Atopy patch test (APT) in the diagnosis of food allergy in children with atopic dermatitis*

Teste de contato atópico (TCA) no diagnóstico de alergia alimentar em crianças com dermatite atópica

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Abstract: BACKGROUND - Atopic Dermatitis is a chronic inflammatory skin disease. Food allergens are important in the pathogenesis in 1/3 of the cases. Several mechanisms are involved in the pathogenesis of Atopic Dermatitis. Immediate reactions are identified by both measurement of specific IgE and skin prick test. Atopy Patch Test seems to be relevant in the investigation of patients with suspected delayed-type reactions. Objectives: To evaluate the standardization of this method concerning allergen concentration, occlusion time and interpretation, and determine the specificity and sensitivity of the Atopy Patch Test according to the skin prick test and specific IgE levels in food allergy diagnosis in children with Atopic Dermatitis. Methods: Seventy-two children, aged 2-12 years were selected and followed at the allergy clinic of the Hospital São Zacharias. Skin prick test, specific IgE and food Atopy Patch Test (cow´s milk, egg, soy and wheat) were carried out. Three groups were submitted to the Atopy Patch Test: (1) Atopic Dermatitis with or without Rhinitis and Asthma; (2) Rhinitis and or Asthma without AD; (3) Healthy individuals. Results: In group 1, 40% of the patients presented positive reactions. The longer the exposure time (48h and 72h), the higher the sensitivity. In group 2, the test was more specific than sensitive for all the extracts, with increased sensitivity the longer the time of exposure (72h). In group 3, 8.3% presented positive tests. Conclusion: APT evidenced a great diagnostic value in late-phase reactions to food, with high specificity. It showed to be a specific and reliable tool in comparison with the healthy group´s results.
Keywords: Dermatitis, atopic; Egg hypersensitivity; Food hypersensitivity; Milk hypersensitivity; Patch tests; Skin tests

Resumo: Fundamentos - A Dermatite Atópica é uma doença inflamatória crônica da pele. Os alimentos são importantes na patogênese da doença em 1/3 dos casos. Diversos mecanismos estão envolvidos na fisiopatogenia da dermatite Atópica. As reações imediatas são identificadas pela dosagem de IgE específica e teste de puntura. O teste de contato atópico parece ter relevância na investigação de pacientes com suspeita de reação tardia. Objetivos: Avaliar a padronização do método com relação à concentração do alérgeno, tempo de oclusão e de interpretação; e determinar a especificidade e a sensibilidade do teste de contato atópico em relação ao teste de puntura e a dosagem de IgE específica, no diagnóstico de alergia alimentar em crianças com dermatite Atópica. Métodos: Setenta e duas crianças com 2 a 12 anos foram submetidas a teste de puntura e dosagem de IgE específicas para alimentos (leite de vaca, ovo, soja, trigo). O teste de contato atópico foi aplicado em 3 grupos: (1) Dermatite Atópica com ou sem Rinite e Asma; (2) Rinite e ou Asma sem Dermatite Atópica; (3) Saudáveis. Resultados: No grupo 1, 40% dos pacientes apresentaram reação positiva. Quanto maior o tempo de exposição, maior foi a sensibilidade. No grupo 2, o teste foi mais específico que sensível para todos os extratos; com aumento da sensibilidade com maior tempo de exposição (72h). No grupo 3, 8.3% apresentaram testes positivos. Conclusão: O teste de contato atópico mostrou ter valor diagnóstico em relação às reações de fase tardia a alimentos, com elevada especificidade. Mostrou-se um teste específico e confiável ao comparar com os resultados do grupo controle.
Palavras-chave: Dermatite atópica; Hipersensibilidade alimentar; Hipersensibilidade a leite; Hipersensibilidade a ovo; Testes cutâneos; Testes do emplastro

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INTRODUCTION

Atopic Dermatitis (AD) is an itchy, chronic inflammatory skin condition, which occurs, in most cases, during early childhood.[1] Although the etiology of AD is still not explained, it is known that there is a correlation with genetic, immunological and environmental factors, including food antigens and aeroallergens.[2]

