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Sensitization to house dust mite allergens might be related to the low sensitivity of ImmunoCAP to pollen allergen

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Abstract

Background: Skin prick test (SPT) and ImmunoCAP are widely used to diagnose allergies. However, previous studies showed discordance between the results of SPT and ImmunoCAP and there remains a lack of research to better understand the differences in results between the two tests.

Objective: We investigated factors that affected the discordance between SPT and ImmunoCAP results.

Methods: We reviewed the medical records of 94 subjects who underwent both SPT and ImmunoCAP for six allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, alder, ragweed, mugwort, and Humulus japonicus). We retrospectively analyzed whether age, sex, body mass index, and allergic sensitization to house dust mite (HDM) or seasonal allergens affected the discordance of results between SPT and ImmunoCAP.

Results: The positivity rates for HDM allergens were similar between the two tests. For seasonal allergens, however, the positivity rates were much higher in the SPT than those in the ImmunoCAP. The concordance rates of the two tests were relatively higher for HDM than seasonal allergens. Moreover, the ratio of the subjects positive by SPT and negative by ImmunoCAP was higher for seasonal allergens. Positivity for HDM allergens by SPT resulted in a higher rate of mismatch between the two tests for seasonal allergens.

Conclusion: The ImmunoCAP test for seasonal antigens showed low positivity rates compared to SPT in cases positive for HDM allergens. This suggests that the results of ImmunoCAP are less sensitive for seasonal allergens compared to the SPT in cases positive for HDM allergens.

Key words: Allergy; Skin prick test; ImmunoCAP; Sensitization; Perennial; Seasonal

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Introduction

The prevalence of allergic diseases has been increasing in developed and developing countries.[1,2] Moreover, socio-economic burden and patient quality of life have become important issues. Allergic diseases are characterized by the production of immunoglobulin E (IgE) specific to allergens. Since allergen sensitization is a key factor for the development of allergic disease, its identification is important for the diagnosis of allergic diseases. Various *in vivo* and *in vitro* allergy tests have been developed to identify allergen sensitization and each test has its own strengths and weaknesses. However, there is no definite diagnosis conclusion about which test is the best diagnostic tool for the of allergic sensitization.

The skin prick test (SPT) is most commonly used to diagnose allergic diseases and has shown the highest predictive value compared to serological tests.[3-5] Furthermore, SPT provides rapid results, high sensitivity, reproducibility, and cost-effectiveness.[4,6] However, several circumstances such as previous medication history, underlying disease such as dermographism, and tester skill may affect the test availability and results. In contrast, *in vitro* tests such as the multiple

allergen simultaneous test (MAST); radioallergosorbent test (RAST); and ImmunoCAP (Phadia, Uppsala, Sweden), a fluorescence enzyme immunoassay, do not have these limitations.[7,8] However, the RAST has a risk of exposure to radioactive materials, whereas the MAST has a lower sensitivity than that of SPT, requires a large serum sample, and has a long testing time.[9] The ImmunoCAP has been reported to exhibit results concordant with those of SPT, with higher sensitivity and specificity than those of previous tests.[9] Nevertheless, *in vitro* tests have been widely used because of the limited invasiveness, convenience of testing for multiple allergens, and safety.

However, many studies have reported discordance in test results between SPT and *in vitro* tests[10] and there remains a lack of research to better understand the differences in results depending on the type of allergen. Therefore, it is important to determine exactly what conditions affect these outcomes for accurate diagnosis. The present study aimed to investigate factors that affected the discordance in results between the SPT and ImmunoCAP.

Materials and methods

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 those younger than 13 years of age; with chronic diseases such as asthma, chronic renal failure or cancer; and with skin diseases such as eczema or dermographism. Furthermore, we excluded patients with histamine skin wheals < 2 mm. The details of the study were explained to patients and written informed consent was obtained. Finally, 94 subjects were enrolled in this study. This study was approved by the Institutional Review Board of Jeju National University Hospital.

Allergy test

Dp and *Df* were considered perennial allergens. Alder, ragweed, mugwort, and *Hj* were considered seasonal allergens. All allergens were purchased from Allergopharma (Reinbek, Germany). SPT was performed using a 23-G fine needle on the back to administer extracts of six allergens. A 1% histamine solution (Allergopharma) 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 a positive result for the SPT. Patient blood samples were also obtained. Serum total IgE and IgEs specific to the six allergens were measured using the ImmunoCAP system (Phadia, Uppsala, Sweden). Specific IgE levels > 0.35 kUA/L were considered positive results in the ImmunoCAP test.

