Forensic Science International 223 (2012) 198–201

Contents lists available at SciVerse ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Comparative analysis of aspartic acid racemization methods using whole-tooth and dentin samples

Ayaka Sakuma [a,*], Susumu Ohtani [b], Hisako Saitoh [a], Hirotaro Iwase [a]

[a] Department of Legal Medicine, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba City, Chiba 260-8670, Japan
[b] Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa City, Chiba 277-8561, Japan

ARTICLE INFO

Article history:
Received 17 April 2012
Received in revised form 19 July 2012
Accepted 23 August 2012
Available online 16 September 2012

Keywords:
Personal identification
Age estimation
Racemization
Aspartic acid
Human teeth
Forensic science

ABSTRACT

One way to estimate biological age is to use the aspartic acid (Asp) racemization method. Although this method has been performed mostly using enamel and dentin, we investigated whether an entire tooth can be used for age estimation. This study used 12 pairs of canines extracted from both sides of the mandible of 12 individuals of known age. From each pair, one tooth was used as a dentin sample and the other as a whole-tooth sample. Amino acids were extracted from each sample, and the integrated peak areas of D-Asp and L-Asp were determined using a gas chromatograph/mass spectrometer. Statistical analysis was performed using the D/L-Asp ratio. Furthermore, teeth from two unidentified bodies, later identified as Japanese and Brazilian, were examined in the same manner. Results showed that the D/L ratios of whole-tooth samples were higher overall than those of dentin samples. The correlation coefficient between the D/L ratios of dentin samples and their age was $r = 0.98$, and that of the whole-tooth samples was $r = 0.93$. The difference between estimated age and actual chronological age was −0.116 and −6.86 years in the Japanese and Brazilian cases, respectively. The use of whole teeth makes the racemization technique easier and can standardize the sampling site. Additionally, using only a few tooth samples per analysis made it possible to reanalyze known-age samples. Although the difficulty in obtaining a proper control sample has prevented racemization from being widely used, the method described here not only ensures the availability of a control tooth, but also enables the teeth to be used for other purposes such as DNA analysis. The use of a whole tooth will increase the application of the racemization technique for age determination.

1. Introduction

Age estimation is essential for the identification of unidentified dead bodies, and teeth and bones are often used for this purpose because they retain morphology in the form of skeletal remains long after soft tissue decomposition following death. Indicators for estimating age up to 25 years old are the developmental stage of the ossification nucleus, the state of diaphysis and epiphysis fusion, and the calcification and eruption state of teeth. With regard to the skeleton of older individuals, indicators may include the degree of fusion of the cranial and orbital sutures, the radial groove of lumbar vertebrae, and the degree of dental attrition and stenosis of the dental pulp cavity. However, these morphological methods for age estimation are often associated with a margin of error due to individual differences, resulting in a wider target age range.

Biological methods for age estimation include amino acid racemization techniques and carbon-14 (C-14) analysis. The use of amino acid racemization in the estimation of age is based on the biological phenomenon that the ratio between the enantiomers of aspartic acid (Asp) increases with age. Helfman and Bada [1] were the first to determine the ratio of Asp enantiomers in tooth enamel and showed a correlation between the Asp ratio and age. Ohtani and Yamamoto [2] reported that estimated age fell within ±3 years of actual age when using the racemization rate of Asp from dentin. On the other hand, radiocarbon dating analysis became popular because of the increased level of radioisotope C-14 in the atmosphere due to nuclear testing conducted around the world in 1960s. In 2005, Spalding et al. used the level of C-14 in tooth enamel, which had been incorporated into the tissue during development, to determine the year of birth and subsequently reported estimated age to within ±1.6 years of actual age [3]. Despite their high accuracy, these biological methods have not been routinely used due to technical and installation issues.

The crown of a tooth is covered by an enamel layer rich in inorganic materials, and the root is covered by periodontal tissue. Accordingly, while an organism is biologically active, teeth are kept

* Corresponding author. Tel.: +81 43 226 2078; fax: +81 43 226 2079.
E-mail address: a.sakuma@chiba-u.jp (A. Sakuma).

