碩士學位論文

통년성 항원감작이 ImmunoCAP검사에서 계절성 항원 검사결과에 미치는 영향

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The effect of perennial allergen sensitization on the results of seasonal allergen in ImmunoCAP

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초록

배경: 알레르기를 진단하기 위해 피부단자검사(SPT)와 ImmunoCAP®가 많이 이용된다. 그런데 이전 연구들에서 피부단자검사와 ImmunoCAP® 사이 일부 결과의 불일치가 보고되었다. 하지만 아직 두 검사간의 결과의 차이를 이해하기 위한 연구는 부족한 실정이다.

목적: 우리는 피부단자검사와 ImmunoCAP® 결과의 불일치에 영향을 주는 인자를 찾기위해 연구하였다.

방법: 94명의 환자를 대상으로 6개의 알레르기 항원(*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, alder, ragweed, mugwort, and *Humulus japonicus*)에 대해 피부단자검사와 ImmunoCAP®을 시행하였고, 나이, 성별, 체질량지수 또는 통년성 항원이나 계절성 항원에의 감작여부가 두 검사간의 결과의 불일치에 영향을 주는지에 대해 분석하였다.

결과: 두 검사간에 통년성 항원에 대한 양성률은 비슷하였다. 하지만 계절정 항원의 경우에는 피부단자검사에서 양성률이 높은 결과를 보였다. 계절성 항원보다 통년성 항원이 두 검사간에 상대적으로 높은 일치율을 보였다. 그리고, 계절성 항원에 있어서 피부단자검사에는 양성, ImmunoCAP®에는 음성을 보이는 비율이 높았다. 흥미롭게도 통년성 항원에 대한 피부단자검사의 양성도가 계절성

항원에 대한 두 검사간의 일치율에 영향을 주는 것으로 확인되었다. 통년성 항원에 대한 피부단자검사가 양성인 경우, 그렇지 않은 경우보다 계절성 항원에 대한 두 검사간의 불일치율이 높은 결과를 보였다.

결론: 본 연구에서 통년성 항원에 대한 피부단자검사가 양성인 경우 계절성 항원에 대한 ImmunoCAP®의 양성률이 피부단자검사와 비교할 때 떨어짐을 확인하였다. 이 결과를 통해 통년성 항원에 알레르기를 보이는 경우 계절성 항원에 대한 ImmunoCAP®이 위음성을 보이는 경향이 있음을 제안하는 바이다.

Introduction

The prevalence of allergic diseases has been increasing in developed and developing countries. Moreover, socio-economic burden and patient's quality of life have become important issues. Allergic diseases are characterized by producing immunoglobulin E (IgE) specific to allergens. Since allergen sensitization is a key factor for the development of allergic disease, it is very important to identify it for diagnosis of allergic diseases. For identifying allergen sensitization, various *in vivo* and *in vitro* allergy tests have been developed, and each test has its own strengths and weaknesses. However, there is no definite conclusion about which test is the best diagnostic tool to diagnose allergic sensitization.

Skin prick test (SPT) is most commonly used to diagnose allergic diseases, and it showed highest predictive value compared to serological tests [1–3]. Furthermore, SPT showed rapid results, high sensitivity, reproducibility, and cost effectiveness [2, 4]. However, several circumstances such as previous medication history, underlying disease such as dermographism, and skill of the tester may

affect availability and results of the test. On the contrary, *in vitro* tests such as multiple allergen simultaneous test (MAST®), radioallergosorbent test (RAST), and ImmunoCAP® (Phadia, Uppsala, Sweden), a fluorescence enzyme immunoassay, do not have these limitations [5, 6]. However, RAST has a risk of exposure to radioactive materials, whereas MAST® has a lower sensitivity than SPT, requires a lot of serum, and has a long testing time [7]. ImmunoCAP® was also reported to exhibit various concordances with SPT even it had higher sensitivity and specificity than previous tests [7]. Nevertheless, *in vitro* tests have been widely used because of the limited invasiveness, convenience of testing for multiple allergens, and safety.

