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The Prevalence of New Positive Patch Test Reactions at 168 Hours: A Retrospective Cross-Sectional Study

Yama Testinde 168. Saatte Ortaya Çıkan Yeni Pozitifliklerin Prevalansı: Retrospektif Kesitsel Bir Çalışma

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ABSTRACT Objective: Some clinics interpret patch test results 48 hours after application and an additional reading is performed at 72 or 96 hours. Reports in the literature describe delayed positive reaction to some allergens in patients who underwent patch testing for suspected allergic contact dermatitis. However, an additional late reading may be neglected in some clinics. In this study we aimed to identify allergens with delayed positive patch test reactions and to determine the relevance of an additional day 7 (168 hours) patch test reading. **Material and Methods:** The data of 101 patients who underwent patch testing for suspected allergic contact dermatitis between January 2015 and July 2016 were analyzed retrospectively. Demographic data and patch test results of the patients were evaluated. Allergens without a positive reaction at the 48th and 96th hours but with a positive reaction at the 168th hour were considered as delayed positive patch test reaction. **Results:** Sixty-four (63.3%) of 101 patients had positive reactions to at least 1 allergen. A total of 125 positive reactions were detected. Of the 125 positive reactions, 85 (68%) were positive at 48 hours reading and 31 (24.8%) turned positive at 96 hours evaluation. Nine (7.2%) of 125 positive reactions were late reactions that turned positive at 168 hours. Nickel sulfate, gold sodium thiosulfate, Cl+Me-isothiazolinone, formaldehyde, tixocortol pivalate and hydrocortisone-17-butyrate, and fragrance mix were detected as allergens causing late positive reactions. **Conclusion:** We think that late reading at the 168th hour should not be neglected.

Keywords: Allergens; allergic contact dermatitis; patch tests

ÖZET Amaç: Bazı kliniklerde yama testi sonuçları, uygulamadan 48 saat sonra değerlendirilir ve 72 veya 96 saatlerde ek bir değerlendirme yapılır. Literatürde, alerjik kontakt dermatit şüphesiyle yama testi yapılan hastalarda bazı alerjenlere karşı gecikmiş pozitif reaksiyonlar bildirilmektedir. Ancak bazı kliniklerde, fazladan bir geç okuma ihmal edilebilmektedir. Bu çalışmada, gecikmiş pozitif yama testi reaksiyonları olan alerjenleri tanımlamayı ve fazladan bir 7. gün (168 saat) yama testi okumasının yeni pozitif sonuçları saptamaya katkısını belirlemeyi amaçladık. **Gereç ve Yöntemler:** Ocak 2015-Temmuz 2016 tarihleri arasında, alerjik kontakt dermatit şüphesiyle yama testi yapılan 101 hastaların verileri retrospektif olarak değerlendirildi. Hastaların demografik verileri ve yama testi sonuçları incelendi. Kırk sekiz ve 96. saatte pozitif reaksiyon göstermeyip, 168. saatte pozitif reaksiyon veren alerjenler gecikmiş pozitif yama testi reaksiyonu olarak değerlendirildi. **Bulgular:** Yüz bir hastanın 64'ünde (%63,3) 1 veya birden fazla alerjene karşı pozitif reaksiyon görüldü. Altmış dört hastada toplam 125 pozitif reaksiyon tespit edildi. Saptanan 125 pozitif reaksiyonun 85'inin (%68) 48. saat değerlendirmesinde, 31'inin (%24,8) 96. saatte yapılan değerlendirmede pozitifleştiği görüldü. Dokuz (%7,2) pozitif reaksiyon ise 48 ve 96. saatlerde negatif olmasına karşın 168. saatte pozitifleşen geç reaksiyonlardı. Nikel sülfat, altın sodyum tiyosülfat, Cl+Me-izotiazolinon, formaldehit, tiksokortol pivalat, hidrokortizon-17-butirat ve parfüm karışımı geç pozitif reaksiyonlara neden olan alerjenler olarak tespit edildi. **Sonuç:** Yüz altmış sekizinci saatte yapılacak geç okumanın ihmal edilmemesi gerektiğini düşünüyoruz.

