

## Bone Loss in Pediatric Survivors of Acute Lymphoblastic Leukemia

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### ABSTRACT

**Objective:** Bone mineral density (BMD) in children may be negatively affected by acute lymphoblastic leukemia (ALL) or its treatment protocol. The aim of our study was to evaluate bone health by measuring BMD after ALL treatment.

**Materials and Methods:** The age, anthropometric measurements, and lumbar spine BMDs were recorded in 39 pediatric survivors of ALL, with no history of relapse, secondary malignancy, or transplantation. The lumbar spine BMD was measured by dual energy x-ray absorptiometry. The BMD risk factors, pubertal status, age at diagnosis, risk category, the time interval from the completion of the chemotherapy, and cranial radiotherapy were investigated. Serum calcium, phosphate, alkaline phosphates, magnesium, parathormone, and 25-hydroxy vitamin D levels were determined.

**Results:** The mean BMD value was calculated as  $0.668 \pm 0.176$  g/cm<sup>2</sup>. Osteopenia and osteoporosis were detected in nine patients (23.1%) and three patients (7.7%), respectively, according to previously published data of healthy age- and sex-related Turkish children's BMD values. The mean age at diagnosis of patients with ALL, having the Z-score above  $-1$  was lower than in patients having bone defect (Z score  $< -1$ ).

**Conclusion:** Early detection and intervention strategies to optimize bone health are essential in pediatric patients with ALL.

**Keywords:** Acute lymphoblastic leukaemia, bone mineral density, osteopenia, osteoporosis

### Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignant disorder in childhood. Since the survival is improved due to modern combined chemotherapy protocols today, long-term complications increase as a result of intensive therapy [1]. Osteopenia and osteoporosis are important but unnoticed problems that might appear due to disease itself and chemotherapeutic agents, most likely corticosteroids, methotrexate, and cranial radiotherapy. Dietary problems and decreased physical activity are additional factors that influence bone health [2, 3]. The aim of our study was to evaluate the bone loss in ALL survivors who had received modified St. Jude Total XIII protocol [4].

### Materials and Methods

#### Study Population

Thirty-nine pediatric ALL survivors were enrolled into this cross-sectional study conducted in the year 2010. All patients had been treated according to a modified St. Jude Total XIII ALL protocol at the Pediatric Hematology Department of Dr. Sami Ulus Obstetrics and Gynecology, Children's Health and Disease Training and Research Hospital between the years 2000 and 2009. A total of 85 patients with ALL were treated during this period. Patients with relapsed leukemia, secondary malignancy, and a history of transplantation were excluded. Patients who had any of the following features were accepted as high risk: age  $< 1$  year or  $> 10$  years; white blood cell (WBC)  $\geq 25 \times 10^9/L$ ; DNA index  $< 1.16$  or  $> 1.60$ ; central nervous system (CNS) leukemia; and the existence of the t(9;22) or t(1;19) in a relationship with pre-B ALL. Low-risk patients received antimetabolite-based therapy including daily 6-mercaptopurine (6-MP) and weekly Methotrexate (MTX) for 3 weeks, alternating with a fourth week of daily prednisone and weekly vincristine. Weekly rotational combination of the chemotherapy and drug medication

**Table I.** Relationship between the Z-score and gender, treatment protocol, craniospinal radiotherapy, pubertal status, body mass index, age at diagnosis, and bone age of patients

Patient (n=39)	Normal Z-score (n=27)	Abnormal Z-score (n=12)	p*
Gender			
Female (18)	13 (72.2%)	5 (27.8%)	0.668
Male (21)	14 (66.7%)	7 (33.3%)	
Treatment protocol			
Low risk (14)	12 (85.7%)	2 (14.3%)	0.151
High risk (25)	15 (60%)	10 (40%)	
Radiotherapy			
Yes (14)	7 (50%)	7 (50%)	0.17
No (25)	20 (80%)	5 (20%)	
Puberty			
Prepubertal (21)	16 (76.2%)	5 (23.8%)	0.50
Pubertal (18)	11 (61.1%)	7 (38.9%)	
BMI (kg/m <sup>2</sup> )	19.2±3.4	20.7±2.8	0.184
Age at diagnosis (year)	5.7±3.4	10.7±3.6	8.5±3.7
Bone age (year)	12.7±3.8	0.035	0.120

