Scientific Research Publishing

Open Journal of Pediatrics, 2022, 12, 815-826
https://www.scirp.org/journal/ojped
ISSN Online: 2160-8776
ISSN Print: 2160-8741

# Serum Vitamin D and Bone Mineral Density in Children with Growing Pain in a Tertiary Hospital of Bangladesh

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**How to cite this paper:** Ali, Md. A., Haque, M., Islam, M.I., Khan, M.Z.I. and Rahman, S.A. (2022) Serum Vitamin D and Bone Mineral Density in Children with Growing Pain in a Tertiary Hospital of Bangladesh. *Open Journal of Pediatrics*, **12**, 815-826.
https://doi.org/10.4236/ojped.2022.125082

**Received:** October 9, 2022
**Accepted:** November 11, 2022
**Published:** November 14, 2022



## Abstract

**Background:** Growing pain (GP) is the most common form of nonspecific, recurrent leg pain in children aged 4 - 12 years. The exact etiology of GP is not known. However, some studies have found an association between vitamin D and Bone Mineral Status (BMD) status with GP in their study. **Objectives:** To assess the serum level of vitamin D, and BMD and to determine their association with growing pain in children. **Methods:** This cross-sectional analytical study was conducted in the Department of Paediatrics, Bangabandhu Sheikh Mujib Medical University (BSMMU). Sixty children between the age of 6 - 12 years were included in the study from March 2020 to August 2021. Children who fulfilled the Evans criteria of GP were enrolled as cases and thirty age and sex matched healthy children were recruited as the control in the study. Informed written consent was obtained from patients and parents. Serum 25-hydroxy-vitamin-D levels and BMD were performed among cases and controls and subsequently compared to see their association in growing pain. A preformed semi-structured questionnaire was completed for each participant which included socio-demographic, clinical and laboratory characteristics. Appropriate statistical tests were applied for data analysis and performed by SPSS version 22. A p-value less than 0.05 was considered as significant at a 95% confidence interval. **Results:** In this study, 96.7% of growing pain patients had hypovitaminosis D and among them, the majority (86.7%) was vitamin D deficient. There was a significant association between vitamin D with GP compared to healthy control. BMD was significantly lower in the lumbar vertebra (L1 - L4) and femoral neck region (both right and left) among GP children compared to the control group. **Conclusion:** From this study, it may be concluded that the majority of children with GP had hypovitaminosis D and low BMD status compared to the control. Vitamin D defi-

ciency and low BMD status were significantly associated with children with growing pain. Institutional Review Board (I.R.B.) Clearance Certificate (*NO. BSMMU/*2020/4503 Date: 15/03/2020) was provided from the office of the Registrar, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.

## Keywords

Growing Pain, Vitamin D, Bone Mineral Density

## 1. Introduction

Growing pain (GP) is the most common form of nonspecific, recurrent leg pain in childhood and a frequent cause of paediatric outpatient visits [1]. The prevalence of GP ranges from 2.6% to 49% [2] and mainly affects children aged 4 - 12 years [3]. It is typically non-articular, intermittent, bilateral, not associated with limited mobility and usually occurs in the evening or during the night. It is located in the muscles and predominantly affects the anterior thighs, shins, calves or backs of the knees [4]. Physical examination does not reveal objective signs of inflammation and laboratory test results are generally normal [5]. There is no single diagnostic test for growing pain and as a result, it continues to be diagnosed on the basis of both inclusion and exclusion criteria. Most recently, Evan 2008 has proposed diagnostic criteria for growing pain which are currently accepted worldwide [2].

Few studies have been done to elucidate the etiology and pathogenesis of GP. It has been suggested that growing pain may be due to strain on muscles attached to growing bones. These muscles may tire easily and give pain [6]. Children with growing pains may have a low-pain threshold [7] and decreased bone strength [8]. An interesting approach would be to consider calcium and vitamin D metabolism in aetio-pathogenesis of GP [9].

