Individual Age Estimation through Analysis of Tooth Aspartic Acid Racemization

Jessica Jessica[1], Ferry Gultom[2]*, Elza Ibrahim Auerkari[2]

1. Faculty of Dentistry, Universitas Indonesia,Jakarta, Indonesia.
2. Department of Oral Biology, Sub-division Forensic Odontology, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.

Abstract

One of the most accurate methods to estimate individual's age is tooth aspartic acid racemization analysis, of which age is estimated by inserting one's tooth aspartic acid racemization ratio into formula gained from previous research on his/her population. Although many researches had been conducted on different population with different formula concluded, but we have not found any research conducted on Indonesian people.

Our research aimed to analyze the correlation between aspartic acid racemization ratio and age of individuals from a part of Indonesian population and to conclude a formula to estimate individual's age using aspartic acid racemization ratio.

Aspartic acid amount of 14 first Premolar teeth from Javanese, North Sumatera tribe and Chinese-Indonesian people were analyzed using Ultra-high Performance Liquid Chromatography (UPLC). Aspartic acid racemization ratio of each tooth was calculated using Microsoft Excel then analyzed with SPSS 23.

No relationship was shown between subjects' age and subjects' teeth aspartic acid racemization ratio, leading to no regression formula could be concluded to estimate subject's age.

Although we could not conclude any formula in this research, but method performed in this research was able to analyze tooth aspartic acid racemization of subjects from Javanese, North Sumatera tribe and Chinese-Indonesian people living in Jakarta, Bogor, Depok, Tangerang and Bekasi. Further exploration on subjects from specific Indonesian tribes is needed to explore the contribution of tribe difference to tooth aspartic acid racemization and to search formula to estimate individual's age on Indonesian population.

Experimental article (J Int Dent Med Res 2023; 16(1): 32-35)

Keywords: Age estimation, aspartic acid, racemization, UPLC, whole-tooth.

Received date: 15 September 2022 **Accept date:** 23 October 2022

Introduction

Individual age can be estimated by analyzing teeth through several methods, e.g. scoring teeth attrition visually, comparing individual's dental developmental status, through histological analysis of tooth cementum (Tooth Cementum Annulation/ TCA method), or through biochemical analysis of tooth (racemization analysis).[1-12]

Teeth attrition analysis on people with clenching or bruxism habit might lead to a wrong age estimation result.[2] Dental developmental analysis could only be performed on individuals with erupting teeth.[7,13] There could be some difficulties on TCA analysis, e.g. incremental lines thickness variation or incremental lines which are not easily seen separated.[10]

Racemization is a spontaneous process where amino acid converts from its L-form (L-enantiomer) to its D-form (D-enantiomer).[14-15] New made human body proteins are usually formed by L-form amino acids which will convert to its D-form on automatic chemical reaction, known as racemization. Racemization is influenced by temperature and pH.[14,16,17] Aspartic acid is one of the amino acids in human body.[18,19] Racemization rate of aspartic acid is highest among other amino acids.[15,20] D-form aspartic acid (D-Asp) is constantly accumulated in organ with low metabolism rate, such as teeth, bone, cartilage, eye lens and brain.[14,15,21]

Tooth aspartic acid racemization analysis is found to be one of the most accurate method to estimate individual's age.[14,22,23] Many researchers had performed different analysis methods and found a strong correlation between tooth aspartic acid racemization ratio with individual's age and concluded different formula to estimate individual's age in the research population correctly,[16,24-31] but we could not find any research conducted on Indonesian people, therefore the aims of our research was to analyze the correlation between teeth aspartic acid racemization ratio and age of subjects from a part of Indonesian population and to conclude a formula to estimate individual's age using tooth aspartic acid racemization ratio.

Our hypotheses are that modified method performed in this research is capable to analyze tooth

Corresponding author:
Ferry Gultom,
Department of Oral Biology, Faculty of Dentistry,
University of Indonesia, Jl. Salemba Raya No. 4,
Jakarta Pusat, Jakarta 10430, Indonesia.
E-mail: gultom_ferry@yahoo.com

aspartic acid racemization of subjects from Javanese, North Sumatera tribe and Chinese-Indonesian people living in Jakarta, Bogor, Depok, Tangerang or Bekasi (Jabodetabek) and can be applied to estimate individual's age.

