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Original Article

Comparative morphometric study for distinguishing between human and non-human mammalian (cow, dog, horse, monkey and pig) long bones

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Abstract The purpose of this study was to determine whether bone morphometry analysis (morphometric index measurements and angular measurements) on long bones would be robust enough of a technique to distinguish between humans and animals (cow, dog, horse, monkey, and pig). The complete long bones utilized in this study were composed of humerus, femur and tibia. Bones from 14 human skeletons and 2-10 skeletons from each mammal species were used in this study. A total of 33 measurements were generated (16 morphometric index and 17 angular measurement). The 16 morphometric index measurements were performed as bone measurements by a classic osteometric method using osteometric board and vernier caliper and then calculated as morphometric indexes. The 17 angular measurements were performed by bone photographs and then calculated the angles by using the Image J program. 18 out of 33 measurements demonstrated a significant difference ($p<0.05$) between human and non-human mammals. Overall, the difference in results between human and non-human mammal bone morphometry may be associated with the difference of bone functions among these species due to the biomechanics of the bipedal and quadrupedal. In conclusion, morphometric measurements of long bones has the potential for use in distinguishing human bones from cow, dog, horse, monkey, and pig species.

Key words: Animal, Human, Long bones, Morphometry

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บทความต้นฉบับ

การเปรียบเทียบความแตกต่างทางสัณฐานวิทยาของกระดูกยาวระหว่างมนุษย์และสัตว์เลี้ยงลูกด้วยนมที่ไม่ใช่มนุษย์ (วัว สุนัข ม้า ลิงและสุกร)

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บทคัดย่อ การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาลักษณะทางสัณฐานวิทยา (การวัดดัชนีเมตริกและการวัดเชิงมุม) ของกระดูกยาวเพื่อใช้แยกความแตกต่างระหว่างมนุษย์และสัตว์ (วัว สุนัข ม้า ลิงและสุกร) กระดูกยาวที่ใช้ในการศึกษาครั้งนี้ต้องมีลักษณะสมบูรณ์ ประกอบด้วยกระดูกต้นขาหน้า/แขน กระดูกต้นขาหลัง/ขา และกระดูกหน้าแข้ง นำมาจากโครงกระดูกมนุษย์จำนวน 14 ร่าง และจากสัตว์เลี้ยงลูกด้วยนมแต่ละชนิด (2 ถึง 10 ร่าง) วัดทั้งหมด 33 ค่า (การวัดดัชนีเมตริก 16 ค่าและการวัดเชิงมุม 17 ค่า) การวัดดัชนีเมตริกทั้ง 16 ค่า วัดโดยใช้กระดานวัดเมตริกและเวอร์เนียร์คาลิปเปอร์ แล้วนำค่ามาคำนวณเป็นดัชนีเมตริก สำหรับการวัดเชิงมุม 17 ค่า วัดโดยการถ่ายภาพกระดูกและวัดมุมจากภาพโดยใช้โปรแกรม Image J ผลการศึกษาพบว่าค่า 18 จาก 33 ค่าที่วัดได้มีความแตกต่างกันอย่างมีนัยสำคัญ (p<0.05) ระหว่างมนุษย์และสัตว์เลี้ยงลูกด้วยนม ความแตกต่างในลักษณะทางสัณฐานวิทยาของกระดูกระหว่างกระดูกมนุษย์และสัตว์เลี้ยงลูกด้วยนมอาจจะเกี่ยวข้องกับความแตกต่างทางชีวกลศาสตร์ของมนุษย์ที่เดินสองเท้ากับสัตว์ที่เดินสี่เท้า ผลการศึกษานี้สรุปได้ว่าการวัดค่าทางสัณฐานวิทยาของกระดูกยาวมีศักยภาพในการแยกกระดูกมนุษย์ออกจากกระดูกวัว สุนัข ม้า ลิงและสุกร

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Introduction

In forensic science, skeletal remains can reside at a site for an undetermined/unknown amount of time, with the more important question begging whether it is of human origin or not? As such, forensic anthropologists are frequently required to verify the human origin of complete and partial skeletal remains. This determination can be difficult for bone fragments with few or no morphological hallmarks, or made even more difficult for post-mortem damage (Ubelaker, 1989). The more fragile cancellous portions of the articular regions of long bones, especially of the humerus, femur and tibia, can often be targeted by carnivores (Haglund et al., 1988) and rodents (Klippel and Synstelien, 2007). The destruction of such potential diagnostic features may, thus, obstruct a quick decision as to whether a bone fragment is human or non-human.

