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Original article

Plasma amyloid β40/42 ratio and its characteristics

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Abstract

Amyloid-β (Aβ) is formed by the cleavage of amyloid precursor protein (APP) and is characterized by the deposition of Aβ in the brain. A high plasma Aβ40/42 ratio is associated with an increased incidence of dementia. In the present study, we analyzed the effect of aging markers on Aβ40/42 from the data of an anti-aging medical check-up conducted in 2019. The subjects included 21 university faculty and staff (FS; 49.3 ± 12.9 years) and 36 independent elderly (Yurin elderly, YE; 79.3 ± 6.4 years) living in the Yurin area of Kyoto, Japan. Statistical analysis was performed using stepwise multiple regression analysis for a total of 13 items, including gender, age, BMI and anti-aging medical check-up items (muscle age, neural age, bone age, blood vessel age, hormone age, glycative stress, oxidative stress, immune stress, physical and mental stress and lifestyle) as independent variables and Aβ40/42 as a dependent variable. For FS, the dependent variable Y (Aβ40/42) was expressed with the equation "Y = −0.131 X1(lifestyle score) + 19.7", where poor lifestyle was a risk factor for elevated Aβ40/42. Neural age was a risk factor for YE, while blood vessel age and glycative stress were risk factors to analyze all subjects for elevated Aβ40/42. We can conclude that neural age, blood vessel age, glycative stress and poor lifestyle may affect the plasma Aβ40/42 ratio and may be risk factors for exacerbating dementia.

***KEY WORDS*:** dementia, amyloid-β (Aβ), arteriosclerosis, glycative stress, Aβ40/42 ratio

Introduction

Dementia is a disease in which the brain is damaged due to some cause and cognitive functions decline, resulting in various symptoms. There are several diseases causing dementia, and in the descending order of the number of patients, 67.6% have Alzheimer's disease (AD), 19.5% have cerebrovascular dementia, 4.3% have Lewy body dementia and 8.6% have other diseases [1]. Most patients suffer from AD, and there is a pressing need to elucidate its pathogenic mechanism.

Pathophysiologically, AD is a disease in which an accumulation of amyloid-β (Aβ) aggregates outside the nerve cells which triggers the accumulation of tau aggregates in the nerve cells, leading to synapse dysfunction and neuronal cell death. These changes in the brain cause cognitive dysfunction, psychiatric symptoms and behavioral disorders. In other words, in AD, Aβ aggregates and accumulates in the brain even before the appearance of cognitive dysfunction. Early diagnosis and intervention may increase the effectiveness of therapies targeting Aβ [2,3]. However, the only available methods to reliably investigate the level of Aβ deposition are the Aβ-PET (positron emission tomography) imaging and measurement of Aβ levels in the cerebrospinal fluid. Both these methods are expensive and are a physical burden on the patients.

Aβ is formed by the cleavage of amyloid precursor protein (APP) and is characterized by deposition in the brain. It has become possible to detect Aβ in trace amounts of blood samples using MALDI-TOF mass spectrometry [4]. A high plasma Aβ40/42 ratio is associated with an increased incidence of dementia [5]. Our laboratory has been conducting health promotion activities in the Yurin area of Kyoto, Japan as part of the Yurin Study since 2008 [6-9]. Also, anti-aging medical check-ups are being conducted for university faculty

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and staff since 2006 [10]. In the present study, we measured the plasma Aβ40/42 ratio in the recipients of the medical check-ups and analyzed the association with background factors.

Method

Subjects

The subjects of the study were 21 university faculty and staff (FS; 18 males, 3 females; 49.3 ± 12.9 years) and 36 independent elderly (Yurin elderly, YE; 16 males, 20 females; 79.3 ± 6.4 years) living in the Yurin area of Shimogyo-ku, Kyoto. In a previous study [6], an intervention study is being conducted since December 2008 under the health promotion program (Kempo-Juku) with pedometers and printed materials for YE, who are the subjects of the present study.

Anti-aging Medical Checkup

The anti-aging medical check-up was conducted to support motivation and confirm the results. The check-up included anthropometric measurements, evaluating the five functional ages for muscle, bone, hormone, nerve and blood vessel, and glycative stress test. The functional age during the anti-aging medical check-up was calculated using Life Style Compass (Nippon Shooter Ltd., Chiyoda-ku, Tokyo), modified from the Age Management Check^{R} system (Ginga Kobo), and the relative functional ages calculated from these databases were shared with each individual [8,10]. The advantage of functional age is that physical functions can be presented as age equivalents, which is easy for the subjects to become familiar with and change their behavior. By sharing the anti-aging medical check-up results with each individual, we expect that the individuals will understand their weaknesses as they age and bring changes in behavior from the awareness to strive for rejuvenation of functional age. An all-purpose version of the software is currently under development [11].

Evaluation of Subjective Symptoms

Subjective symptoms were divided into "physical symptoms" and "psychological symptoms" and were evaluated on a 5-point scale from 1 to 5 using the Anti-Aging QOL Common Questionnaire (AAQol) [12,13].

