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Differences in compact bone tissue microscopic structure between adult humans (*Homo sapiens*) and Assam macaques (*Macaca assamensis*)

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ABSTRACT

This study investigated the osteon structure of adult humans and Assam macaques, which served as a nonhuman primate model, to find an adequate key for species identification. Samples of compact bone from humans ($n = 5$) and Assam macaques ($n = 5$) – including humerus ($n = 20$), radius ($n = 20$), ulna ($n = 20$), femur ($n = 20$), tibia ($n = 20$) and fibula ($n = 20$) – were processed using conventional histological techniques. 100 secondary osteons from each sample were evaluated under light microscopy. Parameter measurements included: diameter, perimeter and area of Haversian canal and osteon; distance between centers of Haversian canals; and ratio between diameter of Haversian canal and osteon. Four parameters, including diameters and areas of Haversian canal and osteon, demonstrated significantly higher ($P < 0.05$) values in humans than in Assam macaques. Therefore, compact bone microstructure could thus be used as a potential tool to differentiate human and nonhuman primates.

1. Introduction

The first question when faced with a bone fragment in forensic anthropology is whether it is human in origin or not? There are various techniques for species identification, such as macroanatomy [1,2], microanatomy [3,4], radiographic imaging [5] and DNA markers [6]. In situations where bone has been degraded or fragmented into small pieces, making it difficult to classify by gross morphology, other techniques can be used. Species differentiation using microanatomy is of increasing interest because this technique is easy to perform, cost-efficient, quick, and does not require expertise on the part of the investigator [3]. Alternately, microanatomical (histological) examination using microscopy is another effective technique for visualizing internal bone structure. Not only does this technique have advantages for forensic anthropology, it is also important for age estimation [7], bioarchaeology [8] and paleopathology [9].

The principal structures normally used to identify nonhuman bone are osteonal banding and plexiform bone, which is usually found in cows, deer, dogs and pigs [10,11]. However, certain bones belonging to some species do not show this specific structure, thus often making it difficult to distinguish between human and nonhuman bone. Other bone features can also be considered as a classification tool. The first report on classifying compact bone from humans and animals was published in 1903 [12]. Since then, many reports have compared human compact bone with avian [13], deer [11,14], sheep [10,15], pig [10], dog [11], rabbit [15] and cow [15] bones; demonstrating that osteon structure has potential as a classification tool which could be used to identify human compact bone.

Although studies have found a significant difference in osteon structure between humans and animals, the difference between humans and nonhuman primates has yet to be reported. *Therefore*, the purpose of this study was to microscopically examine and

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compare histological features found in nonhuman (primate) and human compact bone.

2. Materials and methods

Human bone samples were obtained from the Department of Anatomy, Faculty of Medicine, Chiang Mai University. The following bones, including humerus, ulna, radius, tibia and fibula, from 5 adult human male skeletons, aged between 50 and 65 years old were used. The Assam macaque bone samples were obtained from the Animal Anatomy Museum, Department of Veterinary Biosciences and Public Health, Faculty of Veterinary Medicine, Chiang Mai University. The following bones, including humerus, ulna, radius, tibia and fibula, from 5 adult Assam macaque male skeletons, aged between 3 and 8 years old were used. The compact bones were 0.5–1.0 cm-thick, cross-sectioned from the superficial surface of the bone at the midshaft point of the humerus, ulna, radius, femur, tibia and fibula. All bones obtained for use in this study did not have any anatomical anomalies or pathogenic lesions. Ethical approval for this research was obtained in 2013 from the research ethics board, Faculty of Medicine, Chiang Mai University.

Bone tissues were separately fixed in 10% formalin for 24 h and then decalcified by 10% nitric acid for 8 h. The specimens were cut into 1-mm-thick pieces and placed in plastic cassettes. Briefly, the tissues were processed in 10% formalin for 1 h (two changes), 95% alcohol for 1 h (three changes), isopropyl alcohol for 1 h (two changes), xylene for 1 h (two changes), and paraplast for 1 h (three changes). The tissues were then embedded in paraffin and cut into 5 μm sections.

Sections were deparaffinized in xylene and rehydrated through a graded series of alcohol to water. Tissue sections were then stained with Harris's hematoxylin for 5 min and washed under running tap water for 5 min; differentiated in 1% acid alcohol for 5 s and washed under tap water for 5 min; dipped in saturated lithium carbonate solution for 5 s and washed under tap water for 5 min; and stained with 1% eosin Y for 3 min and washed under running tap water for 5 min. The sections were then dehydrated through 95% alcohol and absolute alcohol, cleared in xylene, and mounted in Permount.

Individual sections were observed at 40× and 100× using a compound light microscope (AxioCam; Carl Zeiss, Oberkochen, Germany) and measured using AxioVision 4.8.2 software. For each bone sample, 10 slides were made and at least 100 secondary/mature osteons were measured from slides. Only mature osteons, which showed a cement line, were circular in shape, and had complete transverse sections, were selected. Oval or non-circular shapes osteons were excluded. The following quantitative parameters were studied (Fig. 1):

- Diameter, perimeter, and area of Haversian canal and osteon.
- Distance between centers of close Haversian canals.
- Index between diameter of Haversian canal and osteon.

