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Unraveling the Microscopic Mysteries: A Comparative Analysis of Haversian System Morphology in Thai Upper Limb Bones

Descubriendo los Misterios Microscópicos: Un Análisis Comparativo de la Morfología de las Osteonas en los Huesos de los Miembros Superiores Tailandeses

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SUMMARY: Sex estimation from skeletal remains is crucial in forensic anthropology, with growing interest in the microscopic differences in bone structure between sexes. This study compared the Haversian canal (Osteonic canal) diameter (Diameter.Hc) and Haversian system (Osteon) diameter (Diameter.Hs) in the humerus, radius, and ulna of adult males and females in a Thai population. Transverse sections from the midshaft of each bone were obtained from 20 cadavers (10 males, 10 females), processed histologically, and stained with hematoxylin and eosin. Results showed no significant differences in Diameter.Hc and Diameter.Hs between sexes or among the three bones within each sex ($p > 0.05$). However, both parameters were larger in humans compared to Assamese macaques, highlighting interspecific variations in bone microstructure. These findings suggest that Haversian canal and system diameters may not be reliable sex indicators in the Thai population. Nonetheless, the observed differences between humans and non-human primates underscore the importance of further research into interspecific variation in bone microstructure and its potential applications in forensic anthropology and comparative anatomy.

KEY WORDS: Haversian system; Sexual dimorphism; Histomorphometry; Upper limb bones.

INTRODUCTION

Forensic anthropology plays a crucial role in the identification of individuals from skeletal remains. Biological profiles, such as age, sex, stature, and ancestry, can be determined through the analysis of skeletal morphology (Passalacqua *et al*., 2021). Among these profiles, sex estimation is considered one of the most important aspects of forensic anthropology, as it can significantly narrow down the pool of potential matches (Krishan *et al*., 2016). Traditionally, sex estimation has been performed using morphological features of the pelvis and skull, which are known to exhibit sexual dimorphism (Kranioti & Paine, 2011). However, in cases where these skeletal elements are not available or are fragmented, long bones of the upper limb have been shown to be reliable indicators of sex (Asala, 2001; Charisi *et al*., 2011). Several studies have investigated the sexual dimorphism of upper limb bones using morphometric analysis. For instance, Asala (2001) found significant differences in the dimensions of the humerus, radius, and ulna between South African males and females. Similarly, Charisi *et al*. (2011) reported that the length and diameter of the humerus, radius, and ulna were significantly larger in Greek males compared to females.

While morphometric studies have provided valuable insights into the sexual dimorphism of upper limb bones, histological analyses have been less explored. Bone histomorphometry, which involves the quantitative assessment of bone microstructure, has been shown to vary between sexes in other skeletal elements. For example, Havill (2004), found that osteon remodeling dynamics in the femur differed between male and female rhesus macaques (*Macaca mulatta*), with males exhibiting larger osteon sizes compared

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to females. Additionally, Nganvongpanit *et al*. (2015), reported differences in the compact bone tissue microstructure between adult humans (*Homo sapiens*) and Assam macaques (*Macaca assamensis*), suggesting that histomorphometric parameters could be used to differentiate between species. Despite these findings, there is a paucity of research investigating the histomorphometric differences in upper limb bones between sexes in humans. Such studies could provide valuable information for forensic anthropologists, as histological features may be preserved in fragmentary or burnt remains where morphometric analysis is not possible (Cummaudo *et al*., 2018). Moreover, understanding the variation in bone microstructure between sexes could provide insights into the underlying biological mechanisms of sexual dimorphism.

Therefore, the present study aimed to compare the histomorphometric parameters of the humerus, radius, and ulna between adult Thai males and females. Specifically, we examined the diameter of the Haversian canal (Osteonic canal) and Haversian system (Osteon) in each bone type and compared these parameters between sexes. We hypothesized that there would be significant differences in the Haversian canal and Haversian system diameters between males and females, reflecting the sexual dimorphism observed in morphometric studies. Additionally, we investigated whether these histomorphometric parameters differed between the three upper limb bones, as previous studies have suggested that bone microstructure may vary across skeletal elements (Cummaudo *et al*., 2018). To our knowledge, this is the first study to examine the histomorphometric differences in upper limb bones between sexes in a Thai population, thus providing novel insights into the forensic and biological aspects of sexual dimorphism.

