Research Articles

The Effect of Duration of Weaning to Bone Markers and Bone Radiographic Diagnosis in Asian Elephant *(Elephas maximus)*

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Abstract

Metabolic bone disease is an important bone disease in elephant calves. This study investigates the effect of the duration of weaning on osteocalcin and C-terminal telopeptide of type-1 collagen with the radiographic diagnosis of limbs of elephants. Ten elephant calves were set into two groups by their duration of weaning. The early-weaned group was weaned before 2 years old, and the normal-weaned group was weaned after 2 years old. Blood was collected from these two groups to measure bone makers and bone-related parameters. Meanwhile, a radiographic diagnosis was performed to evaluate the elephant bone. A statistically significant difference was found in the osteocalcin of the two groups. The Osteocalcin of the early-weaned group was higher than the normal-weaned group (P = 0.028). Other parameters were not significantly different. All radiographic diagnosis images of elephant bone were normal, with no lesion found. The significantly higher osteocalcin in early weaned elephant calves may indicate the high bone turnover in these elephants. This elevated bone turnover was not seen by radiographic diagnosis. Further investigations and adequate management should be carried out to confirm and prevent metabolic bone disease in early weaned elephant calves.

Keywords: elephant, bone marker, metabolic bone disease, radiographic diagnosis

การศึกษาผลของระยะเวลาหย่านมต่อสัญญาณโรคกระดูกของช้างเอเชีย ควบคู่กับการใช้รังสีวินิจฉัยกระดูก

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บทคัดย่อ

โรคกระดูกเมตาโบลิกเป็นโรคกระดูกสำคัญที่พบได้ในลูกช้าง งานวิจัยชิ้นนี้ศึกษาผลของระยะเวลาหย่านมที่มีต่อสัญญาณโรคกระดูก ได้แก่ ออสติโอแคลซิน และซีเทอร์มินอล เทโลเปปไทด์ ไทป์วัน คอลลาเจน พร้อมกับการใช้รังสีวินิจฉัยกระดูกของลูกช้าง โดยแบ่งลูกช้างจำนวน 10 เชือกเป็น 2 กลุ่ม ตามระยะเวลาหย่านม ลูกช้างในกลุ่มหย่านมเร็วมีประวัติหย่านมก่อนสองปี และลูกช้างในกลุ่มหย่านมปกตินั้นมีประวัติหย่านมหลังสองปี ลูกช้างทุกตัวจะทำการเก็บเลือดเพื่อวัดสัญญาณโรคกระดูก และค่าชีวเคมีอื่นๆ ที่เกี่ยวข้อง พร้อมกับการใช้รังสีวินิจฉัยกระดูกเพื่อประเมินภาวะกระดูกของลูกช้าง ผลการศึกษาพบว่ามีความแตกต่างอย่างมีนัยสำคัญของออสติโอแคลซินระหว่างช้างสองกลุ่มโดยที่กลุ่มหย่านมเร็วมีค่ามากกว่ากลุ่มปกติ ($P = 0.028$) ส่วนค่าอื่นๆ ไม่มีความแตกต่างกันอย่างมีนัยสำคัญ ผลของการใช้รังสีวินิจฉัยกระดูกของช้างทุกตัวพบว่าปกติ และไม่พบพยาธิสภาพใดๆ บนกระดูก ผลการเพิ่มขึ้นของออสติโอแคลซินนั้นอาจแสดงถึงภาวะการหมุนเวียนของกระดูกที่เพิ่มมากขึ้น ในลูกช้างกลุ่มที่หย่านมเร็วอาจมีการหมุนเวียนของกระดูกมากกว่าช้างกลุ่มที่หย่านมปกติ โดยที่ไม่สามารถใช้ภาพทางรังสีเพื่อวินิจฉัยการหมุนเวียนของกระดูกที่เพิ่มขึ้นได้ จึงควรมีการวินิจฉัยเพิ่มเติม และมีการจัดการที่เหมาะสมเพื่อป้องกันโรคกระดูกในลูกช้างที่หย่านมเร็ว

คำสำคัญ : ช้าง สัญญาณโรคกระดูก โรคกระดูกเมตาโบลิก รังสีวินิจฉัย

Introduction

Metabolic bone disease is a prominent bone disease in elephant calves (Emanuelson 2006). Metabolic bone disease is caused by the imbalance of minerals which are essential for bone metabolism. The clinical signs of metabolic bone disease in elephant calves are disoriented limbs, varus-valgus, abnormal gait, etc. These signs usually present when elephant calves are 8-9 months old. Most of the elephant calves that presented with the clinical signs often died despite appropriate treatment (Emanuelson 2006). Early diagnosis and treatment prior to the presence of clinical signs are important ways to reduce the mortality rate.