Anthropometric Measurements

Anthropometric measurement included measurement of height, weight and body composition. Body composition was measured using a device (PhysionMD; Nippon Shooter Ltd.) to measure the bioimpedance muscle mass [14].

Blood Biochemistry Tests

Blood biochemistry tests included the measurement of blood levels of plasma Aβ40 and Aβ42, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), fasting blood glucose, hemoglobin A1c (HbA1c) [National Glycohemoglobin Standardization Program (NGSP)], insulin, insulin-like growth factor-I (IGF-I), dehydroepiandrosterone-sulfate (DHEA-s) and cortisol. These were measured by LSI Medience Corporation (Chiyoda-ku, Tokyo). Serum SARS-CoV2 IgG antibodies (MBL, Minato-ku, Tokyo) were measured considering that the study was conducted during the coronavirus pandemic. For the urinary melatonin metabolites, 6-sulfatoxymelatonin (SaMT) [15-17] stored in the urinary bladder during the night was measured.

Muscle Age Measurement

The muscle mass used for muscle age calculation was determined by the bioelectrical impedance method (Physion MD) following the method of previous studies, and muscle age was evaluated using the Weight-Bearing Index (WBI) and basal metabolic rate (kcal/day) as the markers [14].

Bone Age Measurement

The ultrasound method (A-1000; GE Yokogawa Medical Systems, Hino, Tokyo) was used to measure the bone density for bone age calculation, and ankle bone stiffness value and young adult means (% YAM) were used as the indices.

Hormone age Measurement

IGF-I and DHEA-s [18], the blood markers for aging used to calculate the hormone age, were measured by blood biochemistry tests and converted to the hormone age.

Neural Age Measurement

The Wisconsin Card Sorting Test (WCST) [19-21] was used to determine the higher brain functions used to calculate the neural age. The neural age was evaluated by entering the categories achieved (CA), numbers of response cards until the first category achieved (NUCA), preservative errors of Nelson (PEN), preservative errors of Milner (PEM), total errors (TE) and marker data of reaction time in the software [11].

Blood Vessel Age Measurement

Arteriosclerosis used to calculate the blood vessel age was measured with the fingertip photoplethysmogram (Dynapulse SDP-100; Fukuda Denshi, Bunkyo-ku, Tokyo) [22-24].

Glycative Stress

As previously reported, the AGE Reader mu™ (DiagnOptics, Groningen, Netherlands) was used to measure the glycative stress markers and SAF (skin autofluorescence) value, which is the integral data of autofluorescence value of advanced glycation end products (AGEs) was measured [25-27].

Immune Stress

The lymphocyte count was used as the score marker. A lymphocyte count between 2,665～3,188/mm^3 was assigned a score of 90 or more. Lymphocyte count less than 706/mm^3 and 4,191/mm^3 or more were assigned a score of 50 or less [11].

Physical and mental stress

The cortisol/DHEA-s ratio (C/D ratio) was used as the score marker. A C/D ratio between 4.58～5.77 was assigned a score of 90 or more. C/D ratio of 11.07 or more and below 2.99 was assigned a score of 50 or less [11].

Lifestyle

The scores were calculated with exercise habits, alcohol consumption, smoking and sleeping duration as indices [11].

Oxidative stress

Since markers for oxidative stress were not measured in the present study, the values estimated from AAQol were used [11].

Statistical analysis

The results were expressed as average value ± standard deviation. Statistical analysis was performed using stepwise multiple regression analysis with Microsoft Excel for a total of 13 items, including gender, age, BMI and anti-aging medical check-up items (muscle age, neural age, bone age, blood vessel age, hormone age, glycative stress, oxidative stress, immune stress, physical and mental stress and lifestyle) as independent variables and Aβ40/42 as a dependent variable. A risk rate of less than 5% was considered a significant difference.

Ethical Review

This study was conducted in compliance with the Helsinki Declaration (revised at the 2013 WMA General Assembly in Fortaleza) and the Ethical Guidelines for Medical and Health Research Involving Human Subjects (Notification of the Ministry of Education, Culture, Sports, Science and Technology Ministry of Health, Labor and Welfare). When starting this study, subjects were allowed to decide freely to participate in the program. Signed informed consent was obtained from the subjects after explaining that they would not suffer any disadvantages even if they withdraw from participation due to any circumstances. The Ethics Review Committee has approved this research for "Research on Human Subjects" of Doshisha University (Application Number: #14089, 17093).

Results

Changes in the Plasma Aβ40/42 Ratio

Although the age-related changes in the Aβ40/42 ratio appears to gradually increase with age (***Fig.1-a***), a significant correlation with age was not observed ($y = 0.0109x + 7.5934$, $r = 0.15$, $n = 57$).

The subjects of the current study included FS and YE, with each group having different lifestyles, environments and gender composition. Therefore, we analyzed each group (***Table 1, 2***). All subjects were negative for the SARS-CoV2 antibody.

The YE were older than FS, while the functional age of muscle, bone, hormones, nerves and blood vessels was significantly advanced, muscles, hormones and nerves were maintained younger than the actual age when Δ functional age (functional age-actual age) was compared. Regarding the risk factors that accelerate aging, YE had lower scores for physical and mental stress and oxidative stress, and these stresses were more severe.

