Age estimation of living Indian individuals based on aspartic acid racemization from tooth biopsy specimen

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Abstract

Background: Age estimation in living individuals is imperative to amicably settle civil and criminal disputes. A biochemical method based on amino acid racemization was evaluated for age estimation of living Indian individuals. Design: Caries-free maxillary/mandibular premolar teeth (n = 90) were collected from participants with age proof documents and divided into predefined nine age groups. Materials and Methods: Dentine biopsy from the labial aspect of the tooth crown was taken with an indigenously developed microtrephine. The samples were processed and subjected to gas chromatography. Dextrorotatory:levorotatory ratios were calculated, and a regression equation was formulated. Results: Across all age groups, an error of 0 ± 4 years between protein racemization age and chronological age was observed. Conclusion: Aspartic acid racemization from dentine biopsy samples could be a viable and accurate technique for age estimation of living individuals who have attained a state of skeletal maturity.

Key words: Age estimation, aspartic acid racemization, dentine biopsy, gas chromatography, microtrephine

Introduction

Age is the measure of an attribute relative to the chronological age of an average normal individual. Krogman defines chronological age as the birthday or the calendric age. It is based on sidereal time and is constant. Precise estimation of age is important for forensic personnel and anthropologists. It assumes significance in living individuals who do not have valid demographic details and are facing civil and criminal charges. In these circumstances, verification of chronological age is imperative to ascertain whether the concerned person has reached the age of imputability. In India, the age threshold for criminal prosecution is set at 18 years, i.e. below this, they are tried under the juvenile law.

The Study Group on Forensic Age Diagnostics has issued recommendations for determining the age of living participants undergoing criminal proceedings. Accordingly, this is a combination of results of physical examination, X-ray of the hand/wrist, radiological or computed tomographic examination of the clavicles, and dental assessment that records dentition status and evaluates an
Oxidation, isomerization, and racemization are age-related changes that occur in protein. The newly synthesized proteins are normally composed of levorotatory (L) amino acids. Over a period of time, these convert to dextrorotatory (D) by an automatic chemical reaction (racemization), and it correlates highly with protein age. The rate of racemization is influenced by temperature, humidity, and pH. In particular, L-aspartic acid is transformed to D-aspartic acid, and it accumulates in organs with low metabolic rates (bradytrophic tissues). D:L ratio of this amino acid can be analyzed and used for age estimation. At present, based on accuracy, simplicity, and the time required, teeth are the best organ for analysis of aspartic acid racemization.

Helfman and Bada in 1976 focused on aspartic acid racemization. They correlated the ratio of L- and D-amino acids (D:L ratio) in dentin to age and obtained excellent results (correlation coefficient; r = 0.979). Since then, numerous investigators have documented the efficacy and accuracy of this method. However, till date, majority of the studies have applied this technique to postmortem cases as a tooth needs to be extracted to obtain dentin sample for analysis of amino acid racemization. This may not be an ethically viable option for age determination in living individuals.

To identify the age of living individuals without extracting the teeth, Ritz et al. (r = 0.99) developed a dentin biopsy technique. The results were promising and showed a close relationship between the extent of aspartic acid racemization in dentin biopsy specimens and age. However, no follow-up study to standardize and replicate these findings on a larger sample size has been reported.

Due to an increase in the number of cases requiring age estimation in living individuals, it was conceptualized to conduct a study to estimate age of living Indian individuals based on aspartic acid racemization from dentin biopsy specimen.

Materials and Methods

This study was conducted after obtaining the institute’s ethical clearance. The objectives were to indigenously develop a microtrephine, to obtain and biochemically evaluate the dentin biopsy specimen, and to estimate the age of living Indian individuals based on amino acid racemization.

Development of microtrephine

A microtrephine was fabricated from high-speed steel (En 1.04). Its length was 21 mm. It had an outer and inner diameter of 2.8 and 2.0 mm, respectively. The head comprised eight sharp blades with a cutting edge of 0.3 mm. The depth of penetration of the microtrephine was limited to 4.2 mm. For easy retrieval of the dentin block, a 1.0 mm slot was incorporated in the shaft. The cutting surface of the instrument had a stealth finish. A customized stylus was also fabricated [Figures 1-3].

Collection of dentin biopsy sample

To obtain dentin biopsy specimen, 100 caries-free maxillary/mandibular first and second premolar teeth with compromised periodontal status/indicated for extraction as part of orthodontic treatment plan were collected from participants with at least two verifiable valid identification documents. These credentials were collected; number coded and sealed in an envelope.

