Glycative Stress Research

Online edition: ISSN 2188-3610
Print edition: ISSN 2188-3602
Received: April 20, 2019
Accepted: June 4, 2019
Published online: June 30, 2019
doi:10.24659/gsr.6.2_092

Original article

Evaluation of the glycative stress by non-invansive skin AGEs measurement devices.

Yuji Morita [1,2], Masayuki Yagi [2], Kaori Ishizaki [2], Wakako Takabe [2], Takamaru Komatsu [3], Momoe Nakazawa [1], Minako Matsushima [1], Tetsuro Urata [1], Yoshikazu Yonei [2]

1) Urata Clinic/SQOL Kanazawa, Kanazawa, Ishikawa, Japan
2) Anti-Aging Medical Research Center and Glycative Stress Research Center, Faculty of Life and Medical Sciences, Doshisha University, Kyoto, Japan
3) AGE laboratories Co., Ltd, Kyoto, Japan

Abstract

***Purpose*:** The purpose of this experiment is to verify the adaptability of several devices available in Japan for measuring skin AGEs for glycative stress evaluation. Healthy males and females 20 years old or older, and younger than 100, were recruited for this experiment, and the subjects' skin AGEs values were measured using five models of non-invasive skin AGEs measuring devices. The measured values were then compared and examined. Furthermore, in addition to the values of their skin AGEs, the oxidative stress (OS) and antioxidant power (AP) of their blood were measured, and the effect of oxidative stress on the measurement value of skin AGEs was verified.

***Method*:** The subjects who participated in the study were 58 healthy males and females in their 20s to their 80s. The skin AGEs measuring devices used in this experiment were AGE Reader su, AGE Reader mu, TruAge scanner, TruAge scanner mini and AGEs sensor. The correlations among the values of skin AGEs measured by these five devices were compared and verified. Furthermore, in this experiment, blood samples were collected from the subjects, and the oxidative stress (OS) and antioxidant power (AP) were measured. A hematological test and general blood biochemical tests were conducted prior to undergoing the experiment. Ethical approval of the Ethical Review Board for "Research Targeting Humans" was obtained for this experiment.

***Results*:** The skin AGEs values of the right upper arm and right forearm of the same subject measured by AGE Reader su showed a positive correlation. All the results of the measurements of skin AGEs values of the subject measured using AGE reader su, AGE Reader mu, TruAge scanner and TruAge scanner mini showed positive correlation when AGE Reader mu was made a standard. The AGEs values of the left middle finger measured using AGEs sensor did not show positive correlation with that using AGE Reader mu. As the result of measurements of skin AGEs on the right forearm of the subjects using AGE Reader su, AGE Reader mu, TruAge scanner and TruAge scanner mini, a correlation between the measurement values and ages of the subjects was recognized. OS and AP of the blood of the subjects showed a weak negative correlation. No correlation was recognized between measured values of OS and AP of the subjects and their ages. As the results of the classifications of the subjects by the measured values of skin AGEs and OS of blood, they could be classified into the group with high values both in OS and skin AGEs, that with high value either in OS or skin AGEs and that with low values both in OS and skin AGEs.

***Conclusion*:** The values of skin AGEs measured using the five main skin AGEs measuring devices used in Japan were useful for the evaluation of glycative stress of healthy Japanese. Measuring OS of blood and skin AGEs at the same time made it possible to evaluate the effect of oxidative stress on glycative stress of each subject.

***KEY WORDS*:** skin AGEs measurement device, comparison of a measured value, glycative stress, oxidative stress

Contact Address: Professor Masayuki Yagi, PhD
Anti-Aging Medical Research Center / Glycative Stress Research Center,
Graduate School of Life and Medical Sciences, Doshisha University
1-3, Tatara Miyakodani, Kyotanabe, Kyoto, 610-0394 Japan
Phone/FAX: +81-774-65-6394 E-mail: myagi@mail.doshisha.ac.jp
Co-authors: Morita Y, ymorita707@yahoo.co.jp ; Ishizaki K, ko-sei12@mail.doshisha.ac.jp ;
Takabe W, wtakabe@mail.doshisha.ac.jp ; Komatsu T, dezkyoto@gmail.com ;
Nakazawa M, lac@hospy.jp ; Matsushima M, kensa2-k@hospy.jp ;
Urata T, tetsuro.u@hospy.jp ; Yonei Y, yyonei@mail.doshisha.ac.jp

Glycative Stress Research 2019; 6 (2): 092-102

Introduction

Glycative stress is a concept comprehensively capturing the biological stress caused by the loads of reducing sugars such as glucose and aldehyde and the subsequent effect of the formation and accumulation of advanced glycation products (AGEs) [1]. AGEs include various substances such as pentosidine, N^{ε}-(carboxymethyl) lysine (CML) and crossline. There are various formation mechanisms for AGEs, and there are various glycation reaction intermediates such as ketone and aldehyde [2]. If the proteins in the body are glycated due to glycative stress, changes such as browning, acquisitions of fluorescence and the formation of protein-crosslinking occur. Furthermore, if AGEs combine with RAGE (receptor for AGEs), a cell surface receptor, it causes cell inflammation by signaling [3]. The formation and accumulation of AGEs by glycative stress cause physiological and physical damage in the proteins of various systems and organs. Therefore, glycative stress becomes a cause of aging and development of various diseases.

The measurement of AGEs concentration in blood is an evaluation method of the effect of glycative stress [4]. However, at present, there is no versatile method for measuring AGEs in blood at clinical sites and there is little information concerning the significance of the measurements of various AGEs. Recently, several devices have been developed that make it possible to non-invasively measure the fluorescence strength derived from skin AGEs, and their use became possible at clinical sites [5-10]. These devices have the measurement principle that when skin is irradiated with ultraviolet rays, the AGEs accumulated in skin generate their unique fluorescence. In this measurement, the total values of substances having fluorescence out of various AGEs are measured. However, it has not been possible to identify the fluorescent AGEs. Therefore, the values from this measurement principle lack substance specificity. There is no reference material for skin AGEs and it is difficult to calibrate the measured values of several measuring devices. There are several measuring sites for skin AGEs such as forearm, upper arm and fingertips, and the significance in the difference between measured sites is unknown. However, there are advantages in the measurement of skin AGEs using these devices such as that it is non-invasive, that measurement can be conducted within minutes and that the devices are small-sized.

