

Histomorphometric Assessment of Bilaterality from the Cadaveric Femurs: Implications for Guidance of Field Practice for Anthropological Age Estimation

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ABSTRACT The aim of the present study was to assess bilateral differences in the histomorphometry with osteonal remodeling rate of the femur, which has potential implications for methods of age estimation. Thirty-three femoral samples (18 males and 15 females) were obtained from Korean cadavers and used to analyze the histomorphometric parameters. The thin sections (< 100 mm thick) were prepared by manual grinding. Most of the parameters showed no significant differences between the sides using paired-samples t-tests ($p > 0.05$), except for the average osteon area (OA) and diameter (OD) of males ($p = 0.029$ and 0.033 , respectively). However, the histomorphometric parameters derived from them eventually did not show significant differences between the sides in both sexes. From the Bland-Altman plot, > 95% of the data points were located within ± 1.96 SD of the mean difference, so all of the histomorphometric parameters showed a high degree of agreement between the sides.

INTRODUCTION

The histological age estimation has been reported to be a reliable technique and developed from a variety of bones by earlier reports (Ahlqvist and Damsten 1969; Yoshino et al. 1994), including the femur. When compared with previous anthropological methods, Stout et al. (1994) reported that analyzing microscopic changes with age histomorphology offers objective criteria for estimating age at death when applied to fragmentary skeletal remains and various methods have been developed using a variety of bones and populations.

In the case of the femur, despite its large size in the human skeleton, either of the two can be missing or destroyed as is often the case with forensic fields, as well as in an archaeological context. Most femoral age estimation methods studied by earlier reports (Kerley 1965; Thompson 1979; Ericksen 1991), however, utilize one side of the samples that can be confused with the other side of the femur in histomorphometric analyses.

In the majority of cases, it is necessary to make use of the existing femur, regardless of the side, for estimating age at death, which is based on the presupposition that both femurs in each individual will be equal in bone histology. However, bilateral differences in femoral histomorphometry with the osteonal remodeling rate have not been reported.

The aim of this study was to assess bilateral differences in the histomorphometry with osteonal remodeling rate of the femur, which has potential implications for methods of age estimation. After quantification of secondary osteons and fragments, we applied earlier reports (Stout and Paine 1994; Streeter et al. 2010) for preparing histomorphometric variables and parameters.

MATERIALS AND METHOD

Both right and left femoral samples were obtained from 33 dissected cadavers of known age and sex (18 males and 15 females) from the Department of Anatomy at Gachon University and Ewha Womans University in Korea. None of the femurs were obtained from individuals who had died from a primary bone disease or during which the subject would have been bedridden for a long time prior to death. The age range of the males was 45–84 years (mean and standard deviation, 67.8 ± 12.3 years) and the age range of the females was 47–89 years (73.3 ± 14.7 years; Table 1).

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Table 1: Age distribution of the samples

Age range (in years)	Pooled sexes (n)	Male (n)	Female (n)
40–49	3	2	1
50–59	6	3	3
60–69	5	4	1
70–79	8	6	2
Over 80	11	3	8
Total	33	18	15
Means (years)	70.3	67.8	73.3
Standard deviation	13.5	12.3	14.7

For each specimen, a 5 cm long segment was cut by saw from the midshaft of the right and left femur, and the remaining soft tissue and periosteum adhering to the femoral surface were excised carefully. Specimens were processed in 10% formalin and a mixture of chloroform and methanol for a week at room temperature, and then bleached in 2% H₂O₂ solution for 1 day. The specimens were dehydrated at room temperature, and 1 mm thick cross-sections were obtained from the femoral segment using a diamond wheel (Isomet® 1000; Buehler Instruments, Lake Bluff, IL, USA) and thin sections (approximately 100 µm thick) were prepared by manual grinding on graded silicon carbide abrasive papers. Each section was superimposed on a protractor fixed to the vertical axis between the most anterior cortex and the linea aspera, and the margins $> \pm 50^\circ$ were removed using an acrylic cutter according to a previously published report (Han et al. 2009) and mounted on glass slides in the usual manner.

Following preparation, histomorphometric analysis was performed using a polarizing microscope (BX-51, Olympus, Japan) with image analysis solutions (Image-pro Plus 4.5.1; Media Cybernetics, Inc., Silver Spring, MD, USA). The combination of a 10x objective and 10x oculars fitted with a 10 x 10 eyepiece reticule provided a grid area of 1.0 mm².

The following histomorphometric variables and parameters were analyzed and values were compared between the left and right samples per individual. The analysis algorithm was consistent with the methods proposed by Stout and Paine (1994) and Streeter et al. (2010).

