Does type 1 diabetes mellitus affect bone quality in prepubertal children?

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Original Article

Abstract

Objectives: Type 1 diabetes mellitus (T1DM) in children often starts before the achievement of peak bone mass. This may constitute a landmark in predicting bone fracture risk later in their lives. This study aims to determine the serum levels of bone markers in children with T1DM in combination with their bone mineral density (BMD).

Methods: Children diagnosed with T1DM for 3 years or more without signs of puberty were included in the diabetic group. Another group of age-matched healthy non-diabetic controls was recruited from a local school. The serum levels of a group of biochemical markers for bone formation and resorption were determined in both study groups, and BMD was measured by ultrasound absorptiometry.

Results: Thirty six children with T1DM and 39 normal children were included in this study. The results showed that 24/36 (66.7%) diabetic children had a Z score below zero. Of these, five scored below \(-1\). In contrast, 12/39 (30.8%) children from the control group had a Z score below zero, but none had a score below \(-1\). Significantly lower levels of osteocalcin and procollagen N-terminal peptide were detected in the diabetic group. The serum levels of bone resorption markers were significantly higher in the diabetic group.

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Osteoporosis has become an alarming health problem throughout the entire world, and approximately 200 million people in the world are threatened by this deleterious disease. Osteoporosis is often described as a silent disease because it is typically asymptomatic until a fracture occurs. Like osteoporosis, diabetes mellitus (DM) is a pandemic and a chronic metabolic disorder. In fact, 374 million people in the world may suffer from it, and approximately 200 million people in the world are threatened by this deleterious disease.

**Introduction**

Osteoporosis has become an alarming health problem throughout the entire world, and approximately 200 million people in the world are threatened by this deleterious disease. Osteoporosis is often described as a silent disease because it is typically asymptomatic until a fracture occurs. Like osteoporosis, diabetes mellitus (DM) is a pandemic and a chronic metabolic disorder. In fact, 374 million people in the world may suffer from it, and approximately 200 million people in the world are threatened by this deleterious disease. As a chronic condition, DM adversely affects many different parts of the body including bones, nerves, muscles, eyes, and kidneys. As increased rates of bone fractures and osteoporosis have been found among patients with type 1 DM (T1DM), in both children and adults, and because the mechanisms of diabetic effects on bone cells are very complex, several researchers have described different mechanisms that showed how DM induces osteoporosis and bone fractures through multiple pathways.

Bone marrow-derived endothelial progenitor cells (EPCs) play a significant role in bone healing. DM was found to down-regulate the expression of EPCs through different mechanisms and thereby decrease bone formation at fracture sites. DM is also responsible for the deposition of lipid in the bone marrow, thereby leading to the expansion of the marrow cavity and a decrease in the rate of blood flow to the bone, which is required for the transfer of nutrients. The transformation of osteoblast to adipocyte leads to the reduction of osteoblasts available for bone formation. It is known that DM is responsible for the over expression of advanced glycation end products (AGE) and has roles in bone rigidity. Pancreatic β cells also produce other osteoporotic factors including amylin and preptin. Amylin and preptin induce bone formation and sequester bone resorption and reduce the apoposis of osteoblasts. Osteocalcin is a peptide that positively regulates osteogenesis. DM limits the production of osteocalcin through the negative regulation of osteoblasts by decreased synthesis of insulin, amylin, and preptin.

Dual energy X-ray absorptiometry and peripheral quantitative computed tomography may be used to measure bone mineral density (BMD) in children who are exposed to an increased risk of osteoporosis in their adulthood, but both expose children to unnecessarily ionizing radiation, which is a limiting factor for preventive studies in children. Therefore, in recent years, quantitative ultrasound (QUS) methods have been developed to assess bone mineral status in some peripheral skeletal sites such as hand phalanges, tibia and calcaneus. QUS techniques are safe, easy to use, radiation-free, and come with portable devices so they are particularly suitable and indicated to assess bone mineral status in children.

The optimal use of bone marker measurement with assessment of BMD using QUS of the calcaneum in predicting osteoporosis has not yet been established. Therefore, the present study aims to investigate the levels of bone markers in plasma of children with T1DM in combination with their BMD.

**Materials and Methods**

**Setting**

This study was conducted in the Maternity and Children Hospital (MCH), Almadinah Almunawwarah, Kingdom of Saudi Arabia and was approved by the MCH research and ethics committee. The diabetes unit at MCH provides comprehensive services for children with DM up to the age of 12 years, and the city has one of the highest incidences of childhood T1DM in the world. In this study, BMD was measured using QUS of the calcaneum according to the method of Valerio et al. All measurements were converted to Z-scores using a data bank for age-matched speed of sound values supplied by the manufacturer.

**Conclusion**

T1DM decreases BMD and some bone formation and increases some bone resorption biomarkers. BMD and bone markers are useful diagnostic tools for the early detection of alterations in the bone quality of children with T1DM. This, if treated in a timely manner, may decrease future bone fracture susceptibility.

**Keywords:** Bone mineral density; Osteoporosis; Osteocalcin; Quantitative ultrasound; Vitamin D
Biochemical bone markers

Human osteocalcin assay was performed on a multiwell plate. In addition, the levels of the bone formation markers procollagen N-terminal peptide (PINP), and carboxyterminalpropeptide of type I procollagen (PICP), bone resorption markers [tartrate resistant acid phosphatase (TRAP), pyridinoline (PYD), cross-linked carboxy-terminal telopeptide of type I collagen (CTX-I) and deoxypyridinoline (DPD)] and vitamin D were measured using appropriate commercial ELISA kits (MyBioSource Incorporation, San Diego, CA, USA).