Materials and methods

Reagents

O-phthaldialdehyde (OPA) was purchased from "Sigma" (catalogue number 79760), N- acetyl-L-cysteine (NAC) and reference material of L-aspartic acid were purchased from "Sigma-Aldrich" (catalogue number PHR1098 and 51572, respectively), reference material of D- aspartic acid was purchased from "Aldrich" (catalogue number 219096) and boric acid for Sodium borate buffer (0.4 M, pH 9.4) was purchased from "Merck" (catalogue number 1.00165). All other chemicals were reagent grade.

We analyzed 14 non-carious first Premolar teeth extracted from Javanese, North Sumatera tribe and Chinese-Indonesian people (3, 4 and 7 samples, respectively), living in Jabodetabek for orthodontic or periodontal reason. Subjects' age at extraction ranged between 13.73 and 64.20 years old. Teeth included in this research were healthy teeth with no caries, no fracture, no restoration and supplied with record of subject's age at extraction, sex, and tribe.

The research protocol was approved by Dentistry Research Ethical Commission Faculty of Dentistry Universitas Indonesia (Ethical approval number: 1/Ethical Approval/FKGUI/I/2019). The protocol was the modification of Chen's method.[24]

Samples and Standards Preparation

Samples were washed in 0.2 M HCl, distilled water (3 times) and methanol (5 cc and 5 minutes each) in ultrasonicator, then air dried. After samples were dry, samples were powdered with a custom-made steel mortar and pestle manually. 7.5 mg of sample powder was demineralized with 1 cc of Na_2EDTA (0.5 M, pH 7.4) for 2 hours in ultrasonicator and then for 5 minutes in centrifuge (5000 rpm). After supernatant was discarded, samples were centrifuged (5000 rpm) 3 times in 1 cc of distilled water for 5 minutes each, then hydrolyzed using 900 µl HCl (6 M) in microtube immersed in water-filled laboratory glass in 100°C oven for 6 hours. After 6 hours, samples were put in the freezer for overnight. The next morning, samples were dried in freeze dryer. 1.5 mg of sample was dissolved in 10 cc of distilled water. Reference materials of L- and D-Aspartic acid were used as positive control and distilled water as negative control.

OPA-NAC Reagent Preparation

Reagent was prepared by dissolving 13.75 mg O-phthaldialdehyde (OPA) in 1050 µl Liquid Chromatography methanol. 33.5 mg N-acetyl-L-cysteine (NAC) and Sodium borate buffer (0.4 M, pH 9.4) was then added to the mixture until the volume reached 25 cc.

Sample Derivatization

350 µl of samples was mixed with 700 µl OPA-NAC reagent in Waters microvial. After 2.5 minutes, microvials were put in UPLC sample chamber and 10 µl of each mixture was automatically injected to UPLC system twice. The running time was 16 minutes, with 250 µl per minute flow and amino acids were detected at 340 nm and 445 nm wavelength.

Data Analysis

D-Asp & L-Asp peak area from each sample chromatogram (Fig. 1) was calculated using Empower software and aspartic acid racemization ratio was calculated by dividing D- Asp peak area with L-Asp peak area (D/L) using Microsoft Excel 2010. Racemization ratios and subjects' age were then analyzed with SPSS 23 software for normality and correlation test. Shapiro-Wilk test was applied to analyze data normality ($p>0.05$) and Spearman test was applied to analyze the correlation between aspartic acid racemization ratio with age.

Results

No correlation was found between aspartic acid racemization ratio and subjects' age. No formula could be formulated between subjects' age and racemization ratio.

