Skin Research and Technology 2017; 23: 149–154
Printed in Singapore · All rights reserved
doi: 10.1111/srt.12313

© 2016 John Wiley & Sons A/S.
Published by John Wiley & Sons Ltd
Skin Research and Technology

Pilot study on the correlation between skin auto-fluorescence and serum antioxidant enzyme: skin auto-fluorescence is negatively associated with levels of malondialdehyde

J. H. Yim[1], K. H. Jeong[1], J. Y. Kim[2], Y. H. Cho[2], S. J. Bae[3] and M. K. Shin[1]

[1]Department of Dermatology, College of Medicine, Kyung Hee University, Seoul, Korea,
[2]Department of Medical Nutrition, Graduate School of East-West Medical Science, Kyung Hee University, Seoul, Korea and [3]Korea Electrotechnology Research Institute, Ansan, Korea

Background/purpose: Various methods have been used to objectively record skin changes. However, estimating the intrinsic and extrinsic aging of skin remains a challenge. Our objective was to study intrinsic skin aging with respect to patient age and extrinsic photo-aging of human dorsal (photo-exposed) and volar (photo-protected) forearm *in vivo* through skin auto-fluorescence (AF). We also examined the correlations between serum antioxidant enzyme, malondialdehyde (MDA), and skin AF.
Methods: 37 healthy volunteers were enrolled. We measured skin AF and its heterogeneity on the dorsal and volar forearms. We also examined serum concentration of catalase, superoxide dismutase, vitamin E, and MDA levels in every participant.
Results: In photo-protected areas, skin AF intensity in the 40 years or older group was significantly higher compared to the group less than 40 years-old. On the other hand, heterogeneity value was significantly higher in the less than 40 years-old group in photo-protected area. With respect to serum antioxidant enzyme and MDA level, only MDA level showed a negative correlation with skin AF intensity in photo-exposed area.
Conclusion: We determined that skin AF intensity of the photo-protected area reflects intrinsic skin aging. In addition, degree of photo-aging could be indirectly inferred by skin AF of photo-exposed area and serum MDA level.

Key words: skin auto-fluorescence (AF) – Antioxidant enzyme – Malondialdehyde (MDA) – Photo-aging

© 2016 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd
Accepted for publication 21 June 2016

THE AGING of skin is influenced by both intrinsic and extrinsic factors. Cumulative damage from sunlight exposure in human skin is a major extrinsic aging factor that leads to photo-aging, which is characterized by solar elastosis (increased disorganization of elastic fibers in the superficial dermis) and irregular and reduced collagen within the dermis (1, 2). Photo-aging is mediated by the direct impact of ultraviolet radiation (UVR) and UVR-induced matrix metalloproteinases, which degrade collagen and other matrix proteins within the dermis (3, 4). Oxygen-derived free radicals play an important role in this process. In addition to increased free radical production, a decrease in the enzymatic and non-enzymatic antioxidant defenses are observed after exposure to light (5). On the other hand, intrinsic aging occurs within the tissue itself, via reductions in dermal mast cells, fibroblasts, collagen production, and flattening of the dermal-epidermal junction, which are generally under genetic and hormonal influences (6). Various methods have been used to objectively record skin changes with aging such as changes in the micro- and macro-relief, elasticity, and hydration (conductance). Recently, one study estimated photo-aging by measuring the structure of the keratinocytes, collagen fibers, and dermal papillae using reflectance confocal microscopy. These authors found that sun-exposed areas more frequently showed an irregular honeycomb pattern, polycylic papillary contours, coarse collagen, huddled collagen, and curled bright structures with higher epidermal thickness and furrow depth values (7). However, these

methods require complex imaging analysis and expensive equipment.

Our objective was to examine intrinsic and extrinsic skin aging of the human dorsal (photo-exposed) and volar (photo-protected) forearm *in vivo* through skin auto-fluorescence (AF). We also examined the correlations between serum antioxidant enzyme, malondialdehyde (MDA), and skin AF.

