ORIGINAL ARTICLE

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.[1] Krogman defines chronological age as the birthday or the calendric age. It is based on sidereal time and is constant.[2] Precise estimation of age is important for forensic personnels 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

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orthopantomogram.[3] The accuracy of these methods is influenced by gender, race, and environmental conditions, and it decreases with increasing age.[3]

Oxidation, isomerization, and racemization are age-related changes that occur in protein.[4,5] 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.[6] 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 organ with low metabolic rates (bradytrophic tissues).[6] 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.[6]

Helfman and Bada in 1976 focused on aspartic acid racemization.[7] 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.[8-10] 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.[11] 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, Max^{m} 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

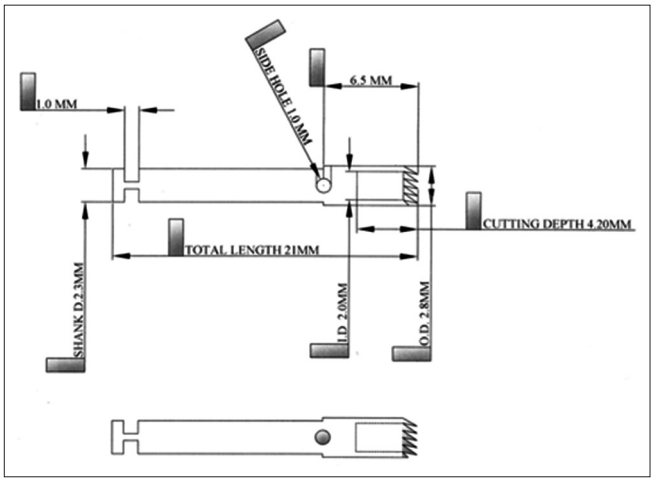

Figure 1: Schematic design of the indigenous microtrephine

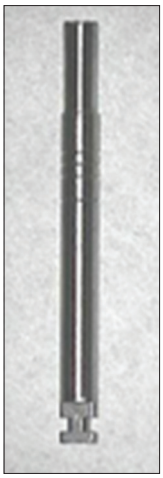

Figure 2: Final Microtrephine

Figure 3: Customized stylus for dentine block retrieval

Figure 4: Schematic representation of dentin biopsy site. The biopsy specimen (black bar) is taken at right angles to the longitudinal axis of the tooth midway between the occlusion plane and the cementoenamel junction; the microtrephine penetrates exactly 2 mm into the dentin. Biopsy locations for investigation of the influence of biopsy layer

microtrephine, and this was retrieved with the aid of the customized stylus. This block of dentin was stored in a centrifuge tube that was coded with a number similar to that marked on the sealed document envelope and the glass vial containing the extracted tooth. It was again stored in a deep freezer.

Chemical analysis of dentin biopsy sample for amino acid racemization

This study followed the methodology documented by Ohtani and Yamamoto[6] for analysis of aspartic acid racemization.

Dentin samples were transported under controlled environment to a laboratory for chemical analysis by gas chromatography (GC). This facility has a certification from the National Accreditation Board for Testing and Calibration and ISO/IEC 17025: 2005. 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 NH_4OH. 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

D:L ratios = ln {(1 + area of D/area of L)/(1- area of D/area of L)}

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 + area of D/area of L)/(1- 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 × x was formulated, where y = 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).

