Indian Journal of Animal Sciences **85** (9): 962–964, September 2015/Article

Identification of species on the basis of histomorphometrical study of compact bone

VARINDER UPPAL[1], NEELAM BANSAL[2] and ANURADHA[3]

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141 004 India

Received: 22 January 2014; Accepted: 26 March 2015

ABSTRACT

The study was conducted on femur of human, cattle, buffalo, horse, dog, deer and poultry birds. The tissues were collected from mid shaft (diaphysis) of femur, decalcified, processed and paraffin section were cut. The cut sections were stained with hematoxylin and eosin, Masson's trichrome, periodic acid Schiff and alcian blue. The species can be identified on the basis of absence of plexiform bone in humans and its presence in animal bone and where plexiform bone is not present, the histomorphometry (Haversian system diameter and Haversian canal diameter) plays an important role in identification. Such type of study should be expanded more so that this data can be applied in forensic science.

Key words: Bone histomorphometry, Forensic science, Species identification

The forensic scientists are usually asked to identify the fragmented skeletal remains of different species in vetero legal or medico legal cases. Sometimes situation comes when human skeletons are presented as highly fragmented, damaged and potentially commingled with the non human skeletal remains as a result of mass disasters, fire and cremation, wars and domestic crimes (Hillier and Bell 2007). If the remains are presented as a whole or partial bones then the different mammalian species can be identified easily based on the gross morphological features. But in the situations where badly degraded and charred fragments of bone are found and genetic information is not sufficiently present, the histological methods are a valuable tool. The histological study of bones include qualitative as well as quantitative analysis i.e. examination of cortical bone tissue to assess the appearance of the bone tissue and histomorphometry of Haversian system diameter (Martiniakova *et al.* 2007). The reports on histomorphometrical studies of bones are very limited in India, so keeping in view the significance of the topic, the present research was planned to study the comparative histomorphometrical and histochemical aspects of femur of human and some of the animals.

MATERIALS AND METHODS

The study was conducted on femur of human, cattle, buffalo, horse, dog, deer and poultry birds (6 in each species except deer in which only 2 animals were used). The tissues were collected from mid shaft (diaphysis) of femur where the compact bone was thick enough and provided a large area for study of bone tissue microstructure. The bone tissue was decalcified, processed and paraffin section were cut (Luna 1968). The cut sections were stained with hematoxylin and eosin, Masson's trichrome, periodic acid Schiff and alcian blue. The micrometrical observations were taken on diameter of Haversian canals and Haversian system of mature osteons in H & E stained slides.

RESULTS AND DISCUSSION

Histologically, the bone can be classified according to the arrangement of collagen fibres in the matrix i.e. woven and lamellar. In woven bone, collagen fibres are arranged in an irregular anastamosing fashion and its production is quick, immature and weak whereas lamellar bone is highly organized bone consisting of parallel lamellae (Martiniakova *et al.* 2006). This type of bone can be further divided into primary bone and secondary bone. Primary bone is produced by bone being laid down onto a pre existing bone surface during growth, such as periosteal surface and contains primary osteons and secondary bone is the immediate replacement of new bone where previous bone has resorbed and contains secondary osteons. Primary bone can have several forms; circumferential lamellar or primary lamellar, plexiform bone, longitudinal, radial and reticular. Plexiform bone is the combination of woven and lamellar i.e. fibrolamellar and is produced by continually creating trabecular networks on the bone surface and lamellae fills the gaps (Hillier and Bell 2007). The name plexiform was derived from the vascular plexus contained within lamellar bone sandwiched by nonlamellar bone and

Present address: [1,3]Associate Professor (v.uppal@yahoo.com, bansal.neelam@rediffmail.com), [2]Professor cum Head (anuradha71@in.com), Department of Veterinary Anatomy.

has an appearance of brick wall. It is more mineralized than woven bone and structurally more stronger than the primary and secondary bone. Martiniakova *et al*. (2006) reported that the human bones can be distinguished from animal bone on the basis of dense Haversian systems whereas the animals from Artiodactyla order have primary vascular plexiform bone pattern. The difference between primary and secondary osteons is that primary osteons are formed during transformation of woven to lamellar bone, contains less lamellae, have smaller vascular channels and no cement line whereas secondary osteons have more lamellae and cement line is present (Martiniakova *et al*. 2007, Hiller and Bell 2007). In the present study, a comparison was made among the femur of different species to understand the lamellar arrangement and type of bone in different species.

