

Comparison of Bone Tissue Elements Between Normal and Osteoarthritic Pelvic Bones in Dogs

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Abstract Physiochemical analysis of bones affected with osteoarthritis (OA) can be used to better understand the etiology of this disease. We investigated the percentage of chemical elements in canine pelvic bone affected with varying degrees of OA using a handheld X-ray fluorescence (XRF) analyzer that discriminates magnesium (Mg^{12}) through bismuth (Bi^{83}). A total of 45 pelvic bones, including both ilium and subchondral acetabular bone plates, were categorized as normal ($n = 20$), mild grade OA ($n = 5$), moderate grade OA ($n = 15$), and severe grade OA ($n = 5$). In normal pelvic, seven elements (P, Ca, Mn, Ag, Cd, Sn, and Sb) differed ($p < 0.005$) in percentage between ilium and acetabulum. Comparisons among the four OA groups found Mn and Fe to be highest in severe grades ($p < 0.05$) in both ilium and acetabulum. Three heavy metals (Ag, Sn, and Sb) were detected in high percentages ($p < 0.05$) in the severe OA group in the acetabulum, but in ilium only Sn was high ($p < 0.05$) in severe OA. In conclusion, the percentages of several elements differed between pelvic types in dogs, and also with increasing severity of OA. The finding of high Mn and Fe in severe grade OA

bone suggests these two elements may be useful in future studies of the etiology and pathophysiology of OA.

Keywords Bone · Dog · Element · Osteoarthritis · Subchondral

Introduction

Osteoarthritis (OA) is one of the most common joint diseases in humans and animals and is characterized by cartilage destruction, subchondral bone sclerosis, and osteophyte formation [1–3]. Symptoms of OA, also known as degenerative joint disease, include general lameness, joint pain, stiffness, and sometimes joint locking. It is the most common form of arthritis and often progresses slowly with age. Research on OA has generally focused on understanding the biology, etiology, and pathology of the disease, finding new techniques for early diagnosis, and developing effective treatments to reinstate proper function of articular cartilage and surrounding tissues [4, 5]. Many studies have evaluated articular cartilage mineralization in patients affected with OA; however, investigations of subchondral bone also are warranted.

Subchondral sclerosis and osteophyte formation are characteristics of chronic OA and widely considered a hallmark of this condition [3]. Subchondral bone consists of bone plates and trabecular bone [3]. Histomorphometric analyses have revealed that the proportion of bone volume and total volume (BV/TV) increases in advanced OA [1], up to about 20 % [6], leading to subchondral bone sclerosis [7]. While articular defects can be limited to the superficial layer of cartilage, often they extend deeper, affecting the underlying subchondral bone as well. Without support from an intact subchondral bed, treatment of the surface chondral lesion is less likely to be effective. Thus, understanding interactions between subchondral

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bone and articular cartilage is important for effective diagnosis and treatment of OA [8].

Determining the elemental profile of tissues affected with OA in relation to differences in disease severity has not been conducted for any species, including dogs. There also are no comprehensive data on the elemental composition of canine bone, either healthy or diseased. So, to increase our knowledge of the pathophysiology of OA, we investigated the percentage of elements in canine pelvic bones with differing degrees of OA using an X-ray fluorescence (XRF) analyzer. X-ray fluorescence is used for routine, relatively nondestructive chemical analyses of rocks, minerals, sediments, and fluids and can provide important information on the elemental components of various biological sample types such as bone [9, 10], teeth [9, 11], and antler [12].

Materials and Methods

Bone Samples

We obtained a total of 45 pelvic bones of unneutered golden retriever ($n = 11$ male, 13 female) and Labrador retriever ($n = 9$ male, 12 female) dogs (age range, 5–12 years) from the Veterinary Cadaveric Center, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand. None of the dogs had reports of hormonal or renal disease or had been treated with corticosteroids within 1 year of death. Bone samples were categorized into four groups adapted from the criteria of Walsh [13]: normal ($n = 20$)—smooth, unbroken surface, homogeneous white to off-white color; mild grade OA ($n = 5$)—cartilage swelling, softening or superficial fibrillation, a light brown homogeneous or white to off-white/light brown in coloration; moderate grade OA ($n = 15$)—deep fibrillation, coarsely broken cartilage surface, dark brown, gray, or red in color, and moderate osteophyte formation at articular cartilage border; and severe grade OA ($n = 5$)—subchondral bone exposure, stippled white and dark brown/red in color, and severe osteophyte formation at the articular cartilage border (Fig. 1).

X-ray Fluorescence Measurement

Bone elemental analyses were conducted using a handheld XRF analyzer (DELTA Premium, Olympus, USA) with a silicon drift detector that can detect elements from magnesium (Mg^{12}) through bismuth (Bi^{83}) on the periodic table. The collimator size was set at 0.3 mm for analysis-area diameter, and operating voltages of 15 and 40 kV were used as the source of incident radiation. Six separate sites on the subchondral bone of acetabulum and 10 sites on the ilium bone were measured for elemental composition [3] (Fig. 2).

Statistical Analysis

The percentage of individual elements in each sample was presented as mean \pm SD. Elemental ratios also were calculated as follows: Ca/P, Ca/Zn, Cd/Zn, Ca/Fe, and Fe/Zn [9, 12, 14]. Differences in elemental percentages and ratios across dogs in the normal bone category were compared using post hoc multiple comparison LSD tests. Differences in elemental percentages and ratios among the four groups were determined by Kruskal-Wallis tests. In cases of statistically significant differences, a Mann-Whitney U test was used. p values < 0.05 were considered significant. The relationship between the element profiles and different degrees of OA was examined using principal component analysis (PCA).

Results

Only 10 elements (Si, P, Ca, Mn, Fe, Zn, Ag, Cd, Sn, and Sb) were detected and are reported as percentages. Light elements (LE) were those with an atomic number lower than Mg (H^1 – Na^{11}) and could not be differentiated as separate elements. LE predominated in bone tissue, which ranged from 60 to 78 % in normal pelvic (Table 1) and 58–82 % in bones affected with OA (Tables 2 and 3).

