

Reactivity to patch tests with nickel sulfate and fragrance mix in infants

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The pattern of patch test reactivity to nickel sulfate and fragrance mix was studied with respect to patch test performance, reproducibility and clinical relevance in a population of unselected infants followed prospectively from birth to 18 months of age. TRUE TestTM patches with nickel sulfate in 3 concentrations, 200, 66 and 22 µg/cm², and fragrance mix 430 µg/cm² were used. A likely case of nickel sensitivity was defined as a reproducible positive reaction with at least homogeneous erythema and palpable infiltration occurring at least 2× and present at both the 12 and 18 months follow-up. 543 infants (268 girls and 275 boys) were tested at least 1×, 304 were tested at both 12 and 18 months. The prevalence of a reproducible positive reaction to nickel was 8.6% (20 girls and 6 boys). A transient positive reaction was observed in 111 children. Clinical relevance of nickel sensitivity was found in only 1 child. No reproducible positive reaction to fragrance mix was found. The high proportion of transient patch test reactivity to nickel sulfate 200 µg/cm² indicates that this standard concentration used for adults cannot be applied to infants. The interpretation of a single positive nickel patch test in infants must be assessed with caution and it is probably of non-specific or irritant nature.

Key words: clinical relevance; fragrance mix; infants; nickel sulfate; patch testing; reproducibility; transient reactivity. © Blackwell Munksgaard, 2004.

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Sensitization to nickel is the most common contact allergy in adolescents and adults. The reported prevalence of nickel allergy varies, depending on age, sex and selection of study population. Even in smaller children nickel allergy is not uncommon (1–4). Further, fragrance allergy is increasingly common and small children are exposed (5).

A nickel exposure regulation was implemented in Denmark in 1992, limiting nickel release from metal objects with prolonged skin contact. The regulation seems to have had a positive effect, as a recent clinical study among 10–14-year-old schoolgirls showed a significant decrease in the frequency of nickel sensitivity after the implementation of the regulation (6). The use of fragrances is not restricted by any regulations.

How and when nickel and fragrance sensitization develop is not clarified in detail and only few investigations have studied contact allergy in an unselected group of small children. The development of the TRUE TestTM patch system (7) has made it possible to perform larger epidemiological patch test studies.

This study in an unselected cohort of infants, patch tested up to 5× during the first 18 months of life, showed that the frequency of positive fragrance mix reactions was very low, whereas nickel patch test reactivity in infancy was common but poorly reproducible and without clinical relevance, leading to the conclusion that nickel patch test reactivity seems to fluctuate in infancy and that patch testing with nickel sulfate in the concentration used for adults cannot be recommended in small children.

Patients and Methods

A total of 562 of 1095 consecutive full-term children born the first 14 days of each month at Odense University Hospital from November 1998 to November 1999 were included in a cohort after parents gave their verbal and written consent. Follow-ups were performed at 3, 6, 12 and 18 months of age. Interview of parents collected information regarding family history and eczema history in relation to exposure to metal

and perfume. Clinical examination and patch testing with nickel sulfate and fragrance mix was performed at each visit.

TRUE TestTM patches (Mekos Laboratories AS, Hillerød, Denmark) of nickel sulfate ($\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$) in 3 concentrations, 200, 66 and 22 $\mu\text{g}/\text{cm}^2$, and fragrance mix 430 $\mu\text{g}/\text{cm}^2$ were used (8, 9). The test tape was fixed on the upper back, removed after 2 days and read at days 2, 3 or 4. Reactions were scored according to the recommendations of the International Contact Dermatitis Research Group (10). 3 investigators performed all readings.

As a result of an unexpectedly frequent occurrence of weak (?+) and transient positive reactions to nickel sulfate 200 $\mu\text{g}/\text{cm}^2$, a likely case of nickel allergy was pragmatically defined as a positive reaction ($\geq +$) occurring at least 2 $\times$ and present at both 12 and 18 months. A palpable erythema (+) occurring 1 $\times$ or 2 $\times$ during the study period but not present at 12 and 18 months of age was considered as an irritant or non-specific reaction. The relevance of a positive test was evaluated in relation to exposure and dermatitis history. *In vitro* tests, such as the lymphocyte transformation test, were not available in this study to document further the presence of nickel allergy.