Materials and Methods

This study was prospectively conducted with volunteers between November 2013 and February 2014 in Kyung Hee Medical Center. 37 healthy Korean volunteers with Fitzpatrick skin type IV and V were included in this study. Inclusion criteria were age between 20 to 80 years-old. All volunteers provided written informed consent.

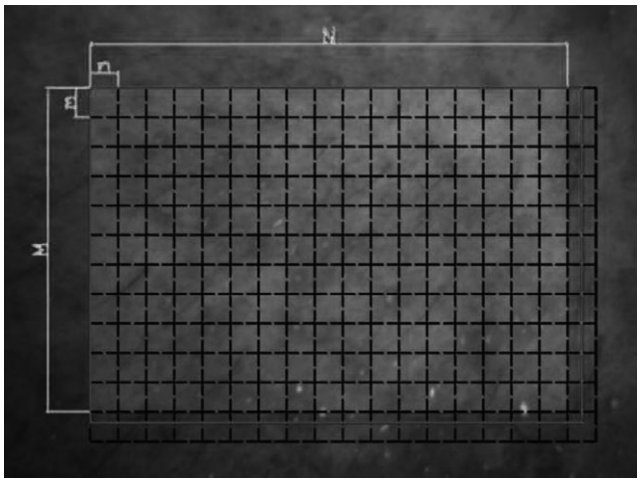

Fig. 1. The region of interest (N, M) was cleaved into 20 × 20 pixels. The parts used in the estimate of aging were set to avoid from the specific area such as scars.

Auto-fluorescence was measured using the EcoSkin® fluorescence video dermatoscope, a hand-held device that yields multispectral images of AF in skin areas 19.3 × 14.5 mm^2 in size with a resolution of 782 × 582 pixels. The fluorescence is excited by an array of LEDs that emit a wavelength of 408 nm. The fluorescence pattern is recorded by means of an RGB television detector with sensitivity maxima in the color channels 620 nm (red channel; R), 530 nm (green channel; G), and 470 nm (brown channel; B), and with heterogeneity (H) of R, G, and B (HR, HG, and HR, respectively).

To calculate the H value that related to the heterogeneity of the skin, we set a region of interest (N, M) and cleave it into 20 × 20 pixels. The parts used in the estimation of aging were set to avoid from the specific area such as scars (Fig. 1). After this, we calculated the average of the signal, $\overline{S_{i,j}}$, of each unit. Red, green, and brown signals were extracted separately to determine the heterogeneity of the skin, as shown in Fig. 2. In order to measure the heterogeneity of the image, the differences between the peripheral units need to be considered. We calculated the value of H, while scanning the sum of the difference between the unit and the left unit, and the unit and the lower unit. Therefore, larger calculated values of H reflected reduced homogeneity of the skin, and thus increased heterogeneity.

We measured skin AF and the heterogeneity of the dorsal forearm (photo-exposed) and volar forearm (photo-protected), as shown in Fig. 3. We also examined the serum concentration of catalase, superoxide dismutase (SOD), vitamin E, and MDA in each participant.

Analysis was performed based on the paired *t*-test and Spearmann's nonparametric test. In

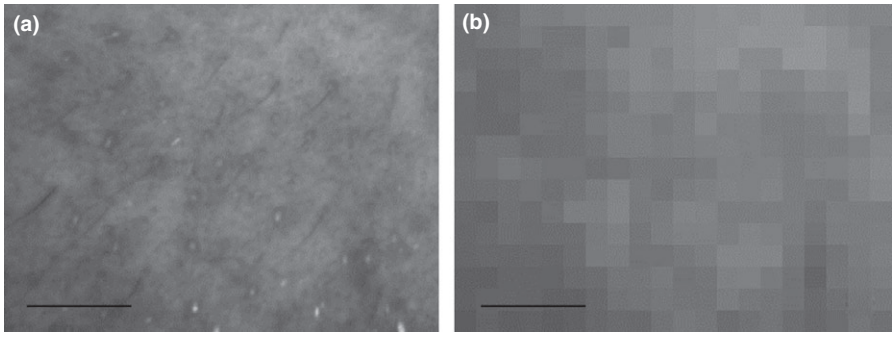

Fig. 2. (a) The extracted image from the green signal. (b) The reconstructed image with the average value, black bars = 5 mm.