Currently, there are various methods available for species discrimination on the basis of skeletal structure, such as morphological, histological, molecular biology and protein radioimmunoassay methods available for species discrimination on the basis of skeletal structure (Chilvarquer et al., 1987; Hillier and Bell, 2007; Imaizumi et al., 2005; Nganvongpanit et al., 2015). However, even with the advent of molecular approaches that potentially offer positive identification accuracy from fragments, they are not without their own specific limitations, especially in terms of cost, time, and in the field real-time application. Undoubtedly, there exists a need for an effective, rapid and inexpensive method for distinguishing human from non-human bone parts.

Qualitative morphological examination remains as one of the easiest methods used to differentiate between human and other mammalian skeletal remains in many aspects (McLain et al., 2002; Saulsman et al., 2010). To determine the biological profiles from human skeletons, classical osteometric techniques have been successfully used to determine sexual dimorphism from long bones such as humerus, femur, tibia or fibula (De Mendonça, 2000; Duyar and Pelin, 2003; Kranioti and Michalodimitrakis, 2009; Purkait, 2005; Rios Frutos, 2005; Wright and Vásquez, 2003). Stature is another biological profile that can be determined by morphometric techniques from long bones (Mahakkanukrauh et al., 2011). In addition, osteometric analysis of long bones has been successfully used to determine the difference between dog breeds (Alpak et al., 2004), red fox and arctic fox (Monchot and Gendron, 2010), sheep and goat (Salami et al., 2010). However, up to now, the identification of human and non-human mammalian bones by osteometric study is quite limited with population specific information. It has been reported on discrimination analysis of long bone morphometrics between humans and quadrupedal (sheep, dog, and pig) and bipedal (kangaroo and emu) animals common to Australia (Saulsman et al., 2010). The results from these study indicated enough variation between species to correctly assign an unknown bone as that of a human or non-human, with cross-validated classification accuracy of 95% or better. More importantly, however, the technique also proved to be accurate if only a fragment of the diaphysis is analyzed; classification accuracy 63–99%. The results of their study, therefore, outline a forensically useful noninvasive method to distinguish human from animal bones.

Our laboratory is interested in determining whether bone morphometry analysis, on long bones from some mammals, would be data to distinguish between humans and non-human species. Therefore, the aim of this study is to compare bone morphometry, using morphometric index measurements and angular measurements, of 3 long bones (humerus, femur and tibia) between human and non-human mammals (monkey, horse, cow, pig and dog). The choice of the animal groups chosen for study was partially influenced by the fact that these animals can be proximal to humans in agricultural areas (cow, pig, and horse), living with humans (dogs), or confused with humans (monkey), thereby having a great chance of being mistaken for human bones.

Materials and Methods

Bone samples

The protocol of this study was approved by Human Ethic Committee, Faculty of Medicine, Chiang Mai University to use human bones and by Animal Ethic Committee, Faculty of Veterinary Medicine, Chiang Mai University to use animal bones in 2013. Human dried bone samples were obtained from the Department of Anatomy, Faculty of Medicine, Chiang Mai University. Animal dried bone samples were obtained from the Animal Anatomy Museum, Department of Veterinary Biosciences and Public Health, Faculty of Veterinary Medicine, Chiang Mai University. The bones used in this study were humerus, femur and tibia. Bones from 14 human skeletons and 2-10 skeletons from each mammal species were used in this study. The age and number of the skeletons from each species is shown in Table 1, however sex was not identified. All bones obtained for use in our study did not have any anatomical anomalies or pathogenic lesions.

Measurements

A total of 33 measurements were generated (16 morphometric index and 17 angular measurement).Morphometric index measurements in this study were modified from previous studies by using either osteometric board, a sliding vernier caliper or a metric tape (Alpak et al., 2004; Bagaria et al., 2012; Bass, 1987; Coussens et al., 2002; Osterhoff et al., 2011; Paley, 2002). The morphometric indexes were calculated as described in Table 2 and Fig. 1. The angular measurements were performed by photographing each bone in its anatomical position and then evaluated the picture by the Image J program as described in Table 3 and Fig. 2. Each measurement was taken three times non-consecutively within one week apart.

Statistical analysis

All measurements are reported as mean and standard deviation. Significant difference between groups was tested by Student's *t*-test, with a *p*-value < 0.05 being an indicator of significance. All analyses were performed using SPSS version 17.

