ISSN 2219-6749

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The Journal of Forensic Odonto-Stomatology

Volume 16, n. 2 - Dec 1998

OFFICIAL PUBLICATION OF THE
INTERNATIONAL ORGANIZATION FOR
FORENSIC ODONTO-STOMATOLOGY

Contents

Research

GENDER DETERMINATION FROM DENTAL PULP BY USING CAPILLARY GEL ELECTROPHORESIS OF AMELOGENIN LOCUS

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ABSTRACT

Gender of dental pulp DNA was determined by the capillary gel electrophoresis (CGE) method with amelogenin locus as a marker. The analysis of the male sample resulted in two peaks, a Y-locus standard sample was added and the CGE method repeated. This resulted in an amplification of the second peak and judging from the difference between the allele sizes of the X and Y loci, it was confirmed that the first peak was for the X and the second for the Y locus. The female sample was then analyzed and one peak was observed. As before the X-locus standard sample was added to this sample and resulted in an amplification of the peak, thus verifying it to be the X locus. The CGE method was conducted for 10 male and 10 female samples and double peaks verified the male sample, while one peak verified the female sample. Gender was thus correctly determined in all cases. **(J Forensic Odontostomatol 1998; 16: 23 - 26)**

Keywords: Gender determination, dental pulp, amelogenin locus, capillary gel electrophoresis.

INTRODUCTION

Up to the present the accepted method for gender determination from the teeth has been performed by slab gel electrophoresis on dental pulp DNA, which used polyacrylamide gel (PAG) or denaturing PAG after having amplified X and Y chromosome-specific alphoid repeat sequences by the polymerase chain reaction (PCR) method.[1,2] In this technique, staining and photographing must be conducted after electrophoresis in order to record the data.

The capillary gel electrophoresis (CGE) method has now been developed to separate various proteins and oligonucleotides, in which crosslinked or linear (non-crosslinked) PAG is contained in the capillary column and the molecular sieve effect applied.[3-5] Recently, application of the CGE method to DNA typing in the field of forensic medicine has invited attention because continuous automatic analyses of multiple samples are possible, staining and photographing are not necessary and data can be input into computers as an electropherogram which can easily be stored.[6-11] This technique however is still in its infancy.

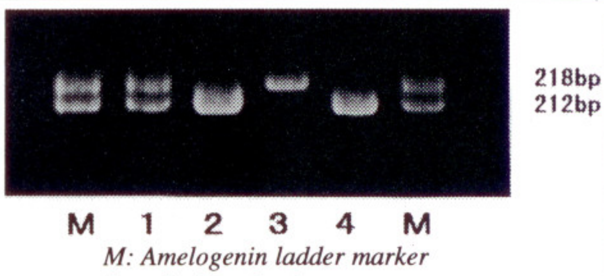
This study describes the determination of gender from DNA extracted from dental pulp by the CGE method with amelogenin locus as a marker.

MATERIALS AND METHODS

DNA was extracted by the phenol/chloroform method from known-age dental pulp obtained from teeth extracted from 10 males and 10 females and stored at room temperature for one year.[12]

The X-chromosome-specific alphoid repeat sequence 212 base pairs (212 bp: X-locus) and Y-chromosome-specific alphoid repeat sequence 218 bp (Y locus) were amplified by the PCR method according to the protocol of Gene Print™ Sex Identification System-Amelogenin kit (Promega)* with dental pulp DNA10 ng as a template DNA. The short tandem repeat (STR) 2 x Loading Solution was then added to obtain PCR products for the CGE method.

The X and Y loci amplified by the PCR method were detected by 10% polyacrylamide gel electrophoresis and silver staining. The individual DNA band was then cut and washed with Tris-HCl, EDTA and DNA was again extracted by the phenol/chloroform method. The extracted DNA was amplified by the PCR method and the amplification of the X and Y loci confirmed (Fig.1).[11] These PCR products were used as standard samples.



M: Amelogenin ladder marker
1: male dental pulp
2: female dental pulp
3: Y–locus standard sample
4: X–locus standard sample

***Fig. 1.** Gender determination from aged dental pulp DNA by the PAGE method of amelogenin locus*

* *Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711-5399, USA*

** *Waters Chromatography Division, Nippon Millipore Limited, 1-3-12 Kitashinagawa Sinagawa-ku, Tokyo 140, Japan*

The Quanta 4000 system** was the CGE equipment used and the capillary column with an inner diameter of 75μm and a length of 35cm was filled with PAG (T=8%, C=0%). After the STR 2 x Loading Solution was added to the PCR products of amelogenin locus in all samples they were introduced into the column by electromigration (200 v/cm for 80 sec.) and electrophoresis was carried out at 260 v/cm for 55 minutes, using the buffer for electrophoresis containing 0.1 M of tris-boric acid and 7 M of urea. X and Y loci were detected by 254nm UV absorption.

RESULTS AND DISCUSSION

1. Identification of each peak in electropherogram

The peaks of the reagents of STR 2 x Loading Solution [dNTP, bromphenol blue (BPB) and xylene cyanol (XCFF)] were observed in addition to those of X and Y loci when the CGE method was performed on PCR products of the sample DNA. We attempted to identify each peak by doing electrophoresis for each reagent individually and Fig.2 shows that several high peaks appeared at about 15 minutes of migration time, and a peak at about 25 minutes when the CGE method was performed for PCR products of the negative control. After only dNTP was tested by electrophoresis, further electrophoresis was carried out for a mixture of dNTP and primer. The results confirmed a first peak for dNTP and a second peak for primer.[9,10]

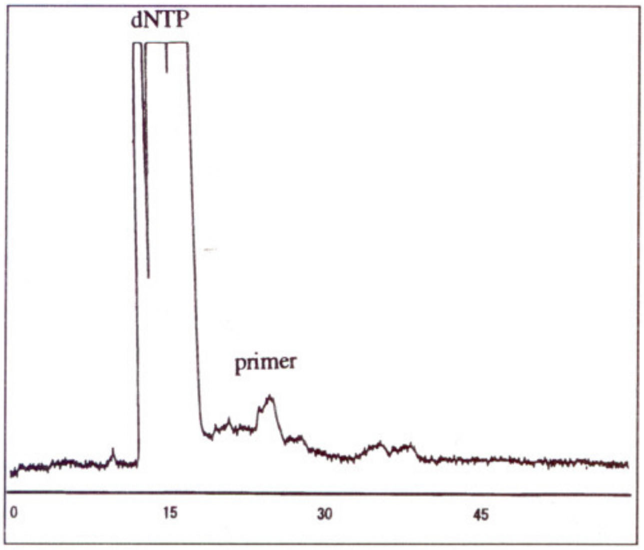


Fig. 2 Electropherogram of negative control

STR 2 x Loading Solution was then added into the PCR products of the negative control. The results showed a high peak at about 20 minutes of migration time, following the primer's peak, and another peak after about 50 minutes. Fig.3 shows that the peaks appearing at about 20 and 50 minutes were for BPB and XCFF respectively[9,10] when dNTP and BPB or XCFF were mixed for electrophoresis.

Because the Quanta 4000 system does not have the function to maintain a constant temperature, an increase

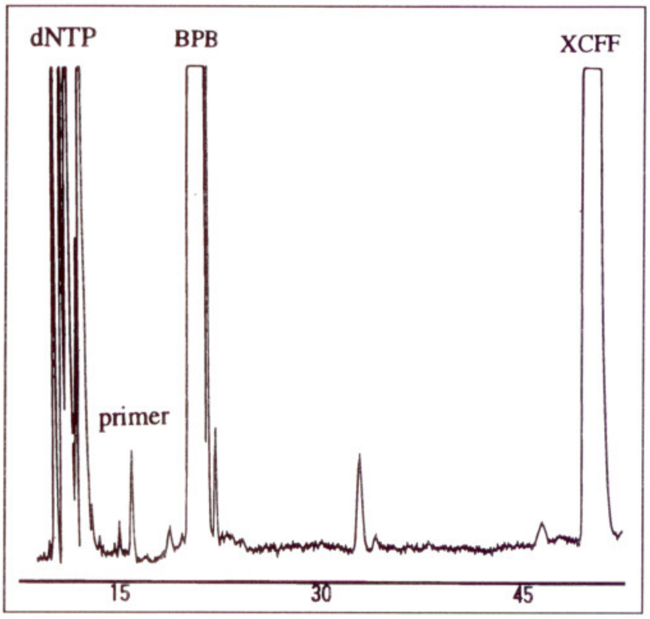


Fig. 3 Electropherogram of negative control and STR 2 x Loading Solution

of temperature of the capillary gel and the buffer for electrophoresis, caused by the flow of electrical current, is unavoidable. Thus, about one minute of difference in the migration time of each peak for reagents occurred during each of the electrophoresis runs, but because each peak did not always overlap and the migration times of BPB and XCFF were observed almost each time, the peaks obtained were considered to be strongly indicative of gender determination,[9] at an optimal temperature of about 24°C.