There have been many studies which reported discordance of test results between SPT and *in vitro* tests [8]. However, there is still a lack of research on understanding the differences in results depending on the type of allergen. Therefore, it is important to know exactly what conditions affect these other outcomes for accurate diagnosis. We aimed to investigate which factors affected the analysis of the discordance between SPT and ImmunoCAP®.

Materials and methods

1. Study subjects

We reviewed the medical records of patients with allergic nasal symptoms (nasal obstruction, watery rhinorrhea, or sneezing) who visited the Department of Otolaryngology, Ajou University Hospital, between June 2012 and May 2013. Among 136 patients who underwent both SPT and ImmunoCAP® for six common allergens in Korea (*Dermatophagoides pteronyssinus (Dp)*, *Dermatophagoides farinae (Df)*, alder, ragweed, mugwort, and *Humulus japonicus (Hj)*), we excluded patients younger than 13 years, with chronic immune-related diseases such as chronic renal failure or cancer, or with skin diseases such as eczema or dermographism. Furthermore, we excluded patients who showed a histamine skin wheal < 2 mm. Finally, 94 subjects were enrolled in this study. This study was approved by the Institutional Review Board of Ajou University Hospital.

2. Allergy test

Dp and *Df* were considered as perennial allergens, and alder, ragweed, mugwort, and *Hj* were considered as seasonal allergens. SPT was performed using a 23G fine needle on the back with extracts of six allergens. A 1% histamine solution was used as positive control and saline was used as negative control. Fifteen minutes after skin pricking, the size of the wheal was measured. A wheal diameter ≥ 3 mm was considered as positive for SPT. Patient bloods were obtained and serum specific IgEs to six allergens were measured using the ImmunoCAP® system (Phadia, Uppsala, Sweden). Specific IgE level > 0.35 kUA/L was considered as positive for ImmunoCAP®.

3. Statistical analysis

We performed the analysis for each allergen individually. In addition, we

categorized all allergens into two groups to perform the analysis between seasonal and perennial allergens. The Student's t-test was used to determine the mean number of sensitized allergens. Linear-by-linear association analysis was used to compare the rate of discordance between perennial and seasonal allergens. Logistic regression analysis was used to confirm the independent effect of the variables. Age, sex, body mass index, and allergen sensitization to perennial allergen or seasonal allergen were included in the analysis. All statistical analyses were conducted with SPSS (17.0; SPSS Inc., Chicago, IL, USA). A p value < 0.05 was considered statistically significant.

Results

Ninety-four patients were enrolled into the study, and 65 (69.1%) were men. Mean age of the patients was 33.53 ± 16.0 years. *Df* showed the highest positive rate (55.3% in SPT and 58.5% in ImmunoCAP®) among the allergens analyzed in both SPT and ImmunoCAP®, followed by *Dp* (54.2% in SPT and 52.1% in ImmunoCAP®). Among seasonal allergens, mugwort had the highest positive rate (30.8 % in SPT and 12.7% in ImmunoCAP®). Positive rates for perennial allergens were similar between both tests. For seasonal allergens, however, positive rates were much higher in SPT than ImmunoCAP® (Table 1).

We divided the patients into two groups: A – same result between SPT and ImmunoCAP® and B – different result between SPT and ImmunoCAP®, and compared the mean number of sensitized allergens between A and B for each allergen. In cases of *Dp* and *Df*, the mean number of sensitized allergens were slightly higher in group B; however, the differences were not significant. The mean number of sensitized allergens for seasonal allergens was significantly

higher in group B than group A (Figure 1).

Concordance rate of the two tests was relatively higher for perennial than seasonal allergens. Figure 2 showed that positive results in both tests were higher for perennial allergens, while negative results in both tests were higher for seasonal allergens. Especially, the ratio of the group with positive results in SPT and negative results in ImmunoCAP® was higher for seasonal allergens (Figure 2).

