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AGE ESTIMATION IN HUMANS THROUGH THE ANALYSIS OF ASPARTIC ACID RACEMIZATION FROM TEETH: A SCOPING REVIEW OF METHODS, OUTCOMES, AND OPEN RESEARCH QUESTIONS

ABSTRACT

Teeth are considered the most resistant structures in the human body. In forensic odontology, teeth are useful for human identification, especially when dental age estimation is necessary. Despite numerous studies, there is no consensus regarding the best methods for dental age estimation. The analysis of aspartic acid racemization, however, has shown promising results. This scoping review aimed to present a descriptive synthesis of the current literature regarding dental age estimation through aspartic acid racemization. Four electronic databases were screened: PubMed, Scielo, Web of Science, and Scopus. Cross-sectional studies published before April 2021 were selected. From 206 articles found, 26 met the eligibility criteria. Several experimental protocols and laboratory settings were detected, but the different protocols did not seem to significantly reduce error rates in dental age estimation.. The analysis of aspartic acid racemization in human dental tissues produced accurate and potentially reliable results for age estimation. Aspartic acid racemization stands out especially in the adulthood – age category in which other methods struggle to deliver proper performances. Studies with larger samples, independent testing, and standardized laboratory procedures are necessary. Equator-like reporting guidelines are encouraged to enable future systematic reviews and meta-analyses.

Keywords: Forensic Dentistry; Age Determination by Teeth; Aspartic acid racemization.

INTRODUCTION

Age estimation plays an essential role in civil and criminal scenarios, with applications in the deceased and the living(1,2). In the former, age contributes to establish the biological profile of unknown victims for human identification. In the latter, age may be used to solve judicial issues that involve children (e.g. adoption, criminal

liability, sexual consent) and adults (e.g. asylum seekers, retirement and pension)(3). As the most resistant structure of the human body, teeth are often used to estimate age(4). Specifically, dental age estimation stands out compared to other techniques mainly because of the resistance of the teeth and their continuous development from early childhood to early adulthood.(6). However, the physiological and chronological ages gradually become more distant over the time. In other words, the increasing distance between physiological and chronological ages can lead to higher error rates(3,5) – in this scenario, optimal methods remain necessary.

Despite the recurrent application of dental age estimation in practice, there is no consensus regarding the best method(7). Depending on the parameters of choice, strategies have been suggested to reach more accurate results. The decision behind the method depends not only on the available facilities/laboratories but also on biological conditions(8). In short, dental age estimation using dental development as a parameter usually reach better performances during childhood, when several teeth develop simultaneously(9). In adults, on the other hand, developmental parameters are scarce or null. Alternative methods based on regressive morphological changes were proposed to overcome this challenge(2,10–12), but their error rates were considerably high.

The biochemical analysis of the human teeth emerged as a powerful tool to estimate the age when developmental parameters end (i.e. in adults)(13,14). In the '70s, Helfman & Bada (1975,1976)(15,16) proposed the study of the racemization reaction of amino acids and found a significant correlation between chronological age and the D / L-aspartic acid ratio present in the enamel and dentin. This method became more robust after subsequent studies and was able to reduce standard error rates (below four years in most of the studies)(5). A drawback of the method is the destructive aspect of laboratory techniques required to analyze pulverized/extracted tooth products(17,18). Hence, from a bioethical perspective, the method is only justified in the deceased.

Amino acids that form biological proteins usually exist in the human body in their L-form, except for some proteins synthesized primarily in their D-form(19). The conversion of L-forms into D-forms is a biochemical reaction known as racemization, and happens over the time. Nonetheless, some human body tissues, such as teeth and other structures i.e. the sclera and vertebral discs have a slow turnover, thus D-

aspartic acid is able to accumulate due to slow protein replacement, allowing biochemical analysis (20–22). Of all the amino acids, the one that has the highest racemization rate is the aspartic acid (3,5,23). Helfman & Bada(16,24) observed, for the first time, the association between the accumulation of (enamel and dentin) D-aspartic acid with age.

At first, D-aspartic acid accumulation was considered solely a stable outcome from the racemization process. Further studies, however, found out that this process is much more complex, and involved spontaneous protein degradation and succinimide formation (67). The evolving knowledge behind the method raised questions that are pertinent to specific situations, i.e. long post-mortem intervals (68).

Cunha et al. (2009)(3) pointed out the existing gap in the forensic literature by highlighting the scarcity of systematic reviews dedicated to age estimation methods. A systematic review was the initial design planned for the present study, but the current literature could not provide enough evidence to answer more focused questions on this matter. In this context, the present study is the first scoping review to describe state of the art regarding main laboratory settings, variables, and covariables in dental age estimation through aspartic acid racemization. The review aims to investigate and map the studies carried out regarding the topic; to clarify the availability of evidence in the field; and to assess methodological research procedures.

MATERIAL AND METHODS

The PECO strategy(25) was used to establish descriptors to maximize the search power. The established parameters were: Population (P): human with known chronological age; Exposure (E): age estimation via racemization of aspartic acid from teeth(15,16); Comparison (C): chronological age (studies should analyze the age difference between the estimated age and the chronological age); and Outcome (O): reliability of the racemization of aspartic acid from human teeth for age estimation (e.g. differences between estimated and chronological age).

