

Serum Osteocalcin, Zinc Nutritive Status and Bone Turnover in Children and Adolescents with Type 1 Diabetes Mellitus

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Introduction

It has been recognized that the alterations in mineral and bone metabolism were associated with DM and that the resulting bone loss is one of the chronic complications of diabetic patients [1]. Osteocalcin, is one of the osteoblast-specific proteins. It is the most abundant bone metabolic protein produced by osteoblast and thus reflects osteoblastic function [2]. Zinc has stimulatory effect on important hormones involved in bone growth such as growth hormone, serum IGF-IGFBP-3, alkaline phosphatase and osteocalcin [3].

This work aimed to study the effect of dietary zinc intake on bone turnover measured through serum Osteocalcin level in patients with T1DM, in addition to define different factors affecting it.

Procedure

The study included 60 children and adolescents with T1DM recruited from Diabetes Clinic, Ain-shams University. Controls consisted of 40 healthy children and adolescents matched in age, gender, BMI and pubertal staging to the study group. A written consent was taken from the parents of both patients and controls. Patients known to take medication that affect bone metabolism was excluded e.g. (Ca, vitamin -D or steroids). All patients presented in the study had normal serum vitamin D level.

Demographic and disease related data were taken, including; age of onset and duration of diabetes, regimen, type and dose of insulin used, history of chronic bone-aches or previous bone fracture on minor trauma and the recorded full dietary intake for 3 days prior to the study [4] to determine the mean dietary zinc intake.

Physical examination including anthropometric measurements and pubertal staging [5] were recorded. Patients with tanner stage

1 are defined as prepubertal and those with tanner stage 2 or above are considered pubertal. Laboratory investigations included were; measurements of the mean fasting and postprandial blood glucose of the last month (at least 30 readings), the mean HbA1c of the last year measurements (4 reading), fasting serum osteocalcin measurement via ELISA and serum zinc assay by direct colorimetric method.

Statistical analysis

The data were processed on computer using SPSS (version 15). Chi-Square test was used to test the association variables for categorical data. Student's t-test was used to assess the difference between two independent samples. Correlation analysis was used to assess the association between two variables. Linear Regression analysis: was used to search for a panel (independent parameters) that can predict the target parameter (osteocalcin). P-values less than 0.05 were considered significant, while at 0.01 or 0.001 were considered highly significant

Results

This study was conducted on 60 children and adolescents with T1DM. They were 25 males and 35 females with a mean age of 12.23± 4.26 years. Patients and controls were well matching regarding demographic characteristics, anthropometric measurements and pubertal staging. Serum osteocalcin was significantly lower in diabetic patients compared to control group (P= 0.00), While there was no significant difference regarding zinc dietary intake and serum zinc (P> 0.05) (Table 1). Serum osteocalcin was significantly higher in prepubertal patients compared to pubertal patients (P= 0.004), while there was no statistical difference in serum zinc and dietary zinc intake as regards pubertal or prepubertal patients (P> 0.05) (Table 2). Serum osteocalcin was lower in patients with HbA1c ≥7.5 compared to patients with HbA1c < 7.5, (P= 0.014). Serum zinc and dietary zinc intake was significantly lower in patients with history of chronic bone aches (P< 0.05), Serum osteocalcin was also lower in patients who had a history of chronic bone aches but of no statistical significant value (P>0.05) (Table 3). Daily dietary zinc intake was positively correlated with height, serum zinc and serum osteocalcin. While serum osteocalcin was significantly positively correlated with serum zinc, and it was significantly negatively correlated with age, age of onset, height, weight, BMI and HbA1c (Figure 1-4).

Variable	Patients (n=60)	Controls (n=40)	Test of sig.	P value
Daily dietary zinc intake (mg/day)	6.24± 2.6	6.2± 2.8	t= 0.07	0.94
Serum zinc (µg/dl)	129.67± 25.85	131.3± 20.88	t= -2.27	0.78
Serum Osteocalcin (ng/ml)	24.52± 10.84	35.69± 11.68	t= -4.89	0.000*

Table 1: Comparison between diabetic patients and control as regards daily dietary zinc intake, serum zinc and serum osteocalcin.

