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Comparison of Intradermal Tests, Total Serum IgE Concentrations and Allergen-Specific IgE Using an Arrayed Protein Chip in Atopic Dogs

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Abstract: Canine atopic dermatitis is a common pruritic skin disease which is most commonly associated with Immunoglobulin (Ig) E immune response to environmental allergens. The aims of this study were to report comparison between total serum IgE concentrations in atopic and non-atopic dogs. Comparison between results of intradermal tests and serum allergen-specific IgE arrayed protein chips was also investigated. Total serum IgE concentrations between 37 atopic dogs (92.5±102.3 μg mL^{-1}) and 34 non-atopic dogs (16.2±30.2 μg mL^{-1}) were significantly different ($p<0.001$). A positive correlation between total serum IgE concentrations and corresponding modified canine atopic dermatitis extent and severity index scores was found ($r = 0.56$, $p<0.001$) in atopic dogs. The cut-off value was 16.8 μg mL^{-1} with the sensitivity of 91.9% and the specificity of 85.3%. The overall sensitivity of specific IgE serum tests was 24%, specificity was 95% and efficacy was 81%. Total serum IgE concentrations could be a screening test and determine severity of atopic dermatitis in dogs with discrete clinical evaluation. However, arrayed protein chip method might not be suitable for screening functional allergen-specific IgE.

Key words: Atopic dermatitis, arrayed protein chip, dog, IgE, serum, screening

INTRODUCTION

Canine atopic dermatitis is a genetically predisposed inflammatory skin disease which is most commonly associated with Immunoglobulin (Ig) E immune response to environmental allergens (DeBoer and Hillier, 2001b). This condition is characterized by pruritus, mainly affecting the ears, eyes, distal extremitiesand truck with a resultant of otitis externa, conjunctivitis and pododermatitis (Scott *et al*., 2001; Favrot *et al*., 2010).

Diagnoses of atopic diseases of dogs are strongly dependent on acquiring accurate medical histories and assessing the corresponding clinical signs including a thorough differential diagnostic process that rules out other (non-related) complications (Willemse, 1986; Prelaud *et al*., 1998; DeBoer and Hillier, 2001a; Hillier and DeBoer, 2001; Scott *et al*., 2001; Favrot *et al*., 2010). In addition, severity of clinical signs associated with atopic dermatitis in dogs can also be semi-quantitated using modified a Canine Atopic Dermatitis Extent and Severity Index (CADESI) (Olivry *et al*., 2007, 2008). Once the clinical diagnosis of atopic dermatitis has been made allergy testing can be performed to identify the offending allergens. This can be achieved using intradermal allergen tests, atopic patch tests or *in vitro* serologic allergy tests (Mueller *et al*., 1999; DeBoer and Hillier, 2001b; Foster *et al*., 2003; Olivry *et al*., 2005). Subsequent selecting offending allergens for immunotherapy is also based on a complete reliable allergen test (Park *et al*., 2000).

Increasing total serum IgE concentrations represents a major empiric risk factor for development of atopic disease and are used as a diagnostic indicator of atopic dermatitis in human patients (Berg and Johansson, 1969; Juhlin *et al*., 1969) whereas diagnostic value of IgE concentrations remains controversial in canine atopic dermatitis (Wilkie *et al*., 1990; Hill *et al*., 1995; DeBoer and Hill, 1999; Roque *et al*., 2011). The aims of this study were firstly to quantitate total serum IgE concentrations in atopic and non-atopic dogs; relationship between total serum IgE concentrations and a modified CADESI in atopic dogs was also evaluated. Secondly, correlation between Intra Dermal Tests (IDT) and allergen-specific IgE was also investigated.

