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A comparison between incremental lines of cementum and aspartic acid racemization in age estimation: histological and biochemical study

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Abstract

Background Age estimation is a vital aspect of the identification of an individual in forensic science. Teeth are one of the toughest structures in the human body and generally survive postmortem destruction. So, they can therefore be used more reliably than any other morphological or histological trait of the adult skeleton for estimating age.

Aim of the study The present work aimed to compare cementum incremental lines and aspartic acid racemization in age estimation among Egyptian subjects.

Methods Sixty-four subjects were assigned to two groups: males and females (each with 32 cases) to determine the effect of sex as a possible factor that might affect age estimation. For each group, in all subjects, age was estimated using histologic examination of ground, unstained section teeth by light and polarized microscopes, in addition to estimating the rate of aspartic acid racemization in the collagen of dentin by using the HPLC method.

Results There was a statistically significant correlation between each method of age estimation and chronological age. However, the strongest degree of correlation was observed for the racemization method, with a correlation coefficient of $r=0.99$ (95% CI 0.98 to 0.99), followed by the light method, $r=0.94$ (95% CI 0.90 to 0.96), and the polarized method, $r=0.93$ (95% CI 0.88 to 0.95).

Conclusions Both methods, cementum incremental lines and aspartic acid racemization, could help in age estimation, but aspartic acid racemization is more reliable and accurate.

Keywords Cementum incremental lines, Aspartic acid, Polarized microscope, Age, Egyptian

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Background

Dental age estimation is a very important aspect of forensic investigations concerned with age estimation from the examination of dental evidence (Acharya & Vimi 2009).

According to Gowda Charan et al. (2014), age estimation is critical to recognizing an individual. Irish & Scott (2016) report that forensic odontology is a reliable and trustworthy way to identify the victims of mass disasters.

Among those undergoing major changes, teeth may be the only means of identification (Gupta et al. 2022). Teeth are considered the most resistant structures in the human body (Matteussi et al. 2022).

Dental age estimation is either based on a well-ordered cascade of changes that occur during the formation and eruption of teeth or on a continuous process that diminishes the quality of dental tissues even when individual growth is complete (Meinl et al. 2008).

Various methods are used to determine age through teething and dentition. These methods can be broken down into multiple categories: clinical, radiographic, histological, chemical, and physical analysis (Alghonamy et al. 2015).

Cement chronology is the counting of incremental lines in tooth-root cementum to estimate an individual's age. Cementum is similar in chemical structure to bone but, unlike the bone, cementum does not undergo a remodeling process and is continually formed throughout life, which is extremely beneficial to the development of methods to estimate age. (Colard et al. 2015).

Cementum incremental lines are a microscopic method for determining a person's age based on the analysis of cementum incremental lines (Radovic 2012).

Alghonamy et al. (2015) used a histological method based on counting the incremental lines of dental cementum to estimate adult age.

Cementum incremental lines are a reliable method for age estimation. It is less reliable for periodontally affected teeth (Dias et al. 2010; Alghonamy et al. 2015). The analysis of cementum incremental lines proves to be a more convenient method of estimating age for young adults, but not for older adults (over 40 years old), as cementum lines become increasingly difficult to differentiate with aging (Gualdi et al. 2022). The method requires a standardized protocol to be followed (Pinto et al. 2022).

Oxidation, isomerization, and racemization are age-related changes that occur in proteins. 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 (Logani et al. 2017).

Aspartic acid racemization (AAR) is thought to be one of the most reliable and accurate ways to figure out a person's age in forensics. Elfawal et al. (2015) estimated the rate of aspartic acid racemization in the collagen of dentin by using HPLC and correlated it with age.

The analysis of aspartic acid racemization in human dental tissues produced accurate and potentially reliable results for age estimation, particularly in the adulthood-age category; however, more research is needed to standardize and validate the biopsy technique. The only drawback of the method is the destructive aspect of laboratory techniques; hence, it is only justified for the deceased (Logani et al. 2017; Matteussi et al. 2022).

While human growth and maturation are unique to everyone, dental age estimation techniques are currently considered the best way to assess chronological age (Lewis & Senn 2015). So, this research aimed to compare between the cementum incremental lines and aspartic acid racemization in age estimation among Egyptian subjects.

Methods

Subjects and sampling

Sixty-four subjects were assigned to two groups: males and females (32 each). Sixty-four first premolar teeth from all subjects were used in this study. Jankauskas et al. (2001) and Kaur et al. (2015) typically used premolars to count cementum. Premolars are single-rooted teeth with increased wall thickness for better examination of the cementum.

They were obtained from the dentist clinic at Zagazig University. Teeth were extracted from patients ranging in age from 20 to 50 years old of both sexes. The teeth that were extracted for orthodontic and periodontal disease are included in the study with the patient's consent. They are common causes of tooth extraction that do not seem to affect the methods of age estimation (Broucker et al. 2016). Teeth with severe pathology or that are broken are not included in the study. Ethical considerations and confidentiality were respected. The study protocol was approved by the Institutional Review Boards (IRB) at Zagazig University's Faculty of Medicine, with approval number ZU-IRB#: 6871/3-8-2022.

Study design

The collected teeth were divided into two equal experimental groups:

- Group 1: 32 males
- Group 2: 32 females

We divided the groups according to the patient's sex to determine the effect of sex as a possible factor that might affect age estimation.

Histological examination was done in the following steps, according to Alghonamy et al. (2015):

Longitudinal ground sections of freshly extracted teeth were prepared. Each tooth was cut into thin sections using a microtomer. The teeth were then ground on Arkansas (carborundum) stone with water to 0.8 mm thickness with uniform digital pressure. The sections were dehydrated in absolute alcohol for 10 min, then cleared with xylene for 10 min, and mounted using dibutyl phthalate in xylene mounting media on glass slides. The ground sections were examined by light (LM) and polarized microscopes (PM) at the middle third of the root to count the incremental lines of the cementum.

