

Comparative study of skin autofluorescence expression in atopic dermatitis and psoriasis: A prospective *in vivo* study

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Background/purpose: Treatment of atopic dermatitis (AD) and psoriasis requires their differentiation from other eczematoid dermatitis and a determination of disease severity. However, both can be clinically difficult and the findings subjectively interpreted. We investigated the utility of *in vivo* autofluorescence (AF) measurements for diagnosis of both diseases, and determination of severity.

Materials and methods: Thirty patients with AD and 30 with psoriasis were recruited, together with sex- and age-matched patients with healthy skin. AF intensity was measured using the EcoSkin® fluorescence video dermatoscope. In AD and psoriasis patients, AF in non-sun-exposed lesional and non-lesional skin was measured. To identify the locations that reflect characteristics of AD, AF was also measured at the other sites in the patients with AD.

Results: AD was associated with lower AF and psoriasis with higher AF intensity peaking around 620 nm. In addition, skin AF intensity of each disease was associated with severity of lesion.

Conclusions: Non-invasive measurement of skin AF *in vivo* can aid in diagnosis of AD and psoriasis as well as in treatment monitoring.

Key words: atopic dermatitis – *in vivo* – psoriasis – skin autofluorescence

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ATOPIC DERMATITIS (AD) and psoriasis are the most common inflammatory diseases of the skin (1, 2). But they differ in their mechanisms of inflammation. The immune response in AD is Th2-mediated, which accounts for the high IgE levels, whereas in psoriasis it is Th1-mediated and associated with local neutrophil infiltration (3, 4). However, as AD progresses to chronic eczema, the scaly thickened patches and plaques resemble psoriasis. Moreover, there are some cases of both diseases occurred in the same patient (5).

There are no specific blood tests or procedures for the diagnosis of AD and psoriasis. In some patients, invasive procedures such as a skin biopsy may be needed to rule out other disorders. The difficulty in distinguishing AD and psoriasis from other skin disorders can lead to a misdiagnosis and inappropriate treatment. A non-invasive tool that aids in the diagnosis of AD and psoriasis and in the verification of

clinical improvement would improve the management of these patients.

Several components of the skin, including nicotinamide adenine dinucleotide, flavin adenine dinucleotide, keratin, lipofuscin, elastin, collagen, tryptophan, melanin, and porphyrins, emit autofluorescence (AF) (6–9). In the epidermis, keratin is the main source of AF, whereas in the dermis, it is connective tissue proteins, such as collagen and elastin cross-links (10). Each of these various fluorophores has a characteristic excitation-emission pattern that reflects the content and biochemical environment of the skin and therefore provides unique and highly sensitive functional information. The advantages of measuring AF have been demonstrated in both healthy and diseased skin. For example, skin AF was investigated to distinguish photoaging from chronological aging, and malignant skin lesions from normal skin (11–15). In an *ex vivo* study, psoriasis was shown to be

associated with high AF intensity, and skin AF values were significantly lower in AD than in other skin diseases (16).

In this study, we asked whether the measurement of AF *in vivo* aids in the diagnosis and differentiation of AD and psoriasis from other skin diseases and in the determination of disease severity.

Materials and Methods

Study design

This study of AD and psoriasis patients was conducted between September 2014 and September 2015. It was approved by the local ethics committee and registered with the Kyung Hee University Hospital Institutional Review Board. Written informed consent was obtained from all participants prior to their enrollment.

Patients

Thirty patients with AD and 30 with psoriasis were recruited together with 60 sex- and age-matched control patients. The inclusion criteria for the AD and psoriasis patients were age 18–50 years and a clinical diagnosis of AD or psoriasis vulgaris determined at the Kyung Hee University (Seoul, South Korea) outpatient dermatology clinic. Eligible participants were those who provided informed consent. Criteria for exclusion included pregnancy, breast-feeding, planning pregnancy within 6 months, the use for at least 1 month of a topical agent containing a steroid, a cosmetic procedure involving the test areas within the last year, and chronic wasting disease that may influence skin AF.

Measurement of skin AF

AF was measured using the EcoSkin® fluorescence video dermatoscope, a hand-held device that yields multispectral images of AF in skin areas $19.3 \times 14.5 \text{ mm}^2$ in size with a resolution of 782×582 pixels. Fluorescence is excited using an array of LEDs with an emission wavelength of 408 nm. The fluorescence pattern is recorded using a 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).

