

Allergic Contact Sensitization in an Unselected Danish Population

The Glostrup Allergy Study, Denmark

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The distribution of allergic contact sensitization was assessed in an unselected population, living in western Copenhagen, Denmark. Ready-to-apply patch tests comprising 23 haptens and mixtures of haptens were mailed to 793 adults, and 567 (71.5%) participated. The tests were read 2 days after application. A total of 111 positive reactions were found among 86 (15.2%) subjects. Sensitization was less frequent in men than in women (11.5% versus 18.8%). Twenty out of the 23 chemicals in the test elicited positive reactions. Positive reactions to nickel and thiomersal were found most frequently (6.7% and 3.4%, respectively). Concerning the other chemicals the frequencies were 1.1% or less. Nickel sensitivity was less frequent in men than in women (2.2% versus 11.1%). The frequencies of sensitization probably represent minimum figures. Regulation of exposure needs to be considered in order to prevent primary sensitization and disease recurrences in those already sensitized. Key words: Patch test; Eczema; Contact dermatitis; Prevention; Epidemiology.

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Allergic contact sensitization is a cellularly mediated delayed type of allergic reaction in the skin. Although individual susceptibility to sensitization has been demonstrated (1), genetic factors seem to be of lesser importance when compared to environmental factors (2).

Our present knowledge of the frequency of allergic contact sensitization is based on data from patients with eczematous skin diseases referred to and examined by dermatologists (3-6), and from skin patch testing performed in defined subgroups of the population (7-10). Because of selection bias, these data may not give a true picture of the occurrence of allergic contact sensitization in the general population.

From these studies (7-10) it appears that 5-10% of women are nickel sensitive, depending on the age group examined. Reliable estimates are not available for the majority of the haptens included in the European Standard Series (11).

In this study, an unselected population comprising 793 adults, living in western Copenhagen, Denmark, was invited for patch testing.

The frequencies of allergic contact sensitization to the particular haptens and mixtures of haptens, and also the frequency of concomitant occurrence of multiple sensitivities are presented.

MATERIALS AND METHODS

Population and invitation

The total population living in the area of The Glostrup Population Studies consisted of 324,931 subjects, which is 54% of the Copenhagen County population and 6% of the total population in Denmark. The representativeness of the sampling area was assessed recently by comparing inhabitants in the sampling area, in Copenhagen County, and in all of Denmark using data from the National Statistics (12). The sampling area was representative of the total Danish population as regards the age and sex distribution and marital status. Regarding industrial categories and employment status, farming and fishing were underrepresented, both in the sampling area and in the Copenhagen County (0.5% versus 6.3% in Denmark), and hence there were fewer self-employed individuals.

A random sample of 8,000, 15- to 69-year-old men and women living in the area of The Glostrup Population Studies participated in a preliminary postal survey about respiratory symptoms, and 6,998 (88.7%) responded (13). Irrespective of their answers, a random sample of 793 respondents were invited for examination (Table I). The invitation procedure has previously been described (13). Briefly, the random sample of respondents were invited to a general health examination by a standard letter containing information about the project and a questionnaire. The subjects were informed that the aim of the survey was to study the distribution of some important disorders in the general population, especially allergy.

Patch test method

The patch tests used were the ready-to-apply TRUE Test (Pharmacia, Sweden) (14-16). The haptens and mixtures of haptens in this test series comprise widely used environmental chemicals such as metals, preservatives, substances in cosmetics, glues, rubber, and medications. At the time of the study formaldehyde was not yet included. The haptens differed further from the European Standard Series (Hermal-Chemi, Germany) by including thiomersal and excluding primine. The TRUE Test was found comparable to routine methods (14-16), and was chosen since mailing of the test was possible. Table II shows the 23 haptens and mixtures of haptens included in the test series. Directions to apply the patch tests to the upper back 2 days before the examination were mailed with the patch test. The tests were read ½-1½ hour after removal. Reactions were classified according to the International Contact Dermatitis Research Group (11). A positive reaction (+) was defined as at least homogenous redness and palpable infiltration in the test area. Reactions not fulfilling these criteria, whether these were follicular reactions, faint erythema, or typical irritant reactions, were classified as non-allergic reactions. If the tests were not applied 2 days before attending, or if the contact with the skin was poor, a new appointment was made when possible. The investigator (NHN) read all reactions. Photos of the test sites were revised by the second author, and in case of disagreement consensus was reached by discussion. The examination took place between February 1990 and February 1991.