The YE had lower IGF-I, DHEA-s and melatonin secretion and a higher C/D ratio than FS. Glycative stress markers SAF and pentosidine were also significantly higher. However, HDL-C was high, and there was no significant difference in the Aβ40/42 ratio between the two groups. This shows that YE is a group with a high fitness level.

The Aβ40/42 ratio for FS was 8.2 ± 0.8 (95% CI: 7.8-8.6), which showed an increasing trend with age and a weak correlation ($r = 0.29$) with age (age range 23-65 years) (***Fig. 1-a***). The percentage of females among FS is small, and changes in the Aβ40/42 ratio mainly reflect the values for males (***Fig. 1-b, c***).

For males, the slope of the regression line of YE was steeper than the regression line of FS, and a downward shift was observed (***Fig. 1-b***). The slope of the regression line was more gradual for women than that of men among YE (***Fig. 1-c***).

Single-correlation Analysis

The results of the single-correlation analysis for the anti-aging medical check-up items with the Aβ40/42 ratio are as follows.

Among FS, a weak correlation was observed for the hormone age ($r = 0.34$) and blood vessel age ($r = 0.30$) (***Table 3***). On the other hand, a negative correlation was observed for neural age ($r = -0.36$), Δ neural age ($r = -0.36$) and Δ muscle age ($r = -0.39$). This is because, among the elderly with a high Aβ40/42 ratio, many tended to be skilled in WCST and have muscle loss. Regarding the risk factors of aging, the Aβ40/42 ratio tended to be higher in subjects with severe stress due to low scores for lifestyle ($r = -0.51$) and physical and mental stress ($r = -0.40$).

A very weak correlation was observed for the Aβ40/42 ratio with neural age ($r = 0.29$) and blood vessel age ($r = 0.25$) in YE. A negative correlation was observed for bone age and hormone age, as the Aβ40/42 ratio tended to be low in females with less hormone secretion and low bone density. Regarding the risk factors of aging, the Aβ40/42 ratio tended to be higher in subjects with severe glycative stress due to a low score for glycative stress ($r = -0.30$). For the elderly, subjects with low scores for physical and mental stress, lifestyle and oxidative stress tended to maintain a lower Aβ40/42 ratio. Activities such as proactive participation in social activities that involve physical and mental stress may prove beneficial. For subjects with a high Aβ40/42 ratio and a tendency for dementia, the markers related to lifestyle scores (sleep duration, smoking and alcohol consumption) were considered to be better.

In the analysis of all subjects, Aβ40/42 ratio tended to be high in individuals with higher blood vessel age ($r = 0.24$). In addition, Aβ40/42 tended to be higher in subjects having severe stress due to a low glycative stress score ($r = -0.28$).

