Osteoprotegerin and C-telopeptide of type I collagen in polish healthy children and adolescents

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Abstract

Purpose: Most metabolic bone diseases are characterized by a disturbances in bone resorption, therefore biochemical markers concerning this process are of special interest. Recently, the novel cytokine osteoprotegerin (OPG), belonging to the tumor necrosis factor receptor family has been established as an endogenous inhibitor of osteoclastogenesis and resorption process. In addition serum C-telopeptide of type I collagen (s-CTX) is one of the resorption markers released into circulation as a result of the osteoclast mediated degradation of type I collagen. However, a clinical application of OPG and s-CTX in children may be difficult by less information of suitable reference data in relation to age, race and sex. The aim of our study was to invastigate serum concentrations of both markers in polish healthy children and adolescents.

Material and methods: We examined 102 healthy children and adolescents in 6-24 years of age, divided on prepubertal, pubertal and postpubertal groups. OPG and s-CTX were determined by ELISA kits from Biomedica (Austria) and Osteometer (Denmark) respectively.

Results: The highest mean values of OPG were in prepubertal girls (4.64±0.57 pmol/L) and boys (4.28±0.86 pmol/L). Next, in older children and adolescents gradually decreased of OPG concentration was observed. We also obtained the decreased of s-CTX concentration in studied children except these in pubertal period. Generally, we obtained significant positive correlation between OPG and s-CTX in all observed groups ($n=102$, $r=0.653$; $p<0.0001$).

Conclusions: We report the age-related decrease in circulating endogenous OPG during childhood and adolescence. Serum OPG concentration in postpubertal period may be similar to those presented in young adults. Prospective studies are needed to investigate the influence of OPG on bone metabolism in children.

Key words: osteoprotegerin, s-CTX, bone resorption, children, adolescents.

Introduction

Biochemical bone markers can provide as a valuable non-invasive tool in the management of metabolic bone diseases [1,2]. They are available to assess both bone formation and bone resorption process. Because most metabolic bone diseases are characterized by disturbances in bone resorption, biochemical markers concerning this process are of special interest [3,4]. Recently, the novel cytokine osteoprotegerin (OPG), belonging to the tumor necrosis factor receptor family has been established as an endogenous inhibitor of osteoclastogenesis and resorption process. OPG binding to RANKL (receptor activator of nuclear factor kappaB ligand) and blocking its interaction with RANK (receptor activator of nuclear factor kappaB) inhibits the proliferation, differentiation, survival and fusion of osteoclastic precursor cells and promotes osteoclasts apoptosis [5,6]. Alternation in this system could form the basis of bone diseases in osteoporosis, renal osteodystrophy, rheumatoid arthritis, Cushing's disease, and human immunodeficiency virus patients [7-9]. Physiologically, the OPG levels demonstrated a positive correlation with age in both sexes. However, these results concerned adult cohorts and ageing women and men [10-11]. Data in females and males younger than 50 years of age show a low variability of serum OPG levels, whereas in accordance with many authors, greater variability was seen in the elderly [5,12]. A steep increase of OPG in females at the sixth decade and in males later at the seventh decade were observed [11].

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Bone consists of a calcified organic matrix, which is composed of 90% type I collagen [13]. During bone resorption, molecule of type I collagen is degraded, and small fragments are liberated into the blood-stream. The amino acid sequence EKAHDGGR found in the C-terminal telopeptides of the α1 chain of type I collagen (CTX), which can undergo β-isomerization, has proven to be a sensitive marker of bone resorption [14]. Higher serum-CTX (s-CTX) levels are associated with lower bone mineral density values in Crohn's disease and postmenopausal osteoporosis [15,16]. S-CTX is associated also with the severity of radiographic findings in patients with rheumatoid arthritis [17]. Clinically, it has been evaluated mainly in postmenopausal women treated with bisphosphonates and hormone replacement therapy [18]. In children, normal serum concentrations of CTX were documented better than OPG. Previously studies shown that serum CTX values reflects the pediatric growth curve similar to patterns observed for other bone formation and resorption markers [19,20].

