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Age estimation in biopsy specimens of dentin

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Abstract Determination of age at death on the basis of aspartic acid racemization in dentin is one of the most reproducible and accurate methods. In Germany, age estimation by this method has so far generally not been applied to living persons, since the extraction of a tooth exclusively for age estimation when it is not medically indicated is regarded as ethically and legally problematic. The development of a biopsy technique applicable to dentin took place against this background. Testing the technique and analysis of dentinal biopsy specimens revealed that the biopsy technique is a low-risk procedure that causes only minor discomfort to the affected person. It is readily practicable and facilitates standardized specimen removal. The relationship between the extent of aspartic acid racemization in dentinal biopsy specimens and age is very close, facilitating age estimation. A prerequisite for accurate results is the performance of biopsies under strictly standardized conditions. If this is guaranteed, age determination on the basis of aspartic acid racemization in dentinal biopsy specimens appears to be superior in precision to most other methods in living persons and can be used for all age groups.

Key words Age determination · Living persons · Aspartic acid racemization · Dentin · Dentinal biopsy

Introduction

Aspartic acid racemization in dentinal proteins is the basis of a remarkably accurate and reproducible method for age estimation (Helfman and Bada 1976; Helfman et al. 1977; Masters 1983, 1985; Ogino et al. 1985; Ohtani 1994; Ohtani and Yamamoto 1987, 1990, 1991, 1992; Ritz et al. 1990, 1993). This method has so far been used almost exclusively for estimation of age at death in unidentified corpses. The analytical procedure involves extraction of one or more teeth. In Germany, age estimation by this method has so far generally not been applied to living persons, since the extraction of a tooth exclusively for age estimation when it is not medically indicated is regarded as ethically and legally problematic. For this reason, a biopsy technique applicable to dentin was developed and the relationship between age and aspartic acid racemization in dentinal biopsy specimen was investigated.

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Materials and methods

A biopsy technique applicable to dentin was developed. 76 dentinal biopsy specimens were taken from the crowns of 62 extracted teeth. In all biopsy specimens the extent of aspartic acid racemization was determined and its relationship to age was tested. Other parameters studied were the influence of pathologic changes or changes in the dentin caused by dental treatment and the importance of biopsy location and layer. The practicability of the biopsy technique in living persons was tested on three patients attending the Clinic for Oral, Maxillary and Facial Surgery at the Christian-Albrechts University in Kiel, Germany.

Biopsy technique

A specially engineered microtrephine (Hager and Meisinger GmbH, Düsseldorf, Germany) with the following characteristics was used to take the biopsies:

Hollow cylinder trephine with internal water-cooling feature for removal of dentine specimens (1 mm in diameter)
Red marking to standardize penetration depth of 1 mm
Diamond coating on cutting face only to minimize cavity dimensions

The trephine was used with a handpiece for internally cooled drills.

The biopsy specimens were removed from the oral or vestibular portions of the crown according to a standardized procedure and were taken midway between the occlusion surface and the enamel-cementum junction (Fig. 1). After removal of the enamel layer with a water-cooled rotating diamond ball reamer (diameter 2 mm), the dentin specimens were taken using the microtrephine with continuous internal and external cooling. Entry was at right angles to the longitudinal axis of the tooth. Penetration into the dentin was exactly 1 mm, as ensured by the red marking on the microtrephine. In teeth with extensively destroyed crowns in which biopsy collection was not feasible within the standardized area, specimens were taken in adjacent regions.

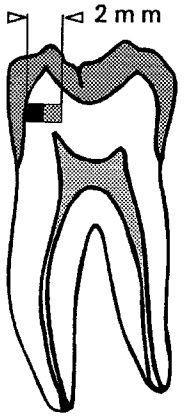

Fig. 1 Standardized biopsy procedure: The biopsy specimen *(black bar)* is taken at right angles to the longitudinal axis of the tooth midway between the occlusion plane and the enamel-cement junction; the trephine penetrates exactly 1 mm into the dentin. Biopsy locations for investigation of the influence of biopsy layer (cf. Fig. 3): The biopsy specimens from the "outer" layers were taken in the standardized way *(black bar)*; the corresponding specimens from the "inner" layers *(dotted bar)* were taken from the adjacent, deeper regions near the pulp

Table 1 Dentin age estimated from Equation 2 for biopsy specimens from different regions (but from a standardized removal height and layer, cf. Fig. 1) of the crowns of 5 teeth (group 1)