Non-attendants for examination

The attendants and non-attendants were compared by using their answers to the preliminary postal questionnaire (13). Over-representation of subjects reporting respiratory symptoms among at-

Table I. The subjects invited for skin patch test, the pattern of participation, the distribution of sensitization to one or more haptens and mixtures of haptens and 95% confidence intervals

Age (years)	Men			Women		
	Invited (n)	Participants (%)	Subjects with one or more sensitivities (%)	Invited (n)	Participants (%)	Subjects with one or more sensitivities (%)
15-34	139	61.2	11.8 (5.8-20.6)	154	72.7	24.1 (17.7-35.2)
35-49	130	73.1	7.4 (3.0-14.6)	138	73.2	18.8 (11.8-28.1)
50-69	123	80.5	15.2 (8.7-23.8)	109	68.8	10.7 (4.7-19.9)
Total	392	71.2	11.5 (7.4-14.7)	401	71.8	18.8 (15.6-25.0)

NOTE: Participation: Age groups. Men: $\chi^2 = 12.24$, $df = 2$, $p < 0.01$; women: $\chi^2 = 0.68$, $df = 2$, $p > 0.7$. Sex. Male vs. female: $df = 1$, $\chi^2 = 0.041$, $p > 0.9$. Sensitivity: Age groups. Men: $\chi^2 = 2.90$, $df = 2$, $p > 0.2$; women: $\chi^2 = 5.33$, $df = 2$, $p > 0.05$. Sex. Male vs. female: $df = 1$, $\chi^2 = 5.29$, $p < 0.05$.

tendants was found. The reported discrepancies between the attendants and the general population was, however, minimal because of a high attendance rate. Out of the 194 non-attendants, 117 (60%) were interviewed by telephone or returned a short postal questionnaire about flexural eczema and a history of eczema at metal contact sites.

Ethics

The study was approved by the ethic committee for Copenhagen County. Pregnant women ($n = 3$) were excluded from the examination.

Statistical analysis

Three age groups were defined: 15-34 years (young), 35-49 years (middle) and 50-69 years (old).

Data were analyzed with the Statistical Package for the Social Sciences (SPSS Inc., Chicago, Ill., U.S). Differences between groups were examined by the chi-square test and the Fisher exact test as appropriate.

The 95% exact confidence intervals of the frequency of sensitization relate to the precision of the population estimate. It is the interval in which we expect the population mean to lie, based on the population sample size (17). The confidence intervals were found in Documenta Geigy.

In any population the overall risk of sensitization by a particular chemical can be measured by the frequency of sensitivity to the chemical. If there were no differences in susceptibility between individuals and no specific associations between chemicals, then the frequency of any combination of sensitizations could be calculated as the product of individual frequencies, i.e. if the frequencies of sensitization to three different chemicals were 1 in x, y and z when these occur singly, then the predicted frequency (and, therefore, the risk of sensitization) would be 1 in xy, xz, yz and xyz when they occurred in combination. The differences between the observed and the calculated number of subjects with multiple sensitizations would, therefore, be a measure of differences in susceptibility to sensitization between subjects or cross reactivity between chemicals. Observed and calculated numbers of subjects were compared by the chi-square test.

Statistical significance was considered to be present if the p value was below 0.05.

RESULTS

Participation pattern

Out of the 793 subjects invited, 599 (75.5%) attended the general health examination. Out of the 793 invited, 567 (71.5%) subjects were patch tested (Table I). Among men the

patch test participation rate was significantly dependent on age. This was due to a low participation rate among the young men, and higher rates among the older men. Among women, the participation rate was not significantly dependent on age. No significant difference was found in participation rate between men and women.

Patch testing

A total of 111 positive reactions were found in 86 (15.2%) subjects. Of these, 44 positive reactions were found in men ($n = 32$), and 67 positive reactions in women ($n = 54$). The distribution of the reactions appears in Table II. No reactions to the negative control were observed. Five subjects returned to the clinic with newly converted reactions during the subsequent days, whereas no reactions appeared after 7 days. The non-allergic reactions (627 non-allergic reactions out of 13,041 individual patch tests) were found to all haptens and mixtures of haptens, 290 in men and 337 in women. The non-allergic reactions were equally represented in all age groups.