However, a clinical application of OPG and s-CTX in children may be difficult by less information of suitable reference data in relation to age, race and sex. The aim of our study was to investigate serum concentrations of OPG and CTX in polish healthy children and adolescents.

Material and methods

Our study group consisted of 102 healthy children and adolescents (52 girls and 50 boys) 6-24 years of age who had been referred to Institute of Mother and Child (Warsaw). Children were divided on subgroups: prepubertal (girls 6-8 y; boys 6-10 y), pubertal (girls 9-13 y; boys 11-15 y) and postpubertal (girls 14--24 y; boys 16-24 y). All individuals showed normal physical development and had no diseases that could affect bone metabolism. None of them was receiving any medication. Informed consent was obtained from the children's parent or from subjects who were 18 years of age. This study had been approved by the Ethics Committee of Institute of Mother and Child.

Non-fasting blood samples were obtained between 8.00 and 9.30. Blood was centrifuged at 1000 x g for 10 min, serum was separated and stored at – 20°C until assay. Osteoprotegerin was determined by enzyme immunoassay (Biomedica, Austria), in which OPG present in the sample, binds to the precoated capture antibody and forms a sandwich with the detection antibody. The intra- and interassay imprecision are 10% and 7% at 5.53 pmol/L.

The s-CTX concentration was measured using Serum Cross-Laps One Step ELISA assay (Osteometer, Biotech, Denmark). This method based on highly specific monoclonal antibody against a β-aspartate isomerized form of the sequence EKAHD--β-GGR derived from the C-terminal telopeptide region of the type I collagen α1-chain. According to manufacturer, the intra- and interassay imprecision (CVs) are 4.9% and 6.6% at 434 ng/L.

All data were compared by Student's t-test. Pearson correlation was computed between s-CTX and OPG concentration and age of studied children. Differences were regarded as statistically significant at $p<0.05$.

Results

Tab. 1 shows the OPG and s-CTX values of studied groups of children in relation to age and sex. The highest mean values of OPG were in girls (4.64±0.57 pmol/L) and boys (4.28±0.86 pmol/L) during prepubertal period. Next, in older children and adolescents gradually decreased of OPG concentration was observed. The significantly lower mean values were obtained in postpubertal girls (3.55±0.70 pmol/L) and boys (3.56±0.36 pmol/L) in comparison to prepubertal and pubertal period (girls, $p<0.05$; boys, $p<0.01$). None statistically significant differences in OPG concentration between girls and boys in particular periods of life were observed. The negative correlation between age and OPG was significant in postpubertal girls, in all groups of boys and girls, and in all studied children (*Tab. 2*).

Mean values of s-CTX were similar in prepubertal children: 2029±361 ng/L for girls, 1883±374 ng/L for boys (*Tab. 1*). The s-CTX levels increased slightly (about 10%) in girls and significantly (about 20%) in boys during puberty to the mean values 2266±368 ng/L and 2281±474 ng/L ($p<0.01$) respectively. After puberty, when bone mineral consolidation occurs, level of s-CTX decreased about 3-fold in girls and 2-fold in boys as compared to the pubertal children. In general, girls showed decreased postpubertal values of s-CTX about 2 years earlier than boys, reflecting the earlier completion of puberty. None statistically significant differences in s-CTX concentration between girls and boys in particular periods of life were observed. The negative correlation between age and s-CTX was significant in all tested groups except prepubertal boys and pubertal children (*Tab. 2*). We also observed significant positive correlation between OPG and s-CTX in group of all tested children ($n=102$, $r=0.653$; $p<0.0001$).

Discussion

We have shown in healthy children and adolescents, that serum OPG levels decreases with age without a gender difference. Moreover, we found a positive association between serum OPG and s-CTX in studied group.