\*difference between groups

of the high-risk patients was the following: etoposide and cyclophosphamide, 6-MP and MTX, etoposide and cytarabine, prednisone and vincristine (with L-asparaginase for 7 doses), and MTX and cytarabine, and high-dose MTX and 6-MP every 8 weeks (the first 56-week period). Finally, high-risk patients received the similar remission induction therapy at the 32nd week as reinduction. All patients were treated for 120 weeks. ALL patients with the initial WBC < 100 × 10<sup>9</sup>/L, patients of T-ALL with WBC < 50 × 10<sup>9</sup>/L, patients with Ph+ ALL and patients with CNS leukemia at diagnosis were received craniospinal radiotherapy.

Informed consent was obtained from parents of the patients, and Ethical Committee approval was obtained from of Dr. Sami Ulus Obstetrics and Gynecology, Children's Health and Disease Training and Research Hospital.

### Study Protocol

On physical examination, height, weight, body mass index (BMI), and pubertal staging were evaluated. Height calculations were done by a Harpenden stadiometer. Weight and height values were calculated three times consequently, and the average values were recorded. The BMI was calculated by dividing the simple index of weight-for-height (in square meter, respectively). Height, weight, and BMI values were analyzed according to Turkish children's standards [5]. The Greulich and Pyle Atlas was used as the methodology for bone age assessment [6]. According to the Tanner-Marshall staging, patients'

pubertal stages were evaluated [7]. In laboratory analyses, serum calcium (Ca, 9.0-10.2 mg/dL), phosphorus (P, 2.0-6.6 mg/dL), magnesium (Mg, 0.7-0.96 mg/dL), alkaline phosphatase (ALP, 42-3823 u/L), parathormone (PTH; 19.2-119.4 pg/mL), and 25-hydroxy (OH) vitamin D levels (11.8-79.2 ng/mL) were noted. Ca, P, Mg, and ALP results were studied in an Beckman Coulter LX20 PRO autoanalyzer, and PTH was studied by the chemiluminescence method in an Immulite 2000 device. 25-OH-Vitamin D was studied by the radioimmunoassay method.

Bone densities of the patients were calculated on the lateral lumbar vertebrae via the Dual Energy X-Ray Absorptiometry (DXA) method (Hologic QDR-4000 [S/N 55530]). Bone mineral density was computed as the g/cm<sup>2</sup> unit. Z-scores were calculated according to the bone marrow density (BMD) values of healthy children by using the following formula:

$$\text{Z-score} = \frac{\text{Patients BMD value} - \text{Age related BMD}}{\text{Standard deviation}}$$

According to the definition by International Society of Clinical Densitometry from 2008, these patients having Z-scores below -2 SD, between -1 and -2 SD, and above -1 SD were accepted as low (osteoporosis), decreased (osteopenia), and normal respectively. Z-scores were classified as < -1 (pathological) and > -1 (normal) when statistical analyses were done [8]. While computing the Z-scores, the average BMD values of Turkish children, which had been deter-

mined in 345 healthy children by Goksen et al. were used [9].

### Statistical Analysis

For statistical analyses, the Statistical Package for the Social Sciences (SPSS Inc.; Chicago, IL, USA) 15.0 program was used. Mean and standard deviations were used for normally distributed variables. The numerical percentage was used for comparison of two numerical groups, and the significance test of difference between two means (t-test) was used for categorical variables. For categorical variables, in-group comparisons chi-squared test (Yates correction or Fisher's exact chi-square) were used. The statistically significant level was accepted as p < 0.05.

### Results

Twenty-one male (53.8%) and 18 female (46.2%) patients with the mean actual age of 11.3±3.8 (range: 5.5-18) years were included into the study. Patients' weight, height, and BMI percentile values were compatible with values of healthy children, and 53.8% of patients were in the prepubertal stages. Eighteen patients (46.2%) were in the first 2-year, and 21 patients were in 2 years after the end of the chemotherapy.