Sensitization was less frequent in men than in women (Table I). Significant differences in the frequencies of sensitization between the age groups were not found among men, nor among women.

Positive reactions to 20 of the 23 haptens and mixtures of haptens in the patch test series were found. The frequencies of reactions to the specific haptens and mixtures of haptens appear in Table II. Sensitization was most common to nickel (38 = 6.7%) and to thiomersal (19 = 3.4%), and less frequently to the other haptens and mixtures of haptens (1.1% or less). The distribution of reactions to nickel appears in Table III. The frequency of sensitization to nickel was lower in men than in women. Among women, the frequency of sensitization was high in young women as compared to the older age groups. No significant differences were found between the sexes comparing sensitization to the other haptens and mixtures of haptens.

The observed number of subjects with multiple reactions, and the number expected from the frequencies of sensitization to the chemicals appear in Table IV. The observed number of subjects with multiple reactions was significantly higher than expected.

Table II. The distribution of sensitization to haptens and mixtures of haptens – percent of subjects with sensitivity (95% confidence intervals)

Haptens and mixtures of haptens	Men (n = 279)	Women (n = 288)	Total (n = 567)
Nickel sulphate	2.2	11.1	6.7 (4.5–9.2)
p-phenylenediamine	0.0	0.0	0.0 (0–0.7)
Neomycin sulphate	0.0	0.0	0.0 (0–0.7)
Potassium dichromate	0.7	0.3	0.5 (0.1–1.4)
Cainemix	0.0	0.0	0.0 (0–0.7)
Fragrancemix	1.1	1.0	1.1 (0.3–2.3)
Colophony	0.4	1.0	0.7 (0.1–1.7)
Epoxy resin	0.4	0.7	0.5 (0.1–1.4)
Thiurammix	0.7	0.3	0.5 (0.1–1.4)
Balsam of Peru	0.7	1.4	1.1 (0.3–2.3)
Ethylenediamine	0.4	0.0	0.2 (0.0–1.1)
Cobalt chloride	0.7	1.4	1.1 (0.3–2.3)
p-t-butylphenol-formaldehyde resin	1.1	1.0	1.1 (0.3–2.3)
Parabens	0.4	0.3	0.4 (0.1–1.4)
Carbamix	0.7	0.0	0.4 (0.1–1.4)
Black-rubbermix	0.4	0.0	0.2 (0.0–1.1)
Isothiazoliones*	0.4	1.0	0.7 (0.1–1.7)
Quaternium 15	0.4	0.0	0.2 (0.0–1.1)
Mercaptobenzothiazole	0.4	0.0	0.2 (0.0–1.1)
Wool alcohols	0.4	0.0	0.2 (0.0–1.1)
Negative control	0.0	0.0	0.0 (0–0.7)
Mercaptomix	0.7	0.0	0.4 (0.1–1.4)
Thimerosal	3.6	3.1	3.4 (2.0–5.4)
Quinolinemix	0.4	0.3	0.4 (0.1–1.4)

NOTE: * 5-chloro-2-methyl-4-isothiazolin-3-one, 2-methyl-4-isothiazolin-3-one (MCI/MI).

Questionnaire

A history of contact eczema (eczema at metal contact sites or hand eczema) was reported by fewer men than women (21% versus 48%). A history of contact eczema was more common among those with as compared to those without a positive patch test (38% versus 18%, and 76% versus 42%, among men and women, respectively).

Non-attendants to examination and non-participants to patch test

A total of 106 subjects (91%) who did not attend the examination answered the questions about flexural eczema and a history of eczema at metal contact sites. Attendance was not significantly dependent on flexural eczema (6% versus 2%, among attendants and non-attendants, respectively). Among women, the frequency of a history of eczema at metal contact sites was significantly higher among attendants than among non-attendants (36% versus 19%, $p < 0.05$). Among men, the difference in attendance rate related to a history of eczema at metal contact sites was not significant.

Patch test participation was not significantly dependent on self-reported flexural eczema, hand eczema or a history of eczema at metal contact sites (5% versus 3%, 20% versus 6%, 22% versus 28%, among participants and non-participants, respectively).

