Age regression for Malaysian males using cortical bone Histomorphometry

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Abstract. Bone microstructures have the capability to record the variations in its structure that occur during the life span. These variations can be analysed to estimate human age at death. This study analysed 30 Malaysian male samples in order to create a regression equation for age estimation. Bone samples were collected from human long bones i.e humerus, radius, ulna, tibia, fibula and femur. Two morphological and five histological variables were selected for analysis. Cortical thickness and medullar cavity diameter did not show any significant relation with increasing age however haversian canal parameters and osteon count showed significant correlation with age. Regression equation with lowest SEE 7.2 was based on Haversian canal area, haversian canal radius and osteon count. The regression equation estimated age at death within 10 years of range for 85% samples. The mean value of the known ages was 39.8 years while the mean value of the estimated ages was 37.03 years. Increasing number of samples including female samples for further research might produce promising results.

1. Introduction
Estimation of human age at death from skeletal remains is one of the main physical aspect in reconstruction demographic profiles. Macroscopic and microscopic analysis of unknown bones might help in estimation of age at death as human skeleton is characterised by both morphological and histological age related changes [1]. Morphological skeletal characteristics are commonly used to estimate age at death in younger individuals [2] while histological features are used in attempt to predict age of older individual [3]. The accuracy of the age estimation techniques is dependent on the diagnosis of several age-related changes in skeleton that occur during the life span. These morphological and histological variation in skeleton are controlled by bone modelling and remodelling. Bone become more porous with increasing age as remodelling results in increases bone resorption and less bone formation. These skeletal changes and growth in sub adults are more controlled and occur over a shorter time span then the breakdown and degeneration of skeleton in adults which means that there is little variation in skeletons of sub adults compare to older adults. This makes age at death estimation of sub adults more accurate while adults age estimation techniques yields estimate with imprecise large ranges. After 50 years of age degeneration patterns in skeleton become unclear to predict their chronological age with fair accuracy. Different factors such as intrinsic and extrinsic genetic factors, environmental and biomechanical factors alter the histology and morphology of the skeleton [4]. Occupation and different physical activity could also lead to distinct bone mass. Factors such as gender, hormones [5], disease, diet [6],
life history [7], ethnicity, nutritional stress [8] and length of daylight [9] influence the rate at which a person’s skeleton breaks down. Several methods based on morphology and histology of bones has been developed to estimate human age at death. Initial macroscopic techniques were qualitative [10],[11],[12] and were dependent of different degenerative stages of skeleton. In latter attempts microscopic histological methods were developed which were applicable to fragments of bones as well. The accuracy of these methods varies with the availability and nature of the samples and age [13],[14]. Jowsey analysed a high amount of bone formation and resorption in young individuals leading to a high rate of bone turnover. In young adults rate of bone turnover is lower while in older adults rate of bone resorption is high [15]. After 70 years of age there is little evidence of increase in bone formation and up to 25% of their endosteal bone surface may be occupied by resorption. Kerley’s histomorphometric techniques that used a complete bone cross section is cited to be the most accurate of all age estimation techniques [15] which reported that numbers of osteons and osteon fragments increases with age, while a gradual decrease occur in percentage of circumferential lamella and number of non-haversian canals. However, the definitions of these variables were uncertain as it was difficult to differentiate between complete osteons and osteon fragments. This uncertainty led to increase the observational error and effected the reliability of results [15]. In later techniques, the average of Kerley’s regression equations estimates was calculated and more reliable age estimates were derived [16]. The original method was corrected by increasing microscopic filed size from 1.25 mm to 1.62 mm and a correction factor was introduced which should be multiplied with count of osteon, fragmented oseon and non-haversian canal [17].

The existence of difference in patterns of bone microstructures has been an important concern in histological age estimation techniques. Bones with different size of osteons and haversian canals exhibits different osteon population densities and will reach to asymptotes at different stages of age [18]. Bone microstructures were identified to have difference with age [19],[20],[21],[22] and activity and varies with population and ancestry [18], [23],[24],[25]. Some researchers reported the difference in bone microstructures especially osteons with gender [26] while other found no such difference [24]. It is vital in classifying the reliability of age estimation techniques to consider the variation of bone microstructures with population. As these variables exhibit difference at same age in two different population due to under or over ageing. Hence age estimation method developed on the samples of one population might not be reliable for other population. The purpose of this study is to explore the variation in bone microstructures of Malaysian males and develop a regression equation for age estimation methods.