When the CGE method was conducted with the capillary column filled with PAG of non-crosslinking at 8% concentration or of 0.5% crosslinking at 5% concentration, almost no difference in separation ability and electrophoresis time was found. However, when the latter PAG was used, degeneration of gel was fast and the separation ability was decreased.11

2. Identification of X- and Y-locus peaks in electropherogram

Fig.4 shows that a peak (arrowed) appeared at about 40 minutes when the CGE method was conducted with known female dental pulp DNA. X-locus standard sample was added in equal volume to this sample for PCR amplification and the CGE method was conducted for resulting PCR products. Fig.5 shows an apparently amplified peak seen at about 40 minutes, which was confirmed as the X-locus.

Fig.6 shows two peaks (arrowed) which appeared about 40 minutes later when the CGE method was conducted with known male dental pulp DNA. Judging from the difference between the allele size of the X- and Y-loci, it was confirmed that the first peak was for the X-locus and the second for the Y-locus. The Y-locus standard sample was added in equal volume to this sample for PCR amplification and the CGE method was conducted for

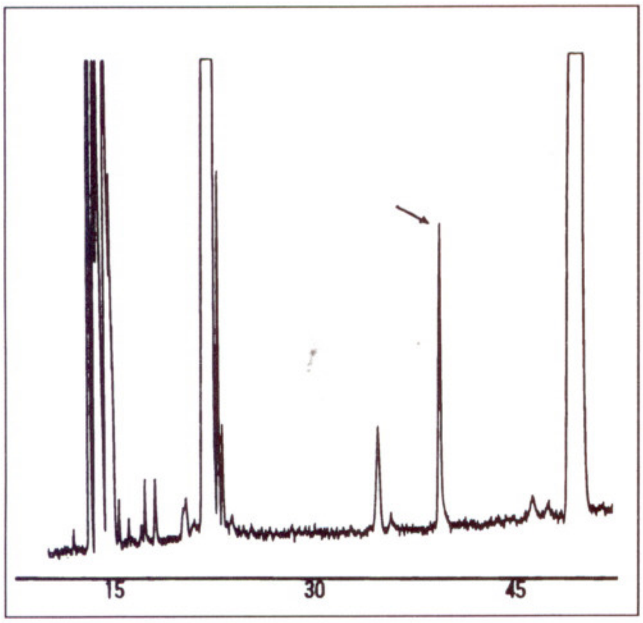


Fig. 4 Electropherogram of female dental pulp DNA

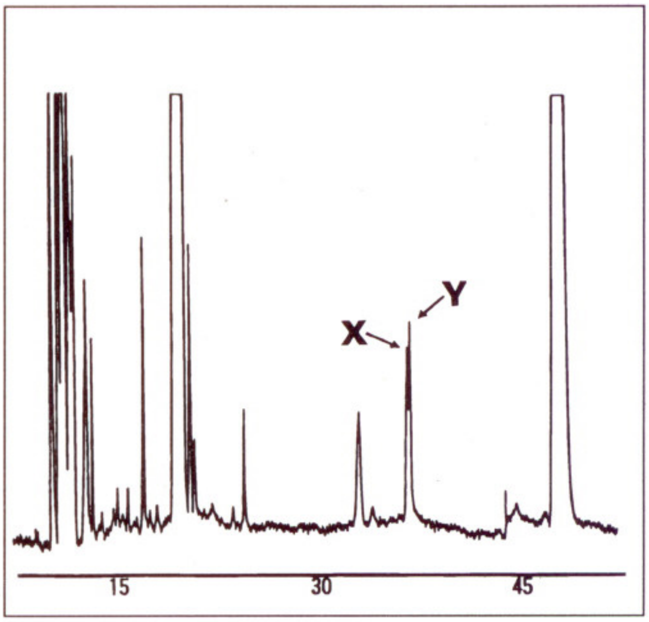


Fig. 6 Electropherogram of male dental pulp DNA

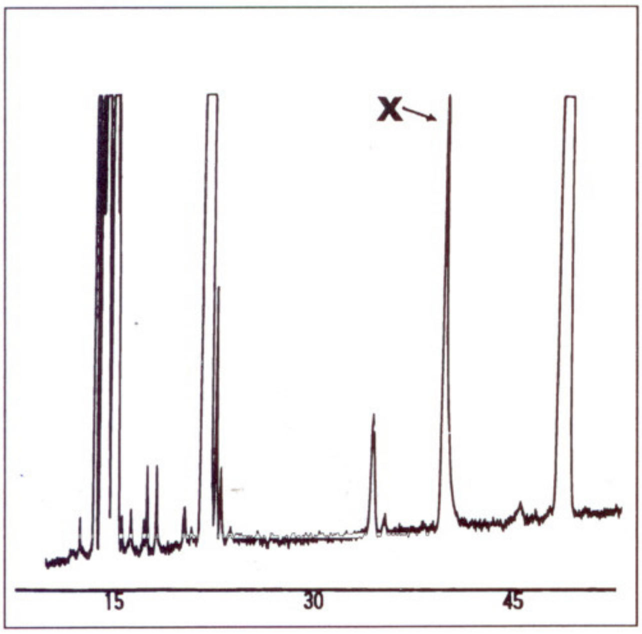


Fig. 5 Electropherogram of female dental pulp DNA and X–locus standard sample

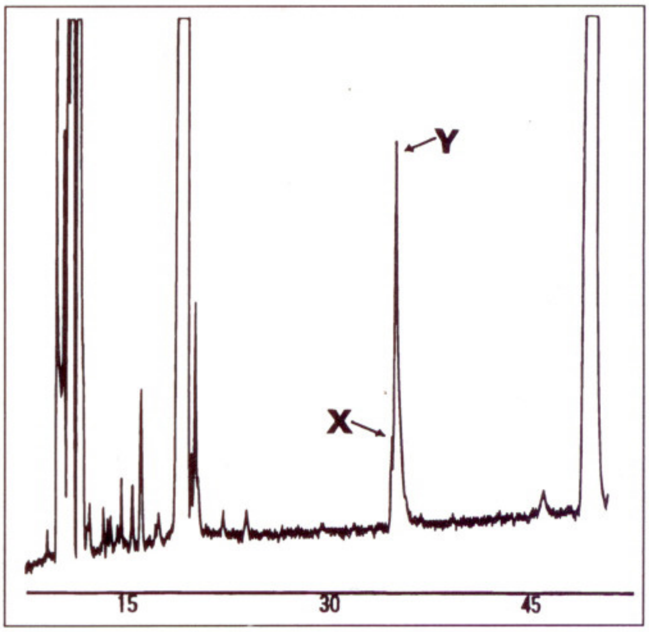


Fig. 7 Electropherogram of male dental pulp DNA and Y–locus standard sample

resulting PCR products. Fig.7 shows an increase in the second peak.

In the VNTR analysis by the CGE method using the Quanta 4000 system, PCR products were subjected to electrophoresis simultaneously with the ladder markers and the alleles evaluated by observing the relative increase in peak height.[8,10] However, as for gender determination, as observed in the results of this study, it was clearly shown that it is not necessary to make simultaneous electrophoresis runs of PCR products and ladder markers and that gender determination from the electrophoretic pattern alone is possible.

