Int J Leg Med (1993) 105:289–293

International Journal of
Legal Medicine

Postmortem estimation of age at death based on aspartic acid racemization in dentin: its applicability for root dentin

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Received September 4, 1992 / Received in revised form November 19, 1992

Summary. The extent of aspartic acid racemization in total dentin and in dentin protein fractions from the *roots* of third molars was determined. In several cases coronal dentin was also investigated. The results of other authors, according to which the racemization of aspartic acid in root dentin apparently proceeds differently than in coronal dentin, could be confirmed. Consequently, the data published so far on age determination based on the extent of aspartic acid racemization in coronal dentin and the "entire dentin of longitudinal sections" cannot be applied to root dentin. In total root dentin and the acid soluble protein of root dentin, a close relationship was observed between the extent of aspartic acid racemization and age. Accordingly, estimation of age at death based on aspartic acid racemization in dentin is also possible for root dentin, apparently with good results. This is important particularly in those cases where a large portion of the coronal dentin is absent, for instance following dental treatment. In the investigation of root dentin, regression equations specific for root dentin must be employed in the estimation of age at death. Corresponding equations for third molars were calculated.

Key words: Estimation of age at death – Aspartic acid racemization – Root dentin – Dentin protein fractions

Zusammenfassung. Der Razemisierungsgrad von Asparaginsäure wurde in Gesamtdentin sowie in Dentinproteinfraktionen aus Zahn*wurzeln* dritter Molaren bestimmt; in einigen Fällen wurde zusätzlich Kronendentin mituntersucht. Die Ergebnisse anderer Autoren, nach denen die Razemisierung von Asparaginsäure in Zahnwurzeldentin offenbar anders verläuft als in Kronendentin, konnten bestätigt werden. Damit können die bislang zur Lebensaltersbestimmung aufgrund des Razemisierungsgrades von Asparaginsäure in Dentin veröffentlichten Daten für Kronendentin und "longitudinale Dentinsegmente" nicht ohne weiteres auf Wurzeldentin übertragen werden. Für Gesamtwurzeldentin und das säurelösliche Wurzeldentinprotein ergab sich eine enge Beziehung zwischen dem Razemisierungsgrad von Asparaginsäure dem Lebensalter. Eine Lebensaltersbestimmung aufgrund des Razemisierungsgrades von Asparaginsäure ist danach auch an Wurzeldentin mit offenbar guten Ergebnissen möglich. Dies ist vor allem in Fällen von Bedeutung, in denen beispielsweise nach zahnärztlichen Maßnahmen ein Großteil des Kronendentins fehlt. Wird Wurzeldentin untersucht, müssen speziell für Wurzeldentin etablierte Regressionsgleichungen für eine Lebensaltersbestimmung herangezogen werden. Entsprechende Daten für dritte Molaren wurden erarbeitet.

Schlüsselwörter: Lebensaltersbestimmung – Razemisierung von Asparaginsäure – Zahnwurzeldentin – Dentinproteinfraktionen

Introduction

A gradual transformation of L-aspartic acid into its D-form (racemization) occurs during life in the dentin protein of permanent human teeth (Helfman and Bada 1976). The extent of aspartic acid racemization in acid soluble dentin proteins is substantially greater than in either total dentin or acid insoluble dentinal protein (Masters 1985; Ohtani and Yamamoto 1990, 1991). The relationship between age and the extent of aspartic acid racemization in total dentin and the acid soluble and insoluble protein fractions is close enough to serve as a basis for estimation of age at death. A number of studies have shown this method to be more exact and of superior reproducibility than most other techniques for postmortem estimation of age at death (Ogino et al. 1985; Ohtani and Yamamoto 1987, 1991; Ritz et al. 1990). Generally, the method can be applied even in cases with long postmortem intervals, since over many years at normal ambient temperatures no substantial racemization occurs postmortem (Ogino et al. 1985).