For the purpose of verifying the adaptability of the these devices, which are available in Japan, to the measurement of skin AGEs for the evaluation of glycative stress, healthy males and females 20 years old or older and younger than 100 years were recruited, and the values of fluorescence strength in skin AGEs of the subjects were measured using the five skin AGEs measuring device models, and compared. Furthermore, in this experiment, in order to evaluate the effect of oxidative stress (OS), one of the factors accelerating glycative stress, oxidative stress (OS) and antioxidant power (AP) of blood were measured at the same time when skin AGEs were measured.

Method

Subjects

Targeting healthy males and females 20 years old or older and younger than 100 years, people with a connection to the Urata clinic/Sqol Kanazawa were recruited as subjects. The ethical approval of the Ethical Review Board for "Research Targeting Humans" of the Society for Glycation Stress Research was obtained for this experiment. The subjects of this experiment were those who had participated in the explanatory meeting of this experiment, agreed to participate in the experiment in writing beforehand, and did not violate the exclusion criteria as follows:

1). Those who suffer some disease and will receive drug therapy at the time of the experiment
2). Those who had a medication-taking habit for the purpose of drug therapy until one month before the practice of experiment (excluding over-the-counter medicine for headache, menstrual pain and cold)
3). Those who have a past or current medical history of a serious disorder in the liver, kidney, heart and blood
4). Those whom the medical doctor in charge of this experiment judged as inappropriate as a subject of this experiment

Fifty-eight subjects who understood the contents of this experiment, and agreed to participate, in writing, and do not violate the exclusion criteria

Experiment Design

This experiment was an in-group observation experiment. The subjects continued living in a normal way until the day of the test. They came to the testing place by themselves and underwent all tests. The tests were conducted at the Urata clinic/Sqol Kanazawa (Kanazawa, Ishikawa, Japan), March 2018.

Physical Measurements

Body height, body weight, body-fat percentage, and body mass index (BMI) were measured.

Blood tests

In this test, a biochemical examination of the blood was conducted from the blood samples collected by venous blood sampling from the subjects. Blood test items were white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet-count (PLT), blood picture (neutrophil (Neutro), lymphochte (Lympho), monocyte (Mono), eosinophil (Eosino) and basophil (Baso), total protein (TP), total albumin (ALB), total bilirubin (TB), indirect bilirubin (IB), aspartate transaminase (AST), alanine amiotranspeptidase (ALT), alkaline phoshotase (ALP), lactate dehydrating enzyme (LDH), γ-glutamyltransferase (γ-GTP), amylase (AMY), total cholesterol (TC), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), triglyceride (TG), blood urea nitrogen (BUN), creatinine (CRE), glomerular filtration rate (eGFR), uric acid (UA), sodium (Na), potassium (K), chloride (Cl),

calcium (Ca), serum iron (Fe), hemoglobin A1c (HbAlc [NGSP]) and insulin (IRI). Blood biochemical tests were assigned to LSI Medience Corporation (Chiyoda-ku, Tokyo, Japan).

Oxidative stress (OS) and antioxidant power (AP) were measured for blood special examination. Spotchem i-Pack Oxystress test (Arkray Inc., Kyoto, Japan) was used for the blood special examination and they were measured immediately after the blood was collected at the testing location[11].

Measurement of Fluorescence of skin AGEs

The strength of fluorescence derived from skin AGEs was non-invasively measured. Five measuring devices of AGE Reader su, AGE Reader mu, (DiagnOptics, Groningen, Netherlands), TruAge scanner, TruAge scanner mini (Morinda, UT, USA) and AGEs sensor (Sharp, Osaka, Japan) were used (***Fig. 1***). The measuring sites tested by the AGE Reader su were the right upper arm and forearm, and that by the AGE reader mu, TruAge scanner and TruAge scanner mini was the right forearm and that by the AGEs sensor was the right middle finger.

Statistical Analysis

The fundamental statistics (average values and standard deviation) were calculated for each data. The correlation analysis was conducted among the skin AGEs values measured by each apparatus and between the skin AGEs values and oxidative stress markers (OS, AP). A statistic soft, Bell Curve for Excel (Companies' Information Service, Shinjuku-ku, Tokyo, Japan), was used for the verification of the statistic results and the correlation of the data was evaluated using Pearson's product-moment correlation coefficient. In the case of $0.4 < |r| \leqq 1.0$, it was determined that there was a correlation, and in the case of $0.2 < |r| \leqq 0.4$, it was determined that there was a weak correlation. As the result of statistical analysis, the risk rate less than 5% was regarded as significant and that less than 10% was regarded as a tendency of risk.

Ethical Review

This experiment was conducted in compliance with the Declaration of Helsinki (revised at the 2013 WMA General Assembly, Fortaleza) and the ethical guidance for medical and health experiments involving humans (notified by the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labor and Welfare). For this purpose, an ethic committee concerning the "research involving humans" was held in the Society for Glycation Stress Research (Shinjuku-ku, Tokyo, Japan) and the validity and ethics of this experiment were deliberated and approved (GSE#2018-003). This experiment was pre-registered as a clinical trial (UMIN#00003133).

Fig. 1. **Skin AGEs measuring device.**

AGEs, advanced glycation end products.

Results

Backgrounds of Subjects

All physical checkup items of the 58 subjects of this experiment were out of the reference interval of Japan Clinical Laboratory Standard Committee [12] and out of the criteria category D (requiring medical treatment) [13] of complete physical examination, and it was proven that all of them were healthy adults. (***Table 1, 2***).The breakdown of the 58 subjects were 5 males and 53 females, and they consisted of four subjects in their 20s. Eleven subjects in their 30s, 23 subjects at their 40s, eight subjects at their 60s, five subjects in their 70s and one subject in the 80s, and the average age was 40.7 ± 11.0 (average value ± standard deviation).