3. Gender determination from dental pulp

The peaks of X- and Y-loci in the male samples and the peak of X-locus in the female samples emerged at about 40 minutes of migration time when the CGE method was conducted for known-age dental pulp DNA in 10 males and 10 females. Gender determination was therefore possible in all cases and these results were identical to those determined by slab gel electrophoresis.

In some cases of slab gel electrophoresis, DNA typing may be difficult because the DNA band may not correspond with the ladder marker, electrophoresis images may be distorted, or the number of bands, for example, numbers one or two, may be unclear. On the other hand the CGE method enables detection of peaks of X- and Y-loci as migration times[10] and facilitates identifying gender. We have also been able to discriminate the differences of six base pairs by the CGE method in contrast with previous work where only one base pair was discriminated.[9,13] This makes the CGE method of gender determination an important addition to the technology in the forensic sciences.

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HUMAN DENTINAL STRUCTURE AS AN INDICATOR OF AGE

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ABSTRACT

Odontological data can constitute essential evidence for age estimation in forensic work. In this research the structural differentiation of dentine was correlated with chronological aging after SEM and EDXA examinations were performed on extracted human teeth ranging in ages between 25 and 74 years. EDXA showed that the spherical structures within the dentine observed were comprised of calcium phosphate. They were then counted and their state of fusion noted and the statistical "Z" test applied to the different age groups. The results demonstrated a correlation between the fusion of calcospherites and age in all layers of the dentine. **(J Forensic Odontostomatol 1998; 16: 27 - 29)**

Keywords: Forensic odontology, age estimation, dentinal age, calcospherites

INTRODUCTION

It is well documented that tooth structure shows some alteration during aging.[1] Gustafson[2] developed statistical methods for age determination based on individual, fully developed and erupted teeth where he looked for changes including attrition, gingival recession, secondary dentine apposition, root resorption, secondary cementum apposition and root transparency, but he excluded the third molars. Chronological age has been determined from the developmental status of third molars,[3] from macroscopically observed attrition[4] and from the increase in the chroma of the tooth. Microscopically observed aging changes are seen in the shrinkage of pulp tissue, the presence of a predentine layer or in the dense dentinal tissue which can be observed by scanning electron microscopy (SEM). The origin of these changes may usually be found in the structural changes of dentine, and the importance of the developing dentition in forensic odontological procedures has been demonstrated.[5]

The purpose of this study was to examine the above-mentioned structural changes in tooth dentine and to correlate them to aging, which could be useful in identification and other aspects of forensic odontology.

MATERIALS AND METHODS

In this study 35 newly extracted human incisal teeth, seven each from five age groups ranging from 25 to 74 years were examined by SEM* coupled to an EDXA.**

All tooth samples were embedded in elastomeric impression material† in a steel mold and sectioned labio-lingually in the middle from the crown to the root. A diamond separating disc,†† guided by a lingitudinal groove in the mold (Fig.1) was used, the sections were then coated with gold-palladium and observed by SEM at 100X magnification. The coronal dentinal area was divided into three parts (outer 1/3, middle 1/3 and pulpal 1/3) and the SEM focus was directed towards the centre of each level of dentine for every tooth observed, then photographed (Fig.2). The mineral composition of the sphere structure was determined by EDXA and in addition the spheres were measured, counted and a ratio for calcospherite fusion calculated using the formula:[6]

$$\text{Calcospherite fusion} = \frac{\text{No. of fused calcospherites}}{\text{Total No. of calcospherites}} \times 100$$

The results were then correlated by a statistical "Z" test with the age of the teeth.

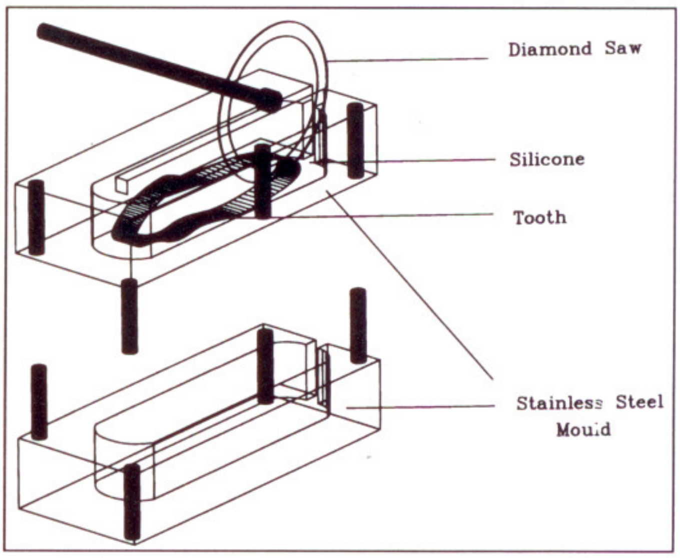

***Fig. 1** Each tooth was sectioned labio-lingually in the middle.*

* *Jeol, JSM-6400 Scanning Microscope, Tokyo, Japan*

** *Tracor Northern TN-5500 Energy Dispersive X-Ray System, Middleton, WI, USA*

† *Optosil P and Xantopren VL. Bayer Dental Levenkusen/Germany*

†† *Komet Dental Gebr. Brasseler GmbH & Co. KG Lemgo, Germany*

RESULTS

The mineral composition of the spheres was demonstrated by EDXA and was found to be principally calcium phosphate (Fig.3). The results of dentinal calcospherite numbers and their correlation with age is given in Table 1.

Table 1: Age/calcospherite correlation in tooth dentine

No. of Group	Age Group	Outer 1/3	Middle 1/3	Pulpal 1/3
1	25-34	C	C	C
2	35-44	C	C-FC	FC
3	45-54	C	FC-NC	NC
4	55-64	C-FC	FC-NC	NC
5	65-74	NC	NC	NC

C : Quantifiable calcospherites
FC : Calcospherite boundaries are not distinguishable
NC : Near-total fusion and unquantifiable individual calcospherites

The results of calcospherite determination showed that in the first age group (25-34 years) they were discrete and quantifiable in all areas and there were no statistically significant differences between all three areas (Fig.4). However, in the second age group (35-44 years) fusion coupled with quantifiable calcospherites (FC) were detected in the middle and pulpal thirds, and this group showed statistically significant differences between the outer and pulpal third areas ($p<0.05$). In the third age group (45-54 years) fusion was observed only in the middle third, but in the fourth age group (55-64 years) fusion of calcospherites occurred both in the outer and middle thirds (Fig.5). Lastly, non-quantifiable (NC) nearly-total fusion was detected in the third and fourth groups in the middle and pulpal thirds while in all parts in the fifth age group (65-74 years), there were no statistically significant differences between the three sections (Fig.6).

When all the groups were correlated for all three sections it was observed that the decrease of quantifiable calcospherites in the pulpal thirds between the first two groups was statistically significant ($p<0.01$) and also that the fusion of calcospherites between the second and third groups was statistically significant ($p<0.01$).

DISCUSSION

Dental structures are composed of calcium phosphate crystals[1,6] and this was confirmed in this study by the SEM and EDXA methods and furthermore that with aging these spheres fuse together to form a mass of matter where the calcospherites become unquantifiable. It was found that in the age group of 25-34 years the calcospherites in all dentinal areas were discrete, but after the age of 35 years fusion of calcospherites began, although some were still discrete. Fusion of the dentinal tissue increased with age and at 65 to 74 years a total fusion was detected in all three areas. In a 50-year period therefore the structure of dentinal tissue changes totally from discrete, quantifiable calcospherite configuration to an amorphous state. A correlation between calcospherite fusion and dentine in vitamin D-resistant rickets has also been reported.

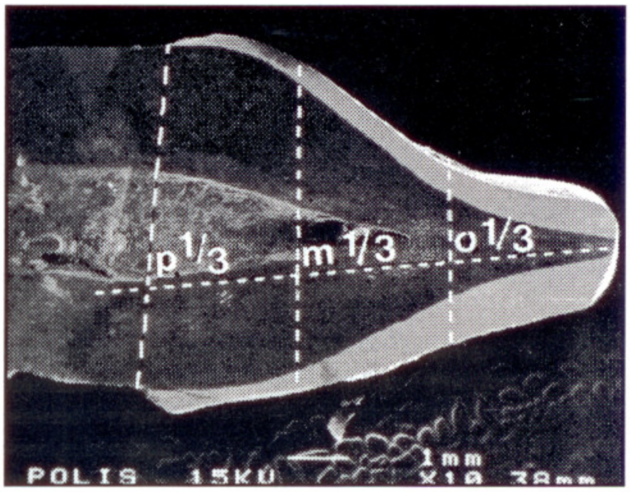

Fig. 2 Dentinal areas (outer 1/3, middle 1/3 and pulpal 1/3).