Statistical analysis

We analyzed each allergen individually. In addition, we categorized the allergens into two groups to assess the differences between seasonal and perennial allergens. Student's t-tests were 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. The agreement between the results of the SPT and ImmunoCAP was evaluated using Cohen's kappa coefficient. Logistic regression analysis was used to confirm the independent effect of the variables. Age, sex, body mass index, and allergen sensitization to perennial or seasonal allergens were included in the analysis. All statistical analyses were conducted using SPSS (17.0; SPSS Inc., Chicago, IL, USA). *P*-value < 0.05 were considered statistically significant.

Results

Of the 94 patients enrolled in the study, 65 (69.1%) were men. The mean age of the patients was 33.53 ± 16.0 years. *Df* showed the highest positivity rates (55.3% in SPT and 58.5% in ImmunoCAP) among the allergens analyzed in both SPT and ImmunoCAP, followed by *Dp* (54.2% and 52.1%, respectively). Among seasonal allergens, mugwort had the highest positivity rate (30.8% in SPT and 12.7% in ImmunoCAP).

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

Characteristics	
Age (years)*	33.53 ±16.0
Sex#	
Male	65 (69.1%)
Female	29 (30.9%)
Skin prick test# positivity rates	
Dp	51 (54.2%)
Df	52 (55.3%)
Alder	18 (19.1%)
Ragweed	22 (23.4%)
Mugwort	29 (30.8%)
Hj	24 (25.5%)
ImmunoCAP# positivity rates	
Dp	49 (52.1%)
Df	55 (58.5%)
Alder	8 (8.5%)
Ragweed	8 (8.5%)
Mugwort	12 (12.7%)
Hj	10 (10.6%)

* mean ± standard deviation.
# number (percentage).
Abbreviations: *Dp*: *Dermatophagoides pteronyssinus*, *Df*: *Dermatophagoides farinae*, *Hj*: *Humulus japonicus*

The positivity rates for perennial allergens were similar between the tests. For seasonal allergens, however, the positivity rate was much higher in the SPT than that in the ImmunoCAP (**Table 1**). The agreements between the SPT and ImmunoCAP results (Cohen's kappa coefficient) were 0.744 for *Dp* ($p < 0.001$), 0.848 for *Df* ($p < 0.001$), 0.302 for alder ($p = 0.001$), 0.238 for ragweed ($p = 0.006$), 0.434 for mugwort ($p < 0.001$), and 0.446 for *Hj* ($p < 0.001$). The concordance rates of the two tests were relatively higher for perennial than for seasonal allergens.

We divided the patient group according to sensitization to *Dp* or *Df* and analyzed the discordance rates between the SPT and ImmunoCAP for each seasonal allergen. The results showed that the discordance rates between the two tests among subjects positive for *Dp* or *Df* by SPT were 29.1% for alder, 27.3% for ragweed, 29.1% for mugwort, and 27.3 % for *Hj*. However, the discordance rates among subjects without sensitization to *Dp* or *Df* by SPT were 2.6% for alder, 15.4% for ragweed, 7.7% for mugwort, and 2.6% for *Hj*. Chi-square tests showed significant differences in concordance and discordance rates between the two tests for seasonal allergens according to *Dp* or *Df* positivity ($p < 0.05$). However, there was no significant difference in discordance rates between SPT and ImmunoCAP for each allergen when we divided subjects according to sensitization to each seasonal allergen (data not shown).

Figure 1 shows that the positive results in both tests were higher for perennial allergens, while the negative results in both tests were higher for seasonal allergens. In particular, the ratio of the group with positive results in SPT and negative results in ImmunoCAP was higher for seasonal allergens.

Therefore, we aimed to identify whether positivity for perennial allergens affected the discordance between SPT and ImmunoCAP results. Following adjustment for confounding variables, we performed a multivariate logistic regression analysis to determine which independent factors might have affected the concordance rate of the two tests. We analyzed the associations between the concordance rates of the two tests for each allergen and age, sex, BMI, and SPT positivity 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. The positivity of SPT for perennial allergens

