P.H. Benhamou[1,3], N. Kalach[1,2], P. Soulaines[1], N. Donne[3], C. Dupont[1]

Ready-to-use house dust mites atopy patch test (HDM-Diallertest®), a new screening tool for detection of house dust mites allergy in children

[1] Université Paris Descartes and Department of Pediatrics-Neonatology, Pediatric Gastroenterology & Nutrition Unit, Cochin-Saint Vincent de Paul Hospital, 82 Avenue Denfert-Rochereau, 75674 Paris Cedex 14, France.
[2] Clinic of Pediatrics Saint Antoine, Saint Vincent de Paul Hospital, Catholic University de Lille, Boulevard de Belfort, 59020 Lille, France.
[3] DBV Technologies, Pépinière Paris Santé Cochin, 27-29 rue du Faubourg Saint Jacques 75014 Paris, France

Key words

Ready-to-use atopy patch test, house dust mites, atopic dermatitis, children

Summary

Aim: *to assess the accuracy and safety of a ready-to-use atopy patch test (HDM-Diallertest®, DBV Technologies, Paris) in the diagnosis of sensitization to house-dust mite (HDM) allergens in children with or without atopic dermatitis.* ***Patients and methods:*** *prospective analysis of a systematic allergic work-up was carried out in 47 children, age 57.4+42 months (mean + SD, range 7 to 176 mo), presenting with isolated or combined atopic dermatitis (AD, n=28) or other symptoms without AD (control group, n=19). Children were routinely tested for specific HDM-IgE [against D. pteronyssinus (DPT) and D. farinae (DF)], and skin testing based on HDM (DPT & DF) skin prick test (SPT) and ready-to-use HDM-ATP (HDM-Diallertest®), with a reading at 72 hours.* ***Results:*** *15 children (31.9%) exhibited specific IgE against both DPT and DF, 16 children (34.04%) exhibited positive SPT against DPT and 17 (36.1%) against DF. HDM-Diallertest® was positive in 15 cases (31.9%). Among these, 9 exhibited with an eczematous reaction showed an excellent correlation with both SPT and specific IgE for DPT and DF, respectively 93.3%, 97.77%, 90.47%, and 90.47%. The different diagnostic techniques of HDM sensitization neither differ between groups, nor correlated specifically with the different clinical manifestations. No side effect was observed during and after patch testing, except for a local reaction without diffusion outside the local test area.* ***Conclusion:*** *The 3 diagnostic techniques exhibited a comparable level of accuracy for the diagnosis of HDM allergens sensitization. The excellent concordance of the highest class reactions of HDM-Diallertest® with the other diagnostic techniques indicates a potential role as a screening tool for the detection of HDM sensitization in infancy.*

Introduction

The atopy patch test (APT) with aeroallergens was introduced by Platts-Mills et al (1) as an experimental model and as a diagnostic tool. These authors showed that the application of house dust mite (HDM) antigen to the epidermis leads to a considerable percentage of positive local reactions in sensitized individuals with atopic dermatitis (AD). However, differences in methodology and lack of gold standard for methodology and interpretation are two major obstacles for development of the APT. The aim of our study was to assess the accuracy and safety of a ready-to-use HDM-APT (HDM-Diallertest®) and the correlation with specific HDM IgE [D. pteronyssinus (DPT) and D. farinae (DF)], and skin prick test (SPT) for DPT and DF, in the assessment of a pediatric allergic population, together with its usefulness in the diagnosis of sensitization to allergens of HDM in children with or without atopic dermatitis (AD).

Methods

Patients

A prospective study of a systematic allergic work-up was carried out between January 2005 and June 2005 in a population of 47 children, age 57.4+42 months (mean + SD, range 7 to 176 months), 18 girls and 29 boys, enrolled following referral to the outpatient clinic of respiratory, cutaneous and food allergy. Patients exhibited AD (n=28, 59.57%), isolated (n=23, 48;9 %), or combined (2 children with digestive manifestations and 3 with pulmonary manifestations), or no AD symptoms, i.e. non AD group, n=19 (40.43%), consisting of patients with digestive manifestations such as colic, vomiting, gastro-esophageal reflux and failure to thrive (n=10, 21.27%), or pulmonary manifestations (n=2, 4.25%), ear nose throat (ENT) manifestations (n=2, 4.25%), angio-edema (n=2, 4.25%), and combined digestive, pulmonary and ENT manifestations (n=3, 6.38%). All abnormal cutaneous, digestive, pulmonary, ENT and angio-edema reactions were analyzed by the parent's child and then validated by the physician. Children were not enrolled if under any food elimination diet, presenting with important skin lesions thus preventing from the possibility of patch testing, and having been treated with antihistamine and oral or cutaneous steroid medications for the last week.