The mean BMD value was calculated as 0.668±0.176 g/cm<sup>2</sup>. Thirty percent of our patients had bone deficit. Osteopenia was detected in 9 patients (23.1%), and osteoporosis was detected in 3 (7.7%). The BMD values of females and males were 0.659±0.13 g/cm<sup>2</sup> and 0.674±0.20 g/cm<sup>2</sup>, respectively. Even though the pathological BMD levels were observed more frequently in males, a statistically significant difference was not determined between the gender and Z-score (p > 0.05). The mean age at diagnosis of the patients was 6.6±3.7 years (1.5-14 years). The age at diagnosis that had a normal Z-score was 5.7±3.4 years, which was significantly lower than in those who had an abnormal Z-score (8.5±3.7 years) (p < 0.05). There was not any significant difference between the period after the chemotherapy discontinuation and Z-score (p > 0.05).

According to patients' histories, 26 patients had not received vitamin D prophylaxis (66.7%) during infancy. Of the 26 patients who had not received vitamin D prophylaxis, Z-scores were above -1, between -1 and -2, and below -2 in 17 (65.4%), 6 (23.1%), and 3 (12.5%) patients, respectively. There was no any statistical significance between the Z-score and vitamin D prophylaxis intake in infancy (p > 0.05).

The relationship between patients' pubertal status, disease risk category, craniospinal radiotherapy history, BMI, and bone age with Z-score is shown in Table 1. According to leukemia risk groups, 14 patients (35.9%) were in low-risk, and 25 patients (64.1%) were in high-risk groups. There was no any statistically significant difference between the Z-score and leukemia risk groups detected. Although the disease risk status was not found to be significant, all 3 patients with osteoporosis were in the high-risk group. To evaluate the effect of puberty on BMD, patients were grouped as pubertal and prepubertal. Of the patients, 21 (53.8%) and 18 (46.2%) were in the prepubertal and pubertal period, respectively. Any statistically significant correlation was not noticed between the Z-scores of pubertal and prepubertal status of survivors.

Serum Ca, P, Mg, ALP, 25-OH-vitamin D, and PTH levels were analyzed, and each parameter was compared with the Z-score. The mean Ca was  $9.6 \pm 0.2$  mg/dL (minimum-maximum: 9.0-10.2), mean P  $4.7 \pm 0.8$  mg/dL (2.0-6.6), mean Mg  $0.8 \pm 0.06$  mg/dl (0.7-0.96), mean ALP  $195.5 \pm 68.6$  u/L (42-3823), mean 25-OH-vitamin D  $33.4 \pm 4.5$  ng/dL (11.8-79.2), mean PTH  $42.8 \pm 22.1$  (19.2-119.4) mg/dl. Vitamin D deficiency was found in 17% of the patients (n: 7), and severe deficiency in 1 patient. Secondary hyperparathyroidism was detected in 7.3% of patients (n=3). These biochemical parameters, 25-OH-vitamin D and PTH levels did not correlate with the Z-score.

## Discussion

According to the International Clinical Densitometry Society definition, of our survivor ALL patients, 23.7% were osteopenic, and 7.7% were osteoporotic. Reduced BMD has been reported in 8%-23% of leukemia survivors who received chemotherapy prior to epiphyseal closure [10]. Although we did not evaluate the BMD before chemotherapy, Swiatkiewicz et al. [11] reported that 30% of leukemia patients did have abnormal BMD values at diagnosis, and more than 70% of patients had abnormal BMD values after intensive chemotherapy prior to the maintenance treatment. Leukemic cell infiltration to the spongy tissue of the bone, or the paracrine excretion of lymphokines and parathormone-related peptides, or parathormon excreted from leukemic cells or ectopically produced, might all explain the low BMD values at the diagnosis [11].

The bone size and BMD increase progressively through the childhood with rapid growth during puberty. Growth hormone, sex steroids, and several exogenous factors such as nutrition and exercise regulate bone growth and remodeling

[12]. The mean age of our patients having the Z-score above  $-1$  was younger than in patients with bone defect ( $p < 0.05$ ). Halton et al. [13] had analyzed the BMD values of 40 patients with ALL at 6-month intervals from the diagnosis, and they found that during the first 2 years of chemotherapy, the BMD values were decreased in patients whose age at diagnosis was above 11, but they were preserved in patients whose age at diagnosis was below 11 years. In another study, patients older than 10 years at diagnosis had the lowest Z-scores for both girls and boys at the lumbar spine and femoral neck [14]. It was assumed that BMD is negatively affected by an increasing age, probably due to a rapid increase of bone turnover in adolescents and during puberty. Therefore, if bone growth in adolescents and puberty is affected, BMD is reduced quickly. Nevertheless, many studies have not confirmed this relationship [15, 16]. We also did not find any correlation between the Z-scores and the pubertal status.

