



Phadiatop Assay와 AdvanSure™ AlloScreen의 임상적 유용성 비교

Comparison of Clinical Utility between Phadiatop Assay and AdvanSure[™] AlloScreen

김민경 · 권민정 · 박효순 · 우희연

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Background: Multiple Allergo-Sorbent Test (MAST) allows simultaneous detection of specific IgE antibodies using multiple allergens, and it is commonly used for allergy screening. Phadiatop assay (Phadia AB, Sweden), including Phadiatop test and Phadiatop Infant test, is a variant of specific IgE test that covers a mixture of common allergens. We compared the clinical utility of Phadiatop assay with that of the MAST AlloScreen (LG Life Science, Korea).

Methods: A total of 218 samples classified by AlloScreen results were collected. Phadiatop test was performed on sera from 61 and 103 aeroal-lergen-positive and -negative subjects. Phadiatop Infant test was performed on sera from 54 and 103 food and aeroallergen-positive and -negative subjects. When the results of AlloScreen and Phadiatop assay were not identical, we confirmed them using ImmunoCAP (Phadia AB).

Results: The concordance rate between AlloScreen and Phadiatop test was 93.2% (κ =0.86, P<0.001). Eleven (6.7%) of 164 specimens showed discrepant results. The results of AlloScreen did not agree with those of ImmunoCAP. The concordance rate between AlloScreen and Phadiatop Infant test was 97.4% (κ =0.945, P<0.001). Four (2.5%) specimens showed negative results in AlloScreen and positive results in Phadiatop Infant test. Three cases were confirmed as positive and one case was not confirmed through ImmunoCAP.

Conclusions: There was excellent agreement between AlloScreen and Phadiatop assay. Phadiatop assay accurately detected sensitization to common food and aeroallergen mixes. Therefore, Phadiatop assay is recommended as a screening test for allergic diseases.

Key Words: Allergy, Immunoassay, Immunoglobulin E, Hypersensitivity

INTRODUCTION

Allergic diseases are commonly referred to as "allergy", and the word was first used by Clemens von Pirquet in 1906 [1]. Allergic

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diseases are caused by hypersensitivity of the immune system to typically harmless substances in the environment. The underlying mechanism involves the binding of immunoglobulin E antibody (IgE) to an allergen and then to mast cells or basophil receptors, where it triggers the release of inflammatory cytokines, such as histamine [2]. Allergy has various manifestations, ranging from mild symptoms, such as red eye and pruritus, to anaphylaxis, which can lead to death [3]. It is important to identify the culprit allergen a patient is sensitized to, as sensitization varies between patients and allergen avoidance is the best treatment.

Allergy screenings and diagnoses are commonly conducted using the skin-prick test (SPT) and allergen-specific IgE (sIgE) tests [4, 5]. SPT is the most common method for confirming the IgE-mediated underlying mechanism of allergic diseases, as it is easy to perform, sensitive, cost-effective, and provides prompt results [6]. However, SPT has several limitations, such as its qualitative-only

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nature, unstandardized amount and choice of allergen, interference from antihistaminergic drugs, and subjective interpretation. Measurement of serum sIgE is a convenient method for patients who cannot discontinue the use of a drug that could interfere with SPT [7]. Among in vitro sIgE assays, ImmunoCAP assay (Phadia AB, Uppsala, Sweden), which is an sIgE test for a specific allergen, is considered the standard method by the Clinical and Laboratory Standards Institute (CLSI). However, it is difficult to perform in routine allergy screenings because of its high cost. Therefore, in laboratories, Multiple Allergo-Sorbent Test (MAST) is commonly performed for allergy screenings [8]. MAST is an enzyme-based immunoassay using an immunoblot technique involving solidphase allergen absorption and immobilization on nitrocellulose [9]. It simultaneously measures serum total IgE and more than 60 sIgEs. However, it is nonspecific and thus requires confirmatory tests. Phadiatop assay (Phadia AB) is an available MAST and a variant of ImmunoCAP assay, the standard sIgE test. There are two types of Phadiatop assays. One is Phadiatop Infant test, which includes food and aeroallergens and targets children younger than four years of age. The other is Phadiatop test exclusively for aeroallergens, which targets adults and children over four years of age. Both tests are based on the ImmunoCAP technology. They are fluorescence immunoassays that measure sIgEs in a mixture of common aeroallergens and food allergens. The AlloScreen assay (LG Life Science, Seoul, South Korea) is a commonly used MAST in Korea [10, 11]. AlloScreen and Phadiatop assay are different in terms of their allergen composition. Although Phadiatop assay does not identify particular sIgEs, it provides information on the presence of sIgEs for the most common allergens.

