

Lack of differentiation of allergic and irritant reactions by skin autofluorescence

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Contact dermatitis is an inflammatory eczematous skin disease that is mostly caused by chemicals or metal ions that exert toxic effects without inducing a T cell response (contact irritants), or by small reactive chemicals that modify proteins and induce innate and adaptive immune responses (contact allergens) (1). Differentiation of acute allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD) is important in order to ensure proper therapeutic and management strategies.

Several non-invasive methods, based on various physical principles, have been used in research to distinguish between allergic and irritant reactions. Among these, reflectance confocal microscopy (RCM) has been shown to be a promising tool for the differentiation of acute ACD and ICD *in vivo* (2).

Skin has many autofluorescent components, or fluorophores, which contain NADH, FAD, keratin, porphyrins, melanin, collagen, and elastin (3). Each fluorophore has characteristic excitation and emission wavelengths, a phenomenon that enables the localization and further quantification of a particular fluorophore. The purpose of the present study was to examine whether the measurement of autofluorescence is helpful in diagnosing and differentiating between ACD and ICD.

Methods

A total of 40 patients, aged between 20 and 64 years (mean: 36 years), with a history of contact dermatitis, were recruited from November 2013 to September 2015. Informed consent was obtained from all patients. This study was approved by the Kyung Hee Medical Hospital Institutional Review Board (KHUHMDIRB 1304-01). The exclusion criteria were immunodeficiency, a history of immunosuppressant use within 6 months before treatment, a cosmetic procedure to test areas within 1 year, and a chronic disease that could influence skin autofluorescence. The ACD group included 30 patients who were patch tested on the upper back for 48 h of occlusion with the TRUE Test® (Mekos Laboratories, Hillerød, Denmark). Readings were performed on day (D) 4, at which

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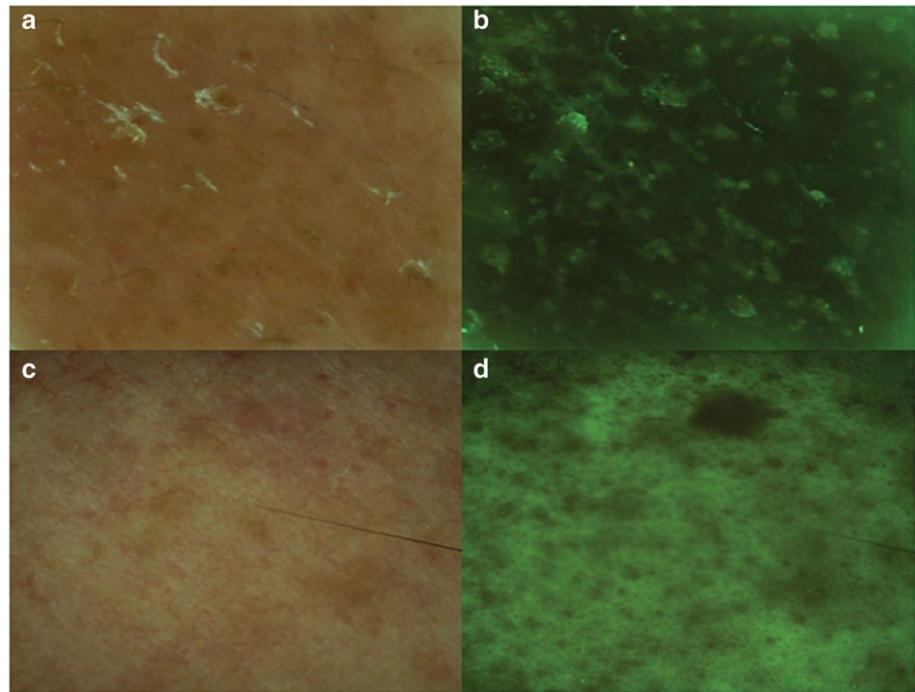


Fig. 1. Representative porphyrin fluorescence images of allergic contact dermatitis [(a) polarized image; (b) autofluorescence image] and irritant contact dermatitis [(c) polarized image; (d) autofluorescence image].

time we measured autofluorescence. The intensity of the reaction was scored and recorded according to the rules of the ICDRG (4). Ten patients with ACD excluded were clinically diagnosed with ICD at our clinic. For cases of ACD or ICD, autofluorescence was measured on a contact dermatitis lesion and on a non-lesion on the opposite side by use of a fluorescence detection device (ECOSkin®; Cubitz, Seoul, Korea) on D4. The fluorescence was excited by an array of LEDs with excitation at 408 nm. The autofluorescence intensities of different groups were compared by measuring fluorescence emission peak intensities at 620 nm (R), 530 nm (G), and 470 nm (B). In order to compare ACD and ICD, the average difference in autofluorescence between lesional and non-lesional skin was calculated. Fluorescence photographs were acquired (Fig. 1), and the resulting digital images were fed into a computer that processed and analysed them with a special program, as in our previous study (5). For statistical analysis, the Mann–Whitney test was performed using IBM SPSS ver. 22 (IBM Corp., Armonk, NY, USA) to identify any significant differences between ACD and ICD. A p -value of <0.05 was regarded as statistically significant.