MATERIAL AND METHOD

Sample collection and preparation. In this study, humerus, radius, and ulna bones were obtained from 20 cadavers (10 males and 10 females) in the Department of Anatomy, Faculty of Medical Science, Naresuan University, Thailand. The cadavers were of known age, sex, and cause of death, and had no history of bone-related diseases or injuries. Only the right upper limb bones were collected to ensure consistency and avoid potential bilateral asymmetry. The bones were cleaned of soft tissue and stored in a dry environment until further processing. Transverse sections of approximately 1.0-1.5 cm thickness were cut from the midshaft of each bone using a low-speed saw, following the protocol described by Beauchesne & Saunders (2006). The sections were then fixed in 10% neutral buffered formalin for 48 hours and decalcified in 20% formic acid for two weeks, with the solution being changed after one week. After decalcification, the samples were dehydrated in a graded series of ethanol, cleared in xylene, and embedded in paraffin block.

Histological processing and analysis. Thin sections of 5 μm thickness were cut from the paraffin-embedded samples using a rotary microtome (RM2235, Leica Biosystems, Wetzlar, Germany) and stained with hematoxylin and eosin (H&E) according to standard protocols. The stained sections were mounted on glass slides and visualized using a light microscope, equipped with a digital camera (DP27, Olympus, Tokyo, Japan). For each bone sample, five microscopic fields were randomly selected and photographed at 10× magnification. The diameter of the Haversian canal (Diameter.Hc) and the diameter of the Haversian system (Diameter.Hs) (as shown in Fig.1) were measured in micrometers (μm) using ImageJ software (version 1.53a, National Institutes of Health, Bethesda, MD, USA). Only complete and circular transverse sections of Haversian canals and systems were included in the analysis to ensure consistency and accuracy.

Statistical analysis. All data were expressed as mean ± standard deviation (SD). Independent t-tests were used to compare the means of Diameter.Hc and Diameter.Hs between males and females for each bone type. One-way analysis of variance (ANOVA) with Tukey's post hoc test was used to compare the means of Diameter.Hc and Diameter.Hs among the three bone types (humerus, radius, and ulna) within each sex. Statistical analyses were performed using SPSS software (version 23.0, IBM Corp., Armonk, NY, USA), with a significance level set at $p < 0.05$.

RESULTS

Histological appearance of Haversian systems. The decalcification process using 20% formic acid and subsequent H&E staining allowed for clear visualization of the Haversian systems in the transverse sections of the humerus, radius, and ulna (Fig. I). The Haversian canals appeared as circular or oval spaces surrounded by concentric lamellae of bone matrix, forming the Haversian systems. The morphology of the Haversian systems varied in size and shape, with some being more circular and others more elongated or irregular.

Comparison of Diameter.Hc and Diameter.Hs between sexes. The mean values of Diameter.Hc and Diameter.Hs for each bone type in males and females are presented in Table I. Independent t-tests revealed no significant differences in Diameter.Hc ($p = 0.51$) or Diameter.Hs ($p = 0.97$) between males and females in the humerus. Similar results were observed in the radius and ulna. Independent t-tests showed no significant differences in Diameter.Hc or

Diameter.Hs between males and females in either the radius (p = 0.22 and p = 0.83, respectively) or the ulna (p = 0.05 and p = 0.32, respectively).

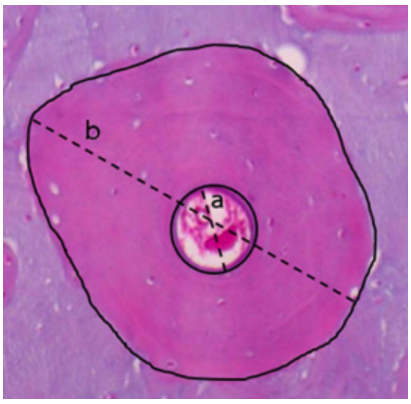

Fig. 1. the quantitative parameters studied are (a) the diameter of the Haversian canal, (b) and the diameter of the Haversian system.

Comparison of Diameter.Hc and Diameter.Hs among bone types. One-way ANOVA was used to compare the mean values of Diameter.Hc and Diameter.Hs among the humerus, radius, and ulna within each sex (Table II). In males, the mean Diameter.Hc in the humerus, the radius, and the ulna, with no significant differences among the three bone types (p = 0.887). Similarly, the mean Diameter.Hs in males in all 3 bone types was no significant differences among the bone types (p = 0.653).