Processing and storage of teeth

Following atraumatic extraction, the tooth was scrubbed with a detergent to remove the adherent patient material. Subsequently, it was immersed in a fresh solution of 5.25% sodium hypochlorite for 20 min. The tooth was washed under running tap water, air-dried, and coded with a number similar to that marked on the sealed document envelope. The extracted teeth were stored in glass vial and kept in a deep freezer (Blue star India) at a temperature of −21°C till further use.

Dentin sample retrieval

The tooth was retrieved from the deep freezer and thawed to room temperature. Barrier protection was undertaken to prevent cross infection. An intra-enamel guiding punch was made with a round diamond bur on the buccal surface, at a right angle to the longitudinal axis of the tooth, midway between the occlusion plane and the cementoenamel junction [Figure 4]. The microtrephine was attached to the handpiece (NSK: NBBW-EC, 1:1 Direct Drive, Latch Type, Maxm Speed 40,000 with Water Nozzle), and a channel was made up to a depth of 2 mm [Figure 4]. A small cylindrical block of dentin was lodged into the hollow shaft of the...
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The laboratory was blinded to the study aim and objectives.

**Armamentarium**

**Instrument**
GC/mass spectrometry (MS) Perkin Elmer (Clarus 500) with Auto sampler (PerkinElmer Life and Analytical Sciences, USA) was used.

**Chemical and reagents used**
D-aspartic acid (Sigma-Aldrich), L-aspartic acid (Sigma-Aldrich), thionyl chloride (SD Fine, Mumbai), isopropanol (Rankem Faridabad), trifluoroacetic anhydride (SD Fine, Mumbai), methanol (high-performance liquid chromatography [HPLC] grade) (SD Fine, Mumbai), purified water (18.2 mega ohm MilliQ), ammonia (25%) (SD Fine, Mumbai), ethanol (SD Fine, Mumbai), hydrochloric acid (HCl) (SD Fine, Mumbai), dichloromethane (SD Fine, Mumbai), and ion-exchange resin (Dowex, 50W-X8, 50-100 mesh Dow Chemical Company, USA) were the chemicals and reagents used.

**Processing of dentin sample**

**Washing**
The block of dentin was taken in a centrifuge tube. One milliliter of 0.2M HCl was added and vortexed for 5 min. The sample was retrieved and placed in a fresh centrifuge tube. To this, 1 ml of purified water (18.2 mega ohm Milli Q) was added, and it was vortexed for 5 min. This step was repeated twice. The sample was retrieved and placed in another centrifuge tube to which 1 ml of ethanol was added. This was vortexed for 5 min. Finally, the sample was placed in a centrifuge tube containing 1 ml of ether and vortexed for a similar period. Subsequent to this, the sample was dried in a hot air oven.

**Hydrolysis**
The dried sample was taken in a glass ampoule. 0.5 ml of 6M HCl was added. The ampoule was sealed and allowed to stand for 6 h at 100°C in a hot air oven. The sample was reconstituted with 5 ml of distilled water.

**Preparation of the column**
The hydrolyzed sample was applied to an ion-exchange resin (Dowex, 50W-X8, 50-100 mesh Dow Chemical Company, USA). The resin was washed with 10 ml of distilled water. Subsequently, the amino acids were eluted from the resin with 5 ml 3N NH₄OH. This fraction was dried by evaporation in a hot air oven at 100°C.

**Derivatization**
One milliliter of 3M thionyl chloride was added to the dried sample, and this was kept in a hot air oven at 100°C for 2 h. The resultant residue was allowed to cool. One milliliter mixture of dichloromethane: trifluoroacetic anhydride (3:1)
was added. This was heated in a hot air oven at 100°C for 20 min. The samples were recooled to room temperature. One milliliter of dichloromethane was added. The sample was vortexed and transferred into a GC vial for application to a GC/MS under the following instrumental conditions: ion source: electron ionization; column: CP-Chirasil-L-Val 25 × 0.25; injector volume: 1 ml; carrier gas: helium, constant flow mode, 1.0 ml/min; and oven program: initial temperature 80°C hold for 2 min, Ramp 1–4 deg/min 190°C hold for 2 min.

