Rom J Morphol Embryol 2011, 52(2):659–668

RJME
Romanian Journal of
Morphology & Embryology
http://www.rjme.ro/

ORIGINAL PAPER

Age assessment from mandible: comparison of radiographic and histologic methods

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Abstract

Age assessment is an integral and important aspect of forensic odontology. The use of long bones and teeth has been documented since decades. The aim of this study was to use both radiographic and histologic methods of age estimation and to determine which method gives a near actual age. Orthopantomograph (OPG) was used to study the radiographic changes and ground sections were made for histologic study. Of the various parameters studied, we concluded that the histologic parameters recorded ages, which were closer to the actual age. Of the histologic parameters, two to three parameters when combined were still better.

***Keywords*:** age assessment, radiographic, histologic, OPG, ground sections.

Introduction

Chronological age assessment is an important part of medico legal practice. The procedures for age determination are complex and involve the consideration of many factors. Changes related to chronological age are seen in both hard and soft tissue. Amongst the hard tissues, bones are important as they undergo a series of changes from prenatal to postnatal life and changes in their composition and structure continue into old age and even after death. Hence, bones form a reliable source of information regarding growth and growth changes. Normally well-defined skeletal development in bones, cranial sutures and teeth take place at specific ages. However, these changes are significantly affected by genetics, general health and other environmental factors [1].

Estimation of age is extremely important, being second only to sex determination in the identification of human remains. The determination of age becomes more difficult as maturity increases. Where fetal material is concerned, a result may be achieved with an accuracy measured almost in days. As age advances, the situation remains fairly satisfactory until about the cessation of growth and especially the cessation of dental changes so that by the age of 20–25 years all growth markers have ceased to be of assistance. As time goes on through adult life into middle age and into old age, matters become progressively more difficult and the margin of error increases [2].

A number of methods for age determination have been proposed. These can be classified in four categories, namely, clinical, radiological, histological and chemical analysis. In the living persons, any or all of the above methods can be used to determine age, in cases where actual age is not known or is to be confirmed. However, in case of a dead person, post-mortem changes such as decomposition, mutilation or skeletonisation may make identification progressively more difficult almost to the point of impossibility [3].

Dental hard tissues and bone are extremely resistant to fire and are usually the only remains after an extended period of burial. As a result, forensic odontology has gained importance as a tool in identifying the skeletal/dental remains. As existing age-at-death estimation techniques have limited precision; researchers have sought to demonstrate age-related changes in the dental hard tissues [4]. The objective of the present study was to evaluate the histological and radiographic changes in the cortical bone with increasing age. The aim was to compare the accuracy of histologic and radiographic methods of age determination from mandible.

Materials and Methods

The present study was conducted in the Department of Oral Pathology and Microbiology, Sharad Pawar Dental College and Hospital. The study protocol was approved by the Institutional Ethical Committee. The sample consisted of 50 mandibles from cadavers of known age who died from natural cause and those that were not affected by any disease altering the structure of the skeleton. Mandibles with fractures or altered cortices were excluded. The ages of the bones ranged from 20–69 years. The samples were divided into five groups according to decades in the age group from 20–69 years as shown in Table 1. Table 1 shows sex distribution of the samples. Out of the total 50 samples 41 (82%) were males and nine (18%) were females.

Table 1 – ***Age and sex distribution of samples***

Age group [years]	Male	Female	Total
20–29	8 (80%)	2 (20%)	10 (100%)
30–39	9 (90%)	1 (10%)	10 (100%)
40–49	7 (70%)	3 (30%)	10 (100%)
50–59	8 (80%)	2 (20%)	10 (100%)
60–69	9 (90%)	1 (10%)	10 (100%)
Total	41 (82%)	9 (18%)	50

Orthopantomograph (OPG) was taken using Planmeca Proline CC 2002 (LBL-X-234A) and following parameters were measured using vernier calipers after tracing the landmarks using an X-ray viewbox: length of ramus (mm), height of body of mandible (mm), distance of lower border of mandible (LB) to inferior margin of mental foramen (IMF) (mm) (left and right), distance of inferior margin of mental foramen (IMF) to crest of alveolar bone (CAR) (mm) (left and right), gonial angle, antegonial (AG) angle, antegonial (AG) depth, width of the cortex at the body and at the antegonial region (TCB at AG).

Ground sections from the region of the body of the mandible (premolar region) were made using the technique described by Balwant R *et al.* (2005) [5].

In each section the number of osteons, diameter of the Haversian canal, average number of concentric lamellae per osteon, area of the Haversian canal (HC area), area of osteon (AR of OS) and Haversian canal perimeter (HC perimeter) were measured on Leica DMLB-2 Research Microscope with Leica Q-win Standard Software and traced using a computer aided image analysis system Leica Qwin ProV3.5.0, Leica Microsystems (Switzerland) Ltd. Histological slides were analyzed at four sites each diametrically opposite to the other under low power (10×) magnification.

Both histological and radiographic readings were taken by two separate observers and the mean was taken. The observations were recorded and subjected to statistical analysis of One-way ANOVA & Stepwise Regression analyses using the program SPSS 16.

Results

In this study, histological evaluation and radiographic analysis of mandibles was carried out on 50 specimens. The samples were divided into five groups; each group spanning a decade and consisting of at least 10 specimens beginning at 20-year-old (Figure 1).

Pooled data from 50 samples (Table 1) when evaluated using regression coefficient demonstrated results with an error of ±3.47 when three histological parameters (number of osteons, average number of lamellae per osteon, Haversian canal diameter) were equated.

The Analysis of Variance for individual groups showed non-significant *p*-value ($p>0.05$) whereas analysis of variance for combined samples (50) gives a significant *p*-value ($p<0.05$).

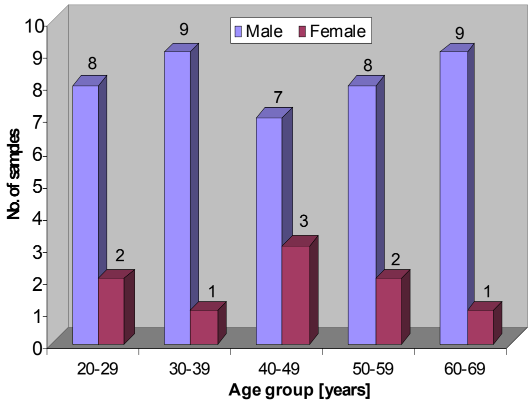

Figure 1 – ***Samples distribution according to age.***

The correlation of histological findings of AR of OS, HC area, HC perimeter to estimated age (Table 2) from all the three parameters show greater degree of error of ±11.98. Hence are of less significance in estimating age-at-death.