Results

Humerus

Mean and standard deviation of 10 humeral measurements (5 humeral morphometric index measurements and 5 humeral angular measurements) are provided in Table 4. Three morphometric indexes (HI1, HI3 and HI4) and 3 angular measurements (HA1, HA4 and HA5) can be potentially used to distinguish the human humerus from non-human mammalian species used in this study ($p<0.05$).

Femur

Mean and standard deviation of 12 femoral measurements (5 femoral morphometric index measurements and 7 femoral angular measurements) are provided in Table 5. Three morphometric indexes (FI1, FI4 and FI5) and 5 angular measurements (FA1, FA2, FA3, FA4 and FA5) can be potentially used to distinguish between the human femur from non-human mammalian species used in this study ($p<0.05$).

Tibia

Mean and standard deviation of 11 tibial measurements (6 tibial morphometric index measurements and 5 tibial angular measurements) are provided in Table 6. Two morphometric indexes (TI3, TI6) and 2 angular measurements (TA2 and TA3) can be potentially used to differentiate the human tibia from other mammalian species used in this study ($p<0.05$)

Table 1. Bone samples according to species.

Species	Age range (years)	Humerus	Femur	Tibia
Horse (*Equus ferus*)	>15	5	7	7
Dog (*Canis familiaris*)	5-10	20	20	20
Bovine (*Bos taurus*)	>7	5	3	4
Pig (*Sus domesticus*)	2-4	8	8	7
Assam macaque (*Macaca assamensis*)	3-8	8	7	8
Human (*Homo sapiens*)	40-65	28	28	28

Table 2. Description of morphometric index measurements.

Measurement	Description
Humerus	
HI1	Humeral robusticity index: The least circumference at distal part of humerus divided by the maximum length from proximal to distal ends of humerus.
HI2	Distal humeral articular index: The width of distal articular surface of the humerus divided by the epicondylar width of the bone.
HI3	Humeral circumference index: The least circumference at the distal part of humeral shaft divided by the maximum circumference at the proximal part of humeral shaft.
HI4	Olecranon fossa index: The width of olecranon fossa divided by the height of olecranon fossa.
HI5	Proximal humeral index: The width of humeral head divided by the length from the proximal end to proximal 1/3 of the bone.
Femur	
FI1	Femoral robusticity index: The least circumference of femoral shaft divided by the maximum length from the proximal to the distal ends of the bone.
FI2	Femoral intercondylar index: The width of the internal surface of the femoral condyles divided by the width from the external surface of femoral epicondyles.
FI3	Femoral platymeric index: The anteroposterior subtrochanteric diameter of the femur divided by the mediolateral subtrochanteric diameter of the bone.
FI4	Femoral epicondylar index: The width of the anterior part of the distal articular surface of femur divided by the epicondylar width of the bone.
FI5	Trochanteric index: The height of greater trochanter divided by the length from the head to proximal 1/3 of the femur.
Tibia	
TI1	Tibial index: The mediolateral diameter of midshaft of tibia divided by the maximum length from the proximal to distal ends of the tibia.
TI2	Tibial platymeric index: The mediolateral diameter at proximal 1/3 of the tibial shaft divided by the anteroposterior diameter at proximal 1/3 of the shaft of the bone.
TI3	Tibial midshaft index: The mediolateral diameter of midshaft of tibia divided by the mediolateral diameter of proximal condyles of the bone.
TI4	Tibial aspect index: The mediolateral tibial width divided by the anteroposterior tibial width.
TI5	Proximal tibial index: The proximal length from the proximal end to the proximal 1/3 of tibia divided by the maximum length from the proximal to the distal ends of the bone.
TI6	Distal tibial index: The anteroposterior diameter of medial malleolus divided by the maximum width of distal end of tibia.

Table 3. Angular measurements of bones as calculated using the Image J program.