1. Intact Osteon Density (P_i , #/mm²), the number of osteons per unit area that had 90% of their Haversian canal perimeters intact.
2. Fragmentary Osteon Density (P_f , #/mm²), the number of osteons per unit area in which 10% or more of the perimeters of their Haversian canals had been remodeled by successive generations of osteons.

3. Osteon Population Density (OPD, #/mm²), total visible osteon density ($P_i + P_f$).
4. Average Osteon Area (OA, mm²), the average area of structurally completed whole osteons for each femoral specimen.
5. Average Osteon Diameter (OD, mm), the average diameter of complete osteons; calculated using the following formula:
 $OD = (4OA/\pi)^{1/2}$
6. Accumulated Osteon Creations (AOC, #/mm²), total number of intact, fragmentary, and missing osteons for a given OPD; expressed as the following formula:
 $AOC = \beta(OPD)$

The scaling operator, β , chosen for estimating missing osteons, is defined as

$$\beta = (1 - \alpha)^{-1}$$

where $\alpha = (OPD)(OPD \text{ asymptote})^{-1}$ and the exponent $\chi^2 = 3.5$, as suggested by earlier reports (Abbott et al. 1996; Mulhern and Van Gerven 1997; Streeter et al. 2010). The OPD asymptote for each sample is estimated using the following formula:

$$OPD \text{ asymptote} = \kappa((OD)^2)^{-1}$$

where κ , the value of intact and fragmentary osteon packing factor, is specific for each bone and is determined by a value of 1.38, as proposed by Abbott et al. (1996) to be more appropriate for the femur based on data from Kerley (1965).

SPSS software (Statistical Package for Social Sciences version 13.0, Inc. Chicago, IL, USA) was used for data analysis. To examine the differences between the left and right histomorphometric parameters, Pearson's correlation analysis and paired-samples t-test were used. Also, a plot of the bilateral differences was done according to method described by Bland and Altman (1986). For this, the difference of paired values on each histomorphometric parameter is plotted against the mean of the two values and the bilateral agreement is accepted when 95% of the data points should lie within ± 1.96 standard deviation (SD) of the mean difference, as they recommend.

RESULTS

Descriptive statistics and results of paired-samples t-test of all parameters for femoral samples are summarized in Table 2. Most of the parameters showed no significant differences between the sides in paired-samples t-test ($p > 0.05$), except for OA and OD of males ($p = 0.029$ and 0.033 , respectively). Even though the OA and OD of

Table 2: Descriptive statistics and differences between the right and left of histomorphometric parameters for samples (mean $\pm$ standard deviation)

Parameter	Males (n = 18)		Females (n = 15)	
	Right	Left	Right	Left
OPD (#/mm ²)	22.877 $\pm$ 4.115	21.845 $\pm$ 4.432	29.058 $\pm$ 6.056	28.412 $\pm$ 5.826
OA (mm ²)	0.023 $\pm$ 0.002*	0.025 $\pm$ 0.002*	0.024 $\pm$ 0.003	0.023 $\pm$ 0.003
OD (mm)	0.174 $\pm$ 0.007*	0.177 $\pm$ 0.009*	0.173 $\pm$ 0.009	0.172 $\pm$ 0.010
AOC (#/mm ²)	25.557 $\pm$ 6.237	24.259 $\pm$ 6.160	37.122 $\pm$ 9.708	35.384 $\pm$ 9.028

OPD, osteon population density; OA, average osteon area; OD, average osteon diameter; AOC, accumulated osteon creations.

* $p < 0.05$ by paired-samples t-test.

males differed between the sides, the histomorphometric parameters derived from them eventually did not show significant differences between the sides in both sexes (Table 2).

As shown in Figure 1, a very strong positive correlation was observed between the left and right OPD with line chart ($r = 0.935$, $p < 0.0001$). To make Bland-Altman plot, the differences between the sides and the mean of both sides for all the paired values of OPD were computed, and, therefore, the mean of the differences is 0.857 and the mean $\pm$ 1.96 SD was -3.349 and 5.063, respectively. From the plot, over 95% of the data points located within the $\pm$ 1.96 SD of the mean difference, so Bland-Altman plot of OPD showed a high degree of agreement between the sides (Fig. 1).

OA also showed a negative correlation between the sides ($r = -0.826$, $p < 0.0001$) and Bland-Altman plot of OA showed a high degree

of agreement between the sides with the mean of the differences (-0.0005) and $\pm$ 1.96 SD (-0.0034 and 0.0024, respectively; Fig. 2).