Comparative Elemental Profile Between Normal Subchondral Acetabulum and Ilium Bone

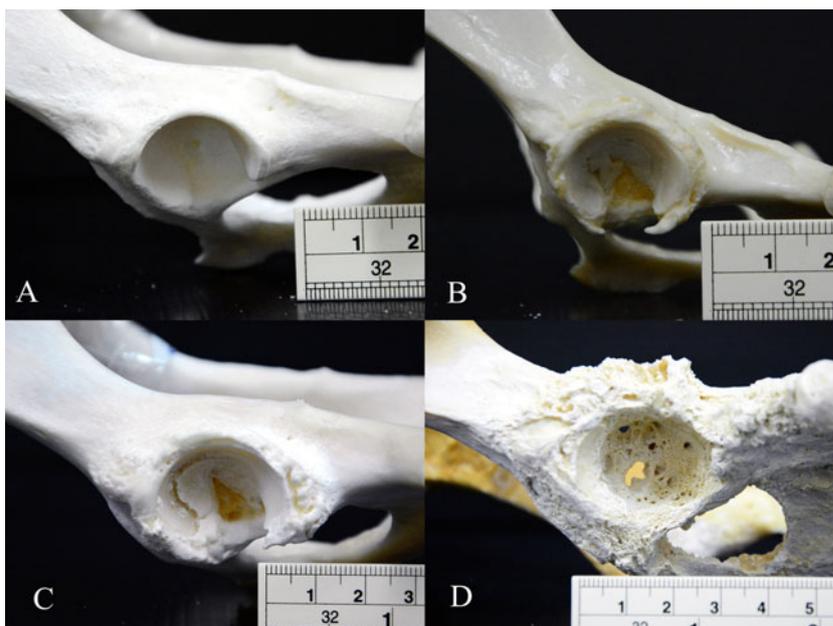
The percentages of elements in acetabulum differed ($p < 0.05$) from those of ilium for seven of the 10 elements and LE (Table 1). A higher ($p < 0.05$) percentage of Mn, Ag, Cd, Sn, Sb, and LE, and lower ($p < 0.05$) percentage of P and Ca, was observed in acetabulum compared to ilium.

Comparative Elemental Profile Among the Four OA Classification Groups

For the subchondral acetabulum, five elements (Mn, Fe, Ag, Sn, and Sb) differed ($p < 0.05$) among the four OA groups (Table 2). The severe group had the highest percentage of Fe, Ag, and Sn compared to the other groups, moderately affected bone had higher percentages of Mn and Fe compared to mild and normal groups, and mild OA had a higher percentage of Mn compared to the normal group ($p < 0.05$).

For ilium, three elements differed significantly among the groups (Table 3): Mn and Fe were higher in the severe and moderate groups compared to mild and normal groups, whereas Sn was highest in the severe OA group compared to the other three groups ($p < 0.05$). We also analyzed the relationship among elements (Ca, P, Si, Mn, Fe, Zn) using principal component analysis (PCA) (Fig. 3) and found acetabulum exhibited a more clear dominant grouping of elements in

Fig. 1 Representative photographs of normal (a), mild (b), moderate (c), and severe (d) grades of osteoarthritis in canine subchondral acetabulum of pelvic



severe OA bone when compared with ilium bone. The altered elements, Si, Mn, and Fe, had a strong correlation with the degree of OA, especially in severe and moderately affected dogs.

Comparative Elemental Ratios

In normal bone, the Ca/P ratio in the ilium was slightly, but significantly, higher than that of the subchondral bone of the acetabulum. None of the other ratios differed between ilium and acetabulum bone ($p > 0.05$) (Fig. 4).

In acetabulum, two elemental ratios differed ($p < 0.05$) among the four OA groups (Fig. 5). The Ca/Fe ratio was lowest in the severe group by the lower percentage than that of the normal group due to a reduced Fe percentage ($p < 0.05$). The ratio in the moderate group was higher than that in the severe group, but only about half that of the mild and normal groups ($p < 0.05$), the latter two of which were similar ($p > 0.05$). By contrast, the Fe/Zn ratio in the severe group was higher ($p < 0.05$) than that in other three groups, again due to differences in Fe percentages. In the moderate group, this

ratio was higher ($p < 0.05$) than in mild and normal groups, with the latter two being similar ($p > 0.05$).

Similar elemental ratio differences were observed in ilium bone (Fig. 6) based on OA severity ($p < 0.05$), again due to the change in Fe percentage. The Ca/Fe ratio was lowest in the severe group ($p < 0.05$) compared to moderate, mild, and normal groups. Moderate and mild OA groups had a lower ($p < 0.05$) Ca/Fe ratio than the normal group, which was similar ($p > 0.05$). Fe/Zn ratios were highest ($p < 0.05$) in the severe group compared to moderate, mild, and normal groups. But there was no difference ($p > 0.05$) in this ratio among moderate, mild, and normal OA groups.

