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Original Article

Comparison of rapid screening immunoassay and intradermal test for canine atopic dermatitis

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The intradermal test (IDT) has been developed for confirming diagnosis of canine atopic dermatitis (CAD). Prior to performing IDT, rapid immunoassay (Allercept E-screen 2nd generation; ES2G) can detect allergen-specific immunoglobulin E (IgE) antibodies in canine serum. The objective of this study was to evaluate agreement between IDT and immunoassay in diagnosis of CAD in domestic atopic dogs. Forty dogs were diagnosed with CAD in accordance with Favrot's criteria. Intradermal testing was performed using 39 selected allergens. ES2G detected IgE antibodies specific for three allergen groups, including indoor allergens, grasses and weeds, and trees. Among 19 dogs diagnosed by IDT, the highest positivity was observed in house dust mites, followed by molds, epidermis and inhalants, house dust, and weeds. A total of 28 atopic dogs were evaluated by rapid ES2G immunoassay. Indoor allergens showed the strongest positive reaction, followed by grasses/weeds and trees. IDT and ES2G were performed concurrently in 17 dogs. The results of ES2G showed slight agreement with those of IDT. Level of agreement was highest for indoor allergens, which showed a predictive positive value of 100% in ES2G. These results indicate that a rapid immunoassay may be valuable for predicting the results of IDT in atopic dogs sensitized to indoor allergens.

Key words: allergen, canine atopic dermatitis, immunoassay, immunoglobulin E, intradermal test

Introduction

Canine atopic dermatitis (CAD) is a common skin disease in dogs defined as inflammatory and pruritic dermatitis with clinical features associated with immunoglobulin E (IgE)-mediated hypersensitivity in response to specific environmental allergens [1-3]. Over the years, several diagnostic tests for identification of offending allergens and subsequent hyposensitization have been extrapolated and adapted [1, 4]. Diagnosis of CAD is based on fulfillment of associated clinical criteria along with elimination of other relevant differential diagnoses. For this, Favrot's criteria has been recommended in dogs [2, 5, 6].

Along with clinical criteria, allergen-specific IgE is routinely identified by either intradermal (IDT) or IgE serological tests (IST) for confirming diagnosis of CAD and determining allergens for immunotherapy [7-9]. Although IDT has been considered as the most accurate method, it has certain disadvantages, as follows: skin reactivity might be affected by previous ingestion of glucocorticoids, antihistamines, or other nonsteroidal anti-inflammatory drugs, sedation is required, large areas of hair have to be shaved, prior or coexisting dermatologic conditions may preclude performance of IDT, and systemic reactions may occur [10]. IST can complement IDT by overcoming these limitations of IDT. However, IDT could not be replaced with IST due to frequent false-positive results, variable reliability and reproducibility, and low sensitivity of IST [11].

Recently, an inexpensive IgE screening immune-assay (Allercept E-screen 2nd generation (ES2G); Heska, Switzerland) showed moderate agreement with IDT and IST. These results have some diagnostic value for veterinarians to decide whether or not to conduct IDT and IST [12, 13].

The distribution of allergens is different between various countries. However, comparison of IDT and immu-

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noassay to diagnose AD in domestic dogs has not been studied in Korea. Therefore, the purpose of this study was to evaluate agreement between IDT and immunoassay in domestic dogs with AD.

Materials and Methods

Cases

Diagnosis of CAD was determined based on a minimum of five Favrot's criteria (1, onset of signs under 3 years of age; 2, dogs living mostly indoor; 3, glucocorticoid-responsive pruritus; 4, pruritus sine materia at onset; 5, affected feet (front and hind); 6, affected ear pinnae; 7, unaffected ear margins; 8, unaffected dorso-lumbar area) and exclusion of other pruritic causes such as adverse food reactions, endocrine diseases, and infectious causes, including bacteria, *Malassezia*, fungi, and ectoparasites [5, 6]. From September 2011 to July 2014, 40 dogs fulfilled Favrot's criteria during the study period, and they were included in this study. Written client consent was obtained prior to examination, and this procedure was performed with the approval of our institutional review board committee. The breed, gender, and age of presentation as well as initial onset were obtained from medical records of the dogs.

Allergen extracts

Intradermal testing was performed using 39 selected allergens (Table 1). These were subdivided into 10 antigen groups, including pollen, weeds, flowers, trees and shrubs, molds, smut, house dust, epidermis and inhalants, house dust mites, and insects. Commercial allergen extracts for IDT were purchased from Greer Laboratories (Lenoir, USA).