The AF in the non-sun-exposed lesional and non-lesional skin was measured. For AD, to

identify the skin locations that best reflect its characteristics, AF at the forehead, cheek, posterior neck, flexural and volar arm surfaces, and dorsal hand was measured. In control patients, AF measured at the volar arm surface served as a reference value (Fig. 1).

Disease severity and measurement area

Clinical data were collected from the patients' medical records. For AD patients, the Scoring Atopic Dermatitis (SCORAD) index was used to evaluate disease severity, with the measured area assigned a grade from 0 to 3 based on the degree of erythema, edema/papulation/induration, oozing/crusting, excoriation, and lichenification. Psoriasis patients were evaluated using the Psoriasis Area Severity Index (PASI) score, which assesses disease severity, grading the measured area from 0 to 3 according to the degree of erythema (redness), thickness (induration), and scale (desquamation).

Statistical analysis

Paired *t*-tests were performed using the Excel 2010 software (v.14.0; Microsoft Co., Redmond, WA, USA) to identify significant differences in the AF in the lesional vs. non-lesional skin of AD and psoriasis patients vs. the normal skin of control patients. Potential correlations among disease severity (SCORAD and PASI), grade of lesion, and AF were determined using Spearman's nonparametric test with SPSS software (v12.0; SPSS Inc., Chicago, IL, USA). For all statistical tests, a $P < 0.05$ was considered to indicate statistical significance.

Results

AF in lesional and non-lesional skin in AD

The 14 females and 16 males with AD had a mean age of 26.27 years (range: 18–48 years). Peaked AF intensity was measured around 620, 530, and 470 nm and the sums of these signals in the lesional and non-lesional skin of the patients and in the healthy skin of the controls are shown in Fig. 2. In AD patients, both the AF intensities peaking around 620, 530, and 470 nm and the sum of these signals were significantly lower in lesional than non-lesional skin ($P = 0.0008, 0.0007, 0.0005, 0.0003$ respectively). The AF intensity peaking around 620 and

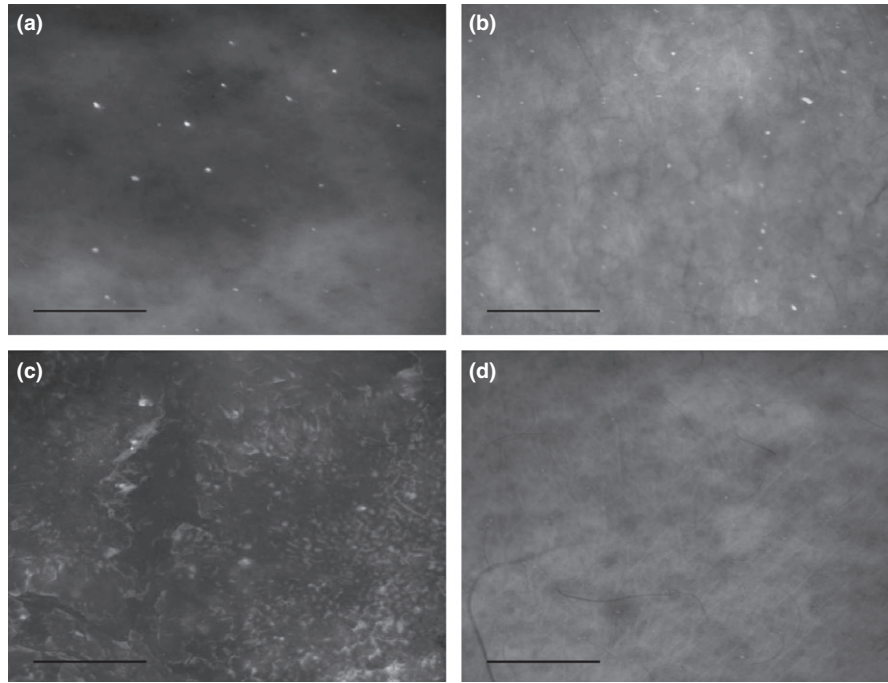


Fig. 1. Autofluorescence (AF) in the lesional and non-lesional skin of patients with atopic dermatitis (AD) and psoriasis. In AD, AF intensity is lower in lesional (a) than in non-lesional skin (b). In psoriasis, red AF distinguishes lesional (c) from non-lesional skin [This is not reported in the results] (d), black bars = 5 mm.