Vitamin D is a fat-soluble vitamin which is synthesized in the skin on exposure to ultraviolet-B radiation from sunlight and only small amounts can be obtained (<10%) from dietary intake [10]. Vitamin D maintains calcium and phosphorus within normal levels which is important for neuromuscular functioning and bone metabolism [11]. Bischoff *et al.* (2001) in their original article in Switzerland have shown that vitamin D receptors are present in both the nuclei and plasma membranes of skeletal muscle cells in mammals, indicating an association between vitamin D and skeletal muscle [12].

A study done in a tertiary care hospital in Northern India found that 100% of children with GP had hypovitaminosis D, 91.1% of them being deficient [13]. A recent study in Bangladesh showed that 92.15% of children had vitamin D deficiency in GP patients with a mean 18.35 ± 6.26 ng/ml, which was significantly lower than the control group [14].

"Dual energy X-ray absorptiometry" (DEXA) is an advanced imaging test that can evaluate "bone mineral density" (BMD) which is a measurement of bone

mass in grams related to the bone area in ($cm^2$) [15]. A decrease in bone mass measured by DEXA may be due to osteopenia, osteomalacia or a reduction in bone size. It can also predict fracture risk and treatment response [15].

Friedland *et al.* (2005) in their original article conducted in USA and Israel reported that BMD was reduced in children presenting with growing pain compared to healthy children [8]. Morandi *et al.* (2014) also found a comparatively lower bone mineral density in growing pain patients by measuring amplitude-dependent speed of sound (AD-SOS) and the bone transmission time (BTT) using quantitative ultrasound assessment (QUS) [16]. Low serum concentrations of 25-OHD with secondary hyperparathyroidism in GP patients lead to decreased bone mineral density and osteopenia [13].

But no such study related to GP associated with vitamin D and BMD status together has been found in our country. The study aimed to assess vitamin D and BMD status and to detect their association with growing pain in Bangladeshi children.

## 2. Materials and Methods

This cross sectional analytical study was conducted at the paediatric rheumatology follow up clinic of Bangabandhu Sheikh Mujib Medical University (BSMMU), from March 2020 to August 2021. Sixty children aged 6 - 12 years, fulfilling the Evans criteria [2] (2008) of growing pain were enrolled for the study. Children with any systemic illness, organic cause of pain, rheumatologic disorders and who had taken vitamin D, calcium, steroid or any other DMARDs within 3 months were excluded. Informed written consent was obtained from parents and Institutional Review Board Clearance Certificate (*NO. BSMMU*/2020/4503 Date: 15/03/2020) was taken before enrollment of the study.

Semi-structured questionniare was formulated and pre-testing was done to validate the questionnaire. This questionnaire was filled up by the investigator regarding demographic variables and characteristics of growing pain. All the participants had undergone investigations to measure serum vitamin D (25- hydroxycholecalciferol) and BMD status by DEXA scan. Age and sex matched healthy siblings of GP patients who did not take any supplements containing vitamin D or calcium in last 3 months were selected as controls in this study. Serum 25(OH)D was determined by chemiluminescent microparticle immunoassay (CMA) technique using SIEMENS ADVIA centaur XPT in laboratory of National Institute of Nuclear Medicine and Allied Science (NINMAS), BSMMU. Depending on their vitamin D level, patients were classified into 3 categories: levels < 20 ng/ml as Vitamin D deficiency; levels 20 to <30 ng/ml as Vitamin D Insufficiency; levels ≥ 30 ng/ml as Vitamin D sufficiency [17]. Hypovitaminosis D comprised of both vitamin D insufficiency and deficiency group of children. BMD was measured in NINMAS, BSMMU by DMS Stratos DR Bone Densitometer Dual Emission X-ray Absorptiometry (DEXA) CE 0120 model manufactured in France. BMD was observed at lumbar vertebra (L1 - 4) & both femoral

neck as bone density in gm/cm² and also expressed in Z score. Low BMD is defined as areal BMD Z-scores less than or equal to −2.0 SD [18]. Statistical analysis was performed by SPSS (statistical program for social science) for version 22. Comparison of vitamin D deficiency, insufficiency and sufficiency level among GP and control group was performed by chi square test. Comparisons of vitamin D level and BMD status between GP children and control group were done with unpaired student t test. A p-value of <0.05 was considered statistically significant.

