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Estimation of age from the femur of Japanese cadavers

Yutaka Watanabe[a], Masayoshi Konishi[a,*], Masahisa Shimada[a], Hidetsugu Ohara[a], Sotaro Iwamoto[b]

[a]*Department of Anatomy, Osaka Medical College, Daigakku-machi 2-7, Takatsuki, Osaka 569, Japan*
[b]*Department of Anatomy, National Defense Medical College, Namiki 3-2, Tokorozawa, Saitama 359, Japan*

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Abstract

The purpose of this study is to estimate the age of cadavers by histomorphometry of the femur. Seventy-two Japanese males ranged from 43 days to 92 years old and 26 females ranged from 2 to 88 years old were used. The thickness of sections was adjusted at 50 to 70 μm by grinding with sand paper. The sections were not decalcificated. They were stained with Villanueva's bone staining powder and with thionin dye. Microradiographs of the sections were obtained by the soft X-ray apparatus. The area, maximum and minimum diameter, and perimeter of the perfect osteon and Haversian canal were measured. In addition, the type II osteon number, osteon fragment number, and area of triangle were also determined. All these parameters were examined by an image analyzer. The parameters of the osteon showed high correlation coefficient with age ($|r|>0.77$), while those of the Haversian canal were low ($|r|<0.11$). All parameters were subjected to multiple regression analysis for producing a multiple regression equation of age estimation. For the stepwise selecting method, the perimeter of osteon, maximum length of the Haversian canal and osteon fragment number were selected for the equation. Their multiple r^2 and standard error of estimation were 0.8874 and 6.39, respectively. For the forward selection method, in addition to the above items, three parameters, the maximum length of Haversian canal, triangle area, osteon fragment number were selected. Their multiple r^2 and standard error of estimation were 0.9484 and 4.884, respectively. Bone staining was useful to clarify the demarcation between osteon and fragment, leading to an increase in the accuracy of age estimation. However, the entire range from birth to 90 years was difficult to cover for precise age estimation.

*Corresponding author.

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1. Introduction

Forensic scientists, physical anthropologists and archeologists have attempted to assign the age of cadavers from such gross-anatomical characteristics as the condition of the suture on the cranium, from pubic symphysis, and from the occlusal surface of the dentine. The discrimination of the stage of cranial suture closing and scoring for ridge of the pubic symphysis might be sophisticated and empirical method for the estimation of cadaver age, however, their results have demonstrated them to be inaccurate methods. Moreover, usually it is hard to obtain a complete skeleton, especially in cases of excavation. Since Kerley [1,2] reported a method for determining cadaver age from thin sections of long bones, age estimation from a piece of bone became an important method in both the forensic and archaeological fields. Following Kerley [1], a number of methods for age estimation were revised. Ahlqvist and Damsten [3], Singh and Gunberg [4] and Thompson [5,6] also modified his method. Iwamoto et al. [7,8] compared the results between the femur and the humerus. Recently, Cool et al., [9] introduced the histomorphometry of the human occipital bone into this field. Yoshino et al., [10] estimated cadaver age by using microradiography of Japanese humerus. Burr [11] stained a thin section of the macaque femur with toluidine blue. Ericksen [12] collected a large number of femur, and combined micro- and macro-measurements.

In the present study, we examined more precise and reliable methods for the age estimation of cadaver, and compared these with the results reported by the previous investigators. Sections were stained by multiple medicaments, and contact micro radiographs were obtained using X-ray apparatus.

2. Materials and methods

The samples used in this study were 98 Japanese cadavers dissected by our medical students, and from the forensic room in 1995 and 1996. The age of 72 males ranged from 43 days to 92 years with a mean ($\bar{x}$) and standard deviation (S.D.) of 50.38 and 21.53 years respectively. The age of 26 females was from 2 to 88 years with 48.85 ($\bar{x}$) and 28.54 (S.D.) years (Table 1).