In females, the mean Diameter. Hc in the humerus, the radius, the ulna there were no significant differences among the bone types (p = 0.704). The mean Diameter.Hs in females in the humerus, in the radius and in the ulna, there were also no significant differences among the bone types (p = 0.553). These results indicate that the histomorphometric parameters of Diameter.Hc and Diameter.Hs did not differ significantly among the humerus, radius, and ulna in either males or females in this Thai population sample.

Table I. Quantitative parameter value comparison of upper limb bones including humerus, radius and ulna in males and females.

Bones	Parameters	Male (n=10)	Female (n=10)	*P*-value
Humerus	Diameter.Hc (µm)	40.94±3.83	37.40±9.52	0.51
	Diameter.Hs (µm)	154.00±16.07	153.56±18.78	0.97
Radius	Diameter.Hc (µm)	42.07±4.50	33.29±4.90	0.22
	Diameter.Hs (µm)	165.50±23.70	170.40±13.89	0.83
Ulna	Diameter.Hc (µm)	40.70±2.88	32.97±7.52	0.05
	Diameter.Hs (µm)	164.07±17.93	148.38±29.04	0.32

Table II. A comparison of the diameter of the Haversian canal and Haversian system in three bones of each sex.

Bones	Male		Female	
	Diameter.Hc (µm)	Diameter.Hs (µm)	Diameter.Hc (µm)	Diameter.Hs (µm)
Humerus	40.94±3.83	154.00±16.07	37.40±9.52	153.56±18.78
Radius	42.07±4.50	165.50±23.70	33.29±4.90	170.40±13.89
Ulna	40.70±2.88	164.07±17.93	32.97±7.52	148.38±29.04
P-value	0.887	0.653	0.704	0.553

DISCUSSION

The use of 20% formic acid as a decalcification agent in this study resulted in clear visualization of the Haversian system and adequate staining of the tissue, consistent with the findings of Bogoevski *et al*. (2019). Formic acid is widely used in anatomical and morphological studies due to its effectiveness in removing minerals while preserving tissue structure. Although, other solutions such as nitric acid, EDTA, and hydrochloric acid can be used for bone decalcification. Thus, the evidence suggests that 20% formic acid is a suitable solution for decalcifying bone in histomorphometric studies of the Haversian system, allowing for clear visualization and analysis of the morphological and histomorphometric characteristics of bone tissue without causing significant damage or distortion.

In this study, we compared the histomorphometric parameters of the Haversian canal diameter (Diameter.Hc) and Haversian system diameter (Diameter.Hs) in the humerus, radius, and ulna between male and female adults in a Thai population. Our results showed no significant differences in Diameter.Hc and Diameter.Hs between sexes for any of the three bone types examined. Additionally, we

found no significant differences in these parameters among the humerus, radius, and ulna within each sex. The lack of sexual dimorphism in Diameter. Hc and Diameter. Hs observed in our study is consistent with the findings of Pfeiffer (1998), who reported no significant differences in osteon size between males and females in a sample of prehistoric Native American femora. Similarly, Mulhern & Van Gerven (1997) found no significant differences in osteon area or Haversian canal area between sexes in a medieval Nubian population. These studies, along with our results, suggest that the histomorphometric parameters of Haversian systems may not be a reliable indicator of sex in skeletal remains. However, our findings contrast with those of Burr *et al*. (1990), who reported larger osteon size in males compared to females in a sample of modern human ribs. The discrepancy between their results and ours may be attributed to differences in the skeletal elements examined, as well as potential population-specific variations in bone microstructure. Furthermore, Britz *et al*. (2009) found that osteon size and Haversian canal size varied along the length of the femur, suggesting that the location of the bone section may influence histomorphometric parameters. Future studies should consider examining multiple skeletal elements and sampling sites to provide a more comprehensive understanding of the factors influencing Haversian system morphology.

The absence of significant differences in Diameter. Hc and Diameter.Hs among the humerus, radius, and ulna in our study is consistent with the findings of Dittmann *et al*. (2006), who reported similar osteon sizes among long bones of the upper limb in a sample of modern humans. This suggests that the mechanical loading environment may be relatively uniform across the upper limb bones, resulting in similar patterns of bone remodeling and Haversian system morphology (Robling & Stout, 2003).

