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Serum Osteocalcin Levels in Girls with Central Precocious Puberty: Relation to the Onset of Puberty

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Osteocalcin is the non-collagenous protein produced by osteoblasts in bone. When it is released into systemic circulation in its uncarboxylated form, it regulates fat and glucose metabolism. Recent studies have shown that osteocalcin is also involved in male fertility. Because the onset of puberty is determined by ethnic, genetic, environmental, and metabolic factors, we focused on determining the role of osteocalcin in the onset of puberty. Central precocious puberty (CPP) is defined as the activation of the hypothalamic-pituitary-gonadal axis before the age of 8 in girls and 9 in boys. CPP is diagnosed when peak luteinizing hormone (LH) reaches ≥ 5.0 IU/l after stimulation with gonadotropin-releasing hormone (GnRH). This retrospective study included 206 girls who showed breast budding before the age of 8 and whose bone age was more advanced than their chronological age. The CPP group included 100 girls who were diagnosed with CPP, and 106 girls were the non-CPP group whose peak LH did not reach ≥ 5.0 IU/l after GnRH stimulation test. Serum osteocalcin levels were measured to investigate the relationship between osteocalcin and the onset of puberty. Our data showed that serum osteocalcin levels were significantly higher in the CPP group (87.7 ± 24.4 ng/ml vs. 68.3 ± 19.5 ng/ml, $P < 0.001$). The multivariate analysis revealed that an increase in bone age and peak LH was significantly associated with the serum osteocalcin level. The results of this study suggest that serum osteocalcin is associated with the onset of puberty in girls.

Keywords: female; luteinizing hormone; osteocalcin; precocious; puberty
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Introduction

Puberty is an important time for developmental processes because it entails dramatic hormonal changes that cause increased growth spurts and bone acquisition. The onset of puberty occurs when the hypothalamic-pituitary-gonadal axis is activated with pulsatile secretion of gonadotropin-releasing hormone (GnRH). Central precocious puberty (CPP) is defined as the activation of the hypothalamic-pituitary-gonadal axis before the age of 8 in girls and 9 in boys.

Precocious puberty has attracted increasing attention due to concerns about early menarche in girls, decreased final height, and psychosocial problems (Klein 1999). Precocious puberty is known to be associated with psychosocial problems, such as premature sexual intercourse and illegal substance use (Carel and Leger 2008). The prevalence of precocious puberty has been increasing, and various factors, including genetics, nutrition, and environmental changes, have been implicated as potential causes.

Recent studies have shown that an increase in calcium utilization is associated with the early physical signs of puberty, but there is insufficient data regarding osteocalcin levels during puberty (Saggese et al. 2002). Moreover, pubertal growth spurts are associated with a rapid increase in the calcium gained by the skeleton. During these periods of accelerated growth, increases in alkaline phosphatase (ALP) and osteocalcin have been shown (Magnusson et al. 1995).

Osteocalcin is the non-collagenous protein produced by the osteoblasts in bone. It is made of three glutamic acid residues that require γ-carboxylation to bind calcium ions. The role of osteocalcin is not yet completely understood, but it is mainly known to regulate bone mineralization and bone turnover (Wei and Karsenty 2015). Osteocalcin levels increase as the peak of growth velocity is reached during puberty (Szulc et al. 2000). The uncarboxylated form of osteocalcin is released into systemic circulation from the osteoblasts, and the metabolic roles of osteocalcin include improving glucose and fat metabolism (Lee et al. 2007). In

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vitro studies have shown that osteocalcin is also involved in the development of male fertility. Osteocalcin stimulates the testes to increase testosterone, but the association between osteocalcin and female fertility has not been confirmed (Oury et al. 2011).

Recent studies have demonstrated that osteocalcin is related to metabolism, but the causal relationship still needs to be investigated (Delmas 1993). The serum levels of osteocalcin are inversely associated with the fasting plasma glucose, and lower osteocalcin levels have been found in overweight and diabetic subjects (Im et al. 2008; Kindblom et al. 2009; Zhou et al. 2009). Therefore, this study was aimed at determining the serum osteocalcin levels in girls with CPP and investigating the relationship between osteocalcin levels and pubertal onset.