The samples from the students were a complete cortical bone ring, and those from the forensic room were a piece of the anterior half. All of samples were sectioned at 5–10 mm thickness in the midshaft of the right femur. The materials were fixed in 10% neutral buffered formalin (formaldehyde) for 7–10 days. The bone rings were washed in running water for 2 days to remove the soft tissue in bone marrow. Then, the materials were defatted in a mixed solution of chloroform and methanol for 7 days, and bleached in 2%-H_20_2 solution for one day. After dehydrating using graded alcohols, materials were embedded in methyl methacrylate (Kishida, Japan) without any decalcifications. They were polymerized for 7–10 days at room temperature. Several thin sections of 1–2 mm thickness were sequentially taken from an original bone mass using a small wedge hand saw. The thin sample was reduced to a regular thickness of 50 to 70 μm by manual

Table 1
Age distribution of the samples

Age cohort (years)	Males (*n*)	Females (*n*)
Under 9	4	4
10–19	1	1
20–29	9	4
30–39	7	0
40–49	12	1
50–59	12	5
60–69	15	4
70–79	6	3
80–89	5	4
Over 90	1	0
Totals	72	26
Means (years)	50.38	48.85
SD	21.53	28.54

grinding on graded sand papers. The section was finally ground on a lapping film with particles under 3 μm. Contact microradiograms of the section were obtained by ultra soft X-ray apparatus (Softex, Japan). Small pieces of sheet film (Kodak, SO-343, USA) were used for the radiograms. Exposure conditions were varied according to thickness of the sample at 10 to 10.5 kVp, 3 to 3.5 mA, 30 to 40 minutes, but the focal film distance was set at 8 cm. After microphotographing with a light microscope, the sections were stained with Villanueva's bone staining powder [13,14] for 72 h. The sections stained were dehydrated with graded alcohols and mounted with Canada balsam on the glass slide in the usual manner. A few sections were stained with thionin as reported by Derkx and Birkehäger-Frenkel [15].

The histomorphometry of the stained sections was carried out with an image analyzing system (SPICCA, Ratoc Co., Japan). The sections were examined using a microscope with a ×10 objective and ×10 CCD Camera lens, resulting in an observed field size of 0.707744 mm^2 (0.81451mm length and 0.86892 mm width) on a monitor of the image analyzing system. Where the boundary of each osteon was unclear and doubtful, the image from Softex film was referred. The measuring region was defined as the midzone between the periosteonal and medullar sites (Fig. 1). The mean values obtained from three fields for one section were used as the measurements of each sample. The parameters were measured as shown in Fig. 2.

The data obtained from each histological parameter were subjected to regression analysis to determine their relation with age and to calculate regression equations for age estimation.

3. Results

3.1. Histochemical comparison between stained and non-stained images

Fig. 3 shows a light microscopic image of the non-stained section. The osteon and its ring appear roundly and the Haversian canal is seen also darkly in its center. The

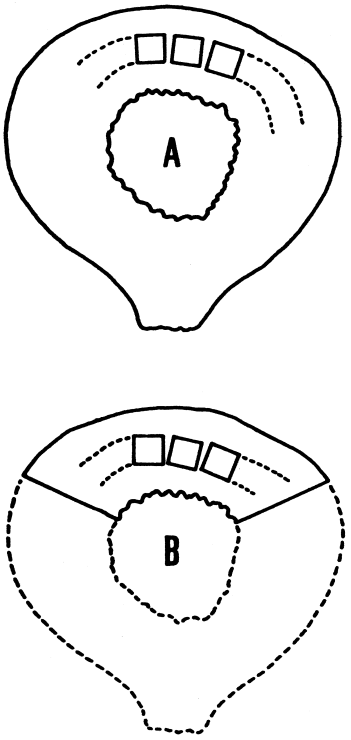

Fig. 1. Measuring field location for histomorphometrics. Box size is 0.7 mm^2 (0.81×0.87 mm), and located mid-zone between the periosteonal and medullar sites. 'A' shows the bone section from the anatomical practice, and 'B' from the forensic room.