Most of metabolic bone disease cases in elephant calf are related to nutritional etiology. Elephant calve may not showed clinical sign of metabolic bone disease immediately, but in several years later they may develop the abnormal gait, other signs of metabolic bone disease, and die (Krajaysri et al., 2003). It is believed that early weaning may related to the metabolic bone disease. There is a study in neonate rats shows that three days early weaning neonate rats have lower bone mass, serum parathyroid hormone, osteocalcin and CTx but higher serum 25(OH)D compared to rat that have free excess to the milk. This indicate breastfeeding is important to bone development and the underlying mechanism might involve alteration of parathyroid hormone and 25(OH)D activities. Normally, wild elephant is weaned at 3 to 5 years old, but in captive elephant, they usually wean at 2.5-3 years old. However, there are several reasons than elephant calves were early separated from their mother. For example, 1) the mother died 2) the owners want to sale the calf for money, etc.

There are multiple ways to diagnose bone diseases, for example, radiographic diagnosis including bone x-ray, computer tomography (CT) and magnetic resonance imaging (MRI) (West 2006; McIlwraith 2005; Greco et al., 2023). However, there are limitations of using radiographic diagnosis in elephants due to their sizeable skeletons. The radiographic diagnosis can only be used in a few parts of long bones, for example, the tibia, fibula, radius, and ulna (West 2006). Another limitation is the difficulties in the early diagnosis of bone disease. Bone lesions are often present in the severe stage of disease progression. Bone markers diagnosis is another method of bone diagnosis which has the advantage of early diagnosis of the imbalance of bone metabolism. Moreover, it can diagnose abnormalities of bone prior to the clinical signs developing (Kilgallon et al., 2008; Udomtanakunchai et al., 2019; Takehana et al., 2020).

Bone marker indicates bone metabolism. Bone metabolism has two major processes; bone synthesis and bone degradation where osteoblast and osteoclast play an important role in these two processes. Abnormalities in bone metabolism cause changes in bone markers. Osteocalcin, the bone synthesis marker, is used in some species such as horses (McIlwraith 2005; Kamr et al., 2020). C-terminal telopeptide of type-1 collagen (CTx), on the other hand, is a bone degradation marker that is used in human (Glendenning et al., 2018).

The study of bone markers in Asian elephants is not widely distributed. There are only a few studies on normal middle-aged elephants (Kilgallon et al., 2008) and variation in healthy at different age (Arya et al., 2015; Takehana et al., 2018). To date, the study about bone markers in early weaned elephant calves which are the risk group for metabolic bone disease is limited. We hypothesized that osteocalcin, CTx, and bone radiographic images were different between early-weaned calves and normal ones.

Materials and Methods

Animals

Ten healthy Asian elephant calves at the age of 4-10 years were comprised in this study. The elephants were divided into two groups according to their age of weaning. Group A was a normal weaned and all of them were weaned at the age of more than two years old. Group B included elephant calves that were weaned before two years of age. All elephants were kept under the same condition, environment, and feed in the National Elephant Institute (NEI), Lampang Province, Thailand. All elephants consume mainly grasses (80-90% of daily diet) and supplement with fruits such as bananas, sugar cane, and concentrate food. Gait and posture were evaluated by the veterinarian at the NEI. All elephants showed normal range of movement, walking stride, and no abnormality of limbs, including asymmetry, swelling, and atrophy noted. Elephant demographic data are shown in the Table 1. This study was approved by the Animal Care and Use Committee (Protocol No. MUVS-2013-19) of the Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, Thailand.

Table 1. Elephants demographic data.

No	Group*	Age		Sex	Body weight (Kg)	Leg (cm.)		Foot (cm.)	
		Year	Month			Circumference	Width	Circumference	Width
1	A	8	9	F	1720	105	18	69	14
2	A	5	11	M	1410	99	19	77	14
3	A	8	8	F	1710	109	17	72	15
4	A	3	10	F	935	85	14	66	12
5	A	5	11	M	895	86	16	61	12
6	B	3	0	M	480	75	15	51	11
7	B	3	0	M	430	76	14	54	11
8	B	5	0	F	345	**	13	47	12
9	B	3	0	F	850	84	16	63	12
10	B	6	0	F	1390	97	18	65	14

* group A is normal weaned elephant calves; group B is early weaned elephant calves.

