Evaluation of patch test with airbone allergic agents in patients with atopic dermatitis

Avaliação do teste de contato com aeroalérgenos em pacientes com dermatite atópica

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Abstract: BACKGROUND: Atopic dermatitis is an inflammatory skin disease that can be triggered by many factors. Several reports confirm the role of airborne allergic agents as aggravating or triggering factors. The patch test with airborne allergic agents or the atopy patch test was suggested to evaluate the role of these allergens in atopic dermatitis.
OBJECTIVE: This study aimed at evaluating the positivity of the atopy patch test in patients with atopic dermatitis.
METHODS: We evaluated 50 patients with atopic dermatitis and 45 with allergic rhinitis, the atopy patch test was performed in these patiennts with extracts of Dermatophagoides pteronissynus, Dermatophagoides farinae and Blomia tropicalis, as well as immediate skin prick tests for the same allergens with cat and dog epithelia and fungi.
RESULTS: It was found that the atopy patch test with dust mites showed higher positivity in individuals with atopic dermatitis when compared to those with allergic rhinitis.
CONCLUSIONS: The atopy patch test shows statistically significant results when performed with dust mites in patients with atopic dermatitis, p = 0.035, odds ratio (OR) = 3.35 and CI (95%) = [1.18, 9, 47].
Keywords: Dermatitis, atopic; Dermatophagoides pteronyssinus; Patch tests

Resumo: FUNDAMENTOS: a dermatite atópica é uma doença inflamatória cutânea que apresenta múltiplos fatores desencadeantes. Há vários relatos de autores que confirmaram os aeroalérgenos como fatores agravantes ou desencadeantes. O teste de contato com aeroalérgenos ou teste de contato atópico foi proposto para avaliar a participação destes alérgenos na dermatite atópica.
OBJETIVO: objetivo deste estudo foi avaliar a positividade do teste de contato atópico em pacientes com dermatite atópica.
MÉTODOS: Avaliamos 50 pacientes com dermatite atópica e 45 do grupo com rinite alérgica, nos quais realizamos teste de contato atópico com extratos de Dermatophagoides pteronissynus, Dermatophagoides farinae e Blomia tropicalis, além de testes cutâneos de leitura imediata para os mesmos alérgenos, acrescidos de epitélio de cão e gato e fungos.
RESULTADOS: verificamos que o teste de contato atópico com ácaros apresentou maior positividade nos indivíduos do grupo de dermatite atópica quando comparado ao grupo de rinite alérgica.
CONCLUSÕES: o teste de contato atópico apresenta resultados estatisticamente significativos quando realizado com ácaros, em pacientes com dermatite atópica, com p=0,035, OR (odds ratio) = 3,35 e IC(95%) = [1,18; 9,47].
Palavras-chave: Dermatite atópica; Dermatophagoides pteronyssinus; Hipersensibilidade

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INTRODUCTION

Atopic dermatitis (AD) is a chronic and recurrent inflammatory dermatosis characterized by eczematous lesions with specific location according to age. In most patients it is associated with asthma or allergic rhinitis (AR). [1]

Most cases start in childhood; 60% occur during the first year of life and 85% by five years of age. The prevalence of this disease in Sao Paulo is around 13% in children between 6 and 7 years of age for both sexes; for children between 13 and 14 years of age the prevalence is 12% for males and 15% for females, according to the ISAAC (International Study of Asthma and Allergy in Children) [2].

The disease presents with acute, subacute or chronic eczematous lesions. In infants lesions are present on the face and extensor surface of limbs; in older children they predominate in flexor regions, and in adults, in various sites, predominantly in flexor regions. [3]

We can say that AD is a complex disease where genetics, the structure of the skin - a characteristic of these patients due to altered barrier function - and immunological and non-immunological disorders interact, with many factors participating in the pathogenesis. [4, 5]

Although the role of allergy in the pathogenesis of AD is still controversial, it is known that contact with allergens, including dust mites, can trigger lesions in some patients. Several authors have shown evidence of involvement of these agents as causes of injuries, both through environmental studies and allergy tests. [6]

The relationship between mites and AD was first reported by Rost* in 1932, with the conclusion that some patients with eczema and also those allergic to dust improved their condition when removed from their homes. [7]

Subsequently, other authors also reported this relationship and observed eczematous lesions after epicutaneous application of airborne allergens into patients with AD, performed through the modified contact test technique called atopy patch test (APT) [8].

