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Assessment of Histomorphological Features of the Sternal End of the Fourth Rib for Age Estimation in Koreans*

ABSTRACT: The aim of this study was to assess the histomorphological features of the fourth rib and to develop age-predicting equations for Koreans. Sixty-four rib samples (36 males and 28 females) obtained from forensic cases were used for developing equations. Two thin sections (<100-μm thick) per sample were prepared by manual grinding. Multivariate analysis of covariance revealed statistically significant differences in age-adjusted histomorphological variables between sexes. Using stepwise regression analysis, osteon population density and average osteon area were correlated with unknown sex (r^2 = 0.826), and sex plus two histomorphological variables provided the best results for an age-predicting equation given the assumption of knowing the sex of a specimen (r^2 = 0.839). Average Haversian canal area had little influence on age estimation for male or female samples, and relative cortical area was not significantly related to age for any specimen.

KEYWORDS: forensic science, forensic anthropology, histomorphometry, age estimation, fourth rib, Korean

Age estimation of unidentified skeletal remains from crime scenes or excavations is of great interest for forensic anthropology. Above all, compared with traditional methods, analyzing microscopic changes with age histomorphology offers objective criteria for estimating age at death when applied to fragmentary skeletal remains (1), and various methods have been developed using a variety of bones and populations.

Since Kerley (2) first reported histological age estimation using long bones, a number of studies have been established, mainly using the femoral midshaft (3–6). Other long bone materials such as the tibia (2,7–10), fibula (2,7,10), humerus (4,11), ulna (4), and radius (10) also have been used. However, most of these long bones must suffer damage to the diaphysis from complete transverse sectioning for histomorphometric analysis, except when the core technique (4) is applied to the femur. In spite of their high accuracy and reliability, histological techniques that use long bones—particularly the femur—place a burden on the anthropologist by missing the opportunity to use other anthropological methods such as osteometry. Unlike the long bones, the ribs are not routinely used for anthropological measurements (1). Of course, utilization of the sternal fragment of the fourth rib could interfere with morphological phase analysis on the sternal rib end, so it needs to be preserved after histological analysis is performed on rib fragment for acquiring additional information of age at death. Moreover, the ribs are less involved than limb bones in biomechanical responses that cause changes in bone mineral density with age (12) and have very small weight-bearing capacity (13). Therefore, they are regarded as an ideal skeletal material for histomorphometry (14,15).

Some studies have reported that population differences have an influence on the reliability of histological age estimation methods when reference equations from one population are applied to another (11,16). Thus, population is one of the many variables in addition to age that can affect bone histology, as different ethnic groups can show differences in bone remodeling (17,18). Consequently, it is necessary that population-specific equations for histological age estimation should be established.

The objective of this study was to assess the histomorphological features of the fourth rib and to develop age-predicting equations for Koreans. For this, we applied the method of Stout and Paine (19) to the sternal end of the fourth rib with some modifications by adding several histomorphological variables introduced by Cho et al. (20), and compared these with the results reported by others.

Materials and Methods

Histomorphological features were assessed from the sternal end of the fourth rib from a wide age range. Rib specimens were obtained from 64 Koreans (36 males and 28 females)—from forensic cases of the Division of Forensic Medicine, National Institute of Scientific Investigation, Seoul, Korea—who died suddenly and had no history of chronic illness. The age range of the males was 22–67 years (mean and standard deviation 44.8 ± 11.7) and the age range of the females was 20–60 years (38.6 ± 11.1 years; Fig. 1). To test the accuracy of histomorphological age estimation in Koreans, a test set was prepared from 19 Korean cadavers (12 males and seven females) from the Department of Anatomy of Konkuk University, Chungju, Korea, and Ajou University School of Medicine, Suwon, Korea.

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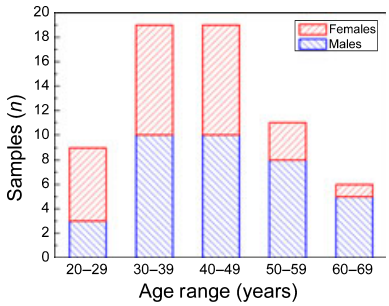

FIG. 1—*Age distribution of samples.*

For each specimen, a 3-cm long segment was cut by saw from the costochondral joint of the left fourth rib to the lateral border, then the intercostal muscles and periosteum adhering to the rib surface were excised carefully. As all specimens required the removal of remaining soft tissue and fat in bone marrow, the preparation protocol introduced by Watanabe et al. (21) was followed. Specimens were fixed in 10% formalin for 7 days, washed in running water for 2 days, defatted in a mixture of chloroform and methanol for 7 days and then bleached in 2% H_2O_2 solution for 1 day. After dehydrating at room temperature, two 1-mm-thick cross-sections were cut serially from each rib segment using a diamond wheel (Isomet® 5000; Buehler Instruments, Lake Bluff, IL). Two thin sections (approximately 100-μm thick) per specimen were prepared for histological analysis by manual grinding on graded silicon carbide abrasive papers according to the manual method modified by Maat et al. (22).