For the first time Buzi F et al. [21] compared serum OPG concentration in a group of 46 normal children (1-14 years old). These authors obtained mean value of OPG 4.05 ± 1.63 pmol/L with no difference between males and females. In children 4-14 years old the level of this marker was similar to those present in young adult men 3.55±0.97 pmol/L [12]. However, our results concerning children 6-14 years old shown higher value for OPG (4.36±0.70 pmol/L) than was reported by Buzi et al. [21]. Moreover, OPG concentrations obtained by us in these prepubertal and pubertal children were also significantly higher ($p<0.0001$) than in adolescents 15-24 years old (4.36±0.70 pmol/L vs 3.48±0.36 pmol/L). Therefore, we think that OPG concentration in postpubertal period may be rather similar to those presented in young adults [12].

High level of OPG was observed in infancy, a decrease to steady levels in childhood and adulthood until 45 years and a further progressive increase until senescence [11]. In accordance with Buzi et al. [21] we obtained an inverse correlation

Table 1. **The concentration of OPG and s-CTX in healthy children and adolescents.**

Gender	Prepubertal groups		Pubertal groups		Postpubertal groups	
	Girls n=14	Boys n=21	Girls n=17	Boys n=19	Girls n=21	Boys n=10
OPG (pmol/L)	4.64±0.57	4.28±0.86	4.28±0.67	4.20±0.48	3.55±0.70•	3.56±0.36••
s-CTX (ng/L)	2029±361	1883±374	2266±368	2281±474*	821±447***	1069±552**

Data are shown as mean value ±SD; •postpubertal girls vs prepubertal and pubertal girls, $p<0.05$; •• postpubertal boys vs prepubertal and pubertal boys, $p<0.01$; *pubertal boys vs prepubertal boys, $p<0.01$; **postpubertal boys vs prepubertal, $p<0.001$ and pubertal boys, $p<0.0001$; ***postpubertal girls vs prepubertal and pubertal girls, $p<0.0001$

Table 2. **Correlation between age and OPG or s-CTX**

Group of children	OPG		s-CTX	
	r	p	r	p
prepubertal girls n=14	-0.216	NS	-0.574	p<0.01
pubertal girls n=17	0.002	NS	0.042	NS
postpubertal girls n=21	-0.461	p<0.05	-0.710	p<0.0001
all girls n=52	-0.649	p<0.0001	-0.786	p<0.0001
prepubertal boys n=21	-0.073	NS	-0.385	NS
pubertal boys n=19	-0.034	NS	0.140	NS
postpubertal boys n=10	-0.466	NS	-0.711	p<0.02
all boys n=50	-0.412	p<0.01	-0.390	p<0.01
all children n=102	-0.517	p<0.0001	-0.619	p<0.0001

of OPG with age. Moreover it seems, that OPG don't reflect exactly the pediatric growth curve. The trend of this marker levels during puberty period appears to be a little different than bone resorption and formation markers. Contrary to OPG we observed the increased (but not very high) of s-CTX concentration leading to a peak in pubertal stage. It is well recognized that bone mass increases with age from infancy to adolescence and that peak bone mass occurs soon after puberty, lasting until 40-45 years, an age after which age-dependent bone loss begins [1]. We also suggest, that puberty period may not affect OPG concentration. It is in accordance with Buzi et al. [21], who found no differences in levels of this marker between normal children and children with early and precocious puberty.

The relationship between serum concentrations of endogenous OPG and bone turnover are still unclear, with different studies yielding different results [22,23]. Some authors suggest that OPG in adults is associated with a profile of bone turnover markers favouring bone formation [24]. Therefore this marker may be protective factor against bone resorption and age-related bone loss.

In conclusion we report an age-related decrease in circulating endogenous OPG in children and adolescents. Serum OPG concentration in postpubertal period may be similar to those presented in young adults. Prospective studies are needed to investigate the influence of OPG on bone metabolism in children.

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