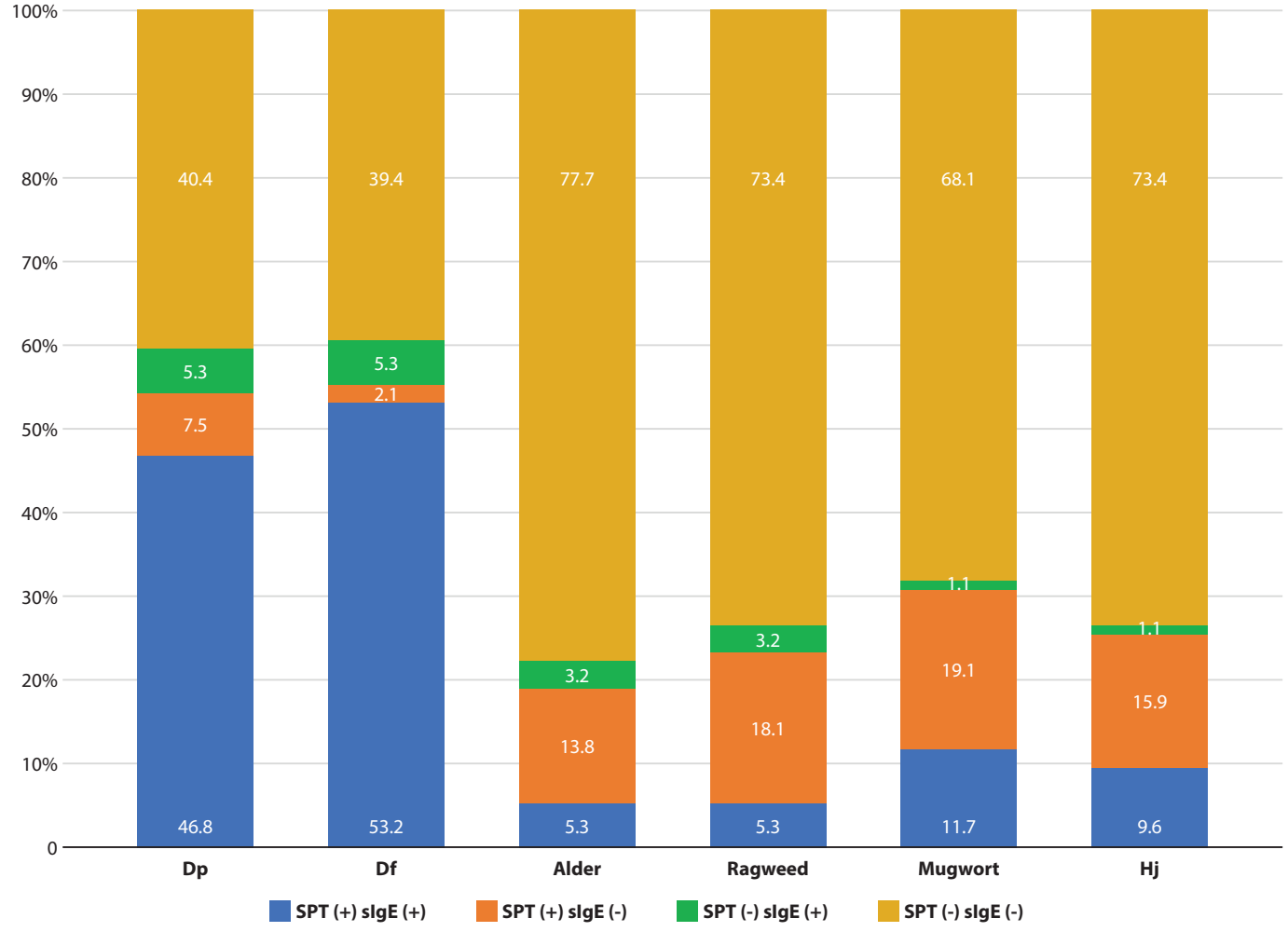

Figure.1 Ratio of groups divided by the results of SPT and ImmunoCAP for each allergen

Abbreviations: *Dp*: *Dermatophagoides pteronyssinus*, *Df*: *Dermatophagoides farinae*, *Hj*: *Humulus japonicus*

Table 2. Multivariate logistic regression analysis.

Variables	Odds ratio (95% confidence interval)					
	Dp	*Df*	Alder	Ragweed	Mugwort	*Hj*
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)	-	-	-	-

Odds ratios were analyzed by multivariate logistic regression using the discordance of results between the skin prick test and ImmunoCAP as the dependent variable.
Bold and italics indicate $p < 0.05$
Abbreviations: *Dp*: *Dermatophagoides pteronyssinus*, *Df*: *Dermatophagoides farina*, *Hj*: *Humulus japonicas*, BMI: body mass index

was shown to affect the concordance rate for seasonal allergens. In other words, perennial allergen positivity by SPT was associated with a higher rate of mismatch for seasonal allergens than otherwise (**Table 2**).

Discussion

The prevalence of allergic diseases such as allergic rhinitis, asthma, and atopic dermatitis has been increasing in recent years.[11,12] Therefore, methods to detect allergens that are important for the diagnosis and treatment of allergic diseases have been developed and evaluated.

