

Increase in concentration of patch test allergen reduces patch test occlusion time to 12 hours without affecting patch test reactivity in patients with parthenium dermatitis

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Abstract:

Background: Patch testing is the standard method to diagnose contact allergy. Patches are applied for 48 hours, which is inconvenient to patients in tropical weather. Therefore, we evaluated different patch test occlusion times with increased concentrations of an allergen to determine if occlusion time can be reduced without compromising on patch test positivity.

Methods: Patch test positive patients with parthenium dermatitis were enrolled and patch tested using 5 different concentrations (10%, 4%, 2%, 1% and 0.5%) of parthenium extract. The patches were applied in triplicate. The first set was removed after 12 hours while the 2nd and 3rd sets were removed after 24 and 48 hours, respectively. Readings were performed at 24, 48 and 96 hours.

Results: Fifty patients with parthenium dermatitis were included. The positive patch test reaction rates were comparable in all three sets at 24 and 48-hours reading irrespective of the occlusion time. All were positive with 10%, 4% and 2% concentrations at 96 hours reading with an occlusion time of 12 hours.

Conclusion: An occlusion time of 12 hours seems adequate to elicit positive patch test reaction at a 96-hour reading if the concentration of patch test allergen can be increased, eg from 1% to 2% in these patients.

Key words: Patch test, occlusion time, parthenium dermatitis

1. Introduction

Contact dermatitis is an inflammatory reaction of the skin due to direct contact with a noxious agent. The diagnosis of contact dermatitis is based on a detailed history and clinical evaluation, particularly the pattern of dermatitis. However, patch testing is the gold standard test to diagnose contact allergy. The conventional patch test procedure involves application of patches on back of patients for 48 hours and the test readings are performed at day 2, day 4 and sometimes on day 6/7 as per ICDRG guidelines.¹ The patients are advised not to take a bath or clean the test site until the

last reading, which is very inconvenient. In tropical weather conditions, 48 hours occlusion is particularly uncomfortable due to sweating and irritation/itching because of this longer occlusion time and more so if the dermatitis develops at the patch test sites due to positive reactions. Many times, the patches come off soon after application due to sweating, especially in patients of lower socioeconomic strata who do not have air-conditioned living and working conditions, and rural areas, making the test wasteful and inconclusive. Some previous studies have shown that the patch occlusion time can be decreased by increasing the concentration of allergen.²⁻⁵ Therefore, in this prospective study we evaluated if patch test occlusion time can be reduced by using higher concentrations of patch test allergen without compromising on the patch test reactivity. Since parthenium is the most common allergen causing contact dermatitis in our setting, we tested this hypothesis in patients of parthenium dermatitis.

2. Materials and methods

2.1 Subjects

Clinically diagnosed and patch test positive patients with parthenium dermatitis, who were earlier patch tested using parthenium acetone extract with 1% and 0.5% concentrations (the standard concentrations used for patch test in parthenium dermatitis) were enrolled in the study after a detailed history and clinical examination. Patients below 18 years of age, with erythroderma or active dermatitis on the back, concurrent bacterial or viral infection at the patch test site, known sensitivity to adhesive plaster, receiving systemic immunosuppressant in the last 4 weeks, or applying topical corticosteroids on the back in the last 1 week were excluded from the study. Ethical approval for the study was obtained from the Institutional ethics board. Written informed consents were obtained before recruitment.

2.2 Preparation of the hapten in different concentrations

Parthenium plant extract and the different concentrations of the allergen were prepared as per the method standardised in our laboratory.⁶ Fully grown and healthy looking leaves of parthenium plant were taken, washed under tap water to remove contaminants and air-dried at room temperature.

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Completely dehydrated and brittle leaves were blended to make into powder. Fifty grams of this powder was mixed with 500 mL of acetone and the flask, covered with aluminium foil or flask paper, was kept at room temperature for 24 hours, with frequent shaking. Then the mixture was filtered with Whatman filter paper no.1 to obtain parthenium acetone extract. These solutions were stored in dark brown bottles with tight containers in refrigerator at a temperature of 4 to 8 degrees Celsius. The stock solution was replaced after every 3 months. For patch testing, the solution thus obtained at a concentration of 10% (weight/volume) was further diluted with acetone to make concentrations of 10%, 4%, 2%, 1% and 0.5%. One microdrop (15 μ L) of extract of each concentration was taken with a micropipette and put on Whatman filter paper no.3 positioned in Finn chambers (Systopic Laboratories, New Delhi, India). These Finn chambers (8 mm diameter) attached to micropore plaster were applied on the patient's back in triplicate sets and occluded with micropore adhesives.