Search Strategy

Two reviewers searched the electronic literature independently in four databases: PubMed/Medline, Web of Science, Scielo and Scopus and in three

languages: Portuguese, English, and Spanish. Descriptors used to build the search strategies were: *((age estimation OR age assessment OR age determination)) AND (aspartic acid OR enantiomer OR enantiomers OR racemization)) AND (teeth OR tooth OR dental)*. The search was restricted to articles published until April 2021. The articles were selected in a three-step process: title, abstract and full-text reading, sequentially. A third reviewer was consulted in case of disagreement. Cross-sectional studies that used the racemization of aspartic acid for dental age estimation were included. Exclusion criteria were: literature reviews, case reports, conference proceedings, studies outside the scope of forensic odontology, letters addressed to other authors or editors, studies involving analysis of non-human materials (animals), experimental studies that evaluated only the influence of laboratory and experimental steps to obtain the amino acids of interest, experimental studies that evaluated the influence of taphonomic changes in the sample, and studies in languages other than Portuguese, English and Spanish.

During the selection of articles, the reviewers were not blind to the authors and journals of publication. All the eligible articles were imported to Mendeley Desktop software, version 1.19.4 (Mendeley Ltd. Elsevier Inc., New York, USA) to enable organization and preliminary exclusion of duplicates.

Data extraction

The following data were extracted: (I) general information: authorship, year of publication and complete citation of the study, the country where the study was carried out, study design, and population; and (II) specific information: objective(s) of the study, sample size, the age interval of the sample (minimum and maximum ages), laboratory protocols, teeth that were analyzed, tissue sampled from the teeth, methodological techniques to separate the enantiomers, authors' scientific remarks and primary outcomes. The experimental and analytical items assessed in our study were based on their relevance to build an adequate methodology(23).

Additionally, the following results were assessed (when available): mean and standard deviation of estimated and chronological ages;; mean error of the method (the difference between estimated and chronological ages); and the standard deviation of the error.

RESULTS

The initial search strategy resulted in 362 articles (Figure 1). Out of the 29 articles selected for full-text reading, four were excluded. These articles did not provide enough detail behind age estimation procedures, or involved samples with taphonomic changes (i.e. pink teeth). An additional article that was not available in full was requested to the authors and received via email(27). Manual search in reference lists resulted in an additional study(16). The final sample of the review consisted of 26 articles that met the eligibility criteria.

All the articles were cross-sectional. Table 1 includes a list of the studies with summarized descriptive data. The distribution of publications over time was uniform, with representative studies in all decades – starting in the 70's(16). Most of the studies were published in journals focused on forensics or anthropology, except for the studies by Alkass et al. (2010), Benesová et al. (2004), and Gillard et al. (1990) published in journals referring to molecular and cell proteomics, analytical chemical separation, and archaeometry, respectively(30-32). Besides, the pioneering articles by Helfman & Bada (1975, 76) were published in a journal belonging to the US National Academy of Sciences(16) and in Nature(15) journal.

Among the studies that reported the origin (geographic population) of the sample, we noticed that Japan was the predominant country based on the number of studies ($n = 8$)(18,33-36). Other populations in Asia were investigated, such as Kuwait(37), India(29,38), and China(4,39). Study samples that were not part of Asia came from Europe, such as Sweden(30), the Czech Republic(31,40), the United Kingdom(32,41), Scandinavia(18), and Poland(28). Studies' sample ranged from $n=2$ (42) to $n=90$ (29). Some studies used training samples to produce regression equations, then applied these equations on testing sets. Independent test sample sets varied their size between $n=5$ (36,43) and $n=90$ (29). Some studies(4,18,29,33–36,38,39) used the same sample size and set to produce and test regression equations. Other studies used independent sets(18,37).

As for descriptive statistics, such as means and standard deviations of chronological and estimated ages, some of the studies did not report the information,

namely the studies by Ohtani et al., 2003 and 2005(35,43), Griffin et al. 2008 and 2009(17,44) and Helman & Bada, 1975 and 1976(16) and Pilin et al.(40). In the remaining articles, descriptive values were collected directly from the text or calculated (indirectly) from the reported figures and graphs. The estimated (dental) ages were constantly very close to the chronological ages.

Regarding standard deviation, the detected values ranged between 4.76 and 23.15 years for the chronological age and between 4.94 and 26.08 years for the estimated age. Despite the evident dispersion in the studied samples, the standard deviation of the chronological and estimated ages generally assumed similar values, which indicates that the distribution of the estimated age is similar to the chronological age.

