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Analysis of Epicutaneous Patch Test Results in Patients with Contact Dermatitis

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ABSTRACT

Background: Allergic contact dermatitis (ACD) is a delayed type of hypersensitivity from contact with a specific allergen to which the patients has developed a specific sensitivity. The aim of the study was to evaluate the results of epicutaneous patch testing with standard series of contact allergen in patients suspected to have ACD. **Methods:** 355 cases of ACD were included in the study. Test substances were applied on the upper part of the patient's back, on clinically uninvolved and untreated skin. All patients were free from therapy with oral antihistamines, steroids and immunosuppressants. The patch test was removed and reaction were evaluated after 48 h and 72 h. Grading of negative (-) to positive (+ to ++++) patch test was done according to the International Contact Dermatitis Research Group. Statistical data analysis was performed by using χ^2 –test. **Results:** Of the 355 cases, 146 patients were male (41.1%) and 209 were female (58.9%). The youngest patients in the study was 16 years of age and the oldest was 67 years of age. The commonest age group affected was 41-50 years. Hands were the most common site of involvement. The occupational character of skin lesions was find in 75 (21.1%). The most common positive reactions were recorded to nickel sulphate 99 (27.8%), cobalt chloride 46 (12.9%), thimerosal 31 (8.7%), colophony 23 (6.5%), carba mix 21 (5.9%), potassium dichromate 20 (5.6%), acid chromici 19 (5.3%), fragrance mix 18 (5%), balsam of Peru 13 (3.7%), formaldehyde 9 (2.5%), and other allergens 26 (7.3%). Females were significantly more likely to show a positive response to two or more allergens ($p<0.05$). There was no statistically significant impact of age, occupation and duration of disease on results of patch testing ($p>0.05$). **Conclusions:** Our results indicate that nickel sulphate, cobalt chloride and thimerosal are the most common allergens responsible for induction of ACD. These findings are crucial in the treatment, long term management, an education of patients with ACD.

Key words: allergen, allergic contact dermatitis, epicutaneous patch test.

1. INTRODUCTION

Allergic contact dermatitis (ACD) is a very common type of skin disorders seen among patients attending dermatology clinics. The prevalence of this disease in the general population ranges from 15% to 28% and is increasing (1, 2). It is a delayed type of hypersensitivity from contact with a specific allergen to which the patients has developed a specific sensitivity. When the antigen contacts the skin, it is processed and presented with HLA-DR on the surface of Langerhans cells, which act as antigen presenting cells in the skin (3). These cells migrate to the regional lymph nodes and the allergen is subsequently processed by the T-lymphocytes. It leads to proliferation of specific T cell clones that circulate through the body and back into the skin. Upon re-exposure of the allergen, CD8+ T-cells response is mediated by the CD4+ T-cell subset. Skin penetration of allergens is facilitated by skin barrier impairment due to dermatitis or trauma. Disruption the integrity of the epidermal barrier appears to be the first step in the events following contact with allergen (4). Clinically, acute ACD is characterized by erythema, swelling and blisters while the more chronic reaction features epidermal reactive changes including lichenification, thick scale and fissuring. A wide range of pictures may evolve representing between these two poles.

Epicutaneous patch tests are tools used in the identification of the etiological agents of allergic contact dermatitis. It is a scientific method of investigation, with internationally defined rules and well-established foundations. The function of the patch test is to produce, in a controlled manner, the elicitation phase

of ACD, and thus determine the etiological agent of this dermatitis (5).It has been recommended that all patients with chronic dermatitis must be patch tested, keeping in view the indefinite course of disease (6). Therefore, the aim of the study was to evaluate the results of epicutaneous patch testing with standard series of contact allergen in patients suspected to have ACD.

2. PATIENTS AND METHODS

This was a descriptive case study conducted at Department of Dermatovenereology , University Clinical Centre Sarajevo, during the 2016-2017 period. 355 patients clinically diagnosed with contact dermatitis were included in the study. After informed consent, relevant history was taken and clinical examination was performed. The following factors were considered: sex, age, duration of disease, location of primary lesions and the relationship between the development of skin lesions and occupational work. All patients were free from therapy with oral antihistamines, steroids and immunosuppressants. Cases with any kind of food sensitivity or any other confirmed skin diseases were also excluded.

Each patient was patch tested with allergens of European Baseline Series (Table 1), manufactured by the Institute of Immunology, Zagreb, Croatia. Test substances were applied on the upper part of the patient's back, on clinically uninvolved and untreated skin with adhesive strips for patch test (Curatest, Lohmann Rauscher, Germany). The patch test was removed and reaction were evaluated after 48 h and 72 h. Patients were instructed to wear the patch for 48 hours without removing it and to avoid contact with water. Grading of negative (-) to positive (+ to +++) patch test was done according to the International Contact Dermatitis Research Group Criteria (7). An irritant response was interpreted as a negative response. Positive patch test results were presented by frequency and percentage. Statistical data analysis was performed by using χ^2 –test.