2.3 Patch test

Patch testing was performed using 5 different concentrations of parthenium acetone extract viz., 10%, 4%, 2%, 1% and 0.5%. The patches were applied in triplicate and were labelled from left to right as set 1, set 2 and set 3, respectively. The first set of patches was removed by the patient him/herself after 12 hours while the 2nd and the 3rd sets were removed after 24 and 48 hours, respectively, under supervision. Patch test readings were performed at 24 hours and 48 hours in all the patients as per ICDRG guidelines while a 96 hour reading was recorded in 22 patients.¹

2.4 Statistical analysis

Data were presented as mean $\pm$ SD or number (%) as appropriate. The shift in the frequency between the two readings at each occlusion time was compared using McNemar's test. The difference in proportions between two occlusion times at each reading was compared using the chi-square test. A *P*-value <.05 was considered statistically significant. Statistical analysis was carried out using Stata12.0 (StataCorp, College Station, Texas, USA).

3. Results

The study included 50 patients with parthenium dermatitis (37 males and 13 females) between 23-80 years of age (mean age: 50.78 ± 11.44 years) having the disease for 2 to 40 years (mean: 10.92 ± 9.03 years). The majority (36%) of them were farmers/laborers and had summer exacerbation of the disease.

Patch test positivity in patches occluded for 12 hours (set 1), 24 hours (set 2) and 48 hours (set 3) with readings at 24 and 48 hours is shown in table 1. On comparison of positive patch test reactions between different sets at 24 hours and 48 hours reading, the values were comparable and statistically not significantly different (Table 1). However, there was a significant increase in patch test positivity from 24 hours to 48 hours reading.

A 96 hours reading in 22 patients showed 100% positivity rate in all 3 sets with higher concentrations (10%, 4% and 2%), irrespective of their occlusion times. The positivity rate reduced to 90.9% (20/22) with lower concentrations (0.5% and 1%) of allergens in set 1. The intensity of patch test reactions with varied concentrations of the allergen at different occlusion times is shown in table 3.

4. Discussion

Contact dermatitis due to parthenium is common in the general population and amongst patients attending contact dermatitis clinics in India.⁷ Patch testing is the gold standard method of diagnosing allergic contact dermatitis. Previous studies have shown that patch test occlusion time can be reduced by increasing the concentration of allergen.²⁻⁵ Bruze et al demonstrated equivalent patch reactions to nickel sulfate when higher concentrations of allergen were used under occlusion for five hours and lower concentrations of allergen were occluded for 48 hours.³ Kosann et al showed that higher concentrations of potassium dichromate were required to elicit patch test reaction at shorter occlusion time of 6 hours versus 48 hours.⁴ However, the ratio of 6-hour to 48-hour minimum elicitation threshold exhibited a wide inter-individual variability in their study; hence, the 6-hour patch test was not recommended for routine testing to diagnose contact allergy to dichromate.⁴

Considering the difference in occlusion time in our study, there was only a marginal increase in positivity rate when occlusion time of patches was increased from 12 hours to 24/48 hours, which was statistically not significant. However, there was a significant increase in positive patch test reactions when the readings were performed at 48/96 hours compared to reading at 24 hours in different sets. The intensity of patch test reaction also increased with an increase in allergen concentration and occlusion time, though this was not uniform.

A 96 hours reading in 22 patients showed 100% positive reactions with all the five concentrations on 24 and 48 hours occlusion while similar results were seen on 12 hours occlusion with higher concentrations only i.e. 10%, 4% and 2%. Therefore, if the concentration of patch test allergen is doubled (from 1% to 2%), the occlusion time of 12-hours may be sufficient to elicit positive patch test reaction at 96-hours reading.

Our results are similar to the study by Hextall et al, where 4 different concentrations (0.01%, 0.1%, 0.3% and 1%) of *p*-phenylenediamine and different occlusion times of 1, 2, 5, 10, 15, 30 and 120 minutes were used in 19 patients who had been previously diagnosed as sensitized to PPD. They observed 69% positive patch test reactions with higher (1%) concentrations of PPD compared to 22% positive reactions with lower (0.01%) concentration when the patches were read at 120 minutes.² In a multicentric study, Bruze et al also showed that higher concentration of MCI/MI produced more patch test reactions when occluded for 48 hours.⁵ Ale et al have also reported equivalent patch test reactions in patches applied in duplicate sets, occluded for 24 hours and 48 hours, and readings performed at 96 hours with a high positive concordance of 85.9% reactions between two sets, but more + reactions were seen in the set occluded for 24 hours and more ++ reactions were seen in the set occluded for 48 hours.⁸ Similarly, our study has shown that higher concentrations of allergen produced more positive reactions at a given occlusion time. However, comparable positive patch test reactions were observed with occlusion times of 12 hours, 24 hours, and 48 hours when readings were performed at 48 hours. Our study had the limitations of small sample size and unsupervised removal of set 1 (12 hours occlusion).