The study protocol was approved by the local ethics committee (# 19980102), and we considered that repeated patch testing with these 2 TRUE TestTM allergens was a safe procedure because patch test sensitization has not been reported for these 2 allergens and due to the widespread exposure to nickel and fragrance containing substances in the daily environment.

Data banking/statistical analysis

All data were entered 2 $\times$ into 2 separate Microsoft Access databases (Office 2000; Microsoft Corporation, Washington, DC, USA). A comparison of raw data was performed. Discrepancies were corrected manually. Statistical analysis was performed with STATA 7.0 for Windows

(STATA Corporation, Texas, USA). Descriptive statistics were used for proportions. Comparison of proportions was done by the chi-squared analysis.

Results

A total of 543 children (268 girls and 275 boys) were patch tested and 304 children (153 girls and 151 boys) were tested at least 2 $\times$ and at both 12 and 18 months. All 5 follow-up sessions were attended by 242 infants (130 girls and 112 boys). In 19 of 562 children, parents either declined patch testing or never appeared for reading. During the study period, 2251 patch tests were performed, equally distributed over the year. In 85 (3.7%) readings (girls 1.6% and boys 2.1%), the test result was excluded because parents failed to remove the patches after 2 days or the tape had loosened, leaving 2166 readings for analysis. Readings were performed on day 2 in 20.5%, on day 3 in 57.8% and on day 4 in 21.8%. The day of reading did not differ between sexes. Spotty macular reactions were noted in 38 (1.8%) readings. On 4 occasions, a tape reaction was registered.

The point prevalence of a positive patch test to nickel among the 543 children tested at least 1 $\times$ is shown in Table 1. The nickel reactivity increased considerably with age. More than 25% of the infants patch tested up to 18 months of age had at some point a positive nickel reaction with erythema and palpable infiltration.

A positive reaction to fragrance mix did not occur until 6 months of age, thereafter the point prevalence was 0.4%, 1.0% and 0% in girls and 1.8%, 1.0% and 1.1% in boys at 6, 12 and 18 months, respectively. No reproducible fragrance mix reaction was seen.

137 of 304 children who were patch tested at least 2 $\times$ and both at 12 and 18 months had a positive reaction at least 1 $\times$, and 48 showed reactivity at least 2 $\times$ (Table 2). However, a positive

Table 1. The point prevalence (%) of a positive patch test reaction ($\geq +$) to nickel sulfate and fragrance mix in infants 0–18 months of age

	0 month		3 month		6 month		12 month		18 month	
	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys
<i>n</i>	250	249	236	227	226	224	200	202	177	175
Nickel sulfate										
200 $\mu\text{g}/\text{cm}^2$	0.8	1.2	5.1	5.3	9.3	7.6	26.0	15.8	29.9	22.9
66 $\mu\text{g}/\text{cm}^2$	0.0	0.0	0.4	0.9	0.9	0.4	2.5	1.5	4.5	4.0
22 $\mu\text{g}/\text{cm}^2$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
Fragrance mix										
430 $\mu\text{g}/\text{cm}^2$	0.0	0.0	0.0	0.0	0.4	1.8	1.0	1.0	0.0	1.1

Table 2. The frequency of a positive patch test ($\geq +$) to nickel sulfate 200 $\mu\text{g}/\text{cm}^2$ in relation to the number of test procedures performed in all children tested at least twice and both at 12 and 18 months ($n = 304$)

Number of patch tests performed per child	2-4	5	Total	≥ 2	5
Number of children tested	62	242	304	304	242
	Frequency			Frequency (%)	
Positive $\geq 1 \times$	27	110	137	45.1	45.5
Positive $\geq 2 \times$	10	38	48	15.8	15.7
Positive at 2 consecutive test occasions	5	25	30	9.9	10.3
Reproducible positive at 12 and 18 months	3	23	26	8.6	9.5

reaction to nickel sulfate occurring at 2 consecutive test occasions was seen in only 30 children, and only 26 children (20 girls and 6 boys) were positive at both 12 and 18 months, resulting in a prevalence of a probable nickel allergy of 8.6%. Already in this age group a probable nickel allergy was significantly more common in girls ($P < 0.05$). In infants not attending both the 12 and 18 months follow-up, 2 had a positive reaction at 2 consecutive occasions prior to 12 months.