Digital images for the incremental lines were taken using LM and PM. Counting the incremental lines was then done manually on digitally enhanced images using the image analysis software program (Mee soft Image analyzer, Version 1.42.1, 2022). The formula for estimation of age at the time of tooth extraction was calculated by adding the average age of tooth eruption in years for each tooth as represented by the London Atlas of Human Teeth (Alqahtani et al. 2010) and the counted number of incremental lines: "estimated age (E) = numbers of incremental lines (n) + eruption age of tooth (t)" (Scott 2012).

Biochemical *studies: aspartic acid racemization (AAR)*

Chemicals

Powders of D- and L-aspartic acid, O-phthaldialdehyde, and *N*-acetyl-L-cysteine (Sigma-Aldrich, Egypt).

Reagent preparation

O-phthaldialdehyde, and *N*-acetyl-L-cysteine reagent was prepared by dissolving O-phthaldialdehyde (8 mg) in methanol (600mL), and then, 500 mL of Na borate buffer and 120 mL of *N*-acetyl-L-cysteine were added. The reagent stock solution was stored at 4°C.

Methods

This was done according to the method of Elfawal et al. (2015): dentin samples remained in the teeth after histological sections were crushed into powder and washed in 15% sodium chloride at 4 °C overnight, then centrifuged (13,000 r/min for 5 min) three times and dried by a high vacuum pump. The dry residue was re-suspended in 1 mL of hydrochloric acid and hydrolyzed for 6 h. The hydrolysates were dried again, and 1 mL of distilled water was added. Finally, 6 mL of ammonium hydroxide solution was added to elute the amino acids in a clean glass tube (the sample solution).

Derivatization was accomplished by mixing 10 mL of the prepared sample solution with 20 mL of O-phthaldialdehyde and *N*-acetyl-L-cysteine reagent. After 2.5 min, 200 mL of Na acetate was added, and then, 10 mL of the derivatized solution was injected into the HPLC system. The HPLC analysis was done at an ambient temperature of 23 °C.

Statistical analysis

Data analysis was performed using the software SPSS (Statistical Package for the Social Sciences) version 28. Quantitative variables were expressed as the mean ± standard error of the mean (SE). The data were statistically analyzed using the mean and standard deviation in the following tests: paired t test, regression analysis, and equation variables. The level of statistical significance was set at $p < 0.05$.

A Bland–Altman plot was used to describe the agreement between two quantitative variables. A scatter plot was used to present the relationship between the two variables.

Results

Histological *results*

Histological study revealed that cementum incremental lines were clearly visible and could be counted under both LM and PM. The age difference was more detectable and clearly visible with the PM, as shown in Figs. 1 and 2 and Table 1.

Biochemical results

The root dentin was analyzed, and the D/L ratio of aspartic was obtained. A linear regression line was established, which is a plot of ln [(1+D/L)/(1-D/L)] of racemization versus the chronological age (Fig. 3) (Table 2).

Statistical *results*

The difference between estimated age and chronological age by different methods of age estimation

A paired t test was used to study the statistical difference between the age estimated by each technique and the chronological age (Table 3). A Bland-Altman plot was used to study the agreement between the age estimated by each technique and the chronological age (Figs. 4, 5, and 6). There was a statistically significant difference between the estimated age and the chronological age in histological methods (LM and PM), but there was no statistically significant difference between the estimated age and the chronological age in biochemical methods (aspartic acid racemization) as follows:

For the LM method, there was a statistically significant difference between the estimated age and the chronological age by an average of 3.36 years (95% CI of the difference: 2.51 to 4.21), $p < 0.001$. The estimated age was lower than the chronological age. The Bland-Altman plot presented in Fig. 4 shows that the difference between the

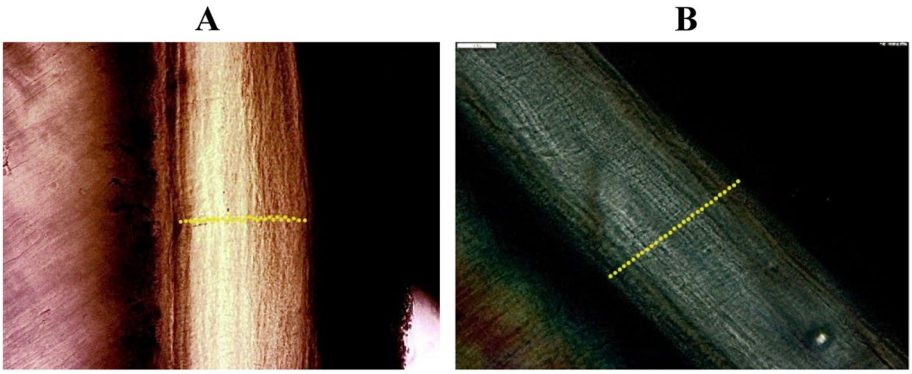

Fig. 1 Micrographs of the premolar of a 43-year-old male patient. **A** LM shows the total number of cementum incremental lines is 31, and **B** PM shows the total number of cementum incremental lines is 29. The estimated ages of LM are 31 + 9 = 40 years, and those of PM are 29 + 9 = 38 years

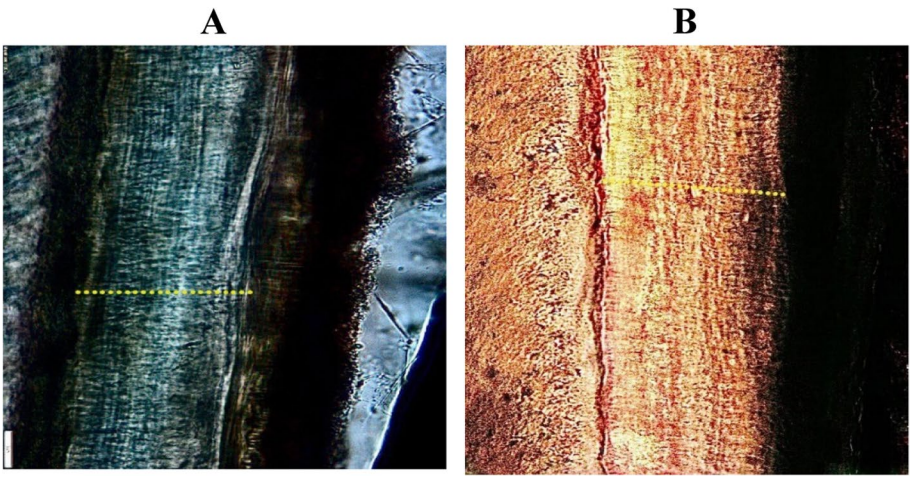

Fig. 2 Micrographs of the premolar of a 34-year-old female patient. **A** LM shows the total number of cementum incremental lines is 25, and **B** PM shows the total number of cementum incremental lines is 22. The estimated ages of LM are 25 + 9 = 34 years, and those of PM are 22 + 9 = 31 years

estimated age and the chronological age increases as the age increases.