Sample No.	Age	Ratio
1	64.20	0.062593
2	55.99	0.072162
3	18.51	0.056587
4	16.70	0.060394
5	25.57	0.070976
6	15.90	0.039085
7	15.90	0.071404
8	15.90	0.102213
9	15.90	0.08922
10	30.76	0.069663
11	30.76	0.110891
12	13.73	0.045116
13	24.84	0.055277
14	14.61	0.080701

Table 1. Data of Subjects Age and Racemization Ratio.

Table 1 shows that different racemization ratio was found on samples which extracted from same subject at the same time, i.e. between samples number 6,7,8, and 9 and between samples number 10 & 11. Normality test of the racemization ratio showed normal distribution ($p=0.736$) while subjects' age did not show normal distribution ($p=0.001$). Spearman correlation analysis between subjects' age and racemization ratio showed no correlation ($p=0.722$), therefore no formula could be formulated between subjects' age and racemization ratio.

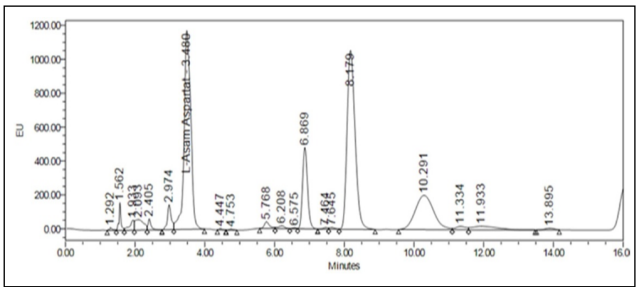

Figure 1. Example of UPLC chromatogram of sample.

Discussion

Previous research had found that tooth aspartic acid analysis is an accurate method to estimate individual's age.[14,22,23] Age is estimated by inserting one's tooth part (except pulp) or whole-tooth aspartic acid racemization ratio into formula concluded in the previous research on his/her population. Analysis can be performed using Gas Chromatography (GC) or High Performance Liquid Chromatography (HPLC).[16,24,25,27,28] UPLC is similar with HPLC but UPLC sample injection and peak area calculation was done automatically, indicating that data collected from UPLC analysis would be more accurate than HPLC. In our research, aspartic acid racemization ratios were different between samples extracted from same subject at the same time, i.e. samples number 6-9 and 10-11. This could be because sample powder taken for analysis since the initial process was not from the same part of tooth. Ohtani found that aspartic acid racemization ratio is different in every part of tooth.[16,31]

Ohtani also found that different tooth parts showed different correlation value between their aspartic acid racemization ratios with subject's age. The highest correlation of aspartic acid racemization ratio of first Premolar teeth with age was seen on dentin (r=0.992), followed by cementum (r=0.988), then enamel (r=0.961).[31] On another study, Sakuma also found a strong correlation between aspartic acid racemization ratio of the whole-tooth with subject's age (r=0.934).[27] We preferred to analyze aspartic acid racemization ratio of the whole-tooth because of some advantages, such as no dentin isolation is needed, much amino acid would be available for analysis -enabling repeatable analysis-, and that the rest of the samples could be preserved for used as control on further study.[27]

Correlation analysis showed no correlation between racemization ratios in this research and subjects' age. This is in contrast with Sakuma who found a strong correlation between racemization ratio from whole-tooth and age.[27] In Sakuma's study, the teeth were powdered using Multi-Beads Shocker and analyzed using Gas Chromatography-Mass Spectroscopy (GC-MS).[27] In our research, we used custom-made steel mortar and pestle to crush the teeth unto powder manually due to research laboratory limitation (we could not find Multi-Beads Shocker in any research laboratory in Jakarta), and we preferred UPLC instead of GC-MS to avoid the risk of additional racemization on sample due to vaporization process on GC sample preparation.[32] The difference of method between our research and Sakuma's could be one of the reasons of the result contradiction, but still need further exploration.

Ohtani stated that error might be differ on different analysis procedure.[16] We performed few modifications on Chen method.[24] Teeth were powdered using custom-made steel mortar and pestle instead of stainless steel mill. We used oven instead of waterbath, and freeze dryer instead of Nitrogen flow. Analysis was performed using UPLC instead of HPLC. These modifications could contribute to our results contradiction, which still need for further exploration.