Allergen	Dilution (%) vechicle	Allergen	Dilution (%) vechicle
Acid chromici	1.0 aqua	Epoxy resin	1.0 vaseline
Cobalt chlorid	1.0 vaseline	Colophony	20.0 vaseline
Asepsol	0.5 aqua	Quaternium	1.0 vaseline
Potassium dichromate	0.5 vaseline	PPD-black rubber mix	0.1 vadeline
Resorcini	2.0 vaseline	Balsam of Peru	25.0 vaseline
Lidocain	1.0 vaseline	Fragrance mix	8.0 vaseline
Nickel sulfate	5.0 vaseline	Paraben mix	15.0 vaseline
Paraphenylene diamine	0.5 vaseline	Ammonium persulfate	2.5 vaseline
Terpentini	10.0 vaseline	Thimerosal	0.1 vaseline
Carba mix	3.0 vaseline	Neomycin sulfate	20.0 vaseline
Mercapto mix	2.0 vaseline	Formaldehyde	1.0 aqua
Thiuram mix	1.0 vaseline	Vaseline	As it is

Table 1. The series of allergens used for patch testing

Allergen	n	%
Nickel sulfate	99	27.8
Cobalt chloride	46	12.9
Thimerosal	31	8.7
Colophony	23	6.5
Carba mix	21	5.9
Potassium dichromate	20	5.6
Acid chromici	19	5.3
Fragrance mix	18	5.0
Balsam of Peru	13	3.7
Formaldehyde	9	2.5
Others	26	7.3

Table 2. Results of epicutaneous patch testing. n= number of positive test results

3. RESULTS

Of the 355 cases, 146 patients were male (41.1%) and 209 were female (58.9%). The youngest patients in the study was 16 years of age and the oldest was 67 years of age. The commonest age group affected was 41-50 years. Minimum duration of disease noted in our study was less than 6 weeks and maximum was 7 years. The majority of the patients had the disease for 6 to 12 months. Hands and face including ears and the neck were the most common site of involvement. The occupational character of skin lesions was find in 75 (21.1%).

Positive patch test results with at least one allergen were obtained for 221 (62.2%) patients, more frequently in women than in men (147 *vs* 74). The frequency of sensitization to allergens used for the study is shown in Table 2. The most common positive reactions were recorded to nickel sulphate 99 (27.8%), cobalt chloride 46 (12.9%), thimerosal 31 (8.7%), colophony 23 (6.5%), carba mix 21 (5.9%), potassium dichromate 20 (5.6%), acid chromici 19 (5.3%) , fragrance mix 18 (5%), balsam of Peru 13 (3.7), formaldehyde 9 (2.5%), Less common were: paraphenyle diamine 6 (1.7%), paraben mix 5 (1.4%) and epoxy resin 3 (0.8%). Reactivity against the rest of the panel was not remarkable. Regarding patch test reactivity, most of the patients had 2+ degree of reaction. Females were significantly more likely to show a positive response to two or more allergens ($p<0.05$). There was no statistically significant impact of age, occupation and duration of disease on results of patch testing ($p>0.05$). The adverse reaction during patch testing were pruritus and tape erythema.

4. DISCUSSION

Epicutaneous patch tests along with history and clinical features are very important steps in the identification of specific causative allergen in patients with ACD. Today, about 3000 antigens are known to act as contact allergens (8). Based on the patch test results, our study identified metals as the most common allergens. In addition to metals, the other common allergens identified included thimerosal, colophony, carba mix, fragrance mix, balsam of Peru and formaldehyde. The frequency of positive patch test results in the patients (62.2%) as well as clear predominance of women among applicants

with eczema, are within the range of results available in literature (9, 10).

Nickel is ubiquitous metal used in a wide variety of products and is the most common allergen encountered worldwide (11). Dermatitis due to contact with nickel was initially described among workers in the nickel-plating industry and was documented as an allergic response in 1925 (12). Frequency on nickel allergy is reported to be continuously increasing in several countries, and represents a major health and socioeconomic problem (13). In our study, 99 (27.8%) patients showed an allergic response to nickel, making it the most prevalent of the allergens identified in this study. Nickel sensitivity was found to be more common in females compared to males with the male female ratio of 1:3. This is in accordance to the studies done by Thilak *et al* (14). Jewellery, spectacle frames, watches and metal components of clothing were the frequent sources of nickel in the study due to prolonged contact with the skin. Nickel salts being soluble in water and sweat easily cause sensitization. The reason for the relatively high prevalence of nickel ACD could be the use of nickel in consumer items that come in direct and prolonged contact with the skin (15). However, exposure may also occur in certain occupational settings generally associated with soluble nickel salts. Oral/intestinal exposure to sufficient doses of nickel ions may trigger systemic allergic dermatitis, with large inter-individual variation related to the elicitation threshold (16). European Union Nickel directive (17) has passed certain legislation with the intention of controlling the use of nickel releasing objects in contact with the skin.

