Original Article

A Survey of Fluorescence Derived from Advanced Glycation End Products in the Skin of Japanese: Differences with Age and Measurement Location

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Abstract

Purpose: To test the validity of glycation as an indicator of aging, using simple devices to measure glycation in skin, and to identify consistent sample locations for skin sampling from a Japanese population.

Methods: The skin autofluorescence (AF) of 780 Japanese people, aged 11-88 years (244 men, mean age: 44.5 ± 18.2 years; 536 women, mean age: 42.8 ± 14.8 years) was measured at two different locations on the fore- and upper arm. The data were used to identify differences in fluorescence with age and sample location.

Results: The coefficient of variation (CV) of skin AF measured from the inside of the upper arm was significantly lower than that measured from the forearms (upper arms 2.68 ± 2.55%, forearms 4.41 ± 4.62%, p = 0.002). The data for skin AF measured from the inside of the upper arm was positively correlated with age for both genders aged from 11 to 69 years (males p < 0.001, r = 0.605, females p < 0.001, r = 0.733); no significant differences were observed between the correlation coefficient for either gender. Skin AF increased from age 10 to 39 years (age 10-19, 1.12 ± 0.19; 20-29, 1.54 ± 0.33; 30-39, 1.89 ± 0.36), but did not change between 40 and 69 years (age 40-49, 2.00 ± 0.38; 50-59, 2.08 ± 0.40; 60-69, 2.01 ± 0.41).

Conclusions: We recommend skin AF in Japanese people be measured at the inside portion of the upper arm. In this measurement location, we found a positive correlation between skin AF and age, and increase with age between 20-39 years gradually. Additional factors should at least be considered in subjects aged 40-69 years.

KEY WORDS: advanced glycation end products (AGEs), skin autofluorescence, aging, forearm, upper arm

Introduction

A survey conducted by the Ministry of Health, Labour and Welfare in 2009 found life expectancy for Japanese was 79.6 years for men and 86.4 for women, a record high for the fourth consecutive year and the highest life expectancy in the world. In the future, most people can expect to live long, healthy lives and to enjoy a relatively high quality of life (QOL). Anti-Aging Medicine seeks to maintain and improve QOL of older persons by diagnosing and treating symptoms of pathological early aging. A key diagnostic tool is the assessment of physical function as an index of functional age 1). An aging index can provide information to patients and clinicians that can be used to identify, prevent, and treat disease 2-4).

Recent research has found that protein glycation increases with age, and researchers have proposed a concept of glycation stress defined as the modification of cell proteins by non-enzymatic/irreversible reactions with reducing sugars 5). Glycation stress includes a number of different chemical reactions that may generate advanced glycation end products (AGEs) through a chain of various intermediate reactions that produce Schiff bases and Amadori products. Given that AGEs have been implicated in the pathology of age-related diseases 6,7), glycation may meet the four characteristics (generality, endogeneity, progression, and harm) advocated by Strehler 8) as an index of aging.

Several AGEs have a characteristic fluorescence, which presents clinicians with a potential method of measuring cumulative AGEs deposition in human skin non-invasively. Although skin autofluorescence (AF) from patients of Caucasian and Chinese ethnicity has been shown to increase with age, AF measurements vary with skin colour 9,10). Here, to assess the effectiveness of skin AF as an aging index and identify a site that might provide consistent results, we measured skin AF in Japanese persons aged 11-88 years at two locations on the arm.
Subjects and Methods

Subjects

A total of 780 Japanese men and women, aged 11 to 88 years (244 men, mean age: 44.5 ± 18.2 years; 536 women, mean age: 42.8 ± 14.8 years) who were not in the hospital at the time of measurement, were selected at random from records kept by the Anti-Aging Medical Research Center, Doshisha University. Subject profiles among men were described in Table 1, and as follows: body mass index, 24.0±3.2 (n=117); systolic/diastolic blood pressure, 129.9±17.7/80.6±12.3 mmHg (n=185); and HbA1c (JDS), 5.2±0.6% (n=62); Subject profiles among women were as follows: body mass index, 22.9±3.5 (n=276); systolic/diastolic blood pressure, 119.8±16.4/72.0±10.9 mmHg (n=246); and HbA1c (JDS) 5.0±0.3% (n=134).