Therefore, we aimed to identify whether positivity for perennial allergen could affect the discordance between results of SPT and ImmunoCAP®. We performed a multivariate logistic regression analysis on the variables that might have affected the concordance rate of the two tests to determine the independent factors following adjustment for the confounding variables. We analyzed the association between concordance rate of the two tests for each allergen and age, sex, BMI and positivity of SPT for perennial or seasonal allergens. In older patients, the rate of mismatch between the two tests was higher for *Dp* and *Df*. Alder was the only allergen for which the concordance rate of the two tests was affected by BMI. Sex was not related to the concordance rate. Interestingly, the positivity of SPT for perennial allergens was shown to affect the concordance rate

for seasonal allergens. When the results of SPT for perennial allergens were positive, the rate of mismatch for seasonal allergens was much higher than otherwise (Table 2).

Discussion

Prevalence of allergic diseases such as allergic rhinitis, asthma and atopic dermatitis has been increasing in recent years [9, 10]. Therefore, methods for detecting allergens that are important in the diagnosis and treatment of allergic diseases have been developed and evaluated.

SPT has been traditionally the most popularly used method [2]. It is an *in vivo* test using the reaction due to degranulation of mast cells combined with IgE antibody [11]. The mean diameter of the wheal greater than or equal to 3 mm, or the diameter of the wheal of tested allergen greater than or equal to that of histamine, is considered as a positive result [7]. A wheal diameter ≥ 3 mm was considered as positive for SPT in this study. With these criteria, SPT can provide cheap and rapid results for sensitized allergens with high sensitivity and specificity. However, SPT has some important limitations. Some circumstances such as previous medication history, underlying disease such as dermographism, and skill of the tester, may affect the test results [2].

In vitro tests using serum such as RAST, MAST® and ImmunoCAP® were free from the limitation mentioned above. Among them, ImmunoCAP® used solid phase material composed of cyanogen bromide-activated cellulose carrier to measure specific IgE in serum. The allergen-binding ability is more than three times higher than that of RAST, which is a conventional paper-disk method; therefore, it easily binds to the sample and the allergen-antibody binding reaches equilibrium within 20 minutes. It can provide rapid results with higher sensitivity and specificity than RAST [12, 13]. Moreover, ImmunoCAP® showed higher sensitivity than MAST® in a recently reported study [14]. Many studies have compared the SPT and ImmunoCAP®. Concordance rate of the two tests was reported to be about 80%, although concordance rate was different according to each allergen [2, 7–9, 11]. This concordance rate was similar to that in our result.

Dp and *Df* were common sensitized allergens in both SPT and ImmunoCAP® in our study. *Dp* and *Df* also exhibited higher concordance rates between both tests compared to seasonal allergens. This result was also consistent with previous reports [2, 7]. When we performed multivariate logistic regression

analysis, the positivity of SPT for perennial allergens was related with decreased concordance rate between the two tests for seasonal allergens. Although we do not know the exact reason for this result, we have hypothesized two theories. First theory is about affinity of the allergens. The cyanogen bromide of ImmunoCAP® requires an amino group to bind to the cellulose allergo-sorbent [13]. Therefore, allergens containing high amounts of carbohydrates (seasonal allergens) compared to those high in amino groups (*Dp* and Df) might be less responsive with the solid phase of ImmunoCAP® [7]. This might affect the result of tree, weed or pollen allergens such as alder, ragweed, mugwort and *Hj* that had shown lower positive rates in ImmunoCAP®. Second theory is associated with fraction of specific IgE among total IgE. If a patient was sensitized by multiple allergens, the amount of total IgE is the sum of that of variable specific IgEs. Thus, when some specific IgE is predominant, serum levels of other specific IgEs can be relatively low and this may be affect the low positive rate of ImmunoCAP® for seasonal allergen when the results for perennial allergens were positive. To prove this theory we had to measure the total IgE from patients, and this can be considered as the limitation of this study. Furthermore, age was related to increased discordance between the two tests for *Dp* and *Df*. Moreover,

a previous study showed a relatively high positive rate of ImmunoCAP® and a decreased positive rate of SPT in relation to old age [15]. BMI was shown to affect the concordance rate of the two tests for alder. This might explain the results of previous studies [16, 17] that sensitization to some specific IgE may be associated with metabolic diseases; therefore, further evaluation of the relationship between obesity and allergy is needed.