Tooth	Actual dentin age (years)	Biopsy region	Estimated dentin age (years)
A	9.1	Mesiopalatinal	10.1
		Mesiobuccal	9.9
		Distobuccal	9.7
		Distopalatinal	9.8
B	11.9	Distolingual	10.5
		Mesiobuccal	11.2
		Distobuccal	10.6
C	44.0	Mesiolingual	43.5
		Distobuccal	43.2
D	42.1	Distobuccal	40.7
		Mesiobuccal	40.3
E	7.4	Mesiobuccal	9.4
		Mesiolingual	9.1
		Distolingual	9.4
		Distobuccal	9.1

Analysis of biopsy specimens from extracted teeth

The extent of aspartic acid racemization was determined in 76 dentinal biopsy specimens from 62 extracted teeth from individuals of known age. In living persons, dentinal biopsy specimens should be taken from posterior teeth. For this reason, only molars (41 third molars, 9 second molars and 12 first molars) were analysed. The teeth involved were provided by dentists and had been extracted from individuals of known age due to medical indications. After washing with water, the teeth were stored dry at 4° C. The teeth were examined within 5 days of extraction.

Of the 62 teeth 32 showed no pathologic changes and the others exhibited fillings and/or were carious to varying degrees. Teeth with crowns or root-canal fillings were not examined. The teeth analysed were divided into 2 groups according to the following criteria:

Group 1 ($n = 57$). Teeth with intact crowns or teeth with small fillings and/or small carious defects in a maximum of 2 surfaces

Group 2 ($n = 5$). Teeth with extensively destroyed crowns (large fillings and/or carious defects in more than 2 surfaces)

The extent of aspartic acid racemization was determined in all biopsy specimens as described earlier (Ritz et al. 1993). The relationship between the extent of aspartic acid racemization in the biopsy specimens of the group 1 teeth (taken under standardized conditions) and dentin age (calculated as proposed by Ogino et al. 1985) was then evaluated by means of linear regression analysis. The values for the group 2 teeth were compared with those for the group 1 teeth.

In some group 1 teeth, several biopsies were performed from different regions and at different layer depths on each tooth:

Two to four specimens were taken from *different regions* (but from a standardized removal height and layer) of the crown in 5 teeth; the different regions are listed in Table 1.

Biopsies were performed at 2 *different layers* (in the same region) in each of 4 teeth (Fig. 1). The biopsy specimens from the "outer" layers were taken according to the standardized method. The corresponding specimens from the "inner" layers were taken from adjacent deeper regions near the pulp (Fig. 1).

After determination of the extent of aspartic acid racemization as described earlier (Ritz et al. 1993), the values for the different regions and layers were compared.

Practicability of the developed biopsy technique in vivo

The practicability of the biopsy technique in living persons was tested on 3 patients attending the Clinic for Oral, Maxillary and Facial Surgery at the Christian-Albrechts-University in Kiel, Germany. The Ethics Commission of the Medical Faculty of the Christian-Albrechts-University in Kiel had approved the study. The patients were informed as required and consented to have the biopsies removed. The biopsy specimens were taken from the mesiobuccal surface of undamaged third molars using the standardized microtrephine technique as described. The cavities were treated with conventional filling materials. The extent of aspartic acid racemization was determined in the biopsy specimens as described earlier (Ritz et al. 1993) and age was estimated using Equation 2.

Results

Dentinal biopsies

The specimens taken by our biopsy technique were approximately 1 mm long, approximately 1 mm in diameter, and approximately 1.2 mg in weight (wet weight). These amounts of dentin proved sufficient for determination of the extent of aspartic acid racemization in all cases.

After biopsy and preparation of the resulting defect for filling, the diameter of the total cavity was approximately 2 mm and its depth about 2 mm. Filling the cavities with conventional plastic materials presented no difficulties. No accidental pulpotomies occurred. The only cases in which the described standardized biopsy technique was not applicable were in teeth with extensively destroyed crowns (group 2 teeth). In these cases, the biopsies had to be performed in deeper dentin layers, often close to filling materials or carious lesions.

Analysis of biopsy specimens from extracted teeth

The relationship between the extent of aspartic acid racemization in the biopsy specimens from the teeth of group 1 and dentin age can be described by the following Equation (1). See also the corresponding regression line in Fig. 2.

$$\ln \frac{(1 + D/L)}{(1 - D/L)} = 0.00124\, t + 0.0472 \quad (r = 0.99) \qquad (1)$$

where D/L = D-aspartic acid/L-aspartic acid, t = dentin age, r = correlation coefficient, SEE = standard error of estimation found for the investigated material (group 1 teeth).