2. Materials and methods
The samples of this study were collected from the mortuaries of Forensic unit, Hospital University Kebangsaan Malaysia and Hospital Kuala Lumpur. Total of 30 men samples were analysed. Medical history including sex, race, ethnicity and age at death of the samples were known. Samples were handled under the Criminal Procedure Code s.331 subsection 2 (CPC s. 331 (2), which stated that: The parts of the human body may be taken and given to the institute for research. Death of most of the samples were reported to occur due to road accidents. The previous medical record reported that all samples were free from pathological conditions. The age range was between 22 to 64 years with mean value of 39.8 years. Only long bones of skeleton were considered in this study, namely humerus, radius, ulna, fibula, tibia and femur. All medical credentials to use bone samples for research analysis were fulfilled.

2.1. Sample preparation and microstructures extraction
Sample preparation technique was based on method described in [4]. A 3 cm length of fragment was cut from the mid shift of long bone and was de-fatted in diethyl ether solution. The fragment was then immersed in mixture of epoxy resin and was placed in vacuum chamber to clear air bubbles and to facilitate resin in fragment of bone. Further sectioning of bone was done with the help of microtome (LEICA SP 1600, Germany). The resulting fragment of bone was glued to a glass slide and was exposed to ultra violet light for 30 seconds. The bone sample was then sectioned at 30 microns. The
images of the resultant sample were taken with the help of a camera mounted on a transmitted light microscope (Nikon Eclipse E600) at 10x magnification. Figure 1 and Figure 2 show the resultant sample and Microscope used for image acquisition.

![Sample of long bone (radius) used in analysis.](image1)

**Figure 1.** Sample of long bone (radius) used in analysis.

![Microscope Nikon Eclipse E600.](image2)

**Figure 2.** Microscope Nikon Eclipse E600

Total of eight images were taken of each sample on both sides of anteromedial, anterolateral, posteromedial and posterolateral shown in figure 3. The location of image acquisition on both sides of line was separated by a square grid of 10x10 µm (100µm²).

![Filed of image acquisition anteromedial (AM), anterolateral (AL), posteromedial (PM), posterolateral (PL), P (posterior), A (anterior), M (Median), L (Lateral).](image3)

**Figure 3.** Filed of image acquisition anteromedial (AM), anterolateral (AL), posteromedial (PM), posterolateral (PL), P (posterior), A (anterior), M (Median), L (Lateral).

![Microscopic image of femur on PM location. Blue circles in image represent osteons and red circles represent haversian canals (HC).](image4)

**Figure 4.** Microscopic image of femur on PM location. Blue circles in image represent osteons and red circles represent haversian canals (HC).

Two morphological variables i.e. cortical thickness (CT) and medullar cavity diameter (MCD) while five histological variables i.e. osteons count (OC), haversian canal number (HCN), haversian canal radius (HCR), haversian canal area (HCA) and haversian canal perimeter (HCP) were taken into consideration. Cortical thickness (CT) and medullar cavity diameter (MCD) were measured using digital Vernier calliper. Osteons having half or more than half of haversian canals present in the visual field were considered in counting. Fragments of osteons present on the periphery of the field were considered as well. Haversian canal parameters were extracted and measured using Cell profiler. Haversian canal area (HCA) was calculated by summing up the area of all haversian canals in eight images of one sample.

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\text{Haversian canal area (HCA)} = \sum_{i=1}^{8} \text{sum of HCA in image}_i
\]
The correlation of morphological and histological variables with age were analysed using Pearson’s correlation (R) and regression equations with different combinations of bone microstructures were developed in SPSS software.

3. Results and discussion
In statistical analysis cortical thickness (CT) in human long bones did not show any significant correlation with age ($P > 0.05$, $R=0.005$). Osteon count showed significant correlation with age at ($P<0.03$) and ($R=0.518$). The haversian canal parameters (HCN), (HCR), (HCA) and (HCP) showed significant correlation with increasing age. Haversian canal area (HCA) showed the most significant positive correlation with age with ($P<0.01$) and ($R=0.526$). Haversian canal radius (HCR) and haversian canal perimeter (HCP) also showed positive correlation with age. Table 1 shows the correlation and significance values of all parameters with age as dependent variable.