** the leg of the calf number 8 is swollen and painful, the circumference and width cannot be measured at that time.

Radiographic diagnosis

Radiographic diagnostic procedures were undertaken following the assessment of gait and posture by a mobile x-ray machine (Hitachi® Sirius Star Mobile, Japan). The Centre of foreleg circumferences and the size of the foot were measured. Two planes of the radiographic image were taken in each elephant. The first plane was a lateromedial view of the left hind limb to assess the long bone. The other plane was dorsoplamar views of the phalange to evaluate growth plate adjustment. kV and mAs for the radiographic technique depended on the thickness of the limbs of each elephant.

All radiographic images were interpreted by Dr. Somkiet Huaijantug and Dr. Petchroi Petchrieng, for abnormal alignments of bones, pathological lesion of soft tissue, cartilage, bone parenchyma and ratio of cortex to bone diameter (CD ratio). The CD ratio was measured manually on computer software (eFilm Workstation® 3.4, Merge Healthcare, Hartland, WI 53029)

Blood parameters

Five millilitres of blood were collected from the auricular vein of each elephant into Plain tube for biochemistry profiles, EDTA tube for N-MID osteocalcin and CTx and heparin tube for haematological parameters. Blood samples were centrifuged at 2000 rpm for 10 minutes and plasma/serum was separated from blood cells and stored at -20^{o}c. for analysis. All elephant blood samples were collected on the same day in the late morning time.

N-terminal mid-fragment osteocalcin (N-MID osteocalcin) and C-terminal telopeptide of type-1 collagen were measured by electrochemiluminescence immunoassay (ECLIA). Calcium, phosphorus, and ALP, CBC, total protein, BUN, and creatinine were measured using automate machine. Hematology and blood biochemistry were analyzed with in 24 hours after blood collection. N-MID Osteocalcin and NTx were sending to the lab and analysis with in a week after blood collection.

Statistical analysis

Comparison of N-MID osteocalcin, CTx, calcium, phosphorus, calcium to phosphorus ratio, ALP, BUN, creatine, total protein, WBC counts, hematocrit, age, CD ratio, and leg circumference of the two groups were undertaken by Mann-Whitney U test. Correlates between all parameters are tested by the Spearman rank test. All statistical analysis was obtained by computerized statistical software, SPSS statistics version 17.0. Probabilities <0.05 were considered significant.

Results

Elephants

All elephants were bright, alert, and responsive from observation and physical examination. There were 3 males and 2 females in each group. The postures and gaits of every elephant were normal. The range of age of the two groups was 4-10 years. There was no significantly difference in age between the two groups ($P = 0.07$, Table 2).

Radiographic diagnosis

There was neither lesion nor abnormal bone alignment found from the tibia and fibula radiographic images (Figure 1). Cartilages and joints on the phalange images were normal and no lesion was found. There was no difference between the tibia CD ratios of the two groups as shown in Table 2 ($P = 0.92$).

Table 2. Comparisons of age, osteocalcin, CTx, calcium, phosphorus, ALP, BUN, creatinine, total protein, WBC counts, hematocrit, CD ratio, and leg circumference between group A (normal weaned elephant calves) and group B (early waned elephant calves). Statistical differences between groups were tested by Mann-Whitney U test. (Reference from Fowler and Mikota 2006).