## 3. Results

A total of 60 patients were studied in the present study. Table 1 shows the demographic data and characteristics of children with GP. Maximum 73.3% patients were more than 10 year old, among them 63.3% were male. There was no statistically significant difference between case and control groups in respect of age and sex.

Table 2 demonstrates the characteristics of growing pain. Fifty five patients (91.7%) complained about calf muscle pain and 66.7% complained pain at night. Pain was relieved after limb massage found in 58.4% patients. Sleep disturbance was found in 25% of patients followed by difficulty in playing and hampered schooling. Headache and abdominal pain observed in 48.3% and 45% of GP patients respectively.

Table 3 depicts that serum vitamin D level was significantly lower in children with growing pain (14.36 ± 6.17 ng/ml) in comparison with control group (27.16 ± 8.20 ng/ml) (p-value < 0.0001).

Figure 1 shows that most of the growing pain patient (86.7%) had vitamin D deficiency and 10% insufficiency with an overall 96.7% hypovitaminosis D and only 3% patients had vitamin D sufficiency. In comparison, among control group 40% had vitamin D sufficiency, 40% had insufficiency and only 20% had deficiency. As hypovitaminosis D comprises of both vitamin D deficiency and insufficiency group, we found that hypovitaminosis D was significantly more prevalent in growing pain patients than in control (96.7% vs 60%, p < 0.0001). (Chi-square test was performed)

Table 1. Demographic data of children with growing pain and control.

| Item | Case (N = 60) n (%) | Control (N = 30) n (%) | p-value |
|---|---|---|---|
| **Age (years)** | | | |
| <10 | 16 (26.7%) | 10 (33.3%) | 0.511 |
| ≥10 | 44 (73.3%) | 20 (66.7%) | |
| **Gender** | | | |
| Male | 38 (63.3%) | 20 (66.7%) | 0.755 |
| Female | 22 (36.7%) | 10 (33.3%) | |

(Chi-square test was performed).

Table 2. Characteristic of growing pain (N = 60).

| Item | Number (N = 60) | Percentage (%) |
|---|---|---|
| **Site of pain** | | |
| Calf muscles | 55 | 91.7% |
| Front of thigh | 36 | 60.0% |
| Back of Knee | 34 | 56.7% |
| **Time of pain** | | |
| Evening | 20 | 33.3% |
| Night | 40 | 66.7% |
| **Pain decrease with** | | |
| Massage | 35 | 58.4% |
| Medicine | 14 | 23.3% |
| Spontaneously | 11 | 18.3% |
| **Hampers activities like** | | |
| Sleep | 15 | 25.0% |
| Playing | 12 | 20.0% |
| Schooling | 4 | 6.7% |
| Does not | 29 | 48.3% |
| **Pain associated with** | | |
| Headache | 29 | 48.3% |
| Abdominal pain | 27 | 45.0% |
| None | 25 | 41.7% |

Table 3. Comparison of S. Vitamin-D level [25-(OH)D in case and control groups (n = 60 + 30).

| Test | Case (n= 60) (mean ± SD) | Control (n= 30) (mean ± SD) | p-value |
|---|---|---|---|
| S. Vitamin D level (ng/ml) | 14.36 ± 6.17 | 27.16 ± 8.20 | <0.0001 |

(Independent-Samples T test was performed).

