

Evaluation of Skin Patch Test Results and Allergen Elimination in Patients with Idiopathic Recurrent Aphthous Stomatitis

İdiyopatik Rekürren Aftöz Stomatit Hastalarında Deri Yama Testi Sonuçlarının ve Allerjen Eliminasyonunun Değerlendirilmesi

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ABSTRACT Objective: The etiology of recurrent aphthous stomatitis (RAS) has not been clarified yet. However, the role of type IV hypersensitivity that was diagnosed by patch tests had been proposed as an etiologic factor in previous studies. The aim of this study is to evaluate the skin patch test (SPT) results of idiopathic RAS patients and to evaluate the effect of allergen elimination on disease progress. **Material and Methods:** The SPT's were applied to 58 patients with idiopathic RAS as well as 40 healthy volunteers and test results were compared. Statistically significant allergens in RAS group were detected for clinical compliance and the presence of these allergens in oral cavity was investigated. Allergenic materials were replaced with allergen-free alternatives and patients were followed up for 12 months. **Results:** The nickel and potassium dichromate positivities were significantly higher in the RAS group when compared to the control group. Ten out of thirteen nickel-positive and 3 out of 11 potassium dichromate-positive RAS patients had a history of dental interventions containing these allergens. In control group no patient had a history of dental procedures. After changing the nickel and potassium dichromate containing dental materials in RAS patients, no relapse was observed in 12 months follow up period. **Conclusion:** The SPT might be considered as a useful test in RAS patients with undetectable etiology. Although no relaps was observed in our study, after changing the allergenic dental materials of RAS patients, further large scaled studies should be performed to clarify the relationship.

Key Words: Patch tests; stomatitis, aphthous

ÖZET Amaç: Rekürren aftöz stomatit (RAS) etiyolojisi henüz tam olarak aydınlatılmamış olup, deri yama testleri (DYT) ile tanı konulan tip 4 hipersensitivite, geçmiş çalışmalarda etiyolojik bir faktör olarak öne sürülmüştür. Bu çalışmada idiyopatik RAS hastalarında DYT sonuçlarının değerlendirilmesi ve kontrol grubu ile karşılaştırılması planlanmıştır. Ayrıca, allerjen eliminasyonunun hastalık gidişatı üzerindeki etkilerinin de incelenmesi amaçlanmıştır. **Gereç ve Yöntemler:** Elli sekiz idiyopatik RAS hastası ve 40 sağlıklı gönüllüye DYT uygulanmış ve sonuçlar iki grup arasında karşılaştırılmıştır. Rekürren aftöz stomatit grubunda istatistiksel olarak anlamlı oranda fazla saptanan allerjenler tespit edilerek, bu allerjenlerin klinik uyumları ve oral mukozadaki varlığı araştırılmıştır. Saptanan allerjenik materyaller allerjen maddeyi içermeyen alternatifleri ile değiştirildikten sonra bu hastalar 12 ay takip edilmiştir. **Bulgular:** İdiyopatik RAS grubunda kontrol grubuna göre nikel ve potasyum dikromat pozitifliği anlamlı oranda yüksek saptanmıştır. Onüç nikel pozitif RAS hastasının 10'unda nikel içeren ve 11 potasyum dikromat pozitif RAS hastasının 3'ünde potasyum dikromat içeren dental girişim öyküsü mevcuttu. Kontrol grubunda hiçbir hastada dental materyal mevcut değildi. Bu hastaların nikel ve potasyum dikromat içeren dental materyalleri nikel ve potasyum dikromat içermeyen alternatifleri ile değiştirildikten sonra yapılan 12 aylık takiplerinde relaps gözlenmemiştir. **Sonuç:** Deri yama testi, nedeni saptanamayan RAS hastalarında faydalı bir test olarak değerlendirilebilir. Çalışmamızda RAS hastalarında allerjenik dolgu maddelerinin değiştirilmesini takiben nüks gözlenmemekle birlikte, bu ilişkinin daha net ortaya konulması için daha geniş ölçekli çalışmalar yapılmalıdır.