Although there have been many reports of inconsistencies between SPT and ImmunoCAP®, there is a lack of reporting instances in which these differences occur. In the present study, we found that the ImmunoCAP® test for seasonal antigens showed low positive rates compared to SPT in cases that were positive for perennial antigens. No significant effect to discordant result between tests was shown in cases that were positive for seasonal allergens. Although we do not know exactly how this occurs, we may need to consider the possibility that the results of ImmunoCAP® might be false negative for seasonal antigens when positive for perennial antigens.

This study has some limitations. The number of subjects was too small to make firm conclusions. And also, multivariate regression analysis should have included

more variables such as cigarette smoking, alcohol consumption, and underlying diseases. Moreover, there may be an error in the interpretation of results because we conducted the analyses on the basis that SPT was considered as the standard diagnostic test. Even though SPT is the most widely used method to diagnose allergic diseases, we cannot be sure that SPT is the standard diagnostic test for allergies. Moreover, we did not consider the symptoms of subjects. Therefore, we should obtain information about symptoms related to sensitized allergens in a further study.

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Table 1. Demographic data of study participants (n=94)

Characteristics	
Age (year)*	33.53 ±16.0
Sex[#]	
Male	65 (69.1%)
Female	29 (30.9%)
Skin prick test[#]	
Positive rate for *Dp*	51 (54.2%)
Positive rate for *Df*	52 (55.3%)
Positive rate for Alder	18 (19.1%)
Positive rate for Ragweed	22 (23.4%)
Positive rate for Mugwort	29 (30.8%)
Positive rate for *H. japonicus*	24 (25.5%)
ImmunoCAP® [#]	
Positive rate for *Dp*	49 (52.1%)
Positive rate for *Df*	55 (58.5%)
Positive rate for Alder	8 (8.5%)
Positive rate for Ragweed	8 (8.5%)
Positive rate for Mugwort	12 (12.7%)
Positive rate for *H. japonicus*	10 (10.6%)

* value was presented as mean ± standard deviation.

[#] value was presented as number (percentage).

(*Dp : Dermatophagoides pteronyssinus, Df : Dermatophagoides farinae, H. Japonicus : Humulus japonicus*)

Table2. Multivariate logistic regression analysis

Variables	Odd ration (95% confidence Interval)					
	Dp	*Df*	Alder	Ragweed	Mugwort	*H.japonicus*
Age (year)	***1.070 (1.022 – 1.121)***	***1.118 (1.038 – 1.204)***	0.997 (0.953 – 1.044)	1.023 (0.983 – 1.065)	1.001 (0.962 – 1.042)	0.994 (0.950 – 1.040)
Sex						
Male	2.179 (0.491 – 9.670)	5.357 (0.527 – 54.463)	0.903 (0.227 – 3.586)	0.910)0.262 – 3.166)	1.101 (0.325 – 3.731)	0.900 (0.233 – 3.469)
Female	Reference	Reference	Reference	Reference	Reference	Reference
BMI	0.950 (0.775 – 1.165)	0.979 (0.745 – 1.285)	***1.215 (1.009 – 1.463)***	1.097 (0.929 – 1.294)	0.918 (0.770 – 1.094)	1.068 (0.894 – 1.275)
Skin prick test						
Positive for perennial allergen	-	-	***19.946 (2.065 – 192.647)***	***7.412 (1.572 – 34.940)***	***4.764 (1.119 – 20.277)***	***13.986 (1.561 – 125.340)***
Positive for seasonal allergen	2.661 (0.611 – 11.593)	6.585 (0.770 – 56.328)	-	-	-	-

Bold and Italic denoted p<0.05

(*Dp : Dermatophagoides pteronyssinus, Df : Dermatophagoides farinae, H. Japonicus* : *Humulus japonicus*)