peripherals of osteon showed lightly dark, and its outer-most layer, called the cement line, is colored moderately. It is hard to differentiate the boundary between the primary osteon and type II osteon. In bone sections stained by Villenueva's stain, the osteoid seams appeared to be dark red or red–violet, while the degree of calcification in the perfect osteon is stained gradually pink. Villanueva [14] described that the reason why some seams were stained green, and others were red to dark red was still open to question. We could not find the green ring in the entire Haversian canal (osteon). Translucent bone showing middle-degree mineralization density was stained orange in color by orange *G*. Translucent bone showing low-degree mineralization density was stained red in color by basic fuchsin. The younger osteon appeared to be red or pink, while the elder was orange or light pink. Where the interstitial layer was highly mineralized, the layer showed unstained or weak orange. In bones of a young age, the staining intensity of osteon and interstitial area was same (Fig. 4a), but in elder bones, the difference of the stain intensity in the both cases was marked as shown in Fig. 4b. The nuclei of osteocytes in the osteon colored variously from light blue to dark purple. Some of the osteocytes located in the peripheral part of osteon appeared large in size, and stained light purple. On the other hand, osteocytes located in the interstitial area

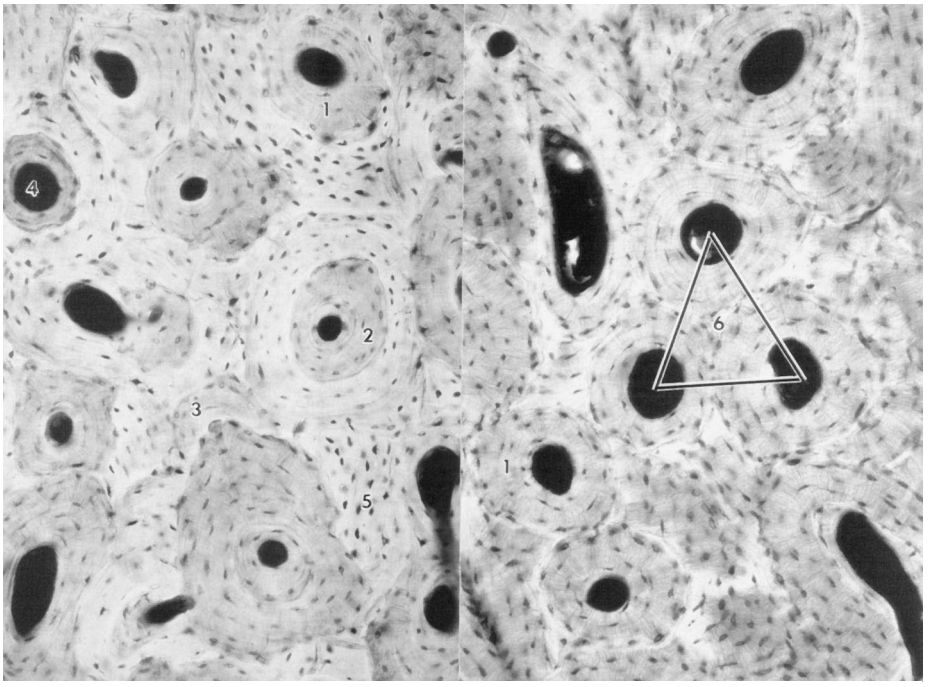

Fig. 2. A light microscopic image of Villanueva's bone stain. Various histomorphological structures were shown, 1: osteon, 2: Type II osteon, 3: osteon fragment, 4: young osteon, 5: interstitial area, 6: area of triangle.

appeared small in size, and stained black or dark purple. Type II osteons showed darker rings than those of the primary one. Therefore, the boundary between primary and secondary osteon was evident, as shown in Fig. 4b.

3.2. Morphological comparison of the X-ray image and the thionin staining samples

In the X-ray image (Fig. 5), an inner ring surrounding the Haversian canal appeared white, indicating high density. The density of the osteons changed sequentially from dark to light with physical development. The density altered gradually from the central to the peripheral portion even within the same osteon. The boundary between the osteon and the interstitial area could be identified easily as in the Villanueva's staining sample. Because the boundary between interstitial areas is unclear, the number of the fragment was uncertain. RNA-presenting rough endoplasmatic reticula were stained violet by the thionin. As shown in Fig. 6, various osteons were found in the observation area. The dark violet osteon was young, while the light one was elder. The preparation stained with the thionin was stored in a cold room for a month, while that of Villanueva's stain was mounted in the Canada balsam and kept for a few months. It was confirmed that thionin staining was disadvantageous for the present measurement. This staining was useful for discriminating the presence of the mineralizing front (Fig. 6).