Variable		25th percentile	Median	75th percentile	p-value	Reference range
Age	Group A	5.50	7.00	9.00	0.07	-
(year)	Group B	3.50	3.50	6.50		
Osteocalcin	Group A	15.50	16.88	24.04	0.03	n/a
(ng/ml)	Group B	25.39	31.03	39.21		
CTx	Group A	2.61	4.11	4.58	0.35	n/a
(ng/ml)	Group B	2.13	2.69	3.93		
ALP	Group A	190	266	291	0.99	60-450
(U/L)	Group B	218	229	234		
Calcium	Group A	10.45	11.00	12.75	0.53	9-12
(mg/dl)	Group B	10.45	10.60	11.10		
Phosphorus	Group A	5.60	6.20	22.10	0.75	4-6
(mg/dl)	Group B	6.15	6.50	6.75		
BUN	Group A	5.50	7.00	9.00	0.14	5-20
(mg/dl)	Group B	3.50	3.50	6.50		
Creatinine	Group A	1.15	1.40	1.55	0.24	1-2
(mg/dl)	Group B	0.80	1/20	1.50		
Total protein	Group A	7.30	8.40	11.55	0.35	6-12
(g/d)	Group B	6.50	7.60	7.90		
Haematocrit	Group A	34.75	37.30	40.15	0.18	30-40
(%)	Group B	38.60	33.50	39.45		
WBC	Group A	15.65	20.20	25.20	0.60	10-18
(x 10^9/L)	Group B	16.90	18.70	21.25		
CD ratio	Group A	0.32	0.43	0.72	0.92	
	Group B	0.35	0.42	0.54		
Leg	Group A	63.50	69.00	74.50	0.05	
circumference	Group B	51.75	58.50	64.50		
(cm.)						

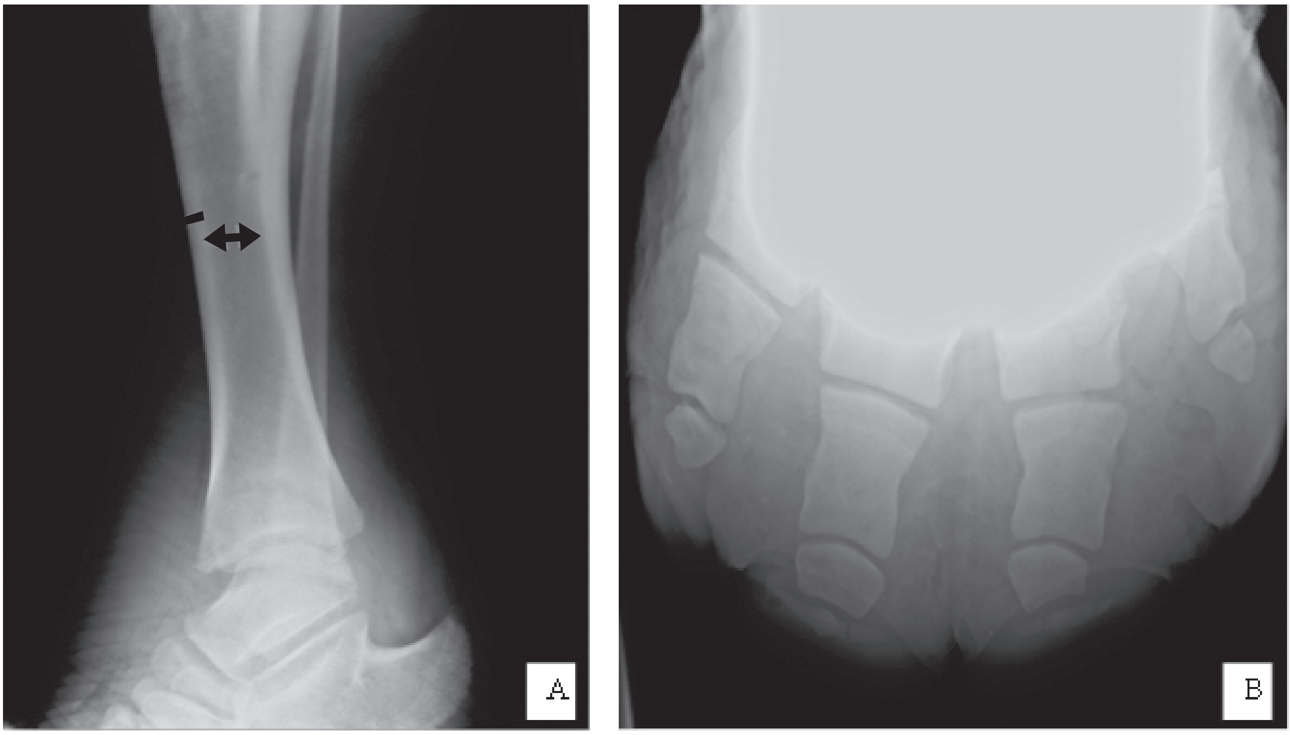

Figure 1. Radiographic images of tibia (A) and phalanges (B) of elephants. There was neither lesion nor abnormal bone alignment found from all radiographic images. In (A), the line indicates tibia cortex and two head arrow indicate diameter for the tibia.

