

Diagnostic utility of skin autofluorescence when patch test results are doubtful

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Abstract

Background: The standard diagnostic test for allergic contact dermatitis is the patch test, which can also be used to identify irritant contact dermatitis. Doubtful reactions (?+) can be often clinically relevant to individuals and can require additional tests.

Objectives: The purpose of this study was to examine whether autofluorescence (AF) measurements in patients with doubtful reactions are helpful in diagnosing contact dermatitis.

Methods: Patients with a history of contact dermatitis were patch tested on the upper back for 48-hours of occlusion using aqueous solutions of 5% sodium lauryl sulfate. Reaction intensity was scored, and AF was measured on reactive lesions and non-lesions. Three dermatologists classified the results as positive or negative using the fluorescence photographs of patients with a doubtful reaction.

Results: Among doubtful reactions, the R/G% values were significantly higher in the AF-based positive group than in the negative group ($P = .0086$). On the other hand, the heterogeneity values of R, G, and B (HR, HG, HB) were significantly lower in the AF-based positive group ($P = .0026, .0046, .0004$ respectively).

Conclusions: Measuring AF along with the clinical readings can help confirm doubtful patch test reactions.

KEYWORDS

allergic contact dermatitis, autofluorescence, doubtful reaction, patch test

1 | INTRODUCTION

Contact dermatitis (CD) is an inflammatory eczematous skin disorder caused by contact with an irritant that induces toxic effects without causing T cell reactions or by contact with an allergen, generally small reactive chemicals, which causes innate and adaptive immune reactions. The standard diagnostic test for allergic contact dermatitis (ACD) is the patch test, which can also be used to identify irritant contact dermatitis (ICD).¹ Patch testing proceeds as follows: Allergens are placed on the skin on the first day, and the skin reaction is analyzed by removing the allergen after about 48 hours. The relevance of the patch test response is determined by the skin reaction following exposure to the causative material. The North American Contact Dermatitis Group

(NACDG) distinguishes skin reactions as follows: (1) negative reaction as macular erythema, (2) erythema, infiltration and possibly papules, (3) erythematous papules or vesicles (or both), (4) spreading blisters or crusts into ulceration (or both), (5) irritation reactions.

However, sometimes allergic patch test results cannot clearly recognize macular erythema, the grade between a negative and positive reaction.² The importance and relevance of doubtful (or faint macular erythema) reactions have been debated. Questionable reactions (?+) can often be clinically relevant to individuals and might require additional tests, such as a repeated patch test with serial concentrations or a use test.³ A questionable reaction might also indicate a mild irritant or sensitizer, such as in a safety assessment screening for cosmetics.

Several components of the skin, including nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide (FAD), keratin, lipofuscin, elastin, collagen, tryptophan, melanin, and porphyrins, emit autofluorescence (AF). Each fluorophore has a unique excitation and emission wavelength, which allows localization and further quantitation of specific fluorophores.⁴ Changes in AF can cause changes in AF expression, reflecting histological changes in CD. Our purpose in this study was to examine whether measuring AF in patients with doubtful patch test reactions is helpful in diagnosing CD.

2 | METHODS

A total of 73 patients with positive findings in cosmetic as is testing were recruited, and informed consent was obtained from all patients. This study was approved by the DERMAPRO Ltd. Institutional Review Board (1-220777-A-N-02-DICN15157). Exclusion criteria were immunodeficiency, history of immunosuppressant use within 6 months of treatment, and a chronic wasting

disease that could influence skin AF. All patients were patch tested on the upper back for 48-hours of occlusion using aqueous solutions of 5% sodium lauryl sulfate (SLS). The squalane patch test was used as a negative control (non-lesion). Readings, including AF, were performed on day 4. Reaction intensity was scored and recorded according to the rules of the International Contact Dermatitis Research Group (ICDRG).⁵ AF was measured on CD lesions and non-lesions on opposite sides using a fluorescence detection device (ECOSkin®; Cubitz, Seoul, Korea). Fluorescence was excited at 408 nm using an array of LEDs. The fluorescence pattern was recorded using an RGB detector with sensitivity maxima in the color channels at 620 nm (red channel; R), 530 nm (green channel; G), and 470 nm (blue channel; B) and with heterogeneity (H) of R, G, and B (HR, HG, and HR, respectively). Fluorescence photographs were obtained (Figure 1), and the resulting digital images were processed and analyzed using a special computer program as explained elsewhere.⁶

