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The extent of aspartic acid racemization in dentin: a possible method for a more accurate determination of age at death?

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Summary. In the current study the objective was to find to what extent a *reliable* determination of age at death is made possible by establishing the degree of aspartic acid racemization in the dentin of teeth. The results of the investigation of 46 teeth are in agreement with the values found by other authors. The method presented makes a reproducible and accurate estimation of age possible. We intend to elaborate and improve this promising method for determination of age at death. The relevant points are presented here.

Key words: Personal identification – Determination of age at death – Aspartic acid racemization in dentin

Zusammenfassung. Es wurde geprüft, inwieweit eine *zuverlässige* Lebensaltersbestimmung durch Feststellung des Razemisierungsgrades von Asparaginsäure in Zahndentin möglich ist. Die Ergebnisse der Untersuchung von 46 Zähnen stimmen gut mit den von anderen Autoren gefundenen Werten überein. Offenbar ermöglicht das vorgestellte Verfahren eine gut reproduzierbare und genaue Lebensaltersbestimmung. Diese vielversprechende Methode zur Altersdiagnose soll jedoch noch ausgearbeitet werden; entsprechende Gesichtspunkte werden vorgestellt.

Schlüsselwörter: Identifikation – Lebensaltersbestimmung – Razemisierungsgrad von Asparaginsäure in Zahndentin

Introduction

For purposes of identification of an unknown corpse, the age at death of a deceased individual is of great interest. With children and adolescents a fairly accu-

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rate age determination is possible by assessing the incomplete process of growth or development, particularly within the skeletal system and the teeth.

Age determination in adults is also generally accomplished through special consideration of the skeletal system and the teeth. However, age determination is more difficult in adults than in children or adolescents. Following completion of the period of growth, age-related changes used in age determination are influenced not only be the age of the individual, but also by numerous endogenous and exogenous factors, such as disease, nutrition and physical strain. It is for this reason that the *biological* age may deviate considerably from the *chronological* age, the latter being of interest in forensic practice.

An optimal method for age determination in adults must therefore be centered around a parameter which measurably changes *only* with increasing (chronological) age and is independent of endogenous and exogenous factors of influence. This requirement is satisfied by the *extent of aspartic acid racemization in the dentin of teeth:*

The human organism uses L-amino acids exclusively in protein synthesis. During the course of a lifetime, however, there is a spontaneous conversion of L-amino acids to their D-form. This racemization process is, above all, temperature-dependent and proceeds slowly and steadily at the relatively constant human body temperature of 37°C. Aspartic acid is characterized by a relatively high rate of racemization compared with other amino acids. Nevertheless, no measurable amounts of D-aspartic acid are to be found in metabolically active tissues, where rapid protein turnover occurs. Conversely, relatively high concentrations exist in those proteins that are synthesized early in human life and are not replaced. Such proteins are found in primary tooth dentin. The relation of D-aspartic acid to L-aspartic acid becomes increasingly larger with advancing tooth age.

The process of aspartic acid racemization can be described with the following equation (Bada and Schroeder 1972, 1975; Smith et al. 1978):

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

where D/L represents the relation of D-aspartic acid/L-aspartic acid, K(Asp.) corresponds to the first-order rate constant of the interconversion of enantiomers, and t represents time.

After Helfman and Bada (1976) had established a close correlation between age of dentin and the extent of aspartic acid racemization in dentin, Ogino et al. (1985) reinvestigated this method of age estimation. According to their results (Ogino et al. 1985) age determination by this method was correct to 4 years in either direction. This method would therefore represent one of the most reliable procedures available for determination of (chronological!) age in adults for the purposes of personal identification. We therefore evaluated the presented method with regard to its reproducibility and practical application.

Materials and methods

A total of 46 teeth extracted from persons of known ages between 13 and 82 years were examined, 35 of which were provided by dentists and 11 of which originated from cadavers. Information about the types of teeth examined can be seen in Table 1.

Table 1. Teeth examined ($n = 46$)

Tooth type	Number	Tooth type	Number
First incisor	4	Second premolar	6
Second incisor	2	First molar	3
Cuspid	4	Second molar	1
First premolar	6	Third molar	20

Examination of the teeth was carried out soon after their extraction. No lengthy post-mortem storage had to be taken into account for those teeth originating from cadavers.