Discussion

We provide new physiochemical information on elemental profiles and ratios in canine bone, and how they are affected by OA. First, elemental content of the subchondral acetabulum bone plate differed from that of ilium bone for several minerals: higher Mn, Ag, Cd, Sn, and Sb; lower Ca and P. Higher percentages of Ca/P ratio in the ilium than in

Fig. 2 Points of measurement on ilium (a) and acetabulum (b) canine pelvic

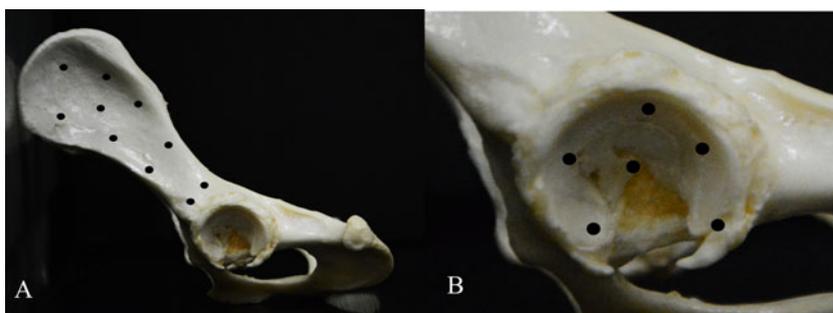


Table 1 The percentage (mean ± SD) of elements present in two types of normal canine pelvic bone: subchondral acetabulum and ilium

Element	Subchondral		Ilium		<i>p</i> value
	Mean ± SD	Range	Mean ± SD	Range	
Si	0.319 ± 0.035	0.175–0.835	0.190 ± 0.026	0.088–0.433	0.297
P	6.912 ± 0.069	4.760–9.860	8.576 ± 0.062	5.730–11.270	0.000
Ca	19.531 ± 0.164	14.870–22.28	21.741 ± 0.135	14.030–28.930	0.018
Mn	0.010 ± 0.002	0.009–0.011	0.007 ± 0.002	0.007–0.008	0.003
Fe	0.017 ± 0.002	0.008–0.045	0.024 ± 0.002	0.010–0.069	0.071
Zn	0.042 ± 0.002	0.009–0.233	0.155 ± 0.002	0.008–2.569	0.302
Ag	0.017 ± 0.001	0.012–0.024	0.012 ± 0.001	0.009–0.020	0.000
Cd	0.022 ± 0.001	0.016–0.030	0.013 ± 0.001	0.006–0.019	0.000
Sn	0.025 ± 0.002	0.017–0.035	0.017 ± 0.001	0.010 ± 0.024	0.000
Sb	0.030 ± 0.002	0.021–0.044	0.019 ± 0.001	0.011–0.029	0.000
LE	73.199 ± 0.223	67.830–78.650	68.840 ± 0.193	60.920–78.360	0.002

acetabulum may provide more strength to that bone, as in other species [15]. Second, we found that the percentage of some elements and their ratios were affected by the severity of OA, most notably Mn and Fe, which were increased significantly.

A finding of elevated Fe percentages in OA dog bones was similar to the higher amount of Fe observed in the femoral head of patients with osteoarthrosis [16]. In that study, a lower

amount of Zn compared to normal patients also was observed, a finding not seen in dogs. In a previous study, analysis of trace elements in serum measured by inductively coupled plasma mass spectrometry found Li and Sn were higher in OA compared to healthy persons [17]; we also observed higher Sn in dogs with OA. By contrast, Kuo et al. [18] found no relationship between concentrations of elements in Taiwanese bone with OA, or

Table 2 The percentage (mean ± SD) and range (in bracket) of elements present in canine subchondral acetabulum bone with different grades of osteoarthritis

Element	Severe	Moderate	Mild	Normal
Si	0.592 ± 0.043 (0.356–1.027)	0.312 ± 0.040 (0.144–0.623)	0.264 ± 0.019 (0.196–0.321)	0.319 ± 0.035 (0.175–0.835)
P	6.446 ± 0.066 (4.070–9.250)	6.308 ± 0.064 (2.817–9.370)	5.635 ± 0.060 (2.152–7.230)	6.912 ± 0.069 (4.760–9.890)
Ca	18.834 ± 0.160 (16.360–22.940)	18.571 ± 0.161 (13.240–20.930)	17.866 ± 0.168 (10.860–21.730)	19.531 ± 0.164 (14.870–22.280)
Mn	0.134 ± 0.009 ^a (0.084–0.185)	0.084 ± 0.007 ^a (0.009–0.142)	0.008 ± 0.004 ^b (0.007–0.008)	0.010 ± 0.002 ^c (0.009–0.011)
Fe	1.027 ± 0.019 ^a (0.623–1.601)	0.268 ± 0.008 ^b (0.016–0.915)	0.019 ± 0.003 ^c (0.014–0.026)	0.017 ± 0.002 ^c (0.008–0.045)
Zn	0.015 ± 0.001 (0.010–0.020)	0.015 ± 0.004 (0.008–0.027)	0.018 ± 0.001 (0.013–0.036)	0.042 ± 0.002 (0.009–0.233)
Ag	0.021 ± 0.001 ^a (0.016–0.025)	0.017 ± 0.001 ^b (0.011–0.023)	0.017 ± 0.001 ^b (0.016–0.019)	0.017 ± 0.001 ^b (0.012–0.024)
Cd	0.025 ± 0.001 (0.021–0.031)	0.021 ± 0.001 (0.013–0.029)	0.018 ± 0.001 (0.009–0.025)	0.022 ± 0.001 (0.016–0.030)
Sn	0.037 ± 0.002 ^a (0.029–0.046)	0.025 ± 0.002 ^b (0.013–0.034)	0.020 ± 0.002 ^b (0.013–0.026)	0.025 ± 0.002 ^b (0.017–0.035)
Sb	0.035 ± 0.002 ^a (0.028–0.040)	0.027 ± 0.002 ^b (0.018–0.036)	0.027 ± 0.002 ^b (0.018–0.037)	0.030 ± 0.002 ^{a,b} (0.021–0.044)
LE	72.712 ± 0.234 (64.320–78.030)	74.216 ± 0.226 (70.630–82.910)	71.396 ± 0.358 (59.090–82.800)	73.199 ± 0.223 (67.830–78.650)

Letters in superscript represent a significant difference (*p* < 0.05) between grade

Table 3 The percentage (mean ± SD) and range (in bracket) of elements present in canine ilium with different grades of osteoarthritis