About 10 to 40% of the patients with atopic dermatitis have allergic reactions to food antigens.[3] Foods often implicated are cow's milk, egg, soy and wheat.[4] Food allergies can be identified by history, skin prick test and specific IgE testing. Oral provocation tests, the double-blind placebo-controlled food challenges, are carried out to confirm the diagnosis. In the absence of an immediate clinical response, food allergy diagnosis becomes difficult. The APT can be a valuable additional method.[3,5]

The first APT descriptions date from 1982 as a skin reaction to food antigens or aeroallergens induced on intact skin.[6]

Several mechanisms are involved in the AD pathogenesis. The IgE-mediated reactions, often associated with immediate reaction, are well characterized. On the other hand, the delayed-type immune responses are poorly understood. A positive skin prick test (SPT) seems to reflect an IgE-mediated reaction to the tested antigen, as well as increased specific IgE levels and provocation test. APT seems to be relevant in the investigation of patients with suspected delayed reaction, with or without the participation of IgE.[4] However, the clinical relevance of positive APT reactions has yet to be proven and standardized.

PATIENTS AND METHODS

Patients

Seventy-two children (34 girls, 38 boys) aged 2 to 12 years old (mean: 6.6) were evaluated. 32 of them had AD (group 1), as defined by Hanifin & Rajka´s criteria.[4] 26 presented a clinical diagnosis of Allergic Rhinitis and/or Asthma, according to the criteria of the PRACTALL (Practicing Allergology Consensus Report) Statement, without AD (group 2), and 12 were control non-atopic patients (group 3).[7] It was an intentional non-probabilistic sample.

METHODS

From April/2007 to March/2010, seventy-two children aged between 2 and 12 years were selected and followed at the Hospital São Zacharias´ allergy clinic. Their parents accepted and signed a Term of Free and Clarified Consent. Specific IgE´s dosages were carried out as well as TCP and APT with food antigens (cow´s milk, egg, soy and wheat).

1) Total IgE and specific IgE measurement

Patients´ blood samples were collected to perform total IgE [*Enzyme Linked Immunosorbent Assay* (ELISA)] and specific IgE testing [ImmunoCAP (Phadia, currently Thermo Fisher)]. The detection limit was 0.35 kU/L IgE. Children were considered sensitized when specific IgE levels were above detection limit.

2) SPT

Cow's milk, egg, soy and wheat (FDA Allergenic, Rio de Janeiro, Brazil) were the extracts used. Histamine base (10mg/ml) was used as positive control and saline solution as negative control. Antisepsis was carried out with 70% alcohol on the healthy skin of the volar region of the forearm, with a 2 cm interval by using a lancet (ALKO, Rio de Janeiro, Brazil).[8] Reading was performed after 20 minutes. The test was considered positive when the wheal had a diameter greater than 3mm. All the patients were advised to avoid the use of oral antihistamine and topical corticosteroid application for at least a week before the test.[9]

3) APT

The APT was carried out with food extracts (cow's milk, egg, soy and wheat) at a 10% concentration (FDA Allergenic, Rio de Janeiro, Brazil) and petrolatum as negative control, using 8 mm diameter aluminum chambers (Finn Chamber on Scampor®, Epitest Ltd Oy, Tuusula, Finland). The tests were performed in duplicate, and applied on the healthy skin of the back. The first patch (APT I) was removed after 24 hours and the second (APT II) after 48 hours. APT I readings were taken at 24, 48 and 72h, and APT II at 48 and 72 hours. The reaction was quantified according to the APT reading criteria of the Revised European Task Force on Atopic Dermatitis (ETFAD) (Chart 1).[8,10]

Chart 1: Recommendations for the APT reading, according to the European Task Force on Atopic Dermatitis (ETFAD)

-	Negative
?	Only erythema, questionable
+	Erythema, infiltration
++	Erythema, small papule
+++	Erythema, several papules
++++	Erythema, vesicle

The presence of +++ or ++++ reaction has a highly positive predictive value to delayed hypersensitivity to the studied food.

1) Statistics

Correlation among specific IgE, SPT and APT in each of the studied group was analyzed, as well as between the atopy (1 and 2) and control (3) groups. The sensitivity and specificity of APT when compared to the specific IgE and to the SPT with a p-value of <0.05 was studied. Sensitivity measured the proportion of positive specific IgE and SPT, which were correctly identified by APT. Specificity measured the proportion of negative specific IgE and SPT, which were correctly identified by APT.