Anahtar Kelimeler: Yama testleri; stomatit, aftöz

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Recurrent aphthous stomatitis (RAS) belongs to the group of chronic inflammatory diseases of the oral mucosa.¹ Although multiple factors such as genetic, trauma, emotional stress, diet, microbial agents, nutritional, hematological defects, hormonal changes, medications, and atopy are implicated in the etiology of RAS, no definitive consensus was found yet.²⁻⁵

The role of type IV hypersensitivity in the etiology of RAS can be explained by the elevated levels of CD8 cytotoxic T cells in the histopathology of the ulcerative stage.⁶ Moreover, it has been suggested that in patients with type IV hypersensitivity, the cytotoxic effect of lymphocytes on the oral epithelium could cause ulcers and this relationship was demonstrated by skin patch tests (SPT) in previous studies.⁷

In the etiologic investigation process of RAS, several laboratory examinations, especially the ones related to nutritional defects, are applied in dermatology clinics as a routine. However, in the situation of unknown etiology, usually symptomatic treatments are being used and recurrence is frequently observed. Since, clarifying the etiology of the disease prior to the treatment, can give clinicians a better insight. Type 4 sensitivity is investigated in patch test and it is applied frequently in cases of recurrent eczema.⁸ However, using patch test for each RAS patient is time-consuming and not feasible. In this study, we aimed to evaluate the results of the SPT of RAS patients with undetectable etiology, and to compare them with control group. In addition, we aimed to investigate the effect of allergen elimination (the allergens detected significantly higher in RAS group than control group) on RAS progress.

MATERIAL AND METHODS

This study was approved by the ethical committee and informed consent was obtained from all patients. Patients who were having a history of oral mucosal aphthae greater than 3 years were enrolled to RAS group. Detailed clinical history and physical examination findings were evaluated and recorded. Moreover, laboratory tests (vitamin B12, iron, iron binding capacity, ferritin, folate, zinc,

complete blood count, liver function tests, kidney function tests, electrolytes, urinalysis, sedimentation, antistreptolysin antibody, C-reactive protein, antinuclear antibody, serum immunoglobulin E) were requested to investigate the etiological aspects. All patients underwent patch test and 24 items European standard series prick test. Patients who have positive prick and patch test results were excluded from the study. In order to exclude secondary causes, such as gastrointestinal diseases, consultations from the Department of Gastroenterology were requested for suspicious cases. A total of 58 patients with RAS having undetected pathology as well as 40 healthy volunteers were included in to the study. Patients who were pregnant, lactating, having systemic diseases, history of systemic medication usage and detectable etiology were excluded from the study. A 21-item European standard patch test series were applied to the backs of the RAS group and the control group. The list of allergens is shown in Table 1. The test material was

TABLE 1: Comparison of positive patch test results to allergens in patients with recurrent aphthous stomatitis group and the control group.

Allergen	RAS group (n=58)	Control group (n=40)	P
Lanolin alcohol	3	1	0.51
Fragrance mixture	4	1	0.33
Thiuram mixture	0	1	0.22
2-Bromo-2-nitropropane 1,3-diol (bronopol)	1	0	0.40
Cobalt chloride	2	1	0.78
Nickel sulfate	13	2	0.019
Methyldibromo glutaronitrile	1	0	0.40
Colophony	2	2	0.70
N-isopropyl-N-phenyl-p phylenediamine	0	1	0.22
Potassium dichromate	11	2	0.045
Mercapto mixture	2	1	0.79
Peru balsam	3	0	0.15
Formaldehyde resin	3	0	0.15
Paraben mixture	1	0	0.40
Cetylstearyl alcohol	2	1	0.79
Bis (diethyldithiocarbamate)- zinc	1	0	0.40
Mercaptobenzotiazol	1	0	0.40
Propolis	2	1	0.79
Bufexamac	2	0	0.23
Lyril	2	1	0.79
Methylchloroisothiazolinone	2	1	0.79

RAS: Recurrent aphthous stomatitis.

opened after a 48 hour period and evaluated after 30 minutes. In addition, patients were re-evaluated on the 72nd hour to check for a late reaction. The test results were evaluated as follows: erythema, edema, and infiltration (1 positive); erythema, infiltration and vesiculation (2 positive); and vesiculebullose (3 positive) were all determined as positive reaction.