Indonesia consists of many tribes. Subjects in our research are not exclusive and came from different tribes (Javanese, Chinese-Indonesian, and North Sumatera tribe). Previous researches on different population found different correlation value and concluded different formula. It still needs further exploration to analyze whether tribe difference contributed to our result.

Racemization is influenced by temperature and pH.[14,16,17] Although subjects in this study live in specific areas, subjects could have different dietary intake which had might contributed to our result. Therefore, we would like to recommend further exploration on subjects with similar dietary intake.

Results in this study had shown us the importance to avoid manual tooth powdering method to minimize the possibility of intra-subject racemization ratio difference. Further studies on subjects from specific Indonesian tribe with similar dietary intake using different powdering method are needed to gain formula for estimating individual's age of the tribe population.

Conclusions

No correlation was found between aspartic acid racemization ratio and subjects' age in our research, therefore no formula could be concluded to estimate individual's age from tooth aspartic acid racemization ratio in this research. Aspartic acid racemization analysis method performed in our research was able to analyze tooth aspartic acid racemization of subjects from Javanese, North Sumatera tribe and Chinese-Indonesian people living in Jakarta, Bogor, Depok, Tangerang, and Bekasi but not applicable to estimate individual's age yet. Further exploration on subjects from specific Indonesian tribes is needed to explore the contribution of tribe

difference to tooth aspartic acid racemization and to search formula applicable to estimate individual's age on Indonesian population.

Acknowledgements

The authors would like to thanks Integrated Laboratory and Research Centre (ILRC) Universitas Indonesia forlending us the freeze dryer machine.

Declaration of Interest

The authors report no conflict of interest.