Table 2 – ***Correlation of age (20–29 years) with histological findings in mandible***

Selection	Dependent variable: age [years]	Multiple R^2	Standard error of estimate
I	-0.16 + 1.01 X1 + 0.12 X2 + 0.12 X3	0.65	1.94
II	16.52 + 0.52 X1 + 0.10 X2	0.49	2.03
III	-0.63 + 1.01 X1 + 0.12 X3	0.64	1.78
IV	25.77 + 0.06 X2 - 0.01 X3	0.07	2.33
V	17.32 + 0.52 X1	0.49	1.88
VI	24.60 + 0.06 X2	0.03	2.16
VII	26.28 - 0.01 X3	0.64	2.16

{No. of osteons (×1); Average no. of lamellae/osteon (×2); Haversian canal diameter (×3)}.

One-way ANOVA					
Source of variation	Sum of squares	Df	Mean sum of square	*F*-value	*P*-value
Regression	8.27	3	2.75	0.49	0.69, NS, *p*>0.05
Residual	33.32	6	5.55		
Total	41.6	9	–		

Comparison of all the parameters of radiographic analysis in 50 specimens of mandible with standard deviation, mean and Pearson's correlation showed significant *p*-values ($p<0.05$) for all the parameters except height of body of mandible, width of cortex at LB and TCB at AG region (Table 3). Of all the parameters showing significant correlation, the distance of IMF to CAR (left and right sides) shows negative correlation with age.

Table 3 – ***Correlation of age (30–39 yrs) with histological findings in mandible***

Selection	Dependent variable: age [years]	Multiple R^2	Standard error of estimate
I	44.04 - 0.39 X1 - 0.09 X2 + 0.007 X3	0.26	1.61
II	44.92 - 0.35 X1 - 0.21 X2	0.23	1.48
III	43.34 - 0.37 X1 + 0.009 X3	0.25	1.47
IV	35.26 + 0.02 X2 + 0.003 X3	0.06	1.52
V	41.16 - 0.25 X1	0.18	1.38
VI	35.93 - 0.04 X2	-0.03	1.41
VII	35.47 + 0.003 X3	0.06	1.41

One-way ANOVA					
Source of variation	Sum of squares	Df	Mean sum of square	*F*-value	*P*-value
Regression	6.39	3	2.13	0.91	0.48, NS, *p*>0.05
Residual	14.00	6	2.33		
Total	20.4	9	–		

Histological findings

Histological examination of each mandible in all the age groups was carried out. Simple and multiple correlation formula were derived, to evaluate the accuracy of single or multiple parameters that produced estimated age closest to the known age.

Table 2 shows regression equations derived for the number of osteons, average no. of lamella/osteon and HC diameter. Among the individual parameters, no. of osteons gives the least error (±1.88) and hence closest estimate to known age. When two or all three parameters were equated, the no. of osteons and HC diameter taken together, gives more accurate estimated age to the error of ±1.78. All the three parameters when taken together give an error up to ±1.94.

Table 3 (age group 30–39 years) shows the same correlation of histological findings to determine the accuracy of estimated age as in age-group 20–29 years.

Table 4 shows that all three parameters when combined or the no. of lamella/osteon gives the least error of ±2.13.

Table 4 – ***Correlation of age (40–49 years) with histological findings in mandible***

Selection	Dependent variable: age [years]	Multiple R^2	Standard error of estimate
I	42.09 - 0.20 X1 + 1.22 X2 + 0.02 X3	0.65	2.13
II	44.61 - 0.16 X1 + 1.23 X2	0.53	2.17
III	45.09 - 0.10 X1 + 0.02 X3	0.40	2.36
IV	37.44 + 0.95 X2 + 0.022 X3	0.55	2.15
V	47.64 - 0.07 X1	0.13	2.36
VI	40.33 + 1.00 X2	0.44	2.13
VII	41.96 + 0.02 X3	0.35	2.23

One-way ANOVA					
Source of variation	Sum of squares	Df	Mean sum of square	*F*-value	*P*-value
Regression	21.78	3	7.26	1.88	0.23, NS, *p*>0.05
Residual	23.11	6	3.85		
Total	44.9	9	–		

Table 5 shows single parameter, i.e., no of lamellae/ osteon gives the least error up to ±2.04. All the parameters when combined give an error of ±2.32.

Table 6 (age group of 60–69 years) shows that Haversian canal diameter is the closest parameter with an error of ±2.18. All the three parameters when taken together give an error of ±2.56.

From the above tables for the five groups, it is found that the parameters to determine the accuracy of estimated age goes on changing in different groups. Amongst the single parameters no of osteons appears to be the closest to known age. All the three parameters in all the age groups give an accuracy up to an error of ±2.56.

Table 5 – ***Correlation of age (50–59 years) with histological findings in mandible***

Selection	Dependent variable: age [years]	Multiple R^2	Standard error of estimate
I	53.15 + 0.14 X1 - 1.27 X2 + 0.006 X3	0.59	2.32
II	55.38 + 0.13 X1 - 1.33 X2	0.58	2.12
III	43.22 + 0.18 X1 + 0.02 X3	0.33	2.47
IV	61.04 - 1.35 X2 - 0.001 X3	0.54	2.20
V	49.71 + 0.13 X1	0.22	2.37
VI	60.72 - 1.33 X2	-0.54	2.04
VII	52.69 + 0.01 X3	0.15	2.40

One-way ANOVA					
Source of variation	Sum of squares	Df	Mean sum of square	*F*-value	*P*-value
Regression	17.69	3	5.89	1.24	0.37, NS, *p*>0.05
Residual	28.41	6	4.73		
Total	46.1	9	–		

Table 6 – ***Correlation of age (60–69 years) with histological findings in mandible***

Selection	Dependent variable: age [years]	Multiple R^2	Standard error of estimate
I	56.48 + 0.08 X1 - 0.29 X2 + 0.03 X3	0.33	2.56
II	70.25 - 0.07 X1 - 0.19 X2	0.19	2.43
III	58.85 + 0.03 X1 + 0.02 X3	0.30	2.36
IV	62.04 - 0.20 X2 + 0.02 X3	0.31	2.34
V	71.07 - 0.10 X1	0.16	2.26
VI	66.31 - 0.30 X2	-0.15	2.26
VII	60.97 + 0.03 X3	0.30	2.18

One-way ANOVA					
Source of variation	Sum of squares	Df	Mean sum of square	*F*-value	*P*-value
Regression	0.49	3	0.16	0.03	0.98, NS, *p*>0.05
Residual	27.60	6	4.60		
Total	28.10	9	–		

Table 7 shows that pooled data from all the 50 samples gives a regression coefficient with an error of ±3.47 when all three parameters were equated together.