Element	Severe	Moderate	Mild	Normal
Si	0.333 ± 0.035 (0.156–0.866)	0.228 ± 0.027 (0.099–0.616)	0.135 ± 0.039 (0.096–0.206)	0.190 ± 0.026 (0.088–0.433)
P	6.741 ± 0.065 (4.960–9.330)	8.829 ± 0.058 (6.000–10.160)	7.492 ± 0.053 (5.430–9.860)	8.579 ± 0.062 (5.730–11.270)
Ca	19.788 ± 0.166 (14.140–24.760)	21.965 ± 0.135 (18.130–26.610)	20.666 ± 0.124 (16.660–23.700)	21.741 ± 0.135 (14.030–28.980)
Mn	0.070–0.006 ^a (0.044–0.100)	0.062 ± 0.005 ^a (0.008–0.111)	0.008 ± 0.004 ^b (0.007–0.008)	0.007 ± 0.003 ^b (0.007–0.008)
Fe	0.462 ± 0.011 ^a (0.235–0.801)	0.189 ± 0.005 ^a (0.009–0.787)	0.045 ± 0.003 ^b (0.014–0.075)	0.024 ± 0.002 ^b (0.010–0.069)
Zn	0.013 ± 0.001 (0.011–0.016)	0.015 ± 0.001 (0.006–0.065)	0.021 ± 0.001 (0.011–0.032)	0.155 ± 0.002 (0.008–2.569)
Ag	0.012 ± 0.065 (0.011–0.015)	0.011 ± 0.001 (0.010–0.014)	0.015 ± 0.001 (0.013–0.018)	0.012 ± 0.001 (0.009–0.020)
Cd	0.010 ± 0.001 (0.008–0.012)	0.014 ± 0.001 (0.009–0.017)	0.014 ± 0.001 (0.010–0.020)	0.013 ± 0.001 (0.006–0.019)
Sn	0.012 ± 0.002 ^a (0.012–0.013)	0.016 ± 0.001 ^b (0.013 ± 0.018)	0.016 ± 0.001 ^b (0.013–0.022)	0.017 ± 0.01 ^b (0.010–0.024)
Sb	0.016 ± 0.003 (0.015–0.016)	0.018 ± 0.001 (0.012–0.023)	0.018 ± 0.001 (0.014–0.026)	0.019 ± 0.001 (0.011–0.029)
LE	71.850 ± 0.342 (65.320–75.050)	69.033 ± 0.212 (63.920–75.030)	71.490 ± 0.174 (66.230–76.380)	69.297 ± 0.191 (60.920–78.360)

Letters in superscript represent a significant difference ($p < 0.05$) between grade

related to femoral neck fractures and ischemic necrosis of the femoral head when compared to control patients.

The two major elements of mineralized tissue, including bone, are Ca and P as hydroxyapatite [Ca₁₀(PO₄)₆(OH)₂], with small amounts of carbonate, Mg, and acid phosphate [19]. These were predominant elements in dog bone samples as well, although an even larger percentage was

made up of light elements that could not be discriminated with our XRF analyzer. A decrease in Ca and P in compact bone reduces bone mineral density (BMD) and is characteristic of osteoporosis [20, 21]. However, neither Ca nor P percentages were reduced in OA samples of either bone type in this study, even in the severe cases. Rather the percentage of Fe was greatly increased

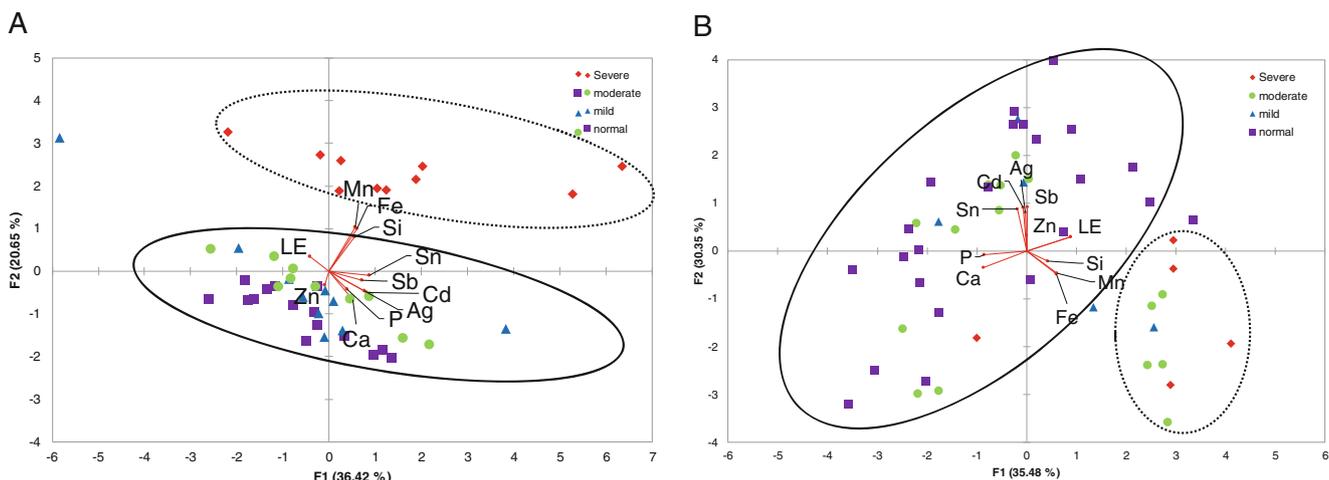
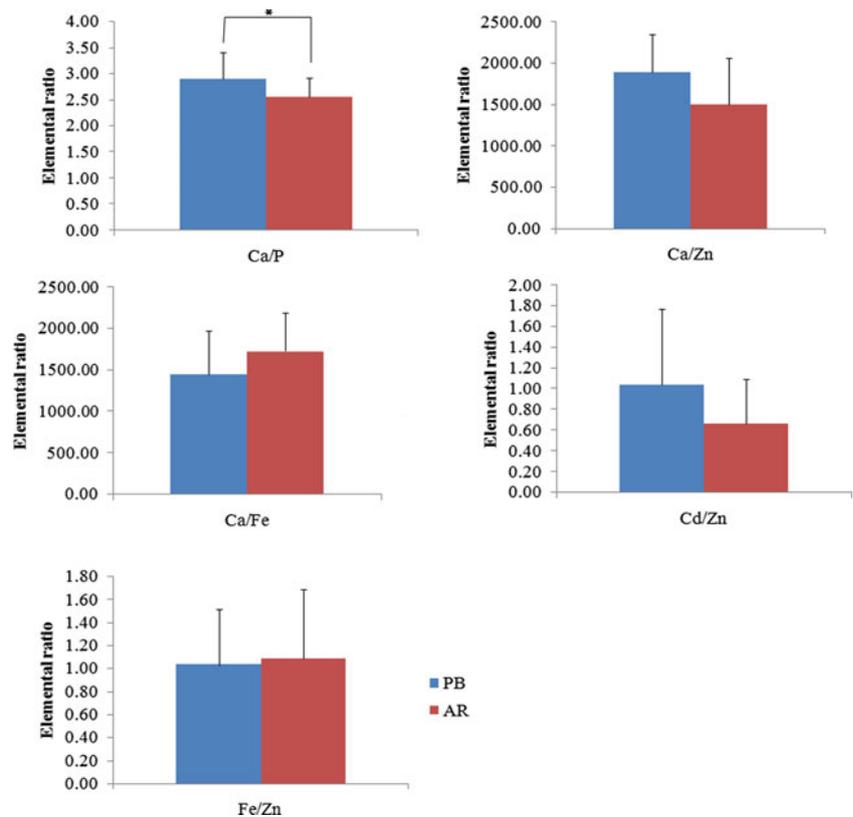