Measurement	Descriptions
Humerus	
HA1	Humeral distal articular angle: The measurement of the angle among three reference points at the anterior side of humerus, from the distal mid-width point of the humerus to the shaft-width point at distal 1/3 of the bone and the most medial point of trochlea.
HA2	Humeral distal condylar angle: The measurement of the angle among three reference points at the anterior side of humerus, from the distal mid-width point of humerus to the shaft-width point at distal 1/3 of the bone and the most medial part of medial epicondyle.
HA3	Humeral shaft angle: The measurement of the angle between two reference lines at the anterior side of humerus, a sagittal line draws from mid-width point at proximal 1/3 of humerus to distal 1/3 of the bone and a horizontal line drawn across the distal 1/3 of the bone, measures from the lateral side of the bone.
HA4	Humeral inclination angle: The measurement of the angle between two reference lines at the anterior side of humerus, a sagittal line drawn from the mid-width point of shaft at mid-shaft and proximal 1/3 of humerus to the proximal end of the bone and an oblique line draws from mid-width point of the head to the first line, measures from the medial side of the bone.
HA5	Humeral tubercular angle: The measurement of the angle between two reference lines at the anterior side of humerus, an oblique line drawn from the tip of greater tubercle to the inferomedial point of humeral head and a horizontal line draws across the inferior part of head of the bone, measures from the lateral side of the bone.
Femur	
FA1	Femoral inclination angle: The measurement of the angle between two reference lines at the anterior side of femur, a sagittal line drawn from the mid-width point of shaft at mid-shaft and the proximal 1/3 of femur to proximal end of the bone and an oblique line drawn from the mid-width point of head to the first line, measures from the medial side of the bone.
FA2	Angle of posterior triangle of femur: The measurement of the angle among three reference points at the posterior side of femur, from the highest point of the greater trochanter to the center of the head and the most medial point of the lesser trochanter.
FA3	Proximal femoral shaft angle: The measurement of the angle between two reference lines at the posterior side of femur, an oblique line drawn from the tip of greater trochanter to the mid-width point at proximal 1/3 of the bone and a horizontal line draws across the proximal 1/3 of the bone, measures from the lateral side of the bone.
FA4	Latero-proximal femoral angle: The measurement of the angle between two reference lines at the anterior side of femur, a sagittal line drawn from the mid-width point at mid shaft and proximal 1/3 of femur to the proximal part of the bone and a horizontal line drawn from the center of the head to the tip of the greater trochanter, then measures the lateral angle.
FA5	Medio-proximal femoral angle: The measurement of the angle between two reference lines at the anterior side of femur, a sagittal line drawn from the mid-width point at mid shaft and proximal 1/3 of femur to the proximal part of the bone and a horizontal line drawn from the center of the head to the tip of the greater trochanter, then measures the medial angle.

Table 3. Angular measurements of bones as calculated using the Image J program (Cont.).

Measurement	Descriptions
FA6	Latero-distal femoral angle: The measurement of the angle between two reference lines at the anterior side of femur, a sagittal line drawn from the mid-width point of the distal end to the mid-width point at distal 1/3 of femur and a horizontal line drawn under the most distal points of the medial and lateral condyles of femur, then measures the lateral angle.
FA7	Medio-distal femoral angle: The measurement of the angle between two reference lines at the anterior side of femur, a sagittal line from the mid-width point of the distal end to the mid-width point at distal 1/3 of femur and a horizontal line drawn under the most distal points of the medial and lateral condyles of femur, then measures the medial angle.
Tibia	
TA1	Angle of tibial tuberosity: The measurement of the angle between two reference lines at the medial side of tibia, a sagittal line drawn parallel to the long axis of the tibia and an oblique line drawn from the highest point of the tibial tuberosity to the most anterior point of the tibial condyle.
TA2	Proximal tibial shaft angle: The measurement between two reference lines at the anterior side of tibia, a horizontal line drawn above the most superior part of tibia and a sagittal line drawn from the mid-width point at the proximal part to the mid-width point at the proximal 1/3 of the bone, measures from the lateral side of the bone.
TA3	Distal tibial shaft angle: The measurement between two reference lines at the anterior side of tibia, a horizontal line drawn under the most inferior part of the tibia and a sagittal line drawn from the mid-width point at the distal part to the mid-width point at the distal 1/3 of the bone, measures from the lateral side of the bone.
TA4	Angle of malleolus: The measurement between two reference lines at the anterior side of tibia, a horizontal line drawn under the most inferior part of the tibia and an oblique line drawn from the tip of medial malleolus to the lateral side of the distal part of the bone.
TA5	Tibial condylar angle: The measurement between two reference lines at the medial side of tibia, an oblique line drawn from the most posterior part of the condyles to the mid-width point at the proximal 1/3 of tibia and a horizontal line drawn across the proximal 1/3 point of the bone, measures from the posterior side of the bone.

Figure 1. The figures indicate the morphometric index measurements used in this study. The humeral index measurements are the humeral robusticity index (HI1), the distal humeral articular index (HI2), the humeral circumference index (HI3), the olecranon fossa index (HI4), and the proximal humeral index (HI5). The femoral index measurements are the femoral robusticity index (FI1), the femoral interconcylar index (FI2), the femoral platymeric index (FI3), the femoral epicondylar index (FI4), and the trochanteric index of femur (FI5). The tibial index measurements were the tibial index (TI1), the tibial platymeric index (TI2), the tibial midshaft index (TI3), the tibial aspect index (TI4), the proximal tibial index (TI5), and the distal tibial index (TI6). The right side of human humerus, femur and tibia is shown, respectively.