Results

In the ACD group, the average fluorescence intensities of each signal (R, G, and B) of the contact dermatitis lesions were lower than those in non-lesional skin ($p = 0.0001$,

$p = 0.0002$, and $p = 0.0001$, respectively). All of the differences were statistically significant (Fig. 2a). In the ICD group, the average fluorescence intensities of each signal of the contact dermatitis lesions were also lower than those of the non-lesional skin ($p = 0.007$, $p = 0.011$, and $p = 0.052$, respectively). Similarly, the differences in R and G were statistically significant. However, the difference in B signals was not statistically significant (Fig. 2b). The average difference in fluorescent intensity of the ACD group was lower than that of the ICD group. None of these differences was statistically significant (Fig. 2c).

Discussion

Acute ACD and ICD are common conditions, and can be remarkably similar both clinically and histologically, despite different pathogenesis. ACD is mediated by memory T lymphocytes, and requires previous sensitization by an allergen, producing a delayed type of hypersensitivity inflammatory reaction. In contrast, a common feature of ICD is external damage to the epidermis, with a subsequent inflammatory reaction. The initial keratinocyte damage can be caused by damage to the cell membrane, removal of surface lipids, or direct cytotoxic effects (6).

The reading of weak positive allergic reactions and some irritant reactions may cause difficulties. Therefore, different non-invasive tests have been used in an attempt to improve diagnostic specificity. Transepidermal water loss has been shown to be higher in ICD than ACD,

