

Bone alkaline phosphatase, osteocalcin and C-terminal telopeptide as bone turnover markers in canine bitches

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ABSTRACT

In clinical veterinary practice the measurement of biochemical markers for assessment of bone metabolism is not yet fully established and is only being used for now as a scientific method in investigations. The purpose of this research is the better understanding of bone metabolism in dogs and assessing the possible utility of biochemical bone markers in routine clinical veterinary practice. The bone markers selected for this study were: bone alkaline phosphatase and osteocalcin as indicators of bone formation, and C-terminal telopeptide of type I collagen as marker of bone resorption. The research was conducted on 58 canine bitches of different breeds. The results indicate increased bone turnover in young animals and decreased turnover in old ones since the highest activities of the investigated bone markers were in the youngest group and they then declined with age. However, elevated activity of bone alkaline phosphatase was noted in older bitches, which has not been detected in other animal species or in healthy humans. Higher bone resorption and lower formation were noticed in heavier dogs.

Key words: bone turnover, dogs, bone alkaline phosphatase, telopeptide, osteocalcin

Introduction

Bone turnover is the main process of bone tissue. It comprises bone cell activities including bone resorption and bone formation. Resorption of the bone and then formation of a new bone are important for the renewal of bone tissue and the reparation of microfractures which appear as a result of stress and metabolic processes. Normally, the

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resorption-formation processes are balanced so there is no net change in bone mass (ALLEN, 2003). During bone formation osteoblasts produce and excrete organic substances of bone matrix: procollagen type I, proteins and enzymes. Osteoclasts are the cells responsible for the resorption of the bone during which they produce enzymes and acids responsible for the dissolving of organic matrix and bone salts. These bone cell products are released into the blood and excreted by urine so they therefore can be used and measured as biomarkers in blood or urine by immunoenzyme (ELISA) or radioimmune (RIA) assays (ALLEN et al., 2000). The advantage of biochemical measurements is the fact that they are noninvasive, inexpensive and can be repeated often because they do not interfere with bone metabolism (WATTS, 1999).

In human clinical practice serum and urinary assays of bone markers provide noninvasive measurements of bone turnover and are useful for a better understanding of the pathogenesis and therefore better monitoring of and treatment selection for certain musculoskeletal or bone disorders, such as postmenopausal osteoporosis (WATTS, 1999; COSMAN et al., 1996; SWAMINATHAN, 2001) Paget's disease (DEFTOIS et al., 1991), and bone tumors (KANAKIS et al., 2004).

In clinical veterinary practice, measurement of biochemical markers for assessment of bone metabolism is not yet fully established. Although interest in using the biomarkers in small clinical practice, especially for dogs, but also for horses (LEPAGE et al., 2001) is increasing, for now they are only being used as a scientific method in investigations.

The purpose of this research was the better understanding of bone metabolism in dogs and assessing the possible utility of biochemical bone markers in routine clinical veterinary practice. It also includes research and measurement of some biochemical parameters of bone turnover, the study of their interconnection as well as their relationship to clinical data.

The bone markers selected for this study were: bone alkaline phosphatase (BALP) and osteocalcin (OC) as indicators of bone formation, and C-terminal telopeptide of type I collagen (CTx) as indicator of bone resorption.

Materials and methods

Animals. Blood samples were obtained during routine diagnostic procedures in 58 canine bitches treated in the outpatients clinic and in the Clinic for obstetrics and reproduction of the Veterinary Faculty in Zagreb. The animals were divided into groups according to age and body weight.

According to age, the dogs were grouped into four groups: <2 years (group 1, n = 25), 3 to 7 years (group 2, n = 19), 8 to 10 years (group 3, n = 8) and dogs over 10 years of age (group 4, n = 6).

According to body weight there were also four groups: <11 kg (group 1, n = 13), 11-15 kg (group 2, n = 15), 16-25 kg (group 3, n = 16) and >25 kg (group 4, n = 14).

There were 22 neutered and 36 non-neutered dogs.

Only healthy dogs were included in the study. Dogs with a history of bone fracture in the past 6 months, pregnant and lactating bitches were excluded from the study.

The samples were collected in vacutainer tubes, centrifuged at 3000 rpm for 15 minutes and then stored at -70 °C until further processing.

Assays for the bone markers. The activities of bone alkaline phosphatase were measured with a commercial human enzyme immunoassay kit (Metra™ BAP EIA kit). The concentrations of osteocalcin and carboxy-terminal telopeptide of type I collagen were measured by use of commercial ELISA assays (N-MID™ Osteocalcin One Step ELISA; Serum CrossLaps™ One Step ELISA).

All three human assays were proved to have good cross-reactivity with canine BALP, OC and CTx (ALLEN, 2003).

