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ORIGINAL PAPER

House dust mite-related allergic diseases: role of skin prick test, atopy patch test, and RAST in the diagnosis of different manifestations of allergy

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Abstract The atopy patch test (APT) was recently defined as an important tool in diagnosis of atopic dermatitis (AD) and also of rhinitis and asthma caused by hypersensitivity to the house dust mites. We evaluated 465 children (279 males and 186 females) aged 0.4–17.6 years (mean 6.6± 3.8 years), by dividing them into four groups: group A, current AD (40 patients); group B, current AD with respiratory symptoms (156 patients); group C, past AD with respiratory symptoms (203 patients); and the control group, respiratory symptoms with no history of AD (66 patients). The APT was significantly more frequently positive in groups with current AD (groups A and B) or past AD (group C) than in the control group, while skin prick test (SPT) and radioallergosorbent test (RAST) were significantly more frequently positive in the control group. With multivariate analysis, for APT, significant differences were found in the comparison between group A vs group B (odds ratio (OR) 1.55) and between group A vs group C (OR 1.81). The mean age was significantly lower in group A than in groups B, C, and the control group and with less significance in groups C vs D. Children sensitized to mites with current or past AD, with or without respiratory symptoms, have a different response to diagnostic tests, which is characterized by a highly significantly more frequent positive APT in comparison with subjects who have respiratory symptoms but a negative history for AD, who show the common response to SPT and RAST.

Keywords Dust mites · Atopic dermatitis · Respiratory symptoms · Atopy patch test · Skin prick test · RAST

Abbreviations

AD	Atopic dermatitis
APT	Atopy patch test
ARIA	Allergic Rhinitis and Its Impact on Asthma
SPT	Skin prick test

Introduction

The house dust mites are a major cause of allergic diseases throughout the world [27]. The most frequently responsible mites are *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* which produce efficient allergens able to induce sensitization and clinical disease [38]. The spectrum of allergic manifestations includes atopic dermatitis (AD) [35], which is particularly frequent in children, rhinitis and asthma [13, 26], and, very rarely, anaphylaxis [10]. The knowledge of the mechanisms underlying the kind of sensitization and the type of clinical expression is far from exhaustive. It was long believed that the load of mite allergens which a subject is exposed to could be an essential factor in determining simple sensitization or

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clinical allergy. However, recent data have indicated that this relationship is not linear, as the highest exposure to dust mites was not directly associated with the risk of allergic outcome [34]. Instead, the mite species seems to have an influence on the type of allergy which subjects develop: In a large group of around 1,700 children, respiratory allergy was associated with both *D. pteronyssinus* and *D. farinae* exposure, while only the latter one was associated with AD [19].

As regards allergy testing, the skin prick test (SPT) or the measurement of specific IgE antibodies in serum is used to indicate sensitization, but they must be combined with the subject's case history to diagnose clinical allergy. The atopy patch test (APT), which consists of applying the suspected allergen to the skin by the same method of patch testing used for contact dermatitis, was introduced as a technique to evaluate sensitization to aeroallergens in subjects with AD [29]. It was later confirmed as a valid test, in particular for dust mites [4, 5, 14, 31, 36]. It is certainly of interest that recent studies have found that the APT can be the only positive test in patients with AD and also with respiratory allergy [6, 11].

We investigated the role of SPT, IgE measurement, and APT in mite allergy by analyzing the relationship between their results and the clinical expression in subjects with different allergic manifestations.

Materials and methods

Patients

From subjects consecutively referred to the Pediatric Allergy Service in San Severo (Foggia, Italy) for AD, 399 patients (239 males and 160 females) aged 0.4–17.6 years (mean ± SD 6.5±4.0; median 5.8) were included in the study. They were referred mostly by pediatricians but also by general practitioners, as they had a history of AD or respiratory symptoms (rhinitis, asthma) with uncertain etiology. The area where the patients lived includes 61 municipalities and 690,992 inhabitants, about 150,000 of whom are in pediatric age; in this area, there are 90 family pediatricians. To be included, the patients had to have a negative history for food allergy and negative skin prick tests to food extracts. The patients who took part in the study were divided into three groups: group A, current AD (40 patients); group B, current AD with respiratory symptoms (156 patients); and group C, past AD with respiratory symptom (203 patients). Sixty-six patients with rhinosinusitis or asthma but without AD (40 males and 26 females) aged 2.8–13.6 years (mean ± SD 6.6±2.8; median 6.5) examined during the same period served as the control group.

