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Original Article

# The proportion of bone mineral density in children with high risk acute lymphoblastic leukemia after 6- and 12-month chemotherapy maintenance phase

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## Abstract

***Background*** Low bone mineral density (BMD) value is one of the current concerns in acute lymphoblastic leukemia (ALL) patients. Some risk factors including use of chemotherapeutic drugs, nutritional status, physical activities, and progression of disease are suspected as the predisposing factors for development of osteopenia and osteoporosis.

***Objectives*** To obtain the proportion of BMD *z*-score, level of calcium ions, and 25(OH)$D_3$ in children with high risk ALL after 6 and 12 months chemotherapy maintenance phase.

***Methods*** We conducted a cross-sectional comparative study from May 2008 to May 2010. Subjects were high risk ALL patients aged 5-18 years old who had completed the 6 or 12 months chemotherapy maintenance phase. We measured 25(OH) $D_3$ level, calcium ion level, and BMD using electro chemi-luminescence immunoassay, ion selective electrode, and dual x-ray absorptiometry, respectively.

***Results*** There were 40 subjects who enrolled this study. The incidence of hypocalcemia and vitamin D deficiency were 33/40 and 40/40, respectively. The mean calcium ion levels, 25(OH)$D_3$ level, and BMD *z-score* values in six months groups were 1.1 (0.1 SD) mmol/L, 21.3 (2 SD) ng/L, -0.7 (0.8 SD), respectively, while in the 12 months group, the values were 1.1 (0.0 SD) mmol/L, 21(2.2 SD) ng/L, -1.7 (0.6 SD), respectively (P=0.478). Body mass index (BMI) and corticosteroid cumulative dose is correlated with the low BMD values in $L_1$-$L_4$.

***Conclusion*** The bone mineral metabolism disorder marked with the low levels of calcium, 25(OH)$D_3$ and osteopenia was observed in ALL patients who underwent chemotherapy. The proportion of the BMD *z*-score value, calcium ion level, and 25(OH)$D_3$ in the two groups were not statistically significant. **[Paediatr Indones. 2010;50:365-70]**.

***Keywords:*** *acute lymphoblastic leukemia , osteopenia, osteoporosis, children*

Leukemia is the most common hematologic malignancy in young children. Owing to the advance in science and medicine, the survival rate of acute lymphoblastic leukemia (ALL) in children increases by 70%.[1,2] On the other hand, survived ALL patients will develop long term side effects from the use of chemotherapeutic drugs and radiotherapy. Current highlighted side effects of intensive chemotherapy are bone mineral density and bone mineral metabolism disorder. Under healthy conditions, bone mineral density (BMD) increases in childhood and adolescence until peak bone mass is reached in the second or third decade. Failure to achieve peak bone mass during childhood is thought to be a major determinant for the subsequent osteoporotic fracture in later life.

The mechanism of osteoporosis in ALL patients is a multifactorial process. Some risk factors, including the use of chemotherapeutic agents such as methotrexate (MTX) and corticosteroid,

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the nutritional status, and disease progression itself are suspected as one of the predisposing factors of osteopenia and osteoporosis.[3,4] Methotrexate (MTX) is a folic acid analog which may cause toxic effects to the bone by the persistent accumulation of MTX-Polyglutamate enzyme.[5,6] The corticosteroid given simultaneously with MTX also has adverse effects to the bone by increasing osteoclast activity and inhibiting osteoblast activity.[7]

Osteoporosis is a condition marked by progressive decrease of bone mass which resulted in the change of bone micro architecture, thus decreasing the bone strength to hold its burden and causing it to be susceptible to fractures, while osteopenia is a milder form of bone mass reduction compared to osteoporosis.[8] The categorization of osteopenia and osteoporosis by BMD measurement in children requires an appropriate *z*-score values based on age and sex. Osteopenia is defined by *z*-score between -1 and -2, while osteoporotic children has *z*-score < -2.[9]

Various studies abroad reported various osteopenia/osteoporosis incidences in ALL patients. Halton[1] reported that the incidence of osteopenia in children with ALL during 6 or 12 months of chemotherapy was 53% and 69% respectively, which increased to 76% after the child had completed the 24 months course of chemotherapy. ALL patients can also develop bone mineral metabolism disorder during the diagnosis of ALL. The disorder is characterized with the reduction of plasma 1,25 Dihydroxyvitamin D and osteocalcitonin.[10]

Studies about bone disorders resulted by chemotherapy in children with ALL have never been conducted in Indonesia. that the aim of this study was to obtain incidence of osteopenia/osteoporosis, and levels of calcium and 25-hidroxy vitamin D (25(OH) $D_3$) in children with ALL in Indonesia.