0379-0738/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.forsciint.2012.08.043

in a relatively stable environment in terms of temperature and humidity, allowing amino acids in the teeth to racemize at a constant rate [4]. In addition, tooth dentin, the major component of a tooth, contains dentin-specific proteins, such as dentin matrix proteins and phosphophoryn, and these proteins contain a large quantity of Asp, which has the fastest racemization rate of amino acids. Tooth enamel [5], dentin [6], and cementum [7] have been used in the Asp racemization method. In particular, the central part of dentin, which is rich in amino acids, is reported to have a superior correlation with age [8]. However, it is not always possible to perform consistent sampling of dentin or to reuse a sample for confirmation of racemization analysis results or other purposes such as DNA testing because of the low quantity per tooth. In addition, more than 4 control teeth of known age and the same tooth type as the corresponding unknown sample are generally required for accurate age estimation [2]. One problem associated with the racemization method, which is thought to be the reason for its poor utilization, is the difficulties associated with control teeth sampling. If a whole tooth is used to extract Asp, the amount is sufficient not only for racemization analysis, but also for use as a control of known age for further racemization analysis. In addition, a leftover sample can be used for other tests such as DNA analysis. Therefore, to simplify racemization steps, standardize sampling, and reuse samples as a control of known age for further analysis, we investigated whether a whole tooth can provide racemization sample material suitable for estimating age. Moreover, to examine the applicability of the method using whole-tooth samples in routine work, the Asp racemization method was performed in the same manner with whole-tooth samples from two unidentified bodies.

2. Materials and methods

This study used 12 pairs ($n = 24$) of canines without severe caries or extensive dental work. Specimens were obtained from both sides of the mandible of 12 individuals of known age. One tooth from each pair was used as a dentin sample ($n = 12$) and the other as a whole-tooth sample ($n = 12$).

A dental handpiece (BL-30, Osada Inc., Tokyo, Japan) and a silicone point (Big Silicone Points; Shofu Inc., Kyoto, Japan) were used to remove blood, calculus, and the periodontal membrane from the tooth surface. To isolate dentin, the center of a tooth was dissected using a low-speed saw (Isomet, Buehler Co. Ltd., Lake Bluff, IL) to obtain a 1-mm thick section (Fig. 1a), and part of the enamel, cementum, and pulp were removed from the section (Fig. 1b). Dentin and whole-tooth samples were each washed for 5 min in 5 ml of 0.2 M hydrochloric acid, 5 ml of deionized water (3 times), 5 ml of ethanol, and 5 ml of ether using an ultrasonicator.

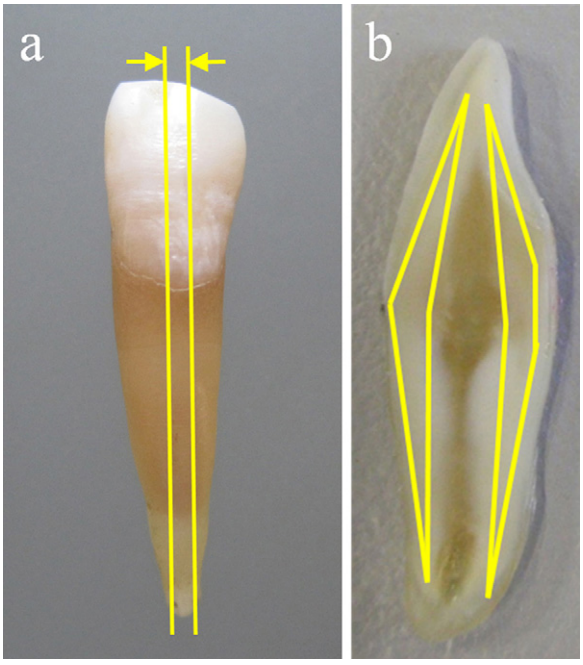

Fig. 1. A 1-mm section was obtained from the center of a mandibular canine (a), and the dentin part of the section was used as a sample after removing the areas of enamel, cementum, and pulp (b).

