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ORIGINAL ARTICLE

Serum allergen-specific and total IgE concentrations in dogs with allergic disorders in South Korea

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Canine atopic dermatitis (CAD) has a genetic basis and is primarily a skin disorder characterized by allergic features. It is caused by an excessive immune response, associated with immunoglobulin E (IgE), to allergens and shows clinical signs of itching and inflammation. The present study evaluated the allergen-specific and total IgE concentration in dogs with allergic disorders. Allergen-specific IgE analysis was performed using a Multiple Allergen Simultaneous Test (MAST) assay, and total IgE analysis was conducted using an ELISA in 82 dogs with CAD. The subjects were divided into two groups: dogs aged ≤3 years and dogs aged >3 years, enabling a comparison of total IgE concentrations between the two age groups. The results showed that the major affecting environmental allergen was *Alternaria alternate*, and the major affecting dietary allergen was raw chestnut. The results revealed that dogs aged ≤3 years had higher total IgE concentrations than dogs aged >3 years with significant difference. The results of this study can contribute to the diagnosis and treatment of CAD in South Korea.

Key Words: Atopic dermatitis, MAST assay, Immunoglobulin E, Allergen-specific IgE, Dogs

INTRODUCTION

Canine atopic dermatitis (CAD) has a genetic basis and is primarily a skin disorder characterized by allergic features. This condition arises from an excessive immune response to environmental allergens and is primarily manifested by itching and inflammation as distinctive clinical symptoms. This is due to its association with immunoglobulin E (IgE) antibodies (Halliwell, 2006). CAD typically develops before the age of 3, can be year-round or seasonal, and may overlap with various other pruritic and inflammatory skin diseases (Bizikova et al, 2015). One of the distinctive characteristics of CAD is its similarity to other skin disorders, meaning that some symptoms may overlap or resemble those of different skin conditions. As a result, cautious approaches and precise clinical assessments to establish an accurate diagnosis are necessary, i.e., the diagnosis of CAD requires consideration of various factors. Genetic factors, particularly in specific dog breeds, can elevate the risk of disease development (Halliwell, 2006). Additionally, the diagnosis of CAD involves clinical signs and in vivo testing, including intradermal testing and in vitro testing for allergen-specific IgE via a Multiple Allergen Simultaneous Test (MAST) assay (Griffin, 2014; Nam et al, 2021). The radioallergosorbent test (RAST) and enzyme-linked immunosorbent assay (ELISA) have been used for measuring serum IgE levels in veterinary medicine. These assays are used to detect allergen-specific IgE antibodies against a panel of allergens considered relevant for the patient (Griffin, 2014). The objective of this study is to investigate the allergens using the MAST assay and contribute to CAD treatment strategies in South Korea.

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MATERIALS AND METHODS

Animals

This study was conducted at Cheongdam Woori Animal Hospital from March 2016 to July 2023 in Seoul. Patients diagnosed with atopic dermatitis and food allergies were enrolled in this clinical study. The study included 82 dogs between the ages of 0.3 and 12 years old. Based on a study indicating that atopic dermatitis has the highest occurrence rate (78.6%) between the ages of 2 and 3 (Wilhem et al, 2010), this research divided the subjects into two groups: dogs aged ≤3 years and dogs aged >3 years (Table 1).

Blood collection

All blood samples were collected from the jugular vein and serum tubes (Vacuette, Greiner Bio-One, KremsmÜnster, Austria) containing the samples were centrifuged for serum collection. The samples were sent to the Veterinary Diagnostic Laboratory (POBANILAB Co., South Korea) for measurement of total IgE and allergen-specific IgE concentrations.

MAST assays

All tests for the detection and quantification of allergen-specific IgE were performed using a MAST assay. The MAST assay was based on a modified protocol from a veterinary diagnostic research laboratory (Mediwiss, Germany). Each patient's serum (250 μL) was allowed to react with allergen-containing nitrocellulose membranes for 45 minutes at room temperature. After washing, biotin-conjugated anti-canine IgE antibodies were added and incubated for 30 minutes at room temperature. Following another washing step, streptavidin conjugated with alkaline phosphatase was added (250 μL), and the mixture was incubated for 20 minutes at room temperature. Unbound conjugates were washed away. NBT/BCIP substrate solution was added, and the test strips were incubated for 20 minutes at room temperature. Subsequently, using software provided by the manufacturer, the fully dried test strips were read, and specific IgE concentrations were assigned a grade within the range of 0.0 to 6.0. In the clinical experiment, allergens producing results above grade 2.5 were considered positive reactions in this investigation. The list of allergens is presented in Table 2.