Following separation from the root of the teeth, the crowns were freed of enamel and the dentin located close to the pulp. The purity of the remaining dentin was assessed under UV light (dentin is luminous under UV light, whereas enamel is not). The dentin samples were then ultrasonicated once in distilled water and twice in 0.7 N HCl, after which they were hydrolyzed for 6 h in 6 N HCl at 100°C. Hydrochloric acid and water were removed in a vacuum and the residue was then esterified with isopropanol/HCl for 1 h at 110°C. This was followed by acetylation with trifluoroacetic anhydride (TFA) for 15 min at 110°C. The amino acids were then present as TFA-isopropyl esters and could be separated and quantified gas chromatographically on a chiral capillary column. A "Permabond L-chirasil-Val" fused silica capillary column (50 m, 0.32 mm inner diameter) from Macherey-Nagel (Düren, FRG) was employed using hydrogen as the carrier gas combined with a flame ionization detector (FID).

Results and discussion

According to Helfman and Bada (1976) and Ogino et al. (1985), the extent of aspartic acid racemization in dentin depends upon the *age of the dentin,* which cannot be equated with *age of the individual.* The difference between individual age and dentin age varies greatly and is dependent on the type of tooth, because the length of the period of dentin synthesis in the first years of life varies from tooth to tooth.

Different tooth types were examined (see Table 1) and in view of the varying correlation between dentin age and individual age (from one tooth to another), the extent of aspartic acid racemization in each case was compared not with individual age but rather with *dentin age*, to enable a simultaneous consideration of all values obtained. In order to compare our results with those of Ogino et al. (1985), the age of the dentin was determined as indicated by these authors (see Table 2), the age of the dentin was then calculated as the difference between individual age and the age at the point of complete tooth crown formation.

Figure 1 presents the values obtained for $\ln [(1 + D/L)/(1\text{-}D/L)]$ in relation to age of dentin.

The regression coefficients calculated by us are in nearly complete agreement with those obtained by Ogino et al. (1985). Accordingly, the equations of the respective linear regression lines are virtually identical:

Equation of the linear regression line according to Ogino et al. (1985)[1]*:*

$$\ln \frac{(1+D/L)}{(1-D/L)} = 0.00120\,t + 0.0319$$

[1] D/L, D-aspartic acid/L-aspartic acid; t, age of dentin

Table 2. Determination of age of dentin. Method of Ogino et al. (1985)

Tooth	Age of dentin
Incisors	Individual age − 4 years
Cuspid	Individual age − 6 years
First premolar	Individual age − 5 years
Second premolar	Individual age − 6 years
First molar	Individual age − 3 years
Second molar	Individual age − 7 years
Third molar	Individual age − 12 years

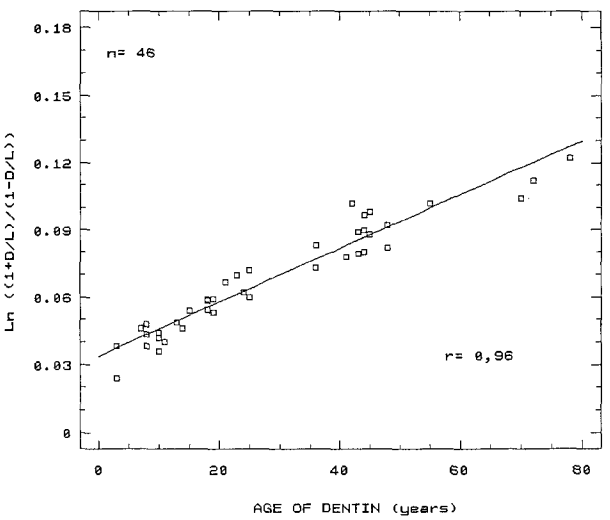

Fig. 1. Ln (1 + D/L)/(1 − D/L) plotted against age of dentin (D/L = D-aspartic acid/L-aspartic acid)

Equation of the linear regression line calculated by us[1]*:*

$$\ln \frac{(1+\mathrm{D/L})}{(1-\mathrm{D/L})} = 0.00120\,t + 0.0337$$

The intersections of the regression lines and the *y*-axis (0.0319 and 0.0337, respectively) differ minimally, with the calculated intersection obtained by Ogino et al. (1985) lying within one standard deviation of the value obtained by us.

Good agreement was found between our results and those of Ogino et al. (1985), although we had simplified the sample processing. However, in consideration of the temperature-dependence of the racemization process the warming times and temperatures given by Ogino et al. (1985) were also adhered to.