Methods

AD was diagnosed according to the criteria set down by Hanifin and Rajka [15], and rhinosinusitis and asthma were diagnosed on the basis of the consensus document Allergic Rhinitis and Its Impact on Asthma [2].

All patients underwent SPT with inhalant allergen extracts (Stallergénes, Antony, France) with test positivity assessed according to the guidelines from the European Academy of Allergology and Clinical Immunology [9] and to APT using house mite extract (Merck, Milan, Italy). In particular, the material used for testing was composed of inert dust mite bodies purified to 20% (D. Pteronyssinus and D. Farinae 1:1), with white vaseline 20% and white mineral oil as excipients. The substance to be tested was applied onto intact skin of the lower back and held firmly in position using adhesive patch tests made up of aluminum Finn chambers 8 mm in diameter, with an area of 50 mm^2 and a volume of about 20 μl. The application period was 48 h. The test was read no less than 30 min after removal to avoid margin effect. Results were interpreted according to the American Academy of Dermatology for APT [17], with a scale ranging from 1+ (weak reaction) to 3+ (very strong reaction). Only

Table 1 Demographic and baseline characteristics of the different groups

	Group A (n=40)	Group B (n=156)	Group C (n=203)	Group D (n=66)	P value
Gender, no. (%)					
M	28 (70)	96 (62)	115 (57)	40 (61)	ns[a]
F	12 (30)	60 (38)	88 (43)	26 (39)	
Age					
Mean ± SD (median)	1.9±1.6 (1.3)	6.5±3.6 (5.8)	7.4±3.9 (6.8)	6.6±2.8 (6.5)	<0.0001[b]
Family history of atopy					
No. (%)	24 (60)	92 (59)	98 (48)	32 (48)	ns[a]

Multiple comparison Bonferroni t test: group A vs group B, group C vs group D

[a] Pearson Chi square test

[b] One-way ANOVA

Table 2 APT, SPT, and RAST results in the different groups

	Group A	Group B	Group C	Control	Value
APT positive to mites, no. (%)					
Overall	22 (55)	113 (72)	121(60)	6 (12)	<0.0001
Familiar atopy present	17	66	67	5	
Males	15	74	75	5	
Females	7	39	46	1	
SPT positive to mites, no. (%)					
Overall	4 (10)	32 (20)	45 (22)	26 (39)	0.002
Familiar atopy present	1	22	29	14	
Males	2	20	30	15	
Females	2	12	15	11	
RAST positive, no. (%)					
Overall	5 (12.5)	29 (19)	44 (22)	28 (42)	0.0001
Familiar atopy present	3	20	22	16	
Males	3	20	29	17	
Females	2	9	15	11	

reactions of 2 and 3+ were considered positive for the purpose of the study.

Specific IgE to dust mites was measured by ImmunoCap (Phadia, Uppsala, Sweden).

Statistical analysis

Data are presented as percentages and as mean ± SD for qualitative and quantitative variables, respectively. Statistical analysis was performed using χ^2 test for categorical variables and analysis of variance (ANOVA) and Bonferroni multiple comparison tests for continuous variable. Contingency tables were used to analyze the association between the categorical variables (sex and positive family history) and the tests used in the study (APT, SPT, and radioallergosorbent test (RAST)) and to perform the univariate analysis of the positivities to APT, SPT, and RAST in the four different groups. Determinants of the single disease were evaluated by means of multivariate analysis performed using logistic regression analysis. We performed several stepwise forward logistic regressions used as dependent variables for each analysis: the control group vs the three atopic eczema/dermatitis syndrome (AEDS) groups, the current AEDS with respiratory group vs the current AEDS group and the past AEDS with respiratory symptom group, respectively, and the past AEDS with respiratory symptom group vs the current AEDS group. In each model, the predictor variables used were age as continuous variable and sex, positive family history, APT, SPT, and RAST as categorical variables. Statistical significance was assumed for $P<0.05$. All statistical analysis was performed using a software program (BMPD version 2007; BMPD statistical software Inc., Los Angeles, CA, USA).