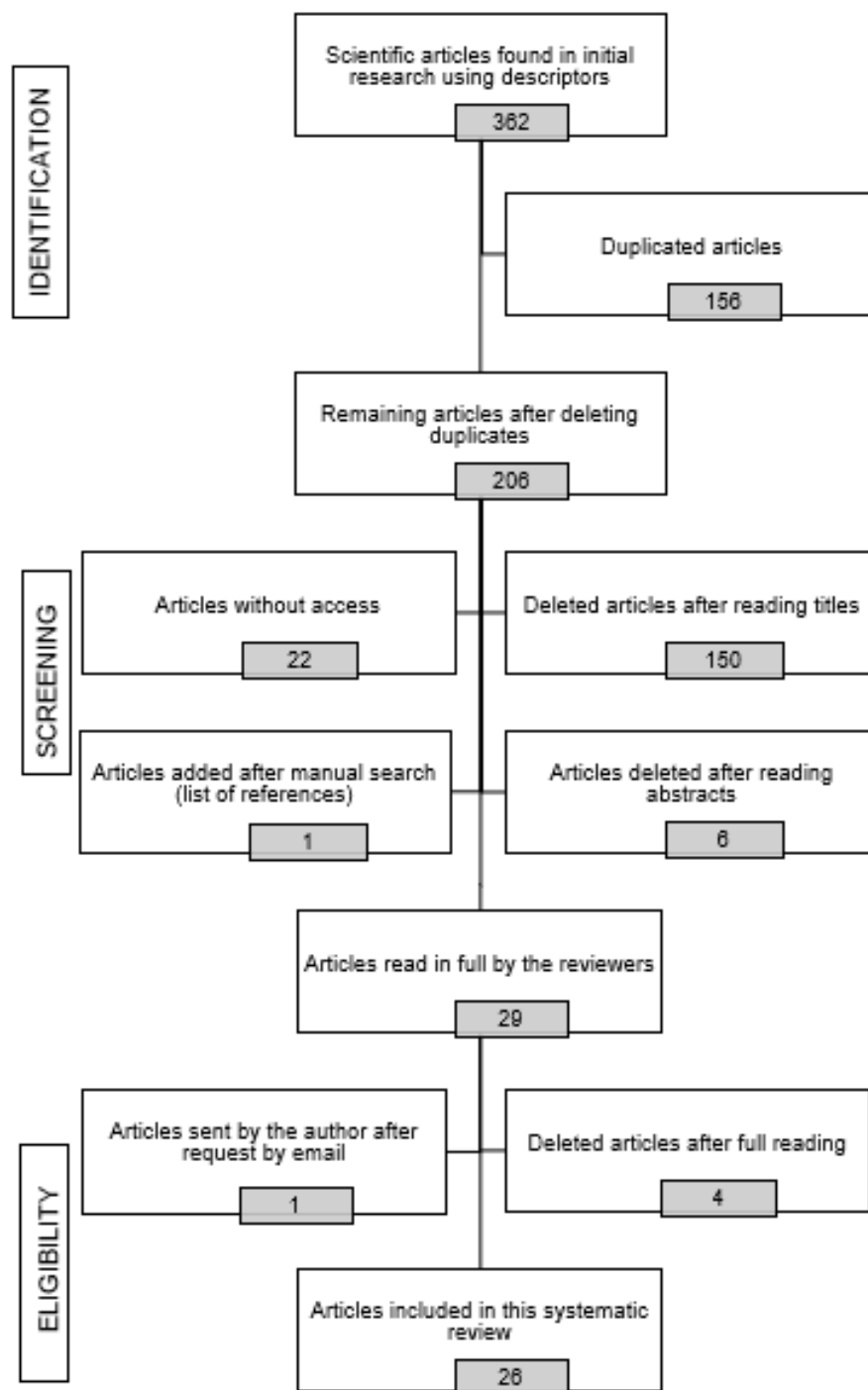


Figure 1: Flowchart of the stages of exclusion and analysis of articles included.

Several tooth types were used to carry out the laboratory analysis. Premolars were more prevalent(28,29,32,37–39,41), followed by canines(31,40,42) and molars(4,47,48). Some studies did not inform which tooth was used for the analysis(15,17,44).

As for the dental tissue analyzed: dentin was the preferred material(4,15,18,28–31,33,35–43,45–49), while the enamel was less frequent: in two studies, a solubilized portion was removed from the enamel surface(17,44); in one study, the enamel was pulverized(16); and in another one, the enamel was removed from 1mm healthy longitudinal sections – it was unclear whether the specimen underwent other experimental interventions(43). In two studies, the whole tooth was used for chromatographic analysis(32,42).

When dentin was the chosen material, in most studies, longitudinal sections of it were retrieved for the analysis(4,18,30,33,35,36,38,42,43). Some studies restricted the examination to coronal dentin only (29,45,46,48,49), while others analyzed only the root portion(28,37). One study separately analyzed both coronal and root dentin for comparison(47).

Addressing laboratory and experimental procedures and settings, fixatives (10% formalin) were used only in 5 studies (28,33,39,45,46). The use of bleaching solution for cleaning samples was uncommon (n = 4)(17,29,32,44). The washing-out step was carried out in most studies; however, it was unclear in some studies if this procedure was performed(18,31,32,38,45). Powdering of the used dental tissue was common, but unclear in a few studies (29,32,43,49).

The protein fraction separated and analyzed differed across studies. The use of the complete sample was predominant(15,16,18,28,29,33,35,38–40,42,45,46), while in four studies (4,31,32,41) only the insoluble portion of these proteins were analyzed. Two studies used only the soluble portion(17,44), and two used both the soluble and insoluble portions separately(47,48). The remaining four did not provide related information(30,37,43,49).

Demineralization protocols have been reported in a few studies. Studies that described these protocols used HCl predominantly(17,32,41,44,47,48), followed by the use of EDTA(4,31,38,40). Regarding the hydrolysis protocols, the opposite occurred. Most studies reported their protocols, with variations in the use of hydrochloric acid as a hydrolytic agent, with slight variations in temperature and time.

Most studies used gas chromatography (GC) (n=16) (16,18,28–30,33,35,36,40,42,46–50), while nine studies used high-performance liquid chromatography (HPLC) (4,15,17,32,37–39,41,44). A single study used both techniques to compare them with each other(31).

A significant number of studies did not report or provide enough information to indicate the values of the mean and standard deviation of the error (difference between the estimated and chronological age)(15–17,44,47–49). For the studies that reported errors, the mean error ranged between 0.72(37) and 12.5(41) years. As for the standard deviations, the values ranged from a minimum of 0.0034(15) to a maximum of 20.2(44) years.

Regarding the sample's eligibility criteria, most of the studies lacked a clear definition (15–18,30–32,35,39,40,44–46,49). Likewise, detailed information about the sample's origin, sex characteristics, and demographics, were absent in most of the studies(15,17,31,32,35,43,44,47–49). We had to be flexible in our analysis since most of the studies had, somehow, prototypical aspects regarding experimental settings, especially the first studies in the field. This is noticeable after a deeper look into the recent studies (4,28,29,37,38,42), which make use of more refined laboratory protocols.