Table 7 – ***Correlation of age [years] with histological findings in mandible***

Selection	Dependent variable: age [years]	Multiple R^2	Standard error of estimate
I	25.81 + 0.74 X1 - 1.45 X2 + 0.01 X3	0.97	3.47
II	28.98 + 0.76 X1 - 1.73 X2	0.96	3.52
III	13.25 + 0.82 X1 + 0.03 X3	0.96	3.77
IV	62.85 - 5.06 X2 + 0.07 X3	0.82	8.15
V	14.95 + 0.90 X1	0.95	4.00
VI	82.03 - 6.77 X2	-0.79	8.66
VII	22.29 + 0.03 X3	0.64	10.20

One-way ANOVA					
Source of variation	Sum of squares	Df	Mean sum of square	*F*-value	*P*-value
Regression	9359.80	3	3119.93	222.5	0.0021, S, *p*<0.05
Residual	645.01	46	14.02		
Total	10004.82	49	–		

In individual groups, analysis of variance shows non-significant *p*-value whereas analysis of variance when combined in all the samples (50) gives a significant *p*-value. Larger sample size gives better Pearson's correlation coefficient value (Figure 2).

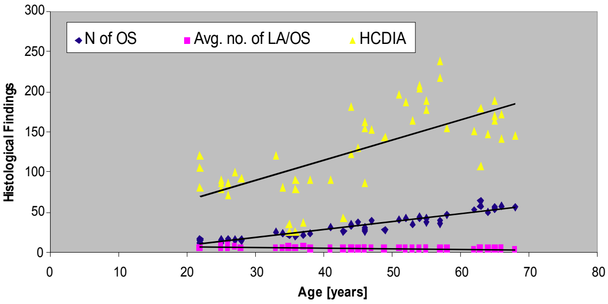

Figure 2 – *Histological parameters against age.*

Table 8 shows the correlation of histological findings of AR of OS, HC area, HC perimeter to estimated age. All the three parameters show greater degree of error to the minimum of ±11.98. Hence are of less significance in estimating age-at-death (Figures 3–5).

Table 8 – *Correlation of age [years] with histological findings in mandible*

Selection	Dependent variable: age [years]	Multiple R^2	Standard error of estimate
I	30.53 - 1.11 X1 - 1.77 X2 + 0.02 X3	0.54	12.20
II	45.81 - 2.43 X1 - 3.44 X2	0.14	14.20
III	30.25 - 0.009 X1 + 0.02 X3	0.53	12.09
IV	30.30 - 0.001 X2 + 0.02 X3	0.53	12.09
V	45.58 - 0.002 X1	-0.09	14.14
VI	45.60 - 0.0034 X2	-0.10	14.12
VII	30.04 + 0.02 X3	0.53	11.98

{AR of OS (×1), HC area (×2), HC perimeter (×3)}.

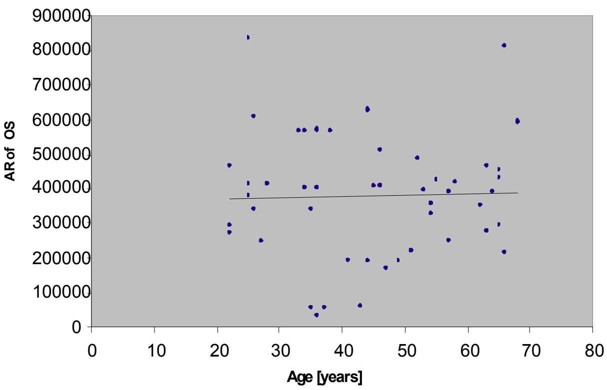

Figure 3 – *Correlation of age [years] with histological findings in mandible (AR of OS).*

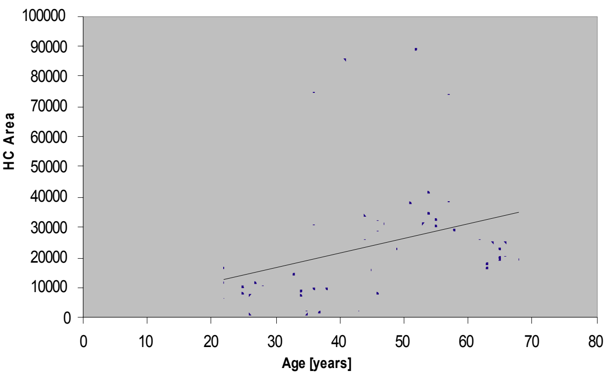

Figure 4 – *Correlation of age [years] with histological findings in mandible (HC area).*

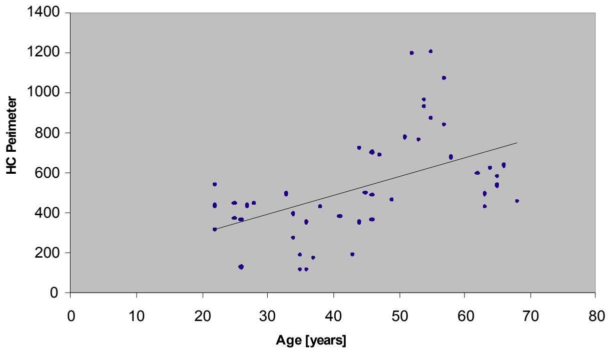

Figure 5 – *Correlation of age [years] with histological findings in mandible (HC perimeter).*

Radiomorphometric analysis

Descriptive statistical analysis (i.e. mean, standard deviation and standard error) was carried out for all the groups in this study for all the parameters. The tables below show descriptive statistics according to age group for various parameters.

