The relationship between osteocalcin levels and sexual stages of puberty in male children

Tolga Altuğ Şen, Orhan Derman, Erol Kınık

Adolescents Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey

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Osteocalcin is a specific and reliable marker which increases with rapid bone turnover and gives data about bone metabolism. The pubertal growth spurt is also known as a good example of rapid bone turnover.

The aim of this study was to determine whether osteocalcin is a useful marker for the pubertal growth spurt period. In this study, osteocalcin levels in male adolescents were examined in relation to their sexual maturation stage and age. The osteocalcin levels and alkaline phosphatase levels were compared during the pubertal growth spurt. Serum osteocalcin and alkaline phosphatase levels were evaluated in 100 eligible healthy male children and adolescents (aged 10 to 17 years). Five groups (n: 20 each) of children and adolescents were formed according to their sexual maturation stages. Finally, the subjects were divided into three main groups in relation to the pubertal growth spurt and sexual maturation stages.

Data were evaluated and compared among these three groups:

First group = Stage 1 (prepuberty) + Stage 2 (early puberty) consisted of 40 (20+20) children and adolescents. Their mean osteocalcin value was 17.2 ± 6.3 ng/ml and alkaline phosphatase 573.8 ± 143.9 IU/L.

Second group: Stage 3 + Stage 4 consisted of 40 (20+20) children and adolescents. These groups were known as the pubertal growth spurt groups. Their mean osteocalcin value was 29.4 ± 10.6 ng/ml and alkaline phosphatase 728.4 ± 233.9 IU/L.

Third group: In this group, there were 20 children and adolescents who reached Stage 5 of sexual maturation and whose pubertal growth spurt was slowing. Their mean osteocalcin value was 15.3 ± 5.8 ng/ml and alkaline phosphatase 435.8 ± 184.8 IU/L.

During the pubertal growth spurt, there is a relationship between bone remodelling and increasing osteocalcin and alkaline phosphatase levels. When sexual maturation reaches Stage 4 at 14 years old, osteocalcin and alkaline phosphatase levels make a peak, associated with the rapid growth in height. As sexual maturation reaches Stage 5, osteocalcin and alkaline phosphatase levels gradually decrease with growth maturation and their levels decline to the level of adults at the completion of this period.

Our study showed that osteocalcin and alkaline phosphatase levels can be used as markers for evaluation of the growth spurt period.

Key words: osteocalcin, pubertal growth, male children.

During bone remodelling, osteocalcin is produced by osteoblasts and its level increases during the events characterized by yrapid bone turnover[1-4]. Osteocalcin is known as a gamma carboxy glutamic acid protein binding calcium. It is rich in bone dentin and other mineralized tissues. Although serum alkaline phosphatase activities and urine hydroxyproline levels are routinely used for evaluating bone metabolism, they are affected by other nonosseous metabolic

events. They are not reliable markers in cases of systemic disorders. Osteocalcin is a bone matrix protein which is specific for bone metabolism and it is not influenced by metabolic bone disorders. In adolescents, the pubertal growth spurt is accompanied very closely by sexual maturation[1,4,5]. During childhood, the rate of gain in height is approximately 5 cm per year; it shows a slow decrease just before the puberty period and after that rapidly increases. It is known as growth blowing or the growth spurt of adolescents[6]. During this period, adolescents gain 15 percent of their adult height, 40 percent of total body mineralization 50 percent of weight and 54 percent of body muscular mass. During the growth spurt, the increase in bone turnover correlates with osteocalcin and alkaline phosphatase levels. There are some diseases which influence bone turnover. For example, osteocalcin levels are increased in primary hyperparathyroidism, hyperthyroidism, with gonadotropin releasing hormone, in chronic renal disease, Paget's disease, rickets, malignant disease, osteoporosis and especially under hemodialysis, and are decreased in liver disease, with estrogen, and in pregnancy, hypothyroidism and hypoparathyroidism. Three vitamins (K, D and C) play an important role in this pathway. Vitamin K plays a major role in glutamic acid production. Vitamin C plays a role in conversion of proline to hydroxyproline, and vitamin D regulates osteocalcin gene transcription[7-8]. Serum osteocalcin plays an important role in the growth of the skeleton correlating with insulin-like growth factor 1 (IGF-1), and it is shown that osteocalcin synthesis in the rat is stimulated by IGF-1. Osteocalcin levels are in correlation with IGF-1 levels[9-10].

The aim of the study was to examine osteocalcin levels in relation with the growth spurt.

Material and Methods

One hundred healthy male children aged between 10 and 17 years who were admitted to the adolescent outpatient clinic were included in the study.

They were chosen according to the following criteria:

1. Absence of growth retardation.
2. No restriction of their normal (physical) activity.
3. Skeletal age appropriate for chronological age.
4. Absence of systemic disease.
5. No medication.