Study design

Children were routinely tested via a blood sample for measurement of HDM specific IgE (DPT and DF), SPT with DPT and DF and ATP, using the ready-to-use technique HDM-ATP (HDM-Diallertest®). All children were randomized for the application of APT on the right or the left side of the back. AD was assessed at enrollment using a routine local score based on the severity and extension of eczematous skin lesions as follows: no, mild, moderate, severe and very severe atopic dermatitis. The physician responsible for the study also did the SPT reading but was blinded to the results of specific IgE. All side effects related to HDM-Diallertest® technique were recorded throughout the study duration, according to a questionnaire explained by the research nurse. A written parental consent was obtained in all cases from both parents.

Methods

DPT & DF specific IgE were analyzed using the RAST Cap System (Phadia, Uppsala, Sweden) calibrated with reference to the World Health Organization standards for IgE. Its specificity was already assessed and its use in children already reported (2). References data for HDM specific IgE were those of the manufacturer with the lowest detection level at 0.35 KU/L, class 1, 0.35-0.70 KU/L, class 2, 0.70-3.5 KU/L, class 3, 3.5-17.50 KU/L, class 4, 17.50-50 KU/L, class 5, 50-100 KU/L, and class 6, > 100 KU/L. (3, 4). A specific HDM-IgE class > was considered as positive.

SPTs were performed with a drop of commercially available extracts of DPT and DF from (ALK®, Copenhagen, Denmark) with a potency of 10 HEP, which, according to the company, is equal to 20 000 BU/ml. The procedures were performed on the children back if aged less than 12 months and on the volar part of the forearm in those above 12 months. Histamine di-hydrochloride (ALK, Copenhagen, Denmark), 10 mg/ml, and glycerosaline were used as positive and negative controls. The wheal size reaction scoring system was class 1 from 3 to 5 mm, class 2 (6-10 mm), class 3 (11-15 mm), and class 4 (>15 mm). A positive reaction implied a wheal diameter 3 mm larger than the negative control after 15 minutes (5).

The ready-to-use HDM ATP (HDM-Diallertest®, DBV-Technologies, Paris, France), 11 mm diameter, consisted of 3 parts, already described (6). A same mixture of pure HDM, 50% DPT and 50% DF was deposited on

the central plastic support in the form of micro granules (5-40 Ìm) mixed with dry powder of glucose forming a homogeneous mono-layer, retained by electrostatics forces. Each HDM-Diallertest® thus contained 300 µg ± 30 µg of DPT & DF. The ready-to-use APT serving as control had the same structure but was deprived of any HDM powder in the central part.

A phone call was given 24h after application of the APTs in order to assess the safety of the HDM-Diallertest®, a specific surveillance of the reaction through the transparent patch membrane being requested from parents The occlusion time was 48 hours, and the results were read by the same investigator 24 hours following removal of the devices, i.e. at 72 hours. Digital pictures were taken at 72 hours and kept into a computer. HDM-Diallertest® reactions were graded according to the criteria used in conventional contact allergy patch testing [International Contact Dermatitis Research Group (ICDRG) rules] with the modifications of the European Task Force on AD (ETFAD) consensus meeting (7, 8); i.e. - as negative; ?, only erythema (class 1); +, erythema and infiltration (class 2); ++, erythema, few papules (up to 3) (class 3); +++ erythema, papules 4 to < many (class 4); ++++, erythema, many or spreading papules (class 5); +++++, erythema, vesicules (class 6). The reaction was considered positive if at 72h the HDM-Diallertest® exhibited a skin reaction graded above that of the negative control, i.e. presence of clear redness and palpable infiltration > class 2, as described by Holm et al et al (9). The same physician evaluated all tests.

Statistical analysis

All statistical tests were performed with the Statview program (Abacus®, Ca, USA). Calculation of mean, median and extremes were done for all quantitative parameters. Differences between qualitative and quantitative parameters were analyzed by the chi-square test and Anova multiple analysis test. A p value of < 0.05 was considered as statistically significant. The concordance test kappa of Cohen was used to assess the degree of concordance between the different diagnostic allergic tests used in this study. The concordance between the different parameters was considered function the value of kappa as; excellent between 0.81 and 1, good 0.61 and 0.8, moderate 0.41 and 0.6, weak 0.21 and 0.4, and finally bad 0 and 0.2.