For the PM method, there was a statistically significant difference between the estimated age and the chronological age by an average of 4.61 years (95% CI of the difference: 3.68 to 5.54), $p < 0.001$. The estimated age was lower than the chronological age. The Bland-Altman plot shows that the difference between the estimated age and the chronological age increases as the age increases (Fig. 5).

For the AAR method, there was no statistically significant difference between the estimated age and the chronological age. The average difference was − 0.14 years (95% CI of the difference: − 0.47 to 0.19), $p = 0.393$, $p < 0.001$. The Bland–Altman plot shows that the difference between the estimated age and the chronological age is symmetrically distributed around zero, and no trends in the difference were observed. Only 4/64 points were outside the limits of agreement, which were − 2.7 and 2.4 (Fig. 6).

Correlation between correlation between chronological and estimated age by each method in both male and female groups

There was a statistically significant correlation between each method of age estimation and the chronological age as calculated using Pearson's correlation, $p < 0.001$. However, the strongest degree of correlation was observed for the racemization method, with a correlation coefficient of $r = 0.99$ (95% CI 0.98 to 0.99), followed by the light method, $r = 0.94$ (95% CI 0.90 to 0.96), and the polarized method, $r = 0.93$ (95% CI 0.88 to 0.95). Scatter plots for the relationships were presented in Figs. 7, 8, and 9. There was no statistical difference between the male and female groups (Table 4).

Table 1 Age estimation by histological methods in the test group

Male group	Actual age	Estimated age by LM	Estimated age by PM
1	25	26	22
2	20	20	20
3	36	36	35
4	50	40	38
5	27	26	24
6	31	30	29
7	36	34	32
8	25	25	24
9	42	33	32
10	39	32	30
11	40	38	36
12	28	27	26
13	39	37	36
14	33	30	28
15	26	25	25
16	29	29	27
17	48	40	39
18	43	40	38
19	37	35	33
20	44	36	35
21	40	34	33
22	37	33	30
23	28	27	25
24	33	32	31
25	22	21	20
26	42	34	32
27	45	40	39
28	40	33	32
29	25	23	22
30	36	35	33
31	29	28	29
32	48	40	38
Female group	**Actual age**	**Estimated age by LM**	**Estimated age by PM**
1	39	37	36
2	40	30	30
3	47	42	41
4	29	29	28
5	26	25	24
6	34	33	31
7	30	28	27
8	27	26	26
9	35	32	31
10	40	34	32
11	43	36	35
12	49	40	39
13	37	35	35
14	50	37	35
15	22	21	22
16	36	35	34
17	27	27	27
18	39	37	36
19	21	21	20
20	41	35	33
21	37	35	32
22	34	34	33
23	27	26	27
24	22	22	22
25	44	39	37
26	37	36	35
27	25	24	22
28	42	33	31
29	36	34	32
30	33	31	31
31	45	40	38
32	49	38	36

Discussion

The identification process is important in the usual work of forensic odontology. In addition, age estimation plays a major role in forensic science in identifying unknown bodies (Aggarwal et al. 2008).

The teeth, as hard tissue, can withstand caries and degradation. This resistance has made teeth a useful exponent to assess changes in diet, the expression of metabolic diseases, and calculating age at the time of death (Gupta et al. 2014).

Regarding the histological methods in this study, longitudinal sections of teeth were used to examine cementum incremental lines under LM and PM and use them for age estimation.

Cementum incremental lines appear under the microscope as pairs of light and dark layers. Adding the count of incremental lines to the age of tooth eruption yields an age estimation of the individual (Gualdi et al. 2022).

In this study, by examining longitudinal sections of teeth under a light microscope and a polarized microscope, cementum incremental lines were seen in most samples from both groups of the study.

This is in accordance with other studies for age estimation conducted by Avadhani et al. (2009) and Mallar et al. (2015), which found that longitudinal sections were better than cross-sections for estimating age.

This was contrary to Maat et al. (2006), who preferred the cross-sections method for age estimation and recommended cutting the sections perpendicular to the exterior of the root (cross-sections) and not to the root axis Wedel (longitudinal).

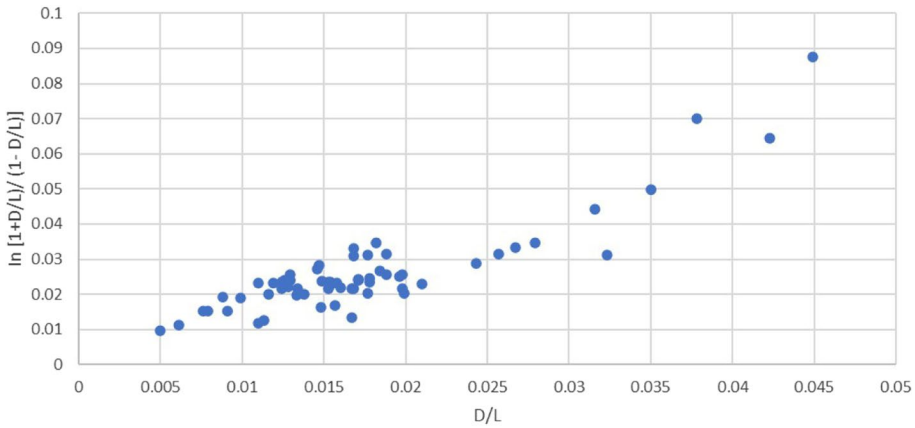

Fig. 3 Graphical presentation of aspartic acid racemization for age estimation

Geetha et al. (2018) preferred longitudinal sections because they gave an opportunity to count lines in both the cellular and acellular cementum on the same tooth with greater reliability of the results.