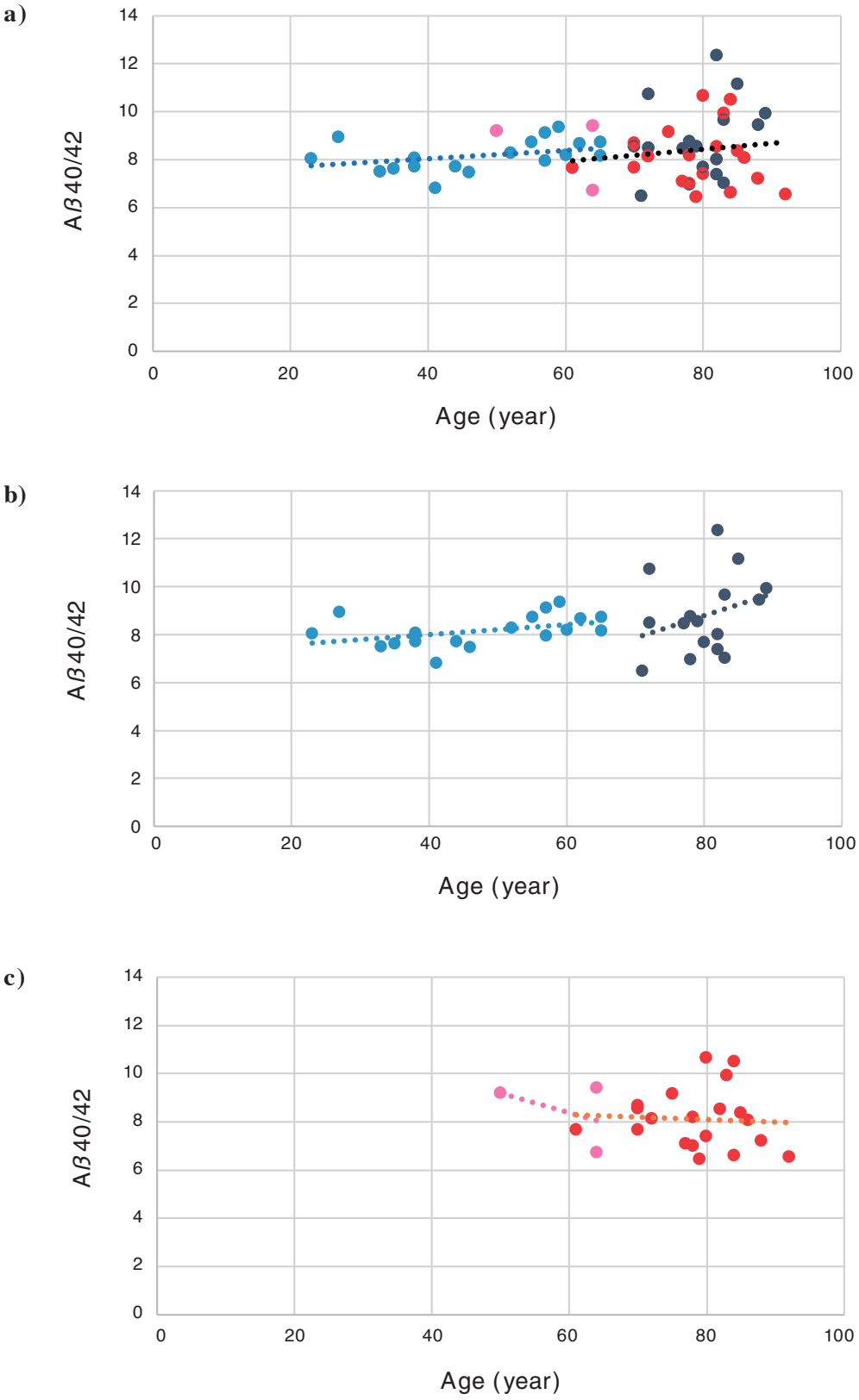

Fig. 1. **Age-related changes in the Aβ40/42 ratio**

Faculty and staff (FS) Male (n = 18), Female (n = 3). Yurin elderly (YE) Male (n = 16), Female (n = 20). **a)** Male and female total. FS: y = 0.0172x + 7.3509, r = 0.29. YE: y = 0.0251x + 6.426, r = 0.11. **b)** Male. FS: y = 0.0208x + 7.1698, r = 0.42, $p < 0.05$. YE: y = 0.0931x + 1.3503, r = 0.30. **c)** Female. FS: y = −0.0811x + 13.244, r = −0.44. YE: y = −0.0102x + 8.911, r = 0.06.

Table 1. Functional age and risk factors for aging.

	Faculty & staff (FS), n = 21			Yurin elderly (YE), n = 36			
	Mean ± SD	95% CI Lower	Upper	Mean ± SD	95% CI Lower	Upper	p value
Age	49.3 ± 12.9	43.3	55.3	79.3 ± 11.6	77.1	81.5	**<0.001**
BMI	23.5 ± 1.6	22.7	24.2	22.6 ± 3.1	21.6	23.7	0.27
【Functional age】							
Muscle age	60.7 ± 13.6	54.4	67.1	79.3 ± 11.6	75.3	83.3	**<0.001**
Bone age	49.2 ± 16.27	41.6	56.8	72.4 ± 19.7	65.7	79.2	**<0.001**
Hormone age	49.6 ± 15.6	42.4	56.9	71.3 ± 8.8	60.5	82.2	**<0.001**
Neural age	43.2 ± 4.3	41.2	45.3	47.3 ± 4.5	45.7	48.8	**<0.001**
Blood vessel age	41.9 ± 11.2	36.7	47.1	67.6 ± 12.0	63.5	71.7	**<0.001**
Δ Muscle age	11.4 ± 9.3	7.1	15.8	0.0 ± 12.0	−4.1	−13.4	**<0.001**
Δ Bone age	−0.1 ± 17.0	−8.0	7.8	−6.9 ± 19.2	−13.4	−0.3	0.19
Δ Hormone age	0.3 11.8	−5.2	5.9	−7.8 11.0	−11.6	−4.0	**0.01**
Δ Neural age	−6.0 14.6	0.8	−12.9	−32.1 5.9	−34.1	−30.0	**<0.001**
Δ Blood vessel age	−7.4 10.9	−12.5	−2.3	−11.7 11.2	−15.5	−7.8	0.17
【Risk factors】							
Glycative stress	63.3 ± 10.2	58.5	68.0	62.3 ± 13.3	57.7	66.8	0.77
Oxidative stress	80.4 ± 7.1	77.0	83.7	70.6 ± 12.5	66.3	74.8	**<0.001**
Physical & mental stress	57.5 16.5	49.8	65.2	48.4 12.5	44.1	52.7	**0.02**
Immune stress	67.8 6.8	64.6	70.9	73.0 14.0	68.2	77.8	0.12
Life styles	87.8 ± 2.9	86.4	89.2	88.8 ± 4.4	87.3	90.3	0.35

Statistical analysis by Student's t test. Δ Functional age defined as "Functional age – Age". BMI, body mass index; 95%CI, 95% confidence interval; SD, standard deviation.

Table 2. Blood and urine chemistry profile.

