Bone histology: A key for human sex determination after death

Hadi Abdullah\textsuperscript{1}, Muhammad Mahadi Abdul Jamil\textsuperscript{1}, Radzi Ambar\textsuperscript{1}, Faridah Mohd Nor\textsuperscript{2}

\textsuperscript{1}Faculty of Electrical and Electronic Engineering, University Tun Hussein Onn Malaysia, Batu Pahat,(86400) Johor, Malaysia.

\textsuperscript{2}Medical centre, University Kebangsaan Malaysia, Bandar Tun Razak, (56000) Batu 9 Cheras, Wilayah Persekutuan Kuala Lumpur, Malaysia

Hadi.uthm@yahoo.com

Abstract. Age at death and sex are the key information required at a crime scene investigation or archaeological exploration. A number of morphological techniques are present to estimate age and sex from human bones. These techniques showed good result when major parts of human bones were present. In cases, where only fractions of bone are present, these techniques become irrelevant. Bone histological techniques can be applied on bone fragments to estimate age and sex. Age estimation using these techniques has been the key focus of research over the last few decades. At the early stages of these techniques, some researchers reported no relation of sex with histological changes. This made sex a by-product of age estimation or was left unconcerned. Bone histology of human changes with region, race and age. This paper presents the history of histological sex determination. Comparison of different researches was given with regards to region of research, selection of bone and microstructural parameter selection. A discussion concludes this paper with overall research done, and what future work can be done to improve histological sex determination.

1. Introduction

Information of age at death and sex play a vital role in crime scene investigation, as well as archaeological exploration. Human bones are the strongest tissue in the body, which takes a longer time to decay. Studies have been carried out to analyze human bones to gather information of the person it belonged to. These studies can be divided into two parts, morphological and histological techniques. Morphological techniques are useful when major parts of bones are present. Skull, pelvis and long bones play vital role in morphological analysis \cite{1}. Figure 1 shows major components of human skeleton, which provide good results in using these techniques. Bone morphology fails when no major part of bones are present or only bone fragments are available. In such cases, histological techniques can be used for microscopic analysis of fragments. Kerley in 1965 was the first to give a relational regression equation of bone microstructures, which can be used to calculate age at death \cite{2}. His work opened doors to new research. Thompson in 1981 continued his work to the next level and developed age-graded contrasts in bone cells for both genders\cite{3}.

Since then, different researches have been conducted on histological analysis of bone with contradicting outcomes\cite{4-6}. This is mainly because humans belonging to different races and regions,
which have different properties of bone structures. These papers are discussed in detail with regards to the history of sex determination from bone histology.

To understand bone histological analysis, one must know the basic structure of bone. Bones are tissues, which provide structural stability to the body, and provide protection to the internal fragile organs. Bones are mainly made of proteins and minerals, making them a reservoir for the body. They have space for bone marrow, and provide perfect environment and protection for bone marrow to provide blood cells to the body.

![Human skull, pelvis and long bones.](image)

**Figure 1.** Human skull, pelvis and long bones.

They consist of living cells, which repair and reconstruct bones according to the body requirement [7]. These cells are osteoblasts, osteoclasts and osteocytes. Osteoblasts are bone-making cells. They make bone structures by secreting bone matrix. These mononuclear cells are small in size compared to osteoclasts, and they stick together through short, slender processes [8]. Osteoclasts, on the other hand are multinucleated cells. These cells perform enzymatic digestion of bone, which is also known as bone resorption. Osteocyte forms the chief cellular component of mature bone. During bone formation, osteocyte is formed from resting osteoblast, which is incorporated into a small space called lacuna in the matrix. Osteocytes have a number of canaliculi, which anastomose with canaliculi from adjacent cells [8] for the exchange of tissue fluids and metabolites between osteocytes and blood capillaries. Bone remodeling is a process, in which osteoclast and osteoblast take part to resorb old bone and make new bone. This process is carried out at multiple locations in human skeleton [8]. Remodeling process produces a haversian canal system consisting of concentric lamellae. This system is known as an osteon system (Figure 2). Bone is continuously under the process of remodeling, which changes the microscopic structures. These structures are analyzed in many histological techniques mainly for age estimation and sex determination.