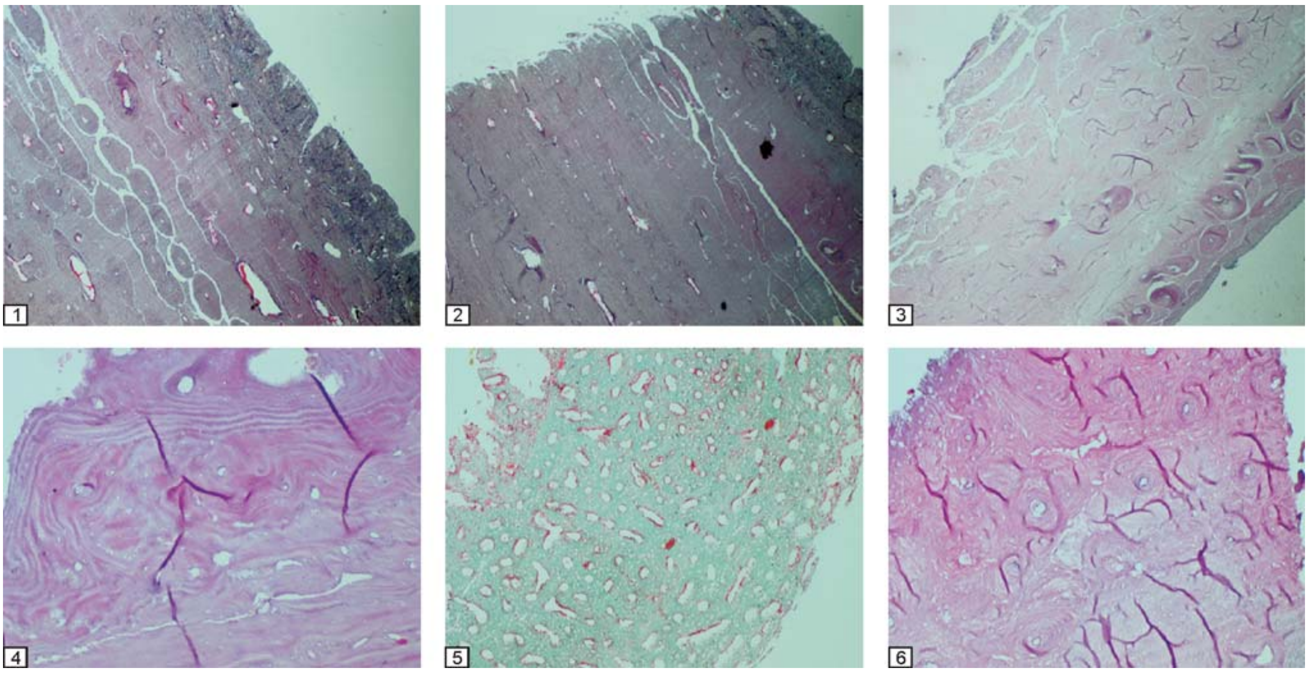
In cattle and buffalo, the femur bone was a combination of plexiform and Haversian bone (Figs 1, 2). Near the periosteal surface it was plexiform bone and in the middle part of laminar bone there was Haversian bone whereas at the endosteal surface there was non vascular bone tissue (Hillier and Bell 2007). The circumferential lamellae near the periosteal and endosteal surface were thin and fragmentary in cow and buffalo. In deer, bone comprises of plexiform and Haversian bone. Hillier and Bell (2007) also observed a combination of plexiform and Haversian bone tissue with plexiform bone near periosteal surface and Haversian bone forming the endosteal surface. Dominguez and Crowder (2012) showed that the plexiform bone is a characteristic feature of non human bones.

In the horse also, the bone (Fig.3) is a combination of plexiform and Haversian (Martiniakova *et al.* 2006 and Hillier and Bell 2007) in long cortical bones. A large number of resorptive spaces were also observed near the endosteal surfaces. The resorptive spaces are formed as a result of calcium deficiency (Jowsey 1965). Large number of resorptive spaces observed in the present study may be due to lack of calcium or may be alteration in the structure of osteons or incomplete mineralization of bone. Thus incomplete mineralization of an osteon may be the most efficient way of maintaining strength in the cortex that is becoming increasingly full of holes because more tissue is being resorbed than is being formed (Jowsey 1966).

In dog, periosteal and endosteal circumferential lamellae were interrupted by Haversian system (Fig.4). Besides this, the interstitial lamellae were very well developed whereas Whiteman (2004) observed well developed periosteal and endosteal circumferential lamellae with various shapes of Haversian canals.

In poultry, the bone was having very dense network of Haversian lamellae and was highly porous (Fig.5).

In human, the typical appearance of long cortical bone consisted of cicumferential lamellae both at the endosteal and periosteal surfaces (Fig.6). The main difference between the human and non human bone was that the plexiform pattern of bone was not observed in the human bones. Dense Haversian system in the middle along with the interstitial lamellae were also observed. The shape of the osteons was generally oval or round. The absence of plexiform bone pattern in human bone was also reported by Hillier and Bell (2007) and Lu *et al.* (2006). Plexiform bone can be present in humans during early fetal development and as primary osteonal formation, in response to injury or inflammation



Figs 1–6. Photomicrographs of paraffin sections of cortical tissue of femur bone showing: **1**. Haversian and plexiform bone in Cattle. Hematoxylin & eosin. × 20; **2**. Haversian and plexiform bone in buffalo. Hematoxylin & eosin. × 20. **3**. Haversian and plexiform bone in horse. Hematoxylin & eosin. × 20; **4**. Well developed interstitial lamellae in dog. Hematoxylin & eosin. × 20; **5**. Porous bone in poultry. Masson's trichrome. × 20; **6**. Well developed Haversian systems in human. Hematoxylin & eosin. ×20.