DISCUSSION

The eligible studies detected in this review showed three main methodological strategies to investigate aspartic acid racemization: : (1) finding more efficient and valid ways to experimentally conduct biochemical analysis on racemization, (2) determining the correlation between altered enantiomer ratios and human chronological age (51), and (3) validating the accuracy of aspartic acid racemization as a reliable age estimation method.

The data assessment performed in this scoping review showed promising results in most studies. Means and standard deviations of the method's errors were frequently below many morphological and developmental methods for dental age estimation(5). Recently, the field of age estimation in adults using dental methods has been supplied with systematic reviews (52,53) Compared to morphological (regressive) and developmental (in general, radiological), aspartic acid racemization seems to reach optimal outcomes.

The correlation values of methods based on computed tomography and magnetic resonance imaging are much lower(52) than those of the aspartic acid racemization(51). Marroquin et al. (2017) conducted a systematic review of several imaging-based age estimation methods in adults(53). According to the authors, there

are only few occasions when imaging-based methods reach outcomes as good as those from aspartic acid racemization. Imaging-based methods(54,55) have a mean error considerably higher than the results reported in our study. Among the volumetric methods, only the method by Tardivo et al. (2014) obtained a mean absolute error (at least) close to some error rates reported with aspartic acid racemization. Tardivo's method was also described by Bjork & Kvaal (2018) as the volumetric method that found the highest correlation coefficient with chronological age (52,56). It is noteworthy that our scoping review found the lowest mean values(37), standard deviations(43,57–61), and standard errors of the estimated age(15) reported amongst all dental age estimation methods in adults (not previously highlighted by any other scoping/systematic review on the topic). A drawback found both in the selection and the analysis of the studies selected in this review was the lack of standardization to report method and results.. Although we observed homogeneity in the statistical analyses used across studies (which would generally be a good sign for a meta-analysis), other aspects had strong heterogeneity, such as the different variables and co-variables studied in the eligible articles.

An important limitation of the eligible studies was the lack of detailed sample description. Samples (individuals with known age) should be large enough to be representative, should have uniform allocation in age categories and balanced sex distribution(62), so the results could be more reliable. In our review, there were cases of small samples(18,31,32,39,41) and cases where the sample number for the regression equation was expressive despite the small test sample(30,42,45,46). Small samples are a significant problem because the “real” reliability of the method is clouded, even if the central concept behind the method is apparently sound.

Outcome reporting was also very obscure Few studies have not directly reported mean error values nor reported sufficient data for their calculation(15–17,44,47–49), but all provided data regarding the standard error of bias or the value of the standard error of the estimates (SEE). This information, combined with the methodological information collected in this review enabled an informative overview of the topic. Hence, we were able to observe how laboratory and procedural variables possibly affected the results.

We observed several settings for the selection of tooth type and analysis, with no evident difference between them regarding results. Even the studies that used

multiple tooth positions (types) obtained satisfactory results(33). It must be noted, however, that studies that obtained exceptional results - with an average error of less than one year(29,37), used only one tooth type for analysis (premolars). The explanation for this phenomenon is given by Ohtani et al. (2003): Different dental types have different rates of dentin formation. Therefore, estimating age based on the extent of racemization of a specific tooth type produces better results than mixing them in the regression analysis(35). This fact was corroborated by Ohtani et al. (2005) and Ohtani et al. (2011) when the researchers observed that the lack of standard related to the tooth type seems to have a more significant influence on age estimation performance than the ethnic origin of the sample(18,43).

Dentin was the dental tissue that was found more frequently in the eligible studies. The methodological decision behind the selection of this tissue (instead of enamel (43)) may be justified by the (more) stable development and reduced susceptibility to environmental changes such as heat and pathological changes(15), compared to enamel. Griffin et al. (2009) suggests that the loss of amino acids to the soil can make the racemization method unfeasible in ancient archaeological specimens(44). . Cementum was analyzed by Ohtani et al. (1995) and figured as a low metabolic tissue in which the racemization rate occurs similarly to the dentin - making the tissue potentially useful in practice. Future studies remain necessary (33).

The analyzed fraction of proteins from dentin or enamel is also part of a methodological decision that seems to have a particular influence in the analysis of racemization rates. In the literature, the fraction were previously pointed out as one of the factors responsible for the inconsistencies in the published error rates(23). While most studies usually use the total fraction of dentin proteins (from the crown or root), some studies detected in our review analyzed the soluble portion of dental enamel(17,44). Despite being a practical material(17) (as it enables fast racemization)(65), some precautions must be taken. Waite et al. (1999) inferred that soluble portions could be lost if this procedure is performed in cadavers - fact that confirmed by Griffin et al. (2009), with archaeological samples(23,44), and Mahlke et al. (2021) (68). Furthermore, the soluble portions are more susceptible to damage by exposure to acid. Ritz et al. (1993) found no significant differences between the analysis of total protein fraction and acid-soluble fraction. The former was slightly more accurate for age estimation compared to the latter. Differently, the insoluble fraction of

proteins performed remarkably worse among the three portions (i.e. soluble, total, insoluble)(47).

The last methodological parameter addressed in this scoping review was the type of chromatographic analysis (HPLC or GC). Both techniques have pros and cons, and one is not necessarily better than the other (Waite et al., 1999). Deciding between both will depend on the researcher's access and experience with units and facilities. Benesová et al. (2004) compared the two techniques and observed favorable results for HPLC based on the higher sensitivity and better results for age estimation using the technique(31). It is worth noting that other studies have obtained better results via GC(18,28,29,33,35,43,45,46). Hence, this parameter (HPLC or GC) does not seem to induce more or less favorable results.