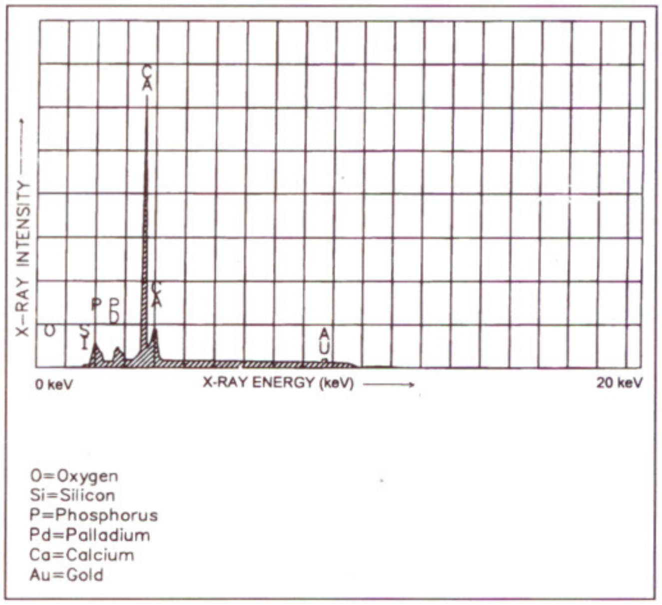

Fig. 3 EDXA spectrum of calcospherite

Kosa *et al.*,[1] in their SEM/EDXA investigation of dentine, found that the Ca/P weight ratio decreases with age and because of mineralization of dentine around the dentinal tubules, they found a narrowing of tubules in time. In other research[7] it was found that Ca, P and Mg ratios determine the mineral deposits in tooth structure with aging.

The present study showed a commonality with the results of these authors, and the statistical results demonstrated that age estimation by the determination of calcospherite fusion could be reliable.

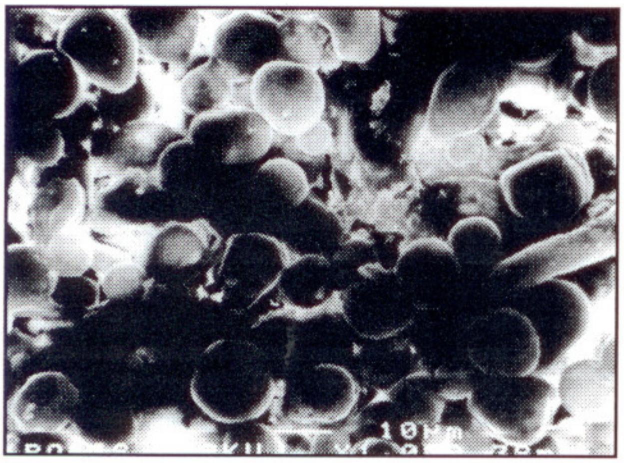

Fig. 4 Quantifiable calcospherites. Age 30 (outer area). X1000

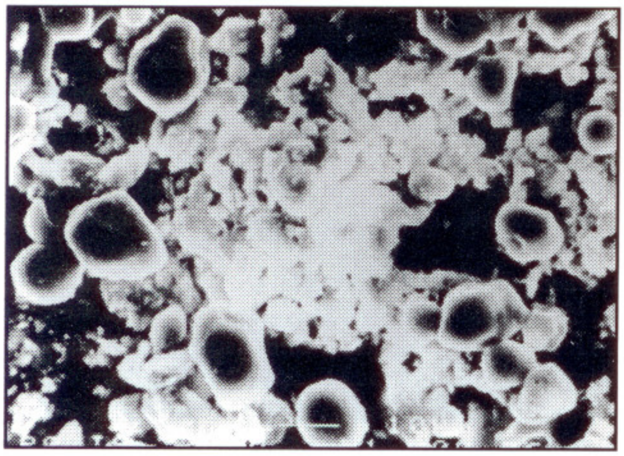

Fig. 5 Fusion coupled with quantifiable calcospherites. Age 37 (pulpal area).

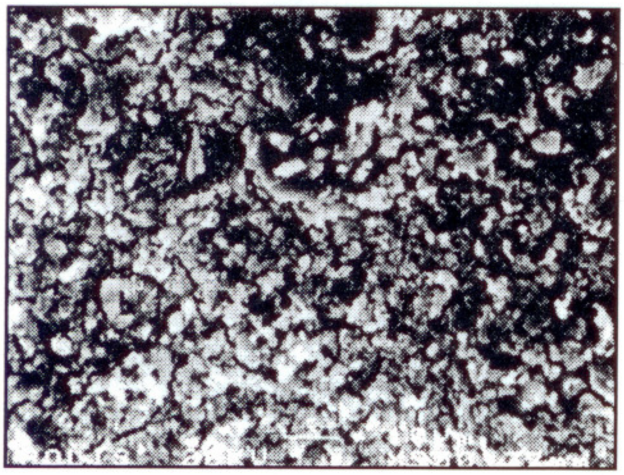

Fig. 6 Total fusion, unquantifiable calcospherites. Age 57 (pulpal area). X1000

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BITEMARKS IN FORENSIC ODONTOLOGY

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ABSTRACT

Person identification is important in criminology, and forensic odontologists are key personnel for identifying a highly individual dentition which could cause a bitemark and which could be used to convict or exculpate a suspect. Bitemarks may be observed in skin, wax, from a dental model indirectly from a photograph, a scanned image of a dental model or food. This paper shows that bitemarks constitute reliable evidence for person identification and that a tolerance of approximately 1mm existed between measurements of a wax bite, dental model, photograph and computer scanned image. **(J Forensic Odontostomatol 1998; 16: 30 - 34)**

INTRODUCTION

Person identification is an important branch of the forensic sciences, and in Turkey finger prints and DNA tests are popular techniques for this purpose, but dental and prosthetic data may also be useful.

Research and practice of forensic odontology have gained momentum since the Second World War and one of the notable earlier cases has been the identification of Adolph Hitler's body from his teeth.[1] It is now widely accepted that tooth alignment, configuration, angulations and occlusion are as individual as fingerprints, and coupled with dental restorations, make dentitions unique so that individuals can be identified with certainty from their teeth.[2,3] Furthermore, in murder and rape cases, criminals often leave bitemarks on the victim, or in food-stuffs such as apple, cheese, chewing gum or chocolate. These bitemarks may show unique characteristics which allow matching of the bitemark to the biter.[2,4]

Sperber[5] identified a murderer from chewing gum and the saliva on it which was the only evidence present. It has also been reported that bitemarks into cloth have been regarded as acceptable evidence.[6] A guilty individual could be identified out of 114 suspects by matching a bitemark with dental casts[7] as was an unknown corpse from dental data and dental casts.[8] In another case[9] a criminal confessed his crime as a result of evidence from bitemarks, dental models and photographs.

Recently computer programs are being used to assist in establishing dental identity producing data which are regarded as sophisticated and reliable.[10-12] In Turkey research in forensic odontology has included investigation of bitemarks and this report reviews techniques currently used for this purpose.

MATERIALS AND METHODS

Two volunteers (1 and 2) bit themselves on the arm and also bit into two different waxes. The bites were photographed and measured and compared with the measurements of casts of the dentitions of the volunteers and also compared with image projections of these models on computer screen.

Skin and photographic records: The photographs were taken by positioning the camera perpendicularly over the bitemarks and taking multiple views with a calliper gauge included as a reference scale. The same instrument was used to measure all the bitemarks (Fig.3). In order to prevent photographic error the dimensions of the calliper gauge in the photograph were reconstituted from the real dimensions of the gauge by using the arithmetical averages of the photographic measurements and calculated from the photographs. As bitemarks fade, the dimensions had to be recorded immediately with the gauge at a 0.02mm level. The mesio-distal measurements of the incisor teeth and the arch widths between the canines and first premolars were recorded from both the photographs and the skin (Figs.2 and 3).

