MICROMETRIC MEASUREMENTS BY SCANNING ELECTRON MICROSCOPE (SEM) FOR DENTAL AGE ESTIMATION IN ADULTS

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ABSTRACT

Determining age from dental structure is a well documented forensic procedure and the data may be used as auxiliary information in identification. In this study 20 measurements of different variables in incisor teeth were obtained using a SEM micrometric scaler and the results were stastically correlated with age by the multiple regression method. A formula was then derived from the calculations for age estimation which gave statistically acceptable results. Gender differences were also investigated and when separated delivered even stronger correlation. **(J Forensic Odontostomatol 2000; 18:22-6)**

Key words: Teeth, SEM, micrometric measurements, age determination, forensic odontology

INTRODUCTION

Age estimation in forensic identification cases following crime and accidents may be accomplished by several methods where teeth are the only remaining evidence.[1] In cases of juvenile teeth age estimation can be carried out by direct dental examination,[2] examination of dental casts[3] and radiographic evidence of various developmental stages of teeth.[2] These do not of course apply to the adult dentition because of the completion of dental maturation[2] but other clues to age can be obtained from macroscopic and microscopic examination of the changes that occur with ageing.

Existing macroscopic procedures include direct oral and dental cast examination and correlating the data with dental records, which has proved to be reliable.[4,5] Existing microscopic and molecular studies include scanning electron microscopy with energy disperse x-ray,[6,7] periapical radiographic parameters,[8] chromatographic analysis[9] and dental pulp DNA testing,[10] all useful in identification.

The aim of the present study was to measure 20 aspects of longitudinal cross-sections of incisor teeth and to develop a formula for calculating age.

MATERIALS AND METHODS

Twenty-four newly extracted human incisor teeth without caries from donors of known age and gender, made up from six from each of four groups in the age range 35 to 70 years were studied (Table 1).

All teeth were embedded in elastometric impression materials* in a steel mould and sectioned labiolingually in the centre from crown to root by means of a diamond separating disc,** guided by a longitudinal groove in the mould. The sections were coated with gold-palladium alloy and observed by SEM† at 100x magnification11 with micrometric scaler (Fig.1).

The tooth landmarks used for measurement are shown in Fig.2.

Least squares multiple regression modelling was used to estimate age using the dental parameters described in Fig.2. Statistical assumptions of constant variance and the predictors were checked for colinearity by VIF (Variance Influence Factor).

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** Comet Dental Gbr. Brasseler Gmbh & Co. KG Lemgo, Germany
† Joel, JSM-6400 Scanning Microscope, Tokyo, Japan