		Faculty & staff (FS), n = 21			Yurin elderly (YE), n = 36			
		Mean ± SD	95% CI Lower	Upper	Mean ± SD	95% CI Lower	Upper	p value
Aβ40/42		8.2 ± 0.8	7.8	8.6	8.4 ± 1.4	7.9	8.9	0.53
IGF-I	(ng/mL)	152.7 ± 49.4	129.6	175.7	82.2 ± 24.8	73.7	90.7	**<0.001**
DHEA-s	(μg/mL)	195.0 ± 123.0	137.7	252.4	77.9 ± 50.0	60.7	95.0	**<0.001**
Cortisol	(μg/mL)	9.9 ± 2.5	8.7	11.0	9.9 ± 2.9	8.9	10.9	0.93
C/D ratio		8.3 ± 7.2	4.9	11.6	18.3 ± 10.9	14.6	22.0	**<0.001**
Urine SaMT	(ng/mL)	62.3 ± 35.6	45.7	79.0	22.6 ± 21.7	15.1	30.0	**<0.001**
FPG	(mg/dL)	88.6 ± 5.9	85.9	91.4	91.0 ± 16.3	85.4	96.6	0.53
HbA1c	(%)	5.5 ± 0.3	5.4	5.6	5.8 ± 0.9	5.5	6.1	0.09
Insulin	(μU/mL)	5.3 ± 1.8	4.5	6.1	6.0 ± 5.3	4.2	7.9	0.55
Pentosidine	(pmol/mL)	23.5 ± 6.3	20.6	26.4	35.0 ± 10.0	31.6	38.5	**<0.001**
SAF		2.1 ± 0.5	1.9	2.3	2.6 ± 0.4	2.4	2.7	**<0.001**
TC	(mg/dL)	215.4 ± 35.4	198.9	231.9	212.6 ± 31.7	201.7	223.4	0.76
LDL-C	(mg/dL)	122.5 ± 29.4	108.8	136.2	119.6 ± 23.5	111.6	127.7	0.69
HDL-C	(mg/dL)	57.4 ± 12.7	51.5	8.6	72.3 ± 19.6	65.6	79.0	**<0.001**
TG	(mg/dL)	97.4 ± 45.0	76.4	118.4	97.5 ± 52.1	79.6	115.4	1.00
Lymphocyte count	(/μL)	1624.0 ± 367.6	1452.5	1795.5	1881.0 ± 702.4	1639.9	2122.0	0.13

Statistical analysis by Student's t test. Aβ40/42, amyloid β40/42 ratio; IGF-I, insulin-like growth factor-I; DHEA-s, dehydroepiandrosterone-sulfate; C/D, cortisol/DHEA-s ratio; SaMT, 6-sulfatoxymelatonin; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c [NGSP]; NGSP, National Glycohemoglobin Standardization program; SAF, skin AGE fluorescence measure by AGE Reader mu; AGE, advanced glycation endproduct; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; TG, triglyceride; 95%CI, 95% confidence interval; SD, standard deviation.

Table 3. Simple correlation analysis.

	Faculty & staff (FS) (n = 21)	Yurin elderly (YE) (n = 36)	Total (n = 57)
Aβ 40/42	1.00	1.00	1.00
Age	0.29	0.11	0.15
Sex (Male 0, Female 1)	0.13	−0.24	−0.12
BMI	−0.18	0.23	0.15
Muscle age	0.01	0.04	0.07
Bone age	0.08	**−0.35**	−0.18
Hormone age	0.34	−0.30	0.00
Neural age	**−0.36**	0.29	0.15
Blood vessel age	0.30	0.25	0.24
Δ Muscle age	**−0.39**	−0.02	−0.12
Δ Bone age	−0.15	**−0.40**	**−0.35**
Δ Hormone age	0.13	−0.33	−0.22
Δ Neural age	**−0.36**	0.09	−0.12
Δ Blood vessel age	−0.04	0.21	0.13
Glycative stress	−0.21	−0.30	−0.28
Oxidative stress	0.04	0.32	0.22
Physical & mental stress	−0.14	0.17	0.05
Immune stress	−0.01	−0.19	−0.15
Life styles	**−0.51**	0.32	0.19
IGF-I	−0.27	0.19	−0.05
DHEA-s	−0.25	0.29	−0.01
Cortisol	0.12	−0.19	−0.12
C/D ratio	**0.36**	−0.17	−0.03
Urine SaMT	−0.32	−0.08	−0.16
FPG	0.14	0.17	0.17
HbA1c	0.24	0.22	0.24
Insulin	−0.01	0.20	0.19
Pentosidine	−0.03	0.20	0.18
SAF	0.14	**0.43**	0.33
TC	−0.16	−0.16	−0.16
LDL-C	−0.10	−0.03	−0.05
HDL-C	−0.20	−0.18	−0.14
TG	0.03	−0.16	−0.12
Lymphocyte count	−0.01	−0.16	−0.12

Statistical analysis by Pearson's correlation analysis. Δ Functional age defined as "Functional age – Age". Aβ40/42, amyloid β40/42 ratio; BMI, body mass index; IGF-I, insulin-like growth factor-I; DHEA-s, dehydroepiandrosterone-sulfate; C/D, cortisol/DHEA-s ratio; SaMT, 6-sulfatoxymelatonin; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; SAF, skin AGE fluorescence measure by AGE Reader mu; AGE, advanced glycation endproduct; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; TG, triglyceride.