Variable	pre pubertal (n=18)	Pubertal (n=42)	Test of sig.	P value
Glycated Hb%	8.21±1.23	9.05± 1.87	1.91	0.062
Zinc daily dietary intake (mg/day)	5.59± 2.74	6.51± 2.53	t= 1.57	0.215
Serum zinc (µg/dl)	124.5± 30.15	132.31± 23.79	t= 1.15	0.287
Serum osteocalcin (ng/ml)	30.53± 9.35	21.94± 10.49	t= 8.97	0.004**

Table 2: Comparison between prepubertal (tanner stage 1) and pubertal (tanner stage >1) diabetic patients as regards glycated Hb%, zinc daily dietary intake, serum zinc and serum osteocalcin.

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Variable	positive history of bone aches (n=35)	negative history of bone aches (n=25)	Test of sig.	P value
Zinc daily dietary intake (mg/day)	5.67± 2.13	7.03± 3.02	t= 2.04	0.046*
Serum zinc (µg/dl)	123.71± 20.06	138.72± 30.59	t= 2.3	0.025*
Serum osteocalcin (ng/ml)	23.44± 11.6	26.02± 9.7	t= 0.91	0.369

Table 3: Comparison between diabetic patients with positive history of boneaches and those with negative history as regards daily dietary zinc intake, serum zinc and serum osteocalcin.

Variable	R square	F	P value
Tanner stage	0.322	27.505	0.000
Zinc daily dietary intake	0.462	24.510	0.000
HbA1c	0.501	18.724	0.000

Table 4: Linear multiregression analysis showing the most important predictors of serum osteocalcin level as a marker of bone turnover.

Regression analysis revealed that the most sensitive predictors for serum osteocalcin level as a marker of bone turnover were tanner staging, zinc dietary intake and HbA1c (Table 4).

Discussion

The changes in the bone mineral density (BMD) and bone turnover markers that occur in the two major clinical types of diabetes (type 1 and type 2) differ because they are associated with different pathogenetic mechanisms. While it is reduction of the BMD that most often occurs in T1DM, in type 2 diabetes various studies, diagnose either a normal, reduced or increased BMD [6].

In our study, patients and controls were well matched regarding age, gender, anthropometric measurements and pubertal staging in order to minimize the influence of age, gender, BMI and pubertal hormonal changes on altered bone metabolism. This study revealed that there was a significant lower serum osteocalcin in diabetic patients compared to controls. These result was in accordance with that reported by Thrailkill et al. [7] and Brandao et al. [8] On the other hand Alexopoulou et al. [9] found that there was no significant difference between patients with T1DM and controls regarding serum osteocalcin level. T1DM has been related to reduce BMD in childhood [10]. In addition, it has been recognized that insulin is essential for osteoblast function, as well as for chondrogenesis and collagen synthesis [11]. In patients with T1DM the impaired bone formation may result from absolute deficiency of insulin and insulin-like growth factor-1 (IGF1), which leads to lower values of peak bone mass [12].

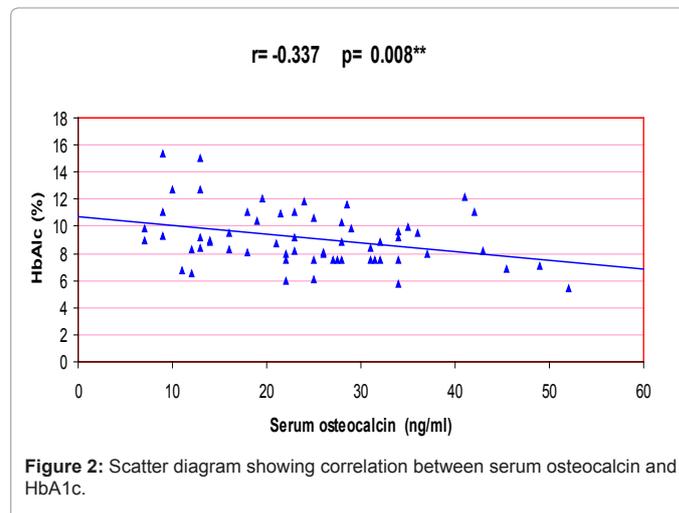
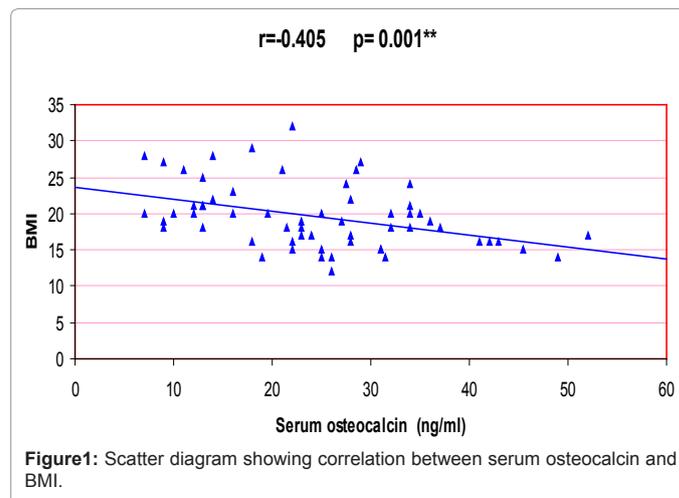
No consensus has been reached as regards normal values for bone markers in healthy children. Bone marker levels increase during the first 3–4 years of life due to increased bone turnover and remains steady until the beginning of puberty, after which they appear to increase parallel to pubertal and height velocity. After 21 years of age, the bone markers return to pre-pubertal levels [13]. Suzuki et al. [1] reported a positive correlation between PTH and osteocalcin, it is speculated that the relative hypofunction of parathyroid gland causes the decreased activity of diabetic osteoblasts. Other study has demonstrated the evidence that the osteoblastic function was decreased whereas the osteoclastic function was conversely elevated in diabetic patients [14]. However, it has been still controversial if the osteoclastic function in diabetes is elevated or not.