Interestingly, our results revealed differences in Diameter. Hc and Diameter.Hs between humans and other mammalian species. Compared to the values reported by Nganvongpanit *et al*. (2015), for Assamese macaques (*Macaca assamensis*), the Haversian canal and Haversian system diameters were larger in both male and female humans in our study. This finding supports the notion that bone microstructure varies among different taxa, likely due to differences in body size, locomotor behavior, and life history (Hillier & Bell, 2007). Comparative studies of bone histomorphometry across a wider range of species may provide valuable insights into the evolutionary and functional significance of Haversian system morphology (Table III).

In conclusion, our study found no significant differences in the Haversian canal diameter and Haversian system diameter between adult males and females or among the humerus, radius, and ulna in a Thai population. These results suggest that histomorphometric parameters of the Haversian system may not be a reliable indicator of sex or bone type in forensic and anthropological contexts. However, the observed differences between humans and other mammalian species highlight the importance of considering interspecific variations in bone microstructure. Future research should aim to expand the sample size, include a wider range of skeletal elements, and explore potential age-related changes in Haversian system morphology to further elucidate the factors influencing bone microstructure in humans and other species.

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Table III. The diameter of the Haversian canal and Haversian system in different animals.

Animals	Study	Diameter.Hs (μm)	Diameter.Hc (μm)
Monkey (*Macaca assamensis*)	Nganvongpanit *et al*. (2015)[a]	153.07±18.63	38.41±6.36
Dog (Golden Retrievers)	Nganvongpanit *et al*. (2017)	174.32±47.33	38.62±20.62
Cat (*Felis catus*)	Morales *et al*. (2012)	49.93±3.29	10.60±1.01
Pig (*Sus scrofadomesticus*)	Morales *et al*. (2012)	114.76±8.19	40.09±14.82
Bovine (Bos taurus)	Morales *et al*. (2012)	118.34±16.40	26.24±3.75
Hen (*Gallus gallus*)	Morales *et al*. (2012)	114.76±8.19	29.49±2.18
Elephant (*Elephas maximus*)	Nganvongpanit *et al*. (2017)[a]	262.00±72.00	61.00±24.00
Human (*Hommosapiens sapiens*)	Nganvongpanit *et al*. (2015)[a]	181.28±23.77	40.79±8.47
Human (*Hommosapiens sapiens*)	This study male[a]	165.50±23.70	42.07±4.50
Human (*Hommosapiens sapiens*)	This study female[a]	170.40±13.89	33.29±4.90

[a] The data was obtained from radius.

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RESUMEN: La estimación del sexo a partir de restos esqueléticos es crucial en la antropología forense, con un interés creciente en las diferencias microscópicas en la estructura ósea entre sexos. Este estudio comparó el diámetro del canal osteónico (Canal de Havers) (Diámetro.Hc) y el diámetro de la osteona (Sistema de Havers) (Diámetro.Hs) en el húmero, el radio y la ulna de hombres y mujeres adultos en una población tailandesa. Se obtuvieron secciones transversales de la diáfisis media de cada hueso de 20 cadáveres (10 hombres, 10 mujeres), se procesaron histológicamente y se tiñeron con hematoxilina y eosina. Los resultados no mostraron diferencias significativas en Diámetro.Hc y Diámetro.Hs entre sexos o entre los tres huesos dentro de cada sexo ($p > 0,05$). Sin embargo, ambos parámetros eran mayores en los humanos en comparación con los monos asamés, lo que destaca las variaciones interespecíficas en la microestructura ósea. Estos hallazgos sugieren que los diámetros del canal osteónico y de la osteona pueden no ser indicadores de sexo confiables en la población tailandesa. No obstante, las diferencias observadas entre humanos y primates no humanos subrayan la importancia de realizar más investigaciones sobre la variación interespecífica en la microestructura ósea y sus posibles aplicaciones en antropología forense y anatomía comparada.

PALABRAS CLAVE: Osteona; Dimorfismo sexual; Histomorfometría; Huesos de las miembros superiores.

REFFERENCES

Asala, S. A. Sex determination from the head of the femur of South African whites and blacks. *Forensic Sci. Int.; 117(1-2)*:15-22, 2001.

Beauchesne, P. & Saunders, S. A test of the revised Frost's 'rapid manual method' for the preparation of bone thin sections. *Int. J. Osteoarchaeol., 16(1)*:82-7. 2006.