Results

The characteristics of patients included in the four study groups are shown in Table 1. Group 1 subjects, i.e., patients

Table 3 Correlation between age categorized in three classes and presence of allergy

	Current AD with and without respiratory symptoms (%)	Current AD without respiratory symptoms (%)	Current AD with respiratory symptoms (%)	Past AD (%)	Current AD with respiratory symptoms (%)	χ^2	*P* value
≤2 years	84			16		30	<0.0001
2< ≤12 years	45.7			54.3			
>12 years	31.6			68.4			
≤2 years		69			31	77.8	<0.0001
>2 years		7.1			92.9		
≤2 years		78.4		21.6		121.6	<0.0001
>2 years		5.3		94.7			
≤2 years			61.9	38.1		5.1	0.07
2< ≤12 years			43.7	56.3			
>12 years			31.6	68.4			

Table 4 Positive results to APT, SPT, and RAST in the different groups, univariate analysis

	Test positivity %	χ^2	OR (95% CI)	*P* value
APT				
A vs B	55 vs 72.4	4.5	2.15 (1.05–4.4)	0.03
D vs A	9.1 vs 55	27.0	12.2. (4.3–34.7)	0.00001
C vs B	59.6 vs 72.4	6.4	1.8 (1.1–2.8)	0.01
D vs B	9.1 vs 72.4	74.8	26.3 (10.6–65.3)	<0.00001
D vs C	9.1 vs 59.6	51.0	14.8 (6.1–35.7)	<0.00001
SPT				
A vs D	10 vs 39.4	10.6	5.8 (2.6–13.2)	0.001
B vs D	20.5 vs 39.4	8.6	2.5 (1.3–4.7)	0.003
C vs D	22.2 vs 39.4	10.9	2.3 (1.3–4.1)	0.006
RAST				
A vs D	12.5 vs 42.4	10.4	5.1 (1.8–14.8)	0.001
B vs D	18.6 vs 42.4	13.8	3.2 (1.7–6.1)	0.0002
C vs D	21.7 vs 42.4	10.9	2.7 (1.5–4.8)	0.0009

with current AD, were significantly younger than subjects of the other three groups. The results of APT, SPT, and RAST in the different groups are reported in Table 2; the APT was significantly more frequently positive in groups with current AD (groups A and B) or past AD (group C) than in the control group, while SPT and RAST were significantly more frequently positive in the control group. Dividing the patients into three different age groups (0–2, 2–12, more than 12 years), a strong association between age group and current AD vs past AD could be seen (Table 3).

Tables 4 and 5 show the results of univariate and multivariate analysis, respectively. As far as univariate analysis was concerned, for APT, a number of significant differences were found between the different study groups, and all the differences between each study group and the control group were highly significant, whereas for SPT and RAST, the significant differences were in favor of the control group vs the three study groups. As regards multivariate analysis, for APT, significant differences were found only in the comparison between group A vs group B (odds ratio (OR) 1.55) and between group A vs group C (OR 1.81).

Discussion

AD has been thoroughly investigated in recent years in its clinical and pathogenetic aspects [1, 20–23]. Despite its denomination, when population studies are performed, the majority of patients with AD in the community do not have atopic sensitization. In the most recent study, which was carried out on 562 children, sensitization to environmental allergens was estimated by specific IgE measurement at about 25% at the age of 3 years [18].