### Table 1. Correlation between morphological and histological microstructures with age

| No | Bone microstructures       | Pearson’s correlation (R) | Significance (two tailed) | Total samples (N) |
|----|---------------------------|---------------------------|---------------------------|-------------------|
| 1  | Cortical thickness        | -.005                     | .980                      | 30                |
| 2  | Medullar cavity diameter  | -.073                     | .700                      | 30                |
| 3  | Osteon count              | .518                      | .001                      | 30                |
| 4  | Haversian canal area      | .526                      | .001                      | 30                |
| 5  | Haversian canal radius    | .511                      | .001                      | 30                |
| 6  | Haversian canal perimeter | .483                      | .002                      | 30                |
| 7  | Haversian canal number    | .491                      | .002                      | 30                |

The correlation of haversian canal parameters shows that HCA increases with age which means that the number of young osteons are present as they have larger haversian canals. The plot diagram of HCA and OC correlation with age is given in figure 5 and figure 6.

![Figure 5. Correlation of HCA with age in $\mu m^2$.](image)

![Figure 6. Correlation of OC with age.](image)

Medullar cavity diameter (MCD) shows very low negative correlation with age which suggests that MCD decreases with a very slow rate. Regression equations based on different combination of microstructures were generated.
The SEE of regression equations shown in table 2 varies between 7.2 and 8.1. As haversian canal area osteon count shows the highest correlation with age in these samples, the equation with the lowest SEE is based on osteon count, haversian canal area and haversian canal radius. Equation 5 in table 2 was tested for cross validation. The estimated age based on this equation varied between 10.4 years for 85% of samples. The estimated age range was within 15 years for samples having age range from 50 to 64. The mean value of the known ages was 39.8 years while the mean value of the estimated ages was 37.03 years. The results of this analysis is in agreement with [4],[2] studies, which were carried on Asians samples. However, the regression equation of this study was based on long bones of male samples only. Including of female samples and increasing the total numbers of sample might help in achieving more accuracy. Further research on behaviour of different microstructures with increasing age would be needed to generate regression equation for Malaysian females and to achieve better accuracy in existing methods.

4. Conclusion
This study analysed 30 Malaysian male samples to developed age estimation regression equation for Malaysian males. Age related changes were studied in morphological and histological microstructures of long bones. The histological microstructures such as osteon count, haversian canal area and haversian canal radius in long bones showed significant correlation with age. The estimated age for individuals up to 50 years was estimated within 9 years, however the estimated range increased when the chronological age of samples exceeded 50 years. Morphological factors such as cortical thickness and medullar cavity diameter did not show any significant correlation with increasing age. Combination of histological and morphological microstructures were used to generate several regression equations however equation with lowest SEE (7.2) was based on osteon count, haversian canal area and haversian canal radius. Study of different parameters of osteons such as osteon density, osteon area, and osteon population might also help in developing better regression equations. Including samples of females might lead to high rate of accuracy. The results of this study are in agreement with the literature which is based on Asian samples. Further research is needed to reduce the difference between chronological age and estimated age.

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| No | Regression equation | $R^2$ | SEE  |
|----|---------------------|------|------|
| 1  | $Y=16.06+0.5(CT)+1.14(MCD)+5.1\times10^{-5}(HCA)-0.007(HCN)-0.21(HCP)+0.44(OC)+3.3(HCR)$ | 0.6  | 8.1  |
| 2  | $Y=16.02+2.41\times10^{-5}(HCA)+0.02(HCN)-0.083(HCP)+0.64(OC)+2.47(HCR)$ | 0.6  | 7.4  |
| 3  | $Y=19.83+2.84\times10^{-5}(HCA)+0.07(HCN)-0.12(HCP)+2.5(HCR)$ | 0.6  | 7.6  |
| 4  | $Y=4.83+1.48\times10^{-5}(HCA)+0.026(HCN)+2.2(HCR)+0.67(OC)$ | 0.5  | 7.3  |
| 5  | $Y=6.66+1.54\times10^{-5}(HCA)+2.33(HCR)+0.74(OC)$ | 0.5  | 7.2  |
5. References

[1] C. D. L. Thomas, M. S. Stein, S. A. Feik, J. D. Wark, and J. G. Clement, "Determination of age at death using combined morphology and histology of the femur," *Journal of Anatomy*, vol. 196, pp. 463-471, 2000.