Pinto et al. (2022), who did a systematic review and meta-analysis, said that a standard protocol should be used to make the method more reliable. He recommended that the histological analysis protocol use cross-sections in the middle third of the tooth root with an approximate thickness of 100 µm, be undecalcified, and be polished carefully to remove any cut marks.

In this study, there was a statistically significant correlation between each method of age estimation and the chronological age as calculated using Pearson's correlation, $p < 0.001$. The positive correlation between estimated and chronological age was stronger with the light microscope than with the polarized microscope. There was a statistically significant difference between the estimated age and the chronological age of an average of 3.36 years in the light microscope and 4.61 years in the polarized microscope.

Pradeep et al. (2021) in their study compared LM, PM, and phase contrast microscopy (PHM). He found that PHM was better than other types of microscopes. It showed a mean difference of 1 year between chronological age and calculated age, while light and polarized microscopy showed a mean difference of 2.88 and 3.34, respectively.

Gupta et al. (2014) found that Pearson's correlation coefficient between estimated age from cemental lines and chronological age was strongly positive using the LM.

Geetha et al. (2018) examined tooth sections under different types of microscopes (LM, PHM, PM, and stereomicroscope) to count cemental annulations. He found that the relationship between chronological age and estimated age using a LM, PHM, and PM had a stronger correlation, while a stereomicroscope showed a weaker correlation.

Pundir et al. (2009) and Alghonamy et al. (2015) revealed a strong positive correlation between estimated and chronological age by either LM or PM in female and male sound single-rooted teeth. They found a strong positive correlation between estimated and chronological age when using PHM and less correlation when using PM and LM.

The result of Avadhani et al. (2009) was near to the results of the current study, which reported that in 18 of 19 specimens examined under the light microscope, the evaluated age varied from chronological age by about 2–3 years.

Aggarwal et al. (2008) found a strong positive correlation between the two variables of estimated age, which was calculated from cemental lines, and chronological age using the PM. The variation between the real and estimated age was found to be in the range of 1–2 years by using PM. From previous studies, it is evident that the LM and PM are both reliable for age estimation from cementum incremental lines. Several studies have shown that the PHM is the best. This was explained by Sanderson (2000), who gave the proper distinction between two types of layers with different optical properties, so it was suitable for viewing colorless and transparent specimens (as cementum).

According to the histological method used in the current study, the difference between the estimated age and the chronological age increases as the age increases, and the accuracy of the tooth cemental annulation method decreases with increasing age.

This agrees with the results of Alghonamy et al. (2015), Swetha et al. (2018), and Sultana et al. (2021), who found that increased age can lead to greater errors,

Table 2 Age estimation by biochemical method (aspartic acid racemization) in the test group

Male group	Actual age	D/L	In [1 + D/L)/(1- D/L)]	Racemization age
1	25	0.0157	0.0169	24
2	20	0.0148	0.0163	20
3	36	0.0099	0.0189	35
4	50	0.0323	0.0312	48
5	27	0.0171	0.0243	27
6	31	0.0184	0.0267	31
7	36	0.0168	0.0216	35
8	25	0.0129	0.0241	25
9	42	0.0076	0.0152	41
10	39	0.0171	0.0241	39
11	40	0.0124	0.0235	39
12	28	0.0127	0.0223	28
13	39	0.0198	0.0215	39
14	33	0.0199	0.0203	32
15	26	0.0113	0.0127	27
16	29	0.0316	0.0442	29
17	48	0.0196	0.0251	50
18	43	0.0167	0.0134	45
19	37	0.0188	0.0256	37
20	44	0.0188	0.0315	43
21	40	0.0210	0.0230	43
22	37	0.0178	0.0236	37
23	28	0.0167	0.0215	28
24	33	0.0279	0.0346	32
25	22	0.0178	0.0246	22
26	42	0.0160	0.0220	43
27	45	0.0149	0.0237	47
28	40	0.0128	0.0221	39
29	25	0.0061	0.0112	25
30	36	0.0449	0.0875	36
31	29	0.0126	0.0241	28
32	48	0.0129	0.0255	48
Female group	**Actual age**	**D/L**	**In [1 + D/L)/(1-D/L)]**	**Racemization age**
1	39	0.0138	0.0199	37
2	40	0.0110	0.0119	41
3	47	0.0153	0.0236	49
4	29	0.0198	0.0256	29
5	26	0.0168	0.0331	25
6	34	0.0168	0.0310	33
7	30	0.0147	0.0283	30
8	27	0.0129	0.0240	27
9	35	0.0350	0.0499	35
10	40	0.0116	0.0201	42
11	43	0.0091	0.0152	43
12	49	0.0158	0.0233	50
13	37	0.0146	0.0273	36
14	50	0.0177	0.0311	52
15	22	0.0243	0.0287	21
16	36	0.0153	0.0217	35
17	27	0.0134	0.0217	29

Table 2 (continued)

18	39	0.0110	0.0231	40
19	21	0.0154	0.0236	21
20	41	0.0257	0.0315	43
21	37	0.0119	0.0232	36
22	34	0.0182	0.0347	36
23	27	0.0050	0.0097	28
24	22	0.0088	0.0191	23
25	44	0.0423	0.0645	41
26	37	0.0154	0.0227	37
27	25	0.0177	0.0203	28
28	42	0.0079	0.0152	40
29	36	0.0133	0.0197	35
30	33	0.0267	0.0334	34
31	45	0.0378	0.0699	45
32	49	0.0124	0.0217	52