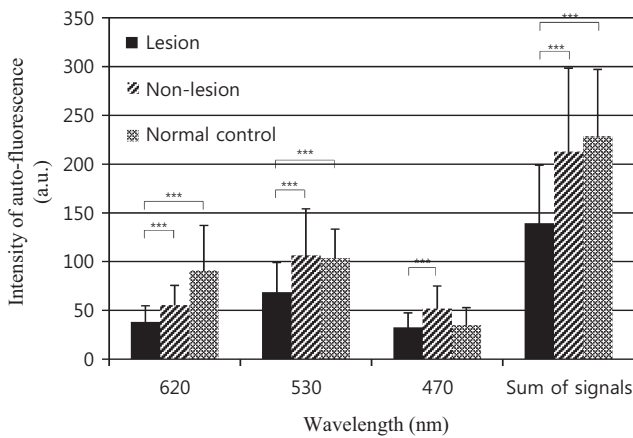


Fig. 2. AF intensity peaking around 620, 530, and 470 nm and the sum of these signals in the lesional and non-lesional skin of AD patients and in healthy control skin. AU, arbitrary units. Error bars represent the standard deviation. *** $P < 0.001$ (paired t -test).

530 nm, but not at 470 nm, and the sum of the signals were significantly lower in the lesional skin of AD patients than in healthy control skin ($P = 0.0000, 0.0000, 0.6322$, and 0.0000).

Regional AF variations in AD

The AF intensity peaking around 530 nm was significantly lower in the forehead and cheek than in non-lesional skin ($P = 0.0007, 0.0071$),

whereas for the AF intensity around 470 nm, the signals in the forehead, cheek, and posterior neck were significantly lower than the non-lesional skin signal ($P = 0.0001, 0.0044, 0.0386$). The AF intensity around 620 nm was significantly lower at the flexural surface of the arm than in non-lesional skin ($P = 0.0108$), whereas the difference in the signals at the volar arm surface and dorsal hand vs. the non-lesional skin signal was not significant (Fig. 3).

Correlations between AF and AD severity

AD severity was determined using the SCORAD index and each measured area assigned by a lesional score ranging from 0 to 3 based on the degree of erythema, edema/papulation/induration, oozing/crusting, excoriation and lichenification. AF intensity was correlated negatively with both the SCORAD index and the lesional score, except for excoriation at 470 nm, but none of the differences were significant statistically (Table 1).

AF in lesional and non-lesional skin in psoriasis

The 12 females and 18 males with psoriasis had a mean age of 40.51 years (range: 20–50 years).

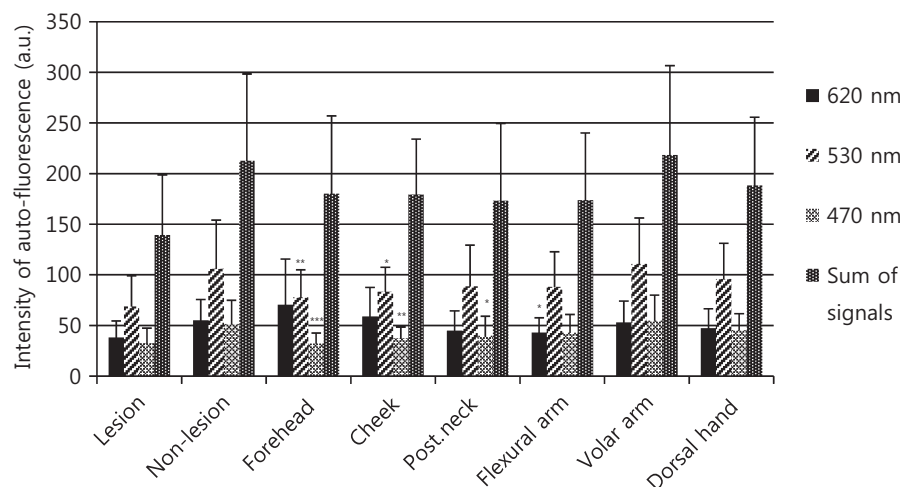


Fig. 3. AF intensities peaking around 620, 530, and 470 nm and the sum of these signals in lesional skin, non-lesional skin, forehead, cheek, posterior neck, the flexural and volar surfaces of the arm, and the dorsal hand of patients with AD. AU, arbitrary units. Error bars represent the standard deviation. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (paired *t*-test).