The results of the single-correlation analysis of measurement items with the Aβ40/42 ratio are as follows.

For FS, the Aβ40/42 ratio tended to be high in subjects with low IGF-I, DHEA-s and melatonin secretion and elevated C/D ratio. For the glycolipid metabolism marker, the Aβ40/42 ratio tended to be high in subjects with elevated HbA1c.

The highest correlation was observed for skin AF levels (r = 0.43) in YE, and the higher the glycative stress, the higher was the Aβ40/42 ratio.

In the analysis of all subjects, the Aβ40/42 ratio tended to be high in subjects with high skin AF levels (r = 0.33) and HbA1c (r = 0.24).

Stepwise Multiple Regression Analysis

The adjusted R^2 was 0.216 from the results of the multiple regression analysis for FS, and a regression equation that could explain 22% of the Aβ40/42 ratio was obtained.

The dependent variable Y (Aβ40/42 ratio) was expressed with the following equation.

Y = −0.131 X 1 (**lifestyle score**) + 19.7

Significance F was at a significance level of less than 5%. In the test showing the coefficient of lifestyle to be 0, p = 0.0194 was obtained. The t-value is also greater than the absolute value 2, indicating that lifestyle influences plasma Aβ. The standard partial regression coefficient was significant at X1, and the contribution rate (e) of the residual (unknown factor) was 0.744. The regression equation showed that the Aβ40/42 ratio decreased with a good lifestyle and high lifestyle score.

The adjusted R^2 was 0.354 from the results of the multiple regression analysis for YE, and a regression equation that could explain 35% of the Aβ40/42 ratio was obtained.

Y = 0.115 X1 (**lifestyle score**) + 0.0372 X2 (**physical and mental stress score**) − 0.0221 X3 (**bone age**) + 0.106 X4 (**neural age**) − 6.68

The significance level was less than 1% from significance F.

The p values for lifestyle, physical and mental stress, bone age and neural age were 0.0154, 0.0333, 0.0338 and 0.0216, respectively, and the coefficients were significantly different from 0. The t-value is also greater than the absolute value 2, showing that each factor influences plasma Aβ. The standard partial regression coefficient was significant at X1, X2, X3 and X4, and the contribution rate (e) of the residual (unknown factor) was 0.579. The regression equation showed that the Aβ40/42 ratio increased with advancing neural age. On the other hand, the Aβ40/42 ratio tended to be high in subjects with good lifestyle markers and low physical and mental stress. This was influenced by the fact that many elderly women with low bone density had a low Aβ40/42 ratio.

The adjusted R^2 was 0.270 from the results of the multiple regression analysis for all subjects, and a regression equation that could explain 27% of the Aβ40/42 ratio was obtained.

Y = −0.0218 X1 (**bone age**) + 0.0346 X2 (**blood vessel age**) + 0.0340 X3 (**oxidative stress score**) − 0.0246 X4 (**glycative stress score**) + 6.74

The significance level was less than 1% from significance F. The p values for bone age, blood vessel age, oxidative stress and glycative stress were 0.00498, 0.000980, 0.00882 and 0.0390, respectively, and significantly different from 0. The t-value is also greater than the absolute value 2, showing that each factor influences plasma Aβ. The standard partial regression coefficient was significant at X1, X2, X3 and X4, and the contribution rate (e) of the residual (unknown factor) was 0.675. The regression equation showed that the more advanced the blood vessel age, the lower the glycative stress score and the more severe the stress, the higher the Aβ40/42 ratio. This was influenced by the fact that many elderly women with low bone density had a low Aβ40/42 ratio.

Discussion

Measurement of the Aβ40/42 Ratio Using MALDI-TOF Mass Spectrometry

Aβ is formed by the cleavage of amyloid precursor protein (APP) and is characterized by deposition in the brain. The deposition level of Aβ can be examined with high reliability using Aβ-PET imaging and measurement of the Aβ level in the cerebrospinal fluid [28,29]. In the present study, the plasma Aβ40/42 ratio was measured using MALDI-TOF mass spectrometry [4]. The association of aging-related markers with the Aβ40/42 ratio was analyzed for FS and YE groups.

The characteristics of the aging markers for FS are shown (***Fig. 2***) [10]. Many FS are characterized by a low bone age and muscle age. The neural age is low for very few subjects at 5%. The reason is that the daily work of FS involves extensive intellectual work, and they have many opportunities to interact with young people such as students and graduate students. The results of the present study showed that physical and mental stress was the most significant risk factor for aging. The lifestyle score is high, with few problems related to smoking, alcohol consumption, exercise and sleep quality.

The characteristics of YE are described below. Our laboratory has been encouraging independent elderly to walk, and data from the accelerometers given to the individuals are checked every month. The average steps per day for men is 10,648, while it is 7,272 for women, which is higher than the average for the elderly in their seventies (men 4,800 steps and women 3,800) [17], and the average monthly activity is 59.1 ± 53.5 Ex [9]. Between 2012 and 2014, AF values indicated a downward trend [17]. The YE is a group who exercise more and have low glycative stress compared to men and women of the same age. In the same way as FS, the lifestyle score is high, with few problems related to smoking, alcohol consumption, exercise and sleep quality.