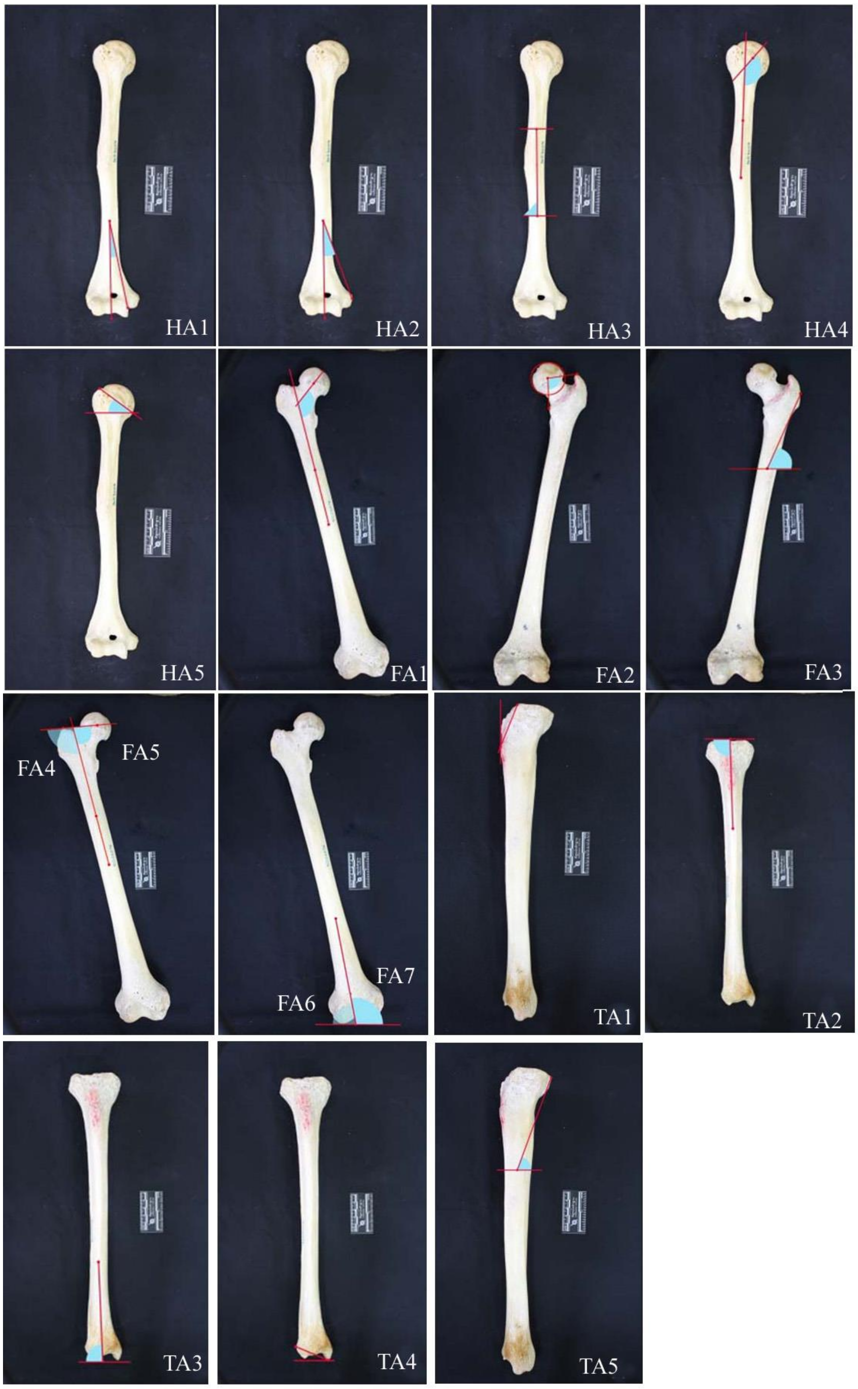

Figure 2. The figures indicate the angular measurements of the bones as calculated by Image J for this study. The humeral angular measurements are humeral distal articular angle (HA1), humeral distal condylar angle (HA2), humeral shaft angle (HA3), humeral inclination angle (HA4), and humeral tubercular angle (HA5). The femoral angular measurements are femoral inclination angle (FA1), angle of posterior triangle of femur (FA2), proximal femoral shaft angle (FA3), latero-proximal femoral angle (FA4), medio-proximal femoral angle (FA5), latero-distal femoral angle (FA6), and medio-distal femoral angle (FA7). The tibial angular measurements were angle of tibial tuberosity (TA1), proximal tibial shaft angle (TA2), distal tibial shaft angle (TA3), angle of malleolus (TA4), and tibial condylar angle (TA5). The right side of human humerus, femur and tibia is shown, respectively.