According to the data on group 1 teeth, dentin age can be calculated from the extent of aspartic acid racemization in dentinal biopsy specimens using the following formula (Eq. 2) when biopsies are performed by the standardized technique:

$$t = 786.89 \ln \frac{(1 + D/L)}{(1 - D/L)} - 36.51 \ (\text{SEE} = 2.9 \text{ years}) \qquad (2)$$

The extents of aspartic acid racemization in the biopsy specimens from the group 2 teeth with extensively destroyed crowns differed substantially from the values for group 1 teeth of the same age. The group 2 dentin ages estimated using Equation 2 deviated considerably from the actual age in each case accordingly (Table 2).

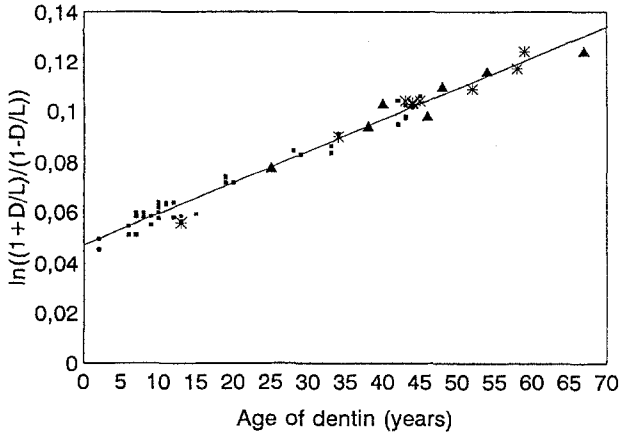

Fig. 2 Extent of aspartic acid racemization {In [(1 + D/L)/(1 − D/L)]} in 57 biopsy specimens taken by the standardized procedure (cf. Fig. 1) in relation to dentin age (group 1 teeth: *stars* first molars, *triangles* second molars, *small squares* third molars)

Table 2 Dentin age estimated from Equation 2 for biopsy specimens from group 2 teeth with extensively destroyed crowns compared with their actual dentin age

Tooth	Actual dentin age (years)	Estimated dentin age (years)
I	15.0	20.6
II	34.5	50.1
III	49.3	65.1
IV	14.8	20.8
V	37.0	50.6

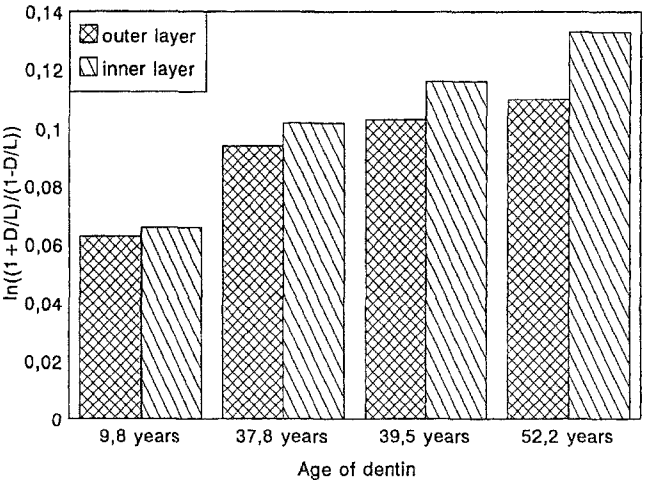

Fig. 3 Extent of aspartic acid racemization {In [(1 + D/L)/(1 − D/L)]} in biopsy specimens taken from "outer" *(checkered bars)* and "inner" *(hatched bars)* dentin layers (cf. Fig. 1) from 4 teeth with different dentin ages

Table 3 Results of age estimation based on the analysis of biopsy specimens taken in vivo from 3 individuals according to the standardized procedure (cf. Fig. 1). The estimated age was calculated from Equation 2

Case	Actual age (years)	Estimated age (years)
1	22.1	23.7
2	34.6	33.9
3	52.7	50.6

Biopsy specimens from different regions (but from a standardized removal height and layer, cf. Fig. 1) of the crown of a single tooth exhibited a nearly identical extent of aspartic acid racemization and the dentin age values estimated from Equation 2 were accordingly also nearly identical (Table 1).

In contrast to this, the biopsy layer had a significant influence on the results. Aspartic acid racemization had clearly progressed further in specimens taken from deeper layers ("inner" layers; Figs. 1, 3) than in biopsy specimens taken by the described standardized technique ("outer" layers; Figs. 1, 3).

Practicability of the biopsy technique in vivo

The biopsy technique also proved readily practicable in vivo. Taking a biopsy and filling the resulting cavity took approximately 15 min. Estimated age (calculated using Eq. 2) and actual age of the 3 investigated patients correlated remarkably well (see Table 3).