This value is comparable with the 22% we found for IgE tests to dust mite—which is the prominent allergen source—while no population studies using the APT are as yet available. As regards the form sustained by hypersensitivity, AD fits at best the concept of systemic disease with multi-organ involvement [28], as made evident by the natural history of atopic disease. Atopic disease is characterized by early presentation with AD and subsequent respiratory manifestations with asthma and rhinitis, which is often summarized using the term “atopic march” [33]. A number of factors are implicated in this complex pathophysiologic development, which includes skin barrier dysfunction, the consequent exposure to allergens, the interaction with dendritic cells

Table 5 Results to APT adjusted for sex and age in the different groups, multivariate analysis

Outcome groups	Covariates	OR	CI 95%	*P* value
A + B vs C	APT	1.09	0.71–1.67	0.17
	APT adjusted for sex and age	1.00	0.94–1.06	1
A vs B	APT	0.51	0.31–0.85	0.007
	APT adjusted for sex and age	1.55	1.00–2.41	0.04
A vs C	APT	0.76	0.47–1.23	0.26
	APT adjusted for sex and age	1.81	1.01–3.25	0.02
B vs C	APT	1.28	1.02–1.62	0.03
	APT adjusted for sex and age	1.01	0.95–1.08	0.65

[23], and the activation of an initially local Th2 but later Th1 response along with a systemic Th2 response inducing isotype switching to IgE synthesis and the involvement of eosinophils [1].

Regarding skin barrier dysfunction, in many examples of recent data, interest has been focused on filaggrin, which consists of filament-associated proteins which are bound to keratin fibers in epidermal cells [30]. It was found that loss-of-function null mutations of the filaggrin gene predispose to AD [24] and that this specific gene is related to sensitization to allergens, to more severe phenotypes of AD [3] and asthma [25]. These mechanisms may explain previous experimental observations in which sensitization by the epicutaneous route leads to localized dermatitis and to bronchial hyperresponsiveness [32].

From a clinical point of view, the best tool to investigate the occurrence of these mechanisms seems to be the ATP, which reveals a reaction with T lymphocyte-mediated allergen-specific immune response [37] rather than referring to just the simple presence in the skin or in the blood of specific IgE, as SPT and RAST do. We recently demonstrated that in patients with asthma or rhinitis, a positive APT to house dust mite was strongly correlated to the presence of current or past AD, while most subjects with respiratory disease but a negative history for AD had instead a positive SPT [12].

In the course of our study, we examined a large population of subjects sensitized to dust mites by dividing them according to their clinical manifestations: current AD, current AD with respiratory symptoms, and past AD with respiratory symptoms, using patients with rhinitis or asthma but with no history of AD as a control group. The differences in rates of positive ATP were highly significant in favor of patients with current or past AD vs control subjects, while concerning SPT and RAST less strong differences were detected in favor of control subjects. The univariate analysis showed for APT significant differences in the rate of positivity in patients with current AD and respiratory symptoms compared with patients with only AD or with past AD and respiratory symptoms and highly significant differences in all single study groups compared with control group. The multivariate analysis including only subjects with current or past AD and adjusted for sex and age showed that APT is a good prognostic factor in the comparison between the group with only AD vs the group with AD and respiratory symptoms and vs the group with past AD and respiratory symptoms. The striking differences in response to APT suggest that different immunologic mechanisms underlie the various manifestations of hypersensitivity to dust mites. It seems conceivable that in subjects with a negative history for AD sensitization occurs by respiratory route and leads to the development of a Th2 pattern of response with ongoing production of specific IgE and consequent positive SPT and RAST. Instead, if mite allergens enter through the skin, as occurs in exposure to common indoor concentrations of the major allergen Der p 1 [16], such entering being facilitated by its proteolytic activity [8] and by the filaggrin-associated skin barrier dysfunction, a different sequence of events is likely to take place. This is ultimately revealed by positive APT and negative SPT and RAST.

Our observations were obtained through the study of a large population of patients with AD referred for allergologic evaluation because of suspected allergic origin of AD or respiratory symptoms, this being a possible bias for the high rate of positive APT we found. In any case, these data need to be confirmed by future studies to gain further importance. If confirmed, the definition of atopy as a genetic predisposition to react to SPT to environmental allergens should be reconsidered, also taking into account recent data on sensitization to arthropods and, in particular, to dust mites [7].