Table 4 demonstrates the comparison of BMD in case and control groups. Here the bone mineral density (BMD) is significantly lower at Lumbar vertebra (L - L4) in children with growing pain (Z score −1.13 ± 1.34) than controls (Z score 0.72 ± 0.82) representing bone mineral status of trabecular bones. This study demonstrates BMD is also significantly lower in children with growing pain in comparison to control in right femoral neck region (Z score: −1.18 ± 0.91 vs 0.71 ± 0.81) and left femoral neck (Z score: −1.15 ± 1.07 vs 0.88 ± 0.89) representing bone mineral status of cortical bones ($p < 0.0001$). (Independent samples T test was performed).

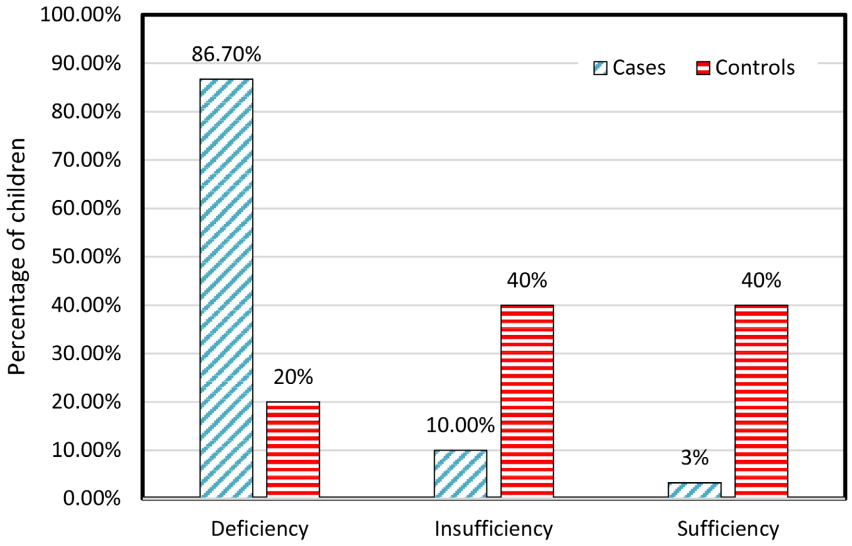


**Figure 1.** Vitamin D status among cases and controls (n= 60 + 30).

**Table 4.** Comparison of BMD between case and control groups (n= 60 + 30).

| Name of Bones at which BMD is measured | Case (n = 60) BMD level (Z score) (mean ± SD) | Control (n=30) BMD level (Z score) (mean ± SD) | 95% CI | p-value |
|---|---|---|---|---|
| Lumbar vertebra (L1 - L4) | −1.13 ± 1.34 | 0.72 ± 0.82 | (−2.38; −1.31) | <0.0001 |
| Right femoral neck | −1.18 ± 0.91 | 0.71 ± 0.81 | (−2.29; −1.50) | <0.0001 |
| Left femoral neck | −1.15 ± 1.07 | 0.88 ± 0.89 | (−2.48; −1.57) | <0.0001 |

(Independent Samples T test was performed).

## 4. Discussion

Recurrent lower limb pains, termed growing pains or growth pains, constitute the most frequent cause of musculoskeletal pain in children [16]. Literature suggested significant association of growing pain with Vitamin D level and bone mineral status in the different studies. Our study aimed to assess the vitamin D and BMD status and to detect their association with growing pain in Bangladeshi children.

Age distribution of growing pain children with growing pain was between 6 - 12 years and highest numbers of cases (73.3%) were found in more than 10 year age group in the present study. Haque *et al.* (2021) in a Bangladeshi community study observed similar findings in their study [14]. They found that about 37.2% of GP children were 10 - 12 years of age groups which was much lower than our study observation. An Australian community study demonstrated observed 37% of GP in children were within 4 - 6 years age group [19]. The reason behind this delayed presentation of the present study could be that many of these patients were initially wrongly diagnosed as a case of Rheumatic fever or JIA and received treatment with Penicillin prophylaxis, NSAIDs and sometimes even ste-

roids. Later on they were diagnosed as growing pain when they came to this tertiary referral center.