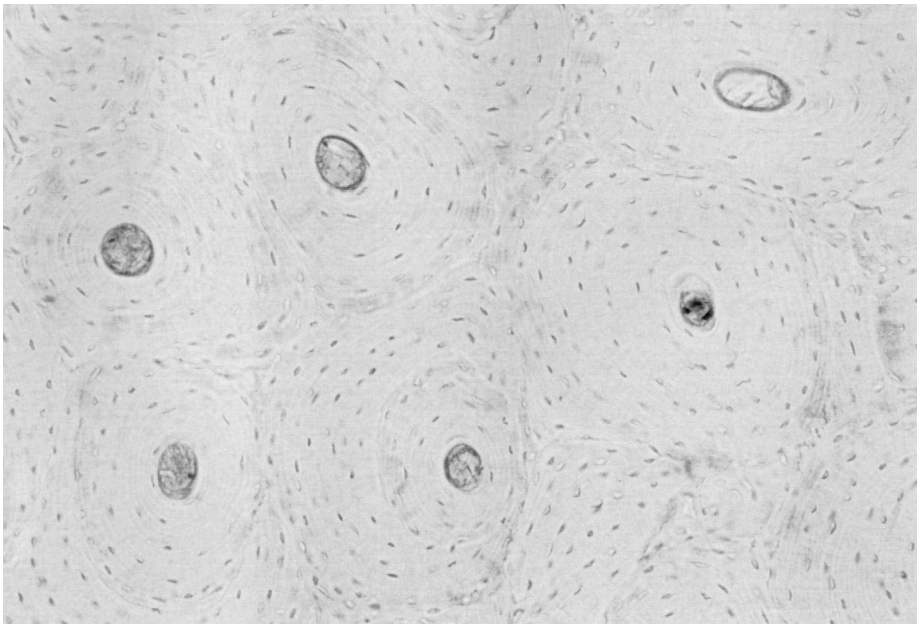

Fig. 3. The light microscopic image of the non-stained section (female, 62 years old).

3.3. Simple regression analysis

By using linear regression analysis of SAS (version 5.0), the osteon perimeter showed the highest correlation coefficient ($r=-0.8351$) with age, followed by the osteon length ($r=-0.8343$), and finally the osteon area ($r=-0.8262$). Such parameters of the Haversian canal as the length, perimeter and width were generally low ($r=-0.068$ to -0.1245). The parameters such as the numbers of osteon fragment and Type II osteons showed middle value. The slope values of the single linear regression formula are shown in Table 2. A number of measuring items showed negative correlation with the age. On the other hand, the number of osteons, fragments and type II osteons showed positive correlation coefficient. Therefore, histologic factors such as the size of the bone decreased with age, while the numeric factors increased with age. The parameters of the Haversian canal did not change.

3.4. Multiple regression analysis

To produce equations for age estimation, all parameters were subjected to the stepwise and forward methods of multiple regression analysis. In the stepwise method, three parameters, i.e., the perimeter of osteon (Pos), length of the Haversian canal (Lh) and the osteon fragment number (frag) were selected. In this case, the multiple correlation coefficient (r) and the r of standard error were 0.8874 and 6.393, respectively. The equation was

