Estimation of Chronological Age from Advanced Glycation End Products Level in Vitreous Fluid

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Abstract

Background: Chronological age estimation is an important process for corpse identification. Advanced glycation end products (AGEs) are accumulated during lifetime and have been used for forensic identification. The AGEs in vitreous humor still lack of information for age estimation. *Objective:* To investigate correlation between the AGEs level contained in vitreous humor and chronological age in postmortem cases. *Methods:* The vitreous humor samples were collected from the postmortem cases (n=142). The AGEs levels were determined by spectrofluorometer and presented in microgram of quinidine hemisulfate equivalent (µg QE/mL) and nanogram of pentosidine equivalent (ng PE/mL). Stepwise linear regression was used to generate the equation. *Results:* The QE and PE showed mild positive correlation (r = 0.350 and 0.195; p <0.001, respectively) while the PE/QE ratio showed moderate negative correlation with age (r = -0.561, p <0.001). The predicted equation showed error value 12 years and gave accurate prediction in range 40-59 years old. *Conclusion:* The AGEs contained in the vitreous humor correlated with chronological age in postmortem cases. The other fluorescence molecules might be interfere during the AGEs measurement then the specific methods should be developed for measuring the AGEs level to decrease the error value.

Key words: *Age estimation, advanced glycation end products, vitreous humor, pentosidine*

Introduction

The formation of advanced glycation end products (AGEs) is a part of normal process in human body. The AGEs ,group of heterogeneous compounds, occurred from reducing sugars that reacted with free amino groups of proteins, lipids, or nucleic acids[1]. The AGEs ,normally pentosidine, ε*N*-carboxymethyllysine, ε*N*-carboxyethyl-lysine and methylglyoxal derivatives are found in the human body[2]. The AGEs play an important role in normal aging process and age-related diseases. Accumulation of the AGEs in tissues including serum, teeth, cartilage and skin are progressive during the lifetime[3]. The AGEs levels have been associated with aging process and correlated with age in volunteers, but AGEs levels in postmortem case is limited[4, 5].

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Vitreous humor is a gelatinous substance and filled in the eyeball where is a close space and is undisturbed by external environments, decomposition process and bacterial contamination[6]. Water, minerals and collagen are major components in the vitreous[7, 8]. The AGEs are found in the vitreous and associated with age in living person[9, 10]. Moreover, the vitreous humor is an alternative material for forensic identification. It was applied in postmortem diagnosis for example postmortem identification, death from hypothermia, sudden infant death, brain damage assessment and intoxication by bleach, but no apply in age estimation[11]. In addition, the process of vitreous humor collection is simple and rapid method.

High performance liquid chromatography (HPLC) coupled with fluorescence detector and enzyme-linked immunosorbent assay (ELISA) are suitable methods for the AGEs level analysis[12]. The HPLC is an expensive instrument and cannot provided in

laboratory. Spectrofluorometer is a basic instrument for determination of fluorescence substances level. An emission and excitation wavelengths are more specific to each substance properties and the pentosidine level was applied by fluorescence spectrometer[13].

In forensic medicine, age estimation is an essential process for person identification both living individuals and postmortem. There are many methods for the age estimation including radiological examination of skeleton and dentine, anthropological method, telomere shortening and chemical substances especially aspartic acid and AGE which are popular chemical substance for age estimation[14]. The AGEs level contained in teeth were highly correlated with chronological age[5]. However, the teeth samples were difficult for sample preparation. Using of the AGEs level contained vitreous humor might be simple and rapid method for age estimation. The aim of the study were to investigate correlation between the AGEs level contained in vitreous humor and chronological age in postmortem cases by spectrofluorometer.

Materials and Methods

One hundred and fourth two male who died within 24 hours were included in this study. All participants aged between 12 to 88 years old. The vitreous humor samples were collected during autopsy at Department of Forensics Medicines, Faculty of Medicine, Chiang Mai University during September 2019 to January 2020. The subjects who had decomposition and not known about gender, age, and race were excluded. The study was approved by the Research Ethics Committee Faculty of Medicine, Chiang Mai University (FOR 2562 06508).