Comparison among Measuring Sites for Measuring Skin AGEs

In this measurement, the values of the skin AGEs at the measuring sites of the right upper arm and the right forearm of the same subject were measured using the AGE Reader su and compared (***Fig. 2***). During measurement, because a problem occurred in the device, the number of the subjects who were measured was 40. The measured values of the upper arm and forearm of the same subjects showed a positive correlation ($y = 0.5876x + 0.7457$, $r = 0.744$. $n = 40$, $p < 0.01$). The slope of primary regression equation was 0.5876 and the forearm was measured higher than the upper arm.

Correlation among the Values of Skin AGEs Measured using Various Devices

In this measurement, the values of the skin AGEs of the same subject were measured using the AGE Reader su, AGE Reader mu, TruAge scanner, TruAge scanner mini and AGEs sensor (***Fig. 3***). The right forearm site was measured using the AGE Reader su, AGE Reader mu, TruAge scanner and TruAge scanner mini and the left middle finger was measured using the AGEs sensor. During measurement, since a problem occurred in the device of TruAge scanner mini, the number of the subjects who were measured using this device was 53. The measured values using AGE Reader mu and AGE Reader su ($y = 0.8547x + 0.2546$, $r = 0.832$, $n = 40$, $p < 0.01$), TruAge scanner ($y = 77.106x + 28.148$, $r = 0.959$, $n = 58$, $p < 0.01$) and TruAge scanner mini ($y = 66.782x + 49.118$, $r = 0.813$, $n = 53$, $p < 0.01$) showed positive correlation. On the other hand, no correlation was observed in the value measured using AGE Reader mu and that measured using AGEs senor.

Relationship between Measured value of Skin AGEs and Age of Subjects

In this measurement, the correlations between the values of skin AGEs measured using each device and the ages of subjects were evaluated (***Fig. 4***). The measured values of skin AGEs and the ages of subjects showed a positive relationship using AGE Reader su ($y = 0.0255x + 1.0523$, $r = 0.702$, $n = 40$, $p < 0.01$), AGE Reader mu ($y = 0.0219x + 1.3238$, $r = 0.567$, $n = 58$, $p < 0.01$), TruAge scanner ($y = 1,6773x + 130.6$, $r = 0.541$, $n = 58$, $p < 0.01$). The measured values using TruAge scanner mini showed a weak correlation with the ages of the subjects ($y = 1.0244x + 151.34$, $r = 0.370$, $n = 53$, $p < 0.01$). On the other hand, no correlation was observed between the measured values using AGEs sensor and the ages of subjects.

Relationship among Measured Value of Skin AGEs, Degree of Blood Oxidization and Antioxidative Activity

In this experiment, in order to evaluate the effect of oxidative stress, one of the factors accelerating glycative stress, OS (oxidative stress) and AP (antioxidant power) of blood were measured. The OS and AP of subjects showed a weak correlation ($y = -10,607x + 2741.3$, $r = 0.304$, $n = 58$, $p < 0.05$, ***Fig. 5***). No correlation between the measured values of the OS and AP of the subjects and their ages was observed (data are not described). Furthermore, the relationships between the measured values of the OS of blood of the subjects and their values of skin AGEs measured by AGE reader mu and AGEs sensor were evaluated (***Fig. 6***). When the correlation diagram plot of the measured values was classified with the measured average value of OS (28.3 ± 6.8 mg/dL, $n = 58$), average value measured by AGE Reader mu (2.2 ± 0.4, $n = 58$) and that by AGEs sensor (0.47 ± 0.09. $n = 58$), it could be classified into a group of subjects with high values both in the OS and skin AGEs, that of those with high value either of OS or skin AGEs and that of those with low values both in OS and skin AGEs. The subjects of each group did not always match in AGE Reader mu and AGEs sensor.

Table 1. Subject background profile.

Inspection item	Unit	Mean	±	SD
Age	years	40.7	±	11.0
Body height	cm	159.4	±	5.7
Body weight	kg	51.4	±	6.3
Body fat	%	40.7	±	11.0
BMI	kg/m^2	20.2	±	1.9

Total subjects, n = 58 (male: 5, female: 53); BMI, body mass index; SD, standard deviation.

Table 2. Results of blood biochemistry and hemogram.

Inspection item	Unit	Reference Interval [1)]	Mean	±	SD
TP (total protein)	g/dL	6.6 - 8.1	7.3	±	0.4
TB (total bilirubin)	mg/dL	0.4 - 1.5	0.6	±	0.3
IB (indirect bilirubin)	mg/dL	–	0.4	±	0.2
AST (aspartate transaminase)	U/L	13 - 30	20.5	±	7.1
ALT (alanine transaminase)	U/L	male: 10 - 42 female: 7 - 23	18.0	±	14.7
ALP (alkaline phosphatase)	U/L	106 - 322	179.6	±	56.4
LDH (lactate dehydrogenase)	U/L	124 - 222	153.9	±	22.7
γ-GTP (γ-glutamyltransferase)	U/L	male: 13 - 64 female: 9 - 32	18.7	±	11.5
AMY (amylase)	U/L	44 - 132	88.3	±	23.9
CK (creatine kinase)	U/L	male: 59 - 248 female: 41 - 153	94.6	±	55.9
BUN (blood urea nitrogen)	mg/dL	8 - 20	13.2	±	3.2
CRE (creatinine)	mg/dL	male: 0.65 - 1.07 female: 0.46 - 0.79	0.6	±	0.1
eGFR (estimated glomerular filtration rate)	mL/min/1.73m^2	≥ 60.0 [2)]	86.5	±	15.4
UA (uric acid)	mg/dL	male: 3.7 - 7.8 female: 2.6 - 5.5	4.3	±	0.9
HbA1c [NGSP]	%	4.9 - 6.0	5.5	±	0.2
IRI (immune reactive insulin)	μU/mL	—	10.7	±	11.6
TC (total cholesterol)	mg/dL	142 - 248	197.7	±	37.6
TG (triglyceride)	mg/dL	male: 40 - 234 female: 30 - 117	75.6	±	44.1
HDL-C (high-density lipoprotein cholesterol)	mg/dL	male: 38 - 90 female: 48 - 103	80.7	±	15.6
LDL-C (low-density lipoprotein cholesterol)	mg/dL	65 - 143	99.0	±	30.8
LDL-C/HDL-C ratio		—	1.3	±	0.5
Na (sodium)	mEq/L	138 - 145	140.3	±	1.4
K (potassium)	mEq/L	3.6 - 4.8	4.0	±	0.3
Cl (chloride)	mEq/L	101 - 108	102.9	±	1.7
Ca (calcium)	mg/dL	8.8 - 10.1	8.8	±	0.3
IP (inorganic phosphorus)	mg/dL	2.7 - 4.6	3.5	±	0.4
Fe (serum iron)	μg/dL	40 - 188	82.7	±	34.8
WBC (white blood cell count)	10^3/μL	3.3 - 8.6	6.1	±	1.5