Intradermal test

Among the 40 dogs included in this study, 19 dogs were examined by IDT, as described previously [10]. Glucocorticoid and anti-histamine treatments were discontinued for at least 4 weeks prior to IDT. Dogs were placed in lateral recumbency under sedation with an intravenous injection using 10 μg/kg of medetomidine (Domitor; Pfizer, South Korea). The hair coat of the lateral thorax was clipped to avoid subsequent skin irritation. Each test site for IDT was marked with a marker pen. Approximately 0.05 mL of each allergen extract was injected into the dermis using an insulin syringe (BD Ultra-Fine; Becton, Dickinson and Company, USA). Histamine phosphate (0.1 mg/mL) and 0.9% phosphate-buffered saline were injected as the positive and negative controls, respectively. The positive control was scored as 4, and negative control sites were assigned scores as 0. Skin reactions were assessed after 15 min of injections and graded from 0 to 4 based on measurement of diameter, degree of

Table 1. A list of allergenic extracts used for IDT

Group	Allergens	Concentration
Pollen	Bermuda grass	1,000 PNU/mL
Weeds	Cocklebur	1,000 PNU/mL
	Goldenrod	1,000 PNU/mL
	Lamb's Quarter	1,000 PNU/mL
	Pigweed, Rough/Red root	1,000 PNU/mL
	Plantain, English	1,000 PNU/mL
	Dandelion	1,000 PNU/mL
	Sage mix	1,000 PNU/mL
	Ragweed mix	1,000 PNU/mL
Trees and Shrubs	Alder, white	1,000 PNU/mL
	Hazelnut, American	1,000 PNU/mL
	Birch mix	1,000 PNU/mL
	Pine mix	1,000 PNU/mL
	11 Tree mix	1,000 PNU/mL
Molds	*Candida albicans*	1,000 PNU/mL
	Acremonium strictum	1,000 PNU/mL
	Trichothecium roseum	1,000 PNU/mL
	Fusarium moniliforme	1,000 PNU/mL
	Fusarium solani	1,000 PNU/mL
	Trichophyton mentagrophytes	1,000 PNU/mL
	Trichophyton rubrum	1,000 PNU/mL
	Aspergillus mix	1,000 PNU/mL
	Penicillium mix	1,000 PNU/mL
	Mucor mix	1,000 PNU/mL
	Rhizopus mix	250 PNU/mL
Smut	Grass Smut mix	1,000 PNU/mL
	Grain Smut mix	1,000 PNU/mL
House dust	Dust, House mixture	100 PNU/mL
Epidermis and Inhalants	Cat epithelia	1,000 PNU/mL
	Cotton seed	1,000 PNU/mL
	Kapok seed	1,000 PNU/mL
	Pyrethrum	1,000 PNU/mL
	Silk	500 PNU/mL
	Mixed feathers	1,000 PNU/mL
House dust mites	*Dermatophagoides farinae*	1:5,000 w/v
	Dermatophagoides pteronyssinus	1:5,000 w/v
Insect	Flea	1:1,000 w/v
	Mosquito	1,000 PNU/mL
	2 Cockroach mix	1,000 PNU/mL
Positive control	Histamine	0.0275 mg/mL
Negative control	0.9% phosphate buffered saline	–

PNU, protein nitrogen units; w/v, weight/volume.

erythema, and induration of the wheal in comparison to control sites. Only reactions graded 2 and stronger were classed as positive, whereas sites with scores of 0 or 1 were classed as negative.

Rapid screening immunoassay

Prior to the test, ES2G reagents were stored at room temperature. ES2G detected IgE antibodies specific for the following allergen groups: 'I' corresponds to indoor allergens such as mites, molds, and fleas, 'GW' to grasses and weeds, and 'T' to tree groups and a control spot containing purified IgE. The reagents sequentially added to the test spot were test serum, biotinylated detection reagent (FcεR1a), streptavidin-alkaline phosphatase, and color development reagent, with a washing reagent every other step. Appearance of the control spot means a valid test, and any other test spots were recorded as positive. This color appearance indicates the presence of one or more detectable allergen-specific IgE against the allergen group in the serum or plasma sample. If only the control spot was visible within 120 sec, the test was negative. ES2G is not a quantitative test, as any visible colored test spot is considered as a positive result [14].