[2] M. Yoshino, K. Imaizumi, S. Miyasaka, and S. Seta, "Histological estimation of age at death using microradiographs of humeral compact bone," *Forensic Sci Int*, vol. 64, pp. 191-8, Feb 1994.

[3] D. Thompson, "Microscopic Determination of Age at Death in an Autopsy Series," 1981.

[4] 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.

[5] P. Zioupos, A. Williams, G. Christodoulou, and R. Giles, "Determining 'age at death' for forensic purposes using human bone by a laboratory-based biomechanical analytical method," *J Mech Behav Biomed Mater*, vol. 33, pp. 109-23, May 2014.

[6] 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.

[7] 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.

[8] G. SM. "The earlier gain and later loss of cortical bone in nutritional perspective.," *Springfield: Charles C.Thomas*, 1970.

[9] O. D. a. S.-E. F. Richman EA, "Differences in intra-cortical bone remodeling in three aboriginal American populations: possible dietary factors.," *Calcif Tissue Int* pp. 209–214, 1979.

[10] W. M. Krogman, "Skeletal changes in young American males," *American Journal of Orthodontics*, vol. 44, pp. 715-716, 1958.

[11] T. W. Todd, "Age Changes in the Pubic Symphysis: VII. The Anthropoid Strain in Human Pubic Symphyses of the Third Decade," *Journal of Anatomy*, vol. 57, pp. 274-294.12, 1923.

[12] S. T. Brooks, "Skeletal age at death: the reliability of cranial and pubic age indicators," *Am J Phys Anthropol*, vol. 13, pp. 567-97, Dec 1955.

[13] E. Kerley, "Forensic Anthropology and Crimes Involving Children," 1976.

[14] S. S. Katzenberg MA, "Ubelaker DH. Methodological considerations in the forensic applications of human skeletal biology. In: eds. New York,NY: Wiley-Liss "Biological anthropology of the human skeleton., pp. 41–67, 2000.

[15] J. J., "Age changes in human bone.," *Clinical Orthopaedics*, vol. 17, pp. 210-218, 1960.

[16] J. Ahlqvist and O. Damsten, "A modification of Kerley's method for the microscopic determination of age in human bone," *J Forensic Sci*, vol. 14, pp. 205-12, Apr 1969.

[17] E. R. Kerley and D. H. Ubelaker, "Revisions in the microscopic method of estimating age at death in human cortical bone," *Am J Phys Anthropol*, vol. 49, pp. 545-6, Nov 1978.

[18] S. Pfieffer, C. Crowder, L. Harrington, and M. Brown, "Secondary osteon and Haversian canal dimensions as behavioral indicators," *Am J Phys Anthropol*, vol. 131, pp. 460-8, Dec 2006.

[19] 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 *Proceedings - 6th IEEE International Conference on Control System, Computing and Engineering. ICCSCE 2016*, 2017, pp. 169-172.

[20] I. Khan, M. M. A. Jamil, T. N. T. Ibrahim, and F. M. Nor, "Automated human age estimation at death via bone microstructures," in *Proceedings - 6th IEEE International Conference on Control System, Computing and Engineering, ICCSCE 2016*, 2017, pp. 580-583.
[21] I. Khan, F. Mohd Nor, and M. M. Abdul Jamil, “A survey of human age estimation techniques from bone microstructures,” in *IFMBE Proceedings*, 2016, pp. 203-207.

[22] H. Abdullah, F. Mohd Nor, and M. M. Abdul Jamil, "Human bone histomorphological pattern differences between genders: A review," in *IFMBE Proceedings*, 2016, pp. 183-187.

[23] H. M. Frost, "Human Haversian system measurements," *Henry Ford Hosp Med Bull*, vol. 9, pp. 145-7, Mar 1961.

[24] H. Cho, S. D. Stout, R. W. Madsen, and M. A. Streeter, "Population-specific histological age-estimating method: a model for known African-American and European-American skeletal remains," *J Forensic Sci*, vol. 47, pp. 12-8, Jan 2002.

[25] Y. Watanabe, M. Konishi, M. Shimada, H. Ohara, and S. Iwamoto, "Estimation of age from the femur of Japanese cadavers," *Forensic Sci Int*, vol. 98, pp. 55-65, Nov 30 1998.

[26] 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.