Results

Among the 47 children, 15 children (31.9 %) exhibited positive specific IgE titers against both DPT and DF, whereas 16 children (34.04%) exhibited positive SPT against DPT and 17 (36.1 %) against DF. The HDM-Diallertest® was positive in 15 cases (31.9%), Table 1. Among these 15 positive HDM-Diallertest®, 9 exhibited an eczematous reaction and showed an excellent concordance with DPT & DF-SPT and specific IgE against DPT & DF, respectively 93.3%, 97.77%, 90.47%, and 90.47%. The different HDM sensitization diagnostic techniques used neither revealed any differences between groups, nor correlated specifically with the different clinical manifestations, Table 1.

Table 1 - Characteristics of AD and non AD patients, n=47

	AD patients	Non AD patients	P	Total
	n=28	n=19		n=47
Female/Male	10/18	8/11	ns*	18/29
Age, months, median (range)	42.3 (7-128)	79.6 (8-176)*	0.002**	49 (7-176)
Positive HDM-Diallertest®, n(%)	11 (39.28)	4 (21.05)	ns*	15 (31.9)
Positive specific DPT-IgE, n(%)	10 (35.7)	5 (26.31)	ns*	15 (31.9)
Positve specific DF-IgE, n(%)	10 (35.7)	5 (26.31)	ns*	15 (31.9)
Positve DPT-SPT, n(%)	11 (39.28)	5 (26.31)	ns*	16 (34.04)
Positive DF-SPT, n(%)	8 (28.5)	9 (47.36)	ns*	17 (36.1)

*According to Chi square test
** According to Anova test
ns : not significant

HDM-Diallertest® exhibited a tendency towards a correlation with the highest RAST and SPT classes, whereas only 7 children exhibited negative HDM-Diallertest® concomitant with RAST-DPT & DF classes above 1. Similarly, only 7 and 8 children exhibited negative HDM-Diallertest® concomitant with SPT-DPT & DF classes above 1, Tables 2 & 3 Children exhibited positive HDM-Diallertest® results concomitant with either positive specific IgE or SPT in 76.4% of cases, whereas those exhibited positive HDM-Diallertest® > grade 3 concomitant with either positive specific IgE or SPT in all cases. The HDM-Diallertest® exhibited also excellent concordant results with both HMD-IgE and SPT against both DPT & DF, kappa between 0.81 and 0.95, Table 4.

Finally, among the 47 children, no side effect was observed during and after patch testing, the reaction being always confined to the local testing area, without diffusion outside.

Discussion

We show in this first pilot and preliminary study carried out in 47 children that the 3 diagnostic techniques exhibited a comparable level of accuracy for the diagnosis of

Table 2 - Correlation between serum specific IgE (RAST classes) to Dermatophagoides pteronyssinus (DPT), Dermatophagoides farinae (DF) and HDM-Diallertest® reactions to HDM extract

RAST classes (DPT), n HDM-Diallertest®	0	1-3	4-6	Not done
Negative	18	4	3	5
Class 1	0	1	1	0
Class 2	6	0	1	0
Class 3	2	0	0	0
Class 4	1	1	1	0
Class 5	0	1	1	0
Class 6	0	0	1	0
RAST classes (DF), n HDM-Diallertest®	0	1-3	4-6	Not done
Negative	18	3	4	5
Class 1	0	1	1	0
Class 2	6	0	1	0
Class 3	1	0	0	1
Class 4	1	1	1	0
Class 5	0	1	1	0
Class 6	0	0	1	0
				0

RAST class of > 1 and APT of > 2 were recognized as positive

Table 3 - Table III : Correlation between skin prick test (SPT) to Dermatophagoides pteronyssinus (DPT), Dermatophagoides farinae (DF) and HDM- Diallertest® reactions to HDM extract

SPT classes (DPT), n HDM-Diallertest®	Negative	Class 1	Class 2	Class 3	Class 4	Not done
Negative	21	2	3	1	1	2
Class 1	0	1	1	0	0	0
Class 2	6	0	0	1	0	0
Class 3	0	2	0	0	0	0
Class 4	1	0	2	0	0	0
Class 5	1	1	0	0	0	0
Class 6	0	1	0	0	0	0
SPT classes (DF), n HDM-Diallertest®	Negative	Class 1	Class 2	Class 3	Class 4	Not done
Negative	20	4	3	1	0	2
Class 1	0	0	1	1	0	0
Class 2	5	1	1	0	0	0
Class 3	2	0	0	0	0	0
Class 4	0	0	3	0	0	0
Class 5	1	0	1	0	0	0
Class 6	0	1	0	0	0	0

SPT of > 1 and APT of > 2 were recognized as positive

HDM allergens sensitization. The excellent concordance of HDM- Diallertest® with other diagnostic techniques during eczematous reactions strongly suggests its use as a reliable non invasive diagnostic tool of HDM sensitization.