Bogoevski, K.; Woloszyk, A.; Blackwood, K.; Woodruff, M. A. & Glatt, V. Tissue morphology and antigenicity in mouse and rat tibia: Comparing 12 different decalcification conditions. *J. Histochem. Cytochem., 67(8)*:545-61, 2019

Britz, H. M.; Thomas, C. D. L.; Clement, J. G. & Cooper, D. M. L. The relation of femoral osteon geometry to age, sex, height and weight. *Bone, 45(1)*:77-83. 2009.

Burr, D. B.; Ruff, C. B. & Thompson, D. D. Patterns of skeletal histologic change through time: comparison of an archaic native American population with modern populations. *Anat. Rec., 226(3)*:307-13, 1990.

Charisi, D.; Eliopoulos, C.; Vanna, V.; Koilias, C. G. & Manolis, S. K. Sexual dimorphism of the arm bones in a modern Greek population. *J. Forensic Sci., 56(1)*:10-8, 2011.

Cummaudo, M.; Cappella, A.; Giacomini, F.; Raffone, C.; Màrquez-Grant, N. & Cattaneo, C. Histomorphometric analysis of osteocyte lacunae in human and pig: exploring its potential for species discrimination. I*nt. J. Legal Med., 133(3)*:711-8, 2018.

Dittmann, K.; Grupe, G.; Manhart, H.; Peters, J. & Strott, N. *Histomorphometry of Mammalian and Avian Compact Bone*. In: Grupe, G. & Peters, J. (Eds.). Microscopic Examinations of Bioarchaeological Remains: Keeping a Close Eye on Ancient Tissues. Rahden, Verlag Marie Leidorf, 2006. pp.48-101.

Havill, L. M. Osteon remodeling dynamics in *Macaca mulatta*: normal variation with regard to age, sex, and skeletal maturity. *Calcif. Tissue Int., 74(1)*:95-102, 2004.

Hillier, M. L. & Bell, L. S. Differentiating human bone from animal bone: A review of histological methods. *J. Forensic Sci., 52(2)*:249-63, 2007.

Kranioti, E. & Paine, R. Forensic anthropology in Europe: an assessment of current status and application. *J. Anthropol. Sci., 89*:71-92, 2011.

Krishan, K.; Chatterjee, P. M.; Kanchan, T.; Kaur, S.; Baryah, N. & Singh, R. K. A review of sex estimation techniques during examination of skeletal remains in forensic anthropology casework. *Forensic Sci. Int., 261*:165.e1-8, 2016.

Morales, J. P.; Roa, H. I.; Zavando, D. & Suazo Galdames, I. Determination of the species from skeletal remains through histomorphometric evaluation and discriminant analysis. *Int. J. Morphol., 30(3)*:1035-41, 2012.

Mulhern, D. M. & Van Gerven, D. P. Patterns of femoral bone remodeling dynamics in a medieval Nubian population. *Am. J. Phys. Anthropol., 104(1)*:133-46, 1997.

Nganvongpanit, K.; Phatsara, M.; Settakorn, J. & Mahakkanukrauh, P. Differences in compact bone tissue microscopic structure between adult humans (*Homo sapiens*) and Assam macaques (*Macaca assamensis*). Forensic Sci. Int., 254:243.e1-5, 2015.

Nganvongpanit, K.; Siengdee, P.; Buddhachat, K.; Brown, J. L.; Klinhom, S.; Pitakarnnop, T.; Angkawanish, T. & Thitaram, C. Anatomy, histology and elemental profile of long bones and ribs of the Asian elephant (*Elephas maximus*). *Anat. Sci. Int., 92(4)*:554-68, 2017.

Passalacqua, N. V.; Pilloud, M. A. & Congram, D. Forensic anthropology as a discipline. *Biology (Basel), 10(8)*:691, 2021.

Pfeiffer, S. Variability in osteon size in recent human populations. *Am. J. Phys. Anthropol., 106(2)*:219-27, 1998.

Robling, A. G. & Stout, S. D. *Histomorphology, Geometry, and Mechanical Loading in Past Populations*. In: Agarwal, S. C. & Stout, S. D. (Eds.). Bone Loss and Osteoporosis: An Anthropological Perspective. Boston, Springer, 2003. pp.189-205.

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