Table 9 (A and B) shows the descriptive analysis (mean and standard deviation) and Pearson's correlation coefficient in group 1 (20–29 years) for all the radiographic parameters. The mean and SD of distance of inferior border of mental foramen to the crest of alveolar ridge is greater than the mean and SD of distance of lower border of mandible to inferior border of mental foramen (Figure 6). This indicates that the height of the body of the mandible is more above the mental foramen in this age group. However, the Pearson's coefficient is not significant (Figure 7).

Table 9A – *Correlation of age (20–29 years) with radiographic parameters in mandible (descriptive statistics)*

Radiographic parameters	N	Minimum	Maximum	Mean	Standard deviation
Age [years]	10	22.00	28.00	24.77	2.27
Length of ramus [mm]	10	52.28	60.70	55.44	2.84
Height of body of mandible [mm]	10	29.76	40.60	34.90	3.80
Distance of LB to IMF (L) [mm]	10	8.52	18.88	12.47	3.08
Distance of IMF to CAR (L) [mm]	10	17.70	26.90	22.59	3.19
Distance of LB to IMF (R) [mm]	10	8.08	15.96	12.33	2.57
Distance of IMF to CAR (R) [mm]	10	17.16	27.42	22.53	3.23
Width of cortex at LB [mm]	10	3.18	6.28	4.81	1.03
AG angle [deg]	10	0.00	171.00	53.50	80.38
AG depth [mm]	10	0.00	2.62	0.65	1.04
Gonial angle [deg]	10	117.00	121.00	119.05	1.64
TCB at AG region [mm]	10	2.16	3.96	3.21	0.55

Table 9B – *Pearson's correlation coefficient*

Parameters	Correlation (*r*)	*P*-value
Length of ramus [mm]	-0.106	0.786, NS, *p*>0.05
Height of body of mandible [mm]	-0.149	0.702, NS, *p*>0.05
Distance of LB to IMF (L) [mm]	-0.179	0.645, NS, *p*>0.05
Distance of IMF to CAR (L) [mm]	0.021	0.958, NS, *p*>0.05
Distance of LB to IMF (R) [mm]	0.149	0.702, NS, *p*>0.05
Distance of IMF to CAR (R) [mm]	-0.281	0.463, NS, *p*>0.05
Width of cortex at IL [mm]	-0.110	0.777, NS, *p*>0.05
AG angle [deg]	-0.484	0.187, NS, *p*>0.05
AG depth [mm]	-0.410	0.273, NS, *p*>0.05
Gonial angle [deg]	-0.179	0.644, NS, *p*>0.05
TCB at AG region [mm]	0.034	0.931, NS, *p*>0.05

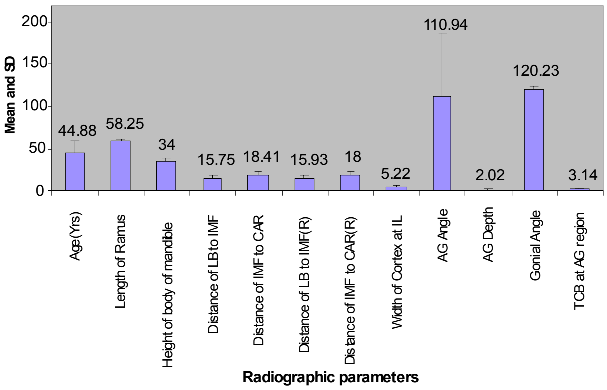

Figure 6 – *Bar diagram showing mean and standard deviations of radiographic parameters.*

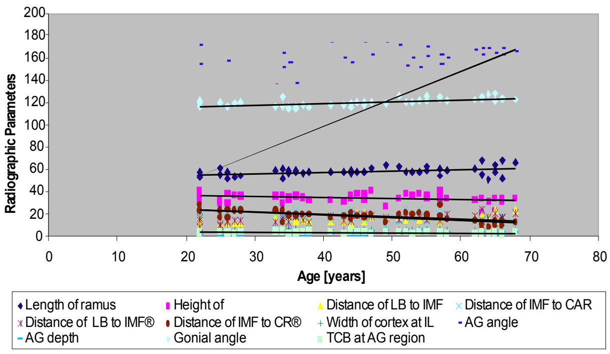

Figure 7 – *Radiographic parameters against age.*

Table 10 (A and B) shows findings of samples in the age group 30–39 years. The value for the length of ramus is higher than that for the previous group. The distance of the IMF to CAR on (left and right) sides is more than the distance of LB to IMF. The Pearson's correlation is non-significant in this age group.

Table 11 (A and B) shows findings of samples in the age group 40–49 years. The value for the length of ramus is higher than that for the previous group. The distance of the IMF to CAR on (left and right) sides is similar to the distance of LB to IMF. The distances IMF to CAR show negative correlation to age. The Pearson's correlation is non-significant in this age group.

Table 10 A – *Correlation of age (30–39 years) with radiographic parameters in mandible (descriptive statistics)*

Radiographic parameters	N	Minimum	Maximum	Mean	Standard deviation
Age [years]	10	33.00	38.00	35.33	1.58
Length of ramus [mm]	10	52.36	60.52	57.64	2.33
Height of body of mandible [mm]	10	30.30	37.54	34.36	2.41
Distance of LB to IMF (L) [mm]	10	13.34	18.54	**15.17**	1.86
Distance of IMF to CAR (L) [mm]	10	14.92	24.00	**19.40**	2.73
Distance of LB to IMF (R) [mm]	10	12.52	18.18	**15.20**	2.17
Distance of IMF to CAR (R) [mm]	10	13.00	22.92	**19.17**	2.87
Width of cortex at LB [mm]	10	3.76	7.14	5.72	1.04
AG angle [deg]	10	0.00	164.00	82.77	78.97
AG depth [mm]	10	0.00	5.44	2.16	2.25
Gonial angle [deg]	10	115.00	125.00	117.22	3.07
TCB at AG region [mm]	10	2.46	4.52	3.37	0.76