Greulich and Pyle's radiographic atlas of skeletal development of the hand and wrist was used for determination of skeletal ages. Alkaline phosphatase levels were measured using spectrophotometric method measuring products of the alkaline phosphatase enzyme and p-nitrophenyl phosphatase reaction. All results were expressed in international unit/litre (IU/L) (BM/Hitochi 917 Systems Pack) All osteocalcin samples were taken after fasting between 8 cm and 9 cm and preserved 30 minutes at room temperature before centrifuge. Osteocalcin levels were measured using ELISA kits (Novo Calcin Metro Biosystems) and results were expressed as ng/ml (nanogram/milliliter). J.M. Tanner's classification[11] was used for sexual maturation staging in the male children. Testicular volumes were evaluated by Proder's orchidometer. Children were divided into five groups according to their sexual maturation stages. Each group consisted of 20 children. At first, clinical and laboratory findings were collected and evaluated in these five groups. Later, some groups were combined according to sexual maturation stages and findings; three groups were formed. Statistical analyses were performed using one way variance analysis ANOVA + Tukey HSD test to compare the osteocalcin levels with the alkaline phosphatase activities in each group. Spearmen correlation test was used to show the relationship between osteocalcin levels and alkaline phosphatase activities.

Results

According to the sexual maturation stages, all data were evaluated and compared in three groups:

Group 1:
Stage 1 (prepuberty) + Stage 2 (early puberty);

Group 2:
Stage 3 + Stage 4 (pubertal growth spurt); and

Group 3:
Stage 5 (late puberty).

The peak levels of osteocalcin and alkaline phosphatase that were determined in Group 2 are given in Table I. Mean alkaline phosphatase levels in Group 2 were significantly higher than in Groups 1 and 3. (728.4 IU/L, 573.8 IU/L, 435.7 IU/L, respectively, $p < 0.05$). Mean osteocalcin level in Group 2 was significantly higher than in Groups 1 and 3 (29.4 ng/ml, 17.2 ng/ml, 15.3 ng/ml, respectively, $P < 0.05$).

There was a significant difference between Groups 1 and 2 and also between Groups 2 and 3, but there was no significant difference between Groups 1 and 3. There was a positive correlation between osteocalcin and alkaline phosphatase levels (Fig. 1), and between osteocalcin levels and sexual maturation stages. Mean osteocalcin levels and sexual maturation stages. Mean osteocalcin levels were highest in the fourth stage (36.3 ng/ml) versus the others: stage 1 18.1 ng/ml; stage 2 16.4 ng/ml; stage 3 22.6 ng/ml; stage 5 15.0 ng/ml, $p < 0.05$. Osteocalcin in adolescents began to rise at about 12 years of age (stage 3), peaked about two years later (stage 4) and then declined toward adult levels at 15 to 16 years of age (stage 5) (Fig. 2).

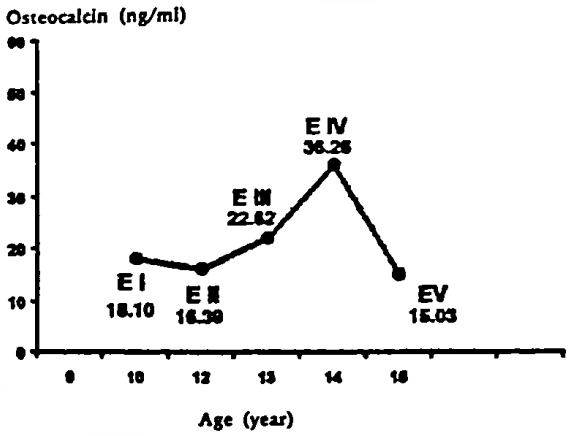

Fig. 2. The changes in mean osteocalcin levels of sexual stages by ages ($p < 0.05$).

Table I. Evaluation of All Results in Relation to Sexual Growth Stage

Variables	Mean values	Standard deviation	Standard error	Number of cases
Group 1 (Sexual Growth Stages 1 and 2)				
Osteocalcin	17.2	6.3	1.0	40
Alkaline phosphatase	573.8	143.9	22.8	40
Testicular volume	4.1	1.1	0.2	40
Skeletal age	135.1	10.0	1.6	40
Chronological age	136.9	10.1	1.6	40
Group 2 (Sexual Growth Stages 3 and 4)				
Osteocalcin	29.4	10.6	1.7	40
Alkaline phosphatase	728.4	233.2	36.9	40
Testicular volume	11.6	4.1	0.6	40
Skeletal age	162.0	10.9	1.7	40
Chronological age	162.6	10.6	1.7	40
Group 3 (Sexual Growth Stage 5)				
Osteocalcin	15.3	5.8	1.3	20
Alkaline	435.7	184.8	41.3	20
Testicular volume	18.2	3.4	0.8	20
Skeletal age	186.3	10.7	2.4	20
Chronological age	186.4	9.4	2.1	20

Osteocalcin (ng/ml); alkaline phosphatase (IU/L); testicular volume (ml); skeletal age and chronological age (month).