## Methods

We conducted a comparative cross-sectional study in children aged 5-18 years with high risk ALL admitted to outpatient Hematology-Oncology Clinic, Department of Child Health Jakarta from May 2008 until May 2010. We included children who had completed 6 months or 12 months (n=73) of chemotherapy maintenance phase. We excluded children who had bone structure abnormalities, including osteogenesis imperfecta and juvenile osteoporosis, kidney disease, inflammatory bowel disease (IBD), persistent or chronic diarrhea, liver cholestasis, received continuous calcium and vitamin D supplementation for 6 months, history of receiving biphosphonate therapy, and lost to follow-up. The subjects who fulfilled the inclusion and exclusion criteria were selected consecutively until the total sample quota is reached. We obtained informed consent from parents.

History and physical examination were conducted to complete the basic information, while supporting data were be acquired from the medical records. All subjects received chemotherapy in accordance with the National Protocol (Jakarta) for high risk ALL. The chemotherapy regiments given during the maintenance phase consisted of intrathecal MTX, oral MTX, oral dexamethasone or prednisone, intravenous vincristine, and oral 6-mercaptopurin (6-MP).

We measured the 25(OH)$D_3$ level using Elecsys Vitamin D3 (25-OH) 2010 cobas e411® kit (*electro chemiluminescence immunoassay method*). The range of measurement is 4-100 ng/mL or 10 – 250 nmol/L with the value lower than detection limit of < 4 ng/mL (< 10 nmol/L) and the value higher than detection limit of >100 ng/mL (>250 nmol/L). We measured calcium ion levels with APL® kit (*ion selective electrode method*). The normal range value is 1.17-1.29 mmol/L. Bone mineral density measurement was conducted using Lunar GE Medical Systems® DEXA (*dual x-ray absorptiometry*). The available reference value for BMD is based on the age, sex and ethnic (for this instance, Asia) starting from the age of 5 years old. Osteopenia is defined as *z*-score between -1 and -2, while osteoporosis is limited for *z*-score BMD < -2.

## Results

Forty children have enrolled this study, and then divided into two groups. Group A was children who had been treated for 6 months (n= 20) and group B who had been treated of 12 months (n=20) chemotherapy maintenance phase. Distribution

of subjects according the socio-demographic and medical characteristic is presented in **Table 1** and **Table 2.** The majority of subjects were 5-8 years old, with male: female ratio of 2:1. Both groups have comparable medical characteristics, except for disease duration (P=0.001) and initial leukocyte count (P=0.043).

**Table 1.** Distribution of subjects according to socio-demographic characteristic of the group

| Characteristics | Group A (n = 20) | Group B (n = 20) |
|---|---|---|
| Sex | | |
| Male | 12 | 15 |
| Female | 8 | 5 |
| Age Groups | | |
| 5-8 yrs | 11 | 15 |
| >9 yrs | 9 | 5 |
| Father's Education Level | | |
| Low | 6 | 8 |
| Medium | 9 | 10 |
| High | 5 | 2 |
| Mother's education Level | | |
| Low | 10 | 12 |
| Medium | 8 | 8 |
| High | 2 | 2 |
| Family Income | | |
| Low | 11 | 12 |
| Medium | 7 | 5 |
| High | 2 | 3 |
| Nutritional Status (BMI) | | |
| Underweight | 7 | 5 |
| Normal/ Overweight | 13 | 15 |
| Puberty Status | | |
| Tanner 1 | 13 | 17 |
| Tanner 2-5 | 7 | 3 |

**Table 3** shows that 33/40 subjects developed hypocalcemia (calcium ion level <1.17 mmol/L). This condition was not accompanied with the clinical manifestations resulted from hypocalcemia. All subjects had low vitamin D level, 13 of the them developed vitamin D insufficiency ($25(OH)D_3$ level of 20-30 ng/mL) while others developed vitamin D deficiency ($25(OH)D_3$ level of <20 ng/mL). Both groups showed statistically insignificant vitamin D and calcium status difference.

