Histomorphometric variance of haversian canal in cortical bone of Malaysian ethnic groups

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Abstract. This research presents a comparison of haversian canal based microstructural parameters between Malaysian citizens. The ages ranges from 22-58 which was divided into four groups of each decade. In each decade comparison was done on haversian canal based parameters between the two Malaysian ethnic groups Chinese-Indian. Parameters in long bones femur, tibia, fibula, humerus, ulna and radius were measured under microscope by obtaining the bone thin section of 30 microns from the mid-shaft. Eight microscopic images were taken from each specimen having an observation region of 1.228mm² for each image. In the third and sixth decade, haversian canals showed relatively larger total covered area in Indian bone specimens. Haversian canal radius was found to be greater in Chinese bone specimen in the fourth decade. The fifth decade showed no differences. Further studies are required to validate the outcomes, compare other ethnic groups and development of automated microstructures identification and measurement tools.

1. Introduction
Bone histomorphological research over the past few decades have developed various methods to extract information from skeletal remain[1, 2]. Morphological analysis involves study of the main structures of skeleton. Skull, jaw, long bones and pelvis are the main key bones for this analysis[3]. Histological analysis on the other hand is not bound to the main skeletal structures. Researches have been carried out to use only fragments of long bone and analyse them to estimate age, sex, race, height and weight[4-7]. Histological analysis of cortical bone for this information started only few decades ago and research conducted on a particular race of humans didn’t show very promising results when applied on others[8-10]. Race and region of human population changes the morphological structures of bone which also effects the microstructures in the cortical bone. This led for stand-alone studies for human bones belonging to various races and region which can be helpful in forensic investigations, archaeological exploration and contribute for future comparisons.

To understand cortical bone microstructures, one must have the knowledge of basic bone structure. Human bones consist of two main types cortical bone and trabecular bone. Cortical bone is the outer hard layer which gives a firm structure to the bones and trabecular bone is the spongy inner part. Cortical bone is build up on basic structures called the osteon systems. The osteon system comprises of blood vessels in the centre called haversian canal and layers of lamella surrounding these canals. Human bones undergo continuous restructuring in the body which is known as bone remodelling. In remodelling process, old bone structure is eaten by cells called osteoclast. Osteoclast leave behind mesh structures which are filled with new bone by the cells known as osteoblast. This process of bone
remodelling changes the microstructures in cortical bones[11-13]. These structural changes are the key observation in bone histological analysis for age and sex estimation. Selection of microstructures and region of microscopic images varies in previous researches (table 1). Most of the microstructures are either observed or derived measurements of osteons and haversian canals. In this study, microstructural parameters related to haversian canal were compared between the two ethnic groups (Chinese-Indian) citizens of Kuala Lumpur, Malaysia. The haversian canal parameters used in this study are mean area (Hcm), total area covered (Hct), mean radius (Hcr), mean perimeter (Hcp) and number (Hcn).

Table 1. Imaging location, bone type and area selected by researchers.

| Reference                  | Region/area                                | Bone                                      | Imaging location   | Purpose                        |
|-----------------------------|--------------------------------------------|-------------------------------------------|--------------------|---------------------------------|
| Kerley, 1965[8]             | USA whites                                 | Femur, tibia, fibula                      |                    | Age regression                 |
| DL Singh, DL Gunberg, 1970[14] | Portland                                   | Femur, tibia                              |                    | Age regression                 |
| DD Thompson, 1979, 1981[10, 15] | USA whites and Eskimos from St. Lawrence, North Alaska and Canada | Femur                                      |                    | population comparison          |
| David B Burr, 1990[16]      | Native American and USA whites             | Femur                                      |                    | Age regression                 |
| Ericksen, 1991[9]           | USA whites                                 | Femur                                      |                    | Age regression                 |
| DM Mulhern, 1997[17]        | Medieval Nubian population from Kulubnarti, Subanese Nubia | Femur                                      |                    | population comparison          |
| KL Bell, 2001[18]           | Australia                                  | Femur                                      |                    | population comparison          |
| C David L Thomas, 2005[19]  | Australia                                  | Femur                                      |                    | population comparison          |
| F Nor, 2009[20]             | Kuala Lumpur, Malaysia                      | Femur, tibia, ulna, radius, Humerus, fibula |                    | Age regression                 |
| HM Britz, 2009[21]          | Australia                                  | Femur                                      |                    | Population comparison          |
| C Hernandez, 2012[22]       | USA whites                                 | Femur                                      |                    | Age regression                 |
| This study                  | Kuala Lumpur, Malaysia                      | Femur, tibia, ulna, radius, Humerus, fibula |                    | Population comparison          |
2. Materials and Methods