Table 1. Characteristics of subjects (N=218)

Characteristics	Values
Age (yr)	40.75 (0.7-82)
Sex (male:female)	98:120
Total IgE (IU/L)	129.57 ± 91.57
Diagnosis	
Allergic rhinitis	9 (4.1)
Asthma	7 (3.2)
Atopic dermatitis	26 (12)
Chronic urticaria	28 (12.9)
Other skin diseases	95 (43.5)
Other respiratory diseases	22 (10.1)
Others	31 (14.2)

Values are presented as mean (range), number (%), or mean \pm standard deviation.

In this study, we compared the clinical utility of Phadiatop assay (Phadiatop test and Phadiatop Infant test) with that of Allo-Screen.

MATERIALS AND METHODS

1. Specimens

This study was performed between December 2018 and June 2019. Serum samples were collected from 218 subjects who were requested for MAST allergy screening. Characteristics of the subjects are described in Table 1. We collected 61 samples that were positive for aeroallergens [Dermatophagoides farinae, Dermatophagoides pteronyssinus, cat epithelium and dander, dog dander, house dust mites, oak, Humulus japonicus, common silver birch, Cladosporium berbarum, Aspergillus fumigatus, Alternaria alternata, candida, cockroach, orchard grass, mugwort, and ragweed allergens] and 103 samples that were negative according to AlloScreen. We also collected 54 samples that were positive for food allergens (milk, egg whites, wheat, soybeans, peanuts, fish, and shrimp). The collected serum specimens were frozen and stored at -80°C before analysis.

This study was approved by the Institutional Review Board of Kangbuk Samsung Hospital (2018-05-060) and conducted in compliance with the World Medical Association Declaration of Helsinki.

2. Allergy screening tests

We used two types of allergy screening tests, and comparison of characteristics between AlloScreen and Phadiatop assays is presented in Table 2.

1) AlloScreen assay

AlloScreen assay is a multiplex test that simultaneously detects

Table 2. Comparison of characteristics between AlloScreen and Phadiatop assay

Characteristics	AlloScreen	Phadiatop assay			
Principle	Immunoblot	Solid-phase fluorescence immunoassay			
Degree of automation	Semi automation	Full automation			
Number of antigens	62	Not published			
Minimal sample volume (μL)	100	40			
Number of tests per run	24	up to 350			
Analysis time per run (hr)	3.5	1.7			
Analysis time per sample (min)	8.75	1.0			



total IgE and sIgE responses against multiple allergens. Total IgE is classified into positive and negative with a cutoff of 100 IU/mL. Test strips were read using AdvanSure AlloScreen (LG Life Science, Korea). The software determined the class (0-6) of sIgE concentrations. In this study, samples were considered positive for an allergen if the sIgE concentration was greater than class 1 (sIgE ≥ 0.35 kU/L) [12].

2) Phadiatop assay

Phadiatop assay, a solid-phase fluorescence immunoassay for serum sIgEs using a mixture of relevant allergens, was analyzed quantitatively in a Phadia 250 System (Phadia AB). This assay provides simultaneous graded determinations of sIgEs for multiple allergens. The manufacturer does not reveal the precise composition of allergens included in Phadiatop assay. According to the published reports, Phadiatop Infant test might include egg white, cow's milk, peanut, wheat, soybean, shrimp, cat epithelium and dander, dog dander, house dust mites, common silver birch, mugwort, and ragweed allergens, whereas Phadiatop test might include D. farinae, D. pteronyssinus, cat epithelium and dander, dog dander, house dust mites, common silver birch, orchard grass, mugwort, and ragweed allergens [7, 13]. Thus, we evaluated Phadiatop Infant test to evaluate its efficacy in detecting food allergens. The results were expressed as Phadia Arbitrary Units/L (PAU/L), which indicated the degree of sensitization. Values of ≥ 0.35 PAU/L were considered to indicate sensitization and were coded as positive.

3, ImmunoCAP Allergen-slgE test

In the case of discrepancies between AlloScreen and Phadiatop assay, we confirmed the results using ImmunoCAP test, which measures sIgE for an individual allergen. Serum samples were analyzed for individual allergen-sIgEs with a Phadia 250 Immunoassay Analyzer (Phadia AB, Sweden). Levels ≥0.35 kUA/L were considered positive.

ImmunoCAP sIgE tests for 16 popular allergens were performed on specimens with negative AlloScreen results and positive Phadiatop assay results. The 16 popular allergens included 9 aeroallergens (D. pteronyssinus, D. farinae, cat epithelium and dander, dog dander, A. alternata, oak, cockroach, ragweed, and mugwort) and 7 food allergens (cow's milk, egg white, wheat, soybean, peanut, fish [cod], and shrimp).