Fig. 3 Principal component analysis (PCA) of elements from subchondral acetabulum (a) and ilium (b) canine pelvic exhibiting mild, moderate, and severe degrees (dash oblong) of osteoarthritis as compared

to normal bone (solid oblong), calcium (Ca), phosphorus (P), silicon (Si), iron (Fe), manganese (Mn), zinc (Zn), silver (Ag), cadmium (Cd), tin (Sn), antimony (Sb), bismuth (Bi), and light element (LE)

Fig. 4 Comparative elemental ratios between ilium pelvic bone (PB) and subchondral acetabulum (AR). Data are presented as mean and standard deviation (*bar*). * $p < 0.05$ indicates a significant difference



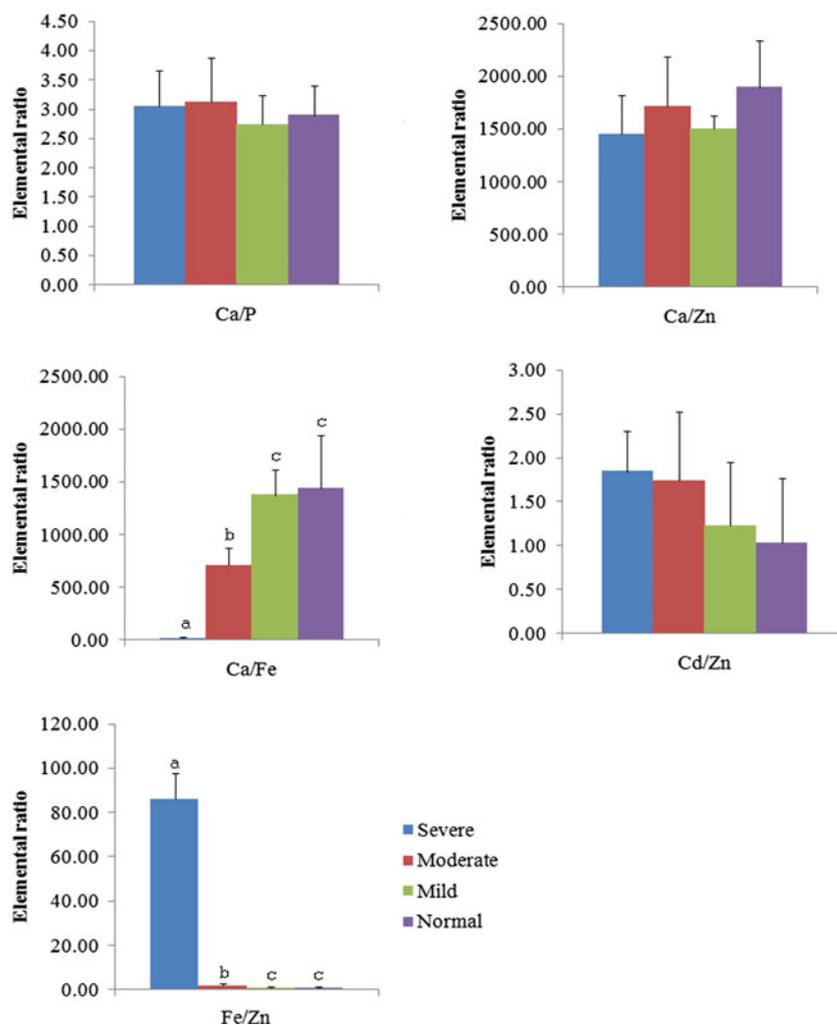
in OA samples, which resulted in altered Ca/Fe and Fe/Zn ratios.