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Most authors have investigated *coronal dentin* only (Helfman and Bada 1976; Ogino et al. 1985; Ritz et al. 1990). Ohtani and Yamamoto (1987, 1991) transversely cut the dentin of teeth of different ages into 8 or 9 blocks each and measured the extent of aspartic acid racemization in each block. In younger teeth the extent of racemization of aspartic acid in the *coronal* dentin tended to be higher than in the corresponding *root* dentin, whereas in older teeth the reverse was true. In addition, different values were observed when labial and lingual portions of one tooth were compared (Ohtani and Yamamoto 1987, 1992). Considering these results, the authors recommended the analysis of the "*entire* dentin of central longitudinal sections" (Ohtani and Yamamoto 1987, 1991, 1992).

In forensic practice the coronal dentin may be largely destroyed or removed (caries, large dental fillings, crowns), whereas the root dentin is usually intact. In such cases it is neither possible to obtain coronal dentin nor "entire dentin of central longitudinal sections" in a reproducible manner. Since Ohtani and Yamamoto (1987, 1991) found different values for the extent of aspartic acid racemization in crown and root of the same tooth, the published results for coronal dentin or the "entire dentin of central longitudinal sections" (Helfman and Bada 1976; Ogino et al. 1985; Ohtani and Yamamoto 1987, 1991; Ritz et al. 1990) cannot be directly applied to root dentin. Ohtani and Yamamoto (1987) investigated exclusively total root dentin in a small number of teeth. Based on their investigations, no conclusions can be reached on the applicability of the method for root dentin.

Hence, we investigated total dentin and the acid soluble and acid insoluble dentin protein fractions of roots to ascertain whether age at death can also be determined on the basis of aspartic acid racemization in cases in which only *root* dentin is available. In some cases coronal dentin was also investigated for comparison.

Materials and methods

1. Preparation of specimens

a. Root dentin. Dental roots of 70 third molars from 70 individuals of known age were examined. Total dentin was analysed in all 70 cases. In addition, the acid soluble and acid insoluble protein fractions of dentin were investigated in 39 of the 70 cases.

Forty-nine teeth were from living persons and were provided by dentists and 21 teeth were obtained from cadavers at autopsy. Postmortem intervals between death and autopsy ranged from a few hours to 6 weeks. The teeth were stored at room temperature for up to 1 year.

Some of the teeth were intact but most had coronal fillings or crowns. Teeth with root fillings were not studied. Root dentin was obtained by mechanically separating the teeth at the enamel-cementum junction, followed by removal of the cementum and pulp tissues. Water cooling was applied in all preparation steps to reduce a possible heating of the samples.

The root dentin was washed at 4°C for 1 h in a 15% NaCl solution, 15 min in ethanol-ether (3:1), 1 h in 2% sodium dodecyl sulphate, and rinsed with water. The specimens were freeze-dried overnight, then pulverized. Aliquots of dentin were used for analysis of total dentin (60 mg) and for acid extraction (150 mg).

b. Coronal dentin. In 13 of the examined third molars coronal dentin was analysed in addition to root dentin. The coronal dentin was prepared as described by Ritz et al. (1990) and subsequently treated the same as the root dentin.

In the coronal dentin samples total dentin was analysed exclusively.

2. Acid extraction

Acid extraction was done on the root dentin of 39 third molars. Each aliquot (150 mg) of dentin was extracted with 5 ml 0.6 N HCl at 4°C for 15 min and centrifuged at 4°C for 10 min. The acid insoluble residue was washed several times in water. The supernatant containing the acid soluble dentin proteins was dried in a vacuum.

3. Measurements

All samples were hydrolysed for 6 h in 6 N HCl at 100°C. HCl and water were removed in a vacuum. The hydrolysate was esterified with isopropanol/sulfuric acid (10:1) for 1 h at 110°C. After removal of the isopropanol by a stream of air, 2 N ammonium hydroxide was added. The samples were alkaline extracted with dichloromethane and dried again. Acetylation was performed with trifluoracetic anhydride (TFA) at 60° for 15 min. The amino acids were now present as TFA-isopropylesters. D- and L-aspartic acid were separated and quantified by gas chromatography on a chiral capillary column (Chirasil-Val) using a flame ionization detector and with hydrogen as carrier gas.