References

1. Suchita S, Kulkarni PG, Keerthi M, Reddy DSP, Rao TM. Occlusal wear - An age determinant. Indian J Dent Adv 2014; 6(2):1523-8. https://doi.org/10.5866/2014.621523
2. Lu CK, Yee MCS, Ravi SB, Pandurangappa R. Forensic age estimation of chinese malaysian adults by evaluating occlusal tooth wear using modified Kim's index. Int J Dent 2017; 2017:41-50. https://doi.org/10.1155/2017/4265753.
3. Kim Y-K, Kho H-S, Lee K-H. Age estimation by occlusal tooth wear. Forensic Sci 2000; 45(2):303-9. https://doi.org/10.1520/JFS14683J
4. Brearley Messer LM, Till MJ. A landmark report on understanding the human dentition. J Am Dent Assoc 2013; 144(4):357-61. http://doi.org/10.14219/jada.archive.2013.0128
5. AlQahtani SJ, Hector M, Liversidge HM. The London atlas of human tooth development and eruption. American Journal of Physical Anthropology 2010; 142(3): 481-490. http://dx.doi.org/10.1002/ajpa.21258
6. Demirjian A. A new system of dental age assessment. Hum Biol 1973; 45(2):211.
7. Lewis AJ, Boaz K, Nagesh K, Srikant N, Gupta N, Nandita K, et al. Demirjian's method in the estimation of age: A study on human third molars. J Forensic Dent Sci 2015; 7(2):153-7. https://doi.org/10.4103/0975-1475.155081
8. Firdaus, Priaminiarti M, Puspitawati R. Third molars as the chronological age estimation indicator at the age of 14-22 years. J PDGI 2013; 62(1):1-6. https://123dok.com/document/yrk45jvz-gigi-molar-tiga-sebagai-indikator-prakiraan-kronologis-tahun.html
9. Gocha TP, Schutkowski H. Tooth cementum annulation for estimation of age-at-death in thermally altered remains. J Forensic Sci 2013; 58(1):1-5. https://doi.org/10.1111/1556-4029.12023
10. Kasetty S, Rammanohar M, Raju Ragavendra T. Dental cementum in age estimation: A polarized light and stereomicroscopic study. J Forensic Sci 2010; 55(3):779-83. https://doi.org/10.1111/j.1556-4029.2010.01363.x
11. Obertová Z, Francken M. Tooth cementum annulation method: Accuracy and applicability. Front Oral Biol 2009; 13(table 1):184-9. https://doi.org/10.1159/000242415
12. Goncharuk-Khomyn M. Modification of Dental Age Estimation Technique among Children from Transcarpathian Region. J Int Dent Med Res 2017; 10(3):851-855.
13. Singh N, Grover N, Puri N, Singh S, Arora S. Age estimation from physiological changes of teeth: A reliable age marker? J Forensic Dent Sci 2014; 6(2):113-21. https://doi.org/10.4103/0975-1475.132541
14. 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. https://doi.org/10.1177/0025802414524383
15. McCudden CR, Kraus VB. Biochemistry of amino acid racemization and clinical application to musculoskeletal disease. Clin Biochem 2006; 39(12):1112-30. https://doi.org/10.1016/j.clinbiochem.2006.07.009
16. 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. https://doi.org/10.1520/JFS2004354
17. 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. https://doi.org/10.1007/s12024-018-9984-8
18. Alberts B, Johnson A, Lewis J, Morgan D, Raff M, Roberts K, et al. Molecular biology of the cell. 6th ed. New York: Garland Science; 2015:109-113.
19. Foulquier E, Ginestoux C, Ouaray Z, Lefranc M. Amino Acids IMGT 2013. Available from: http://www.imgt.org
20. Arany S, Ohtani S. Age estimation by racemization method in teeth: Application of aspartic acid, glutamate, and alanine. J Forensic Sci 2010; 55(3):701-5. https://doi.org/10.1111/j.1556-4029.2010.01324.x
21. Ohtani S. Age estimation by aspartic acid racemization in dentin of deciduous teeth. Forensic Sci Int 1994; 68(2):77-82. https://doi.org/10.1016/0379-0738(94)90305-0
22. Yekkala R, Meers C, Schepdael A Van, Hoogmartens J, Lambrichts I, Willems G. Racemization of aspartic acid from human dentin in the estimation of chronological age. Forensic Sci Int 2006; 159(1):S89-94. https://doi.org/10.1016/j.forsciint.2006.02.022
23. Ohtani S, Yamamoto T. Comparison of age estimation in japanese and scandinavian teeth using amino acid racemization. J Forensic Sci 2011; 56(1):244-7. https://doi.org/10.1111/j.1556-4029.2010.01545.x
24. 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; 266:234-8. http://doi.org/10.1016/j.forsciint.2016.06.010
25. Rajkumari S, Nirmal M, Sunil PM, Smith AA. Estimation of age using aspartic acid racemisation in human dentin in Indian population. Forensic Sci Int 2013; 228(1):38-41. http://doi.org/10.1016/j.forsciint.2013.02.021
26. Hassan Q, Rakha A, Zahid Bashir M. Aspartic acid racemization with correlation to age: A forensic perspective. J Coll Physicians Surg Pakistan 2017; 27(5):283-7.
27. 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. https://doi.org/10.1016/j.forsciint.2012.08.043
28. Fu S-J, Fan C-C, Song H-W, Wei F-Q. Age estimation using a modified HPLC determination of ratio of aspartic acid in dentin. Forensic Sci Int 1995; 73(1):35-40. https://doi.org/10.1016/0379-0738(95)01709-7
29. 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. In: Pollegioni L, Servi S, editors. Unnatural Amino Acids. Methods in Molecular Biology (Methods and Protocols). New Jersey: Humana Press; 2012. p. 265-72.
30. Helfman PM, Bada JL. Aspartic acid racemization in tooth enamel from living humans. Proc Natl Acad Sci 1975; 72(8):2891-4. https://doi.org/10.1073/pnas.72.8.2891
31. Ohtani S, Sugimoto H, Sugeno H, Yamamoto S, Yamamoto K. Racemization of aspartic-acid in human cementum with age. Arch Oral Biol 1995; 40(2):91-5. https://doi.org/10.1016/0003-9969(94)00156-6
32. LTD M. Comparison Table - GC Vs. HPLC. Available from: www.mrclab.com