Location of skin AF measurement site

The equipment maker recommends measurements be taken from the forearm. However, this region is often sunburnt and may change character through the year. To test the influence of location on AF, skin AF was measured at two arm locations in a sub-sample of 81 persons aged 11-69 years selected at random from the total sample population. AF was measured from medial side the forearm below the elbow and from the inside of the upper arm approximately 10 cm above the elbow (Fig. 1). The relative error of measurement and the coefficient of variance (CV [%]) between locations were calculated. From a preliminary analysis of this data, we concluded that the upper arm was the most suitable site to test the influence of age on AF, and took measurements from the upper arm for all 780 persons.

Age structure and group data

Data from all 780 persons were divided by ten year intervals into eight age groups:2) (aged 10-19 [n = 86], 20-29 [n = 81], 30-39 [n = 105], 40-49 [n = 232], 50-59 [n = 182], 60-69 [n = 57], 70-79 [n = 21], 80-89 [n = 16] years).

Statistical analysis

The relative error and CV of skin AF in different test locations (fore and upper arm) were compared by Wilcoxon’s rank test. The difference in skin AF between gender and age group was investigated by correlation analysis using Pearson’s correlation coefficient.

Scatter diagrams were drawn for men and women, as well as one for all data from both genders. A linear regression and the correlation of skin AF and age was calculated for each age group. The mean, standard deviation, and the 95% confidence intervals (95% CIs) were calculated for each age group in both genders. The correlation of age and skin AF was assessed using Pearson’s correlation coefficient, as was the regression of age and skin AF.

The average value, ± standard deviation, and 95% CI were calculated for skin AF each age group and compared by the Steel-Dwass test. Statistics were calculated with SPSS II (IBM

Table 1  Profile for subjects

<table>
<thead>
<tr>
<th></th>
<th>Men mean ± SD</th>
<th>N</th>
<th>Women mean ± SD</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI kg/m²</td>
<td>24.0 ± 3.2</td>
<td>117</td>
<td>22.9 ± 3.5</td>
<td>276</td>
</tr>
<tr>
<td>systolic blood pressure mmHg</td>
<td>129.9 ± 17.7</td>
<td>185</td>
<td>119.8 ± 16.4</td>
<td>246</td>
</tr>
<tr>
<td>diastolic blood pressure mmHg</td>
<td>80.6 ± 12.3</td>
<td>185</td>
<td>72.0 ± 10.9</td>
<td>246</td>
</tr>
<tr>
<td>HbA1c %</td>
<td>5.2 ± 0.6</td>
<td>62</td>
<td>5.0 ± 0.3</td>
<td>134</td>
</tr>
</tbody>
</table>

SD, standard deviation

Measurement of skin AF

The accumulation of AGEs in skin was evaluated with an AGE Reader™ (DiagnOptics, Groningen, Netherlands)9-12. This instrument is designed non-invasively evaluate AGEs accumulated in skin using the principle that several AGEs emit a characteristic AF when excited by UV light. Excitation light (wavelength 300-420 nm) is projected onto 1-cm² of the skin surface, and the intensity of any light (420-600 nm) emitted is measured with a spectrometer. Skin AF (in arbitrary units [AUs] × 100) is calculated from the mean value of the emitted light intensity divided by the excitation light intensity.

![Fig. 1. Location of measurement sites on the upper- and forearm.](image) AF was measured from the medial side of the forearm below the elbow (right) and from the inside of the upper arm approximately 10 cm above the elbow (left). Arrows show the location of measurement sites.
Japan Corp., Chuo-ku, Tokyo, Japan). A p value of 0.05 was considered significant.

Ethical approval

The present study followed guidelines (‘The Ethical Principles Concerning Epidemiologic Study’) published by the Japanese Ministry of Health, Labour and Welfare; Doshisha University Ethical Committee for Clinical Studies approved the study protocol (approval number #0832). Data were not linked to subjects’ personal information.

Results

Difference in skin AF at different arm locations

The mean skin AF measured from the forearm was significantly higher than that measured from the upper arm (forearm 2.14 ± 0.61, upper arm 1.93 ± 0.49, p = 0.002). The CV of the mean skin AF measured from the upper arm was significantly lower than that measured from the forearm (upper arm 2.68 ± 2.55%, forearm 4.41 ± 4.62%, p = 0.002) (Table 2), implying that measurements from the inside of the upper arm will be more consistent. Subsequent measurements were taken from the inside upper arm.