To determine the precision of the method 8–10 dentin samples (total dentin) from each of 4 teeth of different ages were treated and analysed as described above. In all series the coefficient of variation was $< 0.9\%$ (0.67%; 0.69%; 0.73%; 0.88%).

4. Evaluation

The extent of aspartic acid racemization determined in the 13 total dentin samples from crowns was compared with the values of total dentin from the corresponding roots.

Furthermore, the relationship between age and extent of aspartic acid racemization in both total root dentin and the root dentin protein fractions was examined.

The process of racemization of amino acids can be described as (Bada and Schroeder 1972, 1975; Smith et al. 1978):

$$\ln \frac{(1 + D/L)}{(1 - D/L)} = 2\,k\,(\text{Asp.})\;\; t + \text{constant}$$

where D/L represents the proportion of D-aspartic acid to L-aspartic acid, $k(Asp.)$ is the first-order rate constant of the interconversion of enantiomers, and t equals time.

The value $\ln [(1 + D/L)/(1 - D/L)]$ was calculated for each sample. The relationship between age and the extent of aspartic acid racemization, $\ln [(1 + D/L)/(1 - D/L)]$, in total dentin, and in the acid soluble and acid insoluble dentin protein fractions of the investigated roots was evaluated by linear regression analysis.

The extent of aspartic acid racemization is a measure of dentin age. Dentin age, however, does not correspond to the actual age of the individual but to the difference between actual age and the age at which dentin protein was synthesized, which varies according to tooth type. However, in teeth of one type (e.g. third molars) the age at root dentin synthesis – regardless of the range of biological variation – is constant. Therefore, the extent of aspartic acid racemization can be directly related to the *actual age* of individuals, if *exclusively* teeth of one type (e.g. third molars) are investigated. Such an approach was recommended by Ogino et al. (1985) and used in this study.

Results

Figure 1 presents a comparison of the extent of aspartic acid racemization, $\ln [(1 + D/L)/(1 - D/L)]$, in total *root* dentin and total *coronal* dentin of 13 third molars of different ages. While at a young age the extent of racemization in the crown tends to be higher than in the root, this relationship seems to be reversed with increasing age.

Figures 2 and 3 show the values obtained for the extent of aspartic acid racemization in total root dentin ($n = 70$, Fig. 2) and in the acid soluble ($n = 39$) and acid insoluble ($n = 39$) protein fractions (Fig. 3) of root dentin in relation to age.

The following regression equations and correlation coefficients (r) were calculated for the relationship between the extent of aspartic acid racemization in root dentin of third molars and actual age (t):

Total root dentin ($n = 70$; $r = 0.99$):

$$\ln \frac{(1 + D/L)^1}{(1 - D/L)} = 0.00167\, t + 0.00660 \quad (1)$$

Acid soluble protein of root dentin ($n = 39$; $r = 0.99$):

$$\ln \frac{(1 + D/L)}{(1 - D/L)} = 0.00445\, t + (-0.01313) \quad (2)$$

Acid insoluble protein of root dentin ($n = 39$; $r = 0.96$):

$$\ln \frac{(1 + D/L)}{(1 - D/L)} = 0.00107\, t + 0.01515 \quad (3)$$

If root dentin of third molars is analysed, age (t) can be calculated from the following formula (SEE = standard error of estimation found for the investigated material):

Total root dentin (SEE = 2.3 years):

$$t = 580.41 \ln \frac{(1 + D/L)}{(1 - D/L)} - 2.957 \quad (1a)$$

Acid soluble protein of root dentin (SEE = 2.5 years):

$$t = 219.21 \ln \frac{(1 + D/L)}{(1 - D/L)} + 3.667 \quad (2a)$$

Acid insoluble protein of root dentin (SEE = 4.4 years):

$$t = 857.68 \ln \frac{(1 + D/L)}{(1 - D/L)} - 10.460 \quad (3a)$$

Discussion

The organic matrix of dentin consists of approximately 91% collagen and 9% noncollagenous material (Schroeder 1976). Following acid extraction with 0.6 *N* HCl, the acid insoluble dentinal protein fraction is composed chiefly of collagen, while noncollagenous proteins and peptides predominate in the acid soluble fraction (Masters 1985; Takagi and Veis 1984).