Table 1. Specific data of the subjects with atopic dermatitis

Group	N	Age (year)	Sex (N)	Breeds (N)
Age ≤3 years	44	1.79±0.99	Female 10 Spayed female 14 Male 4 Castrated male 4 Unknown 12	Maltese 10 Poodle 6 Bichon frisé 6 Others 22
Age >3 years	38	5.68±1.85	Female 3 Spayed female 17 Male 2 Castrated male 7 Unknown 9	Maltese 12 Poodle 7 Pomeranian 5 Others 14
Total	82	3.59±2.43	Female 13 Spayed female 31 Male 6 Castrated male 11 Unknown 21	

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Table 2. Allergens used to investigate allergen-specific Ig E antibody

	Allergens
1	Citrus mix
2	Kiwi
3	Mango
4	Peach
5	Barley meal
6	Buckwheat meal
7	Hazelnut
8	Peanut
9	Rice
10	Soybean
11	Wheat flour
12	Beef
13	Chicken
14	Pork
15	Cheddar cheese
16	Cow's milk
17	Egg white
18	Anchovy
19	Codfish
20	Crab
21	Mackerel
22	Mussel
23	Salmon
24	Shellfish
25	Shrimp
26	Tuna
27	Cucumber
28	Potato
29	Raw chestnut
30	Tomato
31	Baker's yeast
32	Alternaria
33	Aspergillus
34	*Candida albicans*
35	*Cladosporium*
36	Malassezia
37	Penicillium
38	Cat
39	Sheep wool
40	Bee venum
41	Cockroach mix
42	Flea (Ctenoph.)
43	Pupa
44	*Acarus siro*
45	*D. farinae*
46	*D. pteronyssinus*
47	*Lepidoglyphus destructor*
48	*Tyrophagus putrescentiae*
49	Acacia
50	Ash mix
51	Bermuda grass
52	Birch/Alder mix
53	Ceder, Japan
54	Dandelion
55	English plantain
56	Goldenrod
57	Japanese hop
58	Lamb's quarter
59	Lilac pollen
60	Mugwort
61	Oak, white
62	Orchard grass
63	Ox-eye-daisy
64	Parietaria (Wall pellitory)
65	Pig weed mix
66	Pine
67	Poplar mix
68	Rag weed, short
69	Redtop
70	Reed
71	Russian thistle
72	Rye pollen
73	Sorrel
74	Stinging nettle
75	Swallow willow
76	Sweet vernal grass
77	Sycamore mix
78	Timothygrass
79	Housedust
80	Latex

Total IgE concentration measurements

Total IgE concentrations were quantified using ELISA. ELISA kits equipped with antibodies specifically reacting to canine IgE were used. A 100 ng solution of monoclonal antibodies specific for canine IgE was applied to the wells of an ELISA plate, followed by fixation and blocking. The serums were diluted 1:50 with a serum dilution buffer, and 100 μL of the diluted serum samples was added to each well at room temperature. After a 1-minute incubation period, the wells were washed five times. A secondary antibody specific to IgE from the tested species was applied, followed by incubation with a substrate solution such as TMB (3,3',5,5'-Tetramethylbenzidine) for approximately 10 minutes. The absorbance

was measured to quantify the concentration of canine IgE in each sample by comparing the absorbance with that of canine IgE standard samples.

Statistical analysis

Statistical analysis was performed using a commercial software program (IBM SPSS Statistics 26.0, SPSS Inc., Chicago, IL, USA). The data were normally distributed and represented as means with standard deviations. The data for the IgE concentrations were compared using an independent t-test. A P value<0.05 was considered to be statistically significant.