In conclusion, we found that subjects sensitized to dust mites with current or past AD, with or without respiratory symptoms, have a different response to diagnostic tests, which is characterized by a highly significantly more frequent positive APT in respect to subjects with respiratory symptoms but a negative history for AD, who show the common response to SPT and RAST.

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Conflicts of interest The authors declare no conflict of interest

References

1. Boguniewicz M, Leung DYM (2006) Atopic dermatitis. J Allergy Clin Immunol 117:S475–S480
2. Bousquet J, Van Cauwenberge P (eds) (2001) Allergic Rhinitis and its Impact on Asthma (ARIA). J Allergy Clin Immunol 108 (suppl 5):S146–S150
3. Cork MJ, Robinson DA, Vasilopoulos Y, Ferguson A, Moustafa M, McGowan A et al (2006) New perspectives on epidermal barrier dysfunction in atopic dermatitis: gene–environment interactions. J Allergy Clin Immunol 118:3–21
4. Darsow U, Vieluf D, Ring J (1995) Atopy patch test with different vehicles and allergen concentrations: an approach to standardization. J Allergy Clin Immunol 95:677–684
5. Darsow U, Vieluf D, Ring J (1999) Evaluating the relevance of aeroallergen sensitization in atopic eczema with the atopy patch test: a randomized, double-blind multicenter study. Atopy patch test study group. J Am Acad Dermatol 40:187–193
6. Darsow U, Laifaoui J, Kerschenlohr K et al (2004) The prevalence of positive reactions in the atopy patch test with aeroallergens and food allergens in subjects with atopic eczema: a European multicenter study. Allergy 59:1318–1323
7. Daschner A, Cuellar C, Valls A (2008) Toward a differential definition of atopy: Anisakis simplex and the relationship between parasites and arthropods in respiratory allergy. Parasite Immunol 30:417–424