In the current study, boys were found to be predominant in the GP children. This finding is consistent with the Haque *et al.* (2021) study, where 60.7% were boys [14]. In a Turkish study by Vehapoglu *et al.* (2015) found that most (56.6%) of the GP cases were girls [1]. This dissimilarity proved that boys were getting more care due to socio-cultural context of our country.

In this study, calf muscle pain was the commonest site followed by front of thigh and back of knee. These finding was also similar to Insaf study (2017) where 51.4% experienced calf muscle pain, followed by front of thigh and back of knee pain [17]. All children had experienced pain mostly at night (66.7%) and at evening (33.3%), which is consistent with the studies conducted by Haque *et al.* 2016 [20]. In a study conducted by Haque *et al.* (2016) found 7 - 12 months disease duration was highest (35.9%) followed by 13 - 24 months duration which was matched with the present study [20]. This finding might suggest that many of these patients were initially misdiagnosed as a case of Rheumatic fever or JIA or parents addressed this issue after prolonged duration of illness. Growing pain did not hamper any activities in most of the children in our study. Some of the children experienced sleep disturbance followed by difficulty in playing and schooling. These findings were also in accordance with the study conducted by Haque *et al.* (2016) where 42.5% growing pain patients did not complain of any difficulty in daily activities [20]. This study showed that pain was associated mostly with headache followed by abdominal pain which was similar to the study done by Oster and Neilsen *et al.* (1972) [21]. This observation indicates that these children have increased susceptibility to other pains and aches such as headache and abdominal pain.

Morandi *et al.* in their study reported 33 children with growing pain had low mean vitamin D level which was 15.7 ± 6.9 ng/ml [16]. In another Bangladeshi study also showed mean serum vitamin D level (18.35 ± 6.26 ng/ml) among GP children was significantly lower than the control ($p < 0.0001$) [14]. These studies indicate significant association of serum vitamin D level with growing pain which were similar to the present study.

A study done by Sharma *et al.* in a tertiary hospital of Northern India found that 100% children with GP had hypovitaminosis D, 91.1% of them being deficient [13]. Another study conducted in Pakistan reported that 94% of children with growing pains had hypovitaminosis D and 72% of them had vitamin D deficiency [22]. These findings were similar with observations in our study which signifies that children with GP were predominantly vitamin D deficient. Low vitamin-D status adversely affects bone mass, bone turn-over and muscle strength in children, which could very well contribute to causation of GP in children [13]. In a state of vitamin D deficiency along with PTH stimulation, osteoblasts continue to deposit collagen rubbery matrix on both the endosteal and periosteal surfaces of the skeleton, this matrix expands under the periosteal covering and

could cause an outward pressure on periosteal sensory pain fibers [16].

Bone mineral density (BMD) was significantly lower in lumbar vertebra (L1 - L4), right and left femoral neck among growing pain patients in comparison to control group found in this study ($p < 0.0001$). There is no study performed to detect bone mineral density by DEXA scan in growing pain patients till today. Friedland *et al.* (2005) demonstrated that tibial speed of sound on USG representing bone mineral status was significantly reduced in children with GP compared to controls (Z score −0.546 for boys and −0.891 for girls; $p = 0.004$, $p < 0.001$, respectively) [8]. Another study by Morandi *et al.* (2014) had also found a comparatively lower bone mineral density in growing pain patients by measuring amplitude-dependent speed of sound (AD-SOS) and the bone transmission time (BTT) using quantitative ultrasound assessment (QUS) [16]. Górska *et al.* 2006 found low BMD measured by DEXA scan in the spine region and lower limbs among 50% of the participants in their series of idiopathic chronic musculoskeletal pain syndromes. This finding was similar to our study in relation to low BMD but their cases were diagnosed as idiopathic chronic musculoskeletal pain syndromes whereas in our study we did the DEXA scan only in children with GP. Low serum concentrations of 25-(OH)D with secondary hyperparathyroidism leads to decreased bone mineral density and resultant osteopenia [13].