RBC (red blood cell count)	10^4/μL	male: 4.35 - 5.55 female: 3.86 - 4.92	447.5	±	38.4
Hb (hemoglobin)	g/dL	male: 13.7 - 16.8 female: 11.6 - 14.8	13.3	±	1.5
Ht (hematocrit)	%	male: 40.7 - 50.1 female: 35.1 - 44.4	39.9	±	4.0
MCV (mean corpuscular volume)	fL	83.6 - 98.2	89.3	±	5.5
MCH (mean corpuscular hemoglobin)	pg	27.5 - 33.2	29.8	±	2.3
MCHC (mean corpuscular hemoglobin concentration)	%	31.7 - 35.3	33.3	±	1.0
PLT (platelet count)	10^4/μL	15.8 - 34.8	26.5	±	5.4
Neutro (neutrophil)	%	—	60.8	±	7.6
Lympho (lymphocyte)	%	—	31.1	±	7.2
Mono (monocyte)	%	—	4.9	±	1.5
Eosino (eosinophil)	%	—	2.5	±	1.7
Baso (basophil)	%	—	0.7	±	0.4
OS (oxidative stress)	mg/dL	—	28.3	±	6.8
AP (antioxidant power)	mg/dL	—	2441.3	±	237.6

1) Guideline JSLM2018 of a clinical laboratory test. 2) Criteria category (Revised the Japan Society of Ningen Dock on December 14, 2018). SD, standard deviation

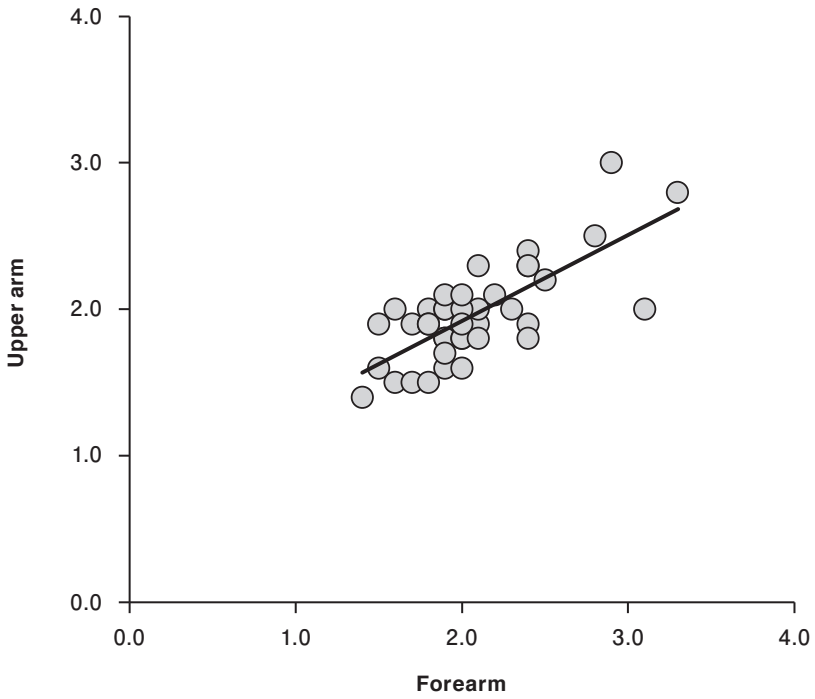

Fig. 2. **Comparison of a measured region.**

Device, AGE Reader su; Region, Right arm; correlation, y = 0.5876x + 0.7457, n = 40, r = 0.744, p < 0.01; Statistical analysis, Pearson product-moment correlation coefficient; AGE, advanced glycation end product.