Table 10 B – *Pearson's correlation coefficient*

Parameters	Correlation (*r*)	*P*-value
Length of ramus [mm]	-0.081	0.835, NS, *p*>0.05
Height of body of mandible [mm]	-0.119	0.760, NS, *p*>0.05
Distance of LB to IMF (L) [mm]	-0.423	0.256, NS, *p*>0.05
Distance of IMF to CAR (L) [mm]	-0.170	0.662, NS, *p*>0.05
Distance of LB to IMF (R) [mm]	0.042	0.915, NS, *p*>0.05
Distance of IMF to CAR (R) [mm]	-0.093	0.813, NS, *p*>0.05
Width of cortex at IL [mm]	-0.401	0.285, NS, *p*>0.05
AG angle [deg]	-0.699	0.036, NS, *p*>0.05
AG depth [mm]	-0.614	0.078, NS, *p*>0.05
Gonial angle [deg]	-0.326	0.392, NS, *p*>0.05
TCB at AG region [mm]	-0.100	0.798, NS, *p*>0.05

Table 11 A – *Correlation of age (40–49 years) with radiographic parameters in mandible (descriptive statistics)*

Radiographic parameters	N	Minimum	Maximum	Mean	Standard deviation
Age [years]	10	41.00	49.00	45.00	2.34
Length of ramus [mm]	10	56.46	63.20	58.87	2.04
Height of body of mandible [mm]	10	27.08	40.84	34.41	4.07
Distance of LB to IMF (L) [mm]	10	10.16	20.36	**16.26**	3.44

Radiographic parameters	N	Minimum	Maximum	Mean	Standard deviation
Distance of IMF to CAR (L) [mm]	10	10.82	23.62	**17.70**	3.81
Distance of LB to IMF (R) [mm]	10	13.96	20.12	**17.77**	2.42
Distance of IMF to CAR (R) [mm]	10	12.82	20.38	**17.26**	2.27
Width of cortex at LB [mm]	10	4.22	6.28	5.64	0.69
AG angle [deg]	10	0.00	173.00	110.77	83.31
AG depth [mm]	10	0.00	4.34	1.92	1.69
Gonial angle [deg]	10	116.00	120.00	118.66	1.32
TCB at AG region [mm]	10	2.08	4.52	3.26	0.64

Table 11 B – ***Pearson's correlation coefficient***

Parameters	Correlation (*r*)	*P*-value
Length of ramus [mm]	0.729	0.026, S, *p*<0.05
Height of body of mandible [mm]	-0.041	0.916, NS, *p*>0.05
Distance of LB to IMF (L) [mm]	0.310	0.417, NS, *p*>0.05
Distance of IMF to CAR (L) [mm]	-0.345	0.364, NS, *p*>0.05
Distance of LB to IMF (R) [mm]	0.028	0.944, NS, *p*>0.05
Distance of IMF to CAR (R) [mm]	-0.315	0.409, NS, *p*>0.05
Width of cortex at IL [mm]	-0.216	0.578, NS, *p*>0.05
AG angle [deg]	0.016	0.967, NS, *p*>0.05
AG depth [mm]	0.029	0.940, NS, *p*>0.05
Gonial angle [deg]	0.443	0.232, NS, *p*>0.05
TCB at AG region [mm]	0.213	0.581, NS, *p*>0.05

Table 12 (A and B) shows findings of samples in the age group 50–59 years. The distance of the IMF to CAR on (left and right) sides is less than the distance of LB to IMF. The Pearson's correlation is not significant for all the parameters in this group.

Table 12 A – ***Correlation of age (50–59 years) with radiographic parameters in mandible (descriptive statistics)***

Radiographic parameters	N	Minimum	Maximum	Mean	Standard deviation
Age [years]	10	51.00	58.00	54.66	2.39
Length of ramus [mm]	10	54.64	62.00	58.83	2.37
Height of body of mandible [mm]	10	33.74	40.60	36.13	2.37
Distance of LB to IMF (L) [mm]	10	13.70	19.36	16.66	1.95
Distance of IMF to CAR (L) [mm]	10	14.38	25.90	18.95	4.41
Distance of LB to IMF (R) [mm]	10	13.40	19.36	16.54	1.83
Distance of IMF to CAR (R) [mm]	10	15.26	27.42	20.05	3.21
Width of cortex at LB [mm]	10	4.38	6.28	5.39	0.75
AG angle [deg]	10	152.00	171.00	160.44	6.96
AG depth [mm]	10	0.88	6.22	3.21	1.89
Gonial angle [deg]	10	120.00	126.00	122.44	2.36
TCB at AG region [mm]	10	2.16	3.78	3.08	0.51

Table 12 B – ***Pearson's correlation coefficient***

Parameters	Correlation (*r*)	*P*-value
Length of ramus [mm]	-0.229	0.554, NS, *p*>0.05
Height of body of mandible [mm]	-0.007	0.986, NS, *p*>0.05
Distance of LB to IMF (L) [mm]	0.057	0.885, NS, *p*>0.05
Distance of IMF to CAR (L) [mm]	0.108	0.783, NS, *p*>0.05
Distance of LB to IMF (R) [mm]	0.209	0.589, NS, *p*>0.05
Distance of IMF to CAR (R) [mm]	-0.027	0.944, NS, *p*>0.05
Width of cortex at IL [mm]	0.155	0.690, NS, *p*>0.05
AG angle [deg]	-0.166	0.670, NS, *p*>0.05
AG depth [mm]	-0.133	0.773, NS, *p*>0.05
Gonial angle [deg]	0.007	0.985, NS, *p*>0.05
TCB at AG region [mm]	-0.608	0.082, NS, *p*>0.05

Table 13 (A and B) shows findings of samples in the age group 60–69 years. The distance of the IMF to CAR on (left and right) sides is distinctly less than the distance of LB to IMF. This is in accordance with the advancing age. The Pearson's correlation is not significant for all the parameters in this group.