The data was analyzed using the SPSS 16.0 software program. Chi-square test was used to compare the RAS group and the control group findings and $p < 0.05$ was considered as statistically significant. Significantly higher allergens in RAS group were detected and the presences of these allergens in patient's oral cavities were investigated. After the allergen elimination, patients were followed up for 12 months.

RESULTS

Of all patients 53 were female, while 45 were male. Control group was consisted of 20 female and 20 male. The mean age of all patients was 31.26 ± 8.78 years. The mean age of control group was $31,85 \pm$

$8,49$ and RAS group was $30,90 \pm 8,95$ years. There was no significant difference between the two groups in terms of age and gender ($p=0.68, 0.50$). Twenty patients were having family history of apthae in RAS group. Twenty patients were having major, 13 patients were having minor and 25 patients were having major and minor apthae together. Bucal mucosa was the most common location of apthae (55%) and the most common symptom was pain.

The evaluation of SPT results showed that 27 patients from the RAS group and 9 patients from the control group tested positive for at least one allergen. Nickel positivity were found in 13 patients in RAS group, and 2 patients in control group. Potassium dichromated positivity were found in 11 patients in RAS group and 2 patients in control group. The positivity against nickel and potassium dichromate was determined to be significantly higher in the RAS group when compared to the control group ($p=0.019, p=0.045$) (Table 1). The degree of skin patch test positivities in RAS and control group were shown in Table 2. Ten out of

TABLE 2: Degrees of skin patch test positivities.

Allergen	RAS group (n=58)	Control group (n=40)
Lanolin alcohol	2 patients ++ and 1 patient +	1 person ++
Fragrance mixture	1 patient +++, 2 patients ++ and 1 patient +	1 person +
Thiuram mixture		1 person +
Bronopol	1 patient ++	
Cobalt chloride	1 patient ++, 1 patient +	1 person +
Nickel sulfate	4 patients +++, 6 patients ++, 3 patients +	1 person ++, 1 person +
Methyldibromo glutaronitrile	1 patient +	
Colophony	2 patient +	2 person ++
N-isopropyl-N-phenyl-p phlyenediamine		1 person ++
Potassium dichromate	4 patient +++, 5 patient ++, 2 patient +,	1 person +++, 1 person +
Mercapto mixture	1 patient +++, 1 patient +	1 person ++
Peru balsam	1 patient +++, 1 patient ++, 1 patient +	
Formaldehyde resin	2 patient ++, 1 patient +	
Paraben mixture	1 patient++	
Cetylstearyl alcohol	2 patient ++,	1 person +
Bis (diethylthiocarbamato)- zinc	1 patient ++	
Mercaptobenzotiazol	1 patient +	
Propolis	1 patient ++, 1 patient +	
Bufexamac	2 patient +	
Lyril	1 patient +++, 1 patient +	1 person ++
Methylchloroisothiazolinone	1 patient ++, 1 patient +	1 person ++

RAS: Recurrent aphthous stomatitis.

TABLE 3: Dental history of the patients with nickel and potassium dichromate positive recurrent aphthous stomatitis and control groups.

	Nickel (+) patients		Potassium dichromat (+) patients	
	Nickel dental history (+)	Nickel dental history (-)	Potassium dichromate dental history (+)	Potassium dichromate dental history (-)
	RAS	10	3	3
Control	0	2	0	2

RAS: Recurrent aphthous stomatitis.

thirteen nickel- positive RAS patients had a history of dental interventions involving nickel. Meanwhile, only 3 out of 11 potassium dichromate- positive RAS patients had a history of dental procedures. In control group no patient had a history of dental procedures (Table 3).

Other allergen positivities in RAS and control groups are shown in Table 1. However in the detailed investigation no clinic relevance was found between test results and examination for these allergens. Since, further investigations were not performed for these allergens.