Changes in skin AF with age

Skin AF was positively correlated with age for both men and women (Fig. 2), and no difference was observed in the correlation coefficient between genders (men r = 0.605, p < 0.001; women r = 0.733, p < 0.001). Regressions of skin AF against age for men, women and for both sexes combined are shown in Fig. 3 (men, y = 0.0224x + 0.9767; women y = 0.0138x + 1.2312; combined data for men and women, y = 0.0174x + 1.1166).

Comparison of age groups

The mean skin AF of each age group increased until the age 30-39 year range, but did not increase from the age 30-39 to 70-89 groups. The standard deviation and 95% CI were smallest in the age 10-19 group, but variation increased in older age groups and was largest in the age 80-89 group (Table 3, Fig. 4).

Table 2 Comparison of skin AF measured in the upper- and forearm

<table>
<thead>
<tr>
<th></th>
<th>Upper arm</th>
<th>Forearm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin AF (mean ± SD)</td>
<td>1.93 ± 0.49</td>
<td>2.14 ± 0.61</td>
</tr>
<tr>
<td>Relative error of Skin AF (mean ± SD)</td>
<td>4.52 ± 3.89</td>
<td>5.50 ± 3.83</td>
</tr>
<tr>
<td>CV of Skin AF (mean ± SD)</td>
<td>2.68 ± 2.55</td>
<td>4.41 ± 4.62</td>
</tr>
</tbody>
</table>

Wilcoxon’s rank test, n = 81
SD, standard deviation; CI, confidence interval; CV, coefficient of variation; AF, auto-fluorescence

![Table 2](image.png)

Fig. 2. Comparison of skin AF measurement taken from upper arm of 60 men and 60 women. AF: auto-fluorescence, r: Pearson’s correlation coefficient. Open circles, men; closed circles, women; solid line, regression line of men; broken line, regression line of women.
Fig. 3. Regressions of skin AF and age in years for Japanese men, women, and for all data.
AF: auto-fluorescence, r: Pearson’s correlation coefficient. Skin AF in Japanese men (a) and women (b), and both sexes (c); solid line, regression line.
Influence of sample location on skin AF measures

Discussion

Caucasian, a previous report showed that AF values obtained in Japanese subjects is the inside of the upper arm. Findings suggest the most suitable location for measuring skin correlation between skin AF and age in Japanese. These measurements are likely to be. Further, we also found a positive correlation of measurements at a location, the more reliable skin AF aging index in Japanese people. We found that the lower CV using an AGE Reader would provide an effective, novel potential inducing glycation stress. Thus these lifestyle factors may promote the accumulation of skin AGES in adults.

Skin AF variation with age

Although skin AF of the upper arm increased with age, most of this increase occurred before age 39 (Fig. 4), and less change occurred between ages 40 and 89. As such, we suspect that additional factors other than age might influence skin AF, with subjects past middle age being more strongly affected than younger individuals. For example, lifestyle changes that often occur from 20 may accelerate glycation, thereby increasing skin AF. These age groups engage in potential behaviors (smoking and drinking) reported to increase glycation; AGES accumulation was higher in the tissue of non-diabetic smokers than non-smokers [15], and alcohol promotes aldehyde production potentially inducing glycation stress. Thus these lifestyle factors may promote the accumulation of skin AGES in adults.

Variation in skin AF in the age 70-89 group may arise from several factors. Many persons older than 70 may present with a disease history that might also influence skin AF although others will be healthy. This variation in disease presentation is likely to increase the variability in skin AF within this age group.

Generation of fluorescence AGES in living tissue

Pentosidine (fluorescence λmax: ex. 335 nm, em. 385 nm) is a typical fluorescent AGE generated by the reaction of ribose with arginine and lysine. The compound is recognized as an early clinical marker for nephropathy and recently has been used as a marker for detecting osteoporosis (bone aging) [16,17]. Found in dermal collagen, concentrations of pentosidine increase with age. When data from a previous study were adjusted for age, the accumulated concentration of pentosidine was much higher in diabetic patients than in healthy persons.

Other fluorescence AGES include crossline (λmax: ex. 379 nm, em. 463 nm) [13] and pyrropyridine (λmax: ex. 370 nm, em. 455 nm) [19]. However, which AGES can be detected using the AGE Reader™ or whether or not the AF intensity is correlated with total AGE concentration in the skin remains unclear. Nevertheless, the AF of immuno-histochemical skin biopsies from patients with diabetes mellitus and nephropathy receiving dialysis is correlated with AGE concentration, such as pentosidine or CML, although CML is a non-fluorescent AGE [20].