Table 4. Humerus measurement index comparison between species

	Human	Monkey	Horse	Cow	Pig	Dog
Morphometric index						
HI1	0.19±0.01 [d]	0.25±0.01 [e]	0.43±0.02 [a]	0.48±0.05 [a,c]	0.41±0.02	0.29±0.01
HI2	0.71±0.02 [d]	0.72±0.02 [d,e]	0.94±0.03 [a]	0.83±0.02	0.73±0.03	0.65±0.03
HI3	0.89±0.03 [d]	0.79±0.04 [e]	0.68±0.02 [a]	0.72±0.01	0.65±0.01	0.75±0.03
HI4	1.17±0.06 [d]	1.33±0.14 [e]	0.93±0.11 [a]	0.92±0.09	0.89±0.06	0.91±0.09
HI5	0.46±0.02 [d]	0.47±0.02 [d, e]	0.96±0.03 [a]	1.11±0.18	1.16±0.08	0.77±0.02
Angle (degree)						
HA1	9.77±1.00 [d]	10.89±0.99 [e]	22.07±2.11 [a]	27.13±2.12	17.87±2.33	12.46±0.99
HA2	19.48±1.15 [d]	20.35±2.16 [b,d,e]	29.88±1.88 [a]	30.78±1.81	30.29±3.69	21.77±1.58
HA3	89.21±0.99 [b,c,d]	89.23±2.02 [b,c,d,e]	81.63±1.50 [a]	88.61±2.67	87.50±1.68	89.25±1.18
HA4	133.95±2.14 [d]	110.31±2.62 [e]	125.83±2.90 [a]	129.94±1.48	121.01±1.99	119.77±3.02
HA5	36.51±1.92 [d]	51.09±1.78 [e]	39.96±3.26 [a]	47.30±2.39	51.52±2.65	73.21±3.60

Values are presented as mean±SD. Different superscripts ([a,b,c,d,e,f]) indicate a significant difference in index mean values between species ($p<0.05$).

Table 5. Femur measurement index comparison between species

Index	Human	Monkey	Horse	Cow	Pig	Dog
Morphometric index						
FI1	020±0.01 [d]	0.24±0.01 [e]	039±0.03 [a]	0.40±0.01 [a,c]	0.36±0.02 [a,f]	0.26±0.01 [b]
FI2	0.27±.0.03 [d]	0.27±0.05 [b,d,e]	0.20±0.02 [a]	0.16±0.00 [c]	0.20±0.02 [a,f]	0.29±0.03 [b]
FI3	0.87±0.06 [b,d]	0.91±0.06 [b,d,e]	1.24±0.10 [a]	1.00±0.01 [c]	1.08±0.06 [f]	0.85±0.05 [b]
FI4	0.47±0.02 [d]	0.44±0.02 [e]	0.58±0.03 [a]	0.43±0.00 [c]	0.39±0.02 [f]	0.36±0.02 [b]
FI5	0.15±0.01 [d]	0.29±0.01 [b, e]	0.71±0.04 [a]	0.73±0.01 [a,c]	0.43±0.02 [f]	0.31±0.01 [b]
Angle (degree)						
FA1	128.24±2.16 [d]	120.31±2.84 [e]	138.01±0.34 [a]	144.50±0.13 [c]	138.46±1.10 [a,b,f]	138.19±4.87 [a,b]
FA2	94.68±4.29 [d]	107.23±2.84 [b,e]	163.00±2.51 [a]	146.02±2.11 [c]	134.60±1.66 [c,f]	109.12±3.08 [b]
FA3	65.68±3.15 [d]	74.08±2.31 [e]	54.24±2.21 [a]	37.71±0.48 [c]	60.59±2.06 [f]	71.18±2.56 [b]
FA4	93.04±5.15 [d]	122.05±2.23 [e]	136.25±5.73 [a]	112.09±0.34 [c]	105.79±2.80 [b,f]	103.43±4.69 [b]
FA5	86.90±5.12 [d]	57.95±2.24 [e]	43.81±5.50 [a]	68.05±0.33 [c]	74.27±2.91 [b,f]	76.62±4.61 [b]
FA6	82.16±1.75 [c,d]	86.52±2.11 [c,e]	90.48±2.04 [a]	91.66±1.52 [a,b,c]	90.25±2.50 [a,c,f]	92.56±2.62 [b]
FA7	97.84±1.75 [c,d]	93.48±2.08 [c,e]	89.52±2.05 [a]	88.20±1.42 [a,b,c]	89.74±2.46 [a,c,f]	87.39±2.59 [b]