![Bone cell at microscopic level.](image)

**Figure 2.** Bone cell at microscopic level [9].
2. Bone microstructural parameters
Most common problem encountered in study of sex determination is the differences in selection of microstructural parameters and their classifications by researchers. The outcome of a study to estimate sex and age is strongly dependent on the selection of bone microstructural parameters. During analysis, bone microstructural parameters can be categorized as observed and derived parameters. This section indicated some microstructural parameters, which were generally used by researchers.

2.1. Observed microstructural parameters
In this study observed parameters are referred to the parameters which are directly analysed from the bone microscopic image. Frequently used observed bone microstructural parameters in previous studies are fragmented osteon (FO), secondary osteon (SO), haversian canal count (HCC), osteon area (OA), osteon diameter (OD), haversian canal area (HCA) and haversian canal diameter (HCD).

2.2. Derived microstructural parameters
Derived parameters are normally estimated from observed parameters. The selection of choosing derived parameters or observed parameters in order to estimate sex depends on the researchers work. The following sections will identify the microstructural parameters of both categories analysed by researchers in the literature. Frequently used derived parameters are Fragmented Osteon Density (FOD), Secondary Osteon Density (SOD), Secondary Osteon Region (SOR), Fragmented Osteon Region (FOR), Haversian canal Region (HCR).

3. Sex determination
This section discussed in detail the history of sex determination. Kerley was the first researcher, who analysed bone microstructures, and gave age estimation regression equation. This opened doors from age estimation to sex determination, height and weight estimation[2].

Kerley developed a regression equation for age estimation by analysing modelling and remodelling stages of bone. Four fields were selected for analysis, namely, anterior, posterior, medial and lateral. A 100-power wide field in each of the four anatomic areas were analysed for the number of osteons and its fragments, amount of lamellar bone and the number of non-Haversian canals. Each microscopic field in the image was 1.25 mm in diameter. The inspection area enclosed by a 10-power objective lens combined with 10-power wide-field oculars. After Kerley’s work on age estimation, the focus diverted to estimate human age in different races. Since many researchers reported that gender of the human do not affect the histology of bone, therefore researchers were more interested in human age estimation leaving a big space in the field of sex determination. Singh and Gunberg developed age estimation method in 1970 from male samples which was also tested on female samples producing satisfying results. However when this method was experimented on additional amount of female specimen by Samson and Branigan, the accuracy of the estimation decreased[10]. In 1979 a sex determination method was developed by DD Thompson which reported that males groups have smaller average haversian canals area than females in similar group (p< 0.05)[11]. However in the fifth and eight decade, haversian canal area in males increased by 133% and in females by 52%. Male were reported to have larger haversian canal area in fifth and eighth decade. Female samples showed greater total area of haversian canal (p< 0.05). Application of the same regression equations to different populations can be done only when sufficient numbers of skeletons of known age at death from each different population are analysed. Using a small core of bone instead of a complete cross-sectional area minimizes the physical damage to a skeleton, and helps ensure access to skeletons, where the question of population-specific, age-related changes in osteon turnover may be addressed directly. The validity of these regressions was based on the New England white population [11]. In 1981, Thompson analyzed the femoral bone cores of adult Eskimo skeletons from Southampton Island (n = 69), St Lawrence Island (n = 53), Baffin Island (n = 44), Kodiak Island (n = 921) and compared the results with the specimen of U.S. whites (n= 144). Males of Southampton Island Eskimo indicated average haversian canal area (HCA) around 0.084mm² / mm² and in females average HCA was 0.105mm² / mm² (p< 0.05) [3]. Nine parameters namely bone mineral index, cortical bone density, cortical thickness, haversian canal and primary osteon area, Secondary osteon lamellae area,
secondary osteon lamellae plus haversian canal area, secondary osteon number, haversian canal perimeter length and secondary osteon perimeter length were analyzed in this study.

