A HISTOLOGICAL PROCEDURE TO DETERMINE DENTAL AGE

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ABSTRACT

Sclerosis of dentine has become one of the well established indicators of ageing and age determination. In this study a new technique was reported where a photomicrographic image of a cross section of sclerotic dentine was converted to a grey scale of 256 tones and then reduced to black and white and read by computer using specially developed software. A regression analysis was applied to a sample of 62 teeth (age range 17-84 years) and an age determination within an error limit of 11 years was obtained. Using a Neural Network software however the error was reduced to 8 years. **(J. Forensic Odontostomatol 2000; 18:1-5)**

Key words: Dental ageing, histology, optic microscope, computer elaboration, Neural Network

INTRODUCTION

Age determination by examining the teeth is one of the useful functions of medico-legal practice in person identification. It is particularly useful as the teeth are scarcely affected by exogenous factors, which, in other procedures, can make the age determination difficult and inaccurate. As a result there has been much research into methods to link age with relevant tooth characteristics.

A milestone in the field of age determination by tooth analysis was the work of Gustafson[1] which takes into account six factors: abrasion, level of periodontosis, secondary dentine shape, cementum apposition, resorption of the root and translucency. These criteria are classified according to particular indices and then combined in a formula which is linked with age. The error of age derived by this method is within 10 years.

Almost half a century later Gustafson's work is still relevant, not only because his methods anticipated modern techniques such as that based on the fuzzy approach[2] used for classifications in various fields of interest, but also because many authors[3,4] have derived their own methods from Gustafson's work.

Generally speaking research has evolved along two routes: a) the search for more objective and measurable parameters and b) the establishment of techniques to preserve the specimens as far as possible.

Thus, Calonius *et al.*[5] analysed bony tissue formation, Boyde[6] observed the increment lines in the dental enamel, Ito[7] proposed that age be determined by a purpose built formula based on the ratio between the sum of the tooth enamel with the pulp cavity thickness and the dentine thickness.

Other papers[8,9,10,11,12,13] report that the increasing transparency of the dentine is associated with increasing age which is a phenomenon caused by the deposition of calcium salts in and around the dentinal tubules, leading to an obliteration of the tubules themselves, and consequently a reduction in their number and an increase of dentinal sclerosis. In this way, the mineralised tubules assume the same refractive index as the dentine and the section appears more transparent.

MATERIALS & METHOD

Sixty-two caries-free, non-endodontically treated teeth with up to 3 roots each were obtained from subjects in the age range 17-84 years (Tables 1 & 2).

They were decalcified, embedded in paraffin wax and the roots sectioned at four points between the lower limit of the crown and the apex of the root, giving four equal parts (Fig. 1). The root portions were then microsectioned at a thickness of 4-5 micrometres at points P1, P2 and P3 (Fig. 1). For two and three rooted teeth the slices were through only one root (a future project will compare observations between the different roots of the same tooth) and the sections for microscopic analysis were all horizontal, following the original cuts.

The sections were then stained by haematoxylin and eosin and observed by optical microscope at 1000x magnification, photographed and processed with a standardized enlargement.

The photographic prints were then transformed into bitmap images and scanned into a PC with software that converts colours into 256 grey tones (Fig. 2).

Gender	Tooth type	Age	RST
M	M	17	6.69
M	C	17	6.53
M	M	17	6.42
F	C	17	4.61
M	PM	19	5.15
M	PM	21	6.96
F	M	21	7.17
M	PM	21	5.02
F	M	24	6.5
F	C	25	7.11
M	C	26	9.17
M	M	27	6.55
M	PM	28	10.22
M	PM	28	10.05
F	M	28	11.22
M	M	28	10.32
F	PM	31	7.76
M	C	32	9.23
F	PM	34	9.12
M	M	35	8.17
F	M	38	8.46
F	PM	39	11.56
M	M	40	9.74
F	M	45	10.9
M	M	46	10.6
M	C	46	10.7
F	M	48	7.97
M	PM	54	9.52
F	C	54	9.5
M	C	54	12.12
M	PM	54	9.55