Approximately, three milliliter of vitreous fluid was collected at eyeball position and the sample was added in sodium fluoride tube. The samples were stored at -80 °C before experimentation. The 0.5 milliliter of vitreous fluid sample was hydrolyzed with one milliliter of 6M hydrochloric acid at 110 °C for 20 hours. The hydrolyzed sample was centrifuged at 12,000 RPM for 10 minutes. Then, ten microliter of the hydrolyzed sample was diluted with 190 microliter of double distilled water.

The AGEs concentration was measured with spectrofluorometer[15]. Quinidine hemisulfate and pentosidine were used as standard substance. The excitation and emission wavelengths was 350/440 nm for quinidine hemisulfate and 328/378 nm for pentosidine, respectively. The results were expressed in microgram of quinidine hemisulfate equivalent per milliliter (μg QE/mL) and nanogram of pentosidine equivalent (ng PE/mL).

The efficiency of the age prediction models were assessed by self-consistency test and bias and inaccuracy value were calculated following equiation:

•Bias = Σ (predicting age – chronological age)/n

•Inaccuracy = Σ|predicting age – chronological age|/n

Data were expressed as mean ± standard deviation (S.D). The correlation between the AGEs levels and chronological age were analyzed by Pearson's correlation. The age prediction models were generated by multiple linear regression. The estimated age and actual age were compared with paired *t*-Test analysis. Independent t-Test was used to compare AGEs levels in cardiovascular disease group and non-cardiovascular diseases group. A *p*-value less than 0.05 ($p<0.05$) was considered significant.

Reults

In this study 142 males who age ranged from 12 to 88 years old were included. We classified the participants into seven groups and the results are showed in Table 1. Cause of death composed of trauma 84 cases (59.2%), sudden unexpected death 41 cases (28.9%), hanging 11 cases (7.7%), and miscellaneous 6 cases (4.2%). The underlying diseases of the participants were obtained from medical record. Hypertension, diabetic mellitus and renal disease found 18 (12.7%), 7 (4.9%), and 4 (2.8%) cases, respectively. The scatter plots between age and the QE, PE, and PE/QE ratio are shown in Figure 1. The value of QE, PE, and PE/QE ratio in these diseases showed similarly levels with the healthy participants or other diseases.

Correlation between the quinidine hemisulfate, pentosidine equivalent and chronological age

The Pearson's correlation was used for determining a correlation between the AGEs concentrations and chronological ages. The correlation between the QE and

PE showed mild positive correlation (r=0.35 and 0.195, respectively). While the PE/QE ratio showed negative correlation (r=-0.561). The correlation of chronological age and the AGEs levels are shown in Figure 1.

Age estimation model

The age estimation equation was generated by linear regression following stepwise method. The formula for age estimation is shown as follow:

Estimated age = 143.404 – (1040.781* PE/QE) + (24.277*PE) – (1.927*QE) (1)

When PE is pentosidine equivalent, QE is quinidine hemisulfate equivalent, and PE/QE is the ratio of pentosidine and quinidine hemisulfate equivalent. The R value of the linear regression equation was 0.607 (R^2 = 0.368, p <0.001). The beta coefficients of QE, PE, and PE/QE ratio in the equation presented -0.975, 1.076, and -0.889 respectively. The standard error of the estimation (SE) showed 15.89.

The self-consistency test was used for testing the efficacy and accuracy of the estimated equation. Bias value of the equation showed 0.028 years and inaccuracy demonstrated 12 years. The estimated age were compared with chronological age in each age range and the results are shown in Table 1. A different value (ΔA) between estimated age and chronological age was plotted with the chronological age. The ΔA in age range 40-59 years old revealed lower inaccuracy value than the other ranges and the graph is shown in Figure 2. The estimated ages were similar with the chronological ages in range 40-59 year old but others ranges were different.

Effect of cardiovascular diseases on the AGEs levels

The cause of death was diagnosed by a forensic pathologist. Coronary artery disease, myocardial infarction, and intracerebral hemorrhage were grouped in cardiovascular disease. For, pneumonia, sepsis, pulmonary embolism, and others were grouped in non- cardiovascular disease. The AGEs level in the cardiovascular disease group was similar level when compared with the level of non- cardiovascular diseases and the results are demonstrated in Table 2.