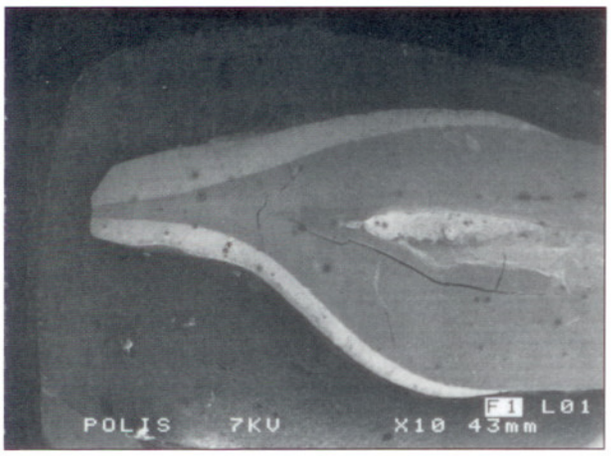

Fig.1: A sagittal section of a tooth from a male subject aged 25 years

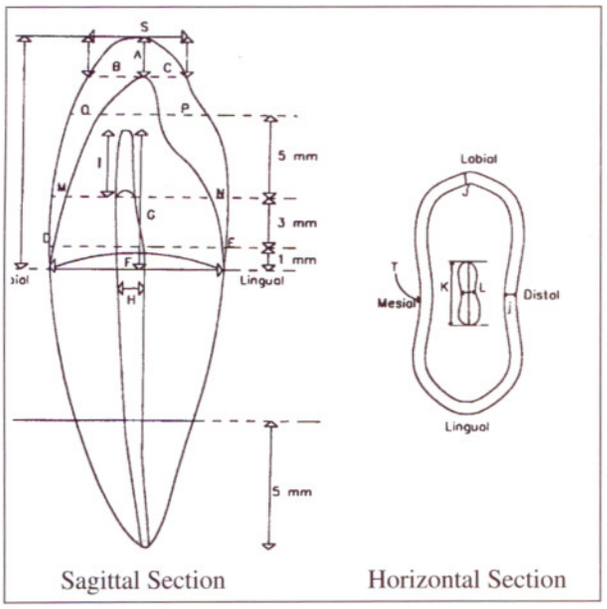

Fig.2:
A: Incisal enamel thickness
B: Incisal, labial enamel thickness
C: Incisal, lingual enamel thickness
D: Labial enamel thickness, 1mm above cervical line
E: Lingual enamel thickness, 1mm above cervical line
F: Cervical total thickness
G: Pulp height from cervical line
H: Pulp width at cervical line
I: Height of predentine over pulp tissue
J: Labial cementum thickness at 5mm over root apex
K: Labio-lingual pulp width at 5mm over root apex
L: Mesiio-distal pulp width at 5mm over root apex
M: Labial enamel thickness, 3mm above cervical line
N: Lingual enamel thickness, 3mm above cerrvical line
O: Labial enamel thickness, 5mm above cervical line
P: Lingual enamel thickness, 5mm above cervical line
R: Maximum crown length
S: Maximum thickness of incisal edge (labio-lingual)
T: Countable cementum layers at 5mm over root apex

RESULTS

The micrometric measurement results for specific points on the teeth were statistically analysed and the descriptive statistics given in Table 2.

The measurements were analysed by the multiple regression method for age estimation and the results for variables T,G,H,F,R,M,O and S showed R^2=0.90 accuracy with the following formula being derived: Age=21.5+2.82T+3.51 G-0.00292 H+3.91 F-2.75 R+0.0278 M-0.00919 O-2.96 S. Standard error prediction of the reported equation is S=4.147. Standard errors and significance of these coefficients are given in Table 3.

When some variables are omitted it is still possible to obtain statistically significant results for age estimation. Thus, when variables T,G,H,F,R and S (R^2=0.882) T,G,H,F and S (R^2=0.857) were tested T,G,H and S (R^2=0.848) and T and S (R^2=0.798) were tested a statistically significant level of $p<0.01$ was obtained.

The reliability of the statistical results may be strengthened by omitting the non-measurable variables and zero measurements i.e. the age dependent factors such as the worn incisal edges or receded pulp. Four cases were then discarded and statistical analyses were repeated for the rest. This time the multiple regression analyses gave R^2=0.95 accuracy for variables T,G,H,F,K,M,D and S ($p<0.01$), R^2=0.84 with variables T,G,H,F and S ($p<0.01$), R^2=0.83 for variables T,G,H and S ($p<0.01$), R^2=0.81 for variables T,G and S ($p<0.01$) and R^2=0.77 for variables T and S ($p<0.01$).

The age estimation calculations were also done for female and male groups separately. The multiple regression analyses for 12 females with the points T,D,S,F,E,O and C showed R^2=0.99 accuracy ($p<0.01$) with the points T,D,S,F,E and D it was R^2=0.99 ($p<0.01$) and with T and D it was R^2=0.85 ($p<0.01$).

The multiple regression analyses for 12 males with the points T,G,H,R,N,M and O was R^2=0.99 ($p<0.01$), with the points T,G,H,R and N was R^2=0.99 ($p<0.01$), and then with the points T and G it was R^2=0.89 ($p<0.01$).