Three dermatologists classified results as positive or negative using the fluorescence photographs of patients with doubtful reactions. The criteria for positive fluorescence were darker lesions

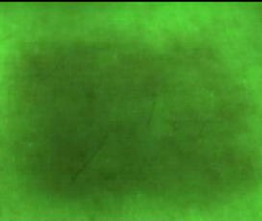
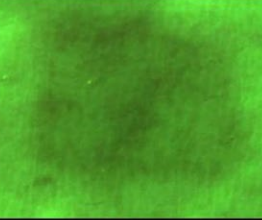
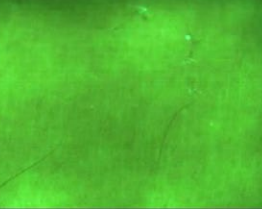
		Representative porphyrin fluorescence images of CD	
Reading of patch test	Reading of AF	Polarized image	AF image
Weak positive (+)			
Doubtful (?+)	+		
	-		
Negative (-)			

FIGURE 1 Autofluorescence (AF) images of contact dermatitis (CD) according to patch test reactions

in three corners or 50%-70% of the area. The agreement of two or more physicians was considered meaningful.

The difference in AF was compared between the positive and negative groups. To calibrate the intensity differences in individual patients, we used the non-lesion intensity minus the AF intensity of the positive or doubtful reaction. The AF intensities of each parameter are presented as mean $\pm$ standard deviation (SD) or median with inter-quartile range (IQR). Average between-group differences were compared using the Mann-Whitney *U* test. AF intensities were compared using the Chi-square and Fisher exact tests. To determine the cut-off point of independent parameters, receiver operating characteristic (ROC) curves were used. The specificity, sensitivity, and accuracy were determined at the cut-off point. Data were analyzed using SPSS for Windows (version 23.0, SPSS Inc., Chicago, IL, USA). For all statistical tests, a *P* value $<.05$ was considered statistically significant.

3 | RESULTS

A total of 73 female patients were enrolled in our study. The mean age was 41.6 ± 6.83 years. A total of 60 positive patch test reactions (+) and 56 doubtful reactions (?+) were observed. Of the doubtful reactions, 40 were classified as positive and 16 were classified as negative using fluorescence photography. AF data from both the positive and negative groups are shown in Table 1.

In the positive group, the R/G% values were significantly higher than they were in the negative group ($P = .0086$). Conversely, the heterogeneity values (HR, HG, HB) were significantly lower in the positive group ($P = .0026, .0046, .0004$, respectively).

Among those parameters, the R/G% parameter showed the highest sensitivity, specificity, and accuracy ($P = .0016$).

4 | DISCUSSION

CD can be suspected based on clinical manifestations of lesions, the distribution of dermatitis, and lack of other causes or associated systemic disease. However, it is clinically difficult to find the trigger for CD when the cause is repeated contact with a mild irritant or weak allergen. Patch testing is the gold standard method for diagnosing ACD and can also be used to identify ICD. Readings of a weak positive allergic reaction or a doubtful reaction can cause difficulties. Therefore, additional tests have been used to improve diagnostic specificity. Intradermal testing is recommended to confirm doubtful patch test reactions, especially a false positive reaction to metals.⁷ In addition, doubling the dose per area can detect significantly more contact allergies to formaldehyde, but it can cause too much irritation at high test concentrations.⁸ Histologically, CD shows spongiosis, acanthosis, and superficial perivascular lymphohistiocytic infiltrates. Because physical and histological examination alone cannot distinguish between

TABLE 1 (A) Autofluorescence (AF) intensity of contact dermatitis (CD) in both positive and negative groups expressed as mean $\pm$ standard deviation (SD) and median (interquartile range). (B) Comparison of the sensitivity, specificity, accuracy, and area under the curve (AUC) of different AF parameters between the positive and negative CD groups