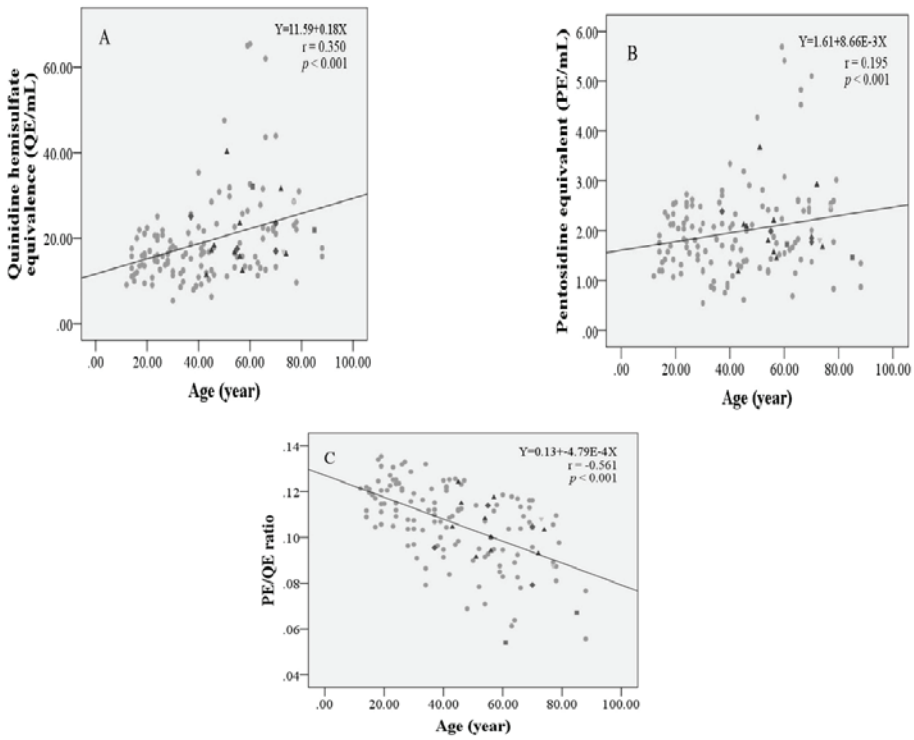

Figure 1. Distribution of the AGEs values in each chronological ages and diseases when p = hypertension, u = Diabetic mellitus q=, renal disease, n = hypertension + diabetic mellitus +renal disease and ˜ = healthy participants. Correlation of the QE, the PE and PE/QE ratio with chronological age are shown in Figure A, B and C, respectively.

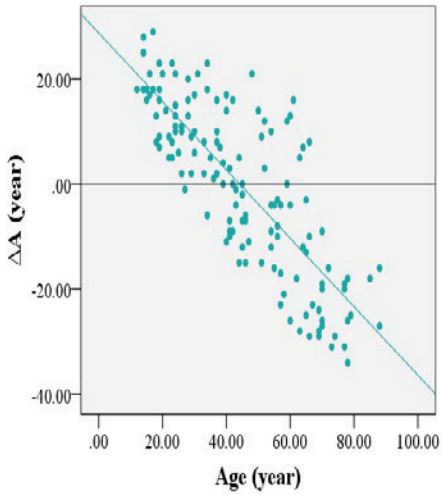

Figure 2. Relative of bias (the difference value of estimated age and chronological age (rA)) and chronological age.