Statistical analysis

Sensitivity, specificity, positive predictive value, negative positive value, and Kappa statistic were calculated to assess the degree of match between IDT and ES2G (Microsoft Excel, USA). Kappa statistic is commonly used for specific statistical methods to assess reliability. It is an indication of the degree of agreement between the results of two diagnostic methods, excluding the possibility of chance. Kappa value of −1.0 indicates perfect disagreement while +1.0 indicates perfect agreement. Strength of agreement for the Kappa coefficient: ≤0=poor, 0.01~0.20=slight, 0.21~0.40=fair, 0.41~0.60=moderate, 0.61~0.80=substantial, and 0.81~1.00=almost perfect agreement [15].

Table 2. Signalments of 40 dogs with AD

Signalments	Classification	Number of dogs (%)
Breed	Shih Tzu	20 (50)
	Maltese	6 (15)
	Boston Terrier	2 (5)
	Cocker Spaniel	2 (5)
	Schnauzer	2 (5)
	Yorkshire Terrier	2 (5)
	Beagle	1 (2.5)
	Golden Retriever	1 (2.5)
	Miniature Pinscher	1 (2.5)
	Mixed breed	1 (2.5)
	Pekingese	1 (2.5)
	Poongsan	1 (2.5)
Gender	Male	22 (55)
	Female	18 (45)
Age of onset	<1y	10 (27)
	1~3y	16 (43.2)
	3~5y	3 (8.1)
	≥5y	8 (21.6)

Results

Cases

Breed, gender, and initial onset age of patients diagnosed as AD according to Favrot's diagnostic criteria are summarized in Table 2. Among the 40 examined dogs, the most common breed was Shih Tzu (50%), followed by Maltese (15%). The mean age of initial onset was 37 months (range: 6 to 96 months) in 37 dogs. Data on three dogs were excluded due to inaccuracy of the information. Percentage of cases of initial onset age under 3 years of age was 70.2%. There was no significant difference in

Table 3. Results of IDT in 19 dogs with AD

Group	Allergens	Number of dogs (%)	Number of dogs (%)
House dust mites	*Dermatophagoides farinae*	11 (17.5)	18 (28.6)
	Dermatophagoides pteryssinus	7 (11.1)	
Molds	*Rhizopus mix*	14 (22.2)	16 (25.4)
	Fusarium solani	1 (1.6)	
	Trichophyton rubrum	1 (1.6)	
Epidermis and Inhalants	Silk	4 (6.4)	9 (14.3)
	Cat epithelia	2 (3.2)	
	Cotton seed	2 (3.2)	
	Mixed feathers	1 (1.6)	
House dust	Dust, House mix	8 (12.7)	8 (12.7)
Weeds	Cocklebur	2 (3.2)	7 (11.1)
	Goldenrod	2 (3.2)	
	Lamb's quarter	2 (3.2)	
	Sage mix	1 (1.6)	
Trees and Shrubs	Hazelnut, America	1 (1.6)	3 (4.8)
	Birch mix	1 (1.6)	
	Pine mix	1 (1.6)	
Insects	Mosquito	1 (1.6)	2 (3.2)
	2 Cockroach mix	1 (1.6)	

gender.

Intradermal test

Among 19 dogs diagnosed by IDT, 18 dogs showed at least one positive response (Table 3). The highest positivity was observed for house dust mites (HDM), followed by molds, epidermis and inhalants, house dust, and weeds. Positive reaction was not detected in the pollen and smut groups. Regarding separate allergens, *Rhizopus* mix showed the highest positivity, followed by *Dermatophagoides farinae*, house dust mixture, and *Dermatophagoides pterinyssinus*.

Rapid screening immunoassay

A total of 28 atopic dogs were evaluated by rapid ES2G immunoassay. Among the 28 dogs, more than one positive response was observed in 19 dogs (67.9%). Positive reaction was the highest against indoors, followed by grasses/weeds and trees (Table 4).

Agreement between IDT and ES2G

Among 40 dogs with AD, 17 dogs (42.5%) were examined by IDT and ES2G concurrently. One dog showed negative reactions in both IDT and ES2G, despite fulfilling Favrot's criteria. Comparison between ES2G and IDT are presented in Table 5. When all allergens were analyzed as one group (altogether group), specificity and positive predictive values were both 100%. The rate of agreement between IDT and the altogether group was slight (Kappa coefficient: 0.206). Among the three allergen mixtures, the highest sensitivity, specificity, and positive predictive values were observed in the indoors group. Especially, specificity and positive predictive values of the indoors group were 100%. For negative predictive values, the highest value was observed in the trees group, followed by the grasses/weeds group and indoors group. Agreement with IDT was strongest for the indoors group (Kappa coefficient: 0.39) and weakest for the trees group (Kappa coefficient: −0.06).