Methods

Study population

This study was performed retrospectively by reviewing the medical records of the study subjects: 206 girls who visited Korea University Ansan Hospital for growth checkups between January 2012 and March 2017. All the subjects showed Tanner breast scores ≥ 2 before the age of 8, and their bone age was more advanced than their chronological age. They showed no organic reasons for having CPP (e.g., brain masses or malformations), and there were no other exclusionary criteria.

A complete history was taken, and a physical examination and routine laboratory tests were conducted in all subjects. A GnRH stimulation test was performed on all subjects in order to diagnose CPP. In a clinical setting, CPP was diagnosed when the peak luteinizing hormone (LH) level reaches ≥ 5.0 IU/l after stimulation with GnRH. Based on the GnRH stimulation test results, the subjects were categorized into two groups: CPP group and non-CPP group. The CPP group included 100 girls who were diagnosed with CPP, and 106 girls were the non-CPP group whose peak LH did not reach ≥ 5.0 IU/l after GnRH stimulation test.

Clinical data collection

The clinical data of the subjects were collected from the medical records through a retrospective review. The data collected included age, height, weight, and bone age. Basal serum samples were obtained for the assessment of the LH and follicle-stimulating hormone (FSH) levels before the GnRH injection. In addition, ALP, insulin-like growth factor I (IGF-I), insulin-like growth factor-binding protein 3 (IGFBP-3), and thyroid hormones were measured.

In order to diagnose CPP, a GnRH stimulation test was performed. GnRH 0.1mg (Gonadorelin acetate 0.105 mg/ml, Handok Pharmaceuticals, Seoul, Korea) was administered intravenously for one minute. The basal and post-stimulation samples were taken at 30, 45, 60, and 90 minutes to measure the LH and FSH levels. CPP was diagnosed when the peak LH level after the GnRH stimulation was above 5 IU/l. The serum osteocalcin levels of the girls in the CPP and non-CPP groups were measured at the same time when the GnRH test was performed using an electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, IN, USA). Before the GnRH was given intravenously, the osteocalcin samples were collected with the basal samples of LH and FSH.

Statistical analyses

The differences between the CPP and non-CPP groups were calculated using a *t*-test to compare the continuous variables. Pearson correlation coefficients were calculated to evaluate the relationship between the osteocalcin concentration and the clinical characteristics, such as age, height, weight, bone age, ALP, IGF-I, IGFBP-3, LH, and FSH levels. A multiple regression analysis was then used to determine which factors were associated with the osteocalcin concentration. A *P* value below 0.05 was considered to be statistically significant, and all of the calculations were performed using IBM SPSS Statistics for Windows, version 20 (IBM Corp., Armonk, NY, USA).

Ethics statement

This retrospective study was approved by the Institutional Review Board at Korea University Ansan Hospital in Ansan, South Korea (approval No. 2017AS015). Informed consent was waived by the board.

Results

Table 1 shows the clinical characteristics and laboratory data. The serum osteocalcin levels for the CPP group were significantly higher than those for the non-CPP group (87.7 ± 24.4 ng/ml vs. 68.3 ± 19.5 ng/ml, $P < 0.001$). Moreover, the serum ALP, IGF-I, LH, and FSH levels in the CPP group were significantly higher than those in the non-CPP group ($P < 0.001$).

The serum osteocalcin levels showed positive correlations with age ($r = 0.252$, $P < 0.001$), height ($r = 0.191$, $P = 0.006$), and bone age ($r = 0.383$, $P < 0.001$). Additionally, the serum osteocalcin levels were positively correlated with the serum ALP ($r = 0.168$, $P = 0.016$), IGF-I ($r = 0.221$, $P = 0.001$), IGFBP-3 ($r = 0.162$, $P = 0.001$), basal LH ($r = 0.264$, $P < 0.001$), peak LH ($r = 0.334$, $P < 0.001$), and basal FSH levels ($r = 0.322$, $P < 0.001$) (Table 2).

As shown in Table 3, the multivariate analysis revealed that bone age and peak LH remained as independent predictors of serum osteocalcin. Moreover, increases in bone age and peak LH were significantly associated with serum osteocalcin levels. However, height, glucose, ALP, and IGF-I were shown to have no independent relationships with serum osteocalcin.