Figures

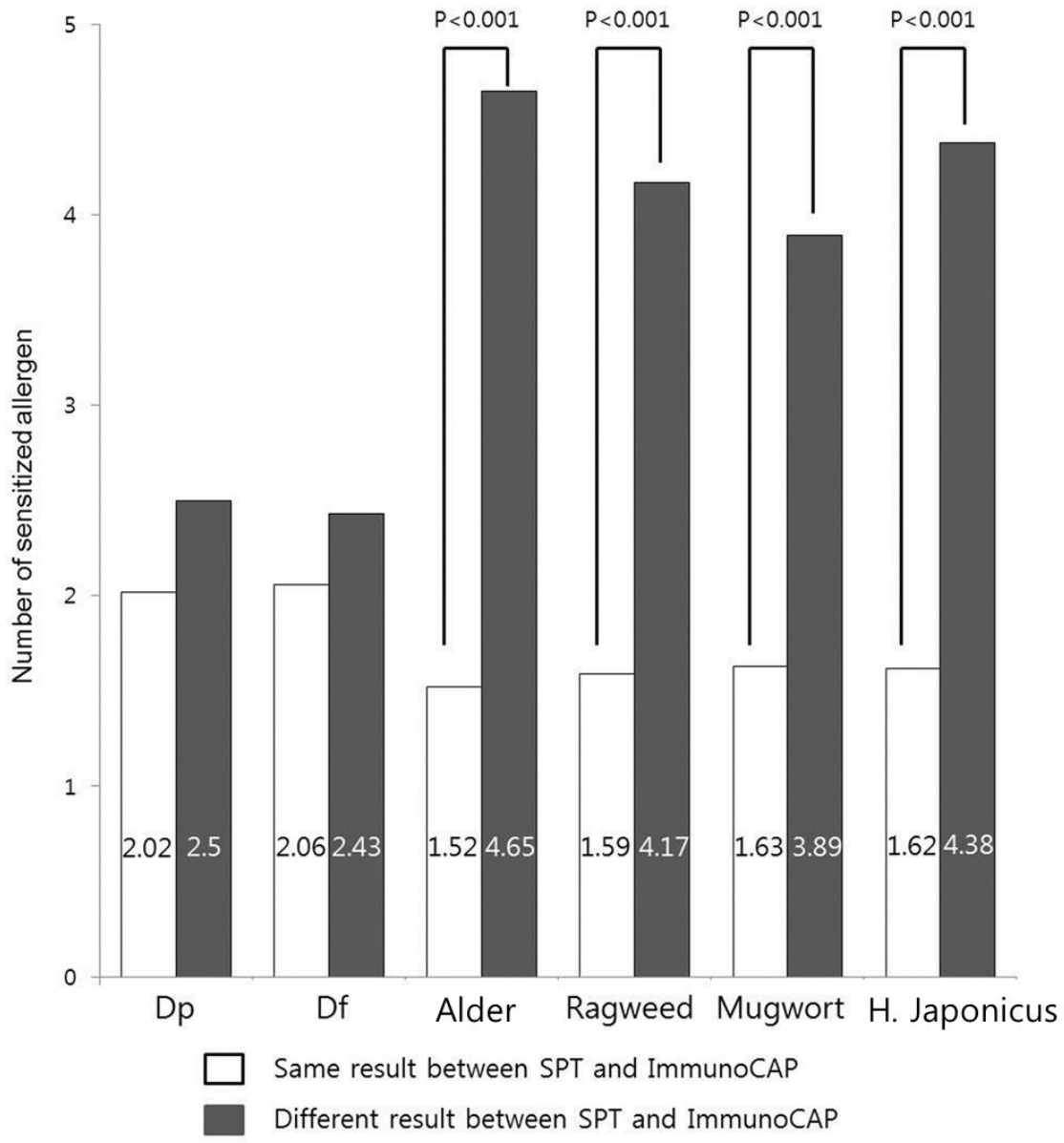

Figure.1 Mean number of sensitized allergen in each allergen according to same or different results between skin prick test and ImmunoCAP® (*Dp : Dermatophagoides pteronyssinus, Df : Dermatophagoides farinae, H. Japonicus : Humulus japonicus*)