Table 3 The difference between age estimated by different methods and actual age

	Mean (SD)	Difference between the actual age and estimated age		*P* value for the paired samples *t* test
		Mean difference	95% Confidence interval for the difference	
Actual age	35.41 (8.17)			
Estimated age (light)	32.05 (5.77)	3.36	2.51 to 4.21	< 0.001
Estimated age (polarized)	30.80 (5.50)	4.61	3.68 to 5.54	< 0.001
Estimated age (racemization)	35.55 (8.41)	− 0.14	− 0.47 to 0.19	0.393

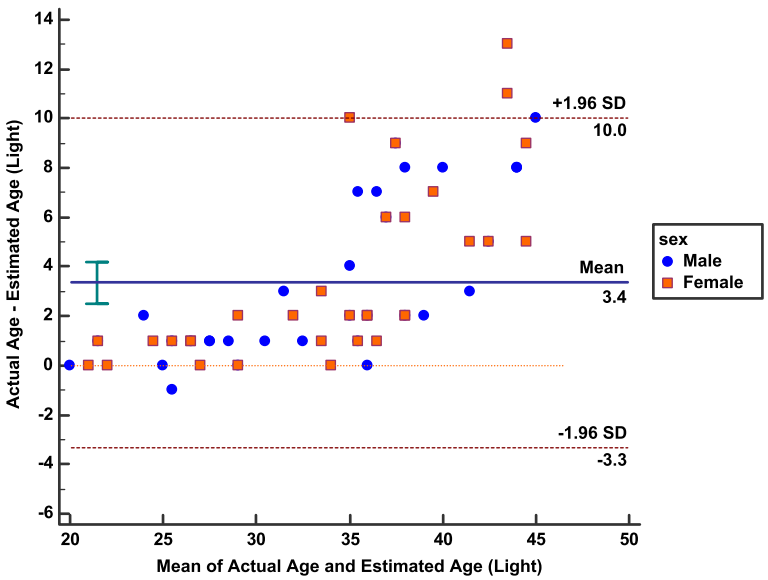

Fig. 4 Bland Altman plot for the agreement between the estimated age using the light method and the actual age (with subgrouping by sex)

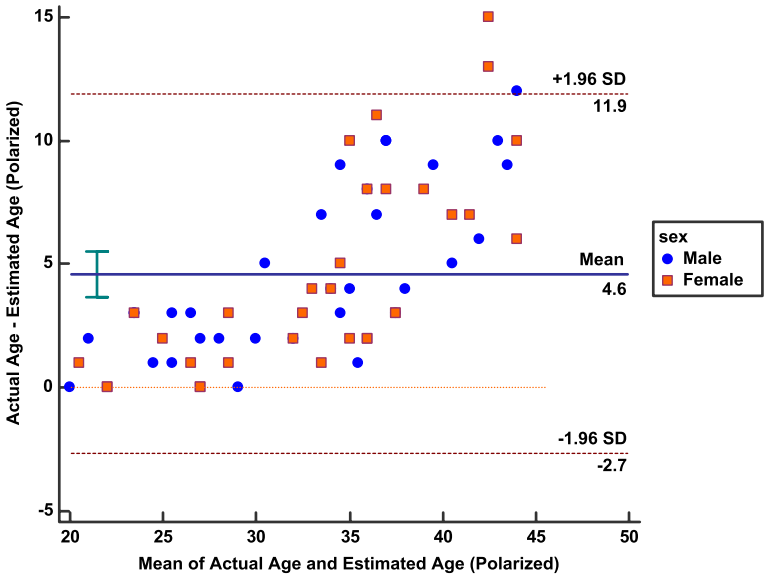

Fig. 5 Bland Altman plot for the agreement between the estimated age using the polarized method and the actual age (with subgrouping by sex)

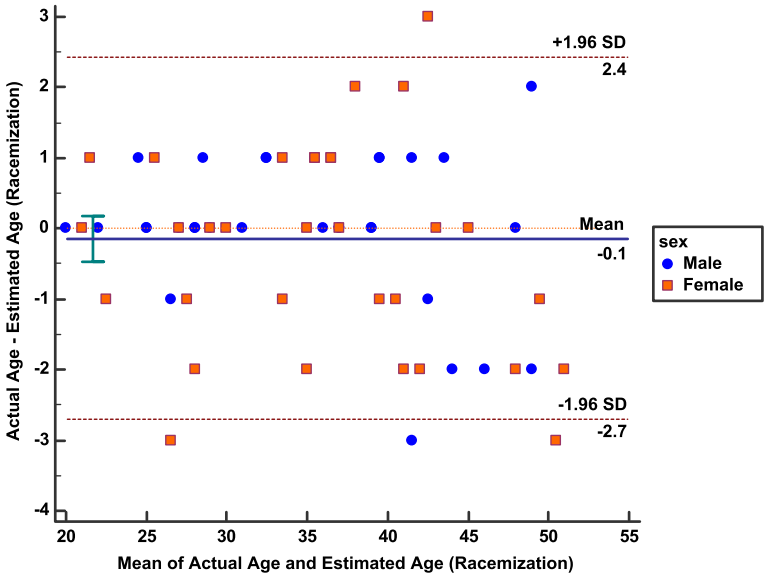

Fig. 6 Bland Altman plot for the agreement between the estimated age using the Racemization method and the actual age (with subgrouping by sex)

and accordingly, age estimation is more precise in teeth younger than 35 years.

The observed age counts by Wittwer et al. (2004) for those above 55 years old showed greater variance from their chronological age. Also, Aggarwal et al. (2008) and Gupta et al. (2014) found that in lower age groups, the correlation coefficients between chronological tooth age and cementum incremental lines were stronger than in the higher age groups. This can be explained by the metabolic disorder of individuals with higher ages in addition to the influence of periodontal regression, dental caries, or other individual characteristics (Kerr 1961).