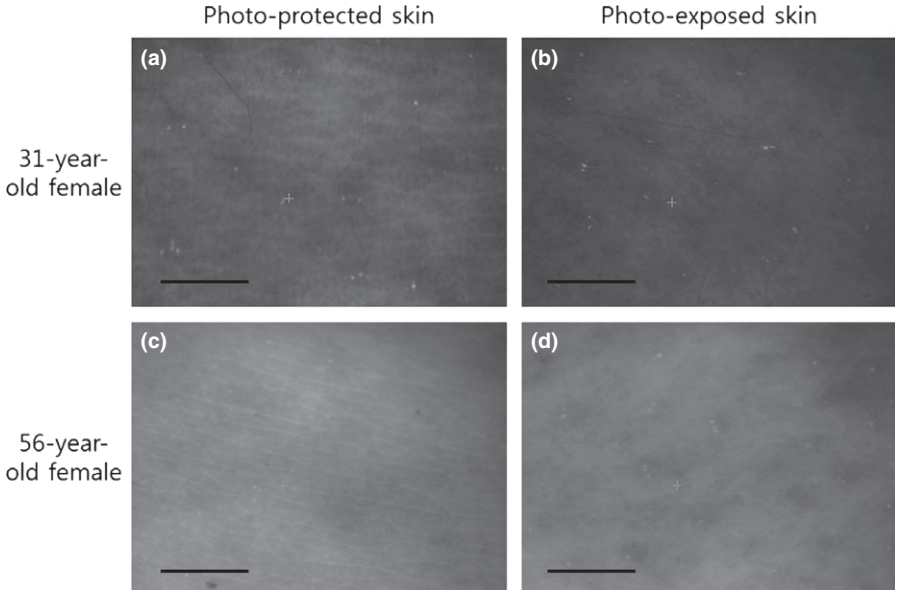

Fig. 3. Representative auto-fluorescence (AF) image of the photo-protected and photo-exposed skin. AF image of the photo-protected skin (a) and photo-exposed skin (b) in a 31-year-old woman. AF image of the photo-protected skin (c) and photo-exposed skin (d) in a 56-year-old woman. AF intensity and homogeneity are significantly higher in the photo-protected skin of a 56-year-old woman (c) than in the photo-protected skin of a 31-year-old woman (a), black bars = 5 mm.

TABLE 1. AF intensity of both the photo-protected and photo-exposed areas classified according to age. Values are presented as means ± SEMs

Photo-protected	R	G	B	HR	HG	HB
Age <40	54.05 ± 14.20	120.21 ± 35.56	54.26 ± 18.12	4.96 ± 0.89	7.71 ± 1.63	9.86 ± 2.52
Age ≥40	66.43 ± 15.37	140.82 ± 26.29	63.11 ± 12.89	4.49 ± 0.59	6.93 ± 0.87	8.51 ± 1.09
	(P = 0.005)*	(P = 0.015)*	(P = 0.031)*	(0.019)*	(0.022)*	(0.009)*
Photo-exposed	**R**	**G**	**B**	**HR**	**HG**	**GB**
Age <40	54.42 ± 19.11	120.05 ± 46.57	54.47 ± 22.42	5.17 ± 1.19	7.71 ± 1.53	10.02 ± 2.19
Age ≥40	65.07 ± 22.11	134.21 ± 31.05	58.79 ± 14.72	5.16 ± 1.02	7.40 ± 1.06	9.17 ± 1.44
	(P = 0.005)*	(P = 0.114)	(P = 0.171)	(0.484)	(0.220)	(0.061)

*P < 0.05 by paired t-test. R, AF intensity peaking around 620 nm; G, AF intensity peaking around 530 nm; B, AF intensity peaking around 470 nm; HR, heterogeneity of R value; HG, heterogeneity of G value; HB, heterogeneity of B value.

all analyses, SPSS software (version 22.0; SPSS Inc., Chicago, IL, USA) was used. Data were valued within a confidence interval of 95%. A P-value less than 0.05 was considered a significant difference.