Glycative Stress

Glycative stress is a series of reactions causing carbonylation of proteins and formation of AGEs due to

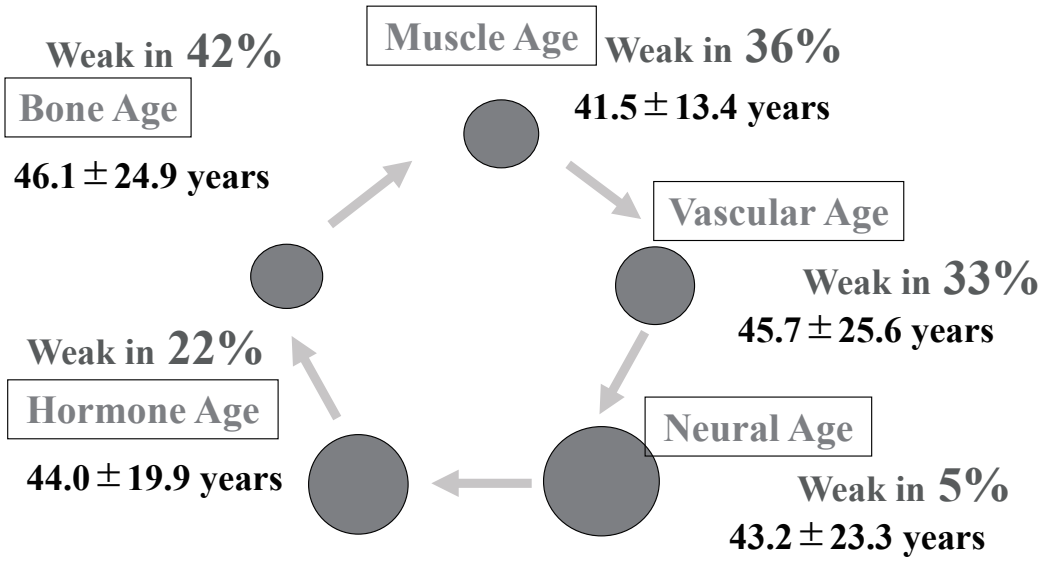

Fig. 2. **Anti-aging medical check-ups in enterprises: university staff and faculty (October, 2006).**

Regarding university staff and faculty (FS, n= 714; male, n = 445; female, n = 269; age, 44.4 ± 26.9 years), weakness due to aging is most seriously observed in bone age, followed by muscle function age. There were few who have weakness due to aging in neural age. The results were expressed as average value ± standard deviation. This figure was cited and modified from Reference 10).

excessive amounts of aldehydes derived from reducing sugars and lipids. Aldehydes act as intermediates in glycation reactions. Diabetic patients who are susceptible to glycative stress are at a greater risk of developing AD [30-33]. Glycation of Aβ and tau proteins may be involved in the onset and progression of AD, with acrolein [34, 35] and methylglyoxal (MGO) [36] as the intermediate aldehydes.

A negative correlation was observed for glycative stress in the analysis results of all the subjects. The reason is considered as follows. The YE belong to Kempo-Juku, a group that walks a higher number of steps per day than the average for their age. According to Kawamoto *et al.* [17], a decrease was observed in the glycative stress marker SAF during the 2-year observation period, leading to the inference that the glycative stress of this group is low. The FS, mainly middle-aged and elderly adults, were not guided on measures to prevent glycative stress, while YE were guided on the measures. This is considered to be the reason for the negative correlation of glycative stress marker with the multivariate regression analysis.

The results of the single-correlation analysis of items related to the glycative stress marker showed that HbA1C, insulin, pentosidine and SAF have a positive correlation with the Aβ40/42 ratio even in YE, which is an appropriate result.

Neural Age

Six indices CA, NUCA, PEN, PEM, TE and reaction time, were used for WCST. We examined the effect of these indices on the Aβ40/42 ratio and found that the higher indices other than CA were, the higher the Aβ40/42 ratio. This suggests that for subjects who answered the questions of many categories correctly and quickly with as few mistakes as possible, the smaller the Aβ40/42 ratio, less accumulation of Aβ in the brain, and has a better cognitive function. Also, an elevated Aβ40/42 ratio is expected to affect the executive functions of the frontal lobe, such as planning, devising and executing actions.

In a previous study that compared the neural age and Δ neural age (Δ neural age = neural age - actual age) of YE with persons requiring support and persons requiring nursing care, the neural age was found to be 71.1 ± 16.7 years for YE, 81.2 ± 9.1 years for persons requiring support and 88.5 ± 5.8 years for persons requiring nursing care, while the Δ neural age was found to be 0.3 ± 11.8 years for YE, 3.2 ± 13.4 years for persons requiring support and 4.8 ± 6.8 years for persons requiring nursing care. The YE exercise more than others in the same age group, which indicates that the neural age of YE is in a younger state than individuals who require support or nursing care. Since WCST has been performed repeatedly for about 10 years, there may be some effect due to familiarity and proficiency. A combination of these factors may have resulted in a lower Aβ40/42 ratio for YE than the other group.