Subsequent to this, well-appreciated peaks of D- and L-aspartic acid were elucidated on a gas chromatogram [Figure 5]. The areas of peaks were calculated by a software Turbo-Mass™, and these values were substituted in the equation

\[ \text{D:L ratios} = \ln \left( \frac{1 + \text{area of D}}{\text{area of L}} \right) / \left(1 - \frac{\text{area of D}}{\text{area of L}} \right) \]

**Results**

**Estimation of age of living Indian individuals based on amino acid racemization**

Coronal dentin specimens from 100 teeth were distributed into nine groups based on age. Accordingly, Group I: 10–15 years (n = 14), Group II: 16–20 years (n = 13), Group III: 20–25 years (n = 5), Group IV: 40–45 years (n = 12), Group V: 46–50 years (n = 9), Group VI: 51–55 years (n = 6), Group VII: 56–60 years (n = 5), Group VIII: 61–65 years (n = 21), and Group IX: 66–70 years (n = 5). Ten teeth could not be analyzed due to incorrect sampling/handling.

The values of D:L ratios of aspartic acid were entered in a Microsoft Excel (2007) spreadsheet. It was then converted into Statistical Package for the Social Sciences (version 16.0) (IBM, SPSS Inc, Chicago, III). A linear regression line was established. This is a graph plotted of ln \((1 + \text{area of D/area of L})/(1- \text{area of D/area of L})\) peaks of aspartic acid versus the actual age [Figure 6]. The slope of the line is “b,” and “a” is the intercept.

The regression equation \(y = a + b \times x\) was formulated, where \(y = \text{protein racemization age; a and b = constant; and x = D:L ratio of aspartic acid. Regression equation, standard error of mean, average error, and regression coefficient (r) were calculated for each group [Tables 1-9].

**Discussion**

Aspartic acid racemization for age estimation has evolved over a period of time and now is an established method.[7-12] However, majority of the studies have been reported from Japan and Germany. The present study is based on the ideology of Ritz et al. and is an attempt to investigate the correlation between aspartic acid racemization and age from tooth biopsy specimen of living individuals in the Indian subcontinent.[11]

The rate of aspartic acid racemization varies with the type of tooth. Accordingly, it is highest for the second molar and decreases in the following order: first molar > second premolar > central incisor > first premolar > lateral incisor > canine.[6,13,14] Ohtani and Yamamoto,[15] Fu et al.,[16] and Yekkala et al.[17] have advocated the use of premolar teeth. In the present study, a combination of maxillary first (n = 25) and second (n = 30) and mandibular first (n = 20) and second premolar (n = 15) teeth was used. Since this class of teeth begins to calcify at the same period (18–24 months),[18] their data can be pooled and a regression equation can be formulated. They are single-rooted (except maxillary first premolar), small in size, yield maximum dentin quantity and have an anterior positioning in the maxillary and mandibular arch,[6,17,19] rendering them more feasible for dentin biopsy in clinical scenario since the ultimate aim is to develop and test a dentin biopsy technique in living adults. In addition, premolar teeth were given preference as these were easily available across all age groups (especially 10–25 years due to extraction as per orthodontic treatment plan).
| Sample code | Gender | Actual age (years) | D: L ratio | Protein racemization age (years) (y*) | Difference (estimated—actual age) (years) |
|-------------|--------|-------------------|------------|--------------------------------------|-------------------------------------------|
| 50          | Male   | 11                | 0.00228    | 12.3                                 | 1.3                                       |
| 85          | Female | 12                | 0.01219    | 13.2                                 | 1.2                                       |
| 88          | Female | 12                | 0.0165     | 13.6                                 | 1.6                                       |
| 93          | Female | 13                | 0.01281    | 14.1                                 | 1.1                                       |
| 96          | Female | 13                | 0.01169    | 13.1                                 | 0.1                                       |
| 48          | Male   | 13.2              | 0.00361    | 12.4                                 | -0.8                                      |
| 49          | Male   | 13.2              | 0.02265    | 14.2                                 | 1.0                                       |
| 43          | Female | 13.5              | 0.00613    | 12.6                                 | -0.9                                      |
| 44          | Female | 13.5              | 0.00613    | 12.6                                 | -0.9                                      |
| 57          | Male   | 14                | 0.01732    | 13.7                                 | -0.3                                      |
| 58          | Male   | 14                | 0.01682    | 13.6                                 | -0.4                                      |
| 26          | Male   | 15                | 0.02863    | 14.7                                 | -0.3                                      |
| 56          | Male   | 15                | 0.01717    | 13.6                                 | -1.4                                      |
| 86          | Male   | 15                | 0.01935    | 13.9                                 | -1.1                                      |