(A) Parameter ^a	Total (N = 116)				Positive (N = 100)				Negative (N = 16)				<i>P</i> -value ^b
	Mean	SD	Median	IQR	Mean	SD	Median	IQR	Mean	SD	Median	IQR	
R	3.40	12.22	3.95	9.97	3.94	12.26	4.00	10.02	−0.01	11.80	0.82	13.68	.1007
G	2.07	5.70	0.00	1.88	1.92	5.43	0.20	1.88	2.98	7.35	0.00	1.89	.4132
B	2.62	22.94	5.83	11.55	2.69	24.31	6.39	11.12	2.16	11.45	1.70	16.61	.1838
R/G%	1.08	13.36	2.17	10.72	1.86	13.83	2.27	10.48	−3.78	8.80	−4.30	12.66	.0086
B/G%	0.30	26.95	2.99	9.13	0.43	28.84	3.08	10.00	−0.51	8.71	0.00	14.38	.0578
B/R%	−0.07	9.20	0.67	4.58	−0.52	9.66	0.00	4.34	2.77	4.81	3.08	5.70	.0561
HR	−9.52	11.54	−7.50	14.86	−10.70	11.64	−8.42	14.49	−2.16	7.72	0.00	9.31	.0026
HG	−39.62	65.89	−19.18	44.46	−43.58	65.26	−21.41	46.15	−14.90	66.50	4.13	47.74	.0046
HB	−10.89	11.02	−10.42	12.99	−12.13	10.65	−11.39	12.50	−3.15	10.44	−1.47	10.48	.0004
(B) Parameter ^a	AUC	95% CI	Cut-off value	<i>P</i> -value ^b	Sensitivity (%)	Specificity (%)	Accuracy (%)	<i>P</i> -value ^b					
R/G%	0.71	0.562-0.848	−4.05	.0086	83.00	56.25	79.31	.0016					
HR	0.74	0.611-0.86	−4.60	.0026	33.00	31.25	32.76	.0062					
HG	0.72	0.556-0.886	−14.08	.0046	36.00	31.25	35.34	.0132					
HB	0.77	0.645-0.903	−6.66	.0004	29.00	25.00	28.45	.0004					

SD, standard deviation; IQR, inter quartile range; AUC, area under the curve; CI, confidence interval; R, AF intensity peaking around 620 nm; G, AF intensity peaking around 530 nm; B, AF intensity peaking around 470 nm; R/G, the ratios of the signal in the R channel to the signals in the G; B/G, the ratios of the signal in the B channel to the signals in the G; B/R, the ratios of the signal in the B channel to the signals in the R; HR, heterogeneity of R value; HG, heterogeneity of G value; HB, heterogeneity of B value.

^aEach parameter = (AF intensity of the non-lesion) - (AF intensity of the positive reaction).

^bA *P*-value less than .05 is considered statistically significant.

ACD and ICD, it is necessary to conduct patch testing and take a history of exposure to contact allergens.⁹ However, specific differences could help to differentiate them. For example, early spongiosis and lymphocytic exocytosis of the follicular infundibulum are common in ACD, but hardly any changes were seen in ICD.¹⁰

In our previous study, lesional skin in CD had lower AF intensity than non-lesional skin.¹¹ Therefore, we here investigated whether measuring AF intensity could help distinguish irritant tendency for allergens that shows a doubtful patch test reaction in CD.

In this study, the parameters showing significant values were R/G%, HR, HG, and HB. The R/G% values were significantly higher and the heterogeneity values (HR, HG, HB) were significantly lower in the positive group. However, because the values given in the results were obtained by subtracting the CD value from the fluorescence value of the non-lesion area, the R/G% is actually lower and heterogeneity is actually higher in the positive group. We hypothesize that spongiosis in the epidermis and dermal cellular infiltration and edema, all of which are characteristics of CD, might have caused lower R values by reducing the fluorescence intensity of endogenous fluorophores such as keratin and collagen. In addition, in CD the skin is temporarily alkalinized, and the number of *Propionibacterium acnes* producing porphyrins decreases, resulting in a decrease in R value. The increased heterogeneity in CD patients could result from an increase in the irregular elongation of the rete ridge as the blood vessels of the dermal papilla expand.

One of the difficulties with research studies of this design is the small number of patients in the negative group. A second limitation is that we could not compare AF intensities without the patch test. Third, the AF spectrum shows considerable overlap between collagen and keratin. We did not analyze each element in each skin layer but instead measured the total surface AF. There is also the possibility of classification bias in the division of doubtful reactions into positive and negative groups.

In conclusion, patch test reactions that are graded as doubtful are common and often clinically relevant. Measuring AF was helpful in classifying doubtful patch test reactions. Large-scale in vivo studies of skin AF will help to clarify the AF characteristics in doubtful reactions.

ACKNOWLEDGEMENTS

This work was supported by a grant from Kyung Hee University in 2018 (KHU-20181041).

CONFLICT OF INTEREST

None.

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How to cite this article: Shin E-J, Seo J-K, Lee EJ, Lee ES, Shin MK. Diagnostic utility of skin autofluorescence when patch test results are doubtful. *Skin Res Technol*. 2018;00:1-4. <https://doi.org/10.1111/srt.12615>