Gender	Tooth type	Age	RST
M	PM	55	12.24
F	PM	55	10.98
F	M	55	9.27
F	M	56	11.97
F	PM	56	10.29
M	C	57	12.76
M	PM	57	13.15
F	C	57	9.25
F	M	58	14.21
M	M	59	11.77
F	PM	60	12.78
M	C	60	13.65
F	C	60	13.26
M	PM	60	12.04
M	M	61	11.11
F	PM	62	13.42
M	M	64	12.18
M	PM	65	11.24
F	M	66	12.78
M	M	66	12.49
F	M	66	10.56
M	M	67	10.57
M	PM	67	11.96
F	C	69	12.02
F	M	70	8.13
M	C	70	11.34
F	PM	70	11.45
M	M	74	13.01
F	M	77	12.23
F	M	84	11.46
F	PM	84	13.12

RST = Ratio of Sclerosis to Tubules; M = Molar; C = Canine; PM = Premolar

Table 1: *Subjects and specimen teeth*

	Male	Female
Molar	13	14
Premolar	12	9
Canine	8	6

Table 2: *Distribution of cases*

This means that the image becomes black and white through a computerized mathematical procedure using special software. This phase consists of a calibration procedure where some parameters are set according to reference diagrams. In Fig. 3 the operation of recognising what is sclerosis and what is dentinal tubule is depicted. After trials by a team of observers, it was possible to establish a threshold at a particular grey tone for what the different observers agreed distinguished a tubule from sclerosis. It is obvious that this threshold mainly depends on a strictly standardized method of photographing, processing and observing the images.

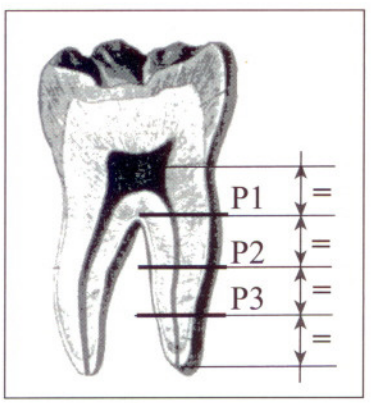

Fig.1: *The root was sectioned at four points and the microsections were obtained at P1, P2 and P3.*

The final result was a black and white image in which what appeared black was sclerosis, and what appeared white was tubule (Fig. 4). The software then automatically read the number of black pixels (representing the dentinal sclerosis area) and the white ones (representing sections through tubules).

RESULTS

The contingency coefficient is a measure of the degree of association of dependence or independence of two samples. The larger the value of this coefficient, the greater the degree of association, but if the coefficient tends to zero, the distribution of cases is random, which suggests that the cases are

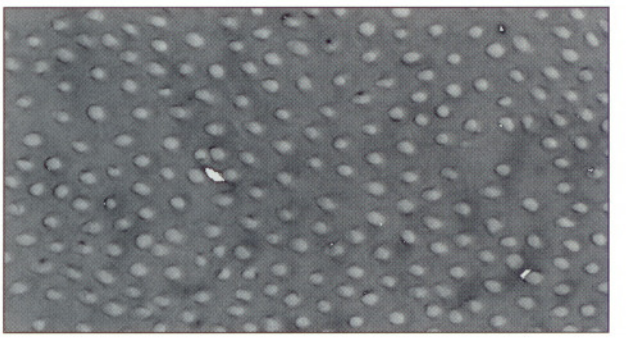

Fig.2: *A colour micrograph of dentine is converted to 256 grey tones.*

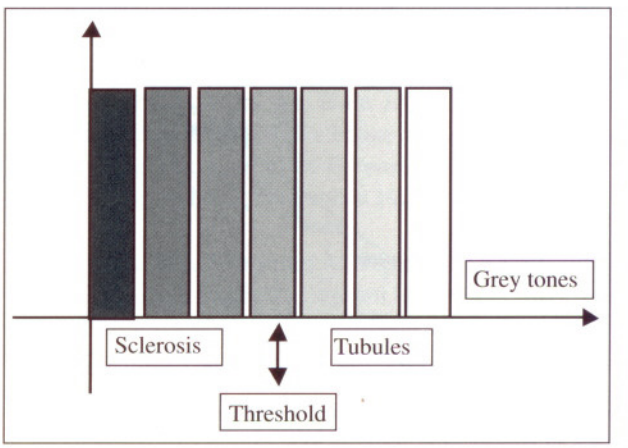

Fig.3: *Using a filter the software user must select the tones that indicate sclerosis or dentinal tubule.*