*Regression equation for age group 10-15 years, *y=22.022+15.239×D:L ratio, average error=1.02
r*(years)=0.52. SE: Standard error=1.32

| Sample code | Gender | Actual age (years) | D: L ratio | Protein racemization age (years) (y*) | Difference (estimated—actual age) (years) |
|-------------|--------|-------------------|------------|--------------------------------------|-------------------------------------------|
| 77          | Male   | 46                | 0.03511    | 47                                   | 1.0                                       |
| 21          | Male   | 47.5              | 0.05225    | 47.6                                 | 0.1                                       |
| 75          | Male   | 47.5              | 0.04964    | 47.5                                 | 0                                         |
| 64          | Male   | 48                | 0.03397    | 46.9                                 | -1.1                                      |
| 101         | Male   | 48.9              | 0.08629    | 48.7                                 | -0.2                                      |
| 3           | Male   | 48.9              | 0.08629    | 48.7                                 | -0.2                                      |
| 5           | Female | 49.1              | 0.0989     | 49.2                                 | 0.1                                       |
| 16          | Female | 49.2              | 0.09938    | 49.2                                 | 0                                         |
| 67          | Female | 49.1              | 0.0989     | 49.2                                 | 0.1                                       |

*Regression equation for age group 46-50 years, *y=45.798+34.549×D:L ratio, average error=0.3, r*=0.86. SE: Standard error=0.55

| Sample code | Gender | Actual age (years) | D: L ratio | Protein racemization age (years) (y*) | Difference (estimated—actual age) (years) |
|-------------|--------|-------------------|------------|--------------------------------------|-------------------------------------------|
| 60          | Female | 51                | 0.05624    | 51.5                                 | 0.5                                       |
| 35          | Female | 51.4              | 0.04969    | 51.1                                 | -0.3                                      |
| 68          | Female | 51.4              | 0.04969    | 51.1                                 | -0.3                                      |
| 76          | Female | 51.4              | 0.05552    | 51.4                                 | 0                                         |
| 31          | Male   | 54                | 0.09326    | 53.8                                 | -0.2                                      |
| 91          | Male   | 54                | 0.09642    | 54                                   | 0                                         |

*Regression equation for age group 51-55 years, *y=48.027+62.462×D:L ratio, average error=0.21, r*=0.97. SE: Standard error=0.34
Dentin formation starts at the dentin–enamel junction and gradually shifts toward the dental pulp and root apex region. The period of dentin formation is variable, and it depends on the type of tooth and the individual. It is documented that 8–10 years or more are required from start to completion, indicating the possibility that the degree of racemization may differ in different parts of the dentin, i.e., coronal or root dentin. For determining racemization rates, crowns are preferred for younger individuals and root for elderly participants. In this study, coronal dentin was used irrespective of the age of the patient, since in clinical situations, dentin biopsy from the root would require extraction.

The sample can be obtained either from the labial or lingual aspect of the crown of the tooth. Literature recommends the latter side because it yields better D/L ratios, suggesting that lingual part may be exposed to higher environmental temperatures. However, in the present study, the labial aspect of the crown of the tooth was used because in clinical condition it would be very difficult to obtain the specimens from the lingual aspect using indigenous microtrephine. In addition, due to an increased surface area on the labial aspect of the crown of the tooth, a substantial amount of dentin sample can be collected. Moreover, Griffin et al. have reported no variation in the yield of D/L ratios from either labial or lingual coronal dentin.

Till date, age estimation by amino acid racemization of dentin has been limited to postmortem cases. In this scenario, the tooth can be extracted for the purpose of dentin retrieval. However, in living individual, extraction of a nondiseased tooth for age estimation may have ethical ramifications. Hence, it was conceptualized to develop a microtrephine for retrieving a dentin sample. The indigenous microtrephine was fabricated from high-speed steel grade composition En 1.04 with stealth coating. This aided in smooth cutting, less heat generation, and atraumatic retrieval of dentin biopsy sample. In addition, it enhances the longevity of the cutting area and prevents tissue adhesion. Since the punch made by the microtrephine is 2 mm in diameter, postbiopsy the tooth can be restored easily with the available tooth-colored adhesive restorative material without affecting aesthetics and functionality of the tooth. Since there are depth indicating markings on the microtrephine, the hazard of excessive cutting and subsequent pulp exposure is minimal. No