The APT is performed with allergens that induce an IgE-mediated reaction in individuals with AD. [8]

The indication of the test is based on the pathophysiology of the disease; it was observed that antigen presenting cells (APC) of these individuals have on their surface high-affinity IgE receptors (FcεRI) that facilitate the capture and internalization of the airborne allergen through the skin. [9]

The association of these immunological characteristics with the change in skin barrier function in these individuals allows airborne allergens to more easily penetrate the epidermis, bind to IgE-FcεRI and be 'presented' to immature T lymphocytes (LT) causing their differentiation into Th2 lymphocytes (LTh2) and subsequently into Th1 lymphocytes (LTh1). [5,6]

Thus, observing the immediate and delayed immune reactions that occur in this disease, the APT was suggested to evaluate a delayed hypersensitivity skin reaction (eczematous lesions), possibly induced by allergens associated with IgE-mediated reactions. Through the APT we attempt to experimentally reproduce the pathophysiological events of AD.

The objective of this study was to evaluate the positivity of the APT, when performed with mites (Dpt, Df and Bt), in patients with AD.

PATIENTS, MATERIALS AND METHODS

The work and the free and informed consent (FIC) were approved by the Ethics Research Committee of the HSPE/IAMSPE. All the research subjects or guardians signed an informed consent to participate.

Ninety-five patients aged over 1 year, of both sexes, from the outpatient Clinics of Dermatology and Allergy and Immunology at "Francisco Morato de Oliveira" State Public Servant Hospital (HSPE) and Allergy Center of the Municipal Government of Santo Andre were selected to participate.

A questionnaire qualifying the disease was administered to all the patients in both groups. After the questionnaire was answered and the patients were submitted to physical examination, they were divided into two groups according to diagnosis:

A. 50 patients with AD, with or without respiratory allergy (rhinitis or asthma); the diagnostic was established according to the Hanifin and Rajka criteria [10] and severity was assessed by SCORAD [11] ("severity score of atopic dermatitis").

B. 45 patients with AR, defined according to the ARIA (ALLERGIC RHINITIS AND ITS IMPACT ON ASTHMA), [12] with or without asthma, but with no evidence of skin disease. The diagnosis of asthma was based on the III Brazilian Consensus on Asthma Management, 2002. [13]

Hemogram, parasitological examination in three samples, total IgE levels, immediate skin test reactivity to mite (Dpt, Df and Bt), cat and dog epithelia, *Alternaria alternata* and fungi III *(astelodami Aspergillus, niger, and terreus)* and IV *(Peniccilinum brevicompactum, expansum and notatum)* were performed.

APT with standardized extract for immediate skin tests for Dpt, Df, Bt and their diluent was used in all patients.

Inclusion criteria:
1. research subjects or guardians who read and signed the consent form.
2. Group A - Patients with AD aged ? 1 year, with or without respiratory allergy.
3. Group B - Patients with AR, with or without asthma, and without skin diseases.
Exclusion criteria:
1. Patients with skin diseases other than AD in both groups;
2. Patients with nonallergic rhinitis;
3. Pregnancy;
4. Younger than one year old;
5. Serious illnesses;
6. Patients using immunosuppressive drugs;
7. Patients using systemic corticosteroids and/or topical drugs at the test site and other medication that might interfere with their performance.
8. Research subjects unable to read or understand the Informed Consent.
9. Those who refused to sign the Informed Consent.

The extracts for the APT (Dpt, Df and Bt) and its diluent were prepared from the extract of immediate skin tests diluted at 30%, using solid petrolatum as vehicle. These were prepared and granted by the FDA ALLERGENIC laboratory.