Possibility of AF as an index of skin functional age

Human skin elasticity reduces with aging, and the skin elasticity curve is shifted downwards in patients with type 2 diabetes compared with that of healthy subjects [21]. This observation implies that glycation stress is a major factor in the reduction of skin elasticity, which is caused by changes in elastic fiber production, collagen, elastin, and extracellular matrix ingredients, such as fibronectin, possibly due to some

Table 3 Mean, SD, and confidence limits for skin AF in each age group

<table>
<thead>
<tr>
<th>Age group</th>
<th>Age range (years)</th>
<th>mean</th>
<th>SD</th>
<th>95% CI</th>
<th>Men (n)</th>
<th>Women (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>teens</td>
<td>10 - 19</td>
<td>1.12 ± 0.19</td>
<td>0.04</td>
<td>38</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>20s</td>
<td>20 - 29</td>
<td>1.54 ± 0.33</td>
<td>0.07</td>
<td>26</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>30s</td>
<td>30 - 39</td>
<td>1.89 ± 0.36</td>
<td>0.07</td>
<td>19</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>40s</td>
<td>40 - 49</td>
<td>2.00 ± 0.38</td>
<td>0.05</td>
<td>56</td>
<td>176</td>
<td></td>
</tr>
<tr>
<td>50s</td>
<td>50 - 59</td>
<td>2.08 ± 0.40</td>
<td>0.06</td>
<td>59</td>
<td>123</td>
<td></td>
</tr>
<tr>
<td>60s</td>
<td>60 - 69</td>
<td>2.01 ± 0.41</td>
<td>0.11</td>
<td>28</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>70s</td>
<td>70 - 79</td>
<td>2.06 ± 0.40</td>
<td>0.18</td>
<td>10</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>80s</td>
<td>80 - 89</td>
<td>2.52 ± 0.85</td>
<td>0.44</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

SD, standard deviation; CI, confidence interval; AF, auto fluorescence

Fig. 4. Comparison of mean and SD of skin AF in each age group for Japanese men and women.

AF, auto-fluorescence. Open circles, mean of skin AF ± standard deviation (SD) via Steel-Dwass’ test.

*p < 0.05 vs. each neighboring age group.

Discussion

Here, we investigated whether or not skin AF measured using an AGE Reader would provide an effective, novel aging index in Japanese people. We found that the lower CV of measurements at a location, the more reliable skin AF measurement are likely to be. Further, we also found a positive correlation between skin AF and age in Japanese. These findings suggest the most suitable location for measuring skin AF in Japanese subjects is the inside of the upper arm.

Influence of sample location on skin AF measures

According to the measurement location of skin AF in Caucasian, a previous report showed that AF values obtained from the forearm or leg are well correlated with diabetes complications [9]. However, several other authors have reported that UV exposure promotes AGE generation in skin, and we therefore posit that AF values were higher in the forearm than in the upper arm because the forearms are more exposed to UV light [13]. In addition, we had found that skin AF values obtained at upper arm in diabetic mellitus patients were higher than those obtained from the same point in non-diabetic subjects [14].
disorder in fibroblast function. Collagen protein can also deteriorate due to oxidation or glycation. These and other biochemical changes cause wrinkling on the face after age 30, and the changes in proteins, elastic fibers, and collagen structure in particular are induced by solar radiation and generated by fibroblasts in the dermis. AGEs accumulate in skin mainly as a modified form of collagen fiber, which plays a role in maintaining skin elasticity in combination with elastin fiber. Amino acids such as lysine or arginine in collagen are modified by glycation to become AGEs, forming cross-links between fibers and thereby reducing skin elasticity. Such AGEs were thought to include pentosidine, crossline, and pyrropyridine, so evaluating the relation between these AGEs and skin AF was needed.

Although briefly described the characteristics of aging is difficult, Strehler suggested common features of aging include universality, internality, progressiveness, and hazardous property. Although the accumulation of AGEs by glycation in living tissues meets these criteria, AGE accumulation may also be induced by other causes unrelated to age.

**Conclusion**

We observed glycation in living tissue by measuring the AF generated from the skin of 780 men and women aged 11-88 years. The upper arm was found to be the most suitable site for AF measurements in both men and women, and we observed a positive correlation between skin AF and age. We also noted a gradual increase in skin AF among individuals aged 11-39 years. Additional factors influencing AF besides simply aging should be considered among individuals aged 40-69 years.

**Conflict of interest statement**

The authors declare no financial or other conflicts of interest in the writing of this paper.

## References