RESULTS

Frequency analysis of major environmental allergens

The analysis of the serum IgE concentration values for each allergen and classification based on the 80 tested allergens resulted in the identification of the most affecting environmental allergen. The results are represented graphically, illustrating the occurrence rates of the top 10 significant environmental allergens provided by POBANILAB (Fig. 1). Typically, mites and molds exhibited high occurrence rates. *Alternaria alternata*, Japanese cedar, and *Tyrophagus putrescentiae* were confirmed to have occurrence rates of over 80%, indicating a very high prevalence, and the frequencies of *Dermatophagoides pteronyssinus*, house dust, *Cladosporium*, *Dermatophagoides farinae*, cockroach mix, goldenrod, and *Malassezia* occurred in decreasing order.

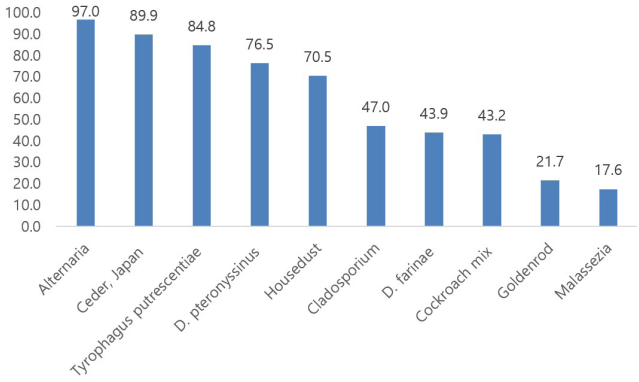

Fig. 1. Frequency of environmental allergens affecting canine atopic dermatitis.

Frequency analysis of major dietary allergens

The analysis of the serum IgE concentration values for each allergen and classification based on the 80 tested allergens resulted in the identification of the most affecting dietary allergen. The results are represented in a graph illustrating the occurrence rates of the top 10 significant dietary allergens provided by POBANILAB (Fig. 2). Raw chestnut, mackerel, and barely meal were confirmed to have the highest occurrence rates compared to other ingredients. The frequencies of mango, buckwheat meal, peanut, kiwi, chicken, wheat flour, and pork occurred in decreasing order.

Total IgE concentrations distribution by age

After undergoing antibody testing through an allergy panel, a total of 82 dogs were examined, with the amounts of total IgE within their serums being measured and analyzed by age group. The results revealed that among 44 dogs aged ≤3 years, the general distribution of total IgE concentrations had an average of 1313.43±759.05 IU Ig E/mL. For 38 dogs aged >3 years, the average IgE concentration was 951.96±710.93 IU Ig E/mL. There was a significant difference (P<0.05) in IgE concentrations between the two groups (Table 3).

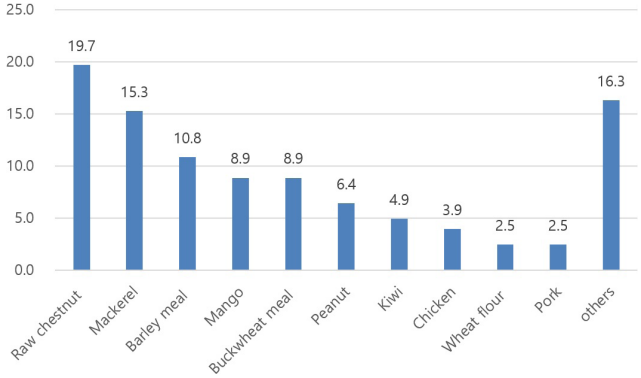

Fig. 2. Frequency of food allergens affecting canine atopic dermatitis.

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Table 3. Distribution of total Ig E concentrations by age group

Group	N	Ig E (IU Ig E/mL)	*P*
Age ≤3 years	44	1313.43±759.05	0.029
Age >3 years	38	951.96±710.93	
Total	82	1145.92±754.73	

DISCUSSION

This study was conducted on dogs in South Korea with allergic diseases such as atopic dermatitis and food allergies. The main objectives of the study were to determine which environmental and dietary allergens have the most significant impact using MAST assays. Additionally, the study aimed to analyze the differences in serum IgE concentrations between two groups based on age (aged ≤3 years/aged >3 years) using the ELISA method.