Values are presented as mean±SD. Different superscripts ([a,b,c,d,e,f]) indicate a significant difference in index mean values between species ($p<0.05$).

Table 6. Tibia measurement index comparison between species

Index	Human	Monkey	Horse	Cow	Pig	Dog
Morphometric index						
TI1	0.06±0.00 ^{d}	0.06±0.00 ^{d,e}	0.11±0.01 ^{a}	0.13±0.01 ^{a,c}	0.12±0.01 ^{a,c,f}	0.08±0.01 ^{b}
TI2	0.72±0.04 ^{d}	0.66±0.07 ^{d,e}	0.98±0.07 ^{a}	1.01±0.01 ^{a,c}	0.78±0.05 ^{f}	0.86±0.06 ^{b}
TI3	0.31±0.02 ^{d}	0.33±0.02 ^{e}	0.43±0.02 ^{a}	0.43±0.03 ^{a,c}	0.38±0.01 ^{b,f}	0.38±0.02 ^{b}
TI4	1.61±0.06 ^{c,d}	1.53±0.07 ^{c,e}	1.72±0.06 ^{a}	1.68±0.12 ^{a,b,c}	1.54±0.05 ^{c,e,f}	1.65±0.06 ^{b}
TI5	0.33±0.02 ^{b,c,d}	0.30±0.02 ^{a,c,e}	0.31±0.01 ^{a}	0.33±0.03 ^{a,b,c}	0.43±0.03 ^{f}	0.33±0.03 ^{b}
TI6	0.66±0.05 ^{d}	0.77±0.03 ^{e}	0.36±0.02 ^{a}	0.56±0.01 ^{b,c}	0.61±0.03 ^{f}	0.55±0.04 ^{b}
Angle (degree)						
TA1	23.99±2.33 ^{a,d}	19.25±1.85 ^{e}	22.33±2.08 ^{a}	32.87±0.70 ^{c}	15.05±1.19 ^{f}	35.22±1.84 ^{b}
TA2	90.42±2.54 ^{d}	93.26±0.80 ^{a,b,e}	94.19±1.26 ^{a}	94.34±0.39 ^{a,c}	96.62±1.38 ^{a,f}	92.81±0.94 ^{b}
TA3	88.34±0.83 ^{d}	93.03±1.61 ^{c,e}	89.23±0.79 ^{a}	93.45±1.11 ^{c}	91.34±1.20 ^{a,f}	87.03±1.14 ^{b}
TA4	30.31±2.41 ^{b,d}	35.12±1.27 ^{c,e}	11.74±1.02 ^{a}	34.82±0.47 ^{c}	31.15±1.07 ^{b,d,f}	30.35±2.33 ^{b}
TA5	65.41±2.27 ^{a,d}	61.07±0.92 ^{e}	64.97±2.13 ^{a}	55.04±2.76 ^{c}	54.90±0.75 ^{c,f}	70.47±2.42 ^{b}

Values are presented as mean±SD. Different superscripts (^{a,b,c,d,e,f}) indicate a significant difference in index mean values between species (p<0.05).

Discussion

Forensic anthropologists generally need to distinguish between human and non-human bone. However, if long bones of non-human origin are close in size to that of humans, the job of determining whether skeletal remains are of human origin or not becomes more difficult. As such, our group was interested in determining whether bone morphometry analysis (morphometric index measurements and angular measurements), on 3 common long bones (humerus, femur and tibia) from animals with similar sizing to humans (cow, dog, horse, monkey, and pig), would be robust enough of a technique to distinguish between humans and non-humans. Out of 33 measurements measured with our long bone samples, 18 measurements from human long bones were significantly different to that of other non-human mammal in this study.