Results

A total of 37 volunteers were enrolled in our study. The volunteers' mean age (±SD) at the time of inclusion was 44.3 (±11.43) years (range 20–76 years). 20 volunteers were female and 17 were male. We also divided volunteers into two groups based on whether they were above or below 40 years of age. There were 19 volunteers less than 40 years-old and 18 participants were 40 years or older.

Auto-fluorescence data from both the photo-protected and photo-exposed areas according to age is shown in Table 1. In the photo-protected area, the R, G, and B values of the 40 years or older group were significantly higher compared to the less than 40 years-old group (P = 0.005, 0.015, 0.031 respectively). On the other hand, the heterogeneity value was significantly higher in the less than 40 years-old group in the photo-protected area (P = 0.019, 0.022, 0.009 respectively). In contrast with the photo-protected area, the AF and heterogeneity values of photo-exposed areas did not show significant differences according to age, with the exception of the R value (P = 0.005).

Correlations between the serum antioxidant enzyme, MDA levels and AF in both the photo-

TABLE 2. AF data of both the photo-exposed and photo-protected areas with statistical analysis according to antioxidant enzyme and MDA levels

	R		G		B	
	Photo-exposed	Photo-protected	Photo-exposed	Photo-protected	Photo-exposed	Photo-protected
Catalase	−0.349 (P = 0.016)*	−0.339 (P = 0.020)*	0.149 (P = 0.920)	−0.230 (P = 0.091)	−0.244 (P = 0.219)	−0.174 (P = 0.134)
SOD	−0.111 (P = 0.413)	0.141 (P = 0.212)	−0.011 (P = 0.942)	−0.117 (P = 0.341)	−0.087 (P = 0.482)	0.051 (P = 0.421)
Vitamin E	0.213 (P = 0.543)	0.147 (P = 0.109)	0.071 (P = 0.712)	−0.133 (P = 0.501)	−0.091 (P = 0.355)	−0.091 (P = 0.497)
MDA	−0.290 (P = 0.048)*	−0.249 (P = 0.091)	−0.360 (P = 0.013)*	−0.292 (P = 0.046)*	−0.325 (P = 0.026)*	−0.226 (P = 0.127)

*P < 0.05 by Spearmann's nonparametric test. R, AF intensity peaking around 620 nm; G, AF intensity peaking around 530 nm; B, AF intensity peaking around 470 nm; HR, heterogeneity of R value; HG, heterogeneity of G value; HB, heterogeneity of B value.

protected and photo-exposed areas are shown in Table 2. There was a negative correlation in the R value and serum catalase level between the photo-exposed and photo-protected areas (P = 0.016, 0.020 respectively). Similarly, the MDA level showed a negative correlation with the R, G, and B values in the photo-exposed area (P = 0.048, 0.013, 0.026 respectively). There was also a negative correlation in the G value and MDA level in the photo-protected area (P = 0.046). However, the SOD and vitamin E level did not show statistical correlations with AF intensity.

Discussion

The potential of using AF to evaluate age-related changes of the skin has been investigated in a number of papers. Experiments in mice have established that the excitation spectra possess bands in the near-UV region that can serve as objective indicators of biological aging and photo-aging (8). In addition, AF can be used to measure the advanced glycation end-products (AGEs), which are related to aging (9).