Discussion

This is the first study of serum osteocalcin levels in a Korean pediatric population. A recent study by Schündeln et al. demonstrated a positive correlation between osteocalcin and testosterone in the male group, but there was no association between osteocalcin and pubertal development in the female group (Schündeln et al. 2017). However, our data showed that the serum osteocalcin levels for each female group were significantly different. Serum osteocalcin showed a positive correlation with bone age and peak LH levels. These findings suggest that the onset of puberty is affected by the levels of serum osteocalcin.

The main role of osteocalcin is as a marker for bone formation, and its levels are elevated in high bone turnover states (Szulc et al. 2000). The highest bone formation

Table 1. Comparison of the characteristics and laboratory data.

Variables	CPP (n = 100)	Non-CPP (n = 106)	P-value
Age (years)	8.22 ± 0.51	8.10 ± 0.59	0.112
Height (cm)	130.0 ± 6.1	129.0 ± 5.3	0.227
Weight (kg)	30.1 ± 6.8	30.0 ± 7.2	0.903
BMI (kg/m^2)	17.7 ± 2.8	17.9 ± 3.1	0.627
Bone age (years)	10.1 ± 0.8	9.4 ± 0.9	< 0.001
Glucose (mg/dl)	90.5 ± 6.2	90.6 ± 5.8	0.832
ALP (IU/l)	256.5 ± 56.2	227.5 ± 59.2	< 0.001
IGF-I (ng/ml)	277.6 ± 103.0	225.8 ± 96.9	< 0.001
IGFBP3 (ng/ml)	2783.8 ± 556.7	2763.1 ± 528.9	0.786
TSH (mIU/l)	2.66 ± 1.25	2.59 ± 1.24	0.716
fT4 (ng/dl)	1.62 ± 2.15	1.52 ± 0.88	0.670
Basal LH (IU/l)	0.31 ± 0.46	0.10 ± 0.81	< 0.001
Basal FSH (IU/l)	2.42 ± 1.24	1.56 ± 0.80	< 0.001
Peak LH (IU/l)	12.43 ± 8.36	3.13 ± 1.10	< 0.001
Peak FSH (IU/l)	14.27 ± 4.29	12.28 ± 3.99	< 0.001
Osteocalcin (ng/ml)	87.7 ± 24.4	68.3 ± 19.5	< 0.001

Data are shown as the mean ± the standard deviation.
CPP, central precocious puberty; BMI, body mass index; ALP, alkaline phosphatase; IGF-I, insulin-like growth factor I; IGFBP3, insulin-like growth factor-binding protein 3; TSH, thyroid-stimulating hormone; fT4, free thyroxine; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

marker concentrations, including osteocalcin and ALP, have been reported in the early stages of puberty, showing that the pubertal stage is a main determinant of bone marker levels (Blumsohn et al. 1994; Abrams et al. 2000; Federico et al. 2003). Rotteveel et al. showed that procollagen I carboxyterminal propeptide (PICP), the collagen produced by osteoblasts, was related to height velocity and the beginning of pubertal stages (Rotteveel et al. 1997). Our results are consistent with those of previous studies regarding bone formation markers. Our data indicated that the onset of CPP was accompanied by increased osteocalcin levels.

Other than its relationship with bone remodeling, osteocalcin has recently been shown to regulate fat mass and glucose metabolism in animal models. For example, Lee at al. (2007) produced osteocalcin-knockout mice with high glucose concentrations and reduced insulin levels. The study suggested that osteocalcin affects insulin sensitivity and energy expenditure (Lee et al. 2007; Booth et al. 2013). Ferron et al. (2012) found that providing these mice with osteocalcin improved their glucose metabolism. The possibility has been raised that the development of metabolic syndrome is related to osteocalcin.

In humans, Kindblom et al. (2009) showed that the osteocalcin level was inversely associated with fat mass and plasma glucose in Swedish men. Additionally, Im et al. (2008) and Zhou et al. (2009) proposed that osteocalcin is related not only to fat and glucose metabolism, but also to lipid metabolism. Jürimäe et al. (2015) showed that osteocalcin was inversely associated with body adiposity and leptin values in Estonian male adolescents. However, there is limited data from the pediatric population about the relationship between osteocalcin and metabolism.