Assay of bone alkaline phosphatase activity

Alkaline phosphatase (ALP) (AlP; EC 3.1.3.1) catalyses the transfer of the phosphate group from para-nitrophenyl phosphate and liberating para-nitrophenol in an alkaline medium. The incubation mixture contained 2-amino-2-methyl-1-propanol (0.35 mol/L), zinc sulphate (1 mmol/L), magnesium acetate (2 mmol/L), pH 10.4, 4-nitrophenylphosphate (12 mmol/L), and 20 microliters of plasma. The liberated para-nitrophenol was measured calorimetrically at 405 nm every minute for three minutes, and the average absorbance per minute was calculated.

Statistical analyses

Statistical analyses were performed using the SPSS statistical software package. Normality and homogeneity of the data were confirmed before ANOVA; differences among the study groups were assessed by one-way ANOVA followed by Duncan’s multiple range tests.

Results

There is no significant difference between the mean age of both groups of the control and diabetic children. As expected, all diabetic children showed higher levels of glycated haemoglobin (HgA1C) than those of control group (Table 1).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Non-diabetic control (n = 39)</th>
<th>Group with Type 1 diabetes mellitus (n = 36)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD (Z score)</td>
<td>0.495 ± 0.169</td>
<td>−0.593 ± 0.230</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>7.89</td>
<td>7.99</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Hg A1C</td>
<td>Normal</td>
<td>10.69 ± 0.392</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>0.0</td>
<td>4.51 ± 0.298</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Figure 1 shows the distribution of Z score versus age among control and diabetic children. It shows that the majority of diabetic children have Z scores below zero (24/36, 66.7% with a mean Z score of −0.593 ± 0.230), and five of them (13.9%) actually have Z scores below −1 (low BMD), whereas only 12/39 (30.8%) of the children in the control group are below zero and no one was below −1 (mean of Z score −0.495 ± 0.169), which indicates that diabetes plays a role in decreasing BMD.

Among bone formation markers, osteocalcin and PINP levels were significantly lower in diabetic children than in the control subjects. Conversely, PICP did not differ significantly (Table 2). Among bone resorption markers in diabetic children, TRAP, PYD and CTX-I levels were found to be significantly higher in diabetics (Table 3). On the other hand, the level of vitamin D (non-diabetic 23.94 ± 0.98, diabetics 26.79 ± 1.05) did not differ significantly (P > 0.05) between the two groups, although the level in diabetic children was slightly higher.

Discussion

Many studies have confirmed that T1DM is associated with decreased BMD. In agreement with these observations, the present study showed that BMD (Z score) was lower in T1DM patients than in the control group. The score in most children was not low enough to be labelled as high risk for fractures at the time of the study, which explains why they did not experience frequent fractures. Conversely, 13.9% of the diabetic children had Z score...
scores below –1, whereas no one from the control group had a Z score below –1. This may explain the higher percentage of osteopenia in older diabetics compared to the general population. Keeping this in mind, one might suggest interfering earlier in diabetic children to prevent future osteopenia and osteoporosis with proper advice regarding exercise and supplementations.

The development of biochemical markers that are specific and sensitive in reflecting the overall rate of bone formation and resorption has markedly improved the non-invasive evaluation of bone turnover in various metabolic bone diseases such as DM. Serum or plasma bone specific ALP (BSAP), osteocalcin, P1CP and P1NP are the most widely measured bone formation markers. The levels of osteocalcin and P1NP in the present study were lower in the serum of diabetic children than that of non-diabetic control. Supporting our finding, it has been found that diabetic osteopenic patients displayed lower serum levels of P1NP, which reflects poor bone formation. P1NP has several functional advantages and has been recommended by the one Marker Standards Working Group due to low inter-individual variability and its stability in serum at room temperature. It is also used as a preliminary biomarker on the effectiveness of a given drug on bone formation.

In the present study, the decreased level of osteocalcin was correlated with low BMD, whereas the activity of ALP was not. However, in other studies, BMD was not correlated with either osteocalcin or ALP. A decrease in bone formation in DM might be due to low levels of serum PTH. In addition, DM reduces osteocalcin levels through the negative regulation of osteoblasts by decreased synthesis of insulin, amylin and preptin.

The majority of bone resorption markers are bone collagen degradation products, with the exception of TRAP. Examples include PYD and DPD, and CTX-1, of which CTX-1 is considered the marker of choice. TRAP in children with T1DM increased gradually and after 12 months was higher than at onset. In the current study, the levels of TRAP, CTX-1 and PYD increased in the serum of diabetic children compared to that of normal children. In other studies, it has been found that levels of both CTX-1 and BMD were lower in T1DM than in the control group. It has been suggested that increased inflammation and induced levels of cytokines are responsible for the acceleration of bone turnover and bone loss in DM patients. Another possible mechanism is that DM is associated with oxidative reactions. Many studies have shown that increases in free oxygen radical levels could induce tissue damage and abnormalities in antioxidant defences in DM.

We conclude that children with T1DM have lower BMD (Z score), low bone formation and high bone resorption markers, which might lead to osteoporosis in the future. Future double-blind studies on the benefits of early intervention to reduce the chances of osteoporosis in diabetics are needed. Moreover, bone marker measurements in combination with BMD are more useful than measuring BMD alone for predicting osteoporosis in diabetic children. In addition, we recommend the QUS devices for their simplicity, the lack of radiation exposure, and the ease of the examination, which can be carried out at a bedside.

Authors contribution

KK and SS designed the study and submitted the grant. MM and AH recruited the patients. SS wrote the first draft and KK, MM and AH were responsible for the critical revision of the manuscript. All authors read and approved the final submitted version.

Conflict of interest

All authors of this manuscript declare that there is no conflict of interest.

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References