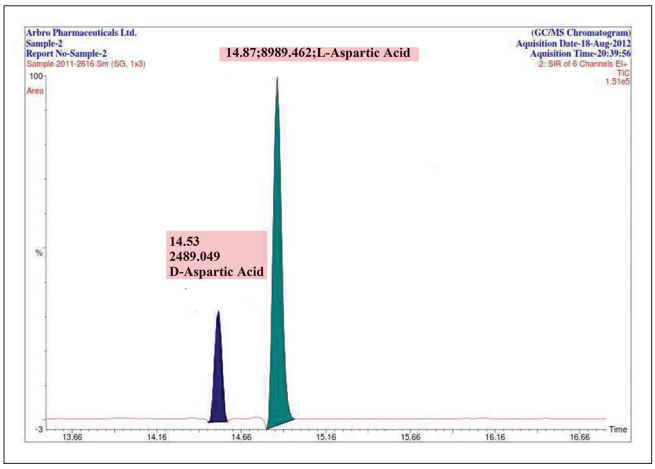

Figure 5: Gas chromatogram showing well-elucidated peak of D and L-aspartic acid

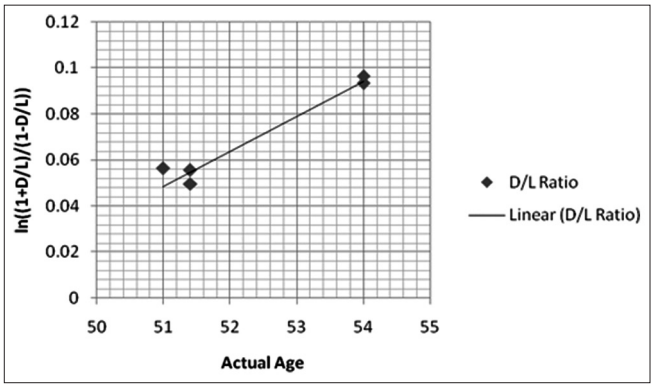

Figure 6: Relationship between racemization rates of aspartic acid and actual age

Table 1: Group I: 10-15 years

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
95	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, #r=Regression coefficient, *y=12.102+92.996×D:L ratio, average error=0.89, #r=0.58. SE: Standard error=1.02

Table 2: Group II: 16-20 years

Sample code	Gender	Actual age (years)	D:L ratio	Protein racemization age (years) (y*)	Difference (estimated−actual age) (years)
80	Male	15.1	0.01977	16.1	1.0
37	Female	15.11	0.03603	16.5	0.6
40	Female	16	0.04248	16.7	0.7
81	Male	16	0.06369	17.3	1.3
82	Male	16	0.0427	16.7	0.7
97	Female	19	0.09811	18.2	−0.8
38	Male	17	0.03633	16.5	−0.5
41	Male	15.2	0.02339	16.2	1.0
33	Male	19	0.11641	18.7	−0.3
98	Female	18	0.09572	18.2	0.2
100	Female	18	0.10423	18.4	0.4
39	Female	19.5	0.04265	16.7	−2.8
42	Female	19.5	0.02791	16.3	−3.2

*Regression equation for age group 16-20 years, *y=15.6+27.2×D:L ratio, average error=1.03, #r=0.53. SE: Standard error=1.54

Table 3: Group III: 21-25 years

Sample code	Gender	Actual age (years)	D:L ratio	Protein racemization age (years) (y*)	Difference (estimated−actual age) (years)
36	Female	21.2	0.0535	22.8	1.6
27	Female	22	0.02215	22.3	0.3
83	Female	24	0.1168	23.8	−0.2
84	Female	24	0.1133	23.7	−0.3
89	Female	24	0.02836	22.4	−1.6

*Regression equation for age group 21-25 years, *y=22.022+15.239×D:L ratio, average error: 0.8, #r=0.52. SE: Standard error=1.32

Table 4: Group IV: 40-45 years

Sample code	Gender	Actual age (years)	D:L ratio	Protein racemization age (years) (y*)	Difference (estimated−actual age) (years)
22	Female	45	0.469	45.2	0.2
12	Male	42	0.063	43.2	−1.2
65	Male	42	0.063	43.2	−1.2
69	Female	45	0.128	43.5	−1.5
70	Female	45	0.134	43.5	−1.5
4	Male	44.4	0.076	43.2	−1.2
66	Male	44.4	0.076	43.2	−1.2
51	Female	45	0.092	43.3	−1.7
92	Female	45	0.086	43.3	−1.7
47	Male	41	0.014	42.9	1.9
73	Male	40	0.035	43	3
2	Female	45	0.569	45.7	−0.7

*Regression equation for age group 40-45 years, *y=42.89+5.056×D:L ratios, average error=1.4, #r=0.48. SE: Standard error=1.7

Table 5: Group V: 46-50 years

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

Table 6: Group VI: 51-55 years

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

The rate of racemization also varies according to the type of dental tissue analyzed. Accordingly, it is the highest in cementum followed by dentin and enamel.[6,20] The higher D/L ratios in cementum may be due to the

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*=0.87. 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	Female	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	Female	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 ratios, average error=0.52, #*r*=0.32. 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.[6] 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.[13] Moreover, the degree of racemization and its correlation with age is highest for dentin.[21] Hence, in this study, dentin specimen was used.

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.[6,22] For determining racemization rates, crowns are preferred for younger individuals and root for elderly participants.[6,17,14] 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.[6,17] However, in the present study, the labial surface of the coronal dentin 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, a substantial amount of dentin sample can be collected. Moreover, Griffin *et al*.[23] 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 ramification. 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 esthetics 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

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.[6] 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.[24] 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.[24] This was also recommend by Waite *et al*.[25] 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 peak 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.[6] 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*.[25] 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[13] 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.[21] Arnany *et al*.[14] 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[26] 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*.[16] 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*.[12] 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*.[27] 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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