2.1. Sample Population

Samples of 33 adults were collocated from Universiti Kebangsaan Malaysia Medical Centre (UKMMC), Kuala Lumpur, Malaysia. The bone samples were collected from individuals having no bone disease in previous medical records. The population of Malaysia is divided into three main ethnic groups Malay, Chinese (from south china) and Indian (from south India)[23]. Among the collected samples 21 were Chinese while 12 were Indian. With respect to sex, 24 samples were males and 9 were females and age of samples ranges from 22-58 The samples are not homogeneously numbered due to the ethnic and religious reservations and availability of cases in forensic medical centre UKMMC. The bone types selected for this work were long bones comprising of humerus, radius, ulna, femur, tibia and fibula. According to previous research conducted on Malaysian samples, haversian canal increases with respect to age. This complies in division of samples into decades for better statistical observation. Figure 1 shows samples orientation with respect to decades and types of bones used.

| Age   | Chinese | Indian |
|-------|---------|--------|
| 20-30 | 5       | 4      |
| 30-40 | 6       | 3      |
| 40-50 | 5       | 3      |
| 50-60 | 5       | 2      |
| Total | 21      | 12     |

Figure 1. Long bones selected for sampling.

2.2. Samples imaging

The bone is cut from mid shaft at 3cm length. To clean the sample and remove fat, it is placed in diethyl ether solution for 24 hours. Epoxy resin is then mixed with bone along with hardener. To remove bubbles from epoxy bone is place in vacuum chamber half hour. Face cut of 30 micros was done using microtome after gluing it on a glass slide[20]. Eight images of each sample are taken as observed region. Figure 2 (a) shows the selected region. These regions were selected based on previous studies on Malaysian samples which observed more microstructure differences at these locations. Nikon eclipse Ts100 microscope was used with Dino Eye camera to obtain microscopic images. Dino capture 2.0 software was used to calibrate obtained images at 4X magnification. The dimensions of observed region are 1.28mm x 0.96mm which gives us a total region area of 1.228mm². Figure 2 (b and c) shows the microscope used and obtained microscopic image. The measurements of haversian can were done manually using measurement tools in Cellprofiler image analysis software.

Figure 2. (a) Location of images from samples. (b) Nikon eclipse Ts100 used to take microscopic
images. (c) Obtained image with Observation area of 1.228mm².

2.3. Inclusion and exclusion criteria
The criteria for inclusion of haversian canal was slightly different from researches which were focused mainly on osteon systems[4, 5, 21]. Since this research focuses on haversian canal not osteon systems so the shape and intactness of osteon was not taken into consideration. The haversian canal present in observation area with more than fifty percent of its area were considered as full haversian canals. In case where haversian canals are connected to Volksmann’s canals, only haverisan canals was measured based on manual observations.

2.4. Statistical tests
The bone samples were divided into four independent age groups of decades from 20 – 60. In each age group five haversian canal based parameters were compared between two sample categories (Chinese-Indian). For comparison of parameters independent samples t-test was used.

3. Results
Measurement of each specimen was done on eight different locations (figure 2 (a)). Mean values of the haversian canal based microstructural parameters measured at these locations are given in table 2. Cumulative fraction distribution was performed to check the contribution of individual haversian canals in total area covered by haversian canals. Figure 3 shows the Hcm cumulative fraction distribution of two groups for ages 20-30. T-test showed significant differences between the two races for Hcm, Hca, Hcr and hcp. It was found that haversian canals in Indians samples were relatively larger than that of Chinese samples (p <0.01), while the number of haversian canals showed no difference (table 3).

| Age groups | Races | Hcm (µm²) | Hca (mm²) | Hcr (µm) | Hcp (µm) | Hcn |
|------------|-------|-----------|-----------|----------|----------|-----|
| 20-30      | Chinese | 1336.9    | 0.16      | 5.8      | 149.1    | 128 |
|            | Indian  | 1833.7    | 0.21      | 6.8      | 173.9    | 117 |
| 30-40      | Chinese | 3816.3    | 0.53      | 9.9      | 257.4    | 142 |
|            | Indian  | 3362.8    | 0.49      | 8.6      | 232.4    | 152 |
| 40-50      | Chinese | 5096.2    | 0.79      | 11.7     | 300.7    | 150 |
|            | Indian  | 4718.8    | 0.69      | 11       | 290.5    | 139 |
| 50-60      | Chinese | 8277.5    | 1.29      | 14.9     | 380.3    | 156 |
|            | Indian  | 9905.7    | 1.52      | 16.5     | 398.4    | 157 |
Figure 3. Cumulative fraction distribution graph demonstrating contribution of haversian canals in the total area covered for two races belonging to ages (20-30).

Table 3. Independent sample t-test of microstructural measurements between Malaysian (Chinese-Indian) bone thin sections.

| Parameter | Hcm  | Hca  | Hcr  | Hcp  | Hcn  |
|-----------|------|------|------|------|------|
| t         | -3.477 | -2.963 | -4.261 | -4.377 | 0.982 |
| Sig.      | 0.00088 | 0.00419 | 0.00006 | 0.00004 | 0.32955 |
| sd        | 700.75 | 10858.1 | 1.034 | 26.99 | 3.47 |

In the decade group of 30-40 independent t-test showed no significance with respect to Hcm, Hct, Hcp and Hcn. Haversian canal radius on the other hand was observed to be larger in Chinese samples (p<0.05). Table 4 shows the independent sample t-test. When compared to the previous decade haversian canal showed less significance. Figure 4 shows the cumulative fraction distribution of haversian canal for this decade group (30-40).