In this study, volunteers in the 40 years or older group had a significantly higher AF intensity compared to the less than 40 years-old group in the photo-protected area. However, the AF value of the photo-exposed area did not show significant differences according to age, with the exception of the R value. In the photo-protected area, AF intensity may increase due to collagen cross-linking with increasing chronologic age (Fig. 4). On the other hand, in the photo-exposed area, the lack of statistical difference between the two groups could be the result of increased elastin deposition due to photo-aging which compensates for the increase in skin AF intensity by collagen cross-linking. Such findings are consistent with other studies that have reported that the intensity of the skin AF increases as chronological age was increased (10).

Photo-aging of the skin can induce dyschromia that results in increasing heterogeneity of skin pigmentation. However, there was no statistical difference in the heterogeneity of AF in the photo-exposed area between the two groups based on age. On the other hand, the photo-protected area showed decreased heterogeneity in the group above the age of 40 as compared to the group below the age of 40. In the areas with intrinsic aging, pigmentary changes due to photo-aging is not clinically prominent. Therefore, we can hypothesize that other factors related to intrinsic aging such as AGEs and flavin adenine dinucleotide have a greater impact than the melanin reflectance, and that the homogeneity of the skin was increased due to

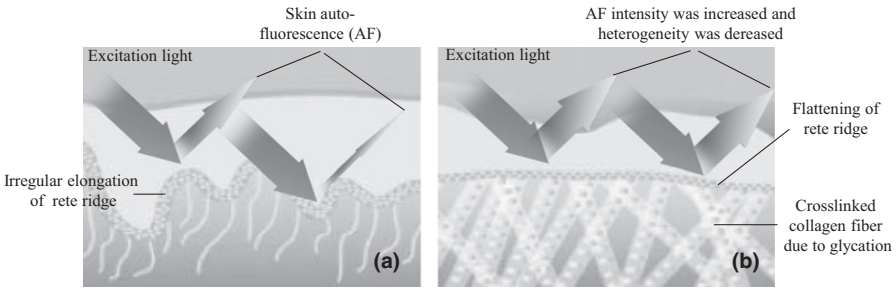

Fig. 4. Diagram of the skin in younger age (a) and in older age (b). As intrinsic skin aging progresses, AF intensity increases due to collagen crosslinking and the heterogeneity of AF decreases because of epidermal flattening caused by a loss of the rete ridges.

the deposition of oxidants related to aging. In addition, it is thought that the statistical significance of HG is the lowest because HG is closest to the wavelength of pigment. On the other hand, for HR and HB, homogeneity related to the aging process seems to increase and heterogeneity seems to decrease. From a structural perspective, it is thought that the heterogeneity of AF increases with the number of dermal papillae. In intrinsic skin-aging, the flattening of the previously undulating epidermis is the most striking change caused by a loss of the rete ridges and reciprocal inter-digitation with capillary-rich dermal papillae (6). Therefore, it is hypothesized that the heterogeneity value of the photo-protected area in the less than 40 years-old group is higher than the 40 years or older group because of these structural changes, as shown in Fig. 4.

Generally, the AF emitted from the skin is increased with skin aging. In contrast, there are significant negative correlations between skin AF and the majority of measures of cumulative UVR doses in photo-aging (11). The skin's enzymatic antioxidant defenses include catalase, SOD, and vitamin E. It is known that chronic and acute photo-damage is mediated by the depletion of these antioxidant enzymes (12). Therefore, we could expect a positive correlation between the antioxidant enzyme level and skin AF intensity of the photo-exposed area. In contrast, we expect a negative correlation between the serum MDA level and skin AF intensity of the photo-exposed area because the lipid peroxidation product MDA accumulates in tissues under conditions of increased oxidative stress. In our study, we found a negative correlation between serum MDA level and skin AF in the photo-exposed area. However, the catalase, SOD and vitamin E levels did not show statistical positive correlations with the AF intensity of the photo-exposed area. Based on these results, we hypothesized that photo-aging is more relevant to serum MDA level than serum antioxidant enzyme level because MDA is directly related with UVR-induced lipid peroxidation while antioxidant enzymes are influenced by various factors such as smoking, BMI, and chronic wasting disease.