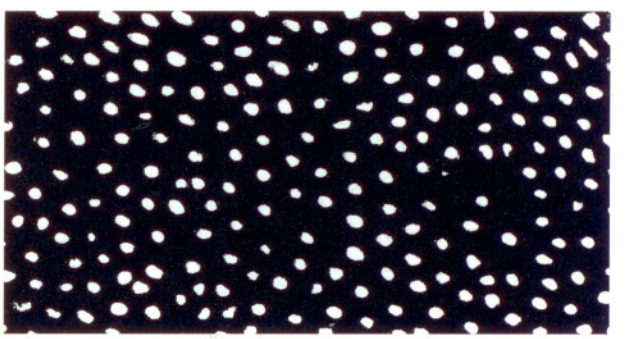

Fig.4: *A micrograph after completion of the filtering procedure.*

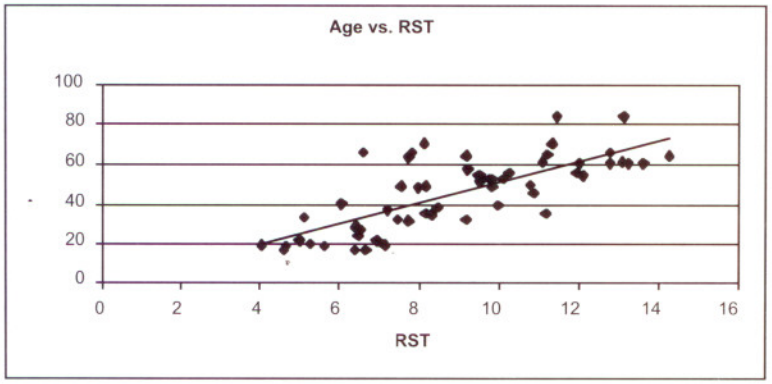

Fig.5: *Distribution of age to RST*

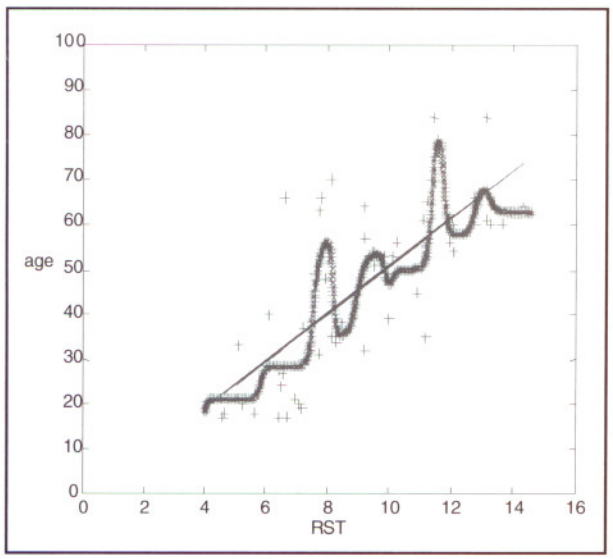

Fig.6: *Distribution of age to RST using the Neural Network software*

well distributed. In this study, the distribution of the two gender groups was well randomized as demonstrated by the contingency coefficient of 0.078.

Dentinal sclerosis was found to increase with age at a statistically significant correlation as the ratio of sclerosis to tubules (RST), representing the average sclerotic area per unit area of tubular dentine, correlated quite strongly with age (r = 0.752). See Fig. 5.

The equation Age = RST x 5.3 – 2.28 displayed the link between age & RST which then delivered an error limit of 11 years.

There was no meaningful difference in the findings between genders. By analysing them separately we found that males had a correlation coefficient r = 0.865 and females had r = 0.699. Comparing the two correlation coefficients statistically, weighted by the number of subjects, a non-statistically significant difference was found (p = 0.0975) and the two groups were therefore considered as uniform.

Three separate analyses were carried out on the three different types of teeth and correlations between RST and age for canines was found to be r = 0.846, for premolars it was r = 0.837 and for molars it was r = 0.721. Once again the differences were very small and the sample could be considered as uniform. The size of the sample however would affect the reliability of the results.

In answer to critics of the linear regression test who question its indiscriminate use in all situations we applied the Neural Network[2] (NN) approach. This is based on a computer programme which is able to interpolate limited data (named "examples" in the NN special language), and then being able to predict additional, collocated data and creating a more complete set. This method then delivered a reduced limit of error for age determination by RST of 8 years.

In addition, the Neural Network calculations gave an indication of the sensitivity of the RST in relation to age. The resulting curve (Fig.6) had horizontal and vertical components and where the vertical showed a wide age range determination the horizontal components delivered more accuracy with less sensitivity in the RST.