The onset of puberty is known to be determined by ethnic, genetic, environmental, and metabolic factors (Parent et al. 2005; Slyper 2006). For instance, obesity with elevated leptin levels and insulin resistance has been associated with early puberty (Shalitin and Phillip 2003; Kelsey and Zeitler 2016). Hannon et al. (2006) and Moran et al. (1999) also showed that insulin sensitivity decreases by ~50% during puberty in otherwise healthy adolescents,

Table 2. Correlation of the characteristics and laboratory data with the osteocalcin concentration.

Variables	*r*	*P*-value
Age	0.252	< 0.001
Height	0.191	0.006
Weight	0.048	0.495
BMI	–0.044	0.531
Bone age	0.383	< 0.001
Glucose	–0.019	0.785
ALP	0.168	0.016
IGF-I	0.221	0.001
IGFBP3	0.162	0.021
TSH	–0.017	0.805
fT4	–0.037	0.595
Basal LH	0.264	< 0.001
Basal FSH	0.322	< 0.001
Peak LH	0.334	< 0.001
Peak FSH	0.104	0.138

Pearson's correlation coefficients are shown for associations with serum osteocalcin concentration.
BMI, body mass index; ALP, alkaline phosphatase; IGF-I, insulin-like growth factor I; IGFBP3, insulin-like growth factor-binding protein 3; TSH, thyroid-stimulating hormone; fT4, free thyroxine; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

Table 3. Multiple linear regression analysis of the factors associated with the osteocalcin concentration.

Variables	Coefficient β	*P*-value
Height	–0.055	0.508
Bone age	0.314	< 0.001
Glucose	0.259	0.651
ALP	0.027	0.420
IGF-I	0.102	0.142
Peak LH	0.238	0.027

Height, bone age, glucose, ALP, IGF1, and peak LH were selected as independent variables.
ALP, alkaline phosphatase; IGF-I, insulin-like growth factor I; LH, luteinizing hormone.

and that increased insulin secretion is accompanied by low insulin sensitivity. However, the factors affecting these changes have not yet been clearly defined.

We showed that serum osteocalcin levels were significantly higher in the CPP group, and that bone age and peak LH levels were independent predictors of serum osteocalcin levels. Since insulin secretion is known to increase during puberty, it is paradoxical that the CPP subjects showed higher serum osteocalcin levels than the non-CPP subjects. It is assumed that the increased insulin secretion in pubertal children is compensated for by elevated osteocalcin; however, the insulin sensitivity of the subjects was not investigated in this study. Further studies about the associations between the serum osteocalcin levels and insulin sensitivity are needed to verify our assumption. In addition, according to the results of the multivariate analysis, peak LH levels are independent predictors of serum osteocalcin levels. Therefore, it is suspected that serum osteocalcin levels are related to pubertal onset, regardless of glucose metabolism.

One limitation of this study was that only total osteocalcin was measured, and blood samples were taken regardless of the fasting time. Animal studies have shown that only the uncarboxylated form of osteocalcin regulates insulin production and sensitivity (Lee et al. 2007). In addition, studies about the reference level of osteocalcin in children are still insufficient. According to a study incorporating 1,634 children (833 boys and 801 girls), serum osteocalcin levels tend to increase with age and decrease at ages 12 and 15 in girls and boys, respectively (Cioffi et al. 1997). Serum osteocalcin levels vary due to multiple factors, including age, ethnic background, smoking status, physical fitness levels, and season of the year (Nimptsch et al. 2007). However, these factors were not considered in this research, and no data about these factors on osteocalcin levels in the Korean population have been published.

Another limitation of this study was that the CPP group was not compared with healthy adolescents. The study population was categorized into two groups: a CPP group consisting of girls diagnosed with CPP, and a non-CPP group consisting of girls who did not show an LH level elevation above 5 IU/l after the GnRH injection. Since the girls in the non-CPP group also showed Tanner breast scores ≥ 2 before the age of 8, further studies are needed to compare osteocalcin levels between CPP patients, sex, and age-matched healthy controls.

In conclusion, our data showed that bone age and peak LH levels were significantly associated with serum osteocalcin levels. Our findings suggest the possible role of osteocalcin in the onset of puberty. Although the results of this study showed that the onset of puberty is associated with osteocalcin, further longitudinal studies are required to understand the underlying relationship between osteocalcin and puberty.

Conflict of Interest

The authors declare no conflict of interest.

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