Table 13 A – ***Correlation of age (60–69 years) with radiographic parameters in mandible (descriptive statistics)***

Radiographic parameters	N	Minimum	Maximum	Mean	Standard deviation
Age [years]	10	62.00	68.00	64.66	1.87
Length of ramus [mm]	10	51.38	68.76	60.45	6.61
Height of body of mandible [mm]	10	21.26	33.74	30.18	4.96
Distance of LB to IMF (L) [mm]	10	14.08	23.04	**18.19**	3.23
Distance of IMF to CAR (L) [mm]	10	9.02	17.98	**13.43**	2.85
Distance of LB to IMF (R) [mm]	10	11.88	24.41	**17.79**	3.78
Distance of IMF to CAR (R) [mm]	10	7.34	14.28	**10.98**	2.57
Width of cortex at LB [mm]	10	2.84	6.92	4.57	1.26
AG angle [deg]	10	0.00	168.00	147.22	55.24
AG depth [mm]	10	0.00	3.76	2.19	1.21
Gonial angle [deg]	10	121.00	128.00	123.78	2.43

Radiographic parameters	N	Minimum	Maximum	Mean	Standard deviation
TCB at AG region [mm]	10	1.56	3.48	2.77	0.56

Table 13 B – ***Pearson's correlation coefficient***

Parameters	Correlation (*r*)	*P*-value
Length of ramus [mm]	0.177	0.649, NS, *p*>0.05
Height of body of mandible [mm]	0.211	0.585, NS, *p*>0.05
Distance of LB to IMF (L) [mm]	0.326	0.392, NS, *p*>0.05
Distance of IMF to CAR (L) [mm]	0.017	0.966, NS, *p*>0.05
Distance of LB to IMF (R) [mm]	0.013	0.974, NS, *p*>0.05
Distance of IMF to CAR (R) [mm]	0.202	0.602, NS, *p*>0.05
Width of cortex at IL [mm]	-0.424	0.255, NS, *p*>0.05
AG angle [deg]	-0.073	0.852, NS, *p*>0.05
AG depth [mm]	0.233	0.545, NS, *p*>0.05
Gonial angle [deg]	-0.018	0.963, NS, *p*>0.05
TCB at AG region [mm]	0.073	0.853, NS, *p*>0.05

Table 14 (A and B) shows comparison of all the parameters of radiographic analysis in 50 specimens of mandible with standard deviation, mean and Pearson's correlation. Table 14B shows significant *p*-values for all the parameters except height of body of mandible, width of cortex at LB and TCB at AG region. Of the parameters showing significant correlation, the distance of IMF to CAR (left and right sides) shows negative correlation with age.

Table 14 A – ***Correlation of age [years] with radiographic parameters in mandible (descriptive statistics)***

Radiographic parameters	N	Minimum	Maximum	Mean	Standard deviation
Age [years]	50	22.00	68.00	44.88	14.31
Length of ramus [mm]	50	51.38	68.76	58.25	3.87
Height of body of mandible [mm]	50	21.26	40.84	34.00	4.04
Distance of LB to IMF (L) [mm]	50	8.52	23.04	15.75	3.29
Distance of IMF to CAR (L) [mm]	50	9.02	26.90	18.41	4.46
Distance of LB to IMF (R) [mm]	50	8.08	24.41	15.93	3.25
Distance of IMF to CAR (R) [mm]	50	7.34	27.42	18.00	4.79
Width of cortex at LB [mm]	50	2.84	7.14	5.22	1.04
AG angle [deg]	50	0.00	173.00	110.94	75.77
AG depth [mm]	50	0.00	6.22	2.02	1.80
Gonial angle [deg]	50	115.00	128.00	120.23	3.29
TCB at AG region [mm]	50	1.56	4.52	3.14	0.62

Table 14 B – ***Pearson's correlation coefficient***

Parameters	Correlation (*r*)	*P*-value
Length of ramus [mm]	0.424	0.004, S, *p*<0.05
Height of body of mandible [mm]	-0.270	0.073, NS, *p*>0.05
Distance of LB to IMF (L) [mm]	0.567	0.000, S, *p*<0.05
Distance of IMF to CAR (L) [mm]	**-0.605**	0.000, S, *p*<0.05
Distance of LB to IMF (R) [mm]	0.545	0.000, S, *p*<0.05
Distance of IMF to CAR (R) [mm]	**-0.669**	0.000, S, *p*<0.05
Width of cortex at IL [mm]	-0.129	0.400, NS, *p*>0.05
AG angle [deg]	0.464	0.001, S, *p*<0.05
AG depth [mm]	0.305	0.041, S, *p*<0.05
Gonial angle [deg]	0.621	0.000, S, *p*<0.05
TCB at AG region [mm]	-0.272	0.070, NS, *p*>0.05

Regression analysis was done for all the parameters in all five groups to arrive at a formula to calculate the predicted age.

Regression equation shown in Table 15 gives an estimated age-at-death of an individual with an error of ±2–3 years.

Table 15 – ***Regression of age with other parameters in 20–29-year-old group***

Parameters	Mean±SD	Correlation (*r*)	Regression equation
Age [years]	24.77±2.27	–	–
Length of ramus [mm]	55.44±2.84	-0.106	32.12 - 0.13 Y
Height of body of mandible [mm]	34.90±3.80	-0.149	33.48 - 0.25 Y
Distance of LB to IMF [mm]	12.47±3.08	-0.179	27.80 - 0.24 Y
Distance of IMF to CAR [mm]	22.59±3.19	0.021	24.10 + 0.03 Y
Distance of LB to IMF (R) [mm]	12.33±2.57	0.149	22.69 + 0.17 Y
Distance of IMF to CAR (R) [mm]	22.53±3.23	-0.281	33.78 - 0.40 Y
Width of cortex at IL [mm]	4.81±1.03	-0.110	25.01 - 0.05 Y
AG angle [deg]	53.50±80.38	-0.484	941.67 - 17.14 Y
AG depth [mm]	0.65±1.04	-0.410	24.89 - 0.19 Y
Gonial angle [deg]	119.05±1.64	-0.179	40.17 - 0.13 Y
TCB at AG region [mm]	3.21±0.55	0.034	24.74 + 0.01 Y

Regression equation shown in Table 16 gives an estimated age-at-death of an individual with an error of ±2–3 years.

Regression equation shown in Table 17 gives an estimated age-at-death of an individual with an error of ±2–3 years.

Regression equation shown in Table 18 gives an estimated age-at-death of an individual with an error of ±2–3 years.

Tables 19 show regression equations for the various parameters on radiographic analysis for the five groups. The regression formula for the five groups with different parameters as shown gives an estimated age-at-death of an individual with an error of ±2–3 years.