Figure 3 depicts high correlation between the sides ($r = 0.926$, $p < 0.0001$) and Bland-Altman plot of AOC showed a high degree of agreement between the sides with the mean of the differences (1.498) and $\pm$ 1.96 SD (-5.778 and 8.775, respectively; Fig. 3).

DISCUSSION

When either of the two femurs to be analyzed for histomorphometry was unavailable or destroyed, Stout (1988) reported that a closely related midshaft of the femur in the majority of cases was considered regardless of the side for estimating age at death, despite concerns for accuracy and reliability. This may assume that both femurs in each individual will be equal in

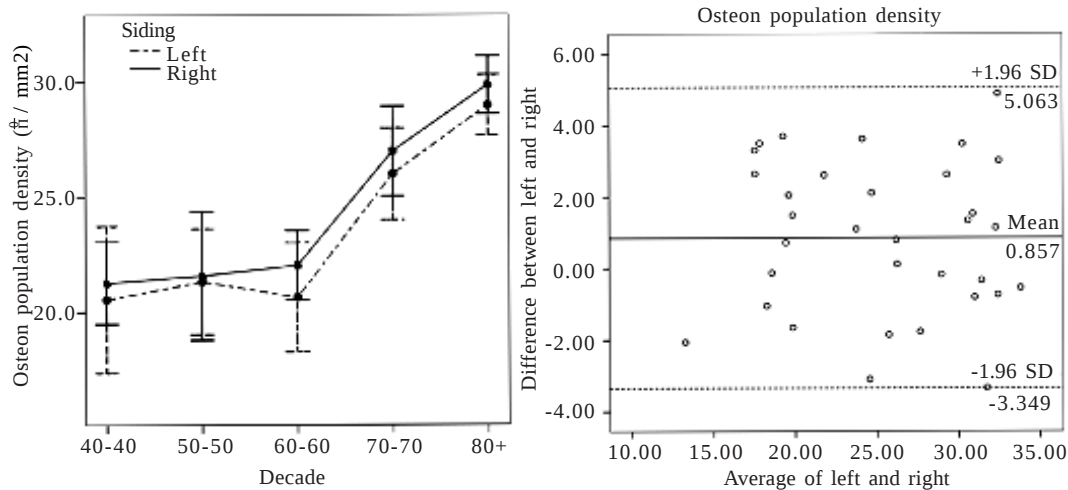


Fig. 1. Osteon population density (OPD) for the left femur compared with the right one in each individual ($r = 0.935$, $p < 0.001$) and Bland-Altman plot representing an agreement of bilateral OPD within the $\pm$ 1.96 standard deviations (SD) of the mean difference

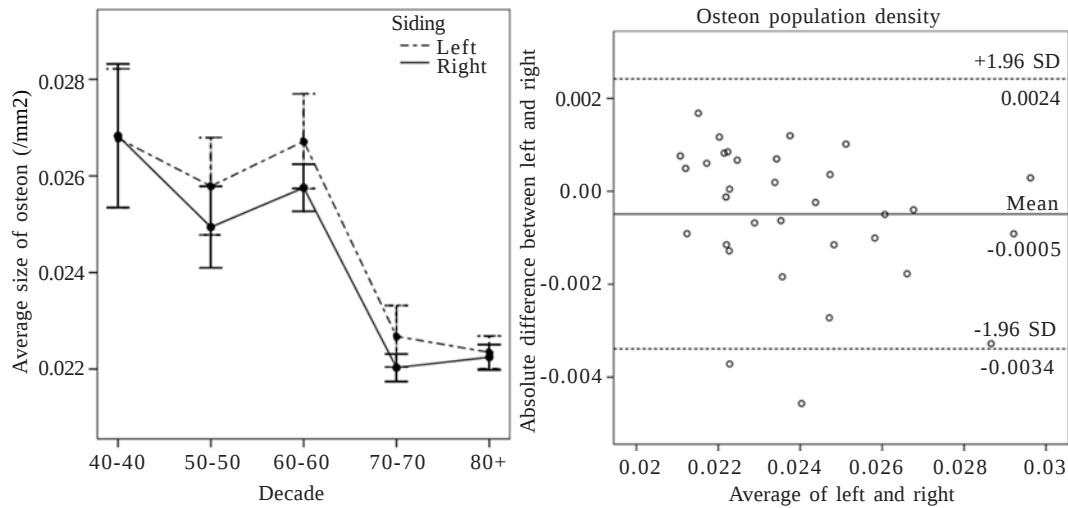


Fig. 2. Average size of osteon (OA) for the left femur compared with the right one in each individual ($r = -0.826$, $p < 0.001$) and Bland-Altman plot representing an agreement of bilateral OPD within the ± 1.96 standard deviations (SD) of the mean difference

bone histology, such as secondary osteons and fragments influencing the osteonal remodeling rate, so it is necessary to confirm the assumption whether or not it is correct.