Statistical analysis. Differences between the study groups were tested by the non-parametric Mann-Whitney test. Spearman correlation coefficients were used to evaluate the correlation between measured biochemical markers of bone turnover as well as their relationship to clinical data. SigmaStat 3.0 for Windows (Jandel Corporation, San Rafael, CA, USA) was used for statistical analysis. The statistical significance between values was set at $P < 0.05$.

Results

BALP activity. The mean values (\pm SE) of BALP activity are presented in Fig. 1. The highest mean (\pm SE) BALP activity was 4.3 (\pm 1.03) U/L in the youngest group, i.e. dogs younger than 2 years. The lowest BALP activity was 0.9 (\pm 0.11) U/L in the second group, dogs between 3-7 years. Significant differences were found between groups 1 and 2 ($P < 0.001$) and groups 2 and 3 ($P < 0.05$).

There were no significant differences in BALP activity between the weight groups (Fig. 2).

OC concentration. Osteocalcin concentration was significantly higher in age group 1; 11.36 (\pm 2.48) ng/mL, compared with other groups (Fig. 1). The lowest concentration 3.0 (\pm 1.11) ng/mL was in the oldest group, i.e. bitches older than 10 years. A significant difference was found between groups 1 and 3 ($P < 0.05$).

We found no significant differences in OC concentration between weight groups (Fig. 2).

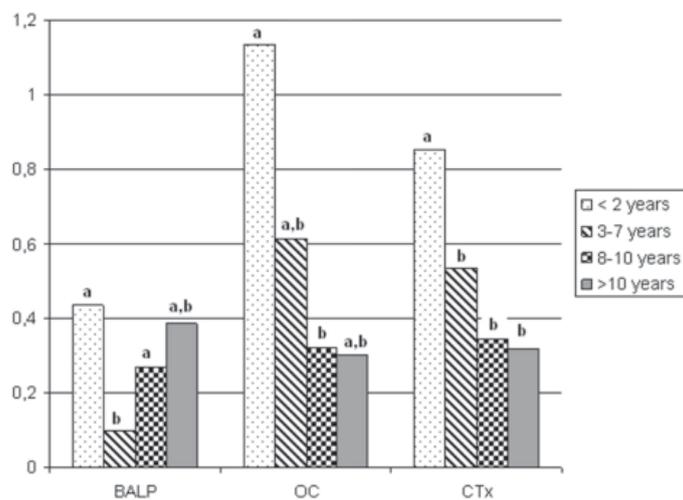


Fig. 1. Mean values of BALP (U/L), OC (ng/mL) and CTx (ng/mL) in various ages. The values of BALP and OC are divided by 10 for the easy reference. For each parameter, bars with different letters (a, b) are significantly different.

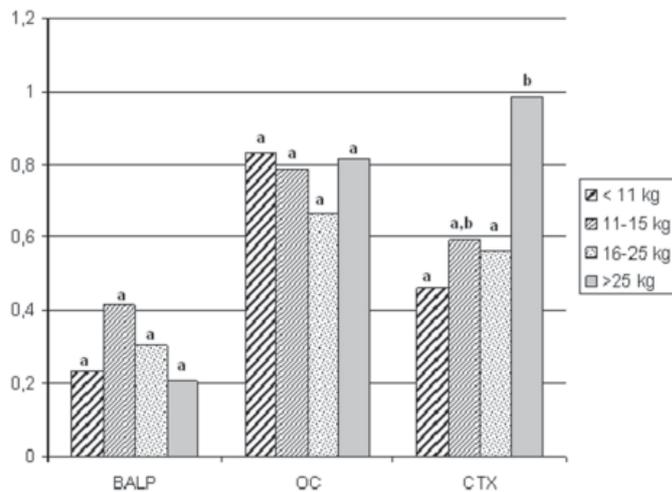


Fig. 2. Mean values of BALP (U/L), OC (ng/mL) and CTx (ng/mL) for various body weights. The values of BALP and OC are divided by 10 for the easy reference. For each parameter, bars with different letters (a, b) are significantly different.