Data analysis was performed using SAS version 8.0 (SAS Institute, Cary, NC). One-way ANOVA and *t*-test were used to test for differential expression. A *P*-value of ≤0.05 was considered statistically significant.

3. Results

In comparing six bones – humerus, ulna, radius, femur, tibia and fibula – within species (Tables 1–8 and Fig. 2), there was a significant difference ($P < 0.05$) for every parameter in human bones. However, in Assam macaques, a significant difference ($P < 0.05$) was observed in only four parameters: diameter of the Haversian canal, perimeter of the Haversian canal, and areas of the Haversian canal and osteon.

The diameter of the Haversian canal in humans (39–47 μm) was significantly larger ($P < 0.05$) than in Assam macaques

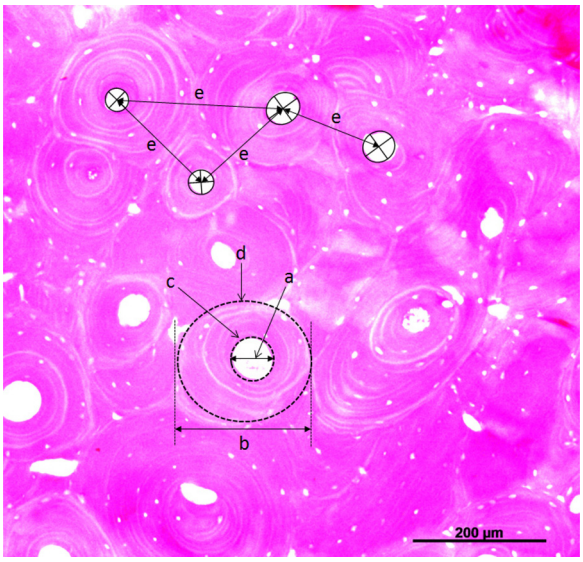

Fig. 1. Quantitative parameters were studied including diameter of Haversian canal (a), diameter of osteon (b), perimeter of Haversian canal (c), perimeter of osteon (d) and distance between centers of close Haversian canals (e) (magnification 50×).

Table 1
Comparative Haversian canal diameter (μm).

	Human	Assam macaque	*P*-value
Humerus	$45.17 \pm 10.63^{a,b,c}$	$36.66 \pm 8.76^{a,b}$	0.000
Ulna	$46.37 \pm 9.42^{b,c}$	$36.71 \pm 7.12^{a,b}$	0.000
Radius	$40.79 \pm 8.47^{a,d}$	38.41 ± 6.36^{b}	0.026
Femur	$42.74 \pm 8.53^{a,b,d}$	$36.64 \pm 6.66^{a,b}$	0.000
Tibia	39.71 ± 7.95^{d}	33.74 ± 6.53^{a}	0.000
Fibula	47.37 ± 11.30^{c}	$35.90 \pm 5.87^{a,b}$	0.000
P-value	0.001	0.000	

Different superscript letters (^{a,b,c,d}) indicate a significant difference ($P < 0.05$) between bones in the same species.

Table 2
Comparative osteon diameter (μm).

	Human	Assam macaque	*P*-value
Humerus	$184.96 \pm 28.63^{a,b}$	149.09 ± 25.96	0.000
Ulna	$197.72 \pm 33.60^{b,c}$	153.14 ± 29.76	0.000
Radius	181.28 ± 23.77^{a}	153.07 ± 18.63	0.000
Femur	175.60 ± 25.68^{a}	150.28 ± 27.53	0.000
Tibia	184.66 ± 28.63^{a}	143.46 ± 26.80	0.000
Fibula	202.95 ± 30.90^{c}	146.74 ± 25.01	0.000
P-value	0.000	0.062	

Different superscript letters (^{a,b,c}) indicate a significant difference ($P < 0.05$) between bones in the same species.

Table 3
Comparative Haversian canal perimeter (μm).

	Human	Assam macaque	*P*-value
Humerus	$141.86 \pm 33.39^{a,b,c}$	$115.12 \pm 27.52^{a,b}$	0.000
Ulna	$145.62 \pm 29.59^{b,c}$	$115.29 \pm 22.37^{a,b}$	0.000
Radius	$128.08 \pm 26.60^{b,c}$	120.61 ± 19.97^{b}	0.026
Femur	$134.22 \pm 26.78^{a,b,c}$	$115.05 \pm 20.963^{a,b}$	0.000
Tibia	124.70 ± 24.98^{d}	105.94 ± 20.50^{a}	0.000
Fibula	148.77 ± 35.51^{c}	112.73 ± 18.44^{a}	0.000
P-value	0.000	0.000	

Different superscript letters (^{a,b,c,d}) indicate a significant difference ($P < 0.05$) between bones in the same species.