### Table 7: Group VII: 56-60 years

| Sample code | Gender | Actual age (years) | D:L ratio | Protein racemization age (years) (y*) | Difference (estimated–actual age) (years) |
|-------------|--------|--------------------|---------|-----------------------------------|-----------------------------------|
| 25          | Male   | 57                 | 0.03898 | 56.9                              | 0.1                               |
| 34          | Male   | 58                 | 0.08365 | 58                                | 0                                 |
| 46          | Male   | 58                 | 0.09859 | 58.4                              | 0.4                               |
| 72          | Female | 59                 | 0.13747 | 59.4                              | 0.4                               |
| 71          | Male   | 60                 | 0.12387 | 59.1                              | −0.9                              |

*Regression equation for age group 56–60 years, *y* = 55.896 + 25.942 × D:L ratio, average error = 0.36, *r* = 0.97. SE: Standard error = 0.63

### Table 8: Group VIII: 61-65 years

| Sample code | Gender | Actual age (years) | D:L ratio | Protein racemization age (years) (y*) | Difference (estimated–actual age) (years) |
|-------------|--------|--------------------|---------|-----------------------------------|-----------------------------------|
| 54          | Female | 60.8               | 0.1071  | 62.5                              | 1.7                               |
| 94          | Male   | 60.8               | 0.1071  | 62.5                              | 1.7                               |
| 15          | Male   | 61.3               | 0.1767  | 62.6                              | 1.3                               |
| 93          | Male   | 62                 | 0.08108 | 62.4                              | 0.4                               |
| 23          | Male   | 62                 | 0.01952 | 62.3                              | 0.3                               |
| 24          | Male   | 62                 | 0.02344 | 62.3                              | 0.3                               |
| 28          | Male   | 62                 | 0.0195  | 62.3                              | 0.3                               |
| 32          | Male   | 62.5               | 0.09523 | 62.4                              | −0.1                              |
| 8           | Female | 64                 | 0.805   | 63.7                              | −0.3                              |
| 9           | Female | 64                 | 0.4139  | 63                                | −1                                |
| 7           | Male   | 63                 | 0.35208 | 62.9                              | −0.1                              |
| 30          | Male   | 65                 | 0.02105 | 62.3                              | −2.4                              |
| 79          | Male   | 62                 | 0.01952 | 62.3                              | 0.3                               |
| 90          | Female | 65                 | 0.02344 | 62.3                              | −2.7                              |
| 10          | Male   | 63                 | 0.41616 | 63                                | 0                                 |
| 13          | Male   | 63                 | 0.23775 | 62.7                              | −0.3                              |
| 18          | Male   | 63                 | 0.31363 | 62.8                              | −0.2                              |
| 19          | Male   | 63                 | 0.49681 | 63.2                              | 0.2                               |
| 17          | Male   | 63                 | 0.29952 | 62.8                              | −0.2                              |
| 20          | Male   | 63                 | 0.44977 | 63.1                              | 0.1                               |
| 61          | Male   | 63                 | 0.52735 | 63.2                              | 0.2                               |

*Regression equation for age group 61-65 years, *y* = 62.31 + 1.795 × D:L ratio, average error = 0.52, *r* = 0.92. SE: Standard error = 0.96

### Table 9: Group IX: 66-70 years

| Sample code | Gender | Actual age (years) | D:L ratio | Estimated age (years) (y*) | Difference (estimated–actual age) (years) |
|-------------|--------|--------------------|---------|--------------------------|-----------------------------------|
| 29          | Male   | 67                 | 0.08772 | 68.4                     | 1.4                               |
| 59          | Male   | 67                 | 0.04203 | 68.1                     | 1.1                               |
| 14          | Female | 70                 | 0.3849  | 70.3                     | 0.3                               |
| 52          | Male   | 70                 | 0.09853 | 68.5                     | −1.5                              |
| 53          | Male   | 70                 | 0.11558 | 68.6                     | −1.4                              |

*Regression equation for age group 66-70 years, *y* = 67.85 + 6.517 × D:L ratio, average error = 1.14, *r* = 0.54. SE: Standard error = 1.8

raised environmental temperature as it is surrounded by periodontal ligament. In addition, it also has a different protein composition. However, cementum is not preferred for age estimation as it is not easily retrievable, and the amount of sample obtained is insufficient. Dentin is surrounded by enamel and cementum. Its moisture content is kept constant with fluid supply through the dentinal tubules. This maintains the ambient temperature. Moreover, the degree of racemization and its correlation with age is highest for dentin. Hence, in this study, dentin specimen was used.
iatrogenic entry into the pulp chamber space was noticed in any of the teeth analyzed. The microtrephine was developed for single use to avoid cross-contamination and prevent dulling of the cutting blades.