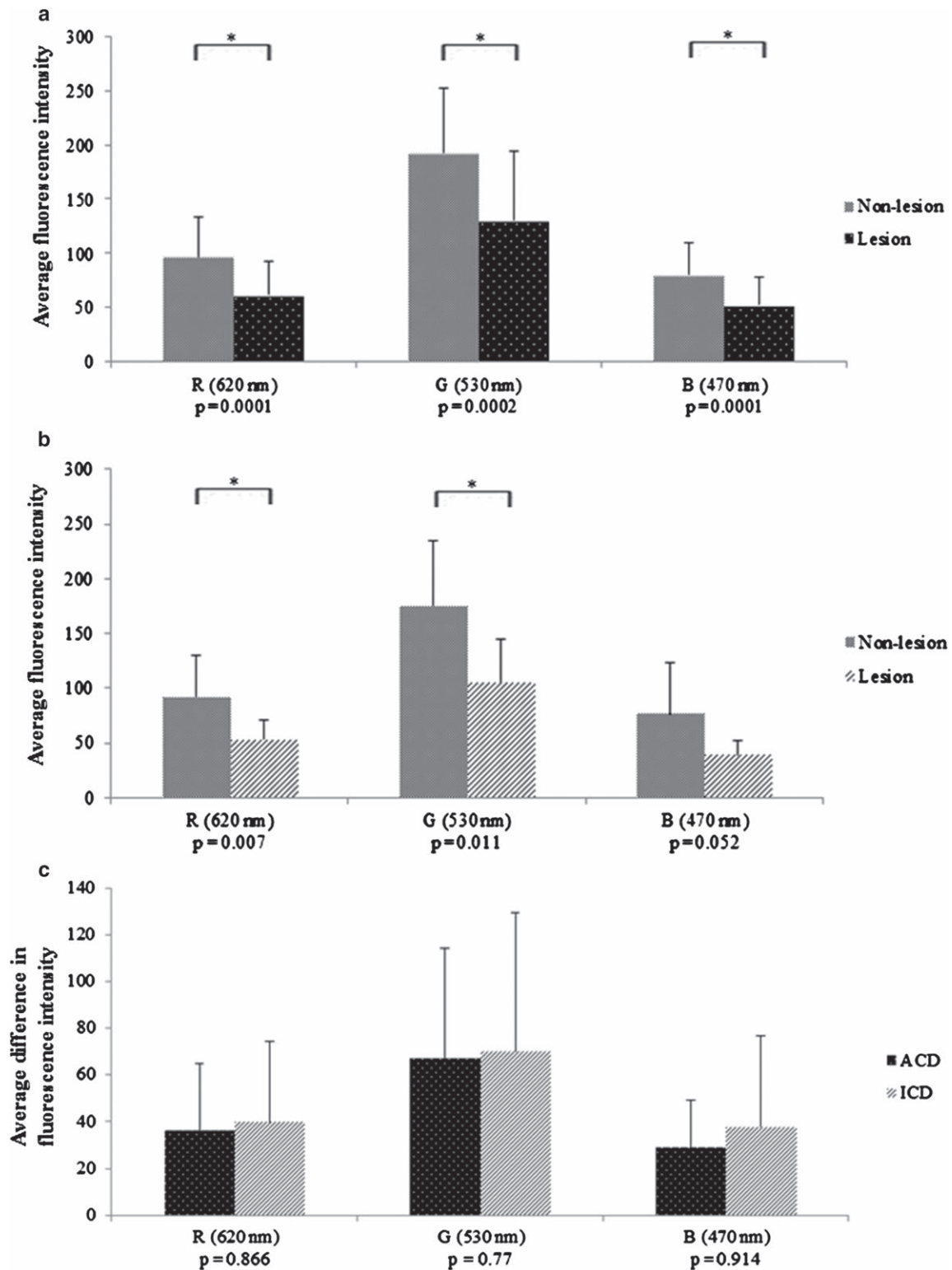


Fig. 2. (a) Average autofluorescence differences in allergic contact dermatitis (ACD) between lesions and non-lesions. (b) Average autofluorescence differences in irritant contact dermatitis (ICD) between lesions and non-lesions. (c) Average difference in autofluorescence intensity between ACD and ICD.

infrared thermography has shown 'hot' spots in ACD but 'cold' spots in ICD, and ultrasound has shown more transcutaneous oedema in ACD and more superficial oedema in ICD (7). The magnitudes of electrical impedance indices was significantly lower in irritant than in allergic reactions (7). *In vivo* RCM has been introduced as a useful tool for the differentiation of ACD and ICD. ICD is characterized by greater disruption of the stratum corneum, a heavier inflammatory infiltrate in the upper epidermal layers and more pronounced parakeratosis than is seen in ACD. Acute ACD, on the other hand, tends to show no or mild stratum corneum changes and more severe vesicle formation, mainly involving the lower epidermal layers (8).

In our study, lesional skin in ACD had lower autofluorescence intensity than non-lesional skin and skin in ICD patients. However, the difference in autofluorescence between ACD and ICD is not significant. In ICD, scaling and reactive epidermal proliferation can increase autofluorescence, because of keratin. However, the dermal infiltrate was deeper and more intense in ACD (8). Therefore, we hypothesized that dermal inflammatory cell infiltration results in lower autofluorescence values, and that decreased amounts of collagen also result in limited autofluorescence.

The main component that influences autofluorescence according to the location of skin is known to be related to the distribution of sebaceous glands and sun exposure (9). To control for these factors affecting skin autofluorescence, autofluorescence of skin lesions was measured in the trunk and was compared with that of non-lesional skin on the opposite side. Although more research is

needed, the difference according to body site should be considered as a factor that affects autofluorescence in dermatological practice.

Two limitations of our study are that the number of patients is small, and we have compared patch test-induced acute ACD with ICD with an unknown cause. Previous data have shown that the morphology of ICD varies with different irritants. For example, the predominant feature of sodium lauryl sulfate-induced ICD is parakeratosis, with spongiosis generally being present. Nonanoic acid, on the other hand, produces tongues of dyskeratotic cells within the granular and spinous layers, whereas croton oil produces spongiosis and exocytosis (10). Other limitations are that the patch test can also lead to ICD, and that the autofluorescence spectrum shows considerable overlap between collagen and keratin. We did not analyse each element in each skin layer, but instead measured the total surface autofluorescence. Future work should therefore evaluate ICD that is induced by the same irritant, and analyse the difference in autofluorescence intensity according to severity.

Although our present study shows that the autofluorescence intensities of contact dermatitis are significantly lower than those in non-lesional skin, there were no significant differences between ACD and ICD. Skin autofluorescence is not suitable for distinguishing between contact reactions of an allergic and irritant nature. Further larger-scale *in vivo* studies on skin autofluorescence will be helpful to clarify the differences between ACD and ICD.

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