DISCUSSION

Initial results have demonstrated that the proposed method appears quite reliable when compared with other methods either reported or directly tested by the authors. In preliminary work [14,15] we compared the present method with two others investigating "tooth translucency" and the "analysis of the cementum thickness" [16,17] and found weaker correlations regarding RST/age determination.

While the results appear good, there is no doubt that the method is difficult to perform, mainly because of the complex procedure including cutting the roots, treating the sections, microscopic observation of the

slices, photographic processing, scanning, computerisation (after parameter calibration). It is furthermore a reality that the correlation is strengthened with an increase in the sample size and that the more frequent the performance of the procedure the more it becomes reliable because of growing confidence in the operators and the growing efficiency of the technique.

The success of the technique has therefore inspired us to pursue this research, not only to consolidate the statistics by increasing the sample size, but also to make efforts to simplify it. For example, to be able to dispense with photography entirely by allowing image scanning directly from microscope into computer and to allow automatic calibration of the grey scale and estimation of age by the Neural Network software should make age estimation simpler, quicker and more accurate.

REFERENCES

1. Gustafson G. Age determinations on teeth. J Am Dent Assoc 1950; 41: 45-54.

2. Kosko B. Neural Networks and Fuzzy Systems - a dynamical systems approach to machine intelligence. Englewood Cliffs, NJ: Prentice Hall, 1992.

3. Johanson G. Age determinations from human teeth. Odont Revy 1971 vol 22 (Supp 22).

4. Dalitz GD. Age determination of adult human remains by teeth examination. J For Sci Soc 1962; 3-11.

5. Calonius PE, Lunin M, Stout F. Histologic criteria for age estimation of the developing human dentition. Oral Surg Oral Med Oral Pathol 1970; 29: 869-76.

6. Boyde A. Estimation of the age at death of young human skeletal remains from incremental lines in the dental enamel. Proceedings 3rd Int. Meeting in Forensic Immunology, Medicine, Pathology and Toxicology, London, 1963.

7. Ito S. Research on age estimation based on teeth. Jap J Leg Med 1972; 26: 31-41.

8. Bang G, Ramm E. Determination of age in humans from root dentine transparency. Acta Odontol Scand 1970; 28: 3-35.

9. Fineschi V, Momicchioli O, Martini P, Buccelli C. Morfologia dentaria e determinazione dell'età: rilievi mediante morfometria quantitativa. Arch Med Leg Ass 1994; 16: 303-39.

10. Chomette G, Auriol M, Koulibaly M, Bellefquh S, Guilbert F, Vaillant M. Approche de détermination de l'age sur critères morfologiques dentaires obtenues en microradiographie, stéromicroscopie et microscopie à balayage. Rev Stomatol Chir Maxillofac 1986; 87: 30-3.

11. Vasiliadis L., Darling A.I., Levers G.H. The hystology of sclerotic human root dentine. Archs oral Biol. Vol. 28. No. 8. Pp. 693-700, 1983, Pergamon Press.

12. Fineschi V., Momicchioli A., Monciotti F., Nigi F., Vannuccini L. Istologia dentaria e stima dell'età: ulteriore contributo metodologico e casistico. Rivista Italiana di Medicina Legale. Anno XIX n.4-5-, pp.983-999, 1997

13. Traub H.R., Altini M., Hille J.J. A Comparison of Radicular Dentinal Tubule Size in Two Different Age Groups. The Journal of Forensic Odonto-Stomatology, Vol. 6, No 2; December 1988.

14. Amariti ML, Fornaciari M, Restori M, De Ferrari F, Paganelli C, Faglia R, Legnani G. Morphologic and quantitative analysis by computer of the dentinal tubules for the age determination. XVII th Congress of the International Academy of Legal Medicine, Dublin, 1997.

15. Amariti ML, Restori M, De Ferrari F, Paganelli C, Faglia R, Legnani G. Age determination by teeth examination: a comparison between different morphologic and quantitative analyses. J Clinical Forensic Medicine, 1999, 6: 85-9.

16. Drusini AG, Calligari C, Furlan C, Crestani VC, Bordigon D, Betti D, Frignani C, Cortivo P. Determinazione dell'età dentaria nell'adulto: metodologie e risultati. Arch Med Leg Ass 1994; 16: Suppl. 61-8.

17. Kashyap VK, Koteswara Rao NR. A modified Gustafson method of age estimation from teeth. Forensic Sci 1990; 47: 237-47.

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