8. Donnelly S, Dalton JP, Loukas A (2006) Proteases in helminth- and allergen-induced inflammatory responses. Chem Immunol Allergy 90:45–64
9. Dreborg S (ed) (1989) Skin tests used in type I allergy testing. Position paper of the European Academy of Allergology and Clinical Immunology. Allergy 44 (suppl 10)
10. Edston E, van Hage-Hamsten M (2003) Death in anaphylaxis in a man with house dust mite allergy. Int J Legal Med 117:299–301
11. Fuiano N, Incorvaia C (2003) Comparison of skin prick test and atopy patch test with dust mite extracts in patients with respiratory symptoms or atopic eczema dermatitis syndrome. Allergy 58:828
12. Fuiano N, Incorvaia C, Prodam F, Procaccini DA, Bona G (2008) Relationship between the atopy patch test and clinical expression of the disease in children with atopic eczema/dermatitis syndrome and respiratory symptoms. Ann Allergy Asthma Immunol 101:174–178
13. Gaffin JM, Phipatanakul W (2009) The role of indoor allergens in the development of asthma. Curr Opin Allergy Clin Immunol 9:126–135
14. Guler N, Kilerleri E, Tamay Z, Ones U (2006) Atopy patch testing in children with asthma and rhinitis symptoms allergic to house dust mite. Pediatr Allergy Immunol 17:346–350
15. Hanifin JM, Rajka G (1980) Diagnostic features of atopic dermatitis. Acta Dermatoven (Stock) 92:44–47
16. Huss-Marp J, Eberlein-Koenig B, Breuer K, Mair S, Ansel A, Darsow U et al (2006) Influence of short-term exposure to airborne Der p 1 and volatile organic compounds on skin barrier function and dermal blood flow in patients with atopic eczema and healthy individuals. Clin Exp Allergy 36:338–345
17. Kanof NB (1977) The American Academy of Dermatology patch test series for contact dermatitis. Int J Dermatol 16:827–829
18. Kjaer HF, Eller E, Andersen KE, Høst A, Bindslev-Jensen C (2009) The association between early sensitization patterns and subsequent allergic disease. The DARC birth cohort study. Pediatr Allergy Immunol (in press)
19. Kramer U, Lemmen C, Bartusel E et al (2006) Current eczema in children is related to Der f 1 exposure but not to Der p 1 exposure. Br J Dermatol 154:99–105
20. Leung DYM (1995) Atopic dermatitis: the skin as a window into the pathogenesis of chronic allergic diseases. J Allergy Clin Immunol 96:302–318
21. Leung DY, Bieber T (2003) Atopic dermatitis. Lancet 361:151–160
22. Leung DYM, Boguniewicz M, Howell MD, Nomura I, Hamid QA (2004) New insights into atopic dermatitis. J Clin Invest 113:651–657
23. Novak N, Bieber T, Leung DYM (2003) Immune mechanisms leading to atopic dermatitis. J Allergy Clin Immunol 112:S128–S139
24. Palmer CN, Irvine AD, Terron-Kwiatkoski A, Zhao Y, Liao H, Lee SP et al (2006) Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet 38:441–446
25. Palmer CN, Ismail T, Lee TH, Terron-Kwiatkoski A, Zhao Y, Liao H et al (2007) Filaggrin null mutations are associated with increased asthma severity in children and young adults. J Allergy Clin Immunol 120:64–68
26. Peroni DG, Piacentini GL, Alfonsi L, Zerman L, Di Blasi P, Visonà G, Nottegar F, Boner AL (2003) Rhinitis in pre-school children: prevalence, association with allergic diseases and risk factors. Clin Exp Allergy 33:1349–1354
27. Platts-Mills TA, Chapman MD (1987) Dust mites: immunology, allergic diseases, and environmental control. J Allergy Clin Immunol 80:755–775
28. Pucci S, Incorvaia C (2008) Allergy as an organ and a systemic disease. Clin Exp Immunol 153(Suppl 1):1–2
29. Ring J, Kunz B, Bieber T, Vieluf D, Przybilla B (1989) The "atopy patch test" with aeroallergens in atopic eczema. J Allergy Clin Immunol 82:195
30. Sandilands A, Sutherland C, Irvine AD, McLean WH (2009) Filaggrin in the frontline: role in skin barrier function and disease. J Cell Sci 122:1285–1294
31. Shah D, Hales J, Cooper D, Camp R (2002) Recognition of pathogenically relevant house dust mite hypersensitivity in adults with atopic dermatitis: a new approach? J Allergy Clin Immunol 109:1012–1018
32. Spergel JM, Mizoguchi E, Oettgen H, Bhan AK, Geha R (1998) Epicutaneous sensitization with protein antigens induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerosolized antigens in mice. J Clin Invest 101:1614–1622
33. Spergel JM, Paller AS (2003) Atopic dermatitis and the atopic march. J Allergy Clin Immunol 112:S128–S139
34. Tovey ER, Almqvist C, Li Q et al (2008) Nonlinear relationship of mite allergen exposure to mite sensitization and asthma in a birth cohort. J Allergy Clin Immunol 122:114–118
35. Tupker RA, de Monchy JG, Coenraads PJ (1998) House-dust mite hypersensitivity, eczema and other non pulmonary manifestations of allergy. Allergy 53:92–96
36. van Voorst Vader PC, Lier JG, Woest TE, Coenraads PJ, Nater JP (1991) Patch test with house dust mite antigens in atopic dermatitis patients: methodological methods. Acta Dermatol Venereol 71:301–305
37. Wistokat-Wulfing A, Schmidt P, Darsow U, Ring J, Kapp A, Werfel T (1999) Atopy patch test reactions are associated with T lymphocyte-mediated allergen-specific immune response in atopic dermatitis. Clin Exp Allergy 29:513–521
38. Zock JP, Heinrich J, Jarvis D et al (2006) Distribution and determinants of house dust mite allergens in Europe: the European Community Respiratory Health Survey II. J Allergy Clin Immunol 118:682–690

The study was conducted according to the Declaration of Helsinki. Being based on routine diagnostic tests, no authorization by local ethical committee was needed.