Table 1. Micrometrical observations (μm) on Haversian system of cortical bone of femur of different species

Species	Diameter	Area	Perimeter
Cattle	72.026±4.336	4401.165±516.560	226.368±13.628
Buffalo	73.568±4.311	4575.832±522.246	231.216±13.549
Horse	72.177±5.171	4555.559±553.093	226.844±16.253
Dog	44.098±3.289	1715.045±242.465	138.596±10.338
Deer	56.343±5.816	3079.51±598.644	177.091±18.281
Poultry	24.959±0.986	506.277±41.068	78.442±3.099
Human	52.666±1.081	2199.608±84.087	165.523±3.399

Table 2. Micrometrical observations (μm) on Haversian canal of cortical bone of femur of different species

Species	Diameter	Area	Perimeter
Cattle	11.228±0.637	106.089±12.139	35.290±2.003
Buffalo	10.160±0.731	90.367±13.106	31.933±2.299
Horse	9.054±0.283	65.787±4.258	28.455±0.886
Dog	10.648±0.723	98.140±13.162	33.467±2.273
Deer	9.429±0.543	74.972±10.065	29.636±1.708
Poultry	5.915±0.233	28.437±2.190	18.592±0.732
Human	8.898±0.653	69.606±11.165	27.9071±2.054

referred to as periostitis. So if a bone fragment is not fetal or pathological in appearance, the identification of plexiform bone tissue identifies it as nonhuman, a determination which is very important to forensic investigator. The human bone was strongly positive for periodic acid Schiff and alcian blue stains as compared to other species indicative of more of neutral and acid mucopolysaccharides in human bone as compared to non human bone

The histomorphometrical data on the diameter of Haversian canal and Haversian systems are summarized in Tables 1 and 2. As this data gave a range of diameter of Haversian canal and Haversian systems, thus, it can provide information about the species of human or non human origin. As previously reported, the size of the osteons present in the femoral cortex remains approximately the same throughout life while the central canal increases in size (Jowsey 1966). Haversian system diameter and Haversian canal diameter were demonstrated as being markedly different between humans and non human species and can both be advocated as measurement to be used for differentiation (Lackey 2001, Benedix 2004).

It may be concluded that differentiation of human cortical bone and large mammals like cattle, buffalo, horse and deer can be identified on the basis of absence of plexiform bone in humans and its presence in non human bone and where plexiform bone is not present the histomorphometry (Haversian system diameter and Haversian canal diameter) plays an important role in identification. Such type of study should be expanded more so that this data can be applied in archeozoology, forensic anthropology and or criminological practice.

REFERENCES

Benedix D. 2004. 'Differentiation of fragmented bone from Southeast Asia: the histological evidence.' Dissertation submitted to University of Tennessee Knoxville.

Dominguez V M and Crowder C M. 2012. The utility of osteon shape and circularity for differentiating human and nonhuman Haversian bone. In proceedings of 81st Annual Meeting of the American Association of Physical Anthropologists, USA.

Hillier L M and Bell L S. 2007. Differentiating human from animal bone: A review of histological methods. *Journal of Forensic Science* **52(2)**: doi: 10.1111/j.1556–4029.

Jowsey J, Kelly P J, Riggs B L, Bianco A J, Scholz D A and Gershon Cohen J. 1965. Quantitative microradiographic studies of normal and osteoporotic bone. *Journal of Bone and Joint Surgery* **47**: 785–806.

Jowsey J.1966. Studies of Haversian systems in man and some other animals. *Journal of Anatomy* **100(4)**: 857–64.

Lu H L, Zheng J, Yao Y N, Wang H P, Chen L X and Guo J Y. 2006. Comparison on Haversian system between human and animal bones by imaging analysis. *Fa Yi Xue Za Zhi* **22(2)**: 97–100.

Lackey W L.2001. Distinguishing between human and canine bone using Haversian system size and Haversian canal diameter. In proceedings of 53rd Annual meeting of the American Academy of Forensic Sciences, Seattle, WA, Colorado Springs, CO, USA.

Luna L G. 1968. *Manual of histological staining methods of Armed Forces Institute of Pathology*. 3rd edn, pp 34–157.McGraw Hill Book Company, New York.

Martiniakova M, Grosskopf B, Omelka R, Dammers K, Vondrakova M and Bauerova M. 2007. Histological study of compact bone tissue in some mammals: A method for species determination. *International Journal of Osteoarchaeology* **17**: 82–90.

Martiniakova M, Grosskopf B, Omelka R, Vondrakova M and Bauerova M. 2006. Differences among species in compact bone tissue microstructure of mammalian skeleton: Use of a discriminant function analysis for species identification. *Journal of Forensic Science* **51(6)**: doi: 10.1111/j.1556–4029.

Whiteman E J. 2004. 'Differentiating between human and nonhuman secondary osteons in human, canine and bovine tissue'. Thesis submitted to Michigan State University, USA.