The microscopic analysis used in this study followed procedures outlined by Stout and Paine (19), who recommended reading a pattern of alternating microscopic fields (checker-board pattern) to insure a representative area of the sternal rib cross-section. We also added some histomorphological variables introduced by Cho et al. (20). The following variables were measured using an Olympus BX-51 light microscope with simple polarizing attachment (BX-POL; Olympus, Tokyo, Japan) and image analysis solutions (Image-pro Plus 4.5.1; Media Cybernetics, Inc., Silver Spring, MD). The combination of 20× objective and 10× oculars fitted with a 10×10 eyepiece reticule provided a grid area of 0.25 mm^2.

1. CORTICAL AREA (CA, mm^2): the sum of the area of cortical bone contained within all of the microscopic fields in the rib cross-section.
2. RELATIVE CORTICAL AREA (RCA): the relative amount of cortical bone in rib cross-sectional area, or the ratio of CA to total area in rib cross-section.
3. INTACT OSTEON DENSITY (P_i, #/mm^2): the number of osteons per unit area that had 90% of their Haversian canal perimeters intact. Intact osteons were counted if half or more of their area fell within the square grid of the eyepiece reticule.
4. FRAGMENTARY OSTEON DENSITY (P_f, #/mm^2): 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. Fragmentary osteons, including interstitial lamellae, were counted if half or more of their area fell within the square grid of the eyepiece reticule.
5. OSTEON POPULATION DENSITY (OPD, #/mm^2): total visible osteon density ($P_i + P_f$).
6. AVERAGE OSTEON AREA (OA, mm^2): the average area of structurally complete osteons for each rib specimen. Complete osteons with Haversian canals that deviated significantly from a circular profile were excluded. A minimum of 25 complete osteons per rib cross-section was measured.
7. AVERAGE HAVERSIAN CANAL AREA (HA, mm^2): the average area of the Haversian canals contained within complete osteons for each rib specimen. As for the OA, a minimum of 25 Haversian canals per rib cross-section was measured.

The histomorphological variables were averaged over two thin sections of each individual, and all statistical analysis was then performed with SPSS version 11.0 (SPSS Inc., Chicago, IL). Multivariate analysis of covariance (MANCOVA) with Wilks' lambda was performed to test the hypotheses that sex (fixed factor) and age (covariates) had a significant effect on the histomorphological variables (dependent variables). Univariate ANCOVA was conducted for each of the parameters. Variance between the sexes was tested using Levene's test, and all variables were satisfied with the assumption of equal variances ($p > 0.05$). Partial eta-squared (η^2) values, which describe the proportion of variability attributable to a parameter, were included to provide an intuitive measure of effect size. Finally, regression analysis was performed to produce age-predicting equations applicable to circumstances of known or unknown sex of specimens.

Results

Descriptive statistics of all histomorphological variables for the pooled and separate sexes with age range are summarized in Table 1. There were significant differences between males and females in 40–49 years age range for all histomorphological variables and in 60–69 years age range for OA and RCA ($p < 0.05$). The means of each histomorphological variable were dissimilar between sexes, so the hypotheses that both sex and age have a significant effect on the histomorphological variables were confirmed.

Multivariate analysis of covariance (Table 2) revealed that age and sex were significant covariates overall ($p = 0.000$). However, ANCOVA showed that age and sex were significant for OPD ($p = 0.000$ and 0.003, respectively), OA ($p = 0.000$ and 0.004, respectively), and HA ($p = 0.001$ and 0.048, respectively), but age was not significant for RCA ($p = 0.081$). Based on partial η^2 values, age accounted for the largest amount of variability (>76% for OPD and OA), and though sex—where significant—accounted for considerably less variability (<26.2% for all parameters), the data for samples needed to be treated with the sexes separately for further regression analysis.