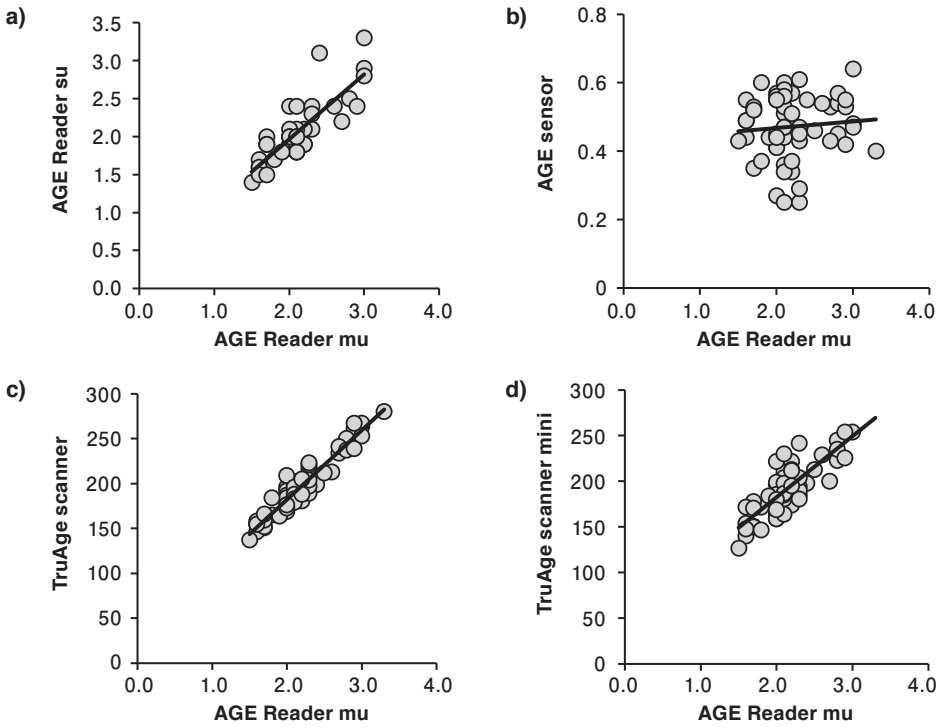

Fig. 3. **Correlation of AGE Reader mu value and other device value.**

Measured region, right forearm; Correlation of AGE Reader mu value, **a)** vs AGE Reader su: y = 0.8547x + 0.2546, n = 40, r = 0.832, p < 0.01, **b)** vs AGEs sensor: y = 0.0192x + 0.4294, r = 0.089, n = 58, **c)** vs TruAge scanner: y = 77.106x + 28.148, r = 0.959, n = 58, p < 0.01, **d)** vs TruAge scanner mini: y = 66.782x + 49.118, r = 0.813, n = 53, p < 0.01; Statistical analysis, Pearson product-moment correlation coefficient; AGEs, advanced glycation end products.

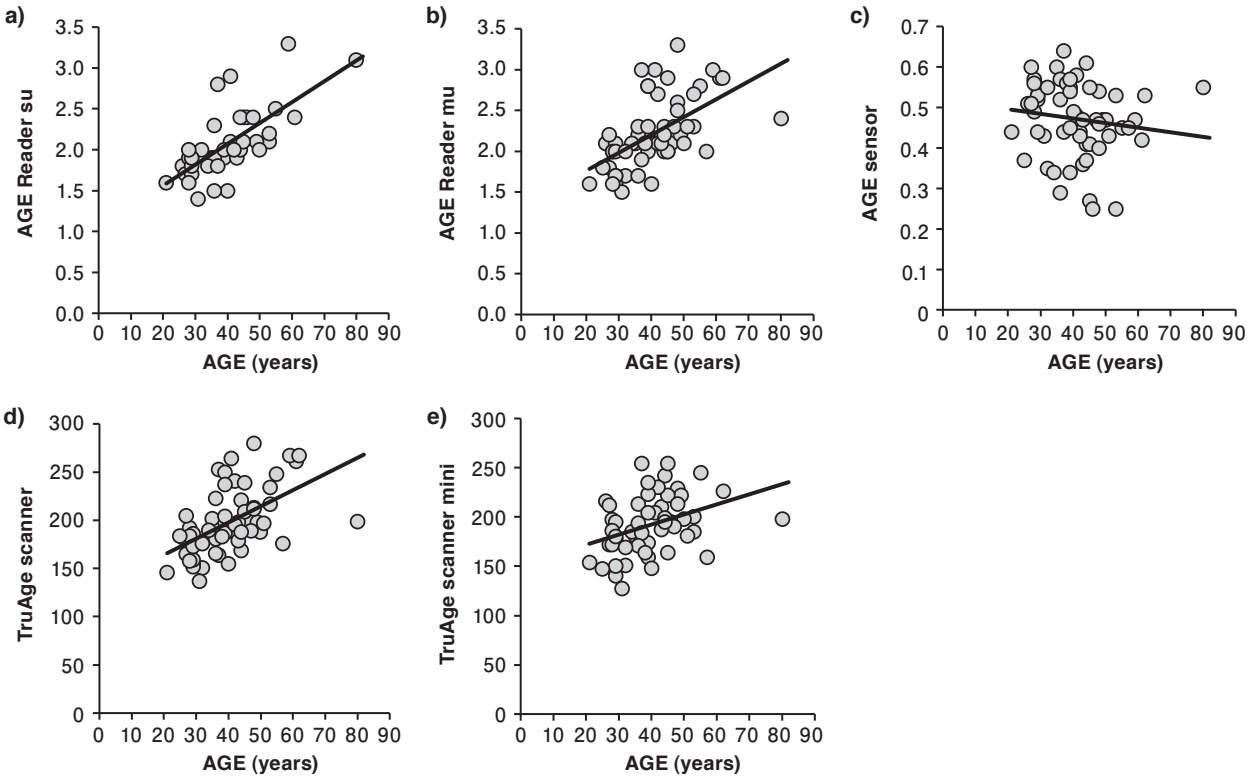

Fig. 4. **Correlation of skin AGEs value and chronological age.**

Measured region, right forearm; Correlation of chronological age, **a)** AGE Reader su: y = 0.0255x + 1.0523, r = 0.702, n = 40, p < 0.01, **b)** AGE reader mu: y = 0.0219x + 1.3238, r = 0.567, n = 58, p < 0.01, **c)** AGEs sensor: y = −0.0011x + 0.5182, r = 0.137, n = 58, **d)** TruAge scanner: y = 1.6773x + 130.6, r = 0.541, n = 58, p < 0.01, **e)** TruAge scanner mini: y = 1.0244x + 151.37, r = 0.370, n = 53; Statistical analysis, Pearson product-moment correlation coefficient; AGEs, advanced glycation end products.