Anahtar Kelimeler: Alerjenler; alerjik kontakt dermatit; yama testi

Contact dermatitis, both irritant and allergic, is an inflammatory cutaneous reaction triggered by direct contact with some chemicals to which people are exposed in daily life.[1] Allergic contact dermatitis (ACD) is a delayed-type hypersensitivity reaction that is revealed when the skin is exposed to a chemical activating antigen specific T cells in a person who has previously been sensitized.[2]

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Patch testing is a regularly used procedure for the diagnosis of ACD resulting from Type IV hypersensitivity. It is used to determine the cause of ACD and intend to stimulate an eczematous reaction to a causative agent applied to the skin.[1,2] Although different protocols are used for patch testing, some clinics interpret results after an occlusion time of 48 hours with an additional reading at Day 3 or Day 4.[1,3,4]

In studies, delayed positive reactions to certain allergens, such as metals, corticosteroids and some preservatives, have been reported in patients undergoing patch testing for suspected ACD. The frequency of delayed positive reactions varied considerably between studies.[1,3,5-9] However, there are some doubts about benefit of the late reading. This requires an additional reading. Therefore some clinics may neglected an additional late reading.[6,7]

In this study, we aimed to determine allergens with late positive patch test results and to find out the relevance of an additional 168 hours patch test reading.

MATERIAL AND METHODS

Ethical approval was received from the Aydın Adnan Menderes University Faculty of Medicine, Non-Interventional Clinical Research Ethics Committee (date: July 28, 2016, no: 2016/922). The study was conducted according to the principles of Declaration of Helsinki.

The data of 101 patients who were admitted to the dermatology outpatient clinic between January 2015 and July 2016 and underwent patch testing for suspected ACD were reviewed retrospectively.

The test was performed by Thin Layer Rapid-Use Epicutaneous Test (T.R.U.E. Test, SmartPractice, Denmark) which included 36 allergens. The patch tests were pasted to the upper back at day 0 (D0) and taken off after 48 hours (D2). Routine interpretations were performed at D2 and D4 (96 hours). An additional reading was performed at D7 (168 hours). The reactions were evaluated with respect to the reading criteria of International Contact Dermatitis Research Group and interpreted as follows: negative reaction (-) shows no reaction; doubtful reaction (?+) shows faint erythema only; weak positive reaction (+) indicates erythema, infiltration, possibly papules; strong positive reaction (++) demonstrates erythema, infiltration, papules, vesicles; finally, extreme positive reaction (+++) indicates intense erythema, infiltrate, coalescing vesicles. A positive patch test result is identified as a reaction which supplies the criteria of at least 1 positive reaction.[1]

The individuals who displayed a negative or doubtful positive reaction at 48 hours and 96 hours, and a positive reaction at 168 hours were interpreted as having late positive patch test reaction.

Statistical analyses were conducted via SPSS 18 (IBM, Armonk, NY). Descriptive analysis, t-test and chi-square test, logistic regression analysis were used in data analysis. In data analysis, $p<0.05$ was considered statistically significant.

RESULTS

Of 101 patients, 64 (63.3%) were female and 37 (36.6 %) were male. The mean age of 101 individuals was 37.6±15.5 years and ranged between 6-74.

Sixty-four (63.3%) patients had positive reactions to at least 1 allergen. Twenty-seven (26.7%) patients had positive reaction to 1 allergen, while 37 (36.6 %) patients had positive reactions to more than 1 allergen. A total of 125 positive reactions were detected in 64 patients. The most common allergens with positive reactions in 101 patients were nickel sulfate (20.8%), thimerosal (13.7%), bacitracin (10.9%), gold sodium thiosulfate (9%), p-phenylenediamine (9%) and cobalt chloride (8%). Of the 125 positive reactions, 85 (68%) were positive at 48 hours reading and 31 (24.8%) turned positive at 96 hours evaluation. Nine (7.2%) of 125 positive reactions were late reactions that became positive at 168 hours (Table 1). Each of the 9 late positive reactions occurred in different patients. In other words, 9 patients displayed new positive reactions on D7. Additionally, 13 reactions (10.4%) which were positive at 48 hours were negative at 96 and 168 hours. Seventeen (13.6%) reactions which were positive at 96 hours were negative at 48 and 168 hours.

There was no difference in age, gender, duration of contact dermatitis and localization of contact der-

TABLE 1: Patch test reaction rates at 48, 96 and 168 hours.