Twenty three subjects in both groups have normal BMD values. However, osteopenia (BMD $L_{1-4}$ z-score value between -1.0 and -2.0) was reported in 15 subjects, and one subject in each group had osteoporosis ( BMD $L_{1-4}$ z-score value of <-2.0). None of the subjects in both groups experienced bone fractures (**Table 4**).

**Table 5** showed a significant negative correlation was observed between BMI and the low BMD $L_{1-4}$ value (r=-0.317, P=0.046). There was a weak positive correlation between the low BMD $L_{1-4}$ with the corticosteroid cumulative dose (r=0.381, P=0.015). Other clinical and laboratory parameters were not proven to correlate with the low BMD $L_{1-4}$ value

## Discussion

The subjects in group B had higher corticosteroid cumulative dose than those in group A, but this difference was not significant (P=0.854). Ten subjects who received dexamethasone weree converted to

**Table 2.** Distribution of subjects according to the medical characteristics of the group

| Variables | Group A Mean (SD) | Group B Mean (SD) | P |
|---|---|---|---|
| Age, yrs | 8.6 (3.2) | 7.8 (2.5) | 0.403* |
| Age on onset, yrs^ | 6.5 (3 to 14) | 5 (3 to 13.5) | * |
| Disease duration, mo | 14.8 (3.4) | 22.3 (2.4) | 0.001 |
| Prednisone, mg/m2# | 4686.6 (1141.8) | 5398.1 (1202.5) | 0.854* |
| Methotrexate, mg/m2# | 3424.5 (825.3) | 3750.4 (461.8) | 0.132 |
| Body weight, kg | 27.4 (12.1) | 28.2 (12) | 0.678* |
| Body height, cm | 125.0 (19.5) | 122.6 (13.2) | 0.657 |
| Initial Hemoglobin, mg/dl | 7.1 (1.9) | 7.6 (3.4) | 0.947* |
| Initial Platelets/mm3 | 67,500 (94,100) | 111,300 (154,600) | 0.718* |
| Initial Leukocyte/mm3 | 15.300 (22,800) | 65,400 (86,900) | 0.043* |
| BMI, kg/m2 | 17.4 (4.2) | 17.9 (3.2) | 0.461 |

Notes: *Mann Whitney; #Cumulative dose; ^ Median, range

**Table 3.** Calcium ion, vitamin D status

| Parameters | Group A (n = 20) | Group B (n = 20) | Σ | P |
|---|---|---|---|---|
| Calcium ion status | | | | |
| Hypocalcemia | 15 | 18 | 33 | 0.407 Ω |
| Normal | 5 | 2 | 7 | |
| Vitamin D status | | | | |
| Insufficiency | 6 | 7 | 13 | 1.000 |
| Deficiency | 14 | 13 | 27 | |
| Calcium Ion (mean,SD mol/L | 1.1 (0.1) | 1.1 (0.0) | | 0.975* |
| 25(OH)D3 (mean,SD) ng/mL | 21.3 (2.2) | 21.0 (2.0) | | 0.710* |

Note: Ω Fisher exact Test; * Independent Samples T-test

**Table 4.** Bone mineral density in children with acute lymphoblastic leukemia

| Parameter | Group A | Group B | P* |
|---|---|---|---|
| BMD $L_1$-$_4$ z-score (mean,SD) | -0.7 (0.8) | -1.7 (0.6) | 0.478 |
| Osteopenia | 7 | 8 | |
| Osteoporosis | 1 | 1 | 0.946 |
| Normal | 12 | 11 | |

Note: * Independent Samples T-test

**Table 5.** The correlation between the clinical and laboratory parameters with the Low BMD $L_{1-4}$ values in high risk ALL patients

| Variables | r θ | P θ |
|---|---|---|
| Subjects' Age | 0.234 | 0.146 |
| BMI value | -0.317 | 0.046 |
| Disease Onset | 0.243 | 0.131 |
| Disease Duration | 0.057 | 0.726 |
| Methotrexate Cumulative Dose | 0.043 | 0.792 |
| Prednisone Cumulative Dose | 0.381 | 0.015 |
| Calcium Ion Level | 0.121 | 0.457 |
| Vitamin D Level | -0.089 | 0.587 |
| Initial Leukocyte Count | 0.278 | 0.083 |

Note: θ Spearmant Test

predsinone by multiplying it with 6.67. In 1987, Strauss et al[11] included ALL patients who received prednisone or dexamethasone on their study. The observed prednisone cumulative dose was 21.241 mg/m2, not significantly different with subjects receiving dexamethasone (21.530 mg/m2). The mean initial leukocyte count in both groups had a statistically significant difference. Until now, the mechanism of the correlation of initial leukocyte count with the low BMD *z*-score in ALL patients has not been understood.