Samples were air-dried and then powdered using a Multi-Beads Shocker (Yasui Kikai, Osaka, Japan). Ten milligrams each of the samples were hydrolyzed in 5 ml of 6N hydrochloric acid for 6 h at 100 °C using a dry bath incubator (EYELA MG-2200, Tokyo Rikakikai, Tokyo, Japan). After hydrolysis, a centrifugal concentrator (CC-105, Tomy Seiko Co. Ltd., Tokyo, Japan) was used to remove hydrochloric acid and the samples were solubilized in deionized water. Solubilized samples were loaded onto a strong acid cation-exchange resin column, and deionized water followed by 2N aqueous ammonia was applied to the column to extract amino acids.

Esterification of amino acids was performed by mixing each sample with 1 ml of acetyl chloride/isopropanol (2:8, v/v) and heating at 100 °C for 30 min. After drying samples using a centrifugal concentrator, acetylation was performed with 400 μl of methylene chloride and 100 μl of trifluoroacetic anhydride at room temperature for 30 min. Samples were dried using a centrifugal concentrator, solubilized in 100 μl of ethyl acetate, and subjected to gas chromatography/mass spectrometry with a built-in optically active capillary column (5975C; Agilent Technologies, CA). The integrated peak areas of D-Asp and L-Asp were used to determine the D-Asp/L-Asp value as the racemization rate.

Statistical analysis using SPSS® Statistics 17.0 (SPSS Inc., Chicago, IL), followed by regression analysis, was performed to compare the results between dentin and whole-tooth samples. The racemization rates of the two samples were compared using the *t*-test.

2.1. Cases

2.1.1. Case 1

A highly decomposed body was found in the suspect's garden and had been buried for up to 6 months. The soft tissue was decomposed and partially skeletonized. Forensic autopsy could not identify the cause of death. Morphology put the estimated age in the late 40–60 s. The estimated sex determined by DNA analysis was female. Many dental treatments performed under health insurance, such as metal inlays and bridges, were evident and congenital absence of the mandibular left canine and conspicuous overjet were noted. However, there were no intraoral findings that could narrow the range of estimated age. For age estimation using the whole-tooth Asp racemization method, a maxillary canine was taken from the unidentified body and four maxillary canines from persons of known age (33, 53, 64, and 71 years old) were used as a control samples.

2.1.2. Case 2

A drowned male body was found floating in a river. The individual had been dead for 4–7 days. Soft tissues were highly decomposed and there was severe bloating. Forensic autopsy could not identify the cause of death, but near-drowning was suspected. Morphology put the estimated age in the 30–50 s. Many dental treatments not covered by health insurance were evident, such as ceramic inlays and porcelain fused to metal bridge from the maxillary right canine to the left second molar. For age estimation using the whole-tooth Asp racemization method, a mandibular canine was taken from the unidentified body and four mandibular canines from persons of known age (44, 50, 61, and 72 years old) were used as control samples.

The study was approved by the Ethics Review Board of the Graduate School of Medicine, Chiba University, Japan.

3. Results

Table 1 presents the racemization rates for the 12 pairs of dentin and whole-tooth samples in descending order of age.

Table 1
Comparison of the racemization rates of 12 pairs of adult canine teeth (24 teeth in total) from individuals of known age.

Age (years)	Racemization rate for dentin	Racemization rate for whole tooth
17	0.017032	0.018006
18	0.017894	0.026087
21	0.022030	0.023958
32	0.025404	0.027167
33	0.032031	0.039776
39	0.030608	0.033819
48	0.035494	0.040996
51	0.038896	0.047451
61	0.040355	0.047120
64	0.045399	0.053099
64	0.042755	0.058867
76	0.048355	0.051746