We measured elements in the acetabulum because osteophyte formation and subchondral bone sclerosis occur in this location in OA [3]. In subchondral acetabulum bone affected with OA, five elements were significantly different: Mn, Fe, Ag, Sn, and Sb. These were 13.4, 60.4, 1.2, 1.48, and 1.1 times higher in the severe OA group compared to normal bone, respectively. In ilium bone, three elements differed: Mn, Fe, and Sn, with Mn and Fe being highest in the severe OA group by 10.0 and 19.2 times over normal, respectively. Mn and Fe both were associated with the severity in the degree of OA progression. Manganese is a cofactor of glycosyltransferases, glutamine synthetase, and superoxide dismutase, which is essential for the formation of glycosaminoglycan and antioxidant mechanism [14, 20, 22]. Intake of Mn has been shown to increase the amount of hexosamine (subunit of glycosaminoglycan) in epiphyseal cartilage of chicks [23]. Also, prolonged Mn deficiency has been associated with osteoporosis in humans [20, 24]. But our study found that Mn was higher, not lower, in all OA bone, even those categorized as mild. It is possible this discrepancy is due to a functional interaction between subchondral bone and articular cartilage, with a high content of glycosaminoglycan in tissue and deposition of Mn in subchondral bone used for glycosaminoglycan formation in bone and cartilage cells. Although Mn has been found to increase bone ash in femurs of mice, the excessive

intake of Mn depressed growth and induced defective bone calcification [25]. Thus, significantly increased Mn in the severe OA group might be associated with synthesis of new subchondral bone and osteophytes [3].

In addition to increased Mn in all grades of OA for both acetabulum and ilium bone, Fe also exhibited a predominate change, again being higher in affected compared to normal bone. Iron is essential for hydroxylation of proline and lysine residues in biosynthetic precursors of collagen in bone tissue [14, 22]. Moreover, Fe is a cofactor in 25-hydroxycholecalciferol hydroxylase, which is involved in the process of converting vitamin D into an active form to aid calcium absorption. In our study, Fe in the severe OA group (subchondral and ilium bone) was higher than that in the normal group by 60.4 times, which could be caused by subchondral bone sclerosis and osteophyte formation with increasing collagen synthesis. In severe OA, subchondral bone is characterized by an elevated apparent density, increased bone volume, and thickening of the subchondral bone plate [26]. Even with an increased bone volume density, the mineralization was reduced and lower than normal. In our study, we also found a lower Ca/Fe ratio in the severe OA group, while the Fe/Zn ratio was highest, again due to the drastic changes in Fe percentage. However, the Ca/P ratio in all grades of subchondral bone was not different, likely because of bone metabolism to maintain a normal

Fig. 5 Comparative elemental ratios at subchondral bone of acetabular between four study groups. Data are presented as mean and standard deviation (*bar*). *a*, *b*, and *c* indicate significant difference at $p < 0.05$



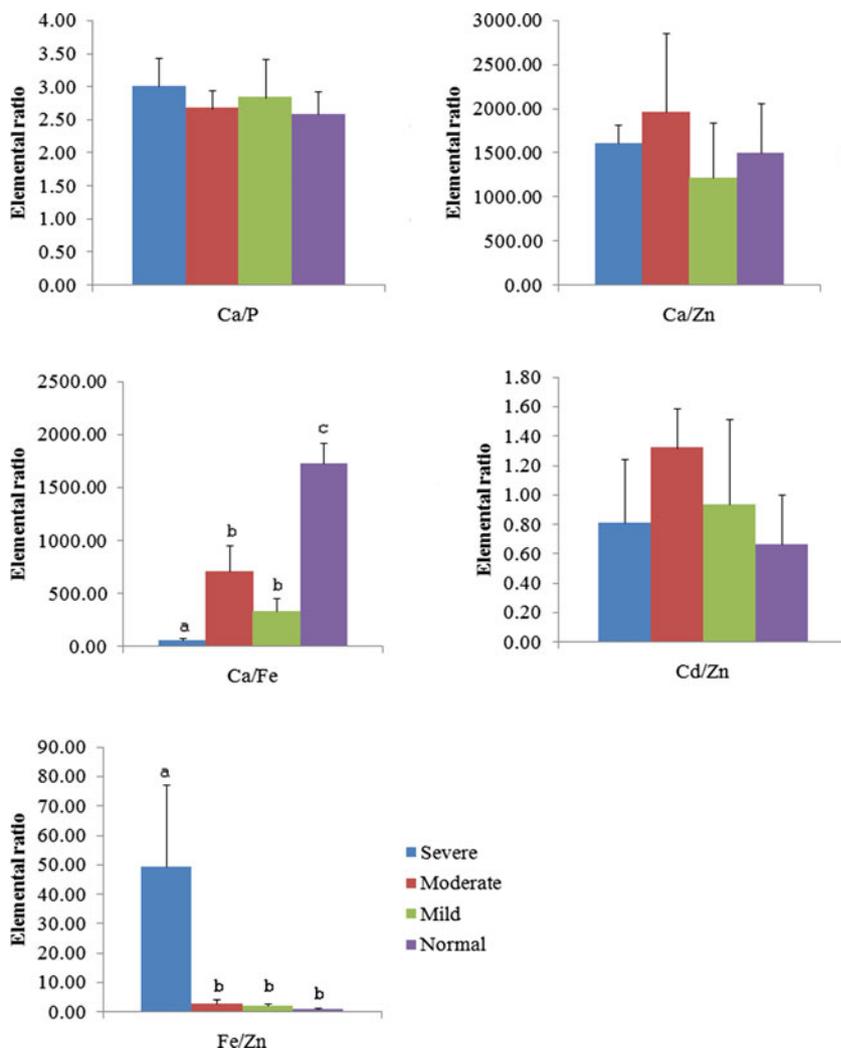
ratio of Ca/P. In 2006, Akesson et al. [26] compared some elements (Se, Cu, Zn, and Fe) in synovial fluid and plasma between normal patients and those with rheumatoid arthritis (RA) or OA. Only synovial fluid Cu and Fe concentration were significantly higher in the OA group. Similar results were reported for Fe, with concentrations in synovial membrane from OA and RA patients being slightly higher than normal [27]. Those are similar to our finding that bone Fe in the severe OA group was significantly higher than normal. Thus, Fe might be an important element involved in OA in dogs and perhaps other species and therefore needs to be studied further.