Table 16 – ***Regression of age with other parameters in 30–39-year-old group***

Parameters	Mean±SD	Correlation (*r*)	Regression equation
Age [years]	35.33±1.58	–	–
Length of ramus [mm]	57.64±2.33	-0.081	42.22 - 0.12 Y
Height of body of mandible [mm]	34.36±2.41	-0.119	41.57 - 0.18 Y
Distance of LB to IMF [mm]	15.17±1.86	-0.423	42.88 - 0.50 Y
Distance of IMF to CAR [mm]	19.40±2.73	-0.170	41.03 - 0.29 Y
Distance of LB to IMF (R) [mm]	15.20±2.17	0.042	34.45 + 0.06 Y
Distance of IMF to CAR (R) [mm]	19.17±2.87	-0.093	38.57 - 0.17 Y
Width of cortex at IL [mm]	5.72±1.04	-0.401	36.84 - 0.26 Y
AG angle [deg]	82.77±78.97	-0.699	292.04 - 34.94 Y
AG depth [mm]	2.16±2.25	-0.614	37.22 - 0.87 Y
Gonial angle [deg]	117.22±3.07	-0.326	108. 86 - 0.63 Y
TCB at AG region [mm]	3.37±0.76	-0.100	35.49 - 0.05 Y

Table 17 – ***Regression of age with other parameters in 40–49-year-old group***

Parameters	Mean±SD	Correlation (*r*)	Regression equation
Age [years]	45.00±2.34	–	–
Length of ramus [mm]	58.87±2.04	0.729	7.59 + 0.64 Y
Height of body of mandible [mm]	34.41±4.07	-0.041	47.45 - 0.07 Y
Distance of LB to IMF [mm]	16.26±3.44	0.310	37.59 + 0.46 Y
Distance of IMF to CAR [mm]	17.70±3.81	-0.345	54.94 - 0.56 Y
Distance of LB to IMF (R) [mm]	17.77±2.42	0.028	44.49 + 0.03 Y
Distance of IMF to CAR (R) [mm]	17.26±2.27	-0.315	50.27 - 0.31 Y
Width of cortex at IL [mm]	5.64±0.69	-0.216	45.36 - 0.06 Y
AG angle [deg]	110.77±83.31	0.016	-18.10 + 0.57 Y
AG depth [mm]	1.92±1.69	0.029	44.96 + 0.02 Y
Gonial angle [deg]	118.66±1.32	0.443	15.35 + 0.25 Y
TCB at AG region [mm]	3.26±0.64	0.213	44.81 + 0.06 Y

Table 18 – ***Regression of age with other parameters in 50–59-year-old group***

Parameters	Mean±SD	Correlation (*r*)	Regression equation
Age [years]	54.66±2.39	–	–
Length of ramus [mm]	58.83±2.37	-0.229	68.02 - 0.23 Y
Height of body of mandible [mm]	36.13±2.37	-0.007	54.91 - 0.01 Y
Distance of LB to IMF [mm]	16.66±1.96	0.057	53.89 + 0.05 Y
Distance of IMF to CAR [mm]	18.95±4.41	0.108	50.88 + 0.20 Y
Distance of LB to IMF (R) [mm]	16.54±1.83	0.209	52.01 + 0.16Y
Distance of IMF to CAR (R) [mm]	20.05±3.21	-0.027	55.39 - 0.04 Y
Width of cortex at IL [mm]	5.39±0.75	0.155	54.40 + 0.05 Y
AG angle [deg]	160.44±6.96	-0.166	132.22 - 0.48 Y
AG depth [mm]	3.21±1.89	-0.133	55.00 - 0.11 Y
Gonial angle [deg]	122.44±2.36	0.007	53.81 + 0.01 Y
TCB at AG region [mm]	3.08±0.51	-0.608	55.06 - 0.13 Y

Table 19 – ***Regression of age with other parameters in 60–69-year-old group***

Parameters	Mean±SD	Correlation (*r*)	Regression equation
Age [years]	64.66±1.87	–	–
Length of ramus [mm]	60.45±6.61	0.177	26.84 + 0.63 Y
Height of body of mandible [mm]	30.18±4.96	0.211	47.77 + 0.56 Y
Distance of LB to IMF [mm]	18.19±3.23	0.326	54.42 + 0.56 Y
Distance of IMF to CAR [mm]	13.43±2.85	0.017	64.31 + 0.03 Y
Distance of LB to IMF (R) [mm]	17.79±3.78	0.013	64.19 + 0.03 Y
Distance of IMF to CAR (R) [mm]	10.98±2.57	0.202	61.61 + 0.28 Y
Width of cortex at IL [mm]	4.57± .26	-0.424	65.97 - 0.29 Y
AG angle [deg]	147.22±55.24	-0.073	382.13 - 2.16 Y
AG depth [mm]	2.19±1.21	0.233	64.33 + 0.15 Y
Gonial angle [deg]	123.78±2.43	-0.018	67.56 - 0.02 Y
TCB at AG region [mm]	2.77±0.56	0.073	64.60 + 0.02 Y

Discussion

Growth of the human beings can be studied as a group of changes taking place from the beginning of prenatal life to senility. Hard tissues (bones and teeth) also undergo changes with growth, which can be a change in shape and/or fusion of ossification centers or after death, these changes remain stable and facilitate ease in estimation of age from hard tissue samples [6–8].

Histologically, bone is a vascular, mineralized connective tissue consisting of cells and an intercellular matrix in which its cells are embedded [9]. According to structure, bone is compact or cancellous bone. Compact bone consists of concentric lamella around a central vascular system, the Haversian system. Bone is in a constant state of remodeling. With age, resorption exceeds formation, resulting in an overall loss of bone [10]. Kerley ER [11] describes fairly consistent changes in the shafts of long bones associated with advancing age. In old age, the long bones become lighter and more brittle owing to these changes and an increase in inorganic constituents [12].

The mandible is amongst the first bones in the body to start ossifying and is unique in that it has both the patterns of ossification (endochondral and intramembranous). The body of mandible is ossified intramembranously whereas the ossification of the coronoid and condyloid processes is endochondral. Until the third decade of life, morphological and dental changes (time and sequence of eruption of teeth) serve as an aid to estimate age [13]. For ages above the third decade, the changes are subtle and have to be studied in greater detail [14].