Among the environmental allergens, *Alternaria alternata* had the most significant impact. *Alternaria alternata* is a type of mold that, according to Martins (2022), is among the most commonly linked mold genera to skin conditions affecting both humans and animals. This is consistent with the results of this study. Japanese cedar is also an important allergen in humans and is a significant cause of atopic dermatitis in dogs (Masuda et al, 2000). Masuda et al. (2000) highlighted that Japanese cedar exposure in dogs leads to a higher occurrence of atopic dermatitis. Similarly, in this study, Japanese cedar exhibited the highest occurrence rate among plant-related allergens. *Tyrophagus putrescentiae* is a type of dust mite and one of the most common inhalant allergens causing allergies in humans. It is also the third most common mite found in Korean households (Choi et al, 2010; Jeong et al, 2012). The high prevalence of *Tyrophagus putrescentiae* in this study is consistent with previous research; however, Song et al. (2022) indicated that atopic dogs had a lower sensitization rate to *T. putrescentiae* allergens compared to *Dermatophagoides farinae*. Song et al. (2022) also showed that *D. farinae*, the predominant species, *D. pteronyssinus*, and *T. putrescentiae* are significant contributors to CAD in South Korea. In this study, however, *T. putrescentiae* showed a higher frequency than *D. farinae* and *D. pteronyssinus*. This difference could be attributed to the environments in which the dogs were kept. Thus, further studies in controlled environments are needed.

Among the dietary allergens, raw chestnut showed the highest prevalence rate. Chestnut is one of the major causes of food-induced allergic symptoms in humans (Bischoff and Crowe, 2005). However, there is little research on whether chestnut triggers allergic reactions in dogs; therefore, further studies are necessary to explore this aspect.

In Adam et al.'s study (2022), mackerel exhibited an occurrence rate of 25.6%. Although this is higher than the 15.3% observed in this study, this disparity in results could be attributed to environmental variations, such as the type of food the dogs were exposed to; accordingly, further research is needed to investigate this aspect with controlled dietary conditions. Helm et al.'s research (2003) showed that, similar to humans, barley also induced allergenic responses in atopic dogs. However, the occurrence rates of other cereal allergens, such as buckwheat and wheat flour, differ from the findings in this article. This discrepancy might be due to differences in the types of diets and treats fed to dogs in South Korea compared to other countries. Further studies are, therefore, necessary to explore this matter in more detail.

In this study, an analysis of average total IgE concentrations revealed that the group aged ≤3 years exhibited higher IgE concentrations compared to the group aged >3 years, with IgE concentrations significantly decreasing with age. Sousa and Marsella (2001) reported that IgE levels in young dogs with atopic dermatitis were not significantly different from those in a healthy control group. This study also confirmed that IgE levels and disease incidence rates do not necessarily correlate in dogs with atopy (Sousa and Marsella, 2001). In the research by Wilhem et al. (2010), the highest occurrence rate of atopic dermatitis was observed in dogs aged 2~3 years at 78.6%, with the rate significantly decreasing

to 7.4% in dogs aged 5 years and older. While few studies compare age-specific total IgE concentrations in dogs with atopic dermatitis, it has been noted that the rate of positive responses decreases with age in humans (Nam and Lee, 2017). Wollenberg et al.'s research (2021) explained that the response associated with the Th2-related environment and IgE antibodies remits spontaneously with age. Similar to humans, the results of this study may be attributed to comparable factors, necessitating further research in this area.

This study's limitations include the potential variations among the individual dogs stemming from diverse living environments, diets, including treats, and the relatively small sample size. Also, the subjects the subjects are composed of various breeds of dogs, and there could be other factors such as breed predisposition that can influence the disease. Furthermore, it should be noted that distinguishing between adverse food reactions and atopic dermatitis clinically is extremely challenging, so in this experiment the differentiation between two diseases was not achieved (Griffin, 2014). Nonetheless, the results of this study can still contribute to identifying the causes or antigens that trigger canine atopic dermatitis and inform treatment approaches in South Korea.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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