Discussion

An optimum method for age determination in living individuals should fulfil the following conditions:

It should facilitate reproducible and sufficiently accurate age determination in all age groups.

The procedure must not impair the health of the affected person.

The methods currently in use are based on radiological or direct assessment of age-related morphological changes in the skeletal system and teeth. The accuracy of these methods is age-dependent. In children and adolescents, fairly accurate age determination is possible as incomplete processes of growth or development can be assessed. In this age group, 95% confidence intervals of approximately ± 2–4 years have been established for radiological methods (Köhler et al. 1994; Mincer et al. 1993; Mörnstad et al. 1994; Staaf et al. 1991). Following completion of the growth period, age estimation by morphological methods becomes difficult and – particularly when nondestructive methods are used – it is not sufficiently accurate (Drusini 1993; Hongwei 1989; Hongwei et al. 1991; Kambe et al. 1991; Mincer et al. 1993; Xiaohu et al. 1992). Previously, the methods of choice in adults were, theoretically, the method according to Gustafson (1950, 1955) and its modifications. However, these methods are destructive, need extracted teeth and have 95% confidence intervals of approximately ± 12 years at best (Dalitz 1962; Endris 1979).

Age determination based on aspartic acid racemization in dentin has been demonstrated to be highly reproducible and accurate; 95% confidence intervals of approximately ± 3–8 years are described (Ogino et al. 1985; Ohtani 1994; Ohtani and Yamamoto 1987, 1990, 1991, 1992; Ritz et al. 1993). The method can be used in all age groups. However, reports published on this method have dealt with age determination by analysing dentin of extracted teeth. We developed a dentin biopsy technique to apply the method to living persons while complying with German ethical and legal regulations.

This biopsy technique is a low-risk procedure comparable to conventional therapy of small carious lesions. It is readily practicable and allows standardized removal of dentinal specimens. Analysis of dentinal biopsy specimens taken by the technique described revealed the following:

1. If dentinal biopsy specimens are taken using the standardized technique described above, the relationship between the extent of aspartic acid racemization and dentin age is close enough to provide a basis for age determination. In accordance with results reported for whole crowns, roots and "longitudinal sections" of extracted teeth (Ogino et al. 1985; Ohtani 1994; Ohtani and Yamamoto 1987, 1990, 1991, 1992; Ritz et al. 1990, 1993), our data for dentinal biopsy specimens indicate remarkably high accuracy levels for this method.

2. A prior condition for reproducible and reliable results is a standardized biopsy technique. The biopsies have to be performed under constant cooling to avoid heat-related racemization of aspartic acid. The layer depth of the biopsies must be maintained within strict boundaries, since the extent of aspartic acid racemization is higher in deep, peripulpar layers than in more superficial layers (Fig. 3). This may be caused by variations in protein composition in deep and superficial dentin layers (Levine 1971; Schumacher and Schmidt 1976), since each protein has its own kinetics of aspartic acid racemization (Brunauer and Clarke 1986; Clarke 1987; Lowenson and Clarke 1988; Stephenson and Clarke 1989). Our biopsy technique ensured removal of biopsy specimens from a defined layer.

3. In teeth with extensively destroyed crowns (group 2 teeth) we were unable to perform biopsies according to the established standardized procedure. The specimens had to be taken from deeper dentin layers, in many cases close to filling materials or carious lesions. The results for these specimens (group 2 teeth) differed substantially from the values obtained for the standardized extracted specimens from group 1 teeth. This was to be expected, since the specimens from group 2 teeth had to be taken from other layers. Furthermore, an influence of iatrogenic and carious lesions on the composition of the surrounding dentin, and thus on aspartic acid racemization (Brunauer and Clarke 1986; Clarke 1987; Helfman et al. 1977; Lowenson and Clarke 1988; Rutsatz et al. 1985; Smith et al. 1978; Stephenson and Clarke 1989) cannot be excluded in these cases. Hence, teeth with extensive crown destruction are not suitable for age determination based on the extent of aspartic acid racemization in biopsy specimens.

The data presented here, together with the findings of other authors on aspartic acid racemization in dentin, permit the following conclusions.

Even if – as in Germany – the extraction of a tooth exclusively for age determination is not permissible from an ethical or legal point of view, our biopsy technique facilitates age estimation based on aspartic acid racemization in dentin in living persons as well. It is of the utmost importance that the biopsies are performed under standardized conditions. If this is guaranteed, the method appears to be superior in precision to most other methods applicable to living persons, especially after completion of the growth period. The method can be used for age determination in all age groups. At present, it appears to be the only method that is accurate enough to be of practical use for age estimations of adults in forensic cases.

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