Blood Vessel Age

The multivariate analysis showed that the blood vessel age was a risk factor for elevating the Aβ40/42 ratio. Arteries are a factor for cerebrovascular dementia since they play an important role in supplying oxygen and nutrients to the brain. Arteriosclerosis is often present in AD patients as well. When a single-correlation analysis is performed for risk factors of arteriosclerosis with the Aβ40/42 ratio, a

weak positive correlation with HbA1c and a weak negative correlation with HDL-C is observed for both FS and YE, which are appropriate results. Since the weightage of TG and LDL-C is high as markers for nutrients in the elderly, their negative correlation suggests that poor nutrition may be a risk factor for increasing the Aβ40/42 ratio.

Lifestyle

The lifestyle scores are at a high level for both groups (82～99, 88.5 ± 3.9). The lifestyle score is comprehensively evaluated from the results of the medical questionnaire for tobacco, alcohol, exercise, sleep duration and water intake. Although the Aβ40/42 ratio increased with a lower lifestyle score for FS, which was an appropriate result, an opposite result was obtained for YE, and the Aβ40/42 ratio increased with a higher lifestyle score. In general, as cognitive function declines in the elderly, there is a decrease in sociability, fewer opportunities to participate in social activities, fewer opportunities to attend night gatherings and fewer opportunities for alcohol consumption. As a result, the lifestyle score starts to decrease. From the perspective of maintaining and preventing a decline in the cognitive function of the elderly, the present study suggests that active participation in social activities may reduce the risk of elevating the Aβ40/42 ratio.

Literature Review for the Aβ42/40 Ratio

Although the Aβ40/42 notation is used in some papers, we have used Aβ40/42 uniformly in this paper.

Regarding the biochemical markers of dementia, it has been reported that plasma protein-coupled acrolein (PC-Acro) and Aβ40/42 ratio can be used to detect mild cognitive impairment (MCI) and AD [37]. Acrolein is one of the intermediates of glycative stress with an aldehyde group. Plasma PC-Acro and the Aβ40/42 ratio of MCI and AD patients were significantly higher in AD patients than healthy individuals [38].

Aβ-PET is a method to detect pathological accumulation of Aβ in the brain, but the procedure is complicated and expensive. The identification performance of the Aβ40/42 ratio for detecting Aβ-PET positive individuals according to the Youden cutoff is sensitivity and specificity of 77.8% and 87.5% respectively, and the Aβ40/42 ratio is useful as a screening method to identify Aβ positivity in the brain during preclinical and prodromal stages of AD [39]. A report that evaluated the cortical Aβ load with positron emission tomography and analyzed its relationship with the Aβ40/42 ratio indicated a positive correlation between the Aβ40/42 ratio and cortical Aβ load [37].

Plasma Aβ 40 and Aβ 42 levels, and the Aβ40/42 ratio increases with age. Age-dependent increase in Aβ42 levels is mitigated by adjustment with APOE-ε4 [40]. APOE-ε4, an allele of apolipo protein E (ApoE), is high in late-onset familial and sporadic AD and has a positive correlation with the deposition of Aβ in the brain.

Regarding association with frailty, a correlation between frailty phenotype (healthy, pre-frail and frail) and the Aβ40/42 ratio is not observed [41]. Subclass analysis after adjustment of APOE-ε4 genotype showed an increasing trend of the Aβ40/42 ratio in frail patients [41].

Like PC-Acro, Aβ40/42 is significantly higher in AD patients than in healthy persons [38], and increases with the decline in cognitive function [42]. The Aβ40/42 ratio also increases in individuals with amnestic mild cognitive impairment (a-MCI) [43].

Although the plasma Aβ40/42 ratio had a weak positive correlation ($r = 0.22$) with the Aβ40/42 ratio of cerebrospinal fluid, a report indicates that the plasma Aβ40/42 ratio alone cannot be used to distinguish AD patients from the control individuals statistically [44]. However, the decrease in Aβ42 level and the increase in Aβ40/42 ratio in the CSF of AD and MCI patients are generally considered changes specific to AD patients [45].

It is suggested that the Aβ40/42 ratio may be low in geriatric depression [46] patients and autistic children [47].

We measured the Aβ40/42 ratio in subjects with poor sleep quality due to bedding that was not suitable and observed no change after one month of improvement in sleep quality [48]. The Aβ40/42 ratio tends to be higher in individuals with poor sleep quality compared with the data of the present study. In an age-adjusted comparison ratio, the Aβ40/42 ratio was significantly higher in individuals with poor sleep quality. The finding suggests that poor "sleep quality" may be a risk factor for increased Aβ40/42 ratio.

Conclusion

The results suggest that neural age, blood vessel age and glycative stress may affect the increase in plasma Aβ40/42 ratio and be a risk factor for exacerbating dementia.

Conflict of Interest Statement

The authors claim no conflict of interest in this study.

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