4. Data analyses

We compared the concordance rate and degree of agreement of AlloScreen and Phadiatop assay. Phadiatop test and Phadiatop Infant test were both evaluated for percent positive agreement (PPA) and percent negative agreement (PNA). When the results of AlloScreen test were concordant with those of Phadiatop assay, the results were considered reference results. When the results of AlloScreen and Phadiatop assay were discrepant, the results of ImmunoCAP assay were considered reference results. We used kappa statistics and McNemar test to compare the degree of agreement between the two screening allergy tests. Kappa values of 0.8-1.0 are considered almost perfect [14]. All statistical analyses were performed using the SPSS software, version 24.0 (SPSS Inc, Chicago, IL, USA). P-values less than 0.05 were considered statistically significant.

RESULTS

A total of 164 samples (61 and 103 samples positive and negative for aeroallergens, respectively) were tested using Phadiatop test and 157 samples (54 and 103 samples positive and negative for food allergens, respectively) were tested with Phadiatop Infant test.

The concordance rate between AlloScreen and Phadiatop test was 93.2% (κ =0.86, P<0.001), and the results of the two tests were not significantly different (P=0.065). PPA was 96.7% and PNA was 91.2%. Among 164 tests, 153 (93.2%) showed identical results between AlloScreen and Phadiatop test: 59 (96.7%, 59/61) showed positive results and 94 (91.2%, 94/103) showed negative results in both tests. Eleven (6.7%) specimens showed discrepant results: 9 (5.5%) were negative in AlloScreen and positive in Phadiatop test, whereas 2 (1.2%) were positive in AlloScreen and negative in Phadiatop. Two cases (I18 and I19) showing AlloScreen positive/Phadiatop negative results were revealed by ImmunoCAP sIgE test to be negative for *D. pteronyssinus* and *D. farinae*. Additional ImmunoCAP assays for cockroach and shrimp allergens, which are known to show cross-reactivity with D. pteronyssinus and D. farinae, also showed negative results. The nine AlloScreen negative/Phadiatop positive specimens were confirmed to be positive for sIgEs of allergens by ImmunoCAP sIgE test (Table 3).

The concordance rate between AlloScreen and Phadiatop Infant test was 97.4% ($\kappa = 0.945$, P < 0.001), and the results of the two



Table 3. ImmunoCAP results of discrepant cases (N=14) between AlloScreen and Phadiatop assay (Phadiatop test and Phadiatop Infant test)

No.	MAST results		ImmunoCAP results, kUA/L (class)						
	AlloScreen (IU/mL)	Phadiatop assay (PAU/L)	d1	d2	w1	w6	f1	f2	f24
N12	Negative (72.46)	Positive (0.38)	0.10 (0)	0.01 (0)	0.21 (0)	0.48 (1)	ND	ND	ND
N32	Negative (30.18)	Positive (0.40)	0.02 (0)	0.01 (0)	0.00 (0)	0.51 (1)	ND	ND	ND
N44	Negative (96.18)	Positive (0.68)	0.18 (0)	0.99 (2)	0.00 (0)	0.00 (0)	ND	ND	ND
N58	Negative (92.21)	Positive (0.43)	0.21 (0)	0.44 (1)	0.00 (0)	0.12 (0)	ND	ND	ND
N68	Negative (84.58)	Positive (1.87)	0.46 (1)	2.54 (2)	0.04 (0)	0.04 (0)	ND	ND	ND
N79	Negative (12.93)	Positive (0.88)	0.20 (0)	0.98 (2)	0.00 (0)	0.00 (0)	ND	ND	ND
N86	Negative (23.70)	Positive (0.35)	0.64 (1)	0.81 (2)	0.01 (0)	0.18 (0)	ND	ND	ND
N100	Negative (77.86)	Positive (0.37)	0.52 (1)	0.39 (1)	0.00 (0)	0.00 (0)	ND	ND	ND
N21	Negative (63.53)	Positive (0.38)*	ND	ND	ND	ND	0.14 (0)	0.27 (0)	0.03 (0)
N60	Negative (56.55)	Positive (0.39)*	0.01 (0)	0.02 (0)	ND	ND	0.17 (0)	0.41 (1)	0.04 (0)
N83	Negative (80.27)	Positive (0.42)*	ND	ND	ND	ND	0.00 (0)	0.03 (0)	1.89 (2)
N87	Negative (3.31)	Positive (0.42) ⁺	1.10 (2)	2.84 (2)	0.00 (0)	0.00 (0)	0.00 (0)	0.01 (0)	0.01 (0)
l18	Positive (248.30)§	Negative (0.31)	0.31 (0)+	0.30 (0)	ND	ND	ND	ND	0.10(0)
l19	Positive (110.80) ^{II}	Negative (0.30)	0.02 (0)+	0.34 (0)	ND	ND	ND	ND	0.03(0)