Table III. Sensitivity to nickel sulphate

Age (years)	Men (%)	Women (%)	Total (%)
15–34	2.4	19.6	12.2
35–49	1.1	7.9	4.6
50–69	3.0	2.7	2.9
Total	2.2	11.1	6.7

NOTE: Sensitivity to nickel. Age groups. Men: $\chi^2 = 0.92$, $df = 2$, $p > 0.6$ (expected frequency below 5 in 50% of cells); women: $\chi^2 = 14.7$, $df = 2$, $p < 0.001$. Sex. Male vs. female: $\chi^2 = 16.8$, $df = 1$, $p < 0.0001$.

DISCUSSION

Patch testing represents a well established technique to diagnose contact allergy to environmental chemicals. A positive patch test does not always imply allergic contact dermatitis. However, subjects with a positive patch test to environmental chemicals represent a group at risk of developing allergic contact dermatitis if exposed to the chemical in question at a concentration exceeding an individual threshold level (17).

One of the main points in the present survey was patch testing of unselected volunteers. To ensure a high participation rate we found it essential that the subjects only needed to come for one examination.

The subjects were invited to a general health examination, with the emphasis on allergy. Therefore, an over-representation of subjects with contact allergy could be expected, or, on the other hand, some subjects with a well recognised contact allergy might refuse patch test. The reported discrepancies were, however, minimal because of the high attendance rate.

The frequencies of sensitization were likely to represent minimum figures for several reasons. Firstly, because of the strict definition of a positive reaction. In most cases it was easy to differentiate between positive reactions and non-allergic reactions, based on these criteria. However, in 5% of the individual patch tests the skin was not completely normal corresponding to the test sites when reading was performed ½–1½ hour after removal of the patches. It is known that the majority of such minor skin changes introduced by patch testing disappear within the following day. Secondly, the frequencies of sensitization were likely to represent a minimum

Table IV. The observed distribution of multiple sensitizations, and the number expected (calculated), assuming random distribution of the sensitizations

Number of positive reactions	Observed (n)	Expected (n)
2	481	464.7
1	69	94.7
2	13	7.9
>2	4	0.4
Total	567	567.0

NOTE: Observed vs expected: $\chi^2 = 43.2$, $df = 3$, $p < 0.0001$.

because only one reading after 2 days was performed. Ideally, patch tests should be read on day 2, 3–4 and 7 (18–19). By performing one reading on day two only, probably 10–20% of the true positive reactions were lost. Conversion from negative to positive test reactions were also observed in the present study. The results concerning neomycin are particularly unreliable, as this test first becomes positive on days 4–7. Finally, the frequencies were likely to represent a minimum, since no additional patch testing was performed with haptens of specific relevance to the individual subject.

Sensitization to nickel was found more frequently than sensitization to the other haptens. The frequency is consistent with those reported from other studies (6–10). The study confirmed that 1 out of 10 women was sensitive to nickel. The frequency of sensitization to thiomersal and to ethylenediamine was similar to figures from other surveys (8, 10).

Most of the haptens and mixtures of haptens elicited positive reactions. This finding indicated that sensitization in the general population was not merely due to a limited number of haptens. However, more than half of the reactions could be ascribed to nickel and thiomersal, whereas the numbers of positive reactions to the other particular haptens and mixtures of haptens were low.

It is generally accepted that multiple sensitivities occur in patients more frequently than might be expected from the occurrence of single sensitivities (1, 21). The present study demonstrated that clustering of multiple sensitivities also occurred in an unselected population. There are two possible explanations for the high frequency of multiple sensitivities to chemically unrelated haptens. One is that subjects who develop multiple allergies do so because they are more susceptible to sensitization than normal subjects, as indicated by the study by Moss et al. (1). The other explanation is that the sensitivities occur because of specific interactions between particular haptens, or concomitant exposure to haptens because the haptens often occur together. The latter mechanism might operate for medicaments and metals such as nickel, chromium and cobalt (1, 21).

The importance of taking preventive measures to avoid clinical disease among sensitized persons as well as sensitization was stressed by the results of this study. The very high frequency (19.6%) of nickel sensitization found in young women supported the need of regulation of nickel exposure such as that introduced in Denmark in 1989 (22). We found that 3.7% of the general population were sensitized to the cosmetics ingredients included in the TRUE Test (fragrance mix, colophony, balsam of Peru, parabenes, MCI/MI, quaternium 15, wool alcohols). Labelling cosmetics with their ingredients, as is already done in the United States, might be beneficial to patients, doctors, and the cosmetic industry.