During the study, 111 children (53 girls and 58 boys) showed an irritant/non-specific reactivity to nickel sulfate 200 $\mu\text{g}/\text{cm}^2$.

The day of reading had no influence on the type of reaction (Table 3). However, irritant or non-specific reactions were less frequent in summer compared with autumn and winter seasons ($P < 0.001$). Stronger positive reactions ($++$) occurred at all seasons and with similar frequency in infants with reproducible positive reactions (17.5%) and with transient reactions (17.2%).

The clinical relevance of a positive test result to nickel sulfate was evaluated in relation to the clinical findings, eczema history after skin contact with metal objects in clothing, metal jewellery, metal toys or ear piercing. Among the 304 infants who attended both the 12 and 18 months follow-up, 9 had a positive eczema history reported by parents and only 1 of these had a reproducible positive nickel sulfate reaction. 4 infants had had their ears pierced before 18 months of age and

none of these were positive. No children had visible eczema suspected to be caused by metal contact at the time of clinical examinations.

Atopic dermatitis was defined in accordance with the Hanifin and Rajka criteria (11), and 40 of the 304 children (girls 9.8% and boys 16.6%) had or had had atopic dermatitis. There was a slight tendency towards an increased frequency of atopic dermatitis in infants with positive and irritant reactivity (Table 4). There was no association between atopy judged by a positive skin prick test and sensitization to nickel.

A positive family history (mother and/or father) of doctor-diagnosed contact dermatitis was present in 37 case children (girls 13.1% and boys 11.3%); and of doctor-diagnosed nickel allergy in 26 case children (girls 6.5% and boys 10.6%). However, there was no association between a reproducible positive nickel patch test in an infant and a positive family history.

Doctor-diagnosed 'atopic diseases', defined as atopic dermatitis, asthma and/or hay fever in the family, were found in 139 children (girls 46.4% and boys 45.0%), again with no association with nickel sulfate sensitization.

Discussion

Patch test studies in series of selected children with suspected contact dermatitis have reported frequencies of positive reactions varying from 14% to 71% of patients (12-17). Nickel was

Table 3. The distribution of type of reaction in relation to day and season of reading characteristics

	<i>n</i>	Reproducible positive reactions	Irritant reactions	Negative reactions
Readings	982	57 (100)	128 (100)	797 (100)
Reading day				
Day 2	179	4 (7.0)	9 (7.0)	166 (20.8)
Day 3	574	36 (63.2)	79 (61.7)	459 (57.6)
Day 4	229	17 (29.8)	40 (31.3)	172 (21.6)
Season				
Spring	269	14 (24.6)	32 (25.0)	223 (28.0)
Summer	195	15 (26.3)	7 (5.5)	173 (21.7)
Autumn	267	14 (24.6)	46 (35.9)	207 (26.0)
Winter	251	14 (24.6)	43 (33.6)	194 (24.3)

The values are given as *n* (%).

Table 4. The distribution of sex, atopic eczema, atopy and family history in subjects with positive, irritant and only negative readings (n = 304)

	Positive (n = 26)	Irritant (n = 111)	Negative (n = 167)	P-value
Sex				
♀	20 (76.9)	53 (47.7)	80 (47.9)	0.02
♂	6 (23.1)	58 (52.2)	87 (52.1)	
Atopic eczema				
Yes	5 (19.2)	19 (17.1)	16 (9.6)	0.12
No	21 (80.8)	92 (82.9)	151 (90.4)	
Atopy*				
Yes	1 (4.2)	9 (8.3)	13 (7.8)	0.79
No	23 (95.8)	100 (91.7)	153 (92.2)	
Family report of contact eczema				
Yes	3 (11.5)	10 (9.0)	24 (14.4)	0.41
No	23 (88.5)	101 (91.0)	143 (85.6)	
Family report of allergy to nickel				
Yes	3 (11.5)	7 (6.3)	16 (9.6)	0.54
No	23 (88.5)	104 (93.7)	151 (90.4)	
Family report of atopic diseases				
Yes	13 (50.0)	26 (23.4)	80 (47.9)	<0.01
No	13 (50.0)	85 (76.6)	87 (52.1)	

Values are given as n (%).