For the correlation between age of dentin and extent of aspartic acid racemization in dentin a correlation coefficient of approximately 0.96 was calculated; the value determined by Ogino et al. (1985) was 0.992. A somewhat larger de-

[1] D/L, D-aspartic acid/L-aspartic acid; *t*, age of dentin

viation from the linear regression line was seen in our results. This could be traced back to a relatively large error caused by the measuring technique, which can be reduced once the equipment is optimized.

An age determination employing our linear regression line is marked by a standard error of estimation of 5.69 years either way. This value can be reduced, however, by optimizing the analytical procedure.

In addition, we plan to eleborate the method presented according to the following points:

1. In our first investigations, each established degree of racemization was related to the *age of dentin,* as the analysis for *different* teeth was to be carried out simultaneously. The determination of the age of dentin as the difference between individual age and the age of completed tooth crown formation (see Table 2) represents, however, a simplification of complex synthesis processes (Schour and Massler 1940; Schroeder 1976). The error associated with this simplification can be avoided as follows: it can be assumed that the duration of dentin synthesis for *one* tooth type (e.g., for the type incisor) is constant, regardless of the range of biological variations. If, however, in further investigations, the analysis for each individual tooth type is carried out separately, then the extent of racemization can be directly related to individual age.

Ogino et al. (1985) also recognized that a separate consideration of individual tooth types leads to more precise values. However, the number of teeth examined by these authors was not sufficient for a satisfactory analysis.

2. The synthesis of crown dentin in permanent teeth requires a period of approximately 4–6,5 years (Schour and Massler 1940). In this process, dentin is built up in layers from the outside moving inwards. The dentin of the outermost layers is therefore several years older than that of the innermost layers. Consequently, in the future we will take the samples from a defined layer.

3. Furthermore, it must be taken into account that the racemization process continues after death. This raises the question as to what extent determination of age at death is possible on a cadaver in the case of longer storage periods.

The racemization of amino acids is a temperature-dependent process; the speed of conversion from L-aspartic acid to the D-form decreases with falling temperature. The average temperature in our latitude is well under the human body temperature of 37°C. If a cadaver is stored at outdoor temperatures (or cooler), post-mortem racemization of aspartic acid in dentin will proceed considerably more slowly than in vivo. According to Ogino et al. (1985), a storage period of 10 years at 15°C results in an error of merely 0.2 years in the age determination using the method presented in the current study. Under normal conditions (storage of a corpse at normal outdoor temperature or cooler), even a period of several years impairs the age estimation only negligibly.

On the other hand, this method is not reliable for corpses which have been exposed to higher temperatures (e.g., fire victims) as previously noted by Masters (1986).

The warming of a corpse, however, causes a more rapid increase in D-aspartic acid concentration in *all* tissues, including those which contain no measurable amounts of D-aspartic acid *in vivo* as a result of a high metabolic protein turnover. By examination of such tissues, the extent of age-independent, *heat-caused* racemization can be established. The values obtained could then be compared with the degree of racemization determined in dentin; the difference between

these two values could be introduced into the determination of individual age at death. This holds true only with the assumption that the "tissue for comparison" exhibits the same speed of aspartic acid racemization as dentin. A suitable "tissue for comparison" should be found with appropriate experiments.

4. In future investigations we will also include root dentin in order to facilitate the application of the presented method in severely destroyed teeth.

In conclusion, we would like to point out the advantages which we think the presented method offers:

– The speed of amino acid racemization is, above all, temperature-dependent. Human body temperature is relatively constant; large temperature fluctuations are not compatible with life. Therefore, the rate of in vivo aspartic acid racemization in dentin cannot be significantly influenced by exogenous or endogenous factors. Thus, the presented method is well-suited for establishment of *chronological* age, which is of interest in forensic practice.

– Special odontological experience is not necessary in order to be able to carry out an age determination with the presented method. However, the achievement of usable results requires that the analysed teeth are reliably assigned to a particular tooth type (e.g. cuspid).

– The results of an age determination by the method presented are reproducible and can thus be checked by any other investigator who has the necessary analytical experience.

The establishment of the extent of aspartic acid racemization in dentin may facilitate a reliable and exact age determination. Further investigations will indicate with what accuracy a determination of age at death is possible using the elaborated and improved method.

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