The continued use of merthiolate in vaccines and rinse solutions for contact lenses as an antimicrobial agent is debatable in the light of our finding of sensitization to thiomersal in 3.4% of the study population. We recommend that the replacement of merthiolate should be seriously considered, since sensitization might be exclusively iatrogenic (23), and due to injections of merthiolate in vaccines (24).

In conclusion, the group at risk of developing allergic con-

tact dermatitis was large, and sensitization was found to a variety of widely used chemicals. Regulation of exposure needs to be considered in order to prevent primary sensitization and disease recurrences in those already sensitized.

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REFERENCES

- Moss C, Friedmann PS, Shuster S, Simpson JM. Susceptibility and amplification of sensitivity in contact dermatitis. *Clin Exp Immunol* 1985; 61: 232–241.
- Menné T, Holm NV. Genetic susceptibility in human allergic contact sensitization. *Sem Dermatol* 1986; 5: 301–306.
- Fregert S, Hjorth N, Magnusson B, et al. Epidemiology of contact dermatitis. *Trans St Johns Hosp Dermatol Soc* 1969; 55: 17–35.
- Schubert H, Berove N, Czernielewski A, et al. Epidemiology of nickel allergy. *Contact Allergy* 1987; 16: 122–128.
- Christoffersen J, Menné T, Tanghøj P, et al. Clinical patch test data evaluated by multivariate analysis. *Contact Dermatitis* 1989; 21: 291–299.
- Agrup G. Hand eczema and other hand dermatoses in South Sweden. *Acta Derm Venereol (Stockh)* 1969 (Suppl 61).
- Peltonen L. Nickel sensitivity in the general population. *Contact Dermatitis* 1979; 5: 27–32.
- Prystowsky SD, Allen AM, Smidth RW et al. Allergic contact hypersensitivity to nickel, neomycin, ethylenediamine, and benzocaine. *Arch Dermatol* 1979; 115: 959–962.
- Menné T, Holm NV. Nickel allergy in female twin population. *Int J Dermatol* 1983; 22: 22–28.
- Seidenari S, Manzini BM, Danese P, Motolese A. Patch and prick test of 593 healthy subjects. *Contact Dermatitis* 1990; 23: 162–167.
- European standard series (ICDRG 1984). *Contact Dermatitis* 1984; 11: 63–64.
- Rasmussen BK, Jensen R, Schroll M, Olesen J. Epidemiology of headache in a general population – a prevalence study. *J Clin Epidemiol* 1991; 44: 1147–1157.
- Nielsen NH, Dirksen A, Madsen F. Can subjects with a positive skin prick test be selected using a short questionnaire? Submitted: *Allergy, European Journal of Allergy and Clinical Immunology*.
- Lachapelle J-M, Bruynzeel DP, Ducombs G, et al. European multicenter study of TRUE Test. *Contact Dermatitis* 1988; 19: 91–97.
- Kreilgård B, Hansen J, Fischer T. Chemical, pharmaceutical and clinical standardization of the TRUE Test caine mix. *Contact Dermatitis* 1989; 21: 23–27.
- Wilkinson J, Bruynzeel DP, Ducombs G, et al. European multicenter study of TRUE Test, Panel 2. *Contact Dermatitis* 1990; 22: 218–225.
- Chinn S. Ranges, confidence intervals, and related quantities: what they are and when to use them. *Thorax* 1991; 46: 391–393.
- Menné T. Relationship between use test and threshold patch test concentration in patients sensitive to 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (MCI/MI). *Contact Dermatitis* 1991; 24: 375.
- Nethercott JR, Holness DL, Adams RM, et al. Results of first and second readings with standard screening trays in North America: 1985 to 1989. *Am J Contact Dermatitis* 1991; 2: 255–259.
- Shehade SA, Beck MH, Hillier VF. Epidemiological survey of standard series patch test results and observations on day 2 and day 4 readings. *Contact Dermatitis* 1991; 24: 119–122.
- Edman B. Computerized analysis of concomitant contact allergens. *Contact Dermatitis* 1991; 24: 110–113.

22. Danish ministry of the environment. Statutory Order No. 472, 1989, June 27.
23. Möller H. Merthiolate allergy: A nationwide iatrogenic sensitization. *Acta Derm Venereol (Stockh)* 1977; 57: 509-517.
24. Förström L, Hannuksela M, Kousa M, Lehmuskallio E. Merthiolate hypersensitivity and vaccination. *Contact Dermatitis* 1980; 6: 241-245.