In this study, for estimating missing osteons to calculate the AOC, the value of the osteon packing factor, κ , was determined by 1.38 to be more appropriate for the femur reported by Abbott et al. (1996) rather than 1.7 of the rib, as proposed

by Stout and Paine (1994). However, some verification studies by Thompson and Gunness-Hey (1981) and Yoshino et al. (1994) have reported that population differences can influence the bone histology for age estimation; further research to prepare specific values of the osteon packing factor for the population as well as the bone element is required.

As expected, the OPD and AOC increased with

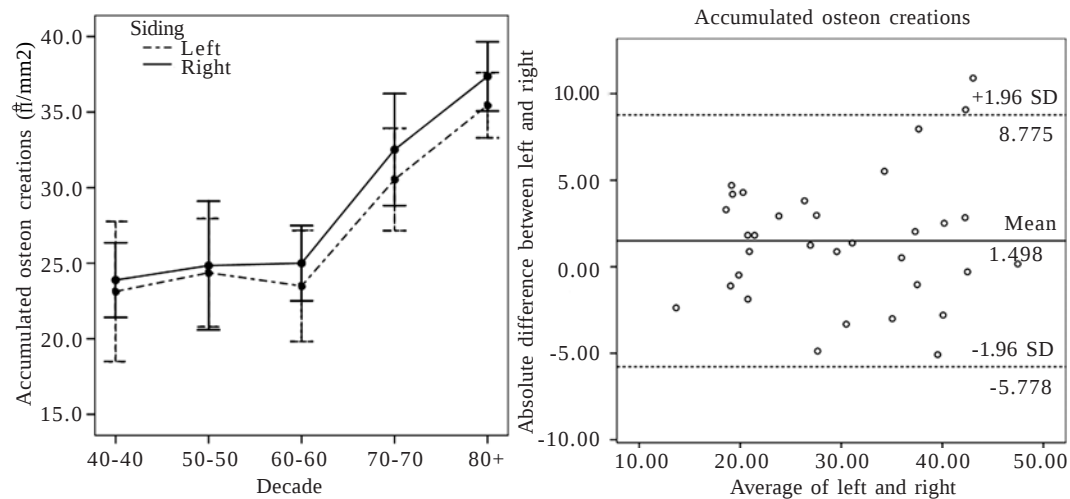


Fig. 3. Accumulated osteon creations (AOC) for the left femur compared with the right one in each individual ($r = 0.926$, $p < 0.001$) and Bland-Altman plot representing an agreement of bilateral AOC within the ± 1.96 standard deviations (SD) of the mean difference

age (Figs. 1 and 3) and the OA decrease with age in the femur (Fig. 2). These patterns have been reported by various studies (Stout and Paine 1994; Mulhern 2000) and provide support for the validity of samples for evaluating bilateral differences, even though there are still some potential biases in this study consisting of cadavers due to the advanced age of the samples (Table 1).

The Bland-Altman plot to describe the agreement between the two quantitative data was introduced by Bland and Altman (1986). In comparison analysis, a high correlation coefficient does not reflect agreement of two quantitative data and paired-samples t-test is not appropriate to evaluate agreement between paired data because the *p*-value is directly affected by the sample size. In the Bland-Altman plot, the difference of the paired data is plotted against the mean of the two data and agreement is accepted when 95% of the data points lie within ± 1.96 SD of the mean difference, as they recommend. The results from this study indicate that all histomorphometric parameters showed agreement between the sides from the Bland-Altman plot (Figs. 1-3).

The development of a bone is influenced by baseline growth in addition to mechanical response, and therefore Frost (1987) and Mulhern (2000) reported that the more mechanical demands of the femur may lengthen the period of cortical drift and rapid bone remodeling. Thus, studies by Stout (1988) reveals that bone histology, as well as the osteonal remodeling rate, can differ between the sides influenced by environmental factors and the probability of bilateral differences may be increased in the elderly for various reasons, such as a disease or fall. Thus, it should be considered and noted the chance of individual variation developed bilateral differences of the femur in practical fields, while the results from this study showed no significant difference between the sides in histomorphometry with the osteonal remodeling rate.

In conclusion, this study demonstrated the importance of evaluating the bilateral differences between the sides for estimating age at death in histomorphometry from the anterior cortex of the femur. Further evaluation to improve the histologic age estimation using osteonal remodeling parameters as regression variables would be needed for absorbing any bias of sampling and measuring differences.

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