommended to show vitamin D status. In this study, we evaluated bone mineral density levels and 25 OH-vitamin D status in children with lymphoma and solid tumors after completion of therapy.

 In our study, vitamin D deficiency and deficiency rate was found to be higher when compared with the normal population. The prevalence of vitamin D deficiencies in the normal population is reported to be 14-49% in our country. In a recent study by Andıran N. *et al.*, 440 children were evaluated in Ankara between the ages of 0-16 and 40% of the cases were observed to have vitamin D levels below 20 ng / ml (5). When compared with healthy children, the rate of low vitamin D levels was significantly higher in our study group with pediatric cancer. 59.6% of the subjects were found to have vitamin D levels less than 20 ng/mL.

 In a recent study by Choudhary *et al.*, 484 patients were evaluated after cancer treatment, and vitamin D deficiency rate was determined in 29% of the patients. In this study, vitamin D deficiency was associated with puberty (Tanner phase 3-5) and race (Non-Hispanic African origin, Asian origin, Hispanic origin). BMI, gender, diagnosis, RT and steroid use were not associated with vitamin D levels (6).

 In another study, Sinha *et al.* reported that vitamin D deficiency was found to be significantly higher in the patient group (21.3%) those who received cancer treatment than in the control group (3.3%). Age, black ethnic origin, winter season and cancer disease were reported as risk factors in the study (Sinha *et al.*, 2011). In an another study by Simmons JH *et al.*, 78 patients were evaluated after the treatment of ALL, and 12% of them was found to be vitamin D deficient (<15 ng / ml) while 53 of them was found to be vitamin D insufficient (15-30 ng / ml). Increasing vitamin D levels in young age, increased dietary vitamin D intake, increased vitamin D support and increased exposure to sunlight were observed to have positive effect while treatment type, race, sex, diagnosis, time after diagnosis and BMI were found to have no effect on outcome (Simmons JH *et al.*, 2011).

 Rossen *et al.* reported that the mean vitamin D value as 29.8 ng / ml; it was observed that vitamin D deficiency was 14.4% and failure was 39.3% during the post-remission study. The decrease in time was found to be 11.4% and this figure was found to be statistically significant (p<0.0001). The results of earlier vitamin D deficiency studies are summarized in Table 4. To our knowledge, in our study, the lowest vitamin D levels was determined in children with cancer compared to other previously published studies from different countries. Another interasting finding in our study is that vitamin D deficiency was unrelated to the treatment content, steroid usage, cumulative chemotherapy dosage and radiotherapy use.

 In our study, the rate of osteopenia was 41.4% and osteoporosis rate was 3.0%. The low bone mineral density ratio was approximately 45% in total. In studies, this rate varies in relation to diagnosis, treatment and time after treatment. Kaste *et al.* reported a low bone mineral density rate of 26% with quantitative computed tomography in 99 patients with a mean duration of 7.2 years. Othman *et al.* reported that the rate of osteopenia was 26% and osteoporosis rate was 16% in 31 patients with Wilms tumor (Othman *et al.*, 2002). Sala *et al.* reported that the rate of osteopenia 45% in patients with lymphoma whose treatment was terminated at least 1 year ago (Sala et al., 2007). Odame *et al.* reported the rate of osteopenia as 44% in 25 patients with brain tumors with a mean follow-up of 7.4 years (Odame *et al.*, 2006).

 Maniadaki *et al*. reported, 17 (group 1) at the beginning of the treatment, 16 (group 2) at the initiation of KT and 10 patients with ALL (10 patients) who had been treated at the end of the study, the BMD z score was -0.74 ± 0.32, -1.59 ± 0.24, -2.03± 0.27 respectively (Maniadaki *et al*., 2006). They found a statistically significant difference between group 1 and 3. The ratio of patients with BMD <-2 was reported to be 17% in group 1, 25% in group 2 and 50% in group 3. Kaste *et al.* reported that the rate of osteoporosis was 44.3% and the rate of osteopenia was 41.4% in 70 ALL patients with a mean follow-up of 4.9 ± 2.5 years (Kaste *et al.*, 2001). In the analysis of risk factors on BMD, daily calcium intake yielded significant results. The duration of treatment, cranial radiotherapy and cumulative steroid dose did not have a significant effect on BMD levels. These studies show that the rate of decrease in BMD levels increases as the duration of treatment increases and it reaches the highest rate at the end of treatment. In our study, dexa evaluation of patients was performed after completion of treatment. The dexa measurements of the patients were not taken before the treatment. Thus, it was not possible to compare the changes at the end of the diagnosis and treatment. However, literature in children and adults shows that oncologic treatment increases BMD. Furthermore, the majority of these studies describe the low rates of BMD in different stages of treatment protocols (Odame *et al*., 2006; Maniadaki *et al.*, 2006).

 When the literature is reviewed, it is seen that low BMD ratio has been reported in a wide range from 25% to 85% (Kaste *et al.*, 2001; Bechard *et al.*, 2015). In the literature, the rate of BMD in children with cancer was mostly investigated in patients with leukemia. The rate of BMD in these patients varies between 21-85% (Kaste *et al.*, 2001; Bechard *et al.*, 2015). The rates in patients with lymphoma and solid tumors are between 26-65% (Bechard *et al.*, 2015). In our study, similarly, the low BMD rate was found to be 44.4%. Therefore, the results should be evaluated carefully. Because it is one of the effective factors in the results of the studies; the methods, diagnostic groups, applied treatments and follow-up periods of BMD vary. In addition, vital differences such as nutrition and activity and hereditary factors, which are factors affecting BMD, have not been addressed in the majority of these studies.

**Table 4.** A comparison of Vitamin D deficiency in studies.

|  |  |  |  |
| --- | --- | --- | --- |
| **Study**  | **No of subjects** | **Age** | **Vitamin D Deficiency** |
| Andıran *et al*., 2012 | 440 | 0-16 | 40% |
| Chaudry *et al*., 2013 | 484 | 0-18 | 29% |
| Sinha *et al*., 2011 | 61 | 1.5- 24 | 21.3% |
| Simmons *et al*., 2011 | 78 | 8-21 | 53% |
| Rossen *et al*., 2013 | 201 | - | 53.7% |
| Artan *et al*., 2021 | 105 | 0-18 | 59.6% |

 Study Limitations: There were some limitations associated with our study. Since we used a retrospective data obtained from hospital records of our study group, there is lack of data regarding BMI, social status, time spent under daylight, dressing habits and daily vitamin D intake of children. The methods, diagnostic groups, applied treatments and follow-up periods of BMD vary. In addition, vital differences such as nutrition and activity and hereditary factors, which are factors affecting BMD, have not been addressed in the majority of these studies. Additionally, this is a one center study held in Ankara, thus it does not reflect the status of all Turkish children.

1. **CONCLUSION**

 In conclusion, vitamin D levels in children with lymphoma and solid tumors are markedly lower than healthy children in our country. Our study is important in terms of reflecting vitamin D status in children with cancer in Turkey. Comparing studies investigating vitamin D status in children with cancer, the lowest vitamin D rate was found in our study population. It was also noticed that bone strength was significantly affected by the children who recovered from cancer. Necessary attention should be given to nutrition, vitamin supplementations and physical activities of these patients from the moment of diagnosis. Continued follow-up in terms of possible complications is necessary to improve their future quality of life.

**Conflict of Interest**

The authors have no potential conflicts of interest regarding this study.

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