The APT technique was performed according to the standardization of the Allergy and Dermatology Department, Brazilian Society of Dermatology (SBD). [14]

The tests were applied using aluminum chambers of 8 mm into the top of the dorsal region of patients, previously cleaned with ether. Immediately before the test, abrasion with micropore tape was done ten times in the same place to facilitate the absorption of antigens, as described in other studies. [15]

The substances, 5 cm apart from one another, were left on the dorsal region for 48 hours and then removed (day 2). The second reading took place 96 hours after the start of the test (day 4) (Figure 1), following the criteria established by the Brazilian Group for the Study of Contact Dermatitis (GBEDC). [14]
(-) negative
(?) uncertain
(+) Erythema with a few papules
(+ +) Erythema, papules and vesicles
(+++) Intense erythema, papules and confluent vesicles.

We considered positive only the reactions of the tests whose intensity in the 96-hour reading was equal to or higher than that obtained after 48 hours; in other words, the results that had an upward direction. The reactions that were less intense in the 96 hour-reading compared to the one obtained after 48 hours were considered irritant and were not relevant to the study.

Commercially available extracts to perform immediate skin tests (Dpt and Df), cat and dog epithelia, *alternaria alternata* and fungi III *(astelodami Aspergillus, niger, and terreus)* and IV *(Peniccilinum brevicompactum, expansum and notatum),* were granted by ALK ABELLÓ Laboratory, and the deBt, by IPI - ASAC Brazil.

The test was performed using the Pepys technique: percutaneously (puncture) on the volar surface of the forearm. After cleaning the area with alcohol 70 ° GL, one drop of each of the extracts above mentioned, the diluent (negative control) and histamine (positive control) were applied. The devices used were disposable puncture needles, one for each substance, introduced perpendicularly to the skin surface, exerting gentle pressure on each drop. The results were read 20 minutes after the beginning of the test by measuring the mean diameter of the papule. Reactions greater than or equal to 3 millimeters in diameter for each substance were considered positive. [16]

Drugs that could interfere with the test results were suspended; patients were advised to discontinue the use of antihistamines 10 days prior to the performance of immediate skin tests. [16]

RESULTS

Of the 95 patients studied, 53% were diagnosed with AD, while 48% had AR.

In the group with AD the median age was 4.9 years; the mean age was 9.3 years in the AR group. In the group with AD, 31 patients (62%) were female and 19 (38%), male, whereas in the AR group, 13 (28.8%) were female and 32 (71.1%), male.

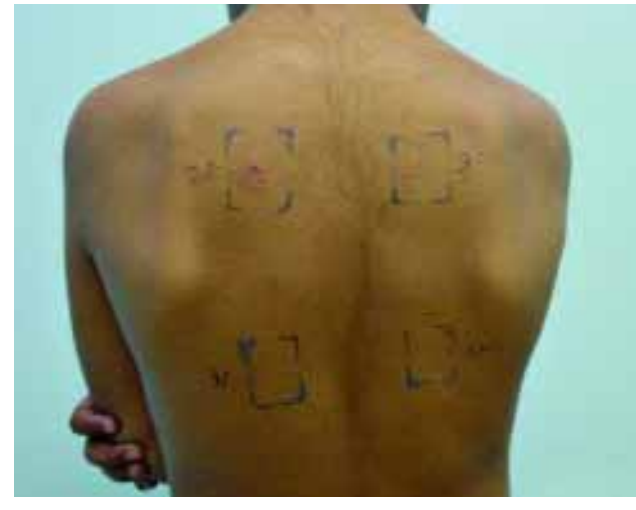

Figura 1: Positive result of the Atopy Patch Test for Dermatophagoides pteronyssinus

In the group with AD, 17 (34%) out of 50 patients had at least one positive APT, while in the group with AR 6 (13.3%) out of 45 patients had at least one positive APT (Table 1).

There were no positive reactions in tests with the diluent in both groups.

There were statistically significant differences between the results for the AD and AR groups ($p < 0.05$).