Beyond the method's technical and scientific aspects there are also logistical and social obstacles to be considered. Specialized staff or training is required due to the complexity of the various stages of sample preparation(66). There must be standardization of the method(23,37). In some countries, especially the least developed ones, the use of complex and potentially expensive laboratory techniques is not feasible on a routine basis(37). However, this should in no way be an argument against its development and use. Additionally, the standardization of the method and studies should be sought as soon as possible to enable the dissemination of the method worldwide, so validation could be encouraged and performed(18)..

Despite the issues related to how eligible studies report methods and results, it is clear that aspartic acid racemization brings an optimistic light to dental age estimation of adults. Future studies with standardized report writing, such as Equator guidelines, are encouraged to enable further systematic reviews, meta-analyses and (consequently) high-level evidence..

CONCLUSION

Contrasting with other methods that struggle to estimate age in adults aspartic acid racemization seems to produce accurate and reliable results. - Given the lack of standardized report writing (for methods and results) among eligible studies, the evidence was not homogeneous enough to enable systematic reviews and meta-analyses. Future studies in the field should benefit from the existing and compiled data of this scoping review to support methodological decisions and optimize research time.

REFERENCES

1. Lewis JM, Senn DR. Dental age estimation utilizing third molar development: A review of principles, methods, and population studies used in the United States. *Forensic Sci Int* [Internet]. 2010;201(1–3):79–83. Available from: <http://dx.doi.org/10.1016/j.forsciint.2010.04.042>
2. Gustafson G. Age determinations on teeth. *J Am Dent Assoc.* 1950;720(July):45–54.
3. Cunha E, Baccino E, Martrille L, Ramsthaler F, Prieto J, Schuliar Y, et al. The problem of aging human remains and living individuals: A review. *FORENSIC Sci Int.* 2009;193(1–3):1–13.
4. Chen S, Lv Y, Wang D, Yu X. Aspartic acid racemization in dentin of the third molar for age estimation of the Chaoshan population in South China. *Forensic Sci Int.* 2016 Sep;266:234–8.
5. Ritz-Timme S, Cattaneo C, Collins MJ, Waite ER, Schütz HW, Kaatsch H-J, et al. Age estimation: The state of the art in relation to the specific demands of forensic practise. *Int J Legal Med.* 2000;113(3):129–36.
6. Alt KW, Rösing FW, Teschler-Nicola M. *Dental Anthropology - Fundamentals, Limits and Prospects.* 1st editio. SpringerWienNewYork; 1998.
7. Lopes LJ, Nascimento HAR, Lima GP, Santos LAN dos, Queluz D de P, Freitas DQ. Dental age assessment: Which is the most applicable method? *Forensic Sci Int* [Internet]. 2018;284:97–100. Available from: <http://dx.doi.org/10.1016/j.forsciint.2017.12.044>
8. Schmeling A, Rudolf E, Vieth V, Dettmeyer R, Geserick G. Forensic Age Estimation: methods, certainty, and the Law. *Dtsch Arztebl Int.* 2016;113:44–50.
9. Lewis J, Senn D. Forensic dental age estimation: an overview. *J CalifDent Assoc.* 2015;43:315–9.
10. Maples WR. An improved technique using dental histology for estimation of adult age. *J Forensic Sci.* 1978 Oct;23(4):764–70.
11. SOLHEIM T. A NEW METHOD FOR DENTAL AGE ESTIMATION IN ADULTS. *FORENSIC Sci Int.* 1993;59(2):137–47.
12. Johanson G. Age determination from human teeth. *Odont Rev.* 1971;22(21):126.
13. Gouveia R, Corte-Real A, Alves S. Estimativa da idade por métodos dentários -

- breve revisão. Identificação em Medicina Dentária. Faculdade de Medicina, Universidade de Coimbra; 2016.
14. Ohtani S, Sugimoto H, Sugeno H, Yamamoto S, Yamamoto K. Racemization of aspartic acid in human cementum with age. *Archs oral Biol.* 1995;40(2):91–5.
 15. Helfman PM, Bada JL. Aspartic acid racemisation in dentine as a measure of ageing. *Nature.* 1976;262(5566):279–81.
 16. Helfman PM, Bada JL. Aspartic acid racemization in tooth enamel from living humans. *Proc Natl Acad Sci U S A.* 1975 Aug;72(8):2891–4.
 17. Griffin RC, Moody H, Penkman KEH, Fagan MJ, Curtis N, Collins MJ. A new approach to amino acid racemization in enamel: Testing of a less destructive sampling methodology. *J Forensic Sci.* 2008;53(4):910–6.
 18. Ohtani S, Yamamoto T. Comparison of age estimation in Japanese and Scandinavian teeth using amino acid racemization. *J Forensic Sci.* 2011 Jan;56(1):244–7.
 19. Iida T, Santa T, Toriba A, Imai K. Amino acid sequence and D/L-configuration determination methods for D-amino acid-containing peptides in living organisms. *Biomed Chromatogr.* 2001;15(5):319–27.
 20. Ritz-Timme S, Laumeier I, Collins M. Age estimation based on aspartic acid racemization in elastin from the yellow ligaments. *Int J Legal Med.* 2003 Apr;117(2):96–101.
 21. Ohtani S. Estimation of age from dentin by utilizing the racemization of aspartic acid: influence of pH. *Forensic Sci Int.* 1995;75(2–3):181–7.
 22. Yamamoto T, Ohtani S. Estimation of chronological age from the racemization rate of L- and D-aspartic acid: how to completely separate enantiomers from dentin. *Methods Mol Biol.* 2012;794:265–72.
 23. Waite ER, Collins MJ, Ritz-Timme S, Schutz H-W, Cattaneo C, Borrman HIM. A review of the methodological aspects of aspartic acid racemization analysis for use in forensic science. *Forensic Sci Int.* 1999;103(2):113–24.
 24. Helfman PM, Bada JL. Aspartic acid racemisation in dentine as a measure of ageing. *Nature* [Internet]. 1976;262(5566):279–81. Available from: <https://www.scopus.com/inward/record.uri?eid=2-s2.0-0017313486&doi=10.1038%2F262279b0&partnerID=40&md5=9d06ee02e9417dcfc9f50b9f11136899>
 25. Maia L, Antonio A. Systematic reviews in dental research. A guideline. *J Clin Pediatr Dent.* 2012;37:117–24.
 26. Moola S, Munn Z, Tufanaru C, Aromataris E, Sears K, Sfectu R. Systematic reviews of etiology and risk. In: Aromataris E, Munn Z, editors. Joanna Briggs