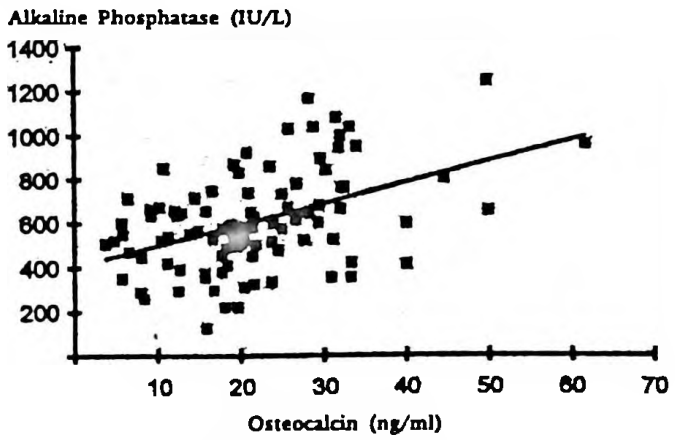

Fig. 1. The relationship between osteocalcin and alkaline phosphatase levels ($r: 0.4187$, $p < 0.001$).

Discussion

The pubertal growth spurt is one of the most prominent signs of puberty. It is characterized by a sharp acceleration in linear growth velocity, a peak period of growth (peak height velocity) and a sharp decline in the yearly growth increment. The growth spurt begins in male adolescents at approximately 12 years of age. The increase in height reaches peak levels at 14 years of age, then declines gradually to the end of puberty. Serum osteocalcin levels parallel physical maturation periods, especially during the first year of life and during the pubertal growth spurt. In neonates, osteocalcin levels

reach 40-60 ng/ml at the end of the first month. These levels are maintained between six and 12 months of age. After the first year, the mean osteocalcin level is 18 ng/ml. It then reaches 25-39 ng/ml during puberty, and declines to adult levels between 17 and 20 years of age in male children. Osteocalcin is the lowest in the morning, gradually increases in the afternoon and reaches its highest level at night. This diurnal rhythm is not influenced by sex, age or season.

Osteocalcin peak levels in the literature reach Stage 4 at 14 years and their relation to the growth spurt is very close. Magnusson et al.[12] reported that osteocalcin levels in male children reached the highest level at 14 years. In other words, osteocalcin achieved its peak level at the peak of height velocity. After 15-16 years of age, osteocalcin levels declined to adult levels with slow growth rate. Magnusson classified cases according to sexual stages: Group 1 = Stage 1 Prepuberty, Group 2 = Stage 2 Early puberty + Stage 3 Pubertal Growth Spurt, Group 3 = Stage 4 Pubertal Growth Spurt + Stage 5 Late Puberty. He did not find any significant correlation of osteocalcin levels and pubertal stage. In that study, the beginning of the pubertal growth spurt (Stage 2) and the decline of the pubertal growth rate (Stage 5) were joined with the pubertal growth spurt periods (Stages 3 and 4), so osteocalcin levels decreased and the expected difference was not found between the groups. This may be due to their having combined the periods of pubertal growth spurt with the beginning and declining growth rate periods, which may have masked any significant changes. Indeed, their study was to have classified sexual stages of puberty as Group 1 = Stage 1 Prepuberty + Stage 2 Early Puberty, Group 2 = Stage 3 + Stage 4 Pubertal Growth Spurt, and Group 3 = Stage 5 Late Puberty, which may explain the lack of significant differences in osteocalcin levels between stages. In the literature, studies of osteocalcin use age, instead of sexual maturation stage, as a criteria, but age does not show correct results due to the relation of different sexual stages. Using the sexual maturation stage was much more suitable than the chronological age for these other studies[5]. Osteocalcin peak levels reached Stages 3 and 4 at 14 years of age[13,14,15]. A relationship between osteocalcin levels and the growth spurt was shown in some studies[16]. A correlation between osteocalcin levels and alkaline phosphatase activities has also been reported in childhood[17]. It is now well established that there is a link between alkaline phosphatase activity and sexual maturity[18-22], which was also confirmed by our results. In conclusion, osteocalcin levels increase with the pubertal growth spurt as do alkaline phosphatase levels. They reach a peak at 14 years of age at Stages 3 and 4, then decrease to the level of adults. There is a very close correlation between the growth spurt and osteocalcin levels. According to our findings, the follow up of osteocalcin levels in relation to sexual maturation stages could be a new method to determine the phase of the pubertal growth spurt. An increase or decrease in osteocalcin levels on consecutive measurements may indicate the child's entering the accelerated or decelerated stages of the growth spurt, respectively. We emphasize that the follow up of adolescent growth is made by the determination of the sexual maturation stage, and not by age. Osteocalcin is a highly specific, reliable and useful marker for evaluation of the growth spurt and is not influenced by nonosseous disorders.

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