TABLE 1. Correlation of AF intensities in AD based on the SCORAD index, erythema, edema/papulation/induration, oozing/crusting, excoriation, and lichenification (Spearman's nonparametric test)

	620 nm	530 nm	470 nm	Sum of signals
SCORAD	-0.224 ($P = 0.223$)	-0.143 ($P = 0.452$)	-0.096 ($P = 0.613$)	-0.175 ($P = 0.355$)
Erythema	-0.165 ($P = 0.384$)	-0.13 ($P = 0.494$)	-0.033 ($P = 0.863$)	-0.148 ($P = 0.435$)
Edema/papulation/induration	-0.086 ($P = 0.653$)	-0.089 ($P = 0.64$)	-0.178 ($P = 0.346$)	-0.106 ($P = 0.576$)
Oozing/crusting	-0.183 ($P = 0.332$)	-0.148 ($P = 0.435$)	-0.041 ($P = 0.828$)	-0.167 ($P = 0.379$)
Excoriation	-0.173 ($P = 0.36$)	-0.087 ($P = 0.646$)	0.037 ($P = 0.846$)	-0.096 ($P = 0.615$)
Lichenification	-0.288 ($P = 0.123$)	-0.227 ($P = 0.228$)	-0.132 ($P = 0.487$)	-0.251 ($P = 0.182$)

AF, autofluorescence; AD, atopic dermatitis; SCORAD, Scoring Atopic Dermatitis.

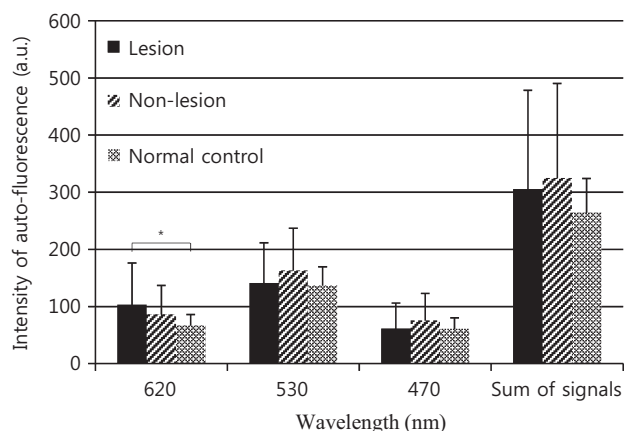


Fig. 4. AF intensities peaking around 620, 530, and 470 nm and the sum of these signals in the lesional and non-lesional skin of psoriasis patients and in the healthy skin of the control group. AU, arbitrary units. The error bars represent the standard deviation. * $P < 0.05$; (paired *t*-test).

The peaked AF intensities around 620, 530, and 470 nm and the sum of these signals in the lesional and non-lesional skin of psoriasis patients and in the healthy skin of the control group is shown in Fig. 4. There was no

significant association in the AF intensity in lesional vs. non-lesional skin, whereas the AF intensity peaking around 620 nm was significantly higher in the lesional skin of psoriasis patients than in healthy control skin ($P = 0.0119$).

Correlations between AF and the PASI and severity scores of lesional skin in psoriasis

Disease activity in psoriasis was determined using the PASI score and each measured area assigned by a lesional score ranging from 0 to 3 based on the degree of erythema, thickness, and scale. AF intensity was correlated positively with all three, as shown in Table 2, but only the association with the degree of erythema was significant ($P = 0.008, 0.007, 0.037, 0.003$ respectively).

Discussion

Atopic dermatitis is one of the 50 most prevalent diseases worldwide and is particularly

TABLE 2. Correlations of psoriasis between AF intensities and the PASI, erythema, thickness, and scale

	620 nm	530 nm	470 nm	Sum of signals
PASI	-0.103 ($P = 0.588$)	0.005 ($P = 0.98$)	-0.152 ($P = 0.422$)	-0.054 ($P = 0.776$)
Erythema	0.472 ($P = 0.008$)**	0.48 ($P = 0.007$)**	0.383 ($P = 0.037$)*	0.52 ($P = 0.003$)**
Thickness	0.247 ($P = 0.189$)	0.15 ($P = 0.43$)	0.158 ($P = 0.404$)	0.252 ($P = 0.178$)
Scale	0.205 ($P = 0.278$)	0.059 ($P = 0.756$)	0.009 ($P = 0.962$)	0.158 ($P = 0.404$)

* $P < 0.05$, ** $P < 0.01$ (Spearman's nonparametric test).