[1] D/L = D-aspartic acid/L-aspartic acid

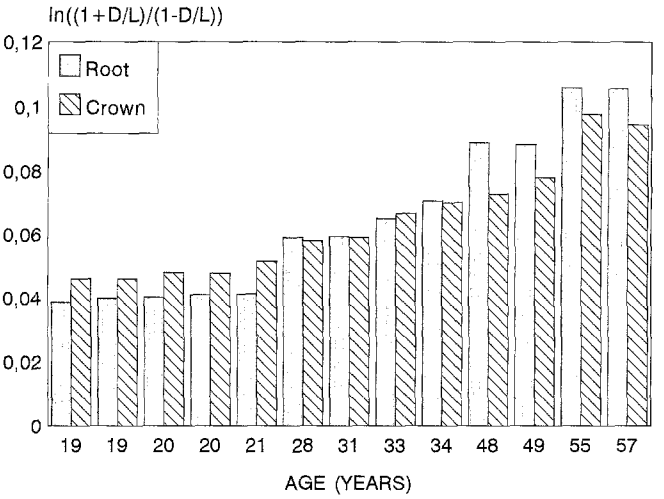

Fig. 1. Comparison of the extent of aspartic acid racemization, $\ln [(1 + D/L)/(1 - D/L)]$, in total *root* dentin and total *coronal* dentin of 13 third molars of different ages

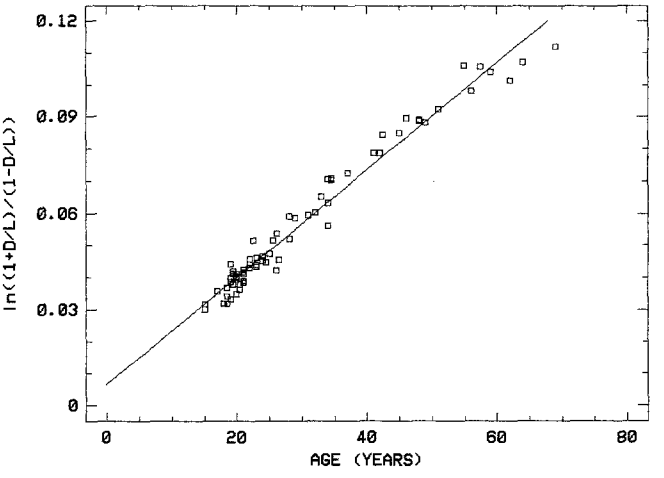

Fig. 2. Extent of aspartic acid racemization, $\ln [(1 + D/L)/(1 - D/L)]$, in *total dentin* of *third molars* ($n = 70$) in relation to actual age

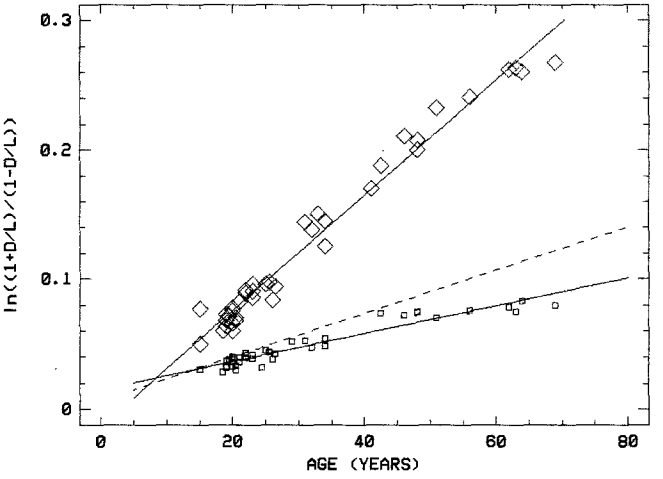

Fig. 3. Extent of aspartic acid racemization, $\ln [(1 + D/L)/(1 - D/L)]$, in *acid soluble* (large rhombi, $n = 39$) and *acid insoluble* (small squares, $n = 39$) *dentinal protein* of *third molars* in relation to actual age; the broken line represents the regression line calculated for total dentin (Fig. 2)