Table 4. Results of ES2G in 28 dogs with AD

Allergen groups	Number of dogs (%)
Altogether	19 (67.9)
Trees	7 (25.0)
Grasses/Weeds	8 (28.6)
Indoors	19 (67.9)

Discussion

The present study evaluated the agreement between IDT and ES2G to determine if a rapid immunoassay could be effective to predict the results of IDT. The most common allergen for CAD appeared to be HDM by IDT. The rate of agreement was slight, and the highest level was observed for indoor allergens among the three allergen groups.

In this study, 12 dog breeds were diagnosed with AD. Among them, Shih Tzu and Maltese were the most commonly affected by CAD. Similar to this result, breeds with a reported predilection for CAD included Shih Tzu, Yorkshire terrier, Miniature pinscher, Cocker spaniel, Maltese, Pekinese, and Schnauzer in Korea [16-18]. These breeds are the most popular and common pure breeds in Korea. Male and female dogs represented 55% and 45% of cases, respectively. These results suggest that there is not a gender predisposition in CAD cases. Generally, most atopic dogs begin manifesting signs between 6 months and 3 years of age [2, 17]. In the present study, initial onset age of clinical signs ranged from 6 months to 8 years with a mean of 3.1 years, and more than 70% of dogs showed clinical signs of AD prior to 3 years of age in this study. These findings are similar to those of previous studies [2, 16-19].

A previous study on IDT demonstrated that the most

Table 5. Comparison of results of IDT and ES2G

Statistical values	Altogether	Trees	Grasses/Weeds	Indoors
Sensitivity	68.8%	0	25%	73.3%
Specificity	100%	93.8%	92.3%	100%
Positive predictive value	100%	0	50%	100%
Negative predictive value	16.7%	93.8%	80%	33.3%
Observed agreement (OA)	70.6%	88.2%	76.5%	76.5%
Chance agreement (CA)	63%	88.9%	70.2%	61.2%
*Kappa=(OA-CA)/(1-CA)	0.206	−0.06	0.209	0.39
Agreement interpretation	Slight	Poor	Slight	Fair

*Kappa coefficient: ≤0=poor, 0.01~0.20=slight, 0.21~0.4=fair, 0.41~0.60=moderate, 0.61~0.80=substantial, and 0.81~1.00=almost perfect agreement.

common type of allergen in Korea is mold while the second most common allergen is house dust, followed by epidermal and inhalant allergens and HDM [16]. In this study, HDM appeared to be the major allergen causing AD, and positive reactions of IDT were frequently observed in the order of molds, epidermis and inhalants, and house dust. A total of 58 atopic dogs living in Seoul were evaluated by IDT in the prior study [16], whereas this study examined 19 dogs living in the Chungcheong area. Differences in area and study population may explain the results of the two studies. Regarding individual allergens, *Rhizopus* was the most frequent and important allergen of CAD in this study, and this result is the same as a previous study [16].

In the present study, 17 dogs were examined by IDT and ES2G concurrently. One dog showing negative reactions in both IDT and ES2G was tentatively diagnosed with undetermined AD or canine atopic-like dermatitis [5, 20]. The results of ES2G appeared to slightly agree with those of IDT, whereas moderate agreement was observed in a previous study on 41 atopic dogs in the United States [13]. This relatively lower rate of agreement in this study could be explained by differences in the study population and distribution of allergens between the United States and Korea. The prevalence of tree and grass allergens is higher in the United States in comparison to Korea [19].

The rate of agreement was highest for indoor allergens, and this result is similar to a previous study [13]. Since the indoor allergen spot contained the major allergen (HDM and molds) of CAD, agreement between IDT and indoor allergens may be strong. Interestingly, this study showed a positive predictive value of 100% against indoor allergens. On the other hand, a positive predictive value of 89% and negative predictive value of 100% were observed in the United States [13]. Although the main cause of this difference is unknown, these findings suggest that true positive reactions were more common in this study in comparison with previous studies [13]. Therefore, IDT could be recommended for clients when positive reactions are noted against indoor allergens. However, negative results should not be used for the purpose of determining whether or not to perform IDT due to a low negative predictive value (33.3%).

In conclusion, rate of agreement was slight between IDT and rapid ES2G immunoassay for detection of allergen-specific IgE. The agreement was strongest for indoor allergens, which showed a predictive positive value of 100%. These results indicate that a rapid immunoassay may be valuable for predicting the results of IDT in atopic dogs sensitized to indoor allergens.

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