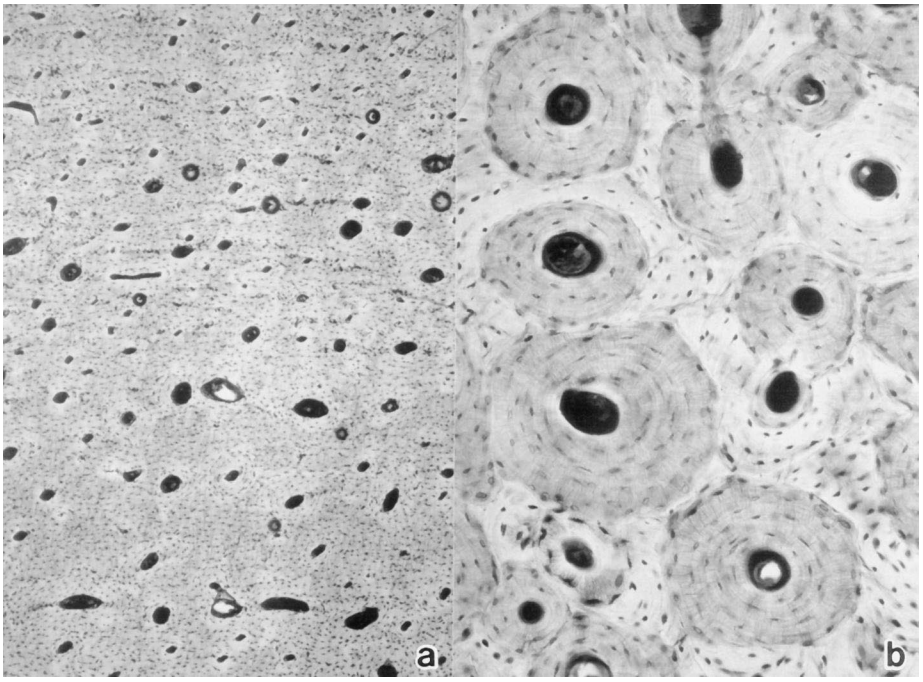

Fig. 4. A light microscopic image of Villanueva's bone stain from a young aged sample (a) and from an elder aged sample (b). Various osteons appear in the elder, but not in the young.

$$\text{Age} = 116.892 - 0.132\ \text{Pos} + 0.338\ \text{Lh} + 1.506\ \text{frag}$$

In the forward method, six parameters, i.e., the length of osteon (Los), width of osteon (Wos), perimeter of osteon (Pos), length of the Haversian canal (Lh), Area of triangle (Tn) and osteon fragment number (frag) were selected. In this case, the multiple correlation coefficient (r^2) and the r^2 of standard error were 0.9484 and 4.884, respectively. The equation was

$$\text{Age} = 105.167 + 0.963\ \text{Los} + 1.123\ \text{Wos} - 0.769\ \text{Pos} + 0.415\ \text{Lh} + 0.0006\ \text{Tri} + 1.608\ \text{frag}$$

The differences between the actual and estimated age were ranged from 0.5 to 7.2 years. The mean value was 4.88 years in this data. In two equations, Pos, Lb and frag were commonly selected.

4. Discussion

The results of age estimation by other workers were shown in Table 3. The standard error of estimation (SEE) was ranged from 3.16 to 11.50 years. Although it is difficult to compare our present data with those previous literature because of the different kinds of