The chance of the test being positive in the group with AD was 3.35 times higher than for the group with AR.

The results showed statistically significant differences between both groups, with $p < 0.05$.

The OR was 2.5, indicating that AD patients had a greater chance of having positive APT.

In relation to the total number of positive tests in each group, we found 25 (16.7%) positive results in the AD group and 10 (7.4%) in the AR group. The total number of positive tests showed greater relevance in the group with AD in relation to those with AR, and this was statistically significant ($p = 0.017$). (Table 2)

Of the 50 patients in the group with AD, 42 (84%) had immediate skin tests positive for at least one allergen; of the 17 patients with at least one positive APT in the same group, 16 (94%) also had immediate skin tests positive for at least one allergen. Of these 16 individuals, 12 (75%) had a positive reaction to the same allergen in both tests.

Statistical methods

The results of the APT for the AD and AR groups were represented by contingency tables and the comparison between the diagnoses in relation to the results of the tests, by Pearson's chi-square test with continuity correction. The odds ratio (OR) and the respective CI - 95% were also calculated.

The significance level was 0.05 ($\cdot$ = 5%) and descriptive levels (p) below this value were considered significant.

DISCUSSION

Atopic dermatitis is a complex disease in which several triggering or aggravating factors, intrinsic and extrinsic, are involved; among these we highlight airborne allergens. [17] Many patients have immediate skin tests positive to airborne allergens. These tests are relevant in relation to respiratory allergic diseases, where the role of airborne allergens is well established. The role of airborne allergens in AD is still controversial because the disease presents with eczematous lesions which are consistent with a delayed reaction mediated by lymphocytes. These tests assess the immediate reaction that does not represent the clinical manifestation of AD and also do not fully evaluate the cutaneous immune response of this disease. [18]

The skin is exposed to environmental influences, including allergens, and it is postulated that they may adhere to the skin surface and repeatedly induce sensitization or elicitation through this organ, based on the pathophysiology of the disease. [19]

All these events prompted some authors to try to reproduce the lesions of atopic dermatitis with triggers such as house dust mites. Since lesions in AD are eczematous, a study model similar to that of allergic contact dermatitis was established. [8]

The first work about the APT was published in 1982 by Mitchell *et al.* and others were subsequently conducted with different methodologies, using various vehicles and concentrations as well as some type of abrasion on the skin in an attempt to achieve standardization. [20]

In a study, Goon observed a positivity of 43.9% for the mite *Dermatophagoides pteronyssinus,* as well as a statistically significant difference when comparing test results in patients with AD and AR. [21]

Jamora *et al.* performed the APT with a mixture of Dpt 50% and Df 50% diluted in petrolatum at 20%. This dilution was fractionated at concentrations equivalent to 1.25%, 1.0%, 0.75%, 0.5%, 0.25% and 0.1%. In their results, the authors showed that 0.1% was better for APT positivity in patients with AD, but the positivity was similar to that of patients with respiratory allergy and non-allergic individuals in

Table 1: Patients who had at least one APT positive for the diagnosis of AD or AR in the 96 –hour reading

	Diagnosis		
Results APT	**Atopic Dermatitis**	**Rhinitis**	**Total**
Positive	17 (34,0%)	6 (13,3%)	23 (24,2%)
Negative	33 (66,0%)	39 (86,7%)	72 (75,8%)
Total	50 (100%)	45 (100%)	95 (100%)
$X^2 = 4,44$	p = 0,035	OR = 3,35	CI (95%)= [1,18 ; 9,47]

Table 2: Total number of positive tests according to the diagnosis of AD or AR

	Diagnosis		
Results APT	**Atopic Dermatitis**	**Rhinitis**	**Total**
Positive	25 (16,7%)	10 (7,4%)	35 (12,3%)
Negative	125 (83,3%)	125 (92,6%)	250 (87,7%)
Total	150 (100%)	135 (100%)	285 (100%)
X^2 = 4,83	p = 0,028	OR = 2,50	CI (95%)= [1,09 ; 5,84]