Assessment of serum vitamin D level and BMD should be done in growing pain children and appropriate measures for correcting these deficiencies should be taken to improve the quality of life and ultimately reduce the chance of future fractures.

This cross-sectional observational study included 60 cases which was determined by appropriate sample size calculation and 30 healthy children were taken as control. Clinical information, biochemical analysis (serum vitamin D level) and BMD status of cases and controls were assessed in this study. Data was collected in the semi-structured questionnaire and analyzed accordingly. There was no obvious limitation of this study.

## 5. Conclusion

In this study, the majority (96.7%) of children with growing pain had hypovitaminosis D and among them, vitamin D deficiency was predominant. Serum vitamin D level and bone mineral density in lumbar vertebra and both femoral neck were significantly lower in children with growing pain in comparison to the control in this study. From this study, it may be concluded that vitamin D deficiency and low BMD status have a significant association with growing pain in children.

## Acknowledgements

I acknowledge all those children who participated in the study and all my teachers in the Department of Paediatrics at Bangabandhu Sheikh Mujib Medical

University, Dhaka, Bangladesh for their guidance, support and encouragements.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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## Appendix 1. Data Collection Sheet

ID No: ☐☐

Date: _____

Name: _____

Age: …….years……months……

Sex: M/F

Father's Name: _____

Contact number: _____

Address: _____

- Limb pain 1. Present 2. Absent
- Involvement of limb 1. Single 2. Both 3. sometimes single, sometimes both
- Duration of pain ……Year……..Month……..Days……..
- When pain start 1. Morning 2. Afternoon 3. Evening 4. Night
- Site of pain 1. Front of thigh 2. Calf muscle 3. Back of knee
- Pain decrease with 1. Message 2. Medicine 3. Sleep 4. Spontaneously
- Limb pain hampers daily activities such as 1. Schooling 2. Playing 3. Awakened from sleep 4. Does not
- Limb pain associated with joint pain & swelling 1. Yes 2. No
- Limb pain associated with fever 1. Yes 2. No
- Limb pain associated with rash over body 1. Yes 2. No
- Limb pain associated with abdominal pain & headache 1. Yes 2. No
- Child is playful or interactive with other peers 1. Yes 2. No
- Any history of trauma 1. Yes 2. No

**Examination Findings:**

Locomotor system examination of lower limb:

Look:

| Joint name | Redness | | Swelling | | Periarticular muscle wasting | | Joint position | | Any deformity | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Rt | Lt | Rt | Lt | Rt | Lt | Rt | Lt | Rt | Lt |
| Knee | | | | | | | | | | |
| Ankle | | | | | | | | | | |
| Small joint of feet | | | | | | | | | | |

Feel and move:

| Joint name | Temp | | Tenderness | | Flactuation test for knee joint | | Patellar tape test for knee joint | | Restriction of joint movement | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Rt | Lt | Rt | Lt | Rt | Lt | Rt | Lt | Rt | Lt |
| Small joint of feet | | | | | | | | | | |
| Ankle | | | | | | | | | | |
| Knee | | | | | | | | | | |
| Hip | | | | | | | | | | |

**Gait:**

**Muscles of lower limb:**

Muscle tenderness: 1.Present 2. Absent

Muscle power: grade

Muscle colour change: 1. Present 2. Absent

**Biochemical parameters:**

| Laboratory parameters | Values |
|---|---|
| S.Vit D (ng/ml) | |

**Bone mineral densitometry data.**

| DEXA | **Value** |
|---|---|
| Lumbar spine (Z score) | |
| Femoral Rt (Z score) | |
| Femoral Lt (Z score) | |