Dentists performed dental procedures to replace the dental materials in these patients to nickel- free and potassium dichromate- free materials for clarifying the effect of allergens on RAS progress. Patients were followed- up for 12 months. Only 1 patient did not come to a follow-up examination. In the follow up period of 9 nickel positive patients and 3 potassium dichromate positive patients, no aphthae formation was seen.

DISCUSSION

The cause of lesions in patients with RAS cannot be explained by a single factor. Therefore, patients should be evaluated in terms of concomitant diseases and factors that may create a predisposition. However, in some cases the cause may still go undetectable and usually performed symptomatic treatment alone cannot prevent relapse and disaffects patient's quality of life.

Neither allergy nor hypersensitivity has been widely investigated as a cause of RAS. However, the identification of exogenous antigens may pro-

vide therapeutic options in the management of RAS. Patch testing is an accepted method of identifying allergens responsible for Type IV allergic reactions of the skin.⁸

There are limited number of studies that have evaluated SPTs in patients with RAS. Nolan et al. applied SPT with food for 21 RAS patients and detected clinically compatible positivity in 12 patients.⁹ In another study involving a large population with oral mucosal disease, 264 patients with RAS underwent European standard series, dental series, and food additives patch tests. The results from these tests were compared with the control group. They determined that the RAS group had a higher positivity against food additives (benzoic acid), chocolate, and fragrance mixtures when compared to the control group.⁸ In our study, we determined that 4 patients with RAS had positivity against the fragrance mixtures but there was no statistically significant difference between control group. Also in the detailed investigation no clinic relevance was found in RAS patients who have positive SPT results for fragrance mix. Torgerson et al. applied SPTs to 331 patients with oral mucosal disease and reported that 148 patients tested positive.¹⁰ The highest rate of positivity was detected for substances such as nickel sulfate, potassium dichromate, and gold sodium thiosulfate. They reported RAS in 3 patients, and only 1 of those 3 patients tested positive against vanillin. They commented that SPT might not be necessary for patients with RAS, however they also noted that further studies are needed due to the small number of RAS patients in their study.

Studies in literature have mostly focused on contact sensitivity towards dental materials and food. Another retrospective study that performed SPTs in 380 patients with RAS reported positivity in 70 patients.¹¹ The authors suggested that exacerbation of RAS in patients with positive patch test results developed independently from contact sensitivity. The author also suggested that the exacerbation may be related to hypersensitivity that developed as a result of swallowing nickel salts present in dental implants. A complete remission was achieved in 28 out of 70 patients with replace-

ment of dental materials.¹¹ In our study, the nickel positivity was significantly higher in patients with RAS when compared to the control group. Moreover, the potassium dichromate positivity was also significantly higher in the RAS group when compared to the control group.

The most common manifestation of nickel allergy is allergic contact dermatitis Type IV T-cell mediated delayed-type hypersensitivity reaction.¹² Nickel is found in certain foods, tap water, cosmetics, and cooking utensils. Another source of nickel is orthodontic appliances. Orthodontic appliances such as bands, brackets, wires etc. can also contain up 50-70% nickel. A variety of non-nickel containing orthodontic devices also exists.^{12,13}

Allergenic materials used in dental procedures can contain amalgam, gold salts, mercury compounds, palladium chloride, methyl methacrylate, potassium dichromate, and nickel.¹⁴ Many of these materials can lead to sensitization, as well as irrita-

tion, and can be triggering factors for diseases such as allergic eczema, oral lichen, burning mouth syndrome, and RAS.¹⁵

In our study, only 10 RAS patients had a history of dental materials containing nickel while 3 patients had a history of dental materials containing potassium dichromate. Despite the small number of patients, no aphthae were detected following a replacement of dental materials to nickel and potassium dichromate-free materials in a 12 month follow-up period, which we believe is a remarkable finding.

In conclusion, according to our study results, SPT might be considered as a useful test for clarifying the etiology and the treatment process of idiopathic RAS. However studies with larger allergen panels including dental series, larger number of patients, and studies particularly focusing on the elimination of allergens on RAS progress might be more enlightening.

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