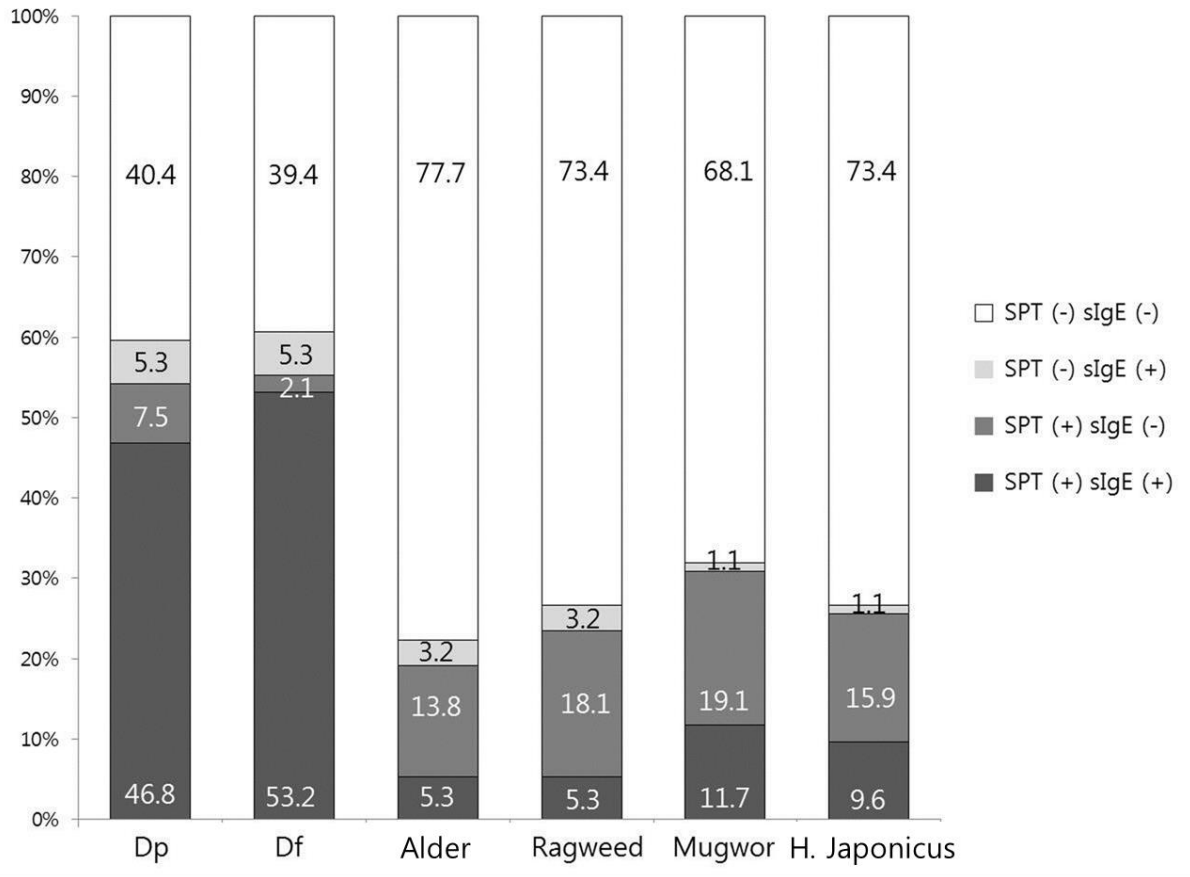

Figure.2 Ratio of group divided by the result of SPT and ImmunoCAP® in each allergens (*Dp : Dermatophagoides pteronyssinus, Df : Dermatophagoides farinae, H. Japonicus : Humulus japonicus*)