This study revealed no difference between diabetic patients and controls regarding zinc dietary intake and serum zinc. Our results were in accordance with that reported by Cunningham et al. [15].

Meanwhile other studies observed that plasma zinc levels were lower in diabetic patients than controls [16,17]. Arreola et al. [16] showed a significant decrease in both bone mineral content and zinc, suggesting that zinc deficiency may be a contributory factor to bone loss in T1DM individuals with poor glycemic control. Zinc plays several roles in bone metabolism, work in cell cultures and animal models have shown stimulation of osteoblasts by zinc [18], while osteoclastic cell formation was inhibited [19]. In addition, the anabolic effect of IGF-I in osteoblasts is enhanced by zinc [20].

Hill et al. [21] showed that zinc stimulates osteoblasts in adults withT1DM. Zinc deficiency, however, impairs DNA synthesis and protein metabolism, negatively impacting bone formation [22]. A number of physiologic systems contribute to zinc homeostasis under different conditions. Changes in absorption of exogenous zinc and gastrointestinal secretion and excretion of endogenous zinc are the primary mechanisms for maintaining zinc homeostasis. Adjustments in renal excretion also occur with low or high intakes of zinc. Tissue and cellular redistribution of zinc may contribute further to the maintenance of zinc homeostasis [23,24].

Serum osteocalcin was significantly higher in patients with HbA1c ≤ 7.5 compared to patients with HbA1c > 7.5. Previous studies suggested that metabolic control plays an important role in bone mineral density in patients with T1DM, so that; poorly glycemic controlled



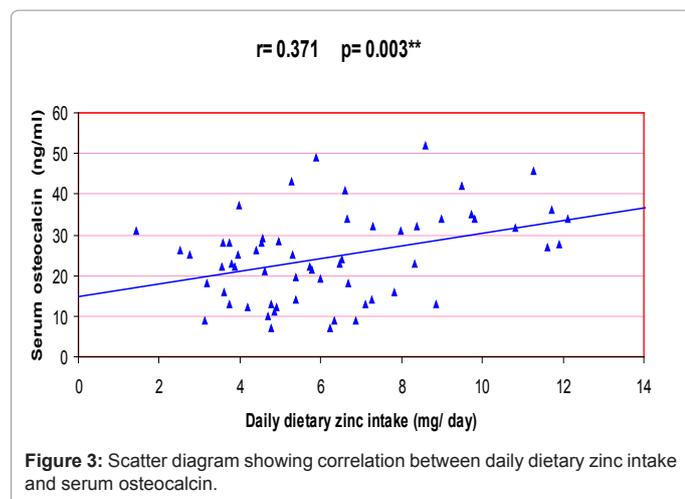


Figure 3: Scatter diagram showing correlation between daily dietary zinc intake and serum osteocalcin.