Age at death estimation in Eskimo skeletons of US whites was done using regression equations which showed that ages obtained from this method exceeded from those obtained by morphological methods. It was specifically true for Eskimos morphologically aged less than 35 years, where it may be assumed that morphological ages are the most accurately determined. Age estimates of white samples were calculated accurately with mean absolute difference of 4.76 years between known and estimated age. The absolute difference between histological and morphological age for Southampton Island skeletons was 15.41 years for females and 14.33 years for males. This degree of error related with the histological method among Eskimos is beyond the error, probably due to variances in the rates of change for morphological indicators between the two populations. Differences of similar degree were reported for histologically and morphologically estimated ages in each of the other Eskimo groups.

David B Burr in 1990 compared the study of skeletal remains of an archaic Native American population (Pecos Indian) with the modern American population. The purpose of his study was to compare changes in an archaic Native American population with those in the modern white populations. His study compared age-related patterns of histologic change in an archaic human population with the modern US and British populations. Secondly, he assessed sexual dimorphisms in histologic structure, and examined whether micro- and macro structural adaptations to aging were related. He reported that males in the Pecos population had smaller osteons than females (p < 0.2)[12]. Osteonal mean wall thickness was reported to be larger in females. Due to the smaller size of osteons in males, they number, and osteon population density was greater compared to females (p < 0.06). This indicates the occurrence of high rate of bone remodelling in males.

MF Erickson in 1991 also studies sex-specified age estimation and divided sex into age-graded groups by decades. This study was conducted in Washington DC [13]. The research comprised 154 female samples with age ranges from 14-97 years and 174 male samples ranging from 16-97 years. Five types of parameters were observed, secondary osteon, type II osteon, fragments, resorption space and non-haversian canal.

Erickson analyzed type II osteons and reported that it had a positive relation with age. However these type II osteons remains constant at a specific stage. Non-haversian canals were reported to have a decrement in both sexes. Furthermore it was reported that in each decade osteon gradually increased in males, while in females, this increase occurred only till sixth decade after which it remained constant. It was reported that in fifth decades of female age, the osteon fragments/mm² increased compared to osteons. But, in males, this ratio did not exceed the number of osteons prior to seventh decade. Erickson also analyzed that in eight decade of females, fragmental bone area increased more than osteonal bone and the area of fragmental bone never increased more than the osteonal bone in males.

DM Mulhern (1997) examined remodeling in femurs of Medieval Nubian Population of 1250–1450 AD. Mulhern observed considerable variations in remodeling patterns of both genders. Eight areas in the sample were analyzed under a microscope. Ten microstructural parameters were used in this research which showed that the number of intact osteons was higher in males compare to females, whereas the area of osteons were larger in females compare to males. He did not find any significant statistics in Haversian canal dimensions between sexes. Intact osteons number showed significant correlation with sex (p < 0.0001). Average intact osteons number in females was (6.73/mm²) and (9.74/mm²) in males [14]. Average fragmented osteons number in females was 4.68/mm² and 2.59/mm² in males, which showed a significant difference. Osteon size also varied with sex (table 1). The mean osteonal cross-sectional diameter and area are different between both sexes (p < 0.05).

KL Bell in 2001 analyzed femoral mid-shaft cross-section of 66 samples and divided them according to their age and sex in groups of two decades each (21-40, 41-60, 61-80, > 80). He reported that the existence of considerable percentage of new young osteon meant that cortical remodeling in female was higher. Females had 34% higher remodeling than males (p = 0.034). When combined, both sex showed no significant difference in parameter of osteon density and between ages[15] (figure 3).
Table 1: Microstructural parameter differences in both sex.

| Parameter                  | Female         | Male          |
|----------------------------|----------------|---------------|
| Intact osteon              | 6.73 ± 0.31    | 9.74 ± 0.39   |
| Fragmented osteon          | 4.68 ± 0.27    | 2.59 ± 0.14   |
| Haversian canal area       | 0.0021 ± 0.0001| 0.0022 ± 0.0002|
| Osteon area                | 0.040 ± 0.001  | 0.036 ± 0.002 |
| Average osteon count       | 12.03 ± 0.47   | 12.79 ± 0.43  |

Figure 3. Evaluation of young osteon based on age groups [18].