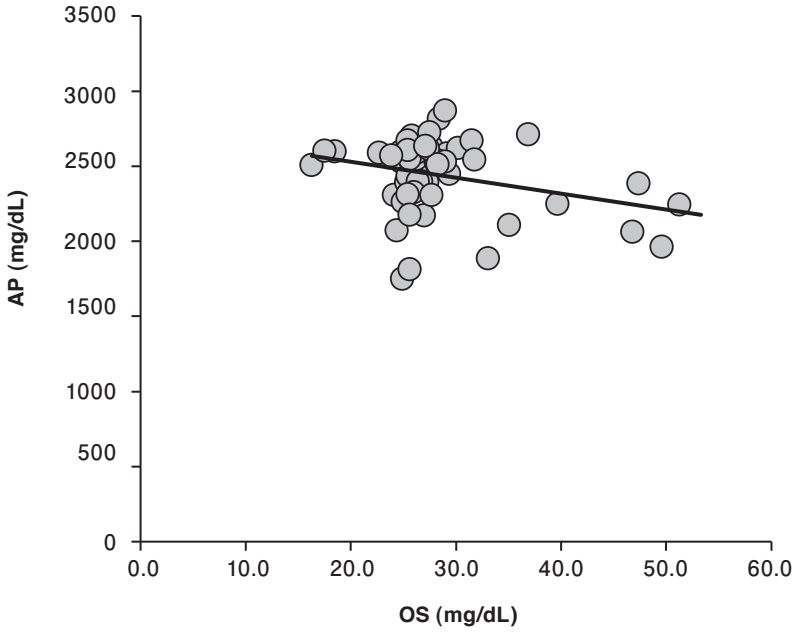

Fig. 5. **Correlation of OS and AP.**

Reagent, Spotchem i-Pack Oxystress test; Correlation, y = –10.607x + 2741.3, r = 0.304, n = 58, $p < 0.05$; Statistical analysis, Pearson product-moment correlation coefficient. OS, blood oxidative stress; AP, antioxidant power.

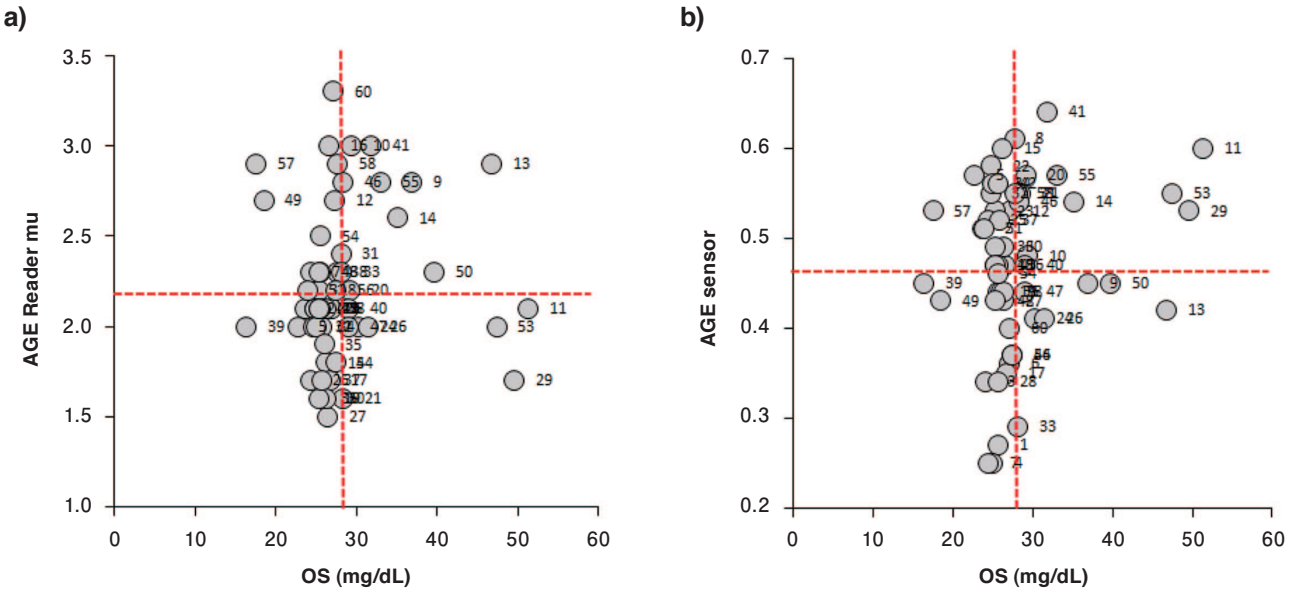

Fig. 6. **Correlation of skin AGEs value and OS.**

Red dotted line, Mean value (mean ± SD), **a)** AGE Reader mu: 2.2 ± 0.4, **b)** AGEs sensor: 0.47 ± 0.09, OS: 28.3 ± 6.8 mg/dL; Number, Subject's ID; AGEs, advanced glycation end products; OS, blood oxidative stress; SD, standard deviation.

Discussion

Difference in Value Measured by Skin AGEs Device

The main skin AGEs devices used in Japan at present are five models of AGE Reader su, AGE Reader mu, TruAge scanner, TruAge scanner mini and AGEs sensor. Each model has the principle that it can measure the accumulation of AGEs in skin by irradiating ultraviolet rays to the skin and measuring the fluorescence unique to AGEs (excitation wavelength: 370 nm and fluorescence wavelength: 440 nm). However, the indicated value is unique with each device and results differ. Furthermore, the sites used for measuring skin AGEs that have been reported are the forearm [5, 8, 10], upper arm [6, 7] and fingertip [9]. In addition, there are no standard substances useful for the adjustment of skin AGEs devices.

In this research, targeting 58 healthy males and females aged from 20 to 80 years old, the skin AGEs values of the subjects were measured at the same time using the five models of skin AGEs measuring devices which are available in Japan, and their adaptabilities to the evaluation of glycative stress were verified. As a result, a positive correlation was recognized in all values measured at the forearm using the four models of AGE Reader mu, AGE Reader su, TruAge scanner and TruAge scanner mini. However, because the indicated values were different via each device, the slope and intercept of regression became different with each combination of the devices. On the other hand, no correlation was recognized between the values measured by AGE Reader mu and AGEs sensor. Even though the measurement principle of these devices for measuring skin AGEs are the same, there is a difference in the measuring sites of right forearm (AGE Reader mu) and left middle finger (AGEs sensor). It was possible that the difference of the measured value was caused by the structural difference of skin tissue such as thickness of blood capillaries, thinness of melanin pigments and epidermal thicknesses such as stratum comeum and stratum lucidum [14].