Haematology and serum biochemistry

WBC count, hematocrit, BUN, creatinine, total protein, ALP, calcium and phosphorus, of all elephants were mostly within normal range. There was no significant difference in these variables between the two groups (Table 2).

Bone markers

N-MID Osteocalcin of group B (early weaned) was significantly higher than group A (normal weaned) (P =0.028). The data of the early-weaned group was all assembled in the same range as well as the normal group. However, one elephant from group A had an N-MID osteocalcin outlier high level (elephant number 2; N-MID osteocalcin = 30.46 mg/dl).

Correlations between parameters

The correlation between all parameters of 10 elephants was shown in the table 3. The correlations between age and leg circumference, total protein and BUN, total protein and creatinine, and phosphorus and calcium to phosphorus ratio, are naturally found. The strong correlation between age and creatinine (0.73; P = 0.03) along with creatinine and leg circumference (0.77; P = 0.02) was explained by the higher muscular mass of the elephant. The higher muscle mass can elevate creatinine levels. There was a strong reverse correlation between N-MID osteocalcin and BUN (-0.82; P = 0.03).

Table 3. Correlations between all parameters of elephants. P-values are shown in parentheses. Marker (*) shows significant correlation at the 0.05 level. The correlation was tested by Spearman Rank test.

	Age	Osteo calcin	CTx	ALP	Ca	P	BUN	Cr	TP	Hct	WBC	CD ratio	Leg Circumference
Age	1.00 (0)	-0.47 (0.18)	0.67*(0.03)	-0.47 (0.22)	-0.04 (0.92)	0.0 (1.0)	0.24 (0.50)	0.73*(0.02)	0.59 (0.07)	0.11 (0.78)	-0.49 (0.89)	0.32 (0.36)	0.77*(0.02)
Osteocalcin		1.00 (0)	-0.02 (0.96)	-0.13 (0.73)	-0.01 (0.97)	-0.30 (0.41)	-0.82*(0.03)	-0.17 (0.65)	-0.53 (0.12)	0.31 (0.39)	-0.09 (0.80)	-0.12 (0.75)	-0.15 (0.70)
CTx			1.00 (0)	-0.47 (0.21)	-0.03 (0.33)	-0.58 (0.08)	0.02 (0.95)0	0.60 (0.07)	0.54 (0.11)	0.36 (0.31)	-0.01 (0.86)	0.13 (0.73)	0.62 (0.08)
ALP				1.00 (0)	0.57 (0.11)	0.2 (0.61)	-0.24 (0.52)	-0.31 (0.41)	-0.44 (0.23)	-0.03 (0.93)	0.59 (0.12)	-0.60 (0.08)	-0.05 90.89)
Calcium					1.00 (0)	0.27 (0.44)	-0.25 (0.49)	-0.23 (0.52)	-0.48 (0.16)	0.12 (0.74)	0.50 (0.14)	-0.23 (0.53)	0.08 (0.83)
Phosphorus						1.00 (0)	-0.06 (0.88)	-0.14 (0.70)	-0.20 (0.58)	-0.30 (0.41)	0.35 (0.33)	0.10 (0.78)	-0.38 (0.31)
BUN							1.00 (0)	0.14 (0.70)	0.69*(0.27)	-0.34 (0.34)	-0.30 (0.40)	0.29 (0.41)	0.12 (0.77)
Creatinine								1.00 (0)	0.65*(0.04)	0.31 (0.39)	-0.11 (0.78)	0.65*(0.04)	0.70*(0.04)
Total Protein									1.00 (0)	0.01 (0.97)	-0.32 (0.36)	0.49 (0.14)	0.35 (0.35)
Hematocrit										1.00 (0)	0.55 (0.09)	-0.25 (0.50)	0.13 (0.73)
WBC											1.00 (0)	-0.43 (0.21)	-0.22 (0.58)
CD Ratio												1.00 (0)	0.22 (0.58)
Leg Circumference													1.00 (0)