C David L Thomas in 2005 studied sex and age differences in 168 samples (73 males and 75 females) of modern Australian population by analyzing porosity in femur samples. Microradiographs of 100 μm sections were analyzed in which pores in bones were extracted with the help image processing method. First, the samples were categorized in three age groups as young, middle and old (20–44 years, 45-64 years and 65+ years) however later he studied the relation between medullar area with sub periosteal area which produced better outcomes. Cortex of the bone was distributed in three radial rings and eight circumferential segments. The porosity in each section was analyzed and it was reported that with the increasing age the porosity in the inner cortex and porosity in the posterior and anterolateral region increases. The porosity patterns in males developed steadily, however in females the porosity patterns were much different in initial age compare to old age. The porosity patterns were regular with continous bone loss alongside a neutral axis of the cortex, [16].

Faridah Mohd Nor in 2009 analyzed microstructural parameters for age and sex determination in 64 Malaysian samples [17]. And reported that there is no significant difference in number of osteon in both sexes. This was in agreement with the study of Pfeiffer in 1996 and 1998. Nor Faridah also reported that females have less but larger osteons and have larger haversian canals compare to males (p< 0.05). These results agreed with Mulhern(1997) and Thompson(1981).

HM Britz in 2009 worked on finding out the relation between weight of the human body and geometry of osteons in human bone. The initial hypothesis of his work stated that weight of human body would be in negative relation with the size of femoral osteon area (On.Ar) and femoral osteon diameter (On.Dm) if the age, sex and height were kept constant. HM Britz also attempted to find out the link of (osteon circularity; On.Cr) with weight. The samples included 45 male and 43 female with age ranging from 17 to 97. Osteons numbers (n= 12,690) were counted in the microradiographs of femur samples. Univariate analysis of covariance was used (n= 87; 1 outlier) with log-transformed age, weight and height as covariates and sex as a fixed factor [18].
It was reported that weight had a negative correlation with On.Dm and On.Ar ($p=0.004$ and $p=0.006$, respectively). There was significant relation between age and osteons, as well as its circularity. Relation of increasing age with On.Dm and On.Ar was negative, however it was positive with On.Cr. The On.Dm and On.Ar were reported to show significant difference between the both genders ($p=0.019$ and $p=0.021$), with males having larger osteons. Sex had no significance relation with On.Cr ($p=0.449$). Height as well, did not show any significant relation with geometric parameters. Considering partial eta-squared values, age had the highest relation with (On.Cr: 30%), (On.Ar: 28%), (On.Dm: 18%), weight had the second highest relation with (On.Dm: 10%) and (On.Ar: 9%) of the variance in geometry. Sex had the lowest relation with (On.Dm: 7%) and (On.Ar: 6%). While other researchers studied relation of osteon size with age/sex, the author of this study believed that these results were the first to describe a link between weight and osteons.

C Hernandez (2012) noted a significant differences in remodeling of femoral cortex in both genders. It was analyzed that with age, fragmented osteon density varies in male more than female by 50%. These variations occurred in medial and anterior region and total cross-section. However the density of fragmented osteon in females was 50% greater than males in the regions of lateral and posterior and total cross-section. In anatomical region of males, 50% or greater intact osteon density was reported. Females had 50% or more intact osteon changes in posterior and complete cross-section. However this study was mainly focused on age estimation which did not present more detail about sex differences [19].

4. Sex determination
In this section, a compilation of research work done on sex determination using bone histology, histomorphology and porosity were compiled and discussed. The main differences in research work were based on the following factors.

- Area and race of human from which samples were selected.
- Selection of part of bone for analysis.
- Selection of microscopic cross-sectional area for analysis.
- Selection of microstructural parameters.

Table 2 shows researchers from different parts of the world with selection of bones from different parts of human bones and selection of microscopic cross-sectional region for analyses and parameters selected by researchers.