For statistical analysis, the 13th version of the SPSS (Statistical Package for the Social Sciences) software was used.

The agreement or not among the results in relation to both APT I and APT II was based on the kappa (κ) coefficient. Kappa coefficient is a statistical measure of inter-rater agreement for qualitative (categorical) items. It is generally thought to be a more robust measure than simple percent agreement calculation since κ takes into account the agreement occurring by chance.

RESULTS

In group 1, 40% of the patients (13/32) showed a positive reaction, as follows: 34% to cow´s milk (11/32), 37% to egg (12/32), 28.2% to soy (9/32) and 28.2% (9/32) to wheat, with more intense reactions to egg; this was the group with the highest specificity. The longer the exposure, the greater the sensitivity. The 48h kappa was significant and presented bilateral agreement at 72h.

In group 2, positive APT was verified as follows: 19% to cow´s milk (5/26), 11.5% to egg (3/26), 15.3% to soy (4/26), and 11.5% to wheat (3/26). The test was more specific than sensitive to all extracts, the sensitivity increasing with longer exposure (72h).

APT I and APT II agreed at 48h and improved at 72h.

In group 3, kappa was negative, indicating the specificity and sensitivity of the APT, with greater sensitivity at the 72h readings.

APT, as compared to serum-specific IgE and skin prick test proved to be a highly specific test, with low sensitivity, though (Tables 1 - 6). The sensitivity to APT increased with greater exposure and later readings (72h) (Tables 1 - 6).

After applying the APT, none of the patients presented immediate reaction (urticaria) nor AD, rhinitis or asthma exacerbation. In group 1 (AD), two patients had local erythematous reaction at 24 hours, which did not continue in other readings, one being reactive to cow´s milk, wheat and egg, and the other to soy. Only one patient showed eczematous reaction after 24 hours to egg, which remained in the other readings. Other patients were reactive only in the second and third readings. In groups 2 and 3, none of the patients showed irritative reaction on the testing site.

DISCUSSION

Atopic dermatitis (AD) is one of the most common chronic childhood diseases. Food allergy plays an important role in the pathogenesis, affecting about 1/3 of the patients. Type I reactions are more easily identified by history, specific IgE and skin prick test.

Table 1: Patients with atopic dermatitis. Patients´ distribution according to the ATP and serum-specific IgE results

Reading Time	Cow´s Milk		Egg		Wheat		Soy	
	Sensit	Spec	Sensit	Spec	Sensit	Spec	Sensit	Spec
L 24h	0,0%	96%	0,0%	92,9%	0,0%	96,2%	0,0%	96,2%
L 48h	0,0%	96%	0,0%	89,3%	0,0%	84,6%	33,3%	88,5%
R 48h	33,3%	84%	0,0%	89,3%	50,0%	88,5%	0,0%	80,8%
L 72h	0,0%	92%	0,0%	92,9%	0,0%	88,5%	0,0%	92,3%
R 72h	33,3%	72%	0,0%	64,3%	50,0%	69,2%	0,0%	69,2%

Table 2: Patients with atopic dermatitis. Patient´s distribution according to the APT and skin prick test results

Reading Time	Cow´s Milk		Egg		Wheat		Soy	
	Sensit	Spec	Sensit	Spec	Sensit	Spec	Sensit	Spec
L 24h	0,0%	95%	0,0%	93,3%	0,0%	96,2%	50,0%	100%
L 48h	10,0%	100%	0,0%	90,0%	0,0%	84,6%	0,0%	85,7%
R 48h	20,0%	85%	0,0%	86,7%	0,0%	84,6%	50,0%	85,7%
L 72h	20,0%	100%	0,0%	93,3%	0,0%	88,5%	0,0%	92,9%
R 72h	30,0%	75%	0,0%	66,7%	50,0%	69,2%	50,0%	75,0%

Table 3: Patients with Rhinitis and/or Asthma. Patients´ distribution according to the APT and serum-specific IgE results

Reading Time	Cow´s Milk		Egg		Wheat		Soy	
	Sensit	Spec	Sensit	Spec	Sensit	Spec	Sensit	Spec
L 24h	0,0%	100%	0,0%	100%	0,0%	100%	0,0%	100%
L 48h	0,0%	100%	0,0%	100%	0,0%	100%	0,0%	100%
R 48h	0,0%	95,5%	0,0%	100%	0,0%	100%	0,0%	100%
L 72h	0,0%	100%	0,0%	100%	0,0%	95%	0,0%	100%
R 72h	0,0%	77,3%	0,0%	86,4%	16,7%	90%	0,0%	83,3%