MATERIALS AND METHODS

Animals: Seventy one dogs admitted to the Dermatological Clinic at the National Taiwan University Veterinary Hospital during 2008 to 2009 were enrolled for

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Table 1: The breeds and numbers of 37 atopic dogs and 34 non-atopic dogs represented in the study

Breeds	Atopic (n = 37)	Non-atopic (n = 34)	Total (n = 71)
Akita inu	0	2	2
Beagle	2	2	4
Bichon frise	1	0	1
Boston terrier	1	0	1
Chihuahua	2	3	5
English bulldog	2	0	2
Golden retriever	4	3	7
Husky	2	0	2
Labrador retriever	2	2	4
Maltese terrier	4	4	8
Miniature dachshund	3	1	4
Miniature schnauzer	2	3	5
Pomeranian	0	3	3
Pug	0	1	1
Shiba inu	2	0	2
Shih Tzu	1	1	2
Toy Poodle	1	3	4
Pembroke Welsh Corgi	1	0	1
West Highland terrier	2	0	2
Mix breed	5	5	10

this study; 37 dogs were categorized as atopic group (23 were males, 14 were females with a mean age of 5.2±3.3 years); 34 dogs that were free from any signs of skin problems were included as a group of non-atopic group (19 were males, 15 were females with a mean age of 8.6±4.2 years). Breeds represented in this study are shown in Table 1. All dogs in this study were regularly medicated with heartworm preventatives, ectoparasitic and endoparasitic preventatives monthly.

Inclusion criteria of atopic dermatitis: Inclusion criteria for atopic disease in this study included clinical signs based upon the fulfillment of Willemse's clinical criteria (Willemse, 1986). Flea hypersensitivity and ectoparasites infection were ruled out by means of standard diagnostic method. All atopic dogs were free from any forms of corticosteroidsand cyclosporine at least for 4 weeks. Fatty acid supplements and non-medicated shampoos or humectants were allowed.

A modified CADESI (Olivry *et al.*, 2007) was applied and scored in all dogs at the time of enrollment. In this modified version, three degrees of severity of clinical signs (erythema, excoriations, papules/pustules, lichenification, self-induced alopecia) was assessed of 40 sties on the body. In this modified version, the highest score theoretically was the maximum CADESI score was $40 \times 5 \times 3 = 600$.

Blood sample collection: Blood samples were taken by venipuncture and coagulated in upright polypropylene tubes in room temperature for 1 h. The samples were then centrifuged at 3000 g for 10 min. The serum was obtained and placed into an aliquotand then stored at -20°C until the time of assay.

Evaluation of total serum immunoglobulin E concentrations using Enzyme-Linked Immuno Sorbent Assay (ELISA): Total serum IgE was quantified using a commercial kit and according to manufacturer's instructions (Bethyl Laboratories, USA). Briefly, microplates were coated with 1 mg mL^{-1} goat anti-dog IgE purified antibody diluted in 100 mL carbonate-bicarbonate buffer at pH 9.6 and apply 100 μL to each well. Phosphate-Buffered Saline (PBS, pH 7.4) with 0.05% Tween 20 (PBS-Tween) containing 1% BSA was used as the blocking buffer and applied for 30 min at 37°C. Serum samples were diluted 1:1000 in blocking buffer and incubated for 1 h at room temperature.

Goat anti-dog IgE conjugated with horseradish peroxidase diluted 1:10000 was used as an indicator. After incubation for 1 h at room temperature, the reactions were visualized by the addition of the enzyme substrate tetramethyl benzidine for 30 min and apply 100 μL 2 M sodium di-hydrogen orthophosphate to stop the reaction, wavelength for tetramethyl benzidine should be 450 nm (Bio-Tek Instruments Inc., USA).

Allergen-specific IgE using arrayed protein chip: Allergens of *Dermatophagoides farinae, Dermatophagoides pteronyssinus*, human dander, cat epithelia, German cockroachand *Acacia* sp. were purified and spotted duplicately on each chip (Chu *et al.*, 2004), canine IgE and actin were also spotted on each chip as positive and negative controls, respectively. The allergen-arrayed proteins chips were immersed for 1 h in 80 μL of the canine serum. The allergen chips were then washed twice with PBS incubated with anti-canine IgE-Cy3 for 1 h were washed twice with PBS and then washed twice with double deionized water at the end of incubation. The reacted chips were scanned by a GenePix 4000B scanner (Axon Instrument Inc, California, USA) to detect fluorescence intensities generated by Cy3-conjugated anti-canine IgE.