Measuring Sites for Skin AGEs and their Significances

The AGE Reader su is the device that made it possible to non-invasively measure the skin AGEs for the first time in the world [5]. The measurement principle of skin AGEs is to measure the fluorescence unique to AGEs (excitation wavelength: 370 nm and fluorescence wavelength: 440 nm) generated when skin is irradiated with ultraviolet rays. It is reported that the AGEs having unique fluorescence include crossline [15], pyrropyridine [16, 17] and vesperlisine [18, 19]. It has been verified by skin biopsy that the measured value of skin AGEs of the forearm using AGE Reader su correlates with the volumes of fluorescence and pentosidine derived from collagen-linked skin AGEs [5]. Furthermore, it has been verified that the measured value of skin AGEs of the forearm using AGE Reader su is useful for the risk assessments of diabetes complications [20, 21], arterial sclerosis [22], cardiovascular disturbance [23], fracture [24], dementia [25, 26] and skin aging [27]. The measurement of skin fluorescence is affected by the skin color of the subject [28]. Therefore, the measurement of the forearm is easily influenced by exposure to the sun's ultraviolet rays, so a subjects' outdoor living habits often cause difficulties in measurements [8]. In order to alleviate this effect, the measurement of the upper arm site is useful [6]. As the result of the measurement of the values of skin AGEs of the upper arm of 780 healthy Japanese subjects, using AGE Reader su, the value of skin AGEs increased along with aging, and also increased by the lifestyle habits such as drinking, smoking and short sleep [7]. Similar results were reported when the values of skin AGEs of 10,946 healthy Japanese subjects were measured using a TruAge scanner [10]. From these facts, it is possible that the measured values of the skin AGEs of the forearm and upper arm have similar significance. Meanwhile, the site to be measured using AGEs sensor is the right middle finger. The measurement of skin AGEs of the fingertip is less affected by melanin than the forearm or upper arm, and it is said that the right middle finger can be most accurately measured [9]. Furthermore, it has been shown that the values measured using the AGEs sensor correlate with the number of complications that a diabetic patient experiences. Furthermore, the value of skin AGEs of the fingertip using AGEs sensor correlates with the concentration of MG-H1 (methylglyoxal 5-hydro-5-methylimidazolones) in blood.

From these facts, the measurements of skin AGEs using the five models of AGE Reader su, AGE Reader mu, TruAge scanner, TruAge scanner mini and AGEs sensor were considered to be significant for the evaluation of glycative stress of subjects, even if the measured sites were different. However, there are possibly differences among the significances of the measured values of skin AGEs of the forearm, upper arm and fingertip. In particular, the accumulation of data is necessary for the verification of the significance of measurement of fingertip using the AGEs sensor.

Skin AGEs and Oxidative Stress

In this experiment, oxidative stress, one of the causes accelerating glycative stress was measured and its relationship with the measured value of skin AGEs was verified. For verification, the OS and AP of the blood of the subjects were measured [11], and the values measured using an AGE Reader mu and AGEs sensor were compared. Since a weak negative correlation between the OS and AP of the blood of the subjects was observed, there was a possibility that the increase of OS led to the decrease of AP. Meanwhile, both OS and AP of the blood of the subjects have no correlation with ages (data are not described). Therefore, the possibility was considered that oxidative stress is apt to be easily influenced by short term lifestyle habits. These results suggested that the degree of blood oxidation and antioxidant power of blood are the main factors influencing the measured value of glycative stress from the viewpoint of the value of skin AGEs.

Measurement of Skin AGEs at Clinical Site

The measured average value of the OS of all subjects (n = 58) was 28.3 ± 6.8 mg/dL and 95% confidence interval (CI) was 1.8. Similarly, the measured average value of AGE Reader mu was 2.2 ± 0.4 mg/dL and 95% CI was 0.1. Furthermore, the measured average value of AGEs sensor was 0.47 ± 0.09 and 95% CI was 0.02. With these measured values as standards, a measured value correlation diagram

plot of the OS and AGE Reader mu or AGEs sensor was classified into four areas, and as a result, the subjects could be classified into the four groups of that with high value both in oxidative stress (OS) and glycative stress (AGE Reader mu or AGEs sensor), and that with a high value in either of them and that of a lower value in both of them. Both glycative stress and oxidative stress are risk factors of aging and diseases. Since the subjects with either high glycative stress or high oxidative stress could be selected, it made it possible to give guidance on the important task for the prevention of aging and diseases [29].

Research Limitation

The purpose of this experiment was to compare the values of skin AGEs measured using the main five models of skin AGEs measuring devices available in Japan, targeting 58 healthy Japanese males and females, and to verify the adaptability of these devices to the evaluation of glycative stress. There have been many reports concerning the use of devices for the measurement of skin AGEs at the forearm and the upper arm. The values measured using these four models of the devices measuring at the forearm and the upper arm in this experiment have correlation among the devices, thus, it was considered that this experiment had similar significance as the skin AGEs measurements that had been reported. Meanwhile, two years have passed since the start of the sale of the device measuring skin AGEs at the fingertip, there have been few reports on said device. In this experiment, it was found that the values of skin AGEs measured at the upper arm and those at the fingertip did not correlate, but the difference in the significance of measurement could not be clarified.

Conclusion

The skin AGEs measurements using the five main models of skin AGEs measuring devices available in Japan at present (AGE Reader su, AGE Reader mu, Tru Age scanner, TruAge scanner mini and AGEs sensor) were useful for the evaluation of glycative stress of healthy Japanese. Measuring oxidative stress (OS) of blood along with skin AGEs makes it possible to evaluate the effects of glycative stress and oxidative stress of each subject at a clinical site and provides appropriate instruction.

Acknowledgement

This study was presented at the 18th Meeting of Japanese Society of Anti-Aging Medicine on May 26th, 2018, Osaka, Japan.

Conflict of Interest Statement

The skin AGEs measurement devices used in this study are provided by the courtesy of Selista Inc. (Chiyoda-ku, Tokyo, Japan) for AGE Reader mu and Morinda Japan Inc. (Shinjuku-ku, Tokyo, Japan) for TruAge scanner mini. The agents for Spotchem i-Pack Oxystress test are provided by the courtesy of Arkray Marketing Inc. (Shinjuku-ku, Tokyo, Japan).