Four heavy metals (Ag, Cd, Sn, and Sb) also were significantly higher by about 40–70 % in subchondral bone as compared to ilium, so this may be a site of accumulation in bone tissue. There are no reports comparing the percentage of heavy metals between ilium and subchondral bone in dogs or any other species, so these are new findings. Recently, Kubaszewki et al. [27] compared trace element concentration between the femoral bone and intervertebral discs in human

and found Pb, Ni, Mo, Mg, and Zn were 2–25.8 times higher in the bone than in the disc; only Cu concentration was higher in the disc.

Cadmium is an element with proven direct and indirect toxic effects on bones, while Zn affects the content of Cd in the human body [28]. These elements have antagonistic interactions; however, the mechanisms of action in the bone are not fully understood. Increased Cd in the bone has been shown to affect the activity of osteoclasts and inhibit osteoblasts, resulting in decreased mineralization of the bone [29]. In humans, chronic exposure to Cd was associated with reduced BMD [30]. In our study, Cd in subchondral acetabulum bone was significantly higher by 1.69 times than that in the ilium. Tin also can have adverse effects on metabolism of essential trace minerals such as Cu, Zn, and Fe, as well as specific effects on Ca content of the bone [31]. In a classic study conducted over 30 years ago, Yamaguchi et al. [32] showed that oral administration of Sn to rats decreased Ca content in the femoral epiphysis but had no effect on serum Ca, intestinal Ca absorption, or urinary and fecal Ca excretion. In our study,

Fig. 6 Comparative elemental ratios at ilium between four study groups. Data are presented as mean and standard deviation (*bar*). *a*, *b*, and *c* indicate significant difference at $p < 0.05$



Sn in subchondral bone was higher by 1.47 times than that in the ilium. Antimony and Ag in subchondral bone also were higher (1.57 and 1.4 times) than those in ilium bone, respectively. However, the effect of Sb and Ag on bone metabolism has not been thoroughly investigated. It is possible that these heavy metals, Cd and Sn in particular, are accumulated in acetabulum bone and may be related to the reduced percentage of Ca and P compared to ilium. Increasing percentage of heavy metals (Ag, Sn, and Sb) in severe OA compared to normal groups might be related to increasing rate of subchondral bone sclerosis and osteophyte formation in the acetabulum. However, a decreased percentage of Sn in ilium bone in the severe OA group cannot be explained in this context and requires further investigation.

Measures of trace elements in living organisms can serve as indirect bioindicators of environmental pollution. Fresh water mollusks, especially gastropods, have been used as bioindicators for heavy metal contamination of water sources [33]. Also, accumulation of heavy metals in animal hair, such as goat, camel, and sheep, can be a good indicator of

environmental pollutants in forage and soil [34]. Hence, the finding of heavy metals in canine bone samples suggests that they may have been exposed to these elements as a result of environmental contamination and that pelvic bone may serve as another indicator of heavy metal contamination.

There were some limitations to this study. First, the XRF unit did not classify light elements with an atomic number lower than 11 (Na). Second, the XRF machine only measures to a depth of 3 mm, and bone tissue is not homogenous, so the percentage of elements obtained does not reflect the total amount of elements in each sample.

Conclusion

In this study, handheld XRF analysis was used to quantitatively measure bone and subchondral trace elements and was found to be useful for evaluating OA disease progression in dogs. Our results showed that several elements, particularly Mn and Fe, were correlated with pathology of OA, with

elevated proportions in subchondral acetabulum and ilium, especially in the severe OA grades. Thus, XRF can be used to increase our understanding of the etiology of OA and study the mechanisms involved in osteophyte formation and subchondral bone sclerosis in diseased patients. We also believe this method can be applied to the study of other diseases afflicting bones and mineralized tissues. Given the prevalence of bone and joint problems in many species, both domestic and nondomestic, XRF has the potential to assist in the diagnosis and treatment of a myriad of conditions.

Element Abbreviation (Atomic Number) *Mg* magnesium (12), *Si* silicon (14), *P* phosphorous (15), *Ca* calcium (20), *Mn* manganese (25), *Fe* iron (26), *Zn* zinc (30), *Ag* silver (47), *Cd* cadmium (48), *Sn* tin (50), *Sb* antimony (51), *Bi* bismuth (83), *LE* light element; hydrogen (1)–sodium (11).

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Compliance with Ethical Standards

Ethical Approval No ethical approval was required for this study.

Conflict of Interest The authors declare that they have no competing interests.

Authors' Contribution Korakot Nganvongpanit designed and performed all the experiments. Kittisak Buddhachat assisted in the experiments and statistical analysis. Korakot Nganvongpanit, Kittisak Buddhachat, and Janine L. Brown assisted in discussions and writing of the manuscript.