Discrepant cases showed values < 0.16 kUA/L by ImmunoCAP assay for the following allergens and data are not described: e1, cat; e5, dog; m6, Alternaria alternata; i6, cockroach; t7, oak; f3, fish (cod); f4, wheat; wheat; f14, soybean; f13, peanut. Phadiatop assay values \geq 0.35 PAU/L, AlloScreen values > 100 IU/mL, and ImmunoCAP values \geq 0.35 kUA/L are considered positive. N21 was not confirmed.

*These are the results of Phadiatop Infant test. †This is a result of both Phadiatop test and Phadiatop Infant test. †Test for hx2 (house dust mixes) also showed negative results. *Positive result for *D. pteronyssinus*; d2, *D. farinae*; w1, ragweed; w6, mugwort; f1, egg white; f2, cow's milk; f24, shrimp, ND; not done.

tests were not significantly different (P=0.125). PPA was 100% and PNA was 96.1%. Among 157 tests, 153 (97.4%) showed identical results between AlloScreen and Phadiatop Infant test: 54 (100%, 54/54) with positive results and 99 (96.1%, 99/103) with negative results in both tests. All four (2.5%) discrepant results were AlloScreen negative/Phadiatop Infant positive. Three specimens were confirmed to be positive for cow's milk, oak, shrimp, D. pteronyssinus, and D. farinae, but one (N21) was not confirmed (Table 3). Sample N21 showed 0.27 kUA/L of cow's milk and 0.14 kUA/L of egg white in ImmunoCAP, and the patient had atopic dermatitis without apparent food-related allergic symptoms. Therefore, we considered that sample N21 showed a false positive result in Phadiatop Infant test.

As the results of Phadiatop test were completely concordant with the gold standard, both the sensitivity and specificity of Phadiatop test were 100%. The sensitivity and specificity of Phadiatop Infant test were 100% and 99%, respectively.

DISCUSSION

We assessed the clinical utility of Phadiatop test and Phadiatop Infant test in comparison with that of AlloScreen, based on ImmunoCAP assay. No study has compared AlloScreen with Phadiatop or Phadiatop Infant tests. Only one study had compared Phadiatop test to RIDA qLine allergy test (R-Biopharm AG, Darmstadt, Germany), which is a MAST, with ImmunoCAP sIgE test as a reference method [7]. A total of 430 consecutive specimens from patients with allergic symptoms were tested. The concordance rate between the two tests was 80.7% ($\kappa = 0.614$, P < 0.001), and the results of the two tests were not significantly different according to McNemar test (P = 0.19). Based on ImmunoCAP assay, RIDA gLine allergy test showed 40 false positive results, whereas Phadiatop test showed only one false positive result. One study has assessed the diagnostic value of Phadiatop Infant test based on sIgE allergens using a Pharmacia CAP System™ (Phadia AB) in young children (N=149) aged 0-4 years with wheezing or eczema [14]. The sensitivity, specificity, positive predictive value, and negative predictive value of Phadiatop Infant test were shown to be 96%, 96%, 92%, and 98%, respectively. The authors concluded that Phadiatop Infant test can help in differential diagnoses of IgE-mediated allergies in young children, but it is a disadvantage that testing for the allergens not included in Phadiatop Infant test could not be performed.

In our study, almost perfect concordance was observed between AlloScreen and Phadiatop test and between AlloScreen and Phadiatop Infant test. There were 11 (6.7%) discrepant results between