The SPT has traditionally been the most commonly used method.[4] It is an *in vivo* test based on a reaction due to the degranulation of mast cells when combined with IgE antibody.[13] A mean wheal diameter greater than or equal to 3 mm, or greater than or equal to that of histamine, is considered a positive result.[9] The present study defined a wheal diameter ≥ 3 mm as a positive SPT result. Using these criteria, SPT can provide cheap and rapid results for sensitized allergens with high sensitivity and specificity. However, the SPT has some limitations. Circumstances such as previous medication history, underlying disease such as dermographism, and tester skill may affect the test results.[4]

In vitro serum-based tests such as the RAST, MAST, and ImmunoCAP are free of the limitations mentioned above. Among them, the ImmunoCAP uses a solid-phase material composed of a cyanogen bromide-activated cellulose carrier to measure the serum levels of specific IgEs. Its allergen-binding ability is more than three times that of the 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. The ImmunoCAP can provide rapid results with higher sensitivity and specificity than those of the RAST.[14,15] Moreover, the ImmunoCAP showed a higher sensitivity than that of the MAST in a recent study.[16] Many studies have compared the SPT and ImmunoCAP. The reported concordance rate of the two was about 80%, although the rates differed according to allergen.[4,9–11,13] This concordance rate was similar to that observed in the present study.

Dp and *Df* were common sensitized allergens in both SPT and ImmunoCAP in our study. These allergens also exhibited higher concordance rates between both tests compared to the concordance rates for seasonal allergens. This result was also consistent with previous reports.[4,9] Multivariate logistic regression analysis revealed that the positivity of SPT for *Dp* and/or *Df* was related to the decreased concordance rate between the two tests for seasonal allergens. We do not know the exact reason for this result. However, the cyanogen bromide of the ImmunoCAP requires an amino group to bind to the cellulose allergo-sorbent.[15] Therefore, allergens containing high amounts of carbohydrates (seasonal allergens) compared to those high in amino groups (*Dp* and *Df*) might be less responsive to the solid phase of the ImmunoCAP.[9] This might affect the results for tree, weed, or pollen allergens such as alder, ragweed, mugwort, and *Hj*, all of which had shown lower positivity rates in the ImmunoCAP. Furthermore, age was related to increased discordance rates for *Dp* and *Df* between the two tests. A previous study also showed a relatively high positivity rate for the ImmunoCAP and a decreased positivity rate for the SPT in relation to old age.[17] In the present study, BMI was shown to affect the concordance rate of the two tests for alder. This might explain the results of previous studies that sensitization to some specific IgE may be associated with metabolic diseases.[18,19] Therefore further evaluation of the relationship between obesity and allergies is required. Finally, total IgE levels did not differ between groups divided by concordant and discordant SPT and ImmunoCAP results (data not shown)

Although there have been many reports of inconsistencies between SPT and ImmunoCAP results, there is a lack of evaluation regarding the circumstances in which these discordant results occur. In the present study, we found low seasonal antigens positivity rates for the ImmunoCAP test compared to those of the SPT in cases that were positive for *Dp* and/or *Df*. No significant effect on the discordant result between the tests was observed in cases that were positive for seasonal allergens. Although we do not the precise reasons for this observation, it is possible that positivity to *Dp* and/or *Df* in the ImmunoCAP might produce false-negative results for seasonal antigens.

This study has some limitations. First, the number of subjects and allergens were too small to make firm conclusions. Second, there might be an error in the interpretation of results because we conducted our analyses on the basis that the SPT was considered to be the standard diagnostic test. Even though the SPT is the most widely used method for the diagnosis of allergic diseases, we cannot be sure that it is the standard diagnostic test for allergies. Third, we did not consider the symptoms of the subjects, especially the nature of symptoms such as perennial or seasonal presentation. Therefore, future studies should obtain information about symptoms related to sensitized allergens. Finally, *Dp* and *Df* are one of most common perennial allergens. In this study, we considered *Dp* and *Df* as perennial allergens for convenience in analysis. However, these two allergens cannot represent all perennial allergens. Therefore, we should consider this limitation and need further study including more allergens (perennial and seasonal) to confirm the effect of other perennial allergen.

Conclusion

Previous reports showed discordance between the SPT and ImmunoCAP test results. However, we still do not know the exact cause and mechanism for the difference in results between the two tests. Our findings showed low positivity rates for the ImmunoCAP test compared to those of the SPT in cases positive for house dust mite antigens. This suggests that the results of ImmunoCAP are less sensitive for seasonal allergens compared to the SPT in patients positive for house dust mite allergens.

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Author contributions

- J.W.K. and M.B.K. designed the research
- M.B.K., Y.S.K., and J.W.K. collected and analyzed the data
- J.W.K. and M.B.K. wrote the paper.
- J.W.K., G.C.L., M.B.K., and J.C.L. revised the manuscript.
- J.W.K. had primary responsibility for the final content. All authors read and approved the final manuscript.

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