Table 1: *Ages and genders of 24 donors*

Age Groups (years)	**35-44**						**45-54**					
Ages	35	36	37	42	42	43	47	54	51	54	54	54
Genders	M	F	M	M	F	M	F	F	F	F	F	M
Age Groups (years)	**57-64**						**65-74**					
Ages	57	57	57	59	60	63	65	65	67	68	70	70
Genders	F	M	M	F	F	M	M	M	M	F	F	M

Table 2: *The descriptive statistics of specific tooth points within the age range of 35-70 yrs*

VARIABLES RECORDED**	N	MEAN	MEDIAN	TRMEAN	STDEV	SEMEAN	MIN	MAX
B	24	787.6	734.2	786.3	225.5	46	295.3	1310
C	24	504.6	503,4	502.4	110.4	22.5	323.4	784.4
D	24	280.3	273.7	279.7	61.2	12.5	168.6	405.9
E	24	238.5	245.8	239.5	71.2	14.5	84.3	370.8
F	24	5.3796	5.445	5.3859	0.4527	0.0924	4.47	6.15
G	24	2.976	2.805	3.002	1.342	0.274	0	5.37
H	24	785.4	734.1	770.9	450.3	91.9	0	1890
I	23*	800	590	747	724	151	0	2710
J	24	108.77	112.21	107.69	46.31	9.45	49.18	192.15
J'	24	77.89	78.32	72.22	47.59	9.71	27.2	253.39
K	24	914.8	837.2	915.7	353.9	72.2	169.1	1640
L	24	184.8	158.5	179.9	79.5	16.2	68.9	407.6
M	24	501.7	522.5	509	107.8	22	169.1	674.6
N	24	420	417.5	419.6	91.8	18.7	224.7	623.9
O	24	726.3	793.9	746.4	254.5	52	0	1010
P	24	442.6	483.9	455.2	156	31.8	0	609.1
R	24	6.659	6.705	6.651	1.386	0.283	3.48	10.01
S	23*	2.14	1.92	2.12	0.652	0.136	1.15	3.55
T	24	7.667	7	7.591	2.988	0.61	4	13
AGE	24	54.58	55.5	54.77	10.86	2.22	35	70

* (I) and (S) could not be measured in 2 subjects
** For key, see Fig.1

Table 3: *Standard errors and significance of coefficients*

Predictor	Coefficient	Standard Error	Significance
Constant	21, 46	14, 28	0, 155
T	2, 8226	0, 4889	0, 001**
G	3, 5137	0, 9958	0, 003**
H	-0.002916	0, 002924	0, 335
F	3, 906	2, 250	0, 105
R	-2.745	1, 405	0, 071
M	0, 02780	0, 01631	0, 110
O	-0.009193	0.006854	0.201
S	-2.957	3, 140	0, 362

** $p<0.01$

Utilising the above data the following formula were derived and the age estimation female regression equation is:
Age: 13.6+1.41 T-0.117 D+12.3 S+7.17 F-0.0402 E+0.00749 O+0.00636 C. Standard error prediction of the reported equation is S=1.297. Standard errors and significance of these coefficients are given in Table 4.

Age estimation for male regression equation is:
Age: 51.6+2.16 T+3.24 G-0.0177 H-3.70 R+0.0405 N-0.0169 M+0.0159 D. Standard error prediction of the reported equation is S=1.377. Standard errors and significance of these coefficients are given in Table 5.