Institute Reviewer's Manual [Internet]. 2017. Available from: <https://reviewersmanual.joannabriggs.org/>

27. Sajdok J, Kotrbová-Kozak A, Pilin A. Age determination using peptide mapping of non-collagenous proteins in human dentin | Možnosti stanovení věku peptidovým mapováním nekolagenních proteinů lidského dentinu. *Soud Lek.* 2001;46(1):5–8.
28. Wochna K, Bonikowski R, Śmigielski J, Berent J. Aspartic acid racemization of root dentin used for dental age estimation in a Polish population sample. *Forensic Sci Med Pathol.* 2018;14(3):285–94.
29. Rastogi M, Logani A, Shah N, Kumar A, Arora S. Age estimation of living Indian individuals based on aspartic acid racemization from tooth biopsy specimen. *J Forensic Dent Sci.* 2017;9(2):83–90.
30. Alkass K, Buchholz BA, Ohtani S, Yamamoto T, Druid H, Spalding KL. Age estimation in forensic sciences: application of combined aspartic acid racemization and radiocarbon analysis. *Mol Cell Proteomics.* 2010 May;9(5):1022–30.
31. Benesová T, Honzátko A, Pilin A, Votruba J, Flieger M. A modified HPLC method for the determination of aspartic acid racemization in collagen from human dentin and its comparison with GC. *J Sep Sci.* 2004;27:330–4.
32. GILLARD RD, POLLARD AM, SUTTON PA, WHITTAKER DK. AN IMPROVED METHOD FOR AGE AT DEATH DETERMINATION FROM THE MEASUREMENT OF D-ASPARTIC ACID IN DENTAL COLLAGEN. *ARCHAEOLOGY.* 1990;32(1):61–70.
33. Ohtani S. Estimation of age from dentin by using the racemization reaction of aspartic acid. *Am J Forensic Med Pathol.* 1995;16(2):158–61.
34. Ohtani S, Ito R, Arany S, Yamamoto T. Racemization in enamel among different types of teeth from the same individual. *Int J Legal Med.* 2005 Mar;119(2):66–9.
35. Ohtani S, Ito R, Yamamoto T. Differences in the D/L aspartic acid ratios in dentin among different types of teeth from the same individual and estimated age. *Int J Legal Med.* 2003 Jun;117(3):149–52.
36. Ohtani S, Yamamoto T. Age estimation by amino acid racemization in human teeth. *J Forensic Sci.* 2010 Nov;55(6):1630–3.
37. Elfawal MA, Alqattan SI, Ghallab NA. Racemization of aspartic acid in root dentin as a tool for age estimation in a Kuwaiti population. *Med Sci Law.* 2015;55(1):22–9.
38. Rajkumari S, Nirmal M, Sunil PM, Smith AA. Estimation of age using aspartic acid racemization in human dentin in Indian population. *Forensic Sci Int [Internet].* 2013;228(1–3):38–41. Available from:

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-84876482287&doi=10.1016%2Fj.forsciint.2013.02.021&partnerID=40&md5=6a5ed0312d3714f9092924c76ce5b967>

39. Fu SJ, Fan CC, Song HW, Wei FQ. Age estimation using a modified HPLC determination of ratio of aspartic acid in dentin. *Forensic Sci Int.* 1995 May;73(1):35–40.
40. Pilin A, Cabala R, Pudil F, Bencko V. The use of the D-, L- aspartic ratio in decalcified collagen from human dentin as an estimator of human age. *J Forensic Sci.* 2001;46(5):1228–31.
41. Carolan VA, Gardner MLG, Pollard AM, Lucy D. Some Considerations Regarding the Use of Amino Acid Racemization in Human Dentine as an Indicator of Age at Death. *J Forensic Sci.* 1997;42(1):14062J.
42. Sakuma A, Ohtani S, Saitoh H, Iwase H. Comparative analysis of aspartic acid racemization methods using whole-tooth and dentin samples. *Forensic Sci Int.* 2012;223(1–3):198–201.
43. Ohtani S, Yamamoto T. 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.* 2005;50(5):1020–7.
44. Griffin RC, Chamberlain AT, Hotz G, Penkman KEH, Collins MJ. Age estimation of archaeological remains using amino acid racemization in dental enamel: A comparison of morphological, biochemical, and known ages-at-death. *Am J Phys Anthropol.* 2009;140(2):244–52.
45. Ogino T, Ogino H. Application to Forensic Odontology of Aspartic Acid Racemization in Unerupted and Supernumerary Teeth. *J Dent Res.* 1988;67(10):1319–22.
46. Ogino T, Ogino H, Nagy B. Application of aspartic acid racemization to forensic odontology: Post mortem designation of age at death. *Forensic Sci Int.* 1985;29(3–4):259–67.
47. Ritz S, Schutz HW, Peper C. Postmortem estimation of age at death based on aspartic acid racemization in dentin: its applicability for root dentin. *Int J Legal Med.* 1993;105(5):289–93.
48. Ritz S, Stock R, Schütz HW, Kaatsch H-J. Age estimation in biopsy specimens of dentin. *Int J Legal Med.* 1995;108(3):135–9.
49. Ritz S, Schutz HW, Schwarzer B. The extent of aspartic acid racemization in dentin: a possible method for a more accurate determination of age at death? *Z Rechtsmed.* 1990;103(6):457–62.
50. Ohtani S, Abe I, Yamamoto T. An application of D- and L-aspartic acid mixtures as standard specimens for the chronological age estimation. *J Forensic Sci.*