Discussion

Osteocalcin is a non-collagenous protein and is mainly secreted by osteoblasts. It is also secreted by megakaryocytes and adipocytes and excreted by the kidney. It has been reported to be an osteoblast-specific product (Allen 2003). The alteration of osteocalcin can be induced by many factors, including high bone turnover, absence from clearance, administration of some therapeutic agents, and collected time (circadian variation) etc. (Arya et al., 2015; Lee et al., 2000). In the present study, plasma N-MID osteocalcin was higher in early weaned elephant calves. This may indicate that higher bone turnover was likely to be involved with the elevation of plasma N-MID osteocalcin in these early-weaned elephant calves, since all elephants in our study were kept under the same environment and health condition. There are many metabolic bone diseases those cause the abnormality in bone turnover. Indeed, high bone turnover caused thinner bone in some bone diseases such as osteomalacia, rickets in children, and osteoporosis. These diseases were known to have higher osteocalcin from previous studies (Oginni et al., 1996; Brown et al., 2022). Further investigations are required to diagnose these diseases in early weaned elephant calves.

Generally, wild elephant calves are weaned gradually at 2-5 years old, whereas captive elephants are normally weaned at 2-2.5 years old (Emanuelson 2006). Therefore, we used two years old as the cut-point value to differentiate normal-weaned from early weaned elephant calves. The claves in the normal weaned group were mostly weaned at 2.5 years old, in accordance with the training program of Thai Elephant Conservation Center. All the early-weaned calves in the present study were confiscated from illegal properties and weaned at less than two years of age. The findings in the present study implied that the early-weaned calves require an adequate management as they may develop the bone turn over higher than the normal calves. The management to prevent the development of the thinner bone could be carried out appropriately, for example, adequate milk replacement, calcium supplements, proper exercise, and regular physical examination.

It is interesting to note that an elephant in the normal-weaned group had the highest bone CD ratio and higher than the normal range. This could be caused by some diseases such as osteopetrosis or scatter interference (Haugeberg 2008). The higher kV and mAs used in radiographic diagnosis of these calves were carried out because of their large limbs. The higher of kV yielded scatter to the images that may be less accuracy in diagnostic interpretation (Voges 2018). The cause of the bone CD ratio in this case is more likely to be from effects of scatter.

There was only one elephant in the normal weaned group that had higher level of N-MID osteocalcin (30.46 ng/ml). Moreover, his bone CD ratio was lower than others (CD ratio = 0.26; median CD ratio in this group = 0.43). Although there was no correlation between the bone CD ratio and N-MID osteocalcin, this elephant had shown negative correlation between them. More interestingly, he was classified into the normal wean elephant calf, but his history was different from others in the group. He was born from artificial insemination and rejected by her mother after calving. However, he had a stepmother and fed him milk until 2.5 years old.

Alkaline phosphatase (ALP) was also measured in this study. There were no significant different between two groups. Still, ALP has many sources including intestine, liver and not specific to the bone. Bone-specific alkaline phosphatase (BLAP) should be an appropriate test to measure. Takehana et al. (2020) compared three bone markers, t-APL, ALP3, and TRAP5b, between a hand reared Asian elephant calf and two mother-reared elephant calves during 3-12 month-old. Their data suggested that although there is no different in weight gain and growth, lower bone markers in hand-reared elephant calf indicate abnormal bone metabolism. However, this data is from the study of three to twelfth month-old elephant and this present study evaluate the value of alkaline phosphatase in older elephant calves (2-10 year-old), and there could be other factors affect the bone metabolism markers

CTx of two elephant groups was not significantly different. CTx is the degradation product from type I collagen, which is the main component in bone (Glendenning et al., 2018). There are many factors affecting serum CTx, including fasting state, circadian variation, and some hormones. These variations are difficult to measure and interpret. The knowledge about serum CTx in elephants are limited and there is no reference interval to compare. Although, there is a study of CTx in osteoarthritis elephant (Chusyd et al., 2023), the level of CTx is lower than reported in this study. It could be the effect of age, as CTx decrease when human become an adult (Diemar et al., 2021). Further investigation is warrant. The experiment of more samples should be done to investigate or confirm these correlations.

Further plan of the study is to include more elephants, multiple bone markers, and bone-related parameters, for example bone alkaline phosphatase, and parathyroid hormone. In addition, the final diagnosis should be confirmed the bone diseases.

Conclusion

The present study showed that N-MID osteocalcin was significantly higher in early weaned elephant calves, which may indicate the high bone turnover in these elephants. This elevated bone turnover was not seen by radiographic diagnosis. Further investigations and appropriate management should be carried out to confirm and prevent metabolic bone disease in early weaned elephant calves.

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