Calcium is a very important mineral to the bone formation process. The hypocalcemia condition in ALL patients has a multifactorial etiology, including the low diet calcium intake, vitamin D malabsorbtion, and the continuous use corticosteroid will cause calcium absorption disorder in the intestine and calcium loss in urine.[12,13,14] This study observed that 33/40 subjects developed hypocalcaemia, similar to the Ariskoki[12] who found that children with ALL had low calcium level [2.3 (0.1 SD) mmol/L] during chemotherapy. Halton et al[1] also reported that the calcium ion level in the 6 months chemotherapy group was higher than the 12 months chemotherapy group (1.26 (1.2- 1.32) mmol/L and 1.23 (1.15- 1.31) mmol/L, respectively).

Vitamin D, a member of the sterol group, plays an important role in calcium and phosphate metabolism in plasma, through the interaction between intestines, kidneys, and bone.[15,16] If the amount of vitamin D in the body is not adequate, the calcium absorption in the intestines is only around 10-15%.[17] All the subjects in this study had low vitamin D levels; thirteen subjects had vitamin D insufficiency, while 27 subjects had vitamin D deficiency. During the maintenance phase, the exposure to corticosteroid occured continuously, thus causing a potential disorder to the vitamin D metabolism. Low 1,25-$(OH)_2$-$D_3$ level was caused by increasing cell turnover resulting from chemotherapy. Malnutrition, vitamin D malabsorption, decreased exposure to sunlight and physical activities in ALL patients is another mechanism is expected to play a role in the reduction 25$(OH)D_3$ level.[12]

Bone mineral density measurement showed BMD *z*-score decrease in children who received 12 months chemotherapy compared to those in 6 months group (P=0.478). Ariskoki et al[12] studied about the BMD condition in 48 children with ALL after completing the course of chemotherapy. They found a significant decrease in BMD *z*-score in lumbal area

[median -0.77 (-1.3 – 0.23 SD)] (P= <0.01) and femur [median -1.02 (-1.52 - -0.53 SD)] (P= <0.01). We found that body mass index and corticosteroid cumulative dose altered bone density in children who received chemotherapy.

Corticosteroid and methotrexate (MTX) is known to have adverse effects to the bone. Although the doses of these two regiments in this study were still considered low, but the continuous exposure may cause bone formation inhibition. Corticosteroid will inhibit 1-α hydroxylation needed for the synthesis of $1,25(OH)_2D_3$ in the kidney. Without this factor, calcium absorption will be inhibited.[18] In addition, corticosteroid may cause reduction of osteocalcin production, a main bone matrix protein and local cytokine which function to inhibit bone resorption. Meanwhile, MTX as an antimetabolite agent, will inhibit the precursor of primitive mesenchyme cells which plays a role in bone mineralization process. Moreover, the polyglutamate MTX accumulation in the cell will increase the toxicity to bone.

The mechanism between BMI with the low bone mineral density is still unclear. However, some references have proposed a theory about the body's mechanical burden to the bones and adipose tissues as the underlying mechanism of BMD reduction. Adipose tissue plays a role in estrogen, leptin, adipolectin, and IL-6 synthesis. These substances are essential products in the bone mineral metabolism.[19] Therefore, a person with a low BMI will have less adipose tissue, thus causing the synthesis of active biological substances becomes inadequate and cause bone mineral metabolism disorder. The result of this study found a positive correlation between BMD and corticosteroid cumulative dose, impying that the higher corticosteroid cumulative dose, the worse toxic effects of corticosteroid to the bones can be exerted.