*Atopy was defined as a positive prick test to at least 1 of 13 food or inhalant allergens (data missing in 5 children).

always the most frequently positive contact allergen. Patch testing of an unselected infant population has not been performed and provides information about the number of individuals sensitized who are potentially susceptible to develop allergic contact dermatitis.

It has been argued that contact dermatitis is rare in infants due to a limited exposure to contact allergens in infancy and to a less susceptible immune system. However, case reports on contact dermatitis in infancy have been published (18, 19). Some authors have commented that patch test reactions in infants were predominantly irritant reactions, especially when testing with nickel sulfate (20, 21). Later studies have recommended the use of the same test concentration in children as used for adults (22, 23).

In the present study, the TRUE TestTM patches provided an exact dosage of allergen with documented stability, even distribution and high bio-availability (7). The risk of a primary sensitization by TRUE TestTM patches with nickel sulfate and fragrance mix was considered extremely low (6, 24, 25).

Only a few population-based patch test studies have been performed concerning contact allergy in children. A Danish study showed sensitization to nickel in 8.6% and to fragrance mix in 1.8% of school children aged 12–16 (9). Weston and co-workers found a sensitization rate of 7.6% to nickel in children younger than 5 years, and reactions to balsam of Peru and colophony were

positive in 1.5% and 1.2%, respectively (2). In a study of 85 asymptomatic children aged 6 months to 5 years, using the TRUE TestTM system for patch testing, Bruckner and co-workers (4) found a sensitization rate of 24.5% to 1 or more allergens, and nickel was the most prevalent allergen, with a frequency of 8.2% at or before 18 months of age, in accordance with our results; no children reacted to fragrance mix. However, they did not report on the number of irritant reactions.

In this study an increasing number of infants were positive to nickel sulfate over the study period. When looking at prevalence measurements, most reactions were transient and probably of irritant or non-specific nature. The common occurrence of a single positive patch test to nickel sulfate suggests that the use of point prevalence measurements as a reflection of sensitization to nickel in infants is not well founded and that the standard patch test concentration of 200 µg/cm² in this age group causes non-specific/irritant reactions and cannot be recommended for routine use. The lower test concentration of 66 µg/cm² also gave poor reproducibility (1/25).

Other recent studies have reported high patch test reactivity in children, especially to nickel, and reactivity seemed to decrease with increasing age (26, 27).

In agreement with earlier studies in older children and adults (9, 28, 29), we found a female predominance of positive reproducible reactions to nickel sulfate in infants (girls 13.1% and

boys 4.0%). Possible sex-related differences in exposure to nickel in infants cannot explain the female preponderance. A genetically determined increased susceptibility to developing nickel reactivity in females may possibly explain this finding (30).

Clinical relevance of a positive test result was found in only 1 infant. It was based on a parental report of erythematous itchy dermatitis when metal was in contact with the child's skin. No visible skin symptoms suggestive of contact dermatitis from nickel were present at the time of clinical examination. The most common cause of sensitization to nickel is ear piercing and jewelry. In this study, none of the children with ear piercing were reproducible positive reactors.

Sensitization to nickel is not necessarily followed by allergic contact dermatitis, but infants with a reproducible positive reaction to nickel sulfate could represent a group at risk of developing clinically manifest nickel dermatitis later in life (31). Only a follow-up study can determine whether this is the case in the nickel-positive infants in this study.

In one report on the variation of nickel patch test reactivity in 20 nickel-allergic women tested 2× with several weeks interval, increased reactivity at the 2nd patch test suggested a booster effect from the 1st patch test procedure (32). However, no booster effect was seen in the present study, as only 1 of 26 infants with reproducible reactivity developed increased nickel sensitivity at a later test occasion. Further, there was no association between the number of patch test procedures performed and the development of nickel reactivity, in agreement with Hindsen and co-workers (Table 2) (33).