**Table 2. Field, bone type and area selected by researchers.**

| Researcher    | Region/area                  | Bone                  | Field location | Microstructural parameters                                      |
|---------------|------------------------------|-----------------------|----------------|-----------------------------------------------------------------|
| Kerley, 1965  | USA whites                   | Femur, tibia, fibula  |                | Number of osteons, Number of fragments, Amount of lamellar bone, Number of non-Haversian canals |
| Lj Singh, DL  | Portland                     | Femur, tibia          |                | Number of osteons, Average number of lamella per osteon, Average haversian canal diameter |
| Gunberg, 1970 | USA whites and Eskimos from St. Lawrence, North Alaska and Canada | Femur | | Cortical thickness, Bone mineral index, Cortical bone density, Secondary osteon lamellae area, Haversian canal and primary osteon area, Secondary osteon number, Secondary osteon lamellae plus Haversian canal area, Secondary osteon perimeter, Haversian canal perimeter. |
| Thompson, 1979, 1981 | USA whites and Eskimos from St. Lawrence, North Alaska and Canada | Femur | | Secondary osteon area, Haversina canal area. Sample area. Osteon wall thickness. Osteon population density. Secondary osteonal bone. Porosity. Percent osteonal refilling. Cortical area. Total area. Cross-sectional inertia. Polar moment of inertia. |
| David B Burr, 1990 | Native American and USA whites | Femur | | |
| Reference                        | Region       | Race            | Bone | Microstructural Parameters                                                                 |
|---------------------------------|--------------|-----------------|------|--------------------------------------------------------------------------------------------|
| Ericksen, 1991                  | USA whites   | Femur           | Secondary osteon. Type II osteon. Fragments. Resorption space. Non-Haversian canal.        |
| DM Mulhern, 1997                | Medieval Nubian population from Kulubnarti, Subanese Nubia | Femur | Intact Osteon. Fragmentary Osteon. Haversian Canal Area. Osteon Area. Mean Osteonal area. Cross Sectional Diameter. Osteon Population. Accumulated Osteon. Net Osteonal Remodeling Percent Osteonal Refilling. |
| KL Bell, 2001                   | Australia    | Femur           | Femoral porosity. Young osteon. Remodeling cluster                                       |
| C David L. Thomas, 2005         | Australia    | Femur           | Intra cortical porosity.                                                                 |
| F Nor, 2009                     | Kuala lumpur, Malaysia | Femur, tibia, ulna, radius, Humerus, fibula | Cortical thickness. Medullary cavity diameter. Osteon count. Osteon diameter. Osteon area. Osteon perimeter. Haversian canal diameter. Haversian canal area. Haversian canal perimeter. Haversian lamellae count. |
| HM Britz, 2009                  | Australia    | Femur           | Osteon Area. Osteon Diameter. Osteon Circularity                                         |
| C Hernandez, 2012               | USA whites   | Femur           | Intact secondary osteons. Fragmentary Secondary Osteons. Treatment of Type II Osteons. Cortical Area Evaluated. Init. Secondary Osteon Density. Fragments Density. Osteon population. Total subperiosteal Area. Endosteal Area. Cortical Area. Relative Cortical Area. |

5. Conclusion
The literature of bone histology does not give strong evidence of changes in bone microstructures based on genders. Several microscopic parameters of different race and regions were analysed by different researchers for sex determination (Table 2). However, the implementation of these methods can be questioned while applying it to all races. The characteristics of bone microstructures varies with race and region. So far, little research has been carried out in the field of human bone histology to determine sex differences and there is little proof of bone microstructural parameters difference between the two genders. No developed method can be applied to identify sex differences in all races, however studies are being conducted to observe sex differences in specific regions and races. Further research is required in this field to be more precise. A group of clearly defined selected parameters should be studied to determine sex. Use of modern technology could produce better results as computers have become good enough to analyse and extract minor details in medical images. Microscopic images of bone cells are still being inspected manually by experts which makes it a tedious and labour-intensive process. Furthermore, manual inspection of medical images often yields biased results that reliant on the skills of the inspector. Analysis of medical images using modern technology will not only be robust and accurate but will save time and cost. It will also allow objective and reproducible results and could reduce human biased errors. Computer vision systems can assist in storage of large data base of bone images and in transfer of the extracted information from various images. Nevertheless, creating practical automatic systems for bone microstructure image analysis and their explanation has been a major challenge.
References

[1] H. Cho, S. D. Stout, and T. A. Bishop, "Cortical bone remodeling rates in a sample of African American and European American descent groups from the American Midwest: Comparisons of age and sex in ribs," *American Journal of Physical Anthropology*, vol. 130, pp. 214-226, 2006.