The APT was introduced to assess sensitization to inhalant allergens in patients with AD (10, 11), and according to the first observations results, it was assumed that, during patch testing, allergens could induce locally eczematous lesions in AD patients, restricted to those who also gave a positive immediate skin reaction to the same allergen (12). From now on, APT is considered the only provocation test currently available with clinical relevance for contact IgE-mediated sensitization in AD patients. (11). Consequently, APT is regarded as specific for AD patients as it was confirmed in numerous studies (5, 7, 8-13). In a recent paper by Fuiano et al. results of HDM-APT were compared with those of SPT in 297 individuals AD and/or respiratory symptoms. The rate of APT positive was significantly higher in AD children and SPT positive in children with respiratory diseases. Interestingly, in the present study, the rate of eczematous reactions after HDM-Diallertest® was not statistically different in AD and non AD patients probably due to the small number of APT positive patients.

The significant correlation both between SPT, specific IgE and HDM-Diallertest® confirms results reported in the literature (8), the highest grades of skin reaction generating the highest degree of correlation. Fuiano and Incorvaia compared HDM SPT and APTs in children and adults with AD and/or respiratory diseases (13). APTs were more frequently positive than SPT not only in AD patients (86.2% vs 21.6%) but also in those with respiratory symptoms only (78.7% vs 34%). The average positivity rate was significantly lower in our study with the ready to use device (36%), in accordance with data other obtained in AD, 26,9% (14) and 47,2% (11). Contrary to Fuaino's results (13), we found a good correlation between HDM-Diallertest® and SPT in both AD and non AD patients, as well as for DPT SPT and DF SPT, with a correlation rate of 73,3 and 66,6% respectively for the lower grades HDM-Diallertest® reactions and 93,3 and 97,7% respectively for the higher grades.

This good correlation between specific IgE, SPT and APT during HDM testing, is not a common rule for other allergens. In AD patients compared to non-atopic controls, Goon et al. tested HDM, cat dander, Bermuda grass and German cockroach: only the HDM APT showed a correlation with specific IgE, APT for cat dander being well correlated with SPT (15). In addition, during food allergy, APT is badly correlated with SPT and specific IgE (6, 16), immediate reaction tending to be correlated with specific IgE and SPT and delayed ones with APT (6, 16),

In terms of potential routine utilisation of a ready to use APT, the more striking result of this is the very good correlation of specific IgE and SPT with the higher classes of HDM Diallertest®. This point could become crucial in a daily clinical use. When considering only the strongest reactions to HDM, i.e. with infiltration, erythema and few papules, the high degree of predictive value of APT may lead to propose this technique as a non invasive first step when HDM sensitization is suspected.

In a recent study in children under 3 years old with AD, Devilliers et al, have a relatively high number of immedi-

Table 4 - Concordance results of the HDM-Diallertest® compared to the other diagnostic techniques, (n=47)

	Discordant results n	Concordant results n(%)
HDM-Diallertest® vs SPT-DPT		
Overall		30 (66.66)d
Class 1 to 2	13	32 (71.11)d
Class 3 to 6	2	43 (95.55)a
HDM-Diallertest® vs SPT-DF		
Overall		29 (64.44)d
Class 1 to 2	13	32 (71.11)d
Class 3 to 6	3	42 (93.33)a
HDM-Diallertest® vs IgE-DPT		
Overall		29 (69.04)d
Class 1 to 2	13	29 (69.04)d
Class 3 to 6	3	39 (92.85)a
HDM-Diallertest® vs IgE-DF		
Overall		26 (63.41)d
Class 1 to 2	13	28 (68.29)c
Class 3 to 6	2	39 (95.12)a

According to the concordance test Kappa of Cohen;
a kappa is considered excellent between 0.81 and 1
b kappa is considered good between 0.61 and 0.8
c kappa is considered moderate between 0.41 and 0.6
d kappa is considered weak between 0.21 and 0.4
e kappa is considered bad between 0 and 0.2

ate types, urticarial APT reaction. These reactions had only been observed in patient with elevated serum levels of HDM- IgE titers (17). In our study population, we did not noticed such side effects even when HDM-IgE level was very high.

Conclusion

In a population of children with AD, isolated or combined to other symptoms or other symptoms without AD, a high correlation was found between SPTs and specific IgE measurements and the HDM- Diallertest®, when considering the highest grades of reactivity. The excellent concordance with higher class reactions of is highly suggestive for its use in the detection of the IgE and non-IgE mediated HDM sensitization mechanisms in children. These first results should be confirmed by further study in adult and children with a high number of patients in order to verify the good level of accuracy and safety of HDM-Diallertest® before its use in practical as a screening tool for detection of HDM sensitization.

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