Figure 2 shows bivariate scatter plots of each of the histomorphological variables according to age, and related regression results for the pooled and separate sexes are summarized in Table 3. All variables showed age-related changes at a 1% level of significance for the pooled sexes, but when separated by sex, HA for males and RCA for males and females were not significantly related to age ($p > 0.05$). In all cases, the strongest associations with age for the pooled sexes and the males and females were OPD ($r^2 = 0.803$, 0.809, and 0.790, respectively) and OA ($r^2 = 0.768$, 0.789, and 0.725, respectively).

Table 4 shows age-predicting equations derived from stepwise regression analysis. In this, two histomorphological variables, OPD and OA, were selected for unknown sexes (the multiple regression correlation r^2 and standard error were 0.826 and 4.971, respectively). Sex, in addition to two histomorphological variables, was selected as providing a more satisfactory result for the assumption of knowing sexes (r^2 and standard error were 0.839 and 4.821, respectively).

TABLE 1—*Descriptive statistics of histomorphological variables for the pooled and separate sexes (mean ± standard deviation).*

	OPD (#/mm^2)	OA (mm^2)	HA (mm^2)	RCA (ratio)
Pooled sexes (n = 64)				
20–29 years	12.448 ± 1.804	0.029 ± 0.002	0.0011 ± 0.0002	0.381 ± 0.071
30–39 years	17.801 ± 3.309	0.024 ± 0.004	0.0009 ± 0.0002	0.340 ± 0.083
40–49 years	22.744 ± 3.750	0.020 ± 0.003	0.0009 ± 0.0002	0.335 ± 0.090
50–59 years	28.294 ± 2.968	0.016 ± 0.002	0.0010 ± 0.0002	0.304 ± 0.094
60–69 years	31.363 ± 3.037	0.014 ± 0.002	0.0007 ± 0.0001	0.290 ± 0.097
Overall age	21.591 ± 6.581	0.021 ± 0.005	0.0009 ± 0.0002	0.333 ± 0.088
Males (n = 36)				
20–29 years	13.059 ± 3.057	0.028 ± 0.001	0.0010 ± 0.0001	0.333 ± 0.035
30–39 years	18.030 ± 4.068	0.024 ± 0.005	0.0010 ± 0.0002	0.306 ± 0.064
40–49 years	25.052 ± 2.462*	0.019 ± 0.002*	0.0010 ± 0.0003*	0.289 ± 0.074*
50–59 years	28.648 ± 2.458	0.016 ± 0.002	0.0010 ± 0.0003	0.281 ± 0.100
60–69 years	31.748 ± 3.227	0.013 ± 0.001*	0.0007 ± 0.0001	0.257 ± 0.058*
Overall age	23.831 ± 6.557	0.019 ± 0.005	0.0010 ± 0.0002	0.291 ± 0.073
Females (n = 28)				
20–29 years	12.142 ± 1.063	0.029 ± 0.002	0.0012 ± 0.0003	0.405 ± 0.074
30–39 years	17.547 ± 2.423	0.024 ± 0.003	0.0009 ± 0.0001	0.377 ± 0.089
40–49 years	20.179 ± 3.282*	0.022 ± 0.002*	0.0008 ± 0.0001*	0.387 ± 0.080*
50–59 years	27.353 ± 4.589	0.017 ± 0.004	0.0008 ± 0.0001	0.367 ± 0.032
60–69 years	29.436 ± 0.000	0.017 ± 0.000*	0.0007 ± 0.0000	0.458 ± 0.000*
Overall age	18.710 ± 5.479	0.024 ± 0.004	0.0009 ± 0.0002	0.388 ± 0.076

OPD, osteon population density; OA, osteon area; HA, Haversian canal area; RCA, relative cortical area.
*Statistically different between males and females at 5% level of significance.

To test the accuracy of histomorphological age estimation, age-predicting equations were applied to the test set (n = 19), and statistics relevant to the accuracy of equations for unknown and known sexes are presented in Tables 5 and 6. The distribution of estimated age in relation to actual age for unknown and known sexes is plotted with each r^2 value in Figure 3. Both age-predicting equations for unknown and known sexes produced estimated ages that were accurate within ±10 years for over 84% of all individuals. However, when we divided actual ages into those older and younger than 60 years, over 75% of individuals who were under 60 years fell within ±5 years, whereas 72% of individuals who were over the cutoff fell outside ±5 years. The overall reliability of the age-predicting equation for known sexes was slightly better than the equation for unknown sexes (r^2 = 0.816 and 0.759, respectively). Furthermore, there was a similar tendency with this overall reliability when the test results of the age-predicting equations were compared with the sexes separately (data not shown). The 80.0% of males and 66.7% of females who were under 60 years in both equations for unknown and known sexes fell within ±5 years, whereas 71.4% of males in both equations, 100.0% of females in equation for unknown sexes, and 75.0% of females in equation for known sexes, who were over 60 years, fell outside ±5 years. The overall reliability for males was slightly better than females of all ages in both age-predicting equations for unknown and known sexes.