Several studies have found that the histological method has lower reliability when applied to the samples of older people (Gauldi et al. 2022; Colard et al. 2015; Le Cabec et al. 2019; Meinl et al. 2008; Bojarun et al. 2003), owing to the difficulty of distinguishing the incremental line, as explained by Colard et al. (2015) and Gualdi-Russo et al. (2022). In

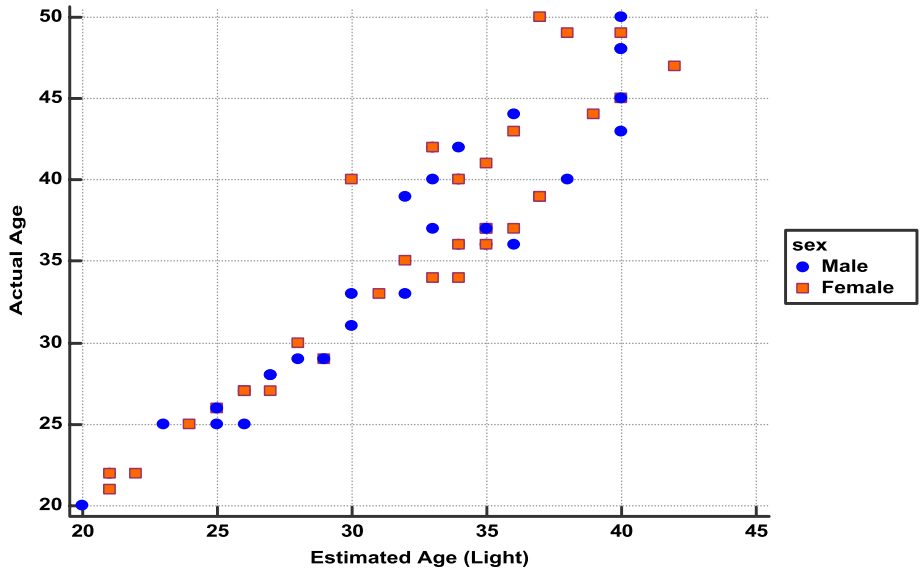

Fig. 7 Scatter plot for the relationship between the estimated age using the Light method and the actual age (with subgrouping by sex)

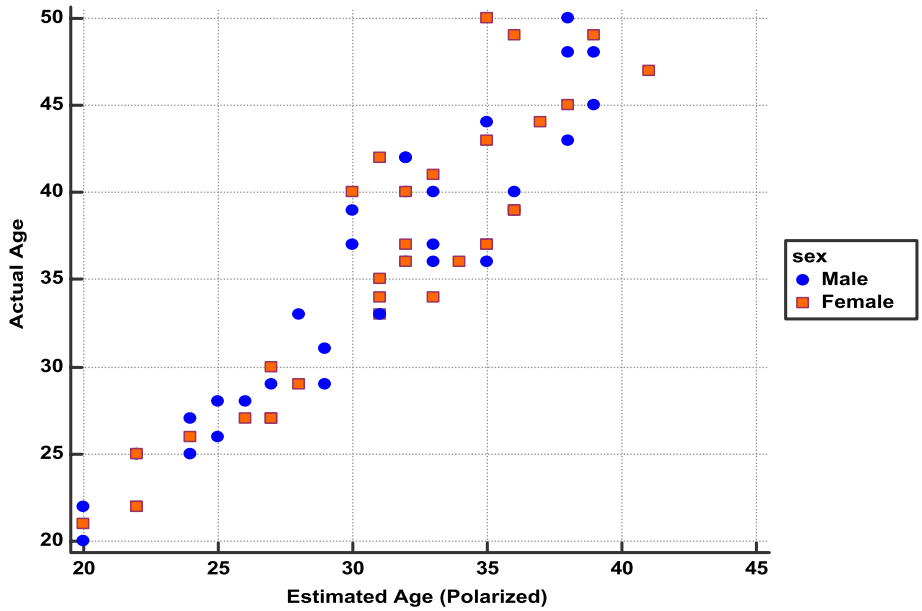

Fig. 8 Scatter plot for the relationship between the estimated age using the Polarized method and the actual age (with subgrouping by sex)

Pinto et al. (2022), a systematic review with meta-analysis, it was found that the method's accuracy decreased with increasing age, possibly because of physiological and pathological alterations associated with aging, but still had considerable reliability. Regarding the biochemical method for age estimation, the current study used aspartic acid racemization for age estimation by HPLC from dentin samples.

High-performance liquid chromatography has been considered an easier and more appropriate method of separating aspartic acid compared to gas chromatography (GC) (Griffin et al. 2010).

Elfawal et al. (2015) have illustrated that the HPLC technique can be used successfully in calculating the chronological age by AAR from dentin samples and can provide dependable results. Other previous studies also used the HPLC method to define AAR in dentin with significant correlation (Fu et al. 1995; Mornstad et al. 1994; Benesova et al. 2004).

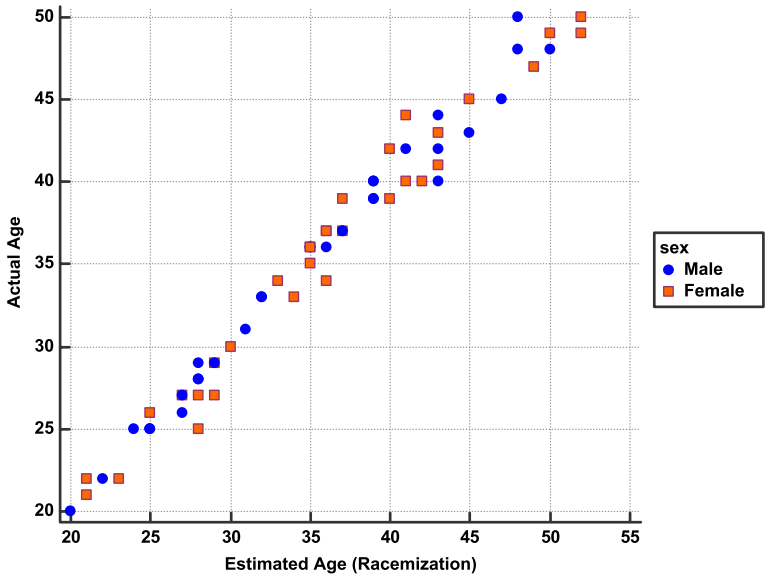

Fig. 9 Scatter plot for the relationship between the estimated age using the racemization method and the actual age (with subgrouping by sex)