Wax records: The bite records were taken in centric relation. In this research two different waxes were used, one was the pink base plate wax* and the other was a rigid blue wax (Surgident Bite Wafers).** The strength of the Surgident Bite Wafers wax was increased by placing a piece of aluminium foil between two layers which also prevented contact between upper and lower teeth. For measuring the wax bitemarks, tooth and arch dimensions were determined with the calliper as before.

* *Dentsply-Detrey, Weybridge, Surrey, UK*
** *Surgident, Columbus Dental, St Louis, USA*

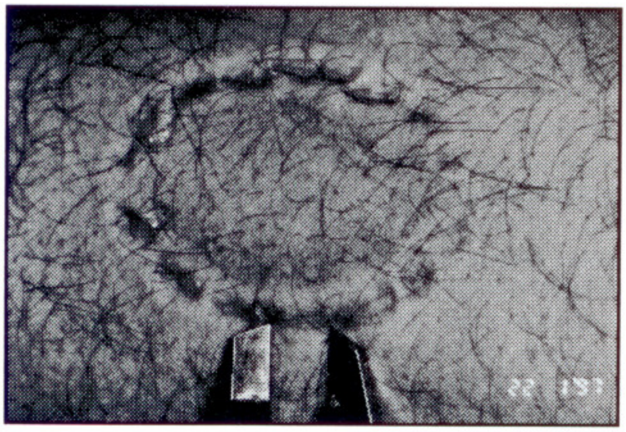

Fig. 1 Bitemark of volunteer 1 showing perfect intercuspal relation.

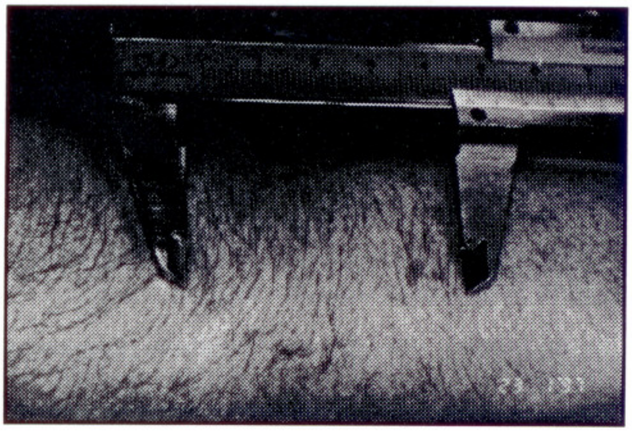

Fig. 2 Bitemark of volunteer 2 with open bite and absence of a maxillary lateral incisor tooth. Caliper gauge used in the measurements is shown.

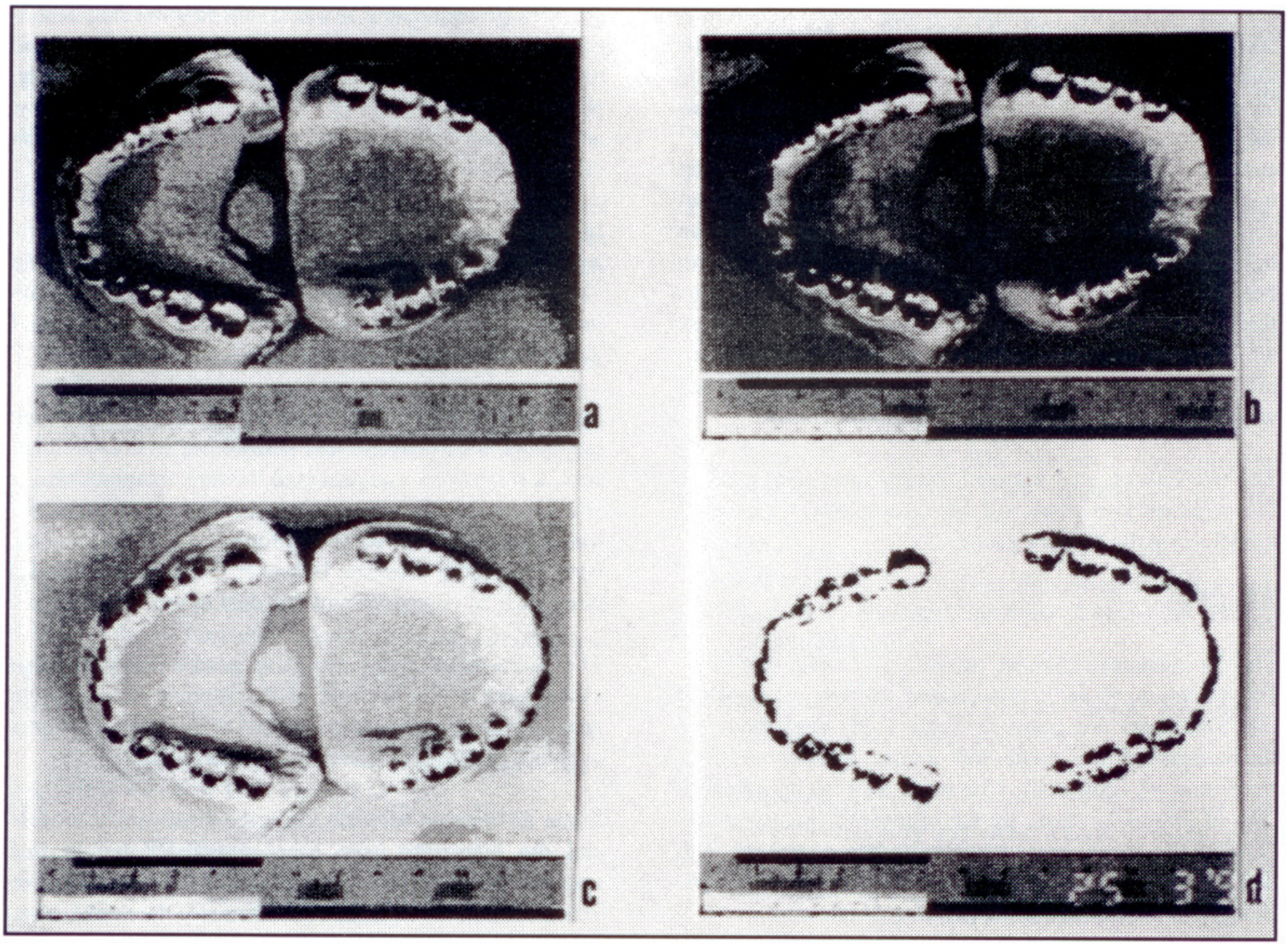

Fig. 3 *a. Transfer of a marked model to the computer by a scanner*
b. "Colour decreasing gray scale program" application over the full colour image.
c. "Gamma correct" command application for colour elimination which causes highlighting of bitemarks.
d. Incisal and occlusal surfaces highlighted by erasing all the colour except the black marks.

Model records: Dental casts were made of the subjects' teeth using alginate impression material† and plaster of Paris. The same mesio-distal dimensions of the teeth and arch widths between canines and first premolar were measured.

The tooth and arch measurements were performed by two observers three times and the arithmetical averages calculated.

Digital transfer of models to a computer program: A computer and scanner (HP Vectra PC and HP 4P Scanner)†† were used to convert a three-dimensional dental model to a two-dimensional digital format.

For imaging the occlusal surfaces differentially from the three-dimensional dental model and to accentuate them, the occlusal surfaces of the teeth were marked in black, then scanned and digitized. A bitmap software was used for determining the highlights of the image, which was taken from the digitized analog image of the scanner. For this purpose Paint Shop Pro 3.0 bitmap software was used†† (Fig.4a, in colour). The colour of the image was then decreased with the "colour decreasing gray scale" command and turned to a black-white coloured bitmap modified duplicate (Fig.4b). By using the gamma-correct command, the gray tones faded away and the image was lightened, which caused the black occlusal marks to become more prominent (Fig.4c). A colour eraser was finally used to clean out all the colours other than black which showed the tooth surfaces clearly. This step provided a clean map of the occlusal markings (Fig.4d) which was further darkened for maximum clarity.