Table 1. Comparison between estimated age and chronological age

Actual age	Number (n)	Mean of actual age (year)	Predicted age range	p-value
12-19	19	16.5	24-48	<0.001
20-29	23	24.8	21-49	<0.001
30-39	19	34.8	26-61	<0.001
40-48	23	43.2	24-64	0.270
50-59	22	54.6	33-66	0.091
60-69	18	64.5	32-75	<0.001
70-88	18	76.3	43-76	<0.001

$p < 0.05$ significant different between actual age and predicted age

Table 2. Comparison of the AGEs levels in cardiovascular diseases and non-cardiovascular diseases

Factors	Cardiovascular diseases (N=24)	Non- cardiovascular diseases (N=17)	p-value
Age	59.6±12.7	57.4±19.1	0.672
Quinidine hemisulfate (μg QE/mL)	23.1±11.7	23.3±13.5	0.948
Pentosidine (ng PE/mL)	2.2±1.0	2.2±1.3	0.888
PE/QE ratio (*10-2)	9.9±2.0	9.5±1.9	0.627

$p < 0.05$ significant different the AGEs level between the cardiovascular disease group and non- cardiovascular diseases group.

Discussion

The AGE formation is a normal process and develops in tissue during aging. The AGEs levels were determined in postmortem for age estimation. Estrogen, female hormone, reduce the AGEs level[16] and gender might effect on pentosidine accumulation in vitreous humor[10].Then only male participants were included in this study to decrease gender effect.

The QE and PE level presented mild positive correlation with chronological age. Both the AGEs and pentosidine in vitreous significant increased depending on age[17]. The PE/QE ratio showed moderate negative correlation with the chronological age. The correlation of the QE and PE level in vitreous were lower than teeth, cartilage, and bone samples[5,18]. The turnover rate of protein might be effect to the AGEs and pentosidine level in tissues. High turnover rate tissue including blood and vitreous humor has a shorter half-life of the AGEs than the low turnover rate tissue[19].

In age ranged 40-59 year old were highly accurate than the others range. The contaminated AGEs in food can absorb through circulation leading to high AGEs levels in blood or accumulate in other tissue[20]. Teenager's lifestyle like to consume about coffee, cocoa, cake, bread and grilled meat leading to highly the AGEs level in blood and might be disposed to the vitreous[21, 22]. Aging is the progressive accumulation of the AGEs from dietary, lifestyle, drug and unknown underlying disease [9, 23]. The AGEs damage to an organism overtime leading to disturbed function on the cellular, tissue and organs. In elderly age, the AGEs level normally higher level than the teenager leading to highly error estimated age.

High level of the AGEs presents in diabetes mellitus and cardiovascular diseases[1, 24]. The cardiovascular disease was only observed with forensic pathologist, but diabetes mellitus could not diagnostic with routine laboratory. The AGEs level in cardiovascular diseases did not different compared with the healthy participants. As same as, the QE, PE and PE/QE ratio in cardiovascular diseases group did not different compared with non-cardiovascular diseases which was discordance with the previous study[25]. In this study showed that cardiovascular diseases did not effect to the QE, PE and PE/QE ratio and might be effect on the age estimation process. However, the fluorescence spectrometry method might be affected by others fluorescence molecules. Then, the specific methods for the AGEs determination should be developed to give the highly predict equation for age estimation.

Conclusion

The PE/QE ratio of vitreous humor in postmortem cases showed moderately correlation with chronological age and suitable for age estimation. The predicted equation presented error value about 12 years and gave accurate range in 40-59 year old. The protein turnover rate and external factors including dietary intake, drugs, and unknown underlying disease might disturb on the AGEs. In the future, teeth, bone, and cartilage samples

should be determined the AGEs for more accuracy in postmortem age estimation. The specific methods should be developed for measuring the AGEs level to decrease the error value.

Conflicts of Interest: The authors declare that they do not have any conflict of interest.