The main therapeutic point according to risk groups was the doses of steroids and methotrexate. High-risk leukemia patients were applied an additional amount of prednisone and methotrexate. The exact mechanism of how methotrexate induces bone mineral loss is still unknown. It is suggested that in methotrexate-treated children, increased bone resorption and excretion can be the cause of increased excretion of the calcium rather than diminished bone formation. [17]. There are different types of mechanisms of glucocorticoid-induced osteoporosis; glucocorticoids prevent vitamin D to form the active metabolite 1.25-dihydroxy vitamin D. The impairment of the intestinal absorption of calcium is caused by disruption of this metabolite production. The inhibited expression of the vitamin D in the bone and production of osteocalcin, along with a decrease of the local production of several cytokines, are caused by glucocorticoid, which normally inhibits bone resorption. [18]. Although our high-risk group patients received more prednisone and methotrexate doses, there were no any significant differences detected between leukemia risk groups. Our study also demonstrated that craniospinal radiotherapy was not affected by the BMD of leukemia survivors. However, in another pediatric study regarding long-term effects of ALL treatment, the BMD values of patients that have received cranial radiotherapy were lower than in those who did not receive it [19]. In the study by Gurney et al. [20] that evaluated the adult survivors of childhood ALL, it was stated that very low BMD was relatively unusual and that the BMD Z-scores tend to improve from adolescence to young adulthood. The primary predictor of suboptimal BMD in

the aforementioned study was a high-dose cranial or craniospinal radiation exposure. Defects in the hypothalamo-pituitary axes, growth hormone deficiency, and direct radiation effect on the bone may affect BMD.

Some of our patients presented with altered biochemical evidence of impaired bone health. Seven patients displayed vitamin D deficiency, and 3 of them with resulting secondary hyperparathyroidism. However, we did not find any significant correlation between the Z-score and Ca, P, ALP, Mg, PTH, and 25-OH-vitamin D levels in our study. Although a slight decrease in BMD has been found, Marinovic et al. [21] reported that serum Ca, P, ALP, 25-OH-vitamin D, 1.25 dihydroxy vitamin D and PTH, and serum osteocalcin and bone ALP levels were normal both at the first evaluation and at the second evaluation, 1 year after the completion of chemotherapy.

Exercise combined with vitamin D and calcium supplementation in healthy children enhances bone mass [22]. Vitamin D and Ca supplementation along with promoted physical activity may ameliorate the bone mass loss in ALL patients. Some authors recommend vitamin D and bisphosphonates treatment with low BMD. However, as the effects of bisphosphonates on leukemia are not well known, this approach deserves marked caution [22, 23].

It is reported that the fracture prevalence is high in patients with ALL and survivors of all ages and is more common in young patients. A significant percentage of these fractures is asymptomatic and associated with a reduced BMD Z-score. Fractures can occur at any time of ALL treatment, frequently during maintenance or shortly after the treatment end [22]. Most significant impact of ALL is at the time of diagnosis and active phase of treatment with substantial recovery following completion of therapy [24].

In conclusion, osteopenia and osteoporosis are important, subtle complications of leukemia treatment. Early detection and intervention strategies to optimize bone health are essential. Thus, all of patients with ALL should be screened for BMD at the time of diagnosis and at various intervals, supportive treatments should be done, and follow-ups should be continued after treatment. Further prospective studies are needed for prevention and progression of osteopenia.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the Ethics Committee of Dr. Sami Ulus Obstetrics and Gynecology, Children's Health and Disease Training and Research Hospital.

**Informed Consent:** Written informed consent was obtained from parents of patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

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**Conflict of Interest:** The authors have no conflicts of interest to declare.

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