5. Conclusion

An occlusion time of 12 hours seems adequate if the concentration of the patch test allergen can be increased, eg from 1% to 2%, to elicit positive patch test reaction at 96 hours (D4) reading in patients of parthenium dermatitis. An occlusion time of 24 hours also seems sufficient to demonstrate patch test positivity at 96 hours (D4) reading even with standard concentration of allergen, ie 1% in these patients. Further studies, however, are needed to confirm our results and similar studies with other allergens are also warranted before reduction in patch test occlusion time can be recommended for routine patch testing.

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Table 1. Patch test positivity in patches with different occlusion times while readings performed at 24 hours and 48 hours (n=44).

Concentration of allergen	SET 1 Occlusion Time 12 hrs			SET 2 Occlusion Time 24 hours			P-value ^a	SET 3 Occlusion Time 48 hours	P-value ^b
	Reading at 24 hours, N (%)	Reading at 48 hours, N (%)	P-value	Reading at 24 hours, N (%)	Reading at 48 hours, N (%)	P-value		Reading at 48 hours, N (%)	
10%	33 (66%)	42 (84%)	.002	38 (76%)	42 (84%)	.045	.27	44 (88%)	.81
4%	28 (56%)	38 (76%)	.007	34 (68%)	39 (78%)	.095	.22	44 (88%)	.27
2%	26 (52%)	31 (62%)	.058	27 (54%)	33 (66%)	.033	.84	38 (76%)	.30
1%	25 (50%)	26 (52%)	.74	23 (46%)	29 (58%)	.033	.69	35 (70%)	.17
0.5%	22 (44%)	24 (48%)	.41	21 (42%)	23 (46%)	.41	.91	33 (66%)	.18

^a Comparison of readings at 24 hours between set 1 and set 2, chi-square test, *P*-value <.05, statistically significant

^b Comparison of readings at 48 hours between set 1, set 2 and set 3, chi-square test, *P*-value <.05, statistically significant

Table 2: Patch test positivity with different occlusion times while readings performed at 24 hours, 48 hours and 96 hours (n=22).

Concentration of allergen	Positive patch test reactions in Set 1 (occlusion time of 12 hours) N (%)				Positive patch test reactions in Set 2 (occlusion time of 24 hours) N (%)				Positive patch test reactions in Set 1 (occlusion time of 48 hours) N (%)		
	Reading at 24 hours	Reading at 48 hours	Reading at 96 hours	P-value	Reading at 24 hours	Reading at 48 hours	Reading at 96 hours	P-value	Reading at 48 hours	Reading at 96 hours	P-value
10%	18 (81.8)	21 (95.4)	22 (100)	.045* .32**	21 (95.4)	21 (95.4)	22 (100)	.32* .32**	22 (100)	22 (100)	-
4%	14 (63.6)	18 (81.8)	22 (100)	.004* .025**	20 (90.9)	21 (95.4)	22 (100)	.16* .16**	22 (100)	22 (100)	-
2%	12 (54.5)	14 (63.6)	22 (100)	.001* .002**	13 (59)	17 (77.2)	22 (100)	.002* .025**	19 (86.3)	22 (100)	.083
	12 (54.5)	13 (59)	20 (90.9)	.004* .001**	11 (50)	13 (59)	22 (100)	.001* .001**	16 (72.7)	22 (100)	.014
	9 (40.9)	11 (50)	20 (90.9)	.001* .001**	9 (40.9)	10 (45.4)	22 (100)	.001* .001**	16 (72.7)	22 (100)	.014

* Comparison of readings at 24 and 48 hours, chi-square test, *P*-value, statistically significant

** Comparison of readings at 48 and 96 hours, chi-square test, *P*-value, statistically significant

Table 3: Grading of severity of patch test reaction with different occlusion times while readings performed at 96 hours (n=22).

Concentration of allergen	Set 1 (occlusion time of 12 hours), N (%)			Set 2 (occlusion time of 24 hours), N (%)			Set 3 (occlusion time of 48 hours), N (%)		
	(+)	(++)	(+++)	(+)	(++)	(+++)	(+)	(++)	(+++)
10%	10 (45.4)	10 (45.4)	2 (9.09)	8(36.3)	13 (59)	1 (4.5)	4 (18.2)	13(59)	5(22.7)
4%	13 (59)	7 (31.8)	2 (9.09)	16 (72.7)	6 (27.2)	0 (0)	11(50)	9 (40.9)	2(9.09)
2%	19 (86.3)	3 (13.6)	0(0)	18 (81.8)	4 (18.2)	0 (0)	13 (59)	7 (31.8)	2(9.09)
1%	18 (81.8)	2 (9.09)	0 (0)	20 (90.9)	2 (9.09)	0 (0)	18 (81.8)	3 (13.6)	1(4.5)
0.5%	19 (86.3)	1 (4.5)	0 (0)	20 (90.9)	2 (9.09)	0 (0)	19 (86.3)	2 (9.09)	1(4.5)