The slope of the calculated regression line for total dentin of roots was less than the slope for acid soluble dentinal proteins but greater than those for acid insoluble dentinal proteins (Fig. 3). Thus, in dentin, aspartic acid racemization is rapid in (acid soluble) noncollagenous proteins or peptides but proceeds only slowly or not at all in the (acid insoluble) collagenous protein fraction. The D-aspartic acid concentrations measured in *total dentin* represent "summary values" derived from the values of the various protein fractions. Since the organic dentin matrix consists predominantly of collagen, the slope of the regression line for total dentin was only slightly greater than that for the acid insoluble protein fraction alone (Fig. 3). Similar findings were reported by Ohtani and Yamamoto (1990, 1991) in studies employing acid extraction of the "entire dentin of central longitudinal sections".

A close relationship between age and the extent of aspartic acid racemization in total dentin and in acid soluble dentinal protein of dental *roots* of third molars was found. This corresponds to the findings in the analysis of coronal dentin and in particular of the "entire dentin of central longitudinal sections" (Ogino et al. 1985; Ohtani and Yamamoto 1987, 1991; Ritz et al. 1990).

Moreover, results of Ohtani and Yamamoto (1987, 1991) were confirmed which indicate different rates of aspartic acid racemization in coronal and root dentin of the same tooth. Values for the extent of aspartic acid racemization in coronal dentin tend to be higher than the corresponding values for root dentin in young teeth, whereas the ratio appears to be reversed with increasing age (Fig. 1). In addition, a comparison of the regression equation calculated for total *root* dentin, equation (1), with the regression equation for total *coronal* dentin of third molars determined by Ogino et al. (1985) reveals that a more rapid gain in the extent of aspartic acid racemization with increasing dental age is found in dental *roots* than in dental *crowns*.

The rate of amino acid racemization depends not only on the ambient temperature but also on the "biochemical environment" (Helfman et al. 1977; Smith et al. 1978). At a relatively constant human body temperature of 37°C the rate of aspartic acid racemization varies in different types of body tissue[2] and in different protein fractions *within the same tissue* (Garner and Spector 1978; Helfman and Bada 1975; Man et al. 1983; Masters 1983, 1985; Masters et al. 1977, 1978; Ohtani and Yamamoto 1990, 1991; Ritz and Schütz 1992). Different rates of aspartic acid racemization in coronal and root dentin could therefore be related to *different ambient temperatures* and/or *different biochemical environments*. Differences in the ambient temperatures of crown and root could play a role, because the ambient temperature of the crown is lower than that of the root. In addition, immunohistochemical investigations indicate a varying distribution of noncollagenous proteins between root and crown (Tung et al. 1985). Thus, the biochemical environment in the root and crown may differ and be responsible for different racemization rates in coronal and root dentin.

The following *conclusions* can be drawn from our findings:

- Since aspartic acid racemization in root dentin apparently proceeds somewhat differently than in coronal dentin, the data published for coronal dentin or the "entire dentin of central longitudinal sections" (Ogino et al. 1985; Ohtani and Yamamoto 1987, 1991; Ritz et al. 1990) cannot be directly applied to root dentin.
- Estimation of age at death based on aspartic acid racemization in *root* dentin is nonetheless possible with apparently good results. This is important in those cases in which the coronal dentin is largely destroyed or removed (caries, dental treatment).
- In the examination of root dentin, regression equations specific for root dentin must be employed for the determination of age at death. Corresponding equations for third molars were calculated. Because third molars are present with considerably higher frequency at a younger age in living persons as well as in corpses, younger teeth predominate in our material. Considering this disadvantage of using third molars as investigative material, data for other tooth types should be collected in further studies.

[2] A measurable *in vivo* racemization of aspartic acid takes place not only in dentin but also in other human and animal tissues with metabolically stable proteins, such as tooth enamel, crystalline lens, the white matter of the brain and intervertebral discs (Fujii et al. 1989; Garner and Spector 1978; Man et al. 1983; Masters et al. 1977, 1978; Muraoka et al. 1987; Ohtani and Yamamoto 1992; Ritz and Schütz 1992)

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