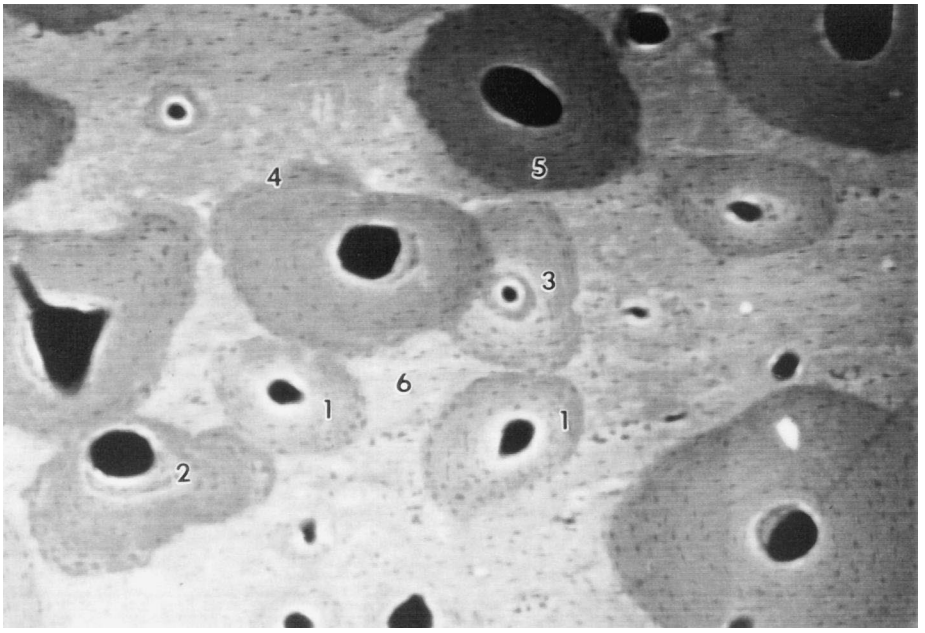

Fig. 5. Microradiographs of cross-sectioned femur (male, 64 years old). 1: osteon, 2: double-zoned osteon, 3: Type II osteon, 4: osteon fragment, 5: low density osteon, 6: interstitial area.

bones, measuring items and number of materials, and age construction, our SEE appears much better. This may be caused by using grinding sections stained by Villanueva bone powder. Through the use of staining, the demarcation among osteons, interstitial layer, type II osteons and osteon fragments were easy to identify. Moreover, the RNA of the rough elastic retinaculum in bone cells was clearly identified by the thionin staining. According to this staining, the young osteons were violet, while the mature were more moderately stained. The clear boundary of osteons may certify the precise measuring line. The range of our age estimation might be more grown up than others. Drisini [16] reported that the estimation error was 3.923 years in femur by using the number of osteons in a given field. The number of his subject was only 24 individuals (15 males and nine females) ranged from 19 to 50 years old. Such good results might have come from a lower age range and fewer number of subjects. In the present study, a fairly high multiple correlation coefficient ($r^2 = 0.9484$) could be obtained by using only six parameters such as length of osteon, width of osteon, perimeter of osteon, length of the Haversian canal, area of triangle, and fragment.

In multiple regression analysis, osteon parameters were highly correlated with age, while those of the Haversian canal were low. Those of the osteon fragment number, osteon, and type II osteon were moderate. In the stepwise method, the area of osteon ranked first, and number of fragments second.

From the following reasons, Stout et al., [17] stated that the rib could be used for reasonable, reliable and accurate estimation of age as compared with that of such long

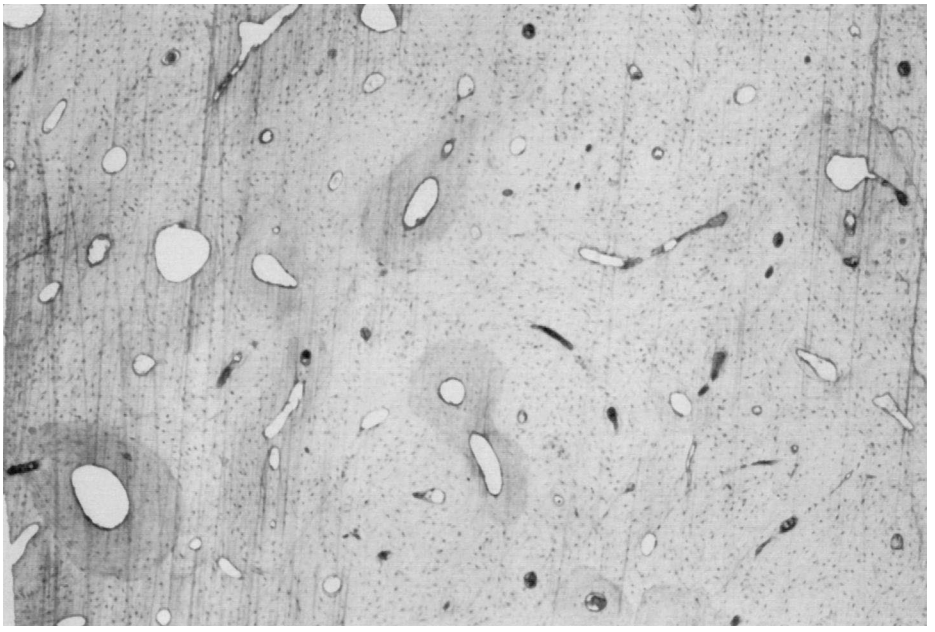

Fig. 6. A light microscopic image of the thionin stain (female, 52 years old). The osteons showing various intensity were recognized.