Table 4: Patients with Rhinitis and/or Asthma. Patients´ distribution according to the APT and skin prick test results

Reading Time	Cow´s Milk		Egg		Wheat		Soy	
	Sensit	Spec	Sensit	Spec	Sensit	Spec	Sensit	Spec
L 24h	0,0%	100%	0,0%	100%	0,0%	100%	0,0%	100%
L 48h	0,0%	100%	0,0%	100%	0,0%	100%	0,0%	100%
R 48h	0,0%	95,7%	0,0%	100%	0,0%	100%	0,0%	100%
L 72h	0,0%	100%	0,0%	100%	0,0%	95,7%	0,0%	100%
R 72h	0,0%	78,3%	0,0%	88%	33,3%	91,3%	0,0%	83,3%

Table 5: Control patients. Patients´ distribution according to the APT and serum-specific IgE results

Reading Time	Cow´s Milk		Egg		Wheat		Soy	
	Sensit	Spec	Sensit	Spec	Sensit	Spec	Sensit	Spec
L 24h	0,0%	100%	0,0%	100%	0,0%	100%	0,0%	100%
L 48h	0,0%	100%	0,0%	100%	0,0%	100%	0,0%	100%
R 48h	0,0%	90,9%	0,0%	100%	0,0%	100%	0,0%	100%
L 72h	0,0%	100%	0,0%	100%	0,0%	100%	0,0%	100%
R 72h	0,0%	100%	0,0%	100%	0,0%	91,7%	0,0%	90,9%

Table 6: Control patients. Patients´ distribution according to the APT and skin prick test results

Reading Time	Cow´s Milk		Egg		Wheat		Soy	
	Sensit	Spec	Sensit	Spec	Sensit	Spec	Sensit	Spec
L 24h	0,0%	100%	0,0%	100%	0,0%	100%	0,0%	100%
L 48h	0,0%	100%	0,0%	100%	0,0%	100%	0,0%	100%
R 48h	0,0%	92,3%	0,0%	100%	0,0%	100%	0,0%	100%
L 72h	0,0%	100%	0,0%	100%	0,0%	100%	0,0%	100%
R 72h	0,0%	100%	0,0%	92,3%	33,3%	100%	50,0%	100%

The gold standard for food allergy diagnosis is still the provocation test, the double-blind placebo-controlled oral challenge. However, there are limitations to its use in clinical practice, either due to its cost, application and interpretation difficulty, or to the risk of anaphylaxis during the test.[3] The diagnosis of delayed hypersensitivity reactions is still a challenge and APT may be used as an alternative method. The macro and microscopic similarity between the positive APT sites and AD lesions indicates that the APT is a valid model to study the AD allergic inflammation.[11]

There is little information in the literature regarding an optimal concentration of APT with food. Niggemann *et al*. suggest the APT performance with *in natura* extract in the concentration of 1:10 so as to exclude false-positive results due to local irritation. They used 12 mm Finn Chambers to reduce the false negatives.[12,13] In this study, all of the tests were per-

formed with a 1:10 solution, and pure Vaseline (diluter) as negative control. There were no cases of irritative reaction to control (false-positives). The chambers were the 8 mm ones, which have already been used in other studies. [3,6]

The APT, as compared to serum-specific IgE and skin prick test proved to be a highly specific test, with low sensitivity, though. Other studies have shown a greater sensitivity; however, those studies were carried out with populations whose previous history reported confirmed or suspected food allergy. [3]

CONCLUSION

- APT proved to have a diagnostic value in relation to late-phase reactions to food (cow's milk, egg, soy and wheat); high specificity confirmed the etiology.

- Positive results were mostly found in readings taken at 48 and 72 hours, especially when the occlusion remained for 48 hours, confirming what was established by EAACI/GA2LEN. Position paper: Present status of the atopy patch test.[14]

- APT can avoid unnecessary elimination diets in children in development stage who have delayed reactions to food.

- APT in AD patients is specific and trustworthy when compared with the control group. ❑

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