The extracted tooth and its corresponding dentin biopsy specimen were sealed in a glass vial and centrifuge tube, respectively. They were coded to avoid mix-up and reduce bias. Racemization is a first-order chemical reaction, and temperature affects the D:L ratio. 1° centigrade increase in temperature results in a 20%–25% increase in the racemization rate. Hence, in the present study, the tooth and dentin specimen were stored in a deep freezer at -21°C. The same ambient temperature was maintained while transporting it to the laboratory.

It is critical to completely separate the D and L-enantiomers of amino acids and to obtain sharp peaks on the chromatogram. This can be achieved by gas (GC) or HPLC. In this study, the former was preferred. Since D and L-enantiomers have identical chemical and physical properties, it is necessary to separate amino acids with one chiral center within a chiral chromatographic system. Moreover, amino acids are usually derivatized as N-trifluoroacetic acid isopropyl esters, and all the amino acids can be separated and quantified on a chiral capillary column in one chromatographic run. According to Johnson and Miller aspartic acid, enantiomers are usually detected by GC. This was also recommend by Waite et al. who suggested that this was a better method because a minute amount of the specimen was sufficient to completely separate the enantiomers and obtain sharp peaks on the chromatogram.

In the present study, the chemical analysis of aspartic acid racemization from dentin biopsy specimen was in accordance with Ohtani and Yamamoto. However, they advocated the pulverization of the sample. This step was omitted in the present methodology because the biopsy sample was minute in size. Waite et al. have recommended the same. The dentin biopsy specimen retrieved by the indigenous microtrephine was sufficient to elucidate sharp well-differentiated peaks on a chromatogram.

One of the essential components of the study was to collect the teeth from individuals with a legitimate age proof document. The following documents were considered valid: passport, birth certificate issued by the municipal corporation, higher secondary school certificate, unique identity card, and driving license. Individuals with either of the two above-mentioned documents were enrolled in the study.

A total of ninety dentin samples were analyzed for aspartic acid racemization. The estimated age was calculated from the regression equation based on least square method. The participants (n = 90) were distributed into nine age groups. Fifty-eight participants had an age range between 40 and 70 years (mean age 41.67 years). The maximum requirements of age estimation in living individuals are in this age group as the accuracy of conventional methods decreases with the advancing age. Fifty-one males and 39 females participated. The estimated age exactly matched the chronological age for six participants. An error of ±1 year was observed in 53 participants. An error of ±2 years was observed in 26 cases. In four participants, the difference between both the parameters was within ±3 years. Only one participant had a difference >3 years. Overall across all age groups, the result of the study demonstrated an error in the range of 0 to ±4 years.

The results were in agreement with studies which applied GC techniques for separation of D:L ratios. Ohtani and Yamamoto evaluated longitudinal sections of dentin of 56 teeth obtained from nine Japanese cadavers with age ranging between 58 and 88 years. The actual and chronological age differed by ±5 years. The same authors evaluated longitudinal enamel sections of 49 teeth from eight Japanese corpses with age range between 58 and 88 years old at death. Their results demonstrated an error range of 0 ± 6 years. Arnany et al. conducted a study on longitudinal sections of dentin of 24 premolars from Japanese individuals ranging from 13 to 88 years. Their error range was ±0.57 year. Ogino and Ogino assessed nine tooth specimens with age range between 12 to 40 years. Their observations demonstrated error range of ±4 years. Our results were also comparable to those studies applying HPLC technique. Fu et al. conducted a study on 28 first premolars from Chinese individuals with age ranging from 14 to 69 years. Tooth crown was ground in a mortar and pestle, and dentin separated with hand under ultraviolet light. Their results yielded an error range of 0 ±4 years. Elfawal et al. carried out a study on root dentin of 89 upper first premolar from Kuwaiti participants with an age range of 10–31 years. The average error was ±1.12 years. Rajkumari et al. conducted a study on buccolingual longitudinal sections of 36 maxillary and mandibular premolar from Indian participants with an age range of 11–70 years. They estimated age between the ranges of ±3 years.

**Conclusion**

The current study is a pilot attempt to investigate aspartic acid racemization for age from tooth biopsy specimen of living individuals. In comparison to the reported literature, the results of the present study are very encouraging. It would be worthwhile to further investigate this technique on a larger sample size/volunteers to standardize and validate the biopsy technique. Collaboration with other investigators would help us to understand ethnic variations as well as effect of laboratory processing conditions.
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Conflicts of interest
There are no conflicts of interest.

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