bone as femur. The rib is used for respiratory purposes, while the femur is locomotion. If the femur changed morphologically by physical development, and by training in the case of bedridden, then the speed of bone resorption might be higher than that of man capable of walking. It is said that body weight does not influence those factors responsible for estimation of age [11].

The compact bones of femur and tibia have mainly been used as subjects for these

Table 2
Linear regression analysis for the relation between age and histomorphometrical items

	Variable	Correlation Coefficient	Intercept	Slope
Osteon	Area	−0.8262	93.62	−0.00102
	Length	−0.8343	132.52	−0.313 12
	Width	−0.7713	133.74	−0.40018
	Perimeter	−0.8351	83.62	−0.04409
Haversian Canal	Area	−0.1059	51.73	−0.00044
	Length	−0.1245	69.56	−0.24263
	Width	−0.0683	57.14	−0.11648
	Perimeter	−0.1145	62.70	−0.05520
	Number osteon	0.6480	−2.31	3.95645
	Number fragment	0.7108	32.23	3.22922
	Number Type II	0.7440	34.26	7.50803
	Area of triangle	−0.6545	97.19	−0.00166

Table 3
Comparison with the previous results

Author(s)	Yrs.	Multiple R^2	SEE (yrs.)	Materials
Singh and Gunberg	1970	0.966	3.16	Mandible
Thompson	1979	0.427	10.57	Ulna
		0.649	7.07	Femur
		0.827	6.21	Humerus
Iwamoto et al.	1978		11.50	Humerus
	1982		8.80	Femur
Drusini	1987	0.864	3.93	Femur
Ericksen	1991	0.670	10.08	Femur
Stout et al.	1994	0.693	10.43	Rib
		0.865	7.18	Combined
Present study		0.948	4.89	Femur

SEE: Standard error of estimation.

methods. The fibula, mandible, humerus, ulna and rib were also used. The femur and tibia, fibula might be loaded the body weight, and are therefore known as weighted bone, while the humerus, ulna and radius are free from body weight. These later bones are used conventionally for examining bone density, because non-weighted bone might be rapidly resorped as compared with the weighted bones. The rapid alteration might generate individual variation in bone tissue. From previous data for age estimation, it is hard to see the difference between weighted and non-weighted bones. The SEE of trunk bones such as the rib and clavicle was larger than that of the extremities. Furthermore, the skeletons of trunk are easily broken by the bacteria and/or high temperature from the outside. In the case of buried skeletons or bleached bones on a mountain for a long time, the rib might often be lost. From these reasons, it might be appropriate to use the compact bone of femur for age estimation of cadaver.

It is suggested that in old age mineral consumption from the osteon may increase with age, because the calcium absorption of intestinal duct may decrease. In middle age, new born osteons could be seen here and there. The size of Haversian canal stained dark red by Villanueva's stain is bigger than the mature one. The number of osteons and fragments increase with age, while the area of osteons decrease. We found that the size of Haversian canal and vascular canal did not change with age.

As to the Haversian canal the parameters selected did not correlate non-significantly to the age. Yoshino et al., [10] stated that the size of Haversian canal appeared to increase with age, but its alteration is age independent. In young age, the capillary network may develop well, so the size of osteon is small. From this fact, it is clear that the remodeling of the bone in young age might be going on at a high speed.