Auto-fluorescence is a non-invasive and rapid tool for skin analysis, but is limited because it is influenced by various factors such as intrinsic and extrinsic skin aging. In order for AF to be effective as a research tool, future research should account for these factors in AF analysis. In this study, we determined that the AF intensity in the photo-protected area reflects intrinsic skin aging. In addition, the degree of photo-aging can be indirectly inferred by the skin AF of the photo-exposed area and serum MDA level.

Funding

This work was supported by a grant from the Next Generation BioGreen 21 Program (no. PJ0090662013), Rural Development Administration, Korea.

Conflicts of Interest

None declared.

References

1. Roberts MS, Dancik Y, Prow TW, Thorling CA, Lin LL, Grice JE, Robertson TA, König K, Becker W. Non-invasive imaging of skin physiology and percutaneous penetration using fluorescence spectral and lifetime imaging with multiphoton and confocal microscopy. Eur J Pharm Biopharm 2011; 77: 469–488.
2. Baillie L, Askew D, Douglas N, Soyer HP. Strategies for assessing the degree of photodamage to skin: a systematic review of the literature. Br J Dermatol 2011; 165: 735–742.
3. Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ. Pathophysiology of premature skin aging induced by ultraviolet light. N Engl J Med 1997; 337: 1419–1428.
4. Chung JH, Seo JY, Choi HR, Lee MK, Youn CS, Rhie G, Cho KH, Kim KH, Park KC, Eun HC. Modulation of skin collagen metabolism in aged and photoaged human skin in vivo. J Invest Dermatol 2001; 117: 1218–1224.
5. Emerit I. Free radicals and aging of the skin. EXS 1992; 62: 328–341.
6. Durai PC, Thappa DM, Kumari R, Malathi M. Aging in elderly: chronological versus photoaging. Indian J Dermatol 2012; 57: 343–352.
7. Haytoglu NS, Gurel MS, Erdemir A, Falay T, Dolgun A, Haytoglu TG. Assessment of skin photoaging with reflectance confocal microscopy. Skin Res Technol 2014; 20: 363–372.
8. Kollias N, Gillies R, Moran M, Kochevar IE, Anderson RR. Endogenous skin fluorescence includes bands that may serve as quantitative markers of aging and photoaging. J Invest Dermatol 1998; 111: 776–780.
9. Mulder DJ, Water TV, Lutgers HL, Graaff R, Gans RO, Zijlstra F, Smit AJ. Skin autofluorescence, a novel marker for glycemic and oxidative stress-derived advanced glycation endproducts: an overview of current clinical studies, evidence, and

limitations. Diabetes Technol Ther 2006; 8: 523–535.
10. Lutgers HL, Graaff R, Links TP, Ubink-Veltmaat LJ, Bilo HJ, Gans RO, Smit AJ. Skin autofluorescence as a noninvasive marker of vascular damage in patients with type 2 diabetes. Diabetes Care 2006; 29: 2654–2659.
11. Sandby-Møller J, Thieden E, Philipsen PA, Heydenreich J, Wulf HC. Skin autofluorescence as a biological UVR dosimeter. Photodermatol Photoimmunol Photomed 2004; 20: 33–40.
12. Sander CS, Chang H, Salzmann S, Müller CS, Ekanayake-Mudiyanselage S, Elsner P, Thiele JJ. Photoaging is associated with protein oxidation in human skin in vivo. J Invest Dermatol 2002; 118: 618–625.

Address:
Min Kyung Shin, MD, PhD
Department of Dermatology
College of Medicine
Kyung Hee University
 # 1 Hoegi-dong, Dongdaemun-gu
Seoul 130-702
Korea
Tel.: +82-2-958-8300
Fax: +82-2-969-6538
e-mail: haddal@hanmail.net