TABLE 2—*Results of multivariate and univariate analysis of covariance for the pooled and separate sexes.*

	Parameter	B	t	Significance	Partial η^2
Multivariate	Intercept	–	–	0.000	0.959
	Age	–	–	0.000	0.830
	Sex	–	–	0.000	0.366
OPD*	Intercept	2.45400	1.695	0.095	0.045
	Age	0.47800	15.576	0.000	0.799
	Sex (F)	−2.22100	−3.083	0.003	0.135
OA*	Intercept	0.03645	28.312	0.000	0.929
	Age	−0.00038	−13.942	0.000	0.761
	Sex (F)	0.00191	2.983	0.004	0.127
HA*	Intercept	0.00133	11.961	0.000	0.701
	Age	−0.00001	−3.385	0.001	0.158
	Sex (F)	−0.00011	−2.021	0.048	0.063
RCA*	Intercept	0.35500	9.313	0.000	0.587
	Age	−0.00144	−1.775	0.081	0.049
	Sex (F)	0.08836	4.656	0.000	0.262

OPD, osteon population density; OA, osteon area; HA, Haversian canal area; RCA, relative cortical area; F, female.
*The parameter of each dependent variable, Sex (male), is set to zero.

Discussion

In the traditional methods of estimating age at death based on gross morphological examination, except for dental analysis such as tooth development and wear (23), there are few methods that provide precise age values with narrow standard errors of estimated age. Even though it is possible, the most useful landmarks of age are often missing or obliterated in fragmented, eroded, or incomplete skeletal compositions (2). In this context, histological age estimation is a good alternative for accurate and reliable age estimation in forensic practice.

The most important constituent of any histological study estimating age at death is the age distribution of samples. Osteonal remodeling and accumulated osteon population are influenced heavily by the chronological age of subjects (14). In this study, individuals who were aged under 20 and over 70 years were excluded (Fig. 1), because transverse cortical drift can result in a significant underestimation of age in juvenile ribs (20) and osteon counts reach an asymptotic value in individuals older than about 60 years (24). Moreover, at the outset of this study, there is a need to eliminate older individuals who may have potential pathological conditions such as spinal deformities causing microstructural changes of rib. Thus, the age-predicting equations were less reliable for individuals over 60 years of age in the test set (Table 6).

In this study, MANCOVA revealed statistically significant differences in age-adjusted histomorphological variables between sexes (Table 2). Sex is an important factor affecting bone remodeling and OPD (14), and bone loss is exacerbated by the increased activity rate of basic multicellular units caused by menopause in middle-aged women (25). Also it is reported that the mean age at

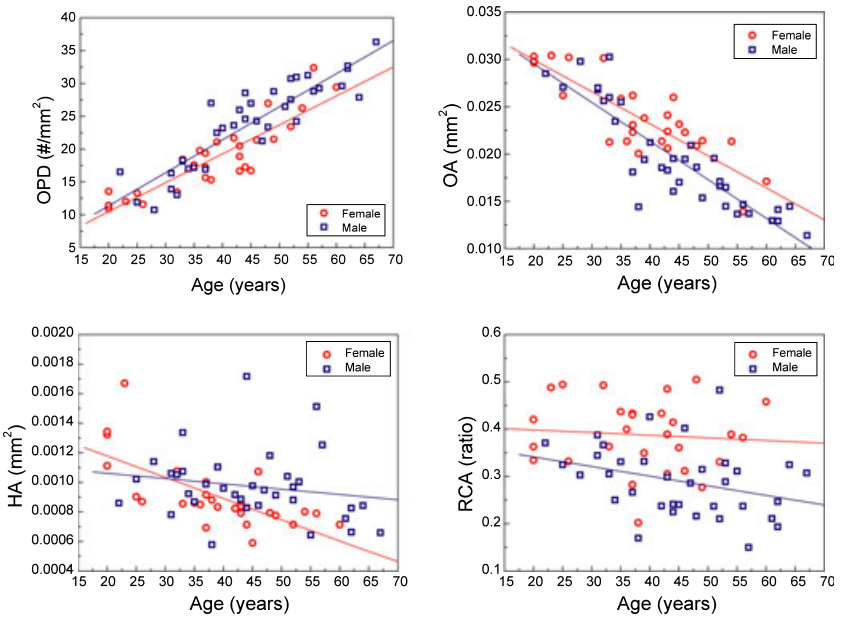

FIG. 2—*Bivariate plots of histomorphological variables with age between sexes. Female samples are represented by open circles (red lines) and male samples by open rectangles (blue lines). The related regression results are summarized in Table 3.*