Table 4 Correlation between each method of age estimation and the actual age in male and female groups

	Correlation with actual age for males			Correlation with actual age for females		
	Correlation coefficient r	95% CI for *r*	*P* value	Correlation coefficient *r*	95% CI for *r*	*P* value
Estimated age (light)	0.95	0.90 to 0.97	< 0.001	0.93	0.86 to 0.96	< 0.001
Estimated age (polarized)	0.94	0.88 to 0.97	< 0.001	0.91	0.83 to 0.96	< 0.001
Estimated age (racemization)	0.99	0.98 to 1.00	< 0.001	0.98	0.97 to 0.99	< 0.001

On the other hand, Logani et al. (2017) used dentine biopsy from the tooth crown of premolars, and the processing of samples was done by GC with high reliability.

Matteussi et al. (2022) concluded that both techniques (HPLC and GC) have pros and cons and that one is not necessarily better than the other; this depends on the researcher's experience with units and facilities.

In this study, by comparing the difference between estimated age and chronological age by the biochemical method, there was no statistically significant difference between both ages (the average difference was −0.14 years). But by using Pearson's correlation, there was a statistically significant correlation between the estimated age and chronological age, $p < 0.001$. There was a stronger degree of correlation between the biochemical and the histological methods.

Elfawal et al. (2015) estimated the individual's age by AAR with an error range within 1 year. He established a significant correlation of the D-/L-Asp ratio with age in a test group of 50 Kuwaiti subjects ($r = 0.97$) using HPLC. He concluded that the validation group consisted of thirty-nine teeth (26 males and 13 females) of recorded age, and all samples showed a significant correlation between estimated age and chronological age.

Logani et al. (2017) concluded that across all age groups in the study, an error of 0 ± 4 years between protein racemization age and chronological age was observed.

All previous results indicate that the AAR technique is more accurate than histological techniques. There was no statistically significant difference between the estimated age and chronological age, with the chronological age having the strongest degree of correlation. This may be explained by the effect of periodontal disease on the effectiveness of cementum incremental lines.

Dias et al. (2010) reported that estimating age by the histological method is a reliable method for teeth without periodontal diseases, with a mean error of 1.6 years for the sample and an average error of 22.6 years for teeth with periodontal disease.

Alghonamy et al. (2015) also reported that periodontal diseases affect the cementum's thickness and hence the reliability of cementum incremental lines.

On the other hand, Broucker et al. (2016) found that periodontal disease did not seem to influence the methodology

negatively when changing the protocols like tooth section thickness and site of sectioning.

On the other hand, tooth dentin is one of the preferable target tissues for precise age estimation when compared with crowns (Ohtani & Yamamoto 2005).

Matteussi et al. (2022) reported that, compared with other methods that are used to estimate age in adults, AAR seems to produce accurate and reliable results. Dentin may be justified as being stable with reduced susceptibility to environmental changes such as heat and pathological changes. The only drawback of the method is the destructive aspect of laboratory techniques; hence, it is only justified for the deceased.

Logani et al. (2017) concluded that AAR from dentine is a viable and accurate technique for age estimation in living individuals who have attained a state of skeletal maturity.

Conclusions

Considering the current study, it was concluded that both cementum incremental lines and aspartic acid racemization are reliable methods for age estimation. The strongest degree of correlation was observed for the racemization method, which was considered better, followed by the light microscope, and then the polarized microscope. The racemization method can be applied in forensic cases as an accurate and reliable method for age estimation.

Abbreviations

AAR	Aspartic acid racemization
D	Dextrorotatory
GC	Gas chromatography
L	Levorotatory
LM	Light microscopes
PHM	Phase contrast microscopy
PM	Polarized microscopes

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Authors' contributions

All authors contributed to this study as follows: taking consent, collecting teeth samples, histological examination of teeth, data collection and retrieval, data capture, statistical analysis of data, and writing sections of the article. All the authors read and approved the final format of the manuscript.

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Availability of data and materials

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Declarations

Ethics approval and consent to participate

The study protocol was approved from the Institutional Review Boards (IRB), Faculty of Medicine, Zagazig University, with the approval number ZU-IRB#: 6871/3–8-2022. Consent was taken from all the participants. Ethical considerations and confidentiality were respected.

Consent for publication

All authors have agreed to the submission and possible publication.

Competing interests

There are no competing interests.

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References

Aggarwal P, Saxena S, Bansal P (2008) Incremental lines in root cementum of human teeth: an approach to their role in age estimation using polarizing microscopy. Indian J Dent Res 19(4):326

Acharya AB, Vimi S (2009) Effectiveness of Bang and Ramm's formulae in age assessment of Indians from dentin translucency length. Int J Legal Med 123:483–488

Alghonamy WY, Gaballah OM, Labah DA (2015) Age estimation in adult human sound and periodontally affected teeth using tooth cementum annulations. Tanta Dent J 12:277–285

AlQahtani SJ, Hector MP, Liversidge HM (2010) Brief communication: the London atlas of human tooth development and eruption. Am J Phys Anthropol 142:481–490

Avadhani A, Tupkari JV, Khambaty A (2009) Cementum annulations and age estimation. J Forensic Dent Sci 1:73e 6

Benesova T, Ales H, Pilin A et al (2004) A modified HPLC method for the determination of aspartic acid racemization in collagen from human dentin and its comparison with GC. J Sep Sci 27:330–334

Bojarun R, Garmus A, Jankauskas R (2003) Microstructure of dental cementum and individual biological age estimation. Medicina (kaunas) 39(10):960–964

de Broucker A, Colard T, Penel G, Blondiaux J, Naji S (2016) The impact of periodontal disease on cementochronology age estimation. Int J Paleopathol 15:128–133

Colard T, Bertrand B, Naji S, Delannoy Y, Bécart A (2015) (2015): Toward the adoption of cementochronology in forensic context. Int J Legal Med 132(4):1117–1124