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References

1. Vlassara H, Palace MR. Diabetes and advanced glycation endproducts. J Intern Med 2002; 251: 87-101.
2. Vistoli G, De Maddis D, Cipak A, et al. Advanced glycoxidation and lipoxidation end products (AGEs and ALEs): an overview of their mechanisms of formation. Free Radic Res 2013; 47: 3-27.
3. Bierhaus A, Humpert PM, Morcos M, et al. Understanding RAGE, the receptor for advanced glycation end products. J Mol Med (Berl) 2005; 83: 876-86.
4. Takahashi M, Oikawa M, Nagano A. Effect of age and menopause on serum concentrations of pentosidine, an advanced glycation end product. The Journals of gerontology series A: biological sciences medical sciences 2000;55: 137-40.
5. Greis F, Reckert A, Fischer K, Ritz-Timme S. Analysis of advanced glycation end products (AGEs) in dentine: useful for age estimation? Int J Legal Med 2018; 132: 799-805.
6. Egger SF, Buxbaum A, Georgopoulos M, et al. Bacterial growth in human vitreous humor. Exp Eye Res 1997; 65: 791-5.
7. Grus FH, Joachim SC, Pfeiffer N. Proteomics in ocular fluids. Proteomics Clin Appl 2007; 1: 876-88.
8. Baniak N, Campos-Baniak G, Mulla A, Kalra J. Vitreous humor: a short review on post-mortem applications. J Clin Exp Pathol 2015; 4: 1-7.
9. Tessier F, Obrenovich M, Monnier VM. Structure and mechanism of formation of human lens fluorophore LM-1. Relationship to vesperlysine A and the advanced Maillard reaction in aging, diabetes, and cataractogenesis. J Biol Chem 1999; 274: 20796-804.
10. Van Deemter M, Ponsioen T, Bank R, et al. Pentosidine accumulates in the aging vitreous body: a gender effect. Experimental eye research 2009; 88: 1043-50.
11. Bévalot F, Cartiser N, Bottinelli C, Fanton L, Guitton J. Vitreous humor analysis for the detection of xenobiotics in forensic toxicology: a review. Forensic toxicology 2016; 34: 12-40.
12. Munch G, Keis R, Wessels A, et al. Determination of advanced glycation end products in serum by fluorescence spectroscopy and competitive ELISA. Eur J Clin Chem Clin Biochem 1997; 35: 669-77.
13. Yanagisawa K, Makita Z, Shiroshita K, et al. Specific fluorescence assay for advanced glycation end products in blood and urine of diabetic patients. Metabolism 1998; 47: 1348-53.
14. Mughal AM, Hassan N, Ahmed A. Bone age assessment methods. A critical review; Pakistan journal of medical sciences 2014; 30: 211.
15. Kalousova M, Skrha J, Zima T. Advanced glycation end-products and advanced oxidation protein products in patients with diabetes mellitus. Physiol Res 2002;51: 597-604.
16. Yan H, Yang W, Zhou F, et al. Estrogen Improves Insulin Sensitivity and Suppresses Gluconeogenesis via the Transcription Factor Foxo1. Diabetes 2019; 68: 291-304.
17. Stitt AW, Moore JE, Sharkey JA, et al. Advanced glycation end products in vitreous: Structural and functional implications for diabetic vitreopathy. Invest Ophthalmol Vis Sci 1998; 39: 2517-23.
18. Verzijl N, DeGroot J, Oldehinkel E, et al. Age-related accumulation of Maillard reaction products in human articular cartilage collagen. Biochemical Journal 2000; 350: 381-7.
19. Verzijl N, DeGroot J, Thorpe SR, et al. Effect of collagen turnover on the accumulation of advanced glycation end products. J Biol Chem 2000; 275: 39027-31.
20. Uribarri J, Woodruff S, Goodman S, et al. Advanced glycation end products in foods and a practical guide to their reduction in the diet. Journal of the American Dietetic Association 2010; 110: 911-6.

21. Snelson M, Coughlan MT. Dietary advanced glycation end products: Digestion, metabolism and modulation of gut microbial ecology. Nutrients2019 ;11: 215.
22. Uribarri J, Cai W, Peppa M, et al. Circulating glycotoxins and dietary advanced glycation endproducts: two links to inflammatory response, oxidative stress, and aging. J Gerontol A Biol Sci Med Sci 2007; 62: 427-33.
23. Luevano-Contreras C, Chapman-Novakofski K. Dietary advanced glycation end products and aging. Nutrients 2010; 2: 1247-65.
24. Singh R, Barden A, Mori T, Beilin L. Advanced glycation end-products: a review. Diabetologia 2001; 44: 129-46.
25. Falcone C, Emanuele E, D'Angelo A, et al. Plasma levels of soluble receptor for advanced glycation end products and coronary artery disease in nondiabetic men. Arteriosclerosis, thrombosis, and vascular biology 2005; 25: 1032-7.