Figure 4. Cumulative fraction distribution graph demonstrating contribution of haversian canals in the total area covered for two races belonging to ages (30-40).
Table 4. Independent sample t-test of microstructural measurements between Malaysian (Chinese-Indian) bone thin sections.

| Parameter | Hcm   | Hca   | Hcr   | Hcp   | Hcn   |
|-----------|-------|-------|-------|-------|-------|
| T         | 1.147 | 0.4638| 2.142 | 1.6458| -1.1452|
| Sig.      | 0.2552| 0.6442| 0.0357| 0.1042| 0.2559|
| Sd        | 2128.65| 41321.66| 2.61  | 70.72 | 3.49  |

There was no significance seen among any parameters in the age group of 40-50 when t-test was applied. Table 5 shows the Independent sample t-test for this age group and figure 5 shows the cumulative fraction distribution of haversian canal.

Figure 5. Cumulative fraction distribution graph demonstrating contribution of haversian canals in the total area covered for two races belonging to ages (40-50).

Table 5. Independent sample t-test of microstructural measurements between Malaysian (Chinese-Indian) bone thin sections.

| Parameter | Hcm   | Hca   | Hcr   | Hcp   | Hcn   |
|-----------|-------|-------|-------|-------|-------|
| T         | 1.016 | 0.889 | 1.502 | 0.859 | 1.154 |
| Sig.      | 0.313 | 0.377 | 0.138 | 0.393 | 0.252 |
| Sd        | 1737.966| 55657.917| 1.99  | 49.16 | 5.589 |

In the fifth decade haversian canals grew larger in size and their number also increased from the previous decades. There was no significant difference found Hcm, Hcn and Hcp, while Hca and Hcr were found to be greater in Indian samples than in Chinese samples (p<0.05) (table 6). Figure 6 shows Cumulative fraction distribution of haversian canal area for the fifth decade.
Figure 6. Cumulative fraction distribution graph demonstrating contribution of haversian canals in the total area covered for two races belonging to ages (30-40).

Table 6. Independent sample t-test of microstructural measurements between Malaysian (Chinese-Indian) bone thin sections.

| Parameter | Hcm  | Hca  | Hcr  | Hcp  | Hcn  |
|-----------|------|------|------|------|------|
| T         | -1.99| -2.01| -2.77| -1.14| 0.16 |
| Sig.      | 0.0516| 0.0492| 0.0075| 0.2559| 0.8723|
| Sd        | 2758.3| 48755.97| 1.928| 50.181| 4.188|

4. Discussion
The haversian canal parameters selected for this research increased in area with each observed decade (table 2). This is in agreement with previous work done[20, 24]. However, their difference with respect to race changes over decades. The third decade showed most differences. In Indian samples haversian canal were relatively larger in size and covered more observed region. The number of haversian canals increased with the progressing decade but showed no difference with respect to races. In the fourth decade haversian canal area in both races showed no differences. Mean radius on the other hand was found to be greater in Chinese samples. The fifth decade showed monotony in all observed parameters. Mean haversian canal radius was found to be greater in Indian samples in the sixth decade. All the samples showed relative measurements except for one Indian sample aged 53. Haversian canals in observation section of AM (figure 2(a)) showed relatively larger haversian canals from any other samples in that decade. Although mean radius was the parameter which showed differences in the third, fourth and sixth decade, the differences did not show continuous significance over the decades.
Cortical bone porosity is generally referred to the network of vascular canals commonly consisting of haversian canals and Volkmann’s canals. Haversian canal is the key component to analyse cortical porosity. In this study haversian canal was measured irrespective of the presence of an osteon system or multiple layer of lamella (also referred as non-haversian canals)[25]. This gives us relation of the cortical bone porosity in which percentage of total area covered by haversian in eight sections can be considered as bone porosity.
5. Conclusion
This research presented a comparison of five microstructures in two Malaysian ethnic groups Chinese and Indian. The result showed maximum differences in third and sixth decades where haversian canal total area covered and mean radius were relatively greater in Indian specimens. Although the results were generally encouraging, it is required to take this work as the founding steps and further continue research to justify and expand the work done, which could include comparison of another ethnic group (Malay). A comparison of age graded sex comparison of the Malaysian samples can also be achieved. Computer aided systems are widely designed for medical imaging applications. A system can be designed to extract microstructures parameters using image processing and analyse the given specimens. This could reduce the time consumed in manual measurements and increase the accuracy.

Acknowledgments
The authors wish to express their gratitude to Office for Research, Innovation, Commercialization and Consultancy Management (ORICC), University Tun Hussein Onn Malaysia for providing postgraduate research Grant vote number (U280) to support this research.

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