2005 Nov;50(6):1298–302.

51. Yekkala R, Meers C, Van Schepdael A, Hoogmartens J, Lambrichts I, Willems G. Racemization of aspartic acid from human dentin in the estimation of chronological age. *Forensic Sci Int*. 2006 May;159 Suppl:S89-94.
52. Bjørk MB, Kvaal SI. CT and MR imaging used in age estimation: A systematic review. *J Forensic Odontostomatol*. 2018;36(1):14–25.
53. Marroquin TY, Karkhanis S, Kvaal SI, Vasudavan S, Kruger E, Tennant M. Age estimation in adults by dental imaging assessment systematic review. *Forensic Sci Int*. 2017 Jun;275:203–11.
54. Kvaal SI, Kolltveit KM, Thomsen IO, Solheim T. Age estimation of adults from dental radiographs. *Forensic Sci Int*. 1995;74:175–85.
55. Cameriere R, Ferrante L, Liversidge HM, Prieto JL, Brkic H. Accuracy of age estimation in children using radiograph of developing teeth. *Forensic Sci Int [Internet]*. 2008 Apr 7 [cited 2020 Jan 29];176(2–3):173–7. Available from: <https://www.sciencedirect.com/science/article/pii/S0379073807007153>
56. Tardivo D, Sastre J, Catherine J-H, Leonetti G, Adalian P, Foti B. Age determination of adult individuals by three-dimensional modelling of canines. *Int J Legal Med*. 2014 Jan;128(1):161–9.
57. Ohtani S, Yamamoto K. Age estimation by amino acid racemization in teeth - A comparison of aspartic acid with glutamic acid and alanine as indicators. *Japanese J Leg Med*. 1991;45(2):119–23.
58. Ohtani S, Yamamoto T. Author response to Waite E.R., Collins M.J., age estimation from racemization rate using heated teeth. *J. Forensic Odontostomatol* 1998; 16:20-1. *J Forensic Odontostomatol*. 1998;16(2):38–40.
59. Ohtani S, Yamamoto T. Comparison of Age Estimation in Japanese and Scandinavian Teeth Using Amino Acid Racemization *. 2011;56(1):244–7.
60. Ohtani S, Yamamoto T. Age estimation by amino acid racemization in human teeth. *J Forensic Sci*. 2010;55(6):1630–3.
61. Ohtani S, Yamamoto K. Estimation of age from a tooth by means of racemization of an amino acid, especially aspartic acid--comparison of enamel and dentin. *J Forensic Sci*. 1992 Jul;37(4):1061–7.
62. Gelbrich B, Lessig R, Lehmann M, Dannhauer K-H, Gelbrich G. Altersselektion in Referenzstichproben. *Rechtsmedizin*. 2010;20(6):459–63.
63. Snee RD. Validation of Regression Models: Methods and Examples. *Technometrics [Internet]*. 1977;19(4):415–28. Available from: <https://www.tandfonline.com/doi/abs/10.1080/00401706.1977.10489581>

64. Sirin N, Matzenauer C, Reckert A, Ritz-Timme S. Age estimation based on aspartic acid racemization in dentine: what about caries-affected teeth? *Int J Legal Med.* 2018;132(2):623–8.
65. Masters PM. Age at death determinations for autopsied remains based on aspartic acid racemization in tooth dentin: Importance of postmortem conditions. *Forensic Sci Int.* 1986;32(3):179–84.
66. Yekkala R, Meers C, Hoogmartens J, Lambrichts I, Willems G, Van Schepdael A. An improved sample preparation for an LC method used in the age estimation based on aspartic acid racemization from human dentin. *J Sep Sci.* 2007;30(1):118–21.
67. Stephenson RC, Clarke S. Succinimide formation from aspartyl and asparaginyl peptides as a model for the spontaneous degradation of proteins. *J Biol Chem.* 1989 Apr 15;264(11):6164-70. PMID: 2703484.
68. Mahlke NS, Renhart S, Talaa D, Reckert A, Ritz-Timme S. Molecular clocks in ancient proteins: Do they reflect the age at death even after millennia?. *Int J Legal Med.* 2021;135(4):1225-1233. doi:10.1007/s00414-021-02522-1