This study had several limitations. First, we did not include healthy children with the same sex and age as control. Second, the reference BMD value was based on US healthy children, because reference BMD values for healthy Indonesian children were not available. The race difference would certainly affect the BMD values. Third, this study did not conduct a detail bone metabolism marker examination because of time and budget limitations. Finally, a diet analysis to assess calcium and vitamin D intake and physical activity assessment were not conducted to the subjects, thus making these became confounding factors.

In conclusion, bone mineral metabolism disorder marked with the low levels of calcium, $25(OH)D_3$ and osteopenia was observed in ALL patients who underwent chemotherapy. The proportion of the BMD *z*-score value, calcium ion level, and $25(OH)D_3$ in the two groups were not statistically significant.

## References


1. Halton JM, Atkinson SA, Fraher L, Webber C, Gill GJ, Dawson S, et al. Altered mineral metabolism and bone mass in children during treatment for acute lymphoblastic leukemia. J Bone Miner Res. 1996;11:1774-83.
2. Sellin RV, Brosnan P, Delpassand A, Zietz H, Klein MJ. Osteopenia in young adult survivors of childhood cancer. J Pediatr Hematol Oncol. 1999;32:272-8.
3. Hogler W, Wehl G, Staa VT, Meister B, Franke AK. Incidence of skeletal complications during treatment of childhood acute lymphoblastic leukemia: comparison of fracture risk with the general practice research database. Pediatr Blood Cancer. 2007;48:21-7.
4. Cohn SL, Morgan ER, Mallete LE. The spectrum of metabolic bone disease in lymphoblastic leukemia. Cancer. 2003;59:346-50.
5. Meister B, Gabner I, Streff W, Dengg K, Fink FM. Methotrexate osteopathy in infants with tumors of the central nervous system. Med Pediatr Oncol. 1994;23:493-96.
6. Blayer WA. The clinical pharmacology of methotrexate: new application of and old drug. Cancer. 1978;41:36-51.
7. Canalis E. Mechanism of glucocorticoid action in bone: implication to glucocorticoid-induced osteoporosis. J Clin Endocrinol Metab. 1996;81:3441-7.
8. Hartman C, Hochberg Z, Shamir R. Osteoporosis in pediatrics. IMAJ. 2003;5:509-15.
9. Steelman J, Zeitler P. Osteoporosis in pediatrics. Pediatr Rev. 2001;22:56-65.
10. Atkinson SA, Halton JM, Bradley C, Wu B, Barr RD. Bone and mineral abnormalities in childhood acute lymphoblastic leukemia: influence of disease, drugs, and nutrition. Int J Cancer Suppl. 1998;11:35-9
11. Strauss AJ, Su JT, Dalton VM, Gelber RD, Sallan SE, Silverman LB. Bone morbidity in children treated for acute lymphoblastic leukemia. J Clin Oncol. 2001;19:3066-72.

12. Ariskoki P, Kroger H, Riikonen P, Parviainen M, Voutilainen R. Disturbance in bone turnover in children with malignancy at completion chemoterapy. Med Pediatr Oncol. 1999;33:455-61.
13. Davies JH, Evans BAJ, Jenney MEM, Gregory JW. Factors influencing bone mineral decrements during treatment of childhood acute lymphoblastic leukemia. Welsh Paediatr J. 2000;24:26-30.
14. Bushinsky AD, Monk RD. Calcium. Lancet. 1998;352:306-11.
15. Ganong WF. Hormonal control of calcium metabolism and the physiology of bone. In: Ganong WF, editor. Review of medical physiology. 17th ed. Connecticut: Appleton and Lange, 1995; p. 352-64.
16. Chesney RW. Metabolic bone disease. In: Behrman RE, Kliegman RM, Jenson HB, Stanton BF, editors. Nelson's textbook of pediatrics. 18th ed. Pennsylvania: Saunders, 2007; p. 2893-8.
17. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;52:266-81.
18. Kaste SC, Wallace DJ, Rose SR, Boyett JM, Lustig RH, Rivera GK. Bone mineral decrements in survivors of childhood acute lymphoblastic leukemia frequency of occurrence and risk factors for their development. Leukemia. 2001;15:728-34.
19. Zhao LJ, Jiang H, Papasian CJ, Maulik D, Drees B, Hamilton J, et al. Corelation of obesity and osteoporosis: effect of fat mass on the determination of osteoporosis. J Bone Miner Res. 2008;23:17-29.