AlloScreen and Phadiatop test, and 4 (2.5%) discrepant results between AlloScreen and Phadiatop Infant tests. Phadiatop assays, including Phadiatop test and Phadiatop Infant test, were revealed to be more accurate than AlloScreen, as confirmed by Immuno-CAP assay. However, the possibility of false negative results caused by low SIgE levels or false positive results due to nonspecific antibody binding cannot be completely excluded, and thus clinical correlation and confirmation tests such as SPT are necessary. Two results (I18 and I19) that were read as positive by AlloScreen and negative by Phadiatop for D. pteronyssinus and D. farinae, which are both house dust allergens, were revealed to be negative by ImmunoCAP. These two patients showed Phadiatop test results between 0.30 PAU/L and the cutoff (0.35 PAU/L) and had symptoms of chronic urticaria requiring close monitoring. According to the manufacturer, allergens with a value of ≥0.2 PAU/L, which corresponds to the limit of quantitation of Phadiatop assay, can be considered positive despite negative ImmunoCAP results if there are clinical symptoms. Therefore, the Phadiatop test results of the two cases can be interpreted as positive according to the judgment of the clinician considering the allergic symptoms. One case (N21) that was not confirmed by ImmunoCAP was determined to be a false positive result by Phadiatop Infant test. Phadiatop Infant test is known to show a positive result if the sum of each allergensIgE value is above the cutoff, and this case might have shown positive results by the sum of the sIgEs for cow's milk (0.27 kUA/L) and egg white (0.14 kUA/L), based on the ImmunoCAP results. However, the patient was a 23-year-old adult who was not indicated for Phadiatop Infant test in principle, which is used for children less than four years old.

In this study, Phadiatop assay provided accurate information about the possibility of allergic diseases. Phadiatop assay can also be helpful in health screening because it has a similar accuracy to ImmunoCAP assay, the gold standard method. However, Phadiatop assay has less allergens than AlloScreen. Moreover, it cannot identify particular sIgEs, and only detects the presence of sIgE for unspecified allergens. Therefore, further examination and a specialist's opinion are required for accurate diagnosis and treatment. Phadiatop test may need more than one sampling for confirmation, as it does not detect which allergen was positive. In contrast, AlloScreen provided an sIgE result for each allergen, and thus would be more suitable for young children, in whom blood sampling is difficult.

There are some limitations in our study. First, the number of specimens was relatively small, and thus further studies are needed for more accurate evaluation. Second, false positivity and false negativity of AlloScreen assay were confirmed based on ImmunoCAP test results, not on clinical manifestation or SPT results. Third, as the panel of allergens for Phadiatop assay is not publically available, we performed ImmunoCAP assay only on common allergens. Finally, the cutoff value for sIgE positivity can differ between allergens [15], but values of ≥0.35 PAU/L were considered indicative of sensitization and were coded as positive in our study.

In summary, compared with AlloScreen, Phadiatop assay can be used to test for fewer allergens and did not provide positive results for specific allergens, but more accurately detected the presence of sIgE for common allergens, such as D. pteronyssinus, D. farinae, mugwort, and birch. Therefore, Phadiatop assay may be useful as a one-shot screening test for subjects with suspected allergic diseases.

요 약

배경: MAST (Multiple Allergo-Sorbent Test)는 여러 알레르겐에 대 한 특이 IgE 항체(sIgE)를 동시에 검출할 수 있어 알레르기 검사의 선별검사로 널리 사용된다. Phadiatop test 및 Phadiatop Infant test (Phadia AB, Sweden)는 특정 IgE검사의 변형 검사로 높은 빈도로 검출되는 알레르겐들의 혼합물에 대한 검사이다. 본 연구에서 MAST인 AlloScreen (LG Life Science, Korea)과 비교하여 Phadiatop assay의 임상적 유용성을 평가하였다.

방법: AlloScreen 검사 결과에 따라 분류된 총 218개의 검체를 수 집하였다. 흡입알레르겐에 대해 양성을 보이는 61개 검체와 103개 의 음성 검체에 대해 Phadiatop test를 시행하였다. 음식 및 흡입알 레르겐에 대해 양성을 보이는 54개 검체와 103개의 음성 검체 대 하여 Phadiatop Infant test 검사를 시행하였다. Phadiatop과 Allo-Screen의 결과가 일치하지 않은 경우, ImmunoCAP (Phadia AB, Sweden)을 사용하여 확인하였다.

결과: AlloScreen과 Phadiatop test 간의 일치율은 93.2% (κ=0.86, P<0.001)이었다. 164개 검체 중 11개(6.7%)가 불일치 결과를 보였 다. AlloScreen의 결과가 ImmunoCAP 결과와 일치하지 않았다. AlloScreen과 Phadiatop Infant test 간의 일치율은 97.4% (к=0.945, P<0.001)이었다. 4개(2.5%)의 검체는 AlloScreen 음성, Phadiatop Infant test 양성이었다. ImmunoCAP 검사에서 3개 검체는 양성으 로 확인되었고 1개 검체는 확인되지 않았다.



결론: AlloScreen과 Phadiatop assay는 높은 일치율을 보였으며 Phadiatop assay는 빈도가 높은 식품 및 흡입 알레르겐 혼합물에 대한 감작을 정확하게 검출하였다. 따라서 Phadiatop assay는 알레르기 질환에 대한 선별 검사로 권장된다.

Conflicts of Interest

None declared.

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