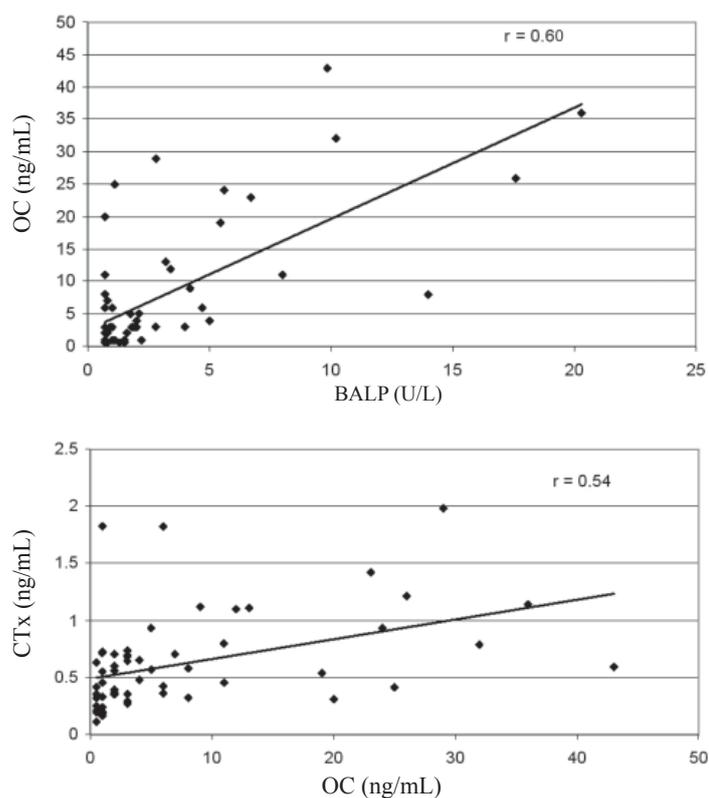


Fig. 3. Positive correlation among bone markers; $P < 0.001$

CTx concentration. According to age, the highest mean CTx concentration $0.85 (\pm 0.08)$ ng/mL was in group 1, while the lowest mean CTx concentration $0.31 (\pm 0.07)$ ng/mL was in group 4 (Fig. 1). There were significant differences between group 1 and groups 2-4 (all $P < 0.001$).

Among the groups formed according to body weight the lowest concentration of CTx, $0.47 (\pm 0.07)$ was in the group where the bitches had body weight lower than 11 kg, i.e. group 1, and the highest CTx concentration $0.98 (\pm 0.15)$ was in the group of the heaviest bitches i.e. bitches heavier than 25 kg (Fig. 2). Among the weight groups there was a significant difference between groups 1 and 4 ($P < 0.001$), and groups 3 and 4 ($P < 0.01$).

There were no significant differences in biomarkers between neutered and non-neutered bitches.

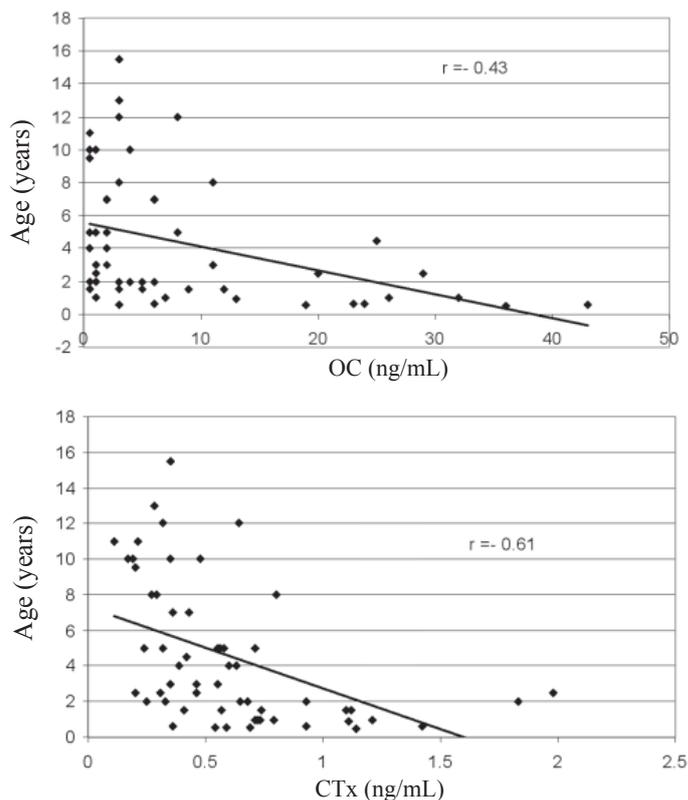


Fig. 4. Negative correlation between bone markers and age; $P < 0.001$

Relation between serum markers. There was a good and positive correlation between BALP and OC ($r = 0.6$) and between OC and CTx ($r = 0.54$) (Fig. 3). A negative correlation was found between OC and age ($r = -0.43$), as well as between CTx and age ($r = -0.61$) (Fig. 4).

Discussion

In this investigation mean BALP activity as well as mean OC and CTx concentrations were lower than the mean values from the previous investigations (ALLEN, 2003; SANECKI et al., 1993; ALLEN et al., 1998) but still within the reference values determined for the marker's activities in dogs (ALLEN et al., 1998). All three measured biomarkers of bone turnover were significantly higher in young bitches compared to the values in adult ones. The result was expected since bone turnover is more intense in young, growing

animals as well as in children (YANG and GREY, 2006). After bone growth, in fully grown, mature bitches, the bone biomarkers' values decreased and were in the referent ranges established for dogs (ALLEN et al., 1998).