In the humerus, 3 morphometric index measurements (HI1, HI3 and HI4) and 3 angular measurements (HA1, HA4 and HA5) can be potentially used to distinguish between human and the 5 non-human mammals used in our study. Regarding morphometric measurements, HI1 designates the size of the humerus, of which HI1 from human samples was lowest amongst the group, indicating humans have the smallest humerus, as compared to the other species examined in this study. Meanwhile, HI3 indicates the shape of the humeral shaft, with the mean value of HI3 from human samples being the highest amongst the group, indicating that human humerus is more cylindrically shaped. Lastly, HI4 indicates the shape of the olecranon fossa of the humerus. The mean value of HI4 from monkey humerus was the highest amongst the group, indicating that monkey olecranon fossa is horizontal-ovoid shaped. With respect to angular measurements, HA1 represents the distal articular angle of the humerus. The mean value of HA1 from human samples was lowest amongst the group, indicating that the distal articular surface of the human humerus is the narrowest when compared to all other mammals used in this study. HA4 represents the inclination angle of the humeral head, and the mean value of HA4 of the human humerus was the highest amongst the group, indicating that the neck of the human humerus is aligned in an obtuse angle with the long axis of the bone. Lastly, HA5 represents the tubercular angle of the humerus. The mean value of HA5 from human humerus was lowest amongst the group, indicating that the plane of head of the human humerus lies almost parallel to the transverse plane of the bone.

In femur, 3 morphometric indexes (FI1, FI4 and FI5) and 5 angular measurements (FA1, FA2, FA3, FA4 and FA5) can be potentially used to distinguish between human and the 5 non-human mammals used in our study. Regarding morphometric measurements, FI1 represents the robusticity index of the femur, which indicates the size of the bone. The mean value of FI1 from human samples was the lowest amongst the group, indicating humans have the smallest femur, as compared to the other species examined in this study. Meanwhile, FI4 represents the epicondylar index of the femur, which indicates the width ratio of the distal part of the bone. The mean value of FI4 from horse samples was the highest amongst the group of samples, reflecting that horses have the widest distal femur surface. Lastly, FI5 represents the trochanteric index of the femur, which indicates the height ratio of the greater trochanter of the bone. The mean value of FI5 from human samples was the lowest amongst the group, indicating that humans have the shortest greater trochanter. With respect to angular measurements, focusing on the greater trochanter and femoral head, the FA4 and FA5 from human samples were close to each other, indicating that the human greater trochanter lies almost parallel to the head of the femur. Alternatively, the greatest difference of FA4 and FA5 was observed in horse samples, indicating that the level of horse greater trochanter is higher that the head of the femur.

In tibia, only 2 morphometric indexes (TI3 and TI6) and 2 angular measurements (TA2 and TA3) can be potentially used to distinguish between human and the 5 non-human mammals used in our study. Regarding morphometric measurements, TI3 represents the ratio of tibial proximal condyles to its shaft. The mean value of TI3 in human samples was the lowest amongst the group, reflecting that humans have the largest surface of the tibial proximal condyle with the thinnest shaft, as compared to the other species examined in this study. TI6 represents the shape of medial malleolus. The mean value of TI6 in horse samples was the lowest amongst the group, implying that horses have ovoid-shaped medial malleolus. With respect to angular measurements, TA2 and TA3 indicate the proximal and distal angles of the tibia shaft. The mean values of TA2 and TA3 from human tibia samples were the lowest values as compared to the other species examined in this study, respectively, which indicates that the tibial angle in humans lie close to a right angle.

Overall, the difference in results between human and non-human bone morphometry in this study may be associated with the difference of bone functions among these species due to the biomechanics of the bipedal and quadrupedal (Saulsman et al., 2010; Ubelaker et al., 2004). Humans are uniquely bipedal with their ranges of movement, from walking, to running. As a result, long bone form and function of bipedal cursors are generally more specialized than quadrupedal cursors. However, the limitation of our study was we could not propose discrimination function due to the limitation of sample used. Moreover, further study need to increase the number of animal species with number of sample of each species to increase the power of discrimination.

In conclusion, there is a necessity to easily differentiate human bones from non-human remains on site/location, within the forensic context. Misidentification can result in further costly, timely and unnecessary investigation. The present study has provided standards (morphometric and angular measurements by image analysis) that allow measurements from any femur, humerus, and tibia to be simply and quickly intercalated without further analysis, to distinguish human long bones from non-human mammal species. The bones were measured by a classic osteometric method using osteometric board and vernier caliper and then calculated as the morphometric indexes. Out of 33 measurements measured with our long bone samples, 18 measurements from human long bones were significantly different to that of other non-human mammal in this study. Hence, there is great potential to use bone morphometry analysis, on long bones, to distinguish between humans and non-humans.

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

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