X^2 (com correção de continuidade) = 4,83 p= 0,028

other concentrations.[22]

Other studies with the APT were conducted to assess its positivity as well as its reproducibility. Ingordo observed greater positivity for this test in the group with AD when compared with non-allergic patients and those with respiratory allergy. The same test was repeated with some patients in the three groups and it showed good reproducibility.[23]

In our study, the APT was conducted with three types of mites (Dpt, Df and Bt) in patients with AD, associated or not with respiratory allergy, and in patients with AR without dermatologic disease. We chose petrolatum as vehicle, based on published data. [20]

When analyzing the total number of positive APT performed with the 3 mites (Table 2), statistically significant differences were found (p = 0.028), as well as when evaluating the number of patients who had at least one positive APT in the AD group as compared to the AR group (table 1, p = 0.035).

These results show a significant association between exposure to mites and onset of eczematous lesions, which explains the report by patients who say their condition worsened after contact with these allergens.

It also explains that AD may precede respiratory allergy, which is called atopic march, as some studies have attempted to demonstrate, because sensitization may start on the skin due to changes in skin barrier function that facilitate the penetration of these allergens and the triggering of the immune response. After differentiation of LT into mature LTh2, some of them will migrate through the bloodstream to other sites, such as lung and nasal mucosa. [5.19]

Later, contact with the same allergen may cause a systemic reaction clinically demonstrated by asthma, rhinitis, and worsening of AD [19].

Reduction in skin barrier function is the "trademark" of AD, and this is usually the first manifestation of allergic disease in childhood, followed by the development of allergic respiratory diseases such as asthma and rhinitis. These diseases have common clinical features, and about 60% of children with AD have asthma and 35% of them, rhinitis. [5.24]

The chromosomal region associated with AD differs from that which is related to allergic respiratory diseases, and this suggests different genetic alterations. Hence, the common etiology that could promote the occurrence of these diseases in the same individual is alteration in the skin barrier due to genetic mutation. [51.8] In this way, allergens, irritant substances and microorganisms can, through the skin, induce immune responses. [5,25,26]

The occurrence of these responses is supported by previous studies that demonstrate immunological characteristics common to both groups of diseases - atopic dermatitis and respiratory allergy - as well as by the observation of higher positivity for the APT in patients with AD when compared with patients with respiratory allergy or non-atopic patients. This fact reinforces the possibility of functional impairment of the skin barrier in these individuals. [9,27]

Therefore, we can say that the relationship of airborne allergens, including mites, with AD is relevant and should be considered in monitoring these patients because, since they show changes in skin barrier function, the penetration of these agents is facilitated.

The performance of the APT and its positivity in some of the individuals with AD show that, besides the relationship with airborne allergens, other antigens, such as food and microorganisms, may participate in the pathophysiology of AD, collaborating with the maintenance or triggering of the lesions. [28,29]

It may also show us that the same antigen can induce different immune responses in the same individual, as we have seen in 12 patients who showed a positive reaction to the same allergens in both tests. Based on these data we observed that the APT is a tool that may assist in the elucidation of triggering agents of AD and in the assessment of its pathophysiology. [29]

On the other hand, works in the literature show that this test does not follow a standardized method;

more research studies need to be conducted to obtain the best sensitivity and specificity for each allergen.[30]

It is known that allergy is not the only etiological factor for this disease. Likewise, hypersensitivity is not the only factor contributing to APT positivity, but this test can identify sensitization to house dust mites, which are able to trigger a delayed immune response, associated with the onset or aggravation of eczematous lesions in AD. The test also supports the concept that IgE and T lymphocytes are involved in the pathophysiology of this disease, as well as the notion that AD is not only a disease with skin barrier disfunction and dry skin, but also an allergic disease. [18]

Given the results of this study and those reported in the literature, the authors of this work consider that the APT holds an important association with AD, but that this test needs to be studied further.

CONCLUSION

We noted that the positivity of the APT was higher in patients with AD as compared to patients with AR.

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