TABLE 3—*Histomorphological variables versus age for the pooled and separate sexes.*

Variable	Intercept	Slope	SEE*	r^2	p
Pooled sexes (n = 64)					
OPD	7.571	1.599	5.255	0.803	0.000
OA	82.837	−1915.055	5.696	0.768	0.000
HA	58.761	−17634.906	11.111	0.119	0.005
RCA	56.384	−42.856	11.210	0.103	0.010
Males (n = 36)					
OPD	6.421	1.608	5.197	0.809	0.000
OA	82.261	−1930.633	5.466	0.789	0.000
HA	53.840	−9343.736	11.694	0.034	0.285
RCA	60.094	−52.731	11.239	0.107	0.051
Females (n = 28)					
OPD	5.144	1.792	5.161	0.790	0.000
OA	89.296	−2140.406	5.901	0.725	0.000
HA	69.233	−33595.580	8.097	0.483	0.000
RCA	43.328	−11.980	11.222	0.007	0.679

OPD, osteon population density; OA, osteon area; HA, Haversian canal area; RCA, relative cortical area.
*SEE, standard error of estimate (years).

TABLE 4—*Age-predicting equations conducted from stepwise regression analysis.*

Equation*	Multiple r^2	SEE†
For unknown sex		
Age = 1.014 (OPD) − 790.651 (OA) + 37.022	0.826	4.971
For known sex		
Age = 1.056 (OPD) − 851.295 (OA) + 2.926 (Sex‡) + 36.132	0.839	4.821

OPD, osteon population density; OA, osteon area.
*Multicolinearity does not exist among independent variables, and the linear regression model was verified by ANOVA at a 1% level of significance.
†SEE, standard error of estimate.
‡Sex = 0 for male, 1 for female.

TABLE 5—*Descriptive statistics of actual age and estimated age for test set (n = 19).*

	Actual age	Estimated age adapted for unknown sex*	Estimated age adapted for known sex*
Mean (years)	59.16	54.66	55.15
Standard deviation	11.65	8.02	8.12
Standard error of mean	2.67	1.84	1.86
Minimum (years)	33	37.19	35.73
Maximum (years)	75	68.83	68.96
Mean difference	–	4.50	4.01
Standard deviation of difference	–	6.10	5.54
Standard error of estimate	–	1.40	1.27

*Estimated ages were based on the age-predicting equations presented in Table 4.

menopause in modern Korean women is 46.9 years (26), and then, in present study, the possible reason for the results showing sex differences in 40–49 years age range for all histomorphological variables (Table 1) could be menopause. On the other hand, no significant differences between sexes in over 50 years except 60–69 years age range for OA and RCA is responsible for relatively small proportion of female samples in this study. In addition to menopause, other physiological factors causing the differences in histomorphological features of bone between males and females (14) must be further explored. In forensic practice, when the sex of skeletal remains can be assumed through other anthropological analysis, the use of the age-predicting equation for a known sex of this study would produce a more reliable result than the equation for unknown sexes (Fig. 3).

Osteon drift is a process that results in a transversely elongated osteon exhibiting a hemicyclic lamellar tail (27). Unfortunately, there are no published criteria for the measurement of drifting osteons. In this study, drifting osteons that fell within the square grid in half or more of their area were counted as intact (P_i), but even

TABLE 6—*Classificational comparison of estimated age resulted in Table 5.*

	Estimated age adapted for unknown sex (n = 19)			Estimated age adapted for known sex (n = 19)		
Age categories	Overall	<60	≥60	Overall	<60	≥60
Estimated ages within ±5 years (%)	42.1	75.0	18.2	47.4	75.0	27.3
Estimated ages within ±5–10 years (%)	42.1	25.0	54.5	36.8	25.0	45.4
Estimated ages greater than ±10 years (%)	15.8	0.0	27.3	15.8	0.0	27.3
Total (%)	100.0	100.0	100.0	100.0	100.0	100.0