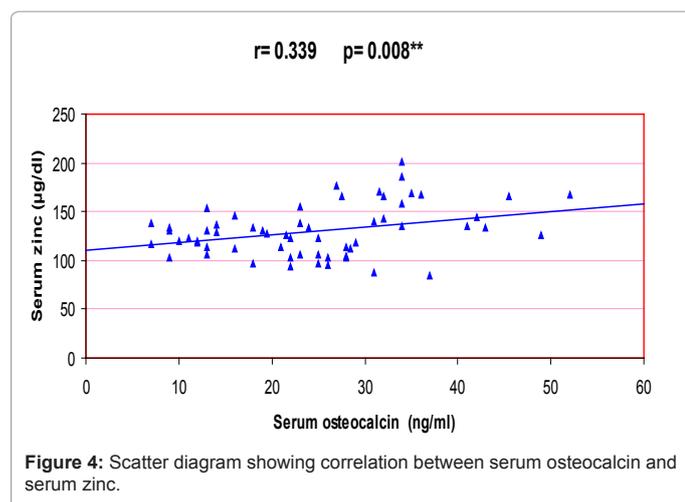


Figure 4: Scatter diagram showing correlation between serum osteocalcin and serum zinc.

patients had significantly lower osteocalcin level than good glycemic controlled patients [8,10]. The persistently poor metabolic control in adolescents with T1DM increases the risk of osteoporosis in adult life [25]. Furthermore, Campos et al. [26] reported that optimization of metabolic control could lead to a cessation of bone destruction after seven years duration of diabetes. Osteocalcin negatively correlated with fasting plasma glucose and HbA1c in both men and postmenopausal women [27]. On the other hand, Alexopoulou et al. [9] found that there was no influence of HbA1c on biomarkers of bone resorption and formation.

Our study revealed that diabetic patients with positive history of bone aches had significantly lower serum zinc and lower daily dietary zinc intake compared to those patients with negative history of boneaches. In addition, serum osteocalcin was lower in patients with history of bone aches compared to diabetic patients without history of boneaches but the results did not reach a statistically significant level. Inadequate intake of zinc has been reported as a risk factor for fractures in men [28]. Ozturk et al. [29] has suggested that zinc deficiency leads to an increase in free radical production. Oxidative stress has been shown to be an independent risk factor for osteoporosis [30].

This study revealed that serum osteocalcin negatively correlated with the duration of diabetes, but the results did not reach a statistical significance. Brandao et al. [8] found no correlation between the

bone markers and the duration of diabetes. The impact of duration of diabetes on bone formation and markers of bone turnover has different explanations. Patients with recent onset T1DM may have impaired bone formation because of the absence of the anabolic effects of insulin, whereas in long-standing type 1 diabetes mellitus, vascular complications may account for low bone mass and increased fracture risk [31].

In our study, serum osteocalcin was negative correlated with age and BMI. These findings are in line with a previous study done by Cifuentes et al. [32] who showed that serum osteocalcin levels were higher in normal weight than in obese diabetic patients. They also reported a negative correlation between osteocalcin and body weight. In addition, Jee-Aee et al. [33] showed that serum osteocalcin level negatively correlated with BMI. Fares et al. [34] found that age and BMI negatively correlated with markers of bone formation.

Serum osteocalcin was significantly higher in prepubertal patients compared to pubertal patients. This is agreed with Brandao et al. [8] who found that osteocalcin is inversely related with pubertal development. Our study showed that dietary zinc intake and serum zinc is positively associated with osteocalcin as a marker of bone turnover in patients with T1DM. The effect of zinc supplementation on endogenous GH secretion, serum IGF-I and IGFBP-3 concentrations, bone formation markers, and linear growth of non-zinc-deficient children with idiopathic short stature showed direct stimulatory effect of zinc on serum alkaline phosphatase and osteocalcin [3]. Maser et al. [36] showed that zinc dietary intake positively correlated with serum osteocalcin. Logistic stepwise multiregression analysis revealed that the most sensitive predictors for serum osteocalcin level as a marker of bone turnover were tanner staging, zinc daily dietary intake and HbA1c respectively.

In conclusion, patients with type 1 diabetes found to have lower serum osteocalcin, which reflect a decreased activity of osteoblasts. Patients with poor glycemic control have lower serum osteocalcin. Normal serum Zinc and good Zinc dietary intake alone is not enough to improve osteoblastic function, but optimization of glucose metabolic profile in addition may help to improve osteoblasts activity and prevent bone complications. Longitudinal studies are required for following-up of bone turnover markers and bone mass in diabetic patients to assess the impact of different minerals intake.

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