OC and CTx concentrations were the lowest in the oldest group, that is, in the group of bitches older than 10 years. Again, this result was expected since bone turnover decreases with age (SANECKI et al., 1993; ALLEN et al., 1998). However, the results also showed elevated activity of BALP in older bitches, that is, in bitches older than 7 years (group 3 and 4). The same result was reported by ALLEN et al. (1998) who investigated a homogenous population of beagle dogs. However, they did not have a good explanation for this increase in BALP activity in older dogs.

In humans, there is a decrease in BALP activity after puberty due to aging. The lowest values of BALP are noted after 40 years of age and later (DELMAS et al., 2000). In women this biomarker is elevated in menopause, and once elevated values in that age mainly stay elevated and do not change with age (KHOSLA et al., 1997). However, since bone turnover is high in that period of life, usually all bone markers of bone turnover are elevated (WATTS et al., 2001), and not only one, as in this investigation. The lack of oestrogen is considered to be one of the major causes of increased bone turnover (KHOSLA et al., 1997) especially in menopause. That is why the activities of bone biomarkers are also high in that period. In this investigation elevated values of BALP activity were found in older bitches, but there were no significant differences for this marker between neutered and non-neutered bitches. This result indicates that there might be some other possible cause for increased bone markers in older dogs, than the lack of oestrogen. Therefore, further investigations into changes in BALP activity with age are necessary.

In the groups formed according to body weight there was only a statistically significant difference in CTx concentration which was higher in the fourth group, that is, the heaviest group (bitches weighing more than 25 kg). Since that group was also the youngest, in which increased bone turnover had already been determined, we believe it is also the reason for the elevated CTx concentration. We do not have an explanation why the remaining two biomarkers were not increased in this group. BREUR et al. (2004) investigated bone formation markers in dogs of different breeds and came to the conclusion that these markers do not show any differences in larger and smaller dog breeds and that their values were in the reference ranges established for beagles.

Although BALP and OC values reflect different phases in osteoblast growth and differentiation (PARTHEMORE et al., 1993; STEIN and LIAN, 1993) in this investigation we found a statistically significant and positive correlation between BALP and OC. The results are in agreement with literature data of bone marker investigation in humans, where a significant correlation was found in children, especially during puberty, and in patients with increased bone turnover (YANG and GREY, 2006; ROSS and KNOWLTON,

1998). These results indicate a connection between bone markers during bone formation since both BALP and OC are osteoblast synthesis products. Positive correlation between OC and CTx is explained by the balance of markers during bone turnover and the connection between processes of bone formation and resorption. The negative correlation between OC and age as well as between CTx and age, indicates decreased bone turnover in older animals.

The results of the biochemical markers of bone turnover are in agreement with literature data and the present knowledge of biochemical markers of bone turnover and bone metabolism in dogs.

Utilisation of bone marker measurement in veterinary medicine could be considered for future investigation and evaluation of bone turnover and the study of bone metabolism. Further work will be required to establish the possible utility of bone markers in clinical practice and clinical investigations.

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SAŽETAK

U veterinarskoj medicini saznanja o biokemijskim pokazateljima koštane pregradnje oskudna su i nedostatna, a njihova primjena je za sada isključivo istraživačka. Cilj ovog istraživanja bio je bolje poznavanje koštanoga metabolizma u pasa na temelju mjerenja biokemijskih pokazatelja koštane pregradnje u krvi te procjena moguće kliničke primjene tih pokazatelja u rutinskoj veterinarskoj kliničkoj praksi. Pokazatelji koštane pregradnje koji su upotrijebljeni u ovom istraživanju jesu: koštana alkalna fosfataza i osteokalcin, kao pokazatelji izgradnje i C-terminalni telopeptid kao pokazatelj razgradnje kosti. Istraživanje je provedeno na 58 kuja različitih pasmina. Rezultati pokazuju pojačanu pregradnju kostiju u mladih životinja i smanjenu u starih životinja budući da su najviše vrijednosti koštanih pokazatelja utvrđene u mladim životinjama, te su opadale s dobi. Međutim, povećana aktivnost koštane alkalne fosfataze zabilježena je u starijih kuja, što nije zapaženo u ostalim životinjskih vrsta niti zdravih ljudi. Također je uočena povećana razgradnja i smanjena izgradnja kosti u pasa veće tjelesne mase.

Ključne riječi: koštana pregradnja, psi, koštana alkalna fosfataza, osteokalcin, telopeptid