Dias P, Beaini T, Melani RF (2010) Age estimation from dental cementum incremental lines and periodontal disease. J Forensic Odontostomatol 28:13–21

Elfawal MA, Alqattan SI, Ghallab NA (2015) Racemization of aspartic acid in root dentin as a tool for age estimation in a Kuwaiti population. Med Sci Law 55(1):22–29

Fu SJ, Fan CC, Song HW et al (1995) Age estimation using a modified HPLC determination of ratio of aspartic acid in dentin. Forensic Sci Int 73:35–40

Geetha HL, Baghisath VP, Vinay HB, Sudheer B, Kumar VJ, Gayathri CH (2018) Age estimation using tooth cementum annulations method by different types of microscope: a comparative study. Int J Oral Health Sci 8:73–80

Gualdi-Russo E, Saguto I, Frisoni P, Neri M, Mongillo J, Rinaldo N (2022) Age estimation using tooth cementum annulations: bias and sources of inaccuracy. Front Biosci (Landmark Ed) 27(5):141

Gowda Charan BK, Reddy SP, Kokila G, Pradeep L (2014) Cemental annulation and phase contrast microscope: tool for age estimation. J SIMLA 6:9–13

Griffin RC, Penkman KEH, Moody H et al (2010) The impact of random natural variability on aspartic acid racemization ratios in enamel from different types of human teeth. Forensic Sci Int 200:148–152

Gupta S, Agnihotri A, Chandra A, Gupta OP (2014) Contemporary practice in forensic odontology. J Oral Maxillofac Pathol 18(2):244

Gupta AA, Kheur S, Alshehri A, Awadh W, Ahmed ZH, Abdul Feroz SM, Khan SS, Mushtaq S, Dewan H, Khurshid Z, Varadarajan S, Sujatha G, Priya V, Patil S (2022) Is palatal rugae pattern a reliable tool for personal identification

following orthodontic treatment? A Systematic Review and Meta-Analysis. Diagnostics 1(2):1–20
Irish JD, Scott GR (2016) A companion to dental anthropology, 1st edn. Wiley Blackwell, UK
Jankauskas R, Barakauskas S, Bojarun R (2001) Incremental lines of dental cementum in biological age estimation. Homo 52(1):59–71
Kaur P, Astekar M, Singh J, Arora KS, Bhalla G (2015) Estimation of age based on tooth cementum annulations: a comparative study using light, polarized, and phase contrast microscopy. J Forensic Dent Sci 7(3):215–221
Kerr DA (1961) The cementum: its role in periodontal health and disease
Le Cabec A, Tang NK, Ruano Rubio V, Hillson S (2019) Nondestructive adult age at death estimation: visualizing cementum annulations in a known age historical human assemblage using synchrotron X-ray microtomography. Am J Phys Anthropol 168:25–44
Lewis JM, Senn DR (2015) Forensic dental age estimation: an overview. J Calif Dent Assoc 43(6):315–319
Logani A, Rastogi M, Shah N, Kumar A, Arora S (2017) Age estimation of living Indian individuals based on aspartic acid racemization from tooth biopsy specimen. J Forensic Dent Sci 9(2):83
Maat GJ, Gerretsen RR, Aarents MJ (2006) Improving the visibility of tooth cementum annulations by adjustment of the cutting angle of microscopic sections. Forensic Sci Int 15(159):S95–S99
Mallar KB, Girish HC, Murgod S, Kumar BY (2015) Age estimation using annulations in root cementum of human teeth: a comparison between longitudinal and cross sections. J Oral Maxillofac Pathol 19(3):396
Matteussi GT, Jacometti V, Franco A, Silva RHA (2022) Age estimation in humans through the analysis of aspartic acid racemization from teeth: a scoping review of methods, outcomes, and open research questions. Forensic Sci Int 331:111154
Meinl A, Huber CD, Tangel S, Gruber GM, Nicola MT, Watzek G (2008) Comparison of the validity of three dental methods for the estimation of age at death. Forensic Sci Int 178(2–3):96–105
Mornstad H, Pfeiffer H, Teivens A (1994) Estimation of dental age using HPLC-technique to determine the degree of aspartic acid racemization. J Forensic Sci 39:1425–1431
Ohtani S, Yamamoto T (2005) Strategy for the estimation of chronological age using the aspartic acid racemization method with special reference to coefficient of correlation between D/L ratios and ages. J Forensic Sci 50(5):1020–1027
Pinto PHV, Fares LC, Silva RHA (2022) Dental age estimation by cementum incremental lines counting: a systematic review and meta-analysis. Forensic Sci Int 341:111492
Pradeep L, Kokila G, Gopinathan PA, Guruswamy S, Nazir SH, Chatterjee A (2021) Age estimation with cemental annulation using light, phase contrast and polarized microscopy. J Microsc Ultrastruct 9:55–60
Pundir S, Saxena S, Aggrawal P (2009) Estimation of age based on tooth cementum annulations using three different microscopic methods. J Forensic Dent Sci 1:82e7
Radovic MB (2012) Aging in the Danube gorges population tooth cementum annulation method. CATAPHAP 12:9–18
Sanderson J (2000) The theory of contrast control in microscope. Quekett J Microsc 38:617–627
Scott AJ (2012) Illusions in regression analysis. Int J Forecast Forthcom 28:689–695
Sultana A, Zainab H, Jahagirdar P, Hugar D (2021) Age estimation with cemental incremental lines in normal and periodontally diseased teeth using phase contrast microscope: an original research. Egypt J Forensic Sci 11(1):1–9
Swetha G, Kattappagari KK, Poosarla CS, Chandra LP, Gontu SR, Badam VR (2018) Quantitative analysis of dental age estimation by incremental line of cementum. J Oral Maxillofac Pathol 22(1):138
Wittwer-Backofen U, Gampe J, Vaupel JW (2004) Tooth cementum annulation for age estimation: results from a large known-age validation study. Am J Phys Anthropol 123(2):119–129

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