The second most common allergen identified in our series was cobalt. Cobalt is a metal found in nature. It is commonly used with nickel for metal plating, and added to alloys to make more robust tools and parts (18). In addition, cobalt may also be found in hair dyes, detergents, antiperspirants, solid soaps, and cosmetics. As some pigments are salts of cobalt, exposure via these may cause ACD. Positive reaction to this allergen occurred in 12.9% of people tested, and this percentage was greater than in other European countries (19). Most patients with positive cobalt chloride tests also had allergies to nickel and potassium dichromate. Approximately 80% of individuals with cobalt sensitivity have co-sensitivity to other metals, with the predominant co-sensitivity being nickel (20). It has been postulated that nickel sensitization and preexisting dermatitis are often prerequisites for cobalt sensitization.

In our study it was a high rate of sensitization to thimerosal. It is an organic mercurial compound of thyosalic acid. Thimerosal has antibacterial and antifungal properties, without irritating the skin and mucous membranes. It is commonly found in cosmetics such as eye shadows, mascaras, lotions, contact lens solution and ophthalmic preparations. This preservative is also used in vaccines and many other products (21). However, many studies clearly indicate that the incidence of positive patch test results with thimerosal is very high (22). Its widespread use may explain the high rate of positive patch test reactions.

Allergic contact dermatitis to colophony was seen in 23 (6.5%) cases. Colophony is a mixture of approximately 100 chemical compounds, 95% of which is abietic acid, the main allergen of rosin. It is used in a wide variety of medical and personal care products. Bajaj *et al* estimated the prevalence of colophony allergy to be 5.7% among 590 patients with a positive skin patch test (23).

Carba mix was the fifth most common allergen in our patients, with a positive reaction recorded in 21 patients (5.9%). The prevalence of carba mix sensitization has also been reported from other countries and have ranged between 2.2 % to 4.4% (24, 25). Carba mix serves as a rubber accelerator added to natural rubber to speed its polymerization. It is used in the manufacture of many rubber products. Examples of such products include health care equipment (medical and utility gloves, tubing, bed sheeting), industrial and safety products (masks, respirators, ear plugs, headphones), office products and sport equipment (16). In addition to rubber products, carba mix may also be found in fungicides, pesticides, hairbrushes, and in some soaps, shampoos, and disinfectants.

Potassium dichromate reactivity was observed in 5.6% of the patients in the present study. Exposure to potassium dichromate is considered to be occupational as it is a major component used in the cement and tile industry. Notably higher prevalence rate of potassium dichromate sensitization of 51% have been reported from India (26)

A mix of eight common fragrances, Fragrance Mix, is commonly used for testing fragrance contact allergy. The mix consists of the cinnamic aldehyde and alcohol, eugenol and isoeugenol, geraniol, hydroxycitronellal, amyl cinnamaldehide, and oak moss (27). In our study, ACD to fragrance mix was seen in 5% cases. A study conducted in Denmark on about 10 000 patients with eczema showed that 5.5% of respondents reported positive reaction after exposure to the mix (28).

Balsam of Peru is also the addition of fragrance in many cosmetics, and its use is wide due to fixative properties. It is a complex substance that contains many potential allergens such as benzoic acid, benzyl acetate, benzyl benzoate, vanillin, nerolidol, and cinnamic acid among others. In addition to its use in fragrances, balsam of Peru can also be found in foods, drinks and medicines. The most common balsam-related foods deemed responsible for causing dermatitis included tomatoes, citrus and spices (29). Among our patients positive patch test with balsam of Peru were found in 3.7% of patients. Most patients with positive balsam of Peru tests also had allergies to fragrance mix.

While formaldehyde itself is not used much as preservative, so-called formaldehyde-releasers are widely used in many cosmetic products, topical medications and household products. Moreover, formaldehyde is found in aminoplastics and phenolic resins, various glues, and textiles. Therefore, the low concentrations of formaldehyde often found in skincare products are sufficient to worsen an existing dermatitis. The prevalence of formaldehyde sensitization has also been reported from other countries and have ranged between 2% and 9% (30). For

consumers allergic to formaldehyde and suffering from any kind of dermatitis, it is very important to know the potential for formaldehyde exposure in order to avoid ACD (31).

ACD is a chain of complex processes of the immune system with response to chemical substances present in the environment. The clinical relevance of positive tests is important in interpreting patch test results, as this enables the differentiation of ACD and contact sensitization. Furthermore, monitoring patients after patch testing is important as regards clinical relevance and treatment evaluation.

5. CONCLUSIONS

Our results indicate that nickel sulphate, cobalt chloride and thimerosal are the most common allergens responsible for induction of ACD. Patch tests are essential for the diagnosis of contact sensitization. These findings are crucial in the treatment, long term management, an education of patients with ACD. In view of more than one-quarter of general population being allergic, improvements of primary prevention of contact allergy need to be enforced.

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- **Conflict of interest:** none declared.

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