Reference

1) Ichihashi M, Yagi M, Nomoto K, et al. Glycation stress and photo-aging in skin. Anti-Aging Med. 2011; 8: 23-29.
2) Nagai R, Mori T, Yamamoto Y, et al. Significance of advanced glycation end products in aging-related disease. Anti-Aging Med. 2010; 7: 112-119.
3) Fukami K, Yamagishi S, Okuda S. Role of AGEs-RAGE system in cardiovascular disease. Curr Pharm Des. 2014; 20: 2395-2402.
4) Yagi M, Yonei Y. Glycative stress and anti-aging: 3. The evaluation of glycative stress: Measurement of advanced glycation end products (AGEs). Glycative Stress Res. 2017; 4: 53-57.
5) Meerwaldt R, Graaff R, Oomen PH, et al. Simple non-invasive assessment of advanced glycation endproduct accumulation. Diabetologia. 2004; 47: 1324-1330.
6) Nomoto K, Yagi M, Arita S, et al. A survey of fluorescence derived from advanced glycation end products in the skin of Japanese: Differences with age and measurement location. Anti-Aging Med. 2012; 9: 119-124.
7) Nomoto K, Yagi M, Arita S, et al. Skin accumulation of advanced glycation end products and lifestyle behaviors in Japanese. Anti-Aging Med. 2012; 9: 165-173.
8) Yamagishi S, Fukami K, Matsui T. Evaluation of tissue accumulation levels of advanced glycation end products by skin autofluorescence: A novel marker of vascular complications in high-risk patients for cardiovascular disease. Int J Cardiol. 2015; 185: 263-268.
9) Yamanaka M, Matsumura T, Ohno R, et al. Non-invasive measurement of skin autofluorescence to evaluate diabetic complications. J Clin Biochem Nutr. 2016; 58: 135-140.
10) Isami F, West BJ, Nakajima S, et al. Association of advanced glycation end products, evaluated by skin autofluorescence, with lifestyle habits in a general Japanese population. J Int Med Res. 2018; 46: 1043-1051.
11) Sato K, Yagi M, Yonei Y. A new method for measuring oxidative stress using blood samples. Glycative Stress Res. 2015; 2: 15-21.
12) Ichihara K, Yomamoto Y, Hotta T, et al. Collaborative derivation of reference intervals for major clinical laboratory tests in Japan. Ann Clin Biochem. 2016; 53(Pt 3): 347-356.
13) Japan Society of Ningen Dock, Criteria category (Revised on April 1, 2018). https://www.ningen-dock.jp/en/other/inspection, 2019/5/1.

14) Alper M, Kavak A, Parlak AH, et al. Measurement of epidermal thickness in a patient with psoriasis by computer-supported image analysis. Braz J Med Biol Res. 2004; 37: 111-117.
15) Obayashi H, Nakano K, Shigeta H, et al. Formation of crossline as a fluorescent advanced glycation end product *in vitro* and *in vivo*. Biochem Biophys Res Commun. 1996; 226: 37-41.
16) Hayase F, Himura H, Asano M, et al. Identification of novel fluorescent pyrrolopyridinium compound formed from Maillard reaction of 3-deoxyglucosone and butylamine. Biosci Biotech Biochem. 1994; 58: 1936-1937.
17) Hayase F. Recent development of 3-deoxyosone related Maillard reaction products. Food Sci Technol Res. 2000; 6: 79-86.
18) Tessier F, Obrenovich M, Monnier VM. Structure and mechanism of formation of human lens fluorophore LM-1. J Biol Chem. 1999; 274: 20796-20804.
19) Nakamura N, Nakazawa Y, Ienaga K. Acid-stable fluorescent advanced glycation end products: Vesperlysines A, B, and C are formed as crosslinked products in the Maillard reaction between lysine or proteins with glucose. Biochem Biophys Res Commun. 1997; 232: 227-230.
20) Hirano T, Iesato Y, Toriyama Y, et al. Correlation between diabetic retinopathy severity and elevated skin autofluorescence as a marker of advanced glycation end-product accumulation in type 2 diabetic patients. J Diabetes Complications. 2014; 28: 729-734.
21) Smita AJ, Gerrits EG. Skin autofluorescence as a measure of advanced glycation endproduct deposition: A novel risk marker in chronic kidney disease. Curr Opin Nephrol Hypertens. 2010; 19: 527-533.
22) Temma J, Matsuhisa M, Horie T, et al. Non-invasive measurement of skin autofluorescence as a beneficial surrogate marker for atherosclerosis in patients with type 2 diabetes. J Med Invest. 2015; 62: 126-129.
23) de Vos LC, Mulder DJ, Smit AJ, Dullaart RP, et al. Skin autofluorescence is associated with 5-year mortality and cardiovascular events in patients with peripheral artery disease. Arterioscler Thromb Vasc Biol. 2014; 34: 933-938.
24) Momma H, Niu K, Kobayashi Y, et al. Skin advanced glycation end-product accumulation is negatively associated with calcaneal osteo-sono assessment index among non-diabetic adult Japanese men. Osteoporos Int. 2012; 23: 1673-1681.
25) Spauwen PJ, van Eupen MG, Köhler S, et al. Associations of advanced glycation end-products with cognitive functions in individuals with and without type 2 diabetes: The Maastricht study. J Clin Endocrinol Metab. 2015; 100: 951-960.
26) Igase M, Igase K. Cognitive impairment and glycative stress. Glycative Stress Res. 2018; 5: 45-49.
27) Corstjens H, Dicanio D, Muizzuddin N, et al. Glycation associated skin autofluorescence and skin elasticity are related to chronological age and body mass index of healthy subjects. Exp Gerontol. 2008; 43: 663-667.
28) Ahdi M, Gerdes VE, Graaff R, et al. Skin autofluorescence and complications of diabetes: Does ethnic background or skin color matter? Diabetes Technol Ther. 2015; 17: 88-95.
29) Yonei Y, Takabe W. Aging assessment by Anti-Aging Medical Checkup. Health Evaluation and Promotion. 2015; 42: 459-464.