[2] E. R. Kerley, "The microscopic determination of age in human bone," *American Journal of Physical Anthropology*, vol. 23, pp. 149-163, 1965.

[3] D. D. Thompson and M. Gunness-Hey, "Bone mineral-osteon analysis of Yupik-inupiaq skeletons," *American Journal of Physical Anthropology*, vol. 55, pp. 1-7, 1981.

[4] I. Khan, F. Mohd Nor, and M. M. Abdul Jamil, "A Survey of Human Age Estimation Techniques from Bone Microstructures," in *International Conference for Innovation in Biomedical Engineering and Life Sciences : ICIBEL2015, 6-8 December 2015, Putrajaya, Malaysia*, F. Ibrahim, J. Usman, M. S. Mohktar, and M. Y. Ahmad, Eds., ed Singapore: Springer Singapore, 2016, pp. 203-207.

[5] I. Khan, M. M. A. Jamil, T. N. T. Ibrahim, and F. M. Nor, "Automated human age estimation at death via bone microstructures," in *2016 6th IEEE International Conference on Control System, Computing and Engineering (ICCSCE)*, 2016, pp. 580-583.

[6] I. Khan, M. M. A. Jamil, T. N. T. Ibrahim, and F. M. Nor, "Analysis of age-related changes in Haversian canal using image processing techniques," in *2016 6th IEEE International Conference on Control System, Computing and Engineering (ICCSCE)*, 2016, pp. 169-172.

[7] D. G. Steele, *The Anatomy and Biology of the Human Skeleton*: Texas A&M University Press, 1988.

[8] L. J. a. J. Carneiro, *Basic Histology: Text and Atlas*, 14 ed.: McGraw-Hill Medical, 2005.

[9] Gary, "Gray’s Anatomy of the Human Body," in [http://www.bartleby.com/107/illus77.h, o. system, Ed., ed, 1918.

[10] B. K. Samson C, "A new method of estimating age at death from fragmentary and weathered bone. In Bodington A, Garland AN, Janaway RC, editors. Death Decay and Reconstruction Approaches to Archaeology and Forensic Science," *Manchester: Manchester University Press*, pp. 101-108, 1987.

[11] D. D. Thompson, "The core technique in the determination of age at death of skeletons," *J Forensic Sci*, vol. 24, pp. 902-15, Oct 1979.

[12] D. B. Burr, C. B. Ruff, and D. D. Thompson, "Patterns of skeletal histologic change through time: comparison of an archaic native American population with modern populations," *Anat Rec*, vol. 226, pp. 307-13, Mar 1990.

[13] M. F. Ericksen, "Histologic estimation of age at death using the anterior cortex of the femur," *Am J Phys Anthropol*, vol. 84, pp. 171-9, Feb 1991.

[14] D. M. Mulhern and D. P. Van Gerven, "Patterns of femoral bone remodeling dynamics in a Medieval Nubian population," *Am J Phys Anthropol*, vol. 104, pp. 133-46, Sep 1997.

[15] K. L. Bell, N. Loveridge, J. Reeve, C. D. Thomas, S. A. Feik, and J. G. Clement, "Superoosteons (remodeling clusters) in the cortex of the femoral shaft: influence of age and gender," *Anat Rec*, vol. 264, pp. 378-86, Dec 1 2001.

[16] C. D. L. Thomas, S. A. Feik, and J. G. Clement, "Regional variation of intracortical porosity in the midshaft of the human femur: age and sex differences," *Journal of Anatomy*, vol. 206, pp. 115-125, 01/05/accepted 2005.

[17] F. M. Nor, R. F. Pastor, and H. Schutkowski, "Age at death estimation from bone histology in Malaysian males," *Med Sci Law*, vol. 54, pp. 203-8, Oct 2014.

[18] H. M. Britz, C. D. Thomas, J. G. Clement, and D. M. Cooper, "The relation of femoral osteon geometry to age, sex, height and weight," *Bone*, vol. 45, pp. 77-83, Jul 2009.

[19] M.-T. J. Cosgriff-Hernandez, "Histomorphometric Estimation of Age at Death Using the Femoral Cortex: A Modification of Established Methods," The Ohio State University, 2012.