The two-dimensional scanned images were then printed, measured and compared with the dimensions of the dental model using the calliper gauge.

RESULTS

The data obtained from the models, wax bites, skin bites, skin photographs and computerized scanned traces of the two volunteers are given in Table 1. As seen from the dimensions of the teeth and arches differences of a maximum of 1mm existed between each technique. The data of bitemarks taken by the reinforced blue wax was close to the model measurements (when compared with soft pink wax) and a discrepancy of 0.2mm was noted, thought to be because of the bending of the wax.

A notable finding during the experiment was the bitemark of the case having perfect intercuspal relationships, which were deep and long lasting (Volunteer 1) (Fig.2). In the case of Volunteer 2 who had an open bite and an absent upper lateral tooth, the bitemarks only occurred where the teeth occluded (Fig.3).

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†† *Hewlett-Packard Co. Singapore*

The result of two-dimensional computer image measurements was reliable compared with three-dimensional model measurements, which were taken with the gauge and calculated by arithmetical ratios, because when measuring the three-dimensional models errors could have arisen due to slipping of the gauge beaks from the reference points. The computer images were particularly reliable when the colour reducing technique was used as in this technique an individual's arch shape and dimensions, tooth arrangement and tooth dimensions could all easily be compared with a photograph, in this case of the bitemark.

DISCUSSION

A bitemark left on a victim's body or in a piece of bitten foodstuff or chewing gum left behind at the scene of a crime can constitute an important item of evidence. However, recording these marks and their evaluation is a complex procedure and as a first step an immediate record of the bitemark is essential, because its form and dimensions may change rapidly.[2,14] Additionally, photographs must be taken without distortion, and they, and computer images must include a scale for calculation of actual dimensions.[2,13]

In this research the camera was positioned horizontally,[2,12] perpendicular to the bitemark and multiple views of the bitemarks taken with the calliper gauge scale included, which assisted in calculating the actual dimensions.

Luntz[15] has stated that wax records cannot reproduce bitemarks accurately. In this study bites in conventional wax, a reinforced wax and in skin were compared and it was found that the waxes gave results very close to the true dimensions but when reinforced, the measurements were even more accurate. However, the results from wax bites must be compared with the data of other techniques.

The three-dimensional model measurements may also cause some errors because of the slipping of the gauge beaks from the reference points and in this project averages of multiple measurements were used.

Some differences between the three-dimensional models and the bitemarks have been observed[16] but in this research the averages of multiple measurements gave variations between 0.1mm and 1.2mm for the model, wax, skin and computer data. The maximum measurement discrepancies occurred when measuring the arch widths in wax. Three-dimensional bitemarks show distortion[3] therefore this author recommends computer graphics for transferring three-dimensional images, on which measurement results close to the skin records are obtained. In this study, the arch measurements of two-dimensional images of models were accurate.

A special computer program called "shape comparison interactive program"[10] for two-dimensional records has been used. It is claimed that when distortion problems of recording and measuring bitemarks are solved they may be used as reliable evidence.

In order to overcome these difficulties and to obtain life-sized records, direct measurements of three-dimensional skin, wax and models were compared with two-dimensional indirect photographic and computer scanner records. The aim was to establish the accuracy of these techniques, and it was shown that when the three-dimensional models were scanned to two-dimensions and the measurements converted by arithmetical means, tooth and arch measurements could be obtained without deformation and the technique of measuring computer images was found to be more accurate when compared to other techniques.

Research into methods of person identification in crimes is an important activity which may assist the courts. Investigations should be multi-disciplinary and should include at least police, forensic pathology, forensic odontology and photography.

CONCLUSIONS

1. Bitemark data obtained directly from skin, wax models, photographs or scanned images can be regarded as reliable evidence in identity determination, and as unique as finger prints.

2. Computer programs for this purpose have been developed using a scanner and an image processing software. A three-dimensional model of a bitten object can be converted to a two-dimensional image which can then be measured.

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Table 1: Measurements of bitemarks from four different media

Volunteer 1	Skin	Wax	Cast	Scanner
Upper right 1	8	8.2	8.3	8.2
Upper left 1	8	8.2	8.1	8.05
Upper right 2	6.8	6.4	6.5	6.7
Upper left 2	absent	absent	absent	absent
Upper right 3	5.8	6.3	6.2	6
Upper left 3	5.9	6.2	6.4	6.3
Upper right 4	6.4	6.9	6.4	6.4
Upper left 4	6.1	6.4	6.1	6.1
Lower right 1	6.5	5.4	5.6	5.7
Lower left 1	5.3	5.3	5.4	5.3
Lower right 2	5.7	5.9	5.9	5.9
Lower left 2	5.6	6	5.9	5.7
Lower right 3	6.2	6.2	6.1	6.3
Lower left 3	5.6	5.6	5.8	5.8
Lower right 4	absent	absent	absent	absent
Lower left 4	6	6.5	6.2	6.2
Upper 3-3	33.3	32.5	32.8	33.3
Upper 4-4	45	44.5	44.8	45
Lower 3-3	29.2	30	28.9	29
Lower 4-4	36.9	36.8	36.4	37

Volunteer 2	Photograph	Wax	Cast	Scanner
Upper right 1	8.2	9.1	9.1	8.9
Upper left 1	8.4	9.3	9.1	9
Upper right 2	6.3	6.5	6.2	6.3
Upper left 2	6.4	6.3	6.1	6.3
Upper right 3	5.9	6.6	6.6	6.2
Upper left 3	6.3	5.9	5.9	6
Upper right 4	Not measurable	7	6.7	6.8
Upper left 4	Not measurable	7	6.8	7
Lower right 1	5.7	5.7	5.6	5.7
Lower left 1	5.6	5.8	5.6	5.6
Lower right 2	5.2	6.2	6	5.9
Lower left 2	5.5	6	5.9	5.9
Lower right 3	6	6.2	6	6
Lower left 3	6.1	6.4	6.2	6.2
Lower right 4	Not measurable	7	6.7	6.8
Lower left 4	Not measurable	7.4	7.1	7.3
Upper 3-3	37.1	37.9	37.2	37.1
Upper 4-4	Not measurable	46.7	45.8	46
Lower 3-3	30.15	30.08	31.3	30.25
Lower 4-4	Not measurable	40.04	39	40

DENTAL CONDITION AND IDENTIFICATION MARKING OF DENTURES IN HOMES FOR THE ELDERLY IN GÖTEBORG, SWEDEN

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ABSTRACT

Denture marking is of crucial importance especially in homes for the elderly where dentures could be misplaced, particularly during cleaning by staff where there is a chance of loss or mix-up. Recent research regarding denture marking in homes for the elderly shows that only about 50% of the dentures were marked and that the issue should receive attention. The aim of this study was to investigate the number of subjects with natural teeth and the number of edentulous subjects in homes for the elderly in Göteborg, Sweden. The results of the present work show that only about 35% of the complete dentures were ID-marked. Thus, even if the number of complete denture wearers is few, in Sweden presently there are predictions that dentures will become more common in the future, and also in other parts of the world owing to the socio-economic conditions of today and likely in the future. **(J Forensic Odontostomatol 1998; 16:35 - 37)**

Keywords: Forensic odontology, edentulism, denture, ID-marking

INTRODUCTION

The importance of denture marking in identification of deceased persons has been pointed out by several authors[1] and according to Keiser-Nielsen[2] it was first mentioned about 70 years ago, while the frequency of ID-marking in Europe at present has been shown to be very variable (2.5 to about 50%).[3-6] It is important to consider that denture marking could have contributed significantly to the identification of deceased in several mass disasters and isolated forensic odontology cases.[3,7-9]

In Sweden the first recommendation from the National Board of Health and Welfare regarding denture marking was issued in 1960 (last revision 1986[10]). Denture marking is of crucial importance especially in homes for the elderly where dentures could be misplaced, particularly during cleaning by staff where there is a chance of loss or mix-up. Recent research regarding denture marking in homes for the elderly shows that only about 50% of the dentures were marked and that the issue should receive attention.[6]

The first aim of this study was to investigate the number of subjects with natural teeth and the number of edentulous subjects in homes for the elderly in Göteborg, Sweden as part of a quality control in Community Dental Care. The second aim was to record the frequency of identification marking of maxillary- and mandibular-complete dentures which is part of a systematic investigation of the technical and clinical aspects of denture marking with special reference to forensic odontology.[11-14]

MATERIAL AND METHODS

The dental condition and frequency of denture marking was studied in 1715 residents (1166 females, 549 men) in 12 nursing homes in Göteborg, Sweden. The number of residents in each facility was between 59 and 214 and the age range was 55 to 105 years. This screening investigation was performed by dental nurses and dental hygienists who had experience in the field of gerodontology under the supervision of the chief of the dental clinic at Wasa Hospital, Community Dental Care (I.S.).