References

- Burr DB (2004) Anatomy and physiology of the mineralized tissues: role in the pathogenesis of osteoarthritis. *Osteoarthritis Cartilage* 12(Suppl A):S20–S30
- Findlay DM, Atkins GJ (2014) Osteoblast-chondrocyte interactions in osteoarthritis. *Curr Osteoporos Rep* 12(1):127–134
- Li G, Yin J, Gao J, Cheng TS, Pavlos NJ, Zhang C, Zheng MH (2013) Subchondral bone in osteoarthritis: insight into risk factors and microstructural changes. *Arthritis Res Ther* 15(6):223
- Pereira D, Ramos E, Branco J (2014) Osteoarthritis. *Acta Med Port* 28(1):99–106
- Malemud CJ (2015) Biologic basis of osteoarthritis: state of the evidence. *Curr Opin Rheumatol* 27(3):289–294
- Fazzalari NL, Parkinson IH (1997) Fractal properties of subchondral cancellous bone in severe osteoarthritis of the hip. *J Bone Miner Res* 12(4):632–640
- Hayami T, Pickarski M, Zhuo Y, Wesolowski GA, Rodan GA, Duong LT (2006) Characterization of articular cartilage and subchondral bone changes in the rat anterior cruciate ligament transection and meniscectomized models of osteoarthritis. *Bone* 38(2):234–243
- Gomoll AH, Madry H, Knutsen G, van Dijk N, Seil R, Brittberg M, Kon E (2010) The subchondral bone in articular cartilage repair: current problems in the surgical management. *Knee Surg Sports Traumatol Arthrosc* 18(4):434–447
- Christensen AM, Smith MA, Thomas RM (2012) Validation of X-ray fluorescence spectrometry for determining osseous or dental origin of unknown material. *J Forensic Sci* 57(1):47–51
- Gonzalez-Rodriguez J, Fowler G (2013) A study on the discrimination of human skeletons using X-ray fluorescence and chemometric tools in chemical anthropology. *Forensic Sci Int* 231(1–3):407.e401–407.e406
- Carvalho ML, Casaca C, Marques JP, Pinheiro T, Cunha AS (2001) Human teeth elemental profiles measured by synchrotron x-ray fluorescence: dietary habits and environmental influence. *X-Ray Spectrom* 30:190–193
- Kierdorf U, Stoffels D, Kierdorf H (2014) Element concentrations and element ratios in antler and pedicle bone of yearling red deer (*Cervus elaphus*) stags—a quantitative X-ray fluorescence study. *Biol Trace Elem Res* 162(1–3):124–133
- Walsh DA, Yousef A, McWilliams DF, Hill R, Hargin E, Wilson D (2009) Evaluation of a photographic chondropathy score (PCS) for pathological samples in a study of inflammation in tibiofemoral osteoarthritis. *Osteoarthr Cartil* 17(3):304–312
- Beattie JH, Avenell A (1992) Trace element nutrition and bone metabolism. *Nutr Res Rev* 5(1):167–188
- Chen QZ, Wong CT, Lu WW, Cheung KM, Leong JC, Luk KD (2004) Strengthening mechanisms of bone bonding to crystalline hydroxyapatite *in vivo*. *Biomaterials* 25(18):4243–4254
- Helliwell TR, Kelly SA, Walsh HPJ, Klenerman L, Haines J, Clark R, Roberts NB (1996) Elemental analysis of femoral bone from patients with fractured neck of femur or osteoarthritis. *Bone* 18(2):151–157
- Zhao T, Chen T, Qiu Y, Zou X, Li X, Su M, Yan C, Zhao A, Jia W (2009) Trace element profiling using inductively coupled plasma mass spectrometry and its application in an osteoarthritis study. *Anal Chem* 81(9):3683–3692
- Kuo HW, Kuo SM, Chou CH, Lee TC (2000) Determination of 14 elements in Taiwanese bones. *Sci Total Environ* 255(1–3)
- Clarke B (2008) Normal bone anatomy and physiology. *Clin J Am Soc Nephrol* 3:S131–S139
- Aaseth J, Boivin G, Andersen O (2012) Osteoporosis and trace elements—an overview. *J Trace Elem Med Biol* 26(2–3):149–152
- Karaaslan F, Mutlu M, Mermerkaya MU, Karaoğlu S, Saçmacı Ş, Kartal Ş (2014) Comparison of bone tissue trace-element concentrations and mineral density in osteoporotic femoral neck fractures and osteoarthritis. *Clin Interv Aging* 18(9):1375–1382
- Palacios C (2006) The role of nutrients in bone health, from A to Z. *Crit Rev Food Sci Nutr* 46(8):621–628
- Leach RMJ, Muenster AM, Wien EM (1969) Studies on the role of manganese in bone formation. II. Effect upon chondroitin sulfate synthesis in chick epiphyseal cartilage. *Arch Biochem Biophys* 133(1):22–28
- Landete-Castillejos T, Molina-Quilez I, Estevez JA, Ceacero F, Garcia AJ, Gallego L (2012) Alternative hypothesis for the origin of osteoporosis: the role of Mn. *Front Biosci (Elite Ed)* 4:1385–1390
- Tal E, Guggenheim K (1965) Effect of manganese on calcification of bone. *Biochem J* 95:94–97
- Ding M (2010) Microarchitectural adaptations in aging and osteoarthrotic subchondral bone issues. *Acta Orthop Suppl* 81(340):1–53
- Kubaszewski U, Ziola-Frankowska A, Frankowski M, Rogala P, Gasik Z, Kaczmarczyk J, Nowakowski A, Dabrowski M, Labeledz W, Miekisiak G, Gasik R (2014) Comparison of trace element concentration in bone and intervertebral disc tissue by atomic absorption spectrometry techniques. *J Orthop Surg Res* 9(1):99

28. Brodziak-Dopierała B, Kwapuliński J, Sobczyk K, Wiechuła D (2015) Analysis of the content of cadmium and zinc in parts of the human hip joint. *Biol Trace Elem Res* 163(1–2): 73–80
29. Akesson A, Bjellerup P, Lundh T, Lidfeldt J, Nerbrand C, Samsioe G, Skerfving S, Vahter M (2006) Cadmium-induced effects on bone in a population-based study of women. *Environ Health Perspect* 114(6):830–834
30. Brzóska MM (2012) Low-level chronic exposure to cadmium enhances the risk of long bone fractures: a study on a female rat model of human lifetime exposure. *J Appl Toxicol* 32(1):34–44
31. Rader JI (1991) Anti-nutritive effects of dietary tin. *Adv Exp Med Biol* 289:509–524
32. Yamaguchi M, Sugii K, Okada S (1982) Tin decreases femoral calcium independently of calcium homeostasis in rats. *Toxicol Lett* 10(1):7–10
33. Gundacker C (2000) Comparison of heavy metal bioaccumulation in freshwater molluscs of urban river habitats in Vienna. *Environ Pollut* 10(1):61–71
34. Rashed MN, Soltan ME (2005) Animal hair as biological indicator for heavy metal pollution in urban and rural areas. *Environ Monit Assess* 110(1–3):41–53