The large number of irritant/non-specific reactions to nickel sulfate 200 µg/cm² may be related to the physiology of the infant skin. Although the skin of the neonate and small children undergoes a process of adaptation and postnatal maturation, it has been shown that full-term neonates and infants have a well-developed epidermis, similar to adult skin with respect to epidermal and stratum corneum thickness, number of keratinocyte layers and transepidermal lipid barrier (34, 35).

Factors that explain skin reactivity to a specific dose of a chemical include dose technique, sex, age, race, climatic conditions, history of atopic diseases and other concomitant diseases. Most likely, non-specific or irritant reactivity is induced by a wide range of events, which together give rise to an inflammatory response to the chemical of concern (36). In this study, sex was not associated with non-specific or irritant reactivity; the test

period, however, seemed to be of relevance, the summer season being less prone to induce irritation.

Inter-individual variations of important proinflammatory mediators such as interleukin-1 (IL-1) and tumour necrosis factor-α (TNF-α) may give rise to variations in the intensity of the inflammatory response. Variation in nickel patch test reactivity has been shown in nickel-allergic patients and some patients demonstrated completely negative test results on at least 1 of more repetitive test occasions (33). In this study, 23 of 26 infants with reproducible reactivity at 18 months attended a follow-up at the age of 36 months. 11 (47.8%) were still positive reactors (10 girls and 1 boy) and 12 negative. Reactions were graded as at least ++ in 7 infants. Whether lack of reactivity in the remaining 12 infants represents inter-individual variation can only be speculation.

Certain clinical phenotypes such as patients with atopic eczema have been shown to react more strongly to irritants, demonstrating significantly higher levels of IL-1 and TNF-α in comparison with the skin of normal individuals. In this study, almost half (19/40) of the infants with atopic eczema developed irritant reactivity to nickel sulfate.

Another important aspect of contact allergy is the properties of the material tested. Nickel is a transition metal. Depending on oxidation state (37), it has the ability to form complexes with different geometries and stability, which either alone or together with other divalent metals can induce or reduce an inflammatory response, i.e. exacerbating effect induced by Fe and Cd or reducing effect exerted by nickel ions combining with Mg, Zn and Mn (38–41). The relatively large number of infants reacting with non-specific and irritant reactivity in this study could be attributed to these very properties of nickel. Irritant reactivity was not prominent when testing with fragrance mix in adult concentration and could partly support this argument.

In a recently published study, it was found that family history (first degree relative) seemed to be a good predictor of nickel allergy (42). 30 symptomatic patients aged 1–18 years were patch tested with nickel sulfate 5%. Symptoms were umbilical dermatitis, wrist dermatitis or an id reaction. All children were tested positive; 24 patients (80%) had a positive family history. In our study, only 3 of 26 positive infants (1 girl and 2 boys) had a positive family history. Our results cannot exclude that a positive family history may be relevant with respect to symptomatic children, but we cannot support relevance with respect to positive reactivity without clinical

symptoms. A family history of atopic disease was no more frequent in the group of positive reactors than in non-reactors, suggesting low correlation.

Fragrance mix

Fragrance mix is an indicator of perfume allergy and detects about 70–80% of contact allergy to perfumed cosmetics. A large number of perfumed products are marketed especially for children (5, 43). No infants were reproducibly positive to fragrance mix in this study. Compared with testing with nickel sulfate, testing with fragrance mix in adult concentration was well tolerated. 2 infants were positive reactors at 18 months of age; the result was not reproduced at the 36 months follow-up. 2 had or had had atopic dermatitis (18.2%); none had a positive family history to perfume allergy. Although allergy to perfume is an increasing problem in adolescents and adults (44), our results suggested that a relevant sensitization to perfume did not occur in early infancy. A restrictive parental attitude towards perfumed products for use by small children could partly explain our findings.

In conclusion, patch tests with nickel sulfate 200 mg/cm² gave a high proportion of non-specific and non-reproducible positive patch tests. This concentration used for adults cannot be recommended in infants; the interpretation of a singular patch test result in small children must be assessed with caution. Further, fragrance mix allergy was not found in this age group.

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