RESULTS

There were 1215 dentures (maxillary and mandibular complete dentures, partial dentures not included) among the 1715 patients and only about 35% of the dentures were marked. Detailed data regarding the patients with natural teeth will be presented elsewhere.

DISCUSSION

The frequency of edentulousness varies from one country to another.[15] In Great Britain edentulism has been reported to be as high as 80-88%[16,17] while in the Nordic countries the prevalence is much lower,[15] but there are some differences within the region.[15,18-21] The reported frequencies are: 2.2-71% (Sweden), 59% (Denmark), 47% (Norway) and 80% (Finland).

Furthermore, the frequency of edentulism is strongly dependent on such factors as age, gender, location and if the person is hospitalized or not. The frequencies mentioned above are therefore difficult to compare and should be considered as a demonstration of a variable population. In a recent study among 88 year-olds Lundgren found that 54% of the elderly who lived in their own homes were edentulous whereas the figure was 71% of institutionalized persons.[22]

It has to be remembered that since a denture is a removable object it is not an absolute proof of identity but a mark can be very helpful to police work as it leads the investigation in the right direction, as well as saving time and money. In a study of fire victims in the Nordic countries, 25 further individuals could have been identified if their dentures had been marked.[9] Of course, it could be argued that major disasters which include many victims receive much more attention than single forensic odontology cases, but it could still be said that each case constitutes 100%.[23] For other ethical reasons it should also be stated that all persons should have the same opportunity to be informed about and offered the possibility of having their dentures marked even though the identification of dentures mainly concerns the living elderly and their daily routines.

Denture marking should not be considered only as a national issue but the international dental profession should be promoting its use among its members worldwide and for the benefit of denture wearers everywhere. In this context it is important to mention projections made[24] of the need for dentures in the future which show that there will still be about 9 million elderly persons in need of complete dentures 25 years from now. Ainamo and Österberg have also predicted the need for dentures in the Nordic countries.[15]

CONCLUSIONS

Only about 35% of the complete dentures (partial dentures not included) in our survey were ID-marked. The issue of denture marking should be considered not only on a national but on an international basis and the world dental profession should be promoting its use for the benefit of all denture wearers. Even if the number of complete denture wearers is few, in Sweden presently there are predictions that dentures will become more common in the future owing to the socio-economic conditions of today and likely in the future.

ACKNOWLEDGEMENT

We hereby gratefully acknowledge the support provided by the Community Dental Care, Göteborg, Sweden. This study has been presented at the IALM'97 (International Academy of Legal Medicine), Dublin, Ireland, August 19-23, 1997.[25]

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AUTHOR RESPONSE TO WAITE ER, COLLINS MJ. AGE ESTIMATION FROM RACEMIZATION RATE USING HEATED TEETH J. Forensic Odontostomatol 1998; 16:20-1

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We believe that practical research is critical for the advancement of science. It is not an accident that age estimation using soluble peptide fractions of heated teeth is similar to estimation using unheated teeth. To confirm this observation, we repeated the experiment using identical left and right teeth taken from the same person. It is well known that very similar D/L ratios are obtained in this way because organogenesis of these teeth occurs almost simultaneously.[1] The left tooth was heated for one hour at 150°C, and the right tooth was not. Although the D/L ratio from the total amino acid fractions of the heated tooth was almost twice that of the unheated tooth, the values from soluble peptide fractions from the heated and unheated teeth were almost the same (the values were 1.10 and 1.03 times higher than those from the unheated teeth; see Table 1).

Table 1: Comparison of D/L ratio in total amino acid and soluble peptide fractions from heated and unheated teeth

Actual age	Total amino acid fraction			Soluble peptide fraction		
	Unheated Right tooth	Heated Left tooth	*Times	Unheated Right tooth	Heated Left tooth	*Times
57 years old	**0.1020**	**0.2084**	**2.04**	**0.2520**	**0.2776**	**1.10**
53 years old	**0.0938**	**0.2048**	**2.18**	**0.2470**	**0.2550**	**1.03**

** (D/L ratio of heated tooth) / (D/L ratio of unheated tooth)*

As a result, we again concluded that it is essential to use soluble peptide fractions for the estimation of age when teeth were exposed to heating. We have performed many experiments with regard to heating and D/L ratios in total amino acid fractions under various temperatures and various times.[2] It is true that heating can cause high D/L ratios but treatment at 150°C for one hour does not increase the estimate by 92 years (Table 2). This tells us there are some phenomena that prevent acceleration of the change from the L-form to the D-form in some way (real block, or cancellation as the questioner suggested,[3] or both), but that cannot be theoretically explained at present.

Table 2: Estimation of age from dentine by racemization of aspartic acid

Actual age	Temp.	Heated time (min.) 0	5	10	15	30	45	60
23	100°C	23.0	-	-	23.3	23.8	24.3	24.5
	200°C	23.1	-	-	127.0	152.8	169.9	183.8
35	100°C	34.9	-	-	35.3	35.8	36.3	36.5
	200°C	35.1	-	-	135.8	161.4	176.3	188.5
44	100°C	44.1	-	-	44.3	44.9	45.4	45.6
	200°C	44.2	-	-	148.1	173.9	187.0	204.8
37	150°C	36.7	39.1	42.0	42.8	48.0	51.2	54.4
	200°C	37.2	92.1	117.0	139.7	164.5	182.6	199.3

At the beginning of our studies, we simply noted that D/L ratios in soluble peptide fractions from heated teeth were not increased in a predictable fashion. Heating up to 160°C caused slight increase in the D/L ratios from soluble peptide fractions, while heating at 180°C caused slight increase in the D/L ratio even from soluble peptide fractions,[2] and after studying this issue we still do not have a convincing explanation for it. As the questioners suggested,[3] collagen which probably shows a low rate of racemization may contribute to it, remembering that *in vivo* experiments always contain inherent differences from *in vitro* experiments. It is true that heating collagen in water causes gelatinization, however, one does not know whether collagen within teeth where it is under very complex organic circumstances compared with water, behaves in the same manner.

Our gas-chromatographic technique also detects relative amounts of L-alanine, glycine, L-proline and L-hydroxyproline in addition to D-aspartic acid and L-aspartic acid. It is true that composition of amino acids in the soluble protein fractions from heated teeth differs from that of unheated teeth (Fig.1) and we agree with the proposal to provide amino acid profiles along with these studies. Figure 2 shows examples of gas-chromatograph performed in our previous paper[4] but one should bear in mind that the amounts determined for these amino acids are relative, not absolute. In this regard, we consider that the absolute amounts of proteins and amino acids detected in samples have only limited significance and we consider age estimation to be a branch of diagnostic science, not pure biochemical science. Although it is better to measure absolute amounts, this would take considerable time, labour and equipment.

Finally, we hope to emphasize two things. First, the detection of D-aspartic acid is very delicate. D/L ratios differ according to grain particle sizes in the substantia compacta of femoral bone[5] and the manner of solubilization and it is therefore very important that all processes are performed in exactly the same way. Secondly, we generally do not need to use soluble peptide fractions, even in a burn victim, as the victim's mouth remains closed and the teeth remain white in many cases. Even when the victim's mouth is open, molars and/or premolars remain white although the front teeth are darkened.[5] In these cases we use total amino acid fractions to estimate age and can obtain a similar degree of accuracy to that obtained from soluble peptide fractions.[6] The treatment at 150°C for one hour causes teeth to become slightly yellowed and our approach is recommended only for cases in which we cannot obtain white teeth from the victim, in which case we still consider it essential to use soluble peptide fractions to estimate age. In other words, there is a method available for age estimation even when usable teeth have changed colour after heating. Of course, we do not consider our method as infallible but we hope it will provide a new insight for age estimation and that further studies will confirm it.