Intradermal allergen tests: The majority dogs included in this study were small breeds and the skin surface for IDT was very limited, selection of allergens was based on the most common local allergens in atopic dogs: *D. farinae, D. pteronyssinus*, human dander, cat epithelia, German cockroach and *Acacia* sp. were included in this study. Results were evaluated after 15 min by assessing the diameter of wheal with a 4 point scale (0, 1+, 2+, 3+ and 4+). Reactions that were ≥2+ were considered positive.

Comparison between allergen-specific IgE and intradermal allergen tests: The allergens that were compared between the two tests were *D. farinae*, *D. pteronyssinus*, human dander, cat epithelia, German cockroach and *Acacia* sp.

Statistics: All statistical analysis was performed using the Statistical Software (Graph Pad Prism 4.0 for Windows, USA). Values of all data were expressed as mean± Standard Deviation (SD). The Mann Whitney test was used for comparison the effect of gender and the difference between total IgE concentrations in atopic and non-atopic dogs. The correlation between total IgE concentrations and age in both groups and CADESI scores in atopic dogs were evaluated by the Spearman's rank test. The reliability of total serum IgE concentrations in diagnosis of AD was assessed by examining the sensitivity and the specificity of the cut-off value. The results of allergen-specific IgE were compared to the results of IDT. All tests were designed as two-tailed test. The $p<0.05$ was considered statistically significant. Using the IDT as the standard, sensitivity, specificity and efficacy of specific-IgE detected by allergen protein chip were calculated according to standard formulas.

RESULTS AND DISCUSSION

Association between non-atopic dogs, dogs with atopic dermatitisand total serum IgE concentration: The most frequent lesions recorded in atopic dogs including various degree of pruritus and self-induced alopecia on periocular, perilabial, lateral pinna, metacarpal and tarsal areas.

The total serum IgE concentrations of non-atopic dogs (16.2±30.2 μg mL^{-1}) and atopic dogs (92.5±102.3 μg mL^{-1}) and were significantly different ($p<0.001$, Fig. 1). A significant correlation ($r = 0.56$, $p<0.001$) was found between the modified CADESI and total serum IgE concentrations in atopic dogs (Fig. 2).

The cut-off value was set at 16.8 μg mL^{-1} with the sensitivity of 91.9% and the specificity of 85.3%. The Receiver-Operating Characteristic (ROC) curve was represented in Fig. 3. The total serum IgE concentrations were not affected by gender in non-atopic dogs ($p = 0.19$) or in atopic dogs ($p = 0.28$). No correlation was found between age and total serum IgE concentrations in either non-atopic dogs ($r = 0.24$, $p = 0.18$) or in atopic dogs ($r = 0.01$, $p = 0.93$).

Comparison between Allergen-Arrayed Chips specific-IgE tests and results of IDST: The sensitivity, specificity and efficacy was varied in among tested allergens (Table 2). The overall sensitivity of allergen-arrayed chip was 24% (ranged 11-50%), specificity was 95% (ranged 89-100%) and efficacy was 81% (ranged 70-96%).

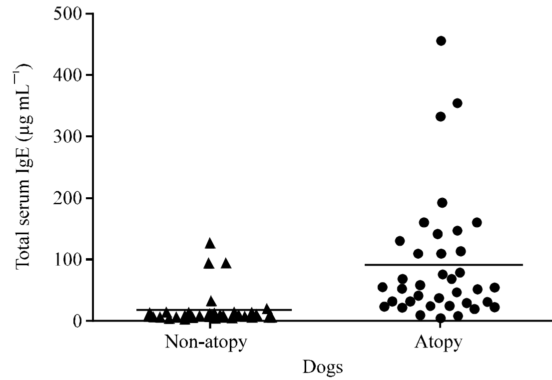

Fig. 1: Scatter plot of total serum IgE concentrations in 34 non-atopic and 37 atopic dogs, bars indicate mean