TABLES

Studies	Year	Design	Population	Sample Size for Age Estimation (Known Age)	Sample Size for producing regression equation (Known Age)	Age range – (Years)	Real Ages Mean (Years)	Real Ages SD	Estimated Ages Mean (Years)	Estimated Ages SD
Alkass et al.	2010	Cross-sectional	Sweden	20	44	13-70 (Estimation)	33,38	21,74	33,04	24,73
Benesová et al.	2004	Cross-sectional	Czech Rep.	3	9	16-84 (Equation) / 25-66 (Estimation)	41	21,93	51,33 (GC)/38(HPLC)	26,08(GC)/21,38(HPLC)
Carolan et al.	1997	Cross-sectional	UK	6	23	11-72 (Equation) / 18-81 (Estimation)	53,83	23,15	56,17	10,83
Chen et al.	2016	Cross-sectional	China	58	58	18-63	31,49	10,56	31,58	10,92
Elfawal et al.	2014	Cross-sectional	Kuwait	39 (Validation group) / 50 (Test group)	50	10-31 (Test group) / 10-30 (Validation)	14,98 (Test group) / 15,1 (Validation)	4,76 (Test group) / 5,16 (Validation)	15,06 (Test group) / 15,21 (Validation group)	4,94 (Test group) / 5,21 (Validation group)

						on group)	on group)	on group)		
Fu et al.	1995	Cross-sectional	China	3 (Cases) / 28 (Test group)	28	14-69 (Test group) / 16-60 (Cases)	35,57 (Test group) / 38 (Cases)	14,84 (Test group) / 21,28 (Cases)	35,64 (Test group) / 39,67 (Cases)	15,01 (Test group) / 22,19 (Cases)
Gillard et al.	1990	Cross-sectional	UK	11 (Archeological data)	21 (Modern data)	9-69,74 (Modern data) / 19-68 (Archeological data tested)	42,27	17,77	48,23	17,31
Griffin et al.	2009	Cross-sectional	UK & Switzerland (Archeological)	100	100	-	-	-	-	-
Griffin et al.	2008	Cross-sectional	UK	31	31	-	-	-	-	-
Helfman & Bada	1975	Cross-sectional	USA	N/A	19	-	-	-	-	-

Helfman & Bada	1976	Cross-sectional	USA	N/A	20	11-75	-	-	-	-
Ogino & Ogino	1988	Cross-sectional	Japan	9	68	12-40	23,33	7,97	20,56	9,04
Ogino, Ogino & Nagy	1985	Cross-sectional	Japan	6	61	14-74 (Equation sample) / 42-89 (Estimation sample)	63,50	16,86	62,17	15,14
Ohtani	1995	Cross-sectional	Japan	24	24	21-82	46,29	15,69	46,33	15,54
Ohtani et al.	2005	Cross-sectional	Japan	49	5-6-8-8-8-6 (Tooth type specific) / 49 (all types of tooth)	58-88	68,50	11,82	-	-
Ohtani & Yamamoto	2010	Cross-sectional	Japan	5	5-5-5-6-5 (Each case used an n)	18-66	40,2	20,9	40,6	21,33

Ohtani , Ito & Yamamoto	2003	Cross-sectional	Japan	56	7-9-9-8-9-5-9 (Type specific) and 56 (whole tooth equation)	58-88	69,3	11,34	-	-
Ohtani & Yamamoto	2011	Cross-sectional	Japan / Scandinavia	11 (Japanese) / 18 (Scandinavian)	11 (Japanese) / 18 (Scandinavian)	21-67 (Japanese) / 13,4-70,6 (Scandinavian)	53,55 (Japanese) / 38,87 (Scandinavian)	13,73 (Japanese) / 22,09 (Scandinavian)	53,55 (Japanese with equation) / 54,05 (Japanese with Scandinavian equation) / 38,88 (Scandinavian with Scandinavian equation) / 38,27 (Scandinavian with Japanese equation)	13,62 (JP with JP equation) / 13,51 (JP with SC equation) / 21,75 (SC with SC equation) / 21,89 (SC with JP equation)
Pilin et al.	2001	Cross-sectional	Czech Republic	71	71 (Total Sample) /46 (Under 60 years old sample only)	15-95	-	-	-	-
Rajkumari et al.	2013	Cross-sectional	India	36	36	11-70	40,64	17,81	40,25	18,10

Rastogi et al.	2017	Cross-sectional	India	90	90	11-70	41,67	20,22	41,64	20,19
Ritz et al.	1993	Cross-sectional	Germany	N/A	70 (total dentin) / 39 (acid soluble & insoluble fractions)	-	-	-	-	-
Ritz et al.	1990	Cross-sectional	Germany	N/A	46	13-82	-	-	-	-
Ritz et al.	1995	Cross-sectional		3	62	-	-	-	-	-
Sakuma et al.	2012	Cross-sectional/Two cases report	Japan	2	24 (12 dentin samples and 12 whole tooth samples)	17-76	43,67 (Equation) / 42,13 (Cases)	20 (Equation)	45,5 (Cases)	-
Wochna et al.	2018	Cross-sectional	Poland	64	75 (Divided into: Group I: Central Incisor (21) / Group II: Lateral Incisor (14) / Group III: Canines (15) / Group IV: First Premolars (14)	30-68 (Group I) / 30-68 (Group II) / 34-68 (Group III) / 20-68 (Group IV)	48,33 (Group I) / 47,71 (Group II) / 51,33 (Group III) / 47,71 (Group IV)	14,72 (Group I) / 14,68 (Group II) / 13,33 (Group III) / 16,29 (Group IV)	48,43 (Group I) / 47,71 (Group II) / 51,33 (Group III) / 47,79 (Group IV)	14,28 (Group I) / 14,25 (Group II) / 13,09 (Group III) / 15,45 (Group IV)

Table 1. Data on study design, population, sample sizes, real and estimated ages means and standard deviations, gathered on the studies selected for the review, when available. SD = Standard deviation.

1. LEGENDS TO FIGURES

Fig. 1 Flowchart of the study selection stages performed in this scoping review