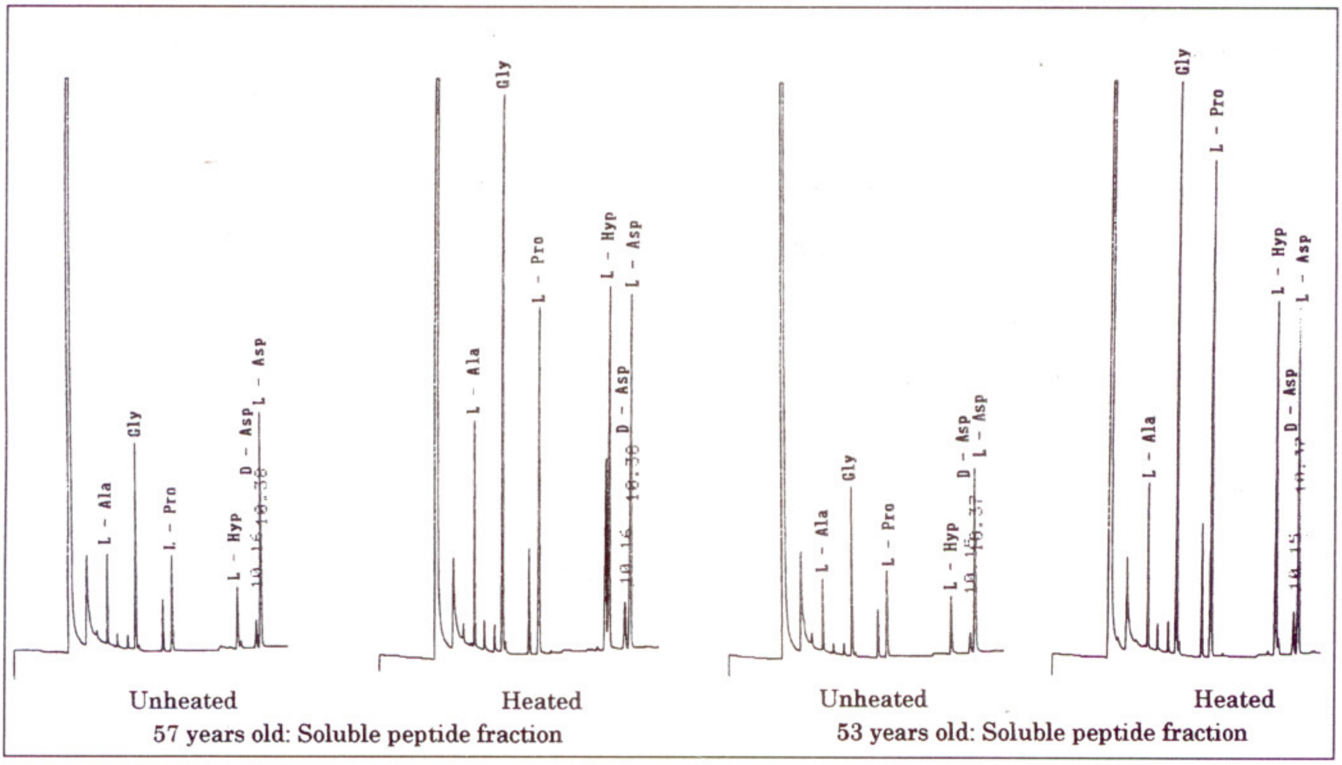

Fig.1 *Gas-chromatographs of soluble peptide fractions from unheated and heated teeth shown in Table 1. Note glycine (Gly), L-proline (L-Pro), and L-hydroxyproline (L-Hyp;) seem to be increased in the heated teeth as the questioners suggested. Abbreviations : L-Ala; L-alanine; D-Asp; D-aspartic acid; L-Asp; L-aspartic acid.*

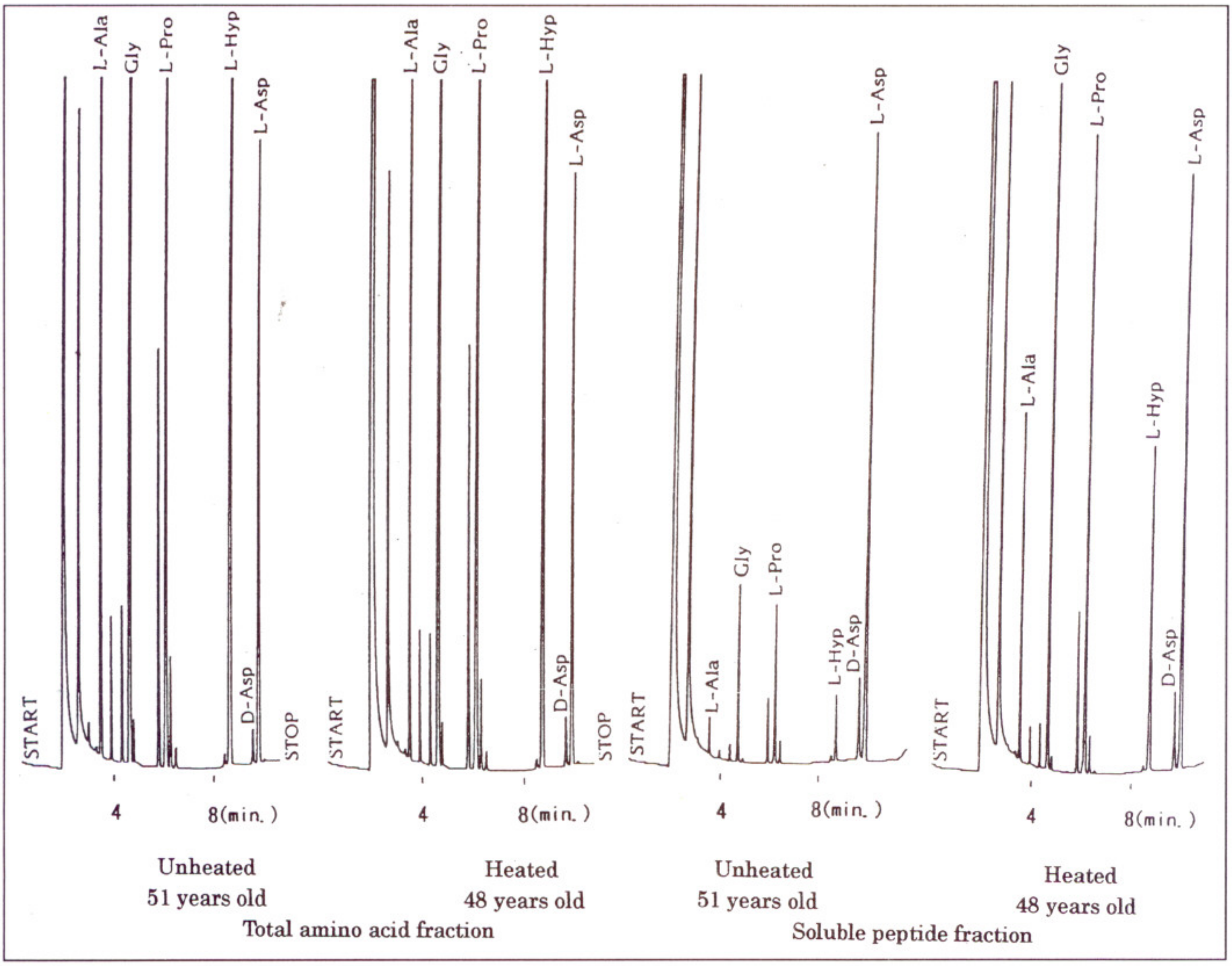

***Fig.2** Examples of gas-chromatograph performed in our pervious paper. 4 D/L ratios are shown in Table 2 of the paper. Abbreviations: see Fig.1.*

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