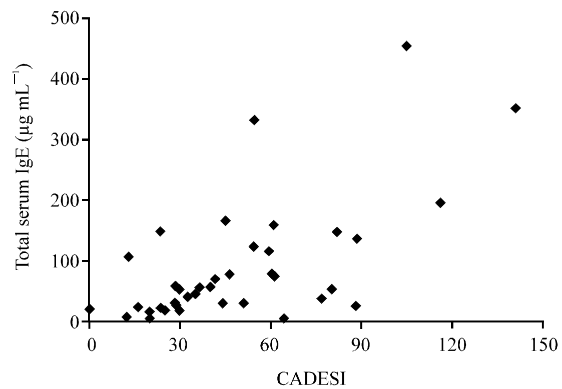

Fig. 2: Total serum IgE concentrations of atopic dogs in relation to their Canine Atopic Dermatitis Extent and Severity Index (CADESI, $r = 0.56$, $p<0.001$)

Table 2: Sensitivity, specificity and efficacy of allergen specific-IgE comparing to intradermal test

Allergen-specific IgE	Sensitivity (%)	Specificity (%)	Efficacy (%)
D. farinae	22	94	70
D. pteronyssinus	11	100	70
Human dander	33	100	93
Cat epithelia	50	100	96
German cockroach	25	96	85
Acacia sp.	29	89	70

Over these 2 decades, clinical value of total serum IgE concentrations in discrimination of atopic dermatitis in dogs remained controversial, range of concentrations was overlapped between atopic and non-atopic dogs (Halliwell and Kunkle, 1978; Hill and DeBoer, 1994;

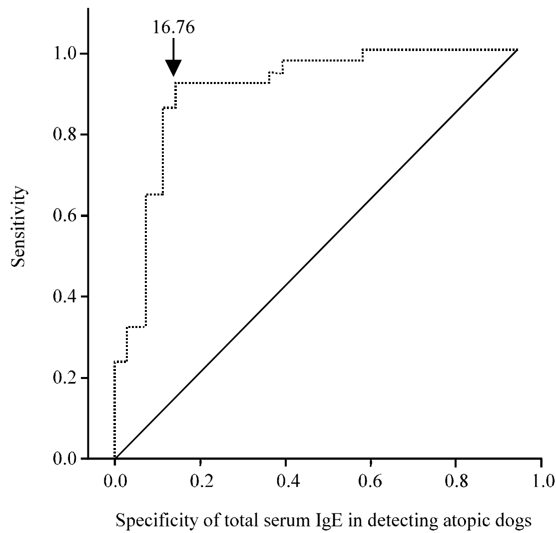

Fig. 3: Receiver-Operating Characteristic (ROC) curve. The cut-off value of total serum IgE concentrations between non-atopic and atopic dogs was set at 16.8 μg mL^{-1}

Hammerling and De Weck, 1998; Zunic, 1998; Park *et al*., 2000; Foster *et al*., 2003; Saevik *et al*., 2003; Olivry *et al*., 2005; Lee *et al*., 2009). Total serum IgE concentrations were affected by many factors such as breeds, age, sex and status of parasite infestation (Hill *et al*., 1995; DeBoer and Hill, 1999; Griot-Wenk *et al*., 1999; Racine *et al*., 1999; Fraser *et al*., 2003; Roque *et al*., 2011). However, relationship between total serum IgE concentrations and CADESI has not been reported in earlier studies.

In this study, total serum IgE concentrations were significantly different between atopic and non-atopic dogs. A positive correlation between total serum IgE concentrations and CADESI was also demonstrated. These findings indicated that total serum IgE concentrations may be applied as a screen test for atopic dermatitis in dogs. Together with discretely clinical evaluation, concentrations of total serum IgE may also determine the severity of atopic dermatitis.

The correlation between the allergen-specific IgE and intradermal allergen test was not satisfied in this study with low sensitivity, high specificity and moderate efficacy. These findings suggested that the method of arrayed protein chip was not detecting functional allergen-specific IgE. Similar results, a low sensitivity (ranged 19.3