In the forensic context, age estimation plays an important role to identify the individual [9]. Bones form an important source for such information as the soft tissues are destroyed after long periods of time. Bones, even fragments, can be used for age evaluation microscopically and provide an approximate age-at-death [11].

Thus in this study, to minimize the drawbacks of the commonly used morphological methods, combination of both radiographic and histologic methods were used. We have also attempted to arrive at the best method/ methods of estimation of age-at-death.

Attempt to find a method or parameter, which gave the closest estimate of age, was made using stepwise regression equation. The first histological parameter studied was the number of osteons, which showed a gradual increase. As the cortex and medullary cavity expand in diameter, mature circumferential bone is formed at a slower rate and encloses/encircles small subperiosteal blood vessels. Osteoclasts burrow longitudinal channels down through the thickening cortex. Later these areas are filled by osteoblasts, which form new bone. New concentric lamella are formed which are slightly smaller than the earlier formed osteons.

The average number of concentric lamellae per osteon, in this study, showed a slight decrease with age. With age, the proportion of bone occupied by osseous tissue also decreases as fatty marrow replaces the normal hematopoietic marrow [12]. This along with decreased osteoblastic activity decreases the rate of formation of new bone and delays mineralization producing lamellae, which are indistinct from each other; this is more pronounced in individuals above 50-year-old.

The Haversian canal diameter increased with increasing age in the present study. This increase in Haversian canal diameter corresponds to those seen in ageing individuals; as there is a shift in balance between the osteoblastic and osteoclastic activity with age. There is greater degree of intra-osteonal remodeling and growth retardation contributing to increased diameter of Haversian canal and hence increasing porosity of cortical bone.

The reason for this could be attributed to the difference in the rate of remodeling in the outer, middle and inner third of the cortex. As bone is apposed slowly in the inner cortex as compared to the outer cortex, the diameter and perimeter of Haversian canal when measured at the inner third gives higher values. Studies like those of Singh IJ and Gunberg DL (1970) [13] using dimensions of outer third of the cortex give lower observed values of Haversian canal dimensions as apposition of bone takes place around the smaller periosteal vessels.

The average area of the osteon, bounded by the cementing line or reversal line, shows an insignificant increase in dimensions with age. According to Ruff CB and Hayes WC (1982) [16] this increase could be due to mechanical stress- and strain-remodeling of bone cortices. Depending on the site used for analysis, i.e., outer, middle or inner third of cortex, the area of the osteon exhibited wide variations in dimensions. Hence, we are in agreement with Thompson DD and Galvin CA (1983) [17] who concluded that osteon areas were poor variables for age estimations. Osteon areas show wide variations in dimensions and are poor indicators of age-at-death estimation.

Quantitative histological methods permit objectivity and offer the advantage of their use for statistical analysis. As there is an acute need for accuracy in the methods for age-at-death estimation, radiographic analysis has been used in conjunction with histological features. Assessment of two different yet related methods to estimate age remove bias and dependence on a single parameter for age estimation.

There were numerous radiographic parameters considered, i.e., length of the ramus, total height of the body of the mandible and height of body of mandible in relation to amount of bone above and below the mental foramen, gonial angle, antegonial angle and depth and width of the cortex at the mental region and at the antegonial region.

The first amongst them was the length of the ramus. The length of the ramus shows an increase in dimensions with increase in age, though it is insignificant. Up to the fifth decade of life, the length of the ramus shows a gradual increase. Thereafter, the length remains more/ less constant and shows very little variation. Other authors have proposed that the mandible does not follow one characteristic pattern throughout life; it is likely that the map of mandibular growth varies with age.

A reduction in the height of the body of the mandible was observed with increase in age. On the radiographs, the mental foramen has been used as a reference point to evaluate height of the body of the mandible. The part above the mental foramen, i.e., the alveolar bone, develops in relation to teeth and shows resorption with loss of teeth. With age, there may be some amount of apposition of bone at lower border of mandible. Thus, in younger individuals the height of body of the mandible above the mental foramen is greater than that below the mental foramen. This situation reverses in the older individuals, when height of the bone above the mental foramen is less than the basal bone due to loss of teeth due to periodontal diseases.

The reason for this reduction is thought to be due to local factors such as masticatory forces leading to skeletal change [18]. Even this served as a poor variable because loss of even single tooth due to caries or periodontal disease caused the observations to be variable.

An increase in the size of the gonial angle was observed in this study. This is in agreement with study of Ohm E and Silness J (1999) [19] who found a close positive association between gonial angle and age. Few authors have attributed this to the presence of teeth. When teeth are present, the muscular activity associated with mastication preserved the angle from any change in size. However, with loss of teeth, the bone undergoes remodeling and consequently an increase in size is seen. Also, other factors affecting this parameter were tooth loss due to lack of awareness, occupation as well as social and attitudinal aspects relating to tooth extraction and early loss of teeth.

The width of cortex at the mental region (MI) and antegonial region (AI) shows a decrease in values with increasing age. This was indicated by a negative and non-significant correlation. This could be attributed to the fact that remodeling of bone continues into old age. As bone remodels, newer bone that is formed is not mineralized completely and neither does it ossify completely, i.e., to the extent it was in adulthood. This lesser mineralized bone is seen on the radiograph as a less dense area at the cortex. Some authors observed that females demonstrated a greater degree of loss of cortical bone with age compared to males. This was attributed to the activity of estrogen, which negated the resorptive effect of parathormone in premenopausal women. Post-menopausal women showed greater bone loss as compared to men in the same age group. As most of our samples were from males, this resulted in insignificant correlation.

The antegonial angle and depth showed a positive correlation to age. This could be attributed to the muscular forces associated with mastication in the dentate individual. In the edentulous individual loss of teeth reduced the masticatory forces.

Conclusions

From this study, we can conclude that histological methods for determining age are a better source of data as compared to radiological and morphological means. Two to three parameters should be considered during evaluation. In cases where the head is decapitated or where only facial bones are found at the crime scene the mandible can be used for determination of age. Even a small fragment of bone (for e.g. in mass disaster cases where everything in messed up) can be subjected to histological assessment with a fair degree of certainty.

The study should be performed in a larger number of samples and staining of the ground sections can also be done.

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Received: October 19th, 2010

Accepted: April 15th, 2011