	48 "+"		48 "-" → 96 "+"		48/96 "-"→168 "+"		Total
Allergen	**n**	**%**	**n**	**%**	**n**	**%**	**n**
Nickel sulfate	14	13.9	5	5.0	2	2.0	21
Lanolin alcohol	2	2.0	1	1.0	0	-	3
Neomycin sulfate	0	-	0	-	0	-	0
Potassium dichromate	5	5.0	0	-	0	-	5
Caine mix	0	-	0	-	0	-	0
Fragrance mix	2	2.0	2	2.0	1	1.0	5
Colophony	0	-	0	-	0	-	0
Epoxy resin	1	1.0	1	1.0	0	-	2
Quinoline mix	1	1.0	0	-	0	-	1
Balsam of Peru	0	-	1	1.0	0	-	1
Ethylenediamine dihydrochloride	0	-	0	-	0	-	0
Cobalt chloride	5	5.0	3	3.0	0	-	8
p-tert-butylphenol formaldehyde resin	1	1.0	0	-	0	-	1
Paraben mix	0	-	0	-	0	-	0
Carba mix	1	1.0	2	2.0	0	-	3
Black rubber mix	1	1.0	0	-	0	-	1
Cl+Me-isothiazolinone	5	5.0	0	-	1	1.0	6
Quaternium-15	1	1.0	0	-	0	-	1
Mercaptobenzothiazole	3	3.0	0	-	0	-	3
p-phenylenediamine	5	5.0	4	4.0	0	-	9
Formaldehyde	3	3.0	0	-	1	1.0	4
Mercapto mix	3	3.0	0	-	0	-	3
Thimerosal	11	10.9	3	3.0	0	-	14
Thiuram mix	5	5.0	0	-	0	-	5
Diazolidinyl urea	1	1.0	1	1.0	0	-	2
Imidazolidinyl urea	0	-	1	1.0	0	-	1
Budesonide	1	1.0	1	1.0	0	-	2
Tixocortol-21-pivalate	0	-	0	-	1	1.0	1
Hydrocortisone-17-butyrate	0	-	0	-	1	1.0	1
Gold sodium thiosulfate	6	5.9	1	1.0	2	2.0	9
Disperse blue 106	0	-	0	-	0	-	0
Bronopol	0	-	2	2.0	0	-	2
Bacitracin	8	7.9	3	3.0	0	-	11
Parthenolide	0	-	0	-	0	-	0
Methyldibromo glutaronitrile	0	-	0	-	0	-	0
Negative control	0	-	0	-	0	-	0
Total	85	68	31	24.8	9	7.2	125

matitis between patients with late positive reactions and those without late positive reactions. The characteristics of the patients in both groups are shown in Table 2. In addition, logistic regression analysis was performed and there was no relationship between late positive reaction and variables (age, gender, duration of contact dermatitis and main location of contact dermatitis).

The frequency of late positive reactions and their relative incidence according to the number of all positive reactions for each agent were shown in Table 3.

DISCUSSION

Despite being used for over 100 years, the patch testing procedure varies worldwide. The heterogeneity of patch test practices raises concerns about the need

TABLE 2: Characteristics of patients with late positive reaction and those without late positive reaction.

	Late positive reaction		p value
	No (n=92)	Yes (n=9)	
Age (years) (mean±SD)	37.8±15.3	35.7±18.5	0.691
Sex , n (%)			
Male	32 (86.5)	5 (13.5)	0.282
Female	60 (93.8)	4 (6.3)	
Duration of contact dermatitis (months) (mean±SD)	47.4±60.6	36.4±26.1	0.593
The main location of contact dermatitis, n (%)			
Hands	77 (92.8)	6 (7.2)	0.198
Others	15 (83.3)	3 (16.7)	

SD: Standard deviation.

TABLE 3: Allergens with late positive patch test reactions and their relative incidence.

Allergen	Number of late positive reaction	Number of total positive reaction	Relative incidence (%)
Nickel sulfate	2	21	9.5
Fragrance mix	1	5	20
Cl+Me-isothiazolinone	1	6	16.7
Formaldehyde	1	4	25
Tixocortol-21-pivalate	1	1	100
Hydrocortisone-17-butyrate	1	1	100
Gold sodium thiosulfate	2	9	22.2

for standardization.[10-12] At least 2 readings are recommended in the European Contact Dermatitis Society protocol for patch testing. However, 3 readings (D2 and D3 or D4 and around D7) are recommended for ideal evaluation.[1] Most of the patch test reactions happen at 72 or 96 hours and many centers interpret the reactions at 48 hours and at 72 or 96 hours. This protocol is adequate to determine most positive patch test results. On the other hand, reports in the literature established late positive patch test results that turn to positive at 168 hours in different proportions of patients.[3,5-8,13] Tanno et al performed a survey with 169 professionals from 47 countries and found that only 36 (21%) of the participants performed 3 readings.[10]

The frequency and distribution of contact allergens show variations from country to country. There are many reports investigating the most common contact sensitizers in our country, in Türkiye.[14-16] However, to our knowledge, there are no studies evaluating the late positive patch test reactions in our country. We found 7.2% late reactions that became positive at 168 hours to metals (nickel sulfate and gold sodium thiosulfate), preservatives (Cl+Me-isothiazolinone and formaldehyde), corticosteroids (tixocortol pivalate and hydrocortisone-17-butyrate), and fragrance mix.