The data of Drusini [16] and, Singh and Gunberg [4] showed very small standard error. Such good results might come from a lower range of age of samples used. They used samples over 20 years of age and a small sample number. Our present data demonstrates a difference between the actual and estimated age of 1.89 years in the range 20 to 39 years old, 2.63 in 40 to 59 years old, 2.62 years in 60 to 79 years old, and 4.15 over 80 years old, respectively. The value of SEE showed high with age. This alteration might correspond with sample number. The variation of young age was small

because the bone of the young age showed almost same equal in histologic structure. In the present study, materials were collected with the same number of samples in each decade.

As shown in Fig. 4, child bone does not yet exhibit osteons. The perfect structure of osteons can be found in the adolescent, as represented by a histologic textbook. Consequently, materials under 20 years old were omitted from the present histomorphometric study.

References

[1] E.R. Kerley, The Microscopic Determination of Age in Human Bone, Am. J. Physic. Anthrop. 23 (1965) 149–164.
[2] E.R. Kerley, D.H. Ubelaker, Revisions in the Microscopic Method of Estimating Age at Death in Human Cortical Bone, Am. J. Physic. Anthrop. 49 (1978) 545–546.
[3] J. Ahlqvist, O. Damsten, A Modification of Kerley's Method for the Microscopic Determination of Age in Human Bone, J. Forensic Sci. 14(2) (1969) 205–212.
[4] I.J. Singh, D.L. Gunberg, Estimation of Age at Death in Human Males from Quantitative Histology of Bone Fragments, Am. J. Physic. Anthrop. 33 (1970) 373–381.
[5] D.D. Thompson, Age-Related Changes in Osteon Remodeling and Bone Mineralization, Ph.D. Dissertation, 1978, University of Connecticut, Storrs.
[6] D.D. Thompson, The Core Technique in the Determination of Age at Death in Skeletons, J. Forensic Sci. 24(4) (1979) 902–915.
[7] S. Iwamoto, M. Konishi, Study on the Age-Related Changes of the Compact Bone and the Age Estimation: Part 3 Determination of the Age Limitation, Med. J. Kinki University 7(1) (1982) 33–40.
[8] S. Iwamoto, E. Oonuki, M. Konishi, Study on the Age-Related Changes of the Compact Bone and the Age Estimation: Part 2 On the Humerus, Acta Medica Kinki University 3(2) (1978) 203–208.
[9] S.M. Cool, J.K. Hendrikz, W.B. Wood, Microscopic Age Changes in the Human Occipital Bone, J. Forensic Sci. 40(5) (1995) 789–796.
[10] M. Yoshino, K. Imaizumi, S. Miyasaka, S. Seta, Histological Estimation of Age at Death using Microradiographs of Humeral Compact Bone, Forensic Sci. Int. 64 (1994) 191–198.
[11] D.B. Burr, Estimated Intracortical Bone Turnover in the Femur of Growing Macaques: Implications for Their Use as Models in Skeletal Pathology, Anatomical Record 232 (1992) 180–189.
[12] M.F. Ericksen, Histologic Estimation of Age at Death Using the Anterior Cortex of the Femur, Am. J. Physic. Anthrop. 84 (1991) 171–179.
[13] A.R. Villanueva, An improved stain for fresh, mineralized bone sections. Useful in the Diagnosis of Certain Metabolic Diseases of the Bone, Am. J. Clin. Path. 47(1) (1967) 78–84.
[14] A.R. Villanueva, A bone stain for osteoid seams in fresh, unembedded, mineralized bone, Stain Technology 49(1) (1974) 1–8.
[15] P. Derkx, D.H. Birkenhäger-Frenkel, A Thionin Stain for Visualizing Bone Cells, Mineralizing Fronts and Cement Lines in Undecalcified Bone Sections, Biotech. Histochem. 70(2) (1995) 70–74.
[16] A. Drusini, Refinements of two Methods for the Histomorphometric Determination of Age in Human Bone, Zeitschrift fur Morphologie und Anthropologie 77(2) (1987) 167–176.
[17] S.D. Stout, W.H. Dietze, M.Y. Iscan, S.R. Loth, Estimation of Age at Death Using Cortical Histomorphometry of the Sternal End of the Fourth Rib, J. Forensic Sci. 39(3) (1994) 778–784.