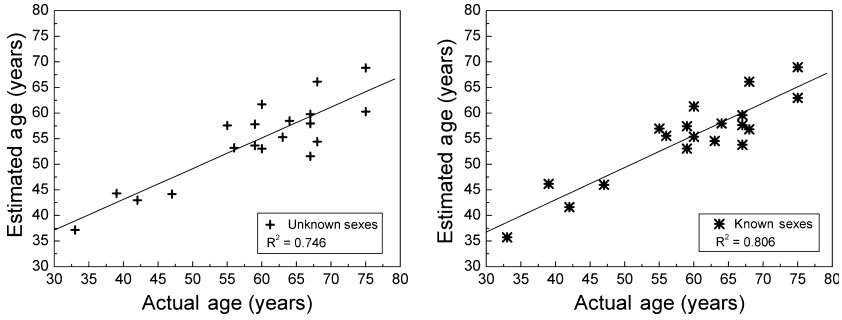

FIG. 3—*The relationship between actual age and estimated age for the test set using the age-predicting equations presented in Table 4. The* r^2*-value of the regression line is shown in each plot.*

TABLE 7—*Comparison with previous studies using ribs.*

Author(s)	Multiple r^2	SEE*	Materials	Site	Population (n)
Stout and Paine (19)	0.776	3.80	Sixth rib	Middle third	Whites and African-Americans in U.S. (40)
Stout et al. (1)	0.865	7.18	Fourth rib	Sternal end	Whites in U.S. (60)
Cho et al. (20)	0.569	12.68	Sixth rib	Middle third	African-American and European-American (103)
Present study	0.839	4.82	Fourth rib	Sternal end	Korean (64)

*SEE, Standard error of estimate.

those contained within the complete cement lines were not measured as the OA. This may be critical to avoid the underestimation of OPD and the overestimation of OA.

In this study, the size of Haversian canals did not change with age for males, but tended to decrease slightly with age for females (Table 3). However, the HA was not selected among independent variables in the stepwise regression analysis (Table 4), and therefore had little influence on the age estimation for either sex. On the other hand, the RCA was not significantly related to age for males or females (Table 3). This is contrary to the results of Cho et al. (20), who reported that the RCA was a significant independent variable for age estimation. It is possible that the reason for this disagreement is the different kind of sampling sites used: the sternal end versus the middle shaft of the rib.

The results of histological age estimation of ribs in other reports are shown in Table 7. Although direct comparison between our present study and the previous studies is difficult because of different kinds of materials, site, population, and the age distribution of samples, our multiple regression correlation (r^2 = 0.839) and standard error of estimate (SEE = 4.82) were considerably better than those reported. It should be noted that Stout and Paine (19) deduced an age-predicting equation by combining the histomorphometry of the rib and clavicle (r^2 = 0.776), and Stout et al. (1) combined costal histomorphometrics and rib phase analysis (r^2 = 0.865). On the other hand, the larger sample size and various source of subjects of Cho et al. (20) produced a relatively low multiple regression correlation (r^2 = 0.569) and a large standard error of estimate (SEE = 12.68), but they produced detailed multiple equations that helped account for a population-specific estimation of rib age.

When age-predicting equations postulated by Stout et al. (1) and Cho et al. (20) were applied to the results of this study, the 29.7% and 17.2% of individual's ages were correctly estimated within ±5 years, 20.8% and 35.2% were within ±5–10 years, and the remaining 49.5% and 47.6% produced estimates with errors greater than ±10 years, respectively. However, the overall reliability of two age-predicting equations was considerably low, regardless of their materials and site. These may imply that there are population differences in histomorphological features between Koreans and such other groups as Americans referring to this case (Table 7). Therefore, it is necessary that population-specific equations for histological age estimation should be established.

It is apparent that estimating the age at death of unknown skeletal remains needs to use as many indicators as possible to postulate a final age estimation (15,28). As stated above, Stout et al. (1) combined histological and morphological analysis and produced good results for age prediction using ribs. Similarly, we expect that more accurate and reliable age estimation of ribs will be possible when additional morphological phase analysis can be used.

In conclusion, histomorphological analysis of the sternal end of the fourth rib is valid for age estimation in Korean subjects and has the advantage of harmony with other forensic anthropological analysis, that is, minimal invasiveness of skeletal remains. Above all, it offers high accuracy for estimating the age at death.

Acknowledgments

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