AF, autofluorescence; PASI, Psoriasis Area Severity Index.

common in children (17). Psoriasis, characterized by erythematous papules and plaques covered with silvery white scales, is a recurrent inflammatory skin disease affecting 0–11.8% of the population (18). Both diseases display wide-ranging clinical heterogeneity and may resemble a number of other skin disorders, such that the correct diagnosis of AD and psoriasis is often clinically challenging. This is especially the case for variants of AD such as patchy pityriasiform lichenoid eczema (the follicular type of AD) and juvenile plantar dermatosis, both of which can be clinically similar to psoriasis (19). Moreover, AD can progress to chronic eczema, characterized clinically by scaly thickened patches and plaques on the skin and histopathologically by hyperkeratosis and epidermal hyperplasia, as in psoriasis. Also, pustules of Kogoj and Munro's microabscess, both of which are pathognomonic for psoriasis, are not always observed in pathologic specimens. Thus, there is a need for additional, preferably non-invasive diagnostic procedures, such as that offered by AF measurement.

We measured the AF intensity of the lesional and non-lesional skin of AD and psoriasis patients and compared the results with those of patients with healthy skin. In both AD and psoriasis, there were significant differences in the AF values. The AF intensity in the lesional skin of AD patients was lower than that of non-lesional skin, healthy control skin, and the skin of psoriasis patients. Thus, the measurement of AF in lesional skin may help to differentiate patients with AD and those with psoriasis although further studies are needed to determine whether AD can be differentiated from other eczematous conditions, such as allergic contact dermatitis. Spongiosis in the epidermis and dermal cellular infiltration and edema, all of which are characteristics of AD, may have caused the lower AF values, by reducing the fluorescence intensity of endogenous fluorophores such as keratin and

collagen. Oxidative stress in response to skin inflammation or the changes in skin lipids associated with impairment of the skin barrier may also have reduced AF intensity, by decreasing flavin levels.

Several studies have shown that not only the lesional but also the non-lesional skin of AD patients reflects the characteristics of this disease. For example, trans-epidermal water loss is significantly higher in both the lesional and non-lesional skin of AD patients than in healthy skin. This finding was corroborated in our study by measurements of other skin areas that best reflect the AF properties of AD. Thus, in AD patients, the AF intensity of the forehead, cheek, posterior neck, and flexural surface of the arm was significantly lower than that of non-lesional skin. However, whether patients can be screened for AD by measuring AF at these sites, in the absence of active lesions, needs to be determined in studies involving a larger number of patients.

Autofluorescence values were highest in psoriasis, which is a hyperproliferative skin disease. According to Bissonnette et al. (20), the fluorescence emission peak at 635 nm in native psoriatic plaques is due to the elevated levels of protoporphyrin IX within the scales. These findings were demonstrated within the plaques of 34 of 75 (45%) patients with psoriasis, and they are consistent with the results of this study, which showed statistically higher AF intensity peaked around 620 nm in the lesional skin of psoriasis patients than in the healthy skin of the controls. A possible explanation for these findings is that the silvery scales, abnormal keratin expression, and increased involucrin levels in psoriasis increase the AF in the corneal layer. Portugal-Cohen et al. (10) also reported significantly higher AF in the lesional and non-lesional skin of psoriasis patients than in healthy skin of controls. The higher fluorescence was attributed to tryptophan moieties in both

the lesional and non-lesional skin of the patients. An increased tryptophan signal in psoriasis was described previously and was found to be an indicator of the proliferation and increased thickness of the epidermis (21, 22). An increase in collagen synthesis in psoriasis was demonstrated in both *in vitro* and *in vivo* models of the disease (23). In biopsies obtained from lesional and non-lesional skin areas of psoriatic patients, the rate of collagen synthesis was higher than that in skin of healthy volunteers. Therefore, it is likely that the increased synthesis of collagen in psoriasis increases dermal AF.

A correlation analysis between severity parameters and the AF intensity in the lesional skin of AD and psoriasis patients was conducted with the goal of establishing an objective disease-monitoring tool. AF was correlated negatively with the SCORAD index, erythema, edema/papulation/induration, oozing/crusting, and lichenification in AD and positively with the degree of erythema, thickness, and scale in psoriasis. In the latter disease, the intensity of AF increased significantly as the degree of erythema increased. Therefore, non-invasive measurements of skin AF *in vivo* may serve not

only as a complementary analysis to improve diagnostic accuracy but also as a monitoring tool for AD and psoriasis.

Conclusion

A low AF intensity was shown to be associated with AD, and a high AF intensity peaking around 620 nm with psoriasis. In addition, in both diseases, AF intensity was associated with severity of lesion. Therefore, skin AF measurement *in vivo* offers a non-invasive tool to improve diagnostic accuracy and monitor treatment in patients with AD and psoriasis.

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Conflicts of Interest

None declared.

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