Higgins et al found new relevant late reactions in 12.8% of 203 patients at 168 hours to cobalt chloride, disperse blue mix, preservatives, mercury, colophony, fragrances and gentamycin sulfate indicating the need for 168 hour evaluation.[6] Madsen et al. reported that they detected 6,509 positive reactions in 9,997 patients and 13.5% of 6,509 positive reactions were found to be late reactions. The most frequent allergens giving late positive reactions were neomycin, budesonide and hydrocortisone-17-butyrate. They stated that late reading is particularly important because some positive reactions would be missed if only one D3/4 reading had been performed.[8]

Davis et al reviewed 135 patients who underwent patch testing with series of corticosteroid allergens. They found only 2 patients (both patients had a late reaction to budesonide) with late positive reaction to corticosteroids. Therefore, they concluded that

late readings were limiting value in detecting corticosteroid allergies.[17] Davis et al. then conducted another study and found that late readings (D7 or afterwards) were helpful when evaluating reactions to metals and topical antibiotics. However, it did not help to diagnose reactions to other agents, including corticosteroids.[3] Chaudhry et al reviewed 298 patients who received patch testing with metals and corticosteroids. They concluded that additional readings after D7 are helpful to determine reactions to metals (gold, cobalt, palladium, beryllium), preservatives (propolis, dodecyl gallate), and neomycin. However, it was not found useful in detecting delayed reactions to topical corticosteroids.[18] In contrast to these studies, we found late positive reaction to topical corticosteroids (tixocortol-21-pivalate, hydrocortisone-17-butyrate).

Jonker et al. reported that 8.2% of 760 individuals displayed late-positive reactions. The most common allergens that cause late positive reactions were nickel sulfate, neomycin sulfate, butylphenol formaldehyde resin, tixocortol-21-pivalate and Cl+Me isothiazolinone. They concluded that it is worthwhile to perform an additional reading.[7] Considering the reported late reactions in adults, Matiz et al studied this matter in 38 children and determined 13% of the reactions as being late reactions. They also pointed out the significance of assessment for late reactions in children.[19] Recently, van Amerongen et al evaluated 3,292 patients, and 13.6% of the individuals displayed new positive reactions on D7. In this way, it supported the significance of an additional late reading, specifically for neomycin sulfate and corticosteroids.[20] In contrast, we did not find any delayed reaction to antibiotics in our study.

Cantwell et al investigated the allergens with negative results on D5 yet positive on D7 or afterwards and found that delayed reactions occurred in 29.7% of the allergens. The most often delayed reactions occurred with metals or metal alloys and also acrylates.[21] In our study, 7.2% of the allergens were late reactions that became positive at 168 hours. Since acrylates were not present in our pacth test, we could not evaluate the delayed reaction to acrylates.

In earlier studies, various parameters such as higher age, female gender, negative irritant control containing sodium lauryl sulphate and allergen groups of corticosteroids, topicals, metals, frangrances, resins were determined as predictive factors for late positive reaction.[8,20,22,23] However, the influence of gender and age on late positive reaction were not found in our study. In addition, late positive reaction was not associated with duration of contact dermatitis and main location of contact dermatitis in our study.

The limitation of our study is that it was not investigated whether the late positive reaction was clinically relevant or not. Another limitation was the probability of not detecting some late positive reactions due to the small number of our cases.

CONCLUSION

We found that most of the positive test reactions were determined at 48 and 96 hour reading. However 8.9% of patients display late positive reactions, which would be missed unless 168 hours readings were performed. Our results confirm previous reports detecting late reactions to some metals, corticosteroids, some preservatives and fragrance mix. However, any delayed allergy to antibiotics was not detected in our study which may be due to the small size of our patient group. The avoidance of allergens is the only cure for ACD and late reacting preservatives and metals are widely present in the objects around us. Therefore, we suggest that 168 hour late reading should not be neglected in clinics. A prospective multicenter study with the same technical factors and methodology (test systems, allergens, vehicles, concentration, occlusion and reading time) involving more patients will help to make a standardization in patch testing for patients with contact dermatitis.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise,

working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Kübra Eren Bozdağ, Münevver Güven;* ***Design:*** *Münevver Güven, Kübra Eren Bozdağ, Neslihan Şendur;* ***Control/Supervision:*** *Kübra Eren Bozdağ, Münevver Güven;* ***Data Collection and/or Processing:*** *Münevver Güven, Kübra Eren Bozdağ;* ***Analysis and/or Interpretation:*** *Münevver Güven, Kübra Eren Bozdağ;* ***Literature Review:*** *Kübra Eren Bozdağ, Münevver Güven;* ***Writing the Article:*** *Münevver Güven, Kübra Eren Bozdağ;* ***Critical Review:*** *Münevver Güven, Kübra Eren Bozdağ;* ***References and Fundings:*** *Münevver Güven, Kübra Eren Bozdağ;* ***Materials:*** *Münevver Güven, Kübra Eren Bozdağ.*

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