DISCUSSION

In this project the differentiating factors required for age estimation were identified and a formula proposed based on human incisor teeth extracted for periodontal reasons. It is however suggested that teeth extracted for other reasons such as from cadavers and forensic cases might reflect biological variations better than homogeneous sources.[12] In this study, in order to establish an age calculation formula and to ensure standardisation, it was preferred to measure tooth layers from the same teeth which were extracted for the same reasons.[13]

Measurements of enamel, dentine, predentine, pulp layers of the crown portion and dimensions of root pulps and cementum layers and in addition the number of cementum layers were recorded. The age correlation formula derived from the statistical analysis of these results varied from R2=0.77-0.94 when gender variables were omitted ($p<0.01$), $R^2=0.90$ when B,C,D,E,I,K,L,N and P variables were also

Table 4: *Standard errors and significance of coefficients*

Predictor	Coefficient	Standard error	Significance
Constant	13,619	8.762	0.195
T	1,4142	0,2374	0,004**
D	-0.1171	0,01067	0,001**
S	12,282	1,944	0,003**
F	7,173	1,046	0,002**
E	-0.040184	0.007352	0,005**
O	0,007492	0,003385	0,091
C	0,006361	0,005147	0.284

**p<0.01

Table 5: *Standard errors and significance of coefficients*

Predictor	Coefficient	Standard error	Significance
Constant	51,626	4,875	0,001**
T	2,1573	0,1903	0,001**
G	3,2418	0,4242	0,002**
H	-0.017748	0.00255	0,002**
R	-3.6996	0,6406	0,004**
N	0.040468	0,009251	0.012
M	-0.016859	0.008257	0,111
D	0,015949	0,009976	0.185

**p<0.01

omitted and R^2=0.94 when additionally J,O and R variables were omitted.

The determination coefficient 'R^2' showed that this method was close to the accepted age estimation results obtained by Gustafson[14] where R^2=0.83 (r=0.91) or others at R^2=0.76 (r=0.87),[15] R^2=0.72 (r=0.85),[16] and R^2=0.64 (r=0.80)[17] and R^2=0.83 (r=0.91).18 Bang and Ramm[18] further found R2=0.37-0.69 (r=0.61-0.83) for intact teeth and R^2=0.25-0.86 (r=0.50-0.93) for sectioned teeth (r is given as a correlation factor).

The age estimation calculations in this research were also applied to the male and female groups separately where the multiple regression analyses for 12 females were applied using the measurement point T,D,S,F,E,O and C showed R^2=0.99 accuracy (p<0.01). The gender variables were chosen, depending on the statistical determination coefficient, derived from different gender sample measurements. Where the gender is known, statistical analysis makes the age estimation becomes more accurate and in female tooth samples the following measurements were important: T (countable cementum layers), D (labial enamel thickness 1mm above the cervical line), S (maximum thickness of incisal edge), F (cervical total thickness), E (lingual enamel thickness 1mm above the cervical line), O (labial enamel thickness 5mm above the cervical line), C (incisal, lingual enamel thickness). In male tooth samples: T,G,H,R,N,M and D were specific measurements, where T (countable cementum layers), G (pulp height from cervical line), H (pulp width at cervical line), R (maximum crown length), N (lingual enamel thickness 3mm above the cervical line), M (labial enamel thickness 3mm above the cervical line), and D (labial enamel thickness 1mm above the cervical line).

These results showed that a minimum of 8 variables were essential for an accurate age estimation calculation. However, some of the current age correlation formulae use only 3-6 variables[14,17] which probably means that some important information is overlooked. This survey established that labial and incisal enamel thickness at different levels, width and height of pulp tissue amount, and the number of cementum layers must be taken into account when age is to be determined. However this research is performed on 24 samples, and should be regarded as a pilot study.

CONCLUSIONS

1. Human age can be determined from SEM measurements of sections of incisor teeth, by using a formula proposed in this study.
2. When gender is known two different age estimation formulae can be used, which will deliver more reliable statistical results.
3. The ageing factors of teeth i.e. changes of enamel layer thickness, amount of pulp tissue recession, and apposition of cementum layers, may be reliable age estimation factors.

The variables directly related to the ageing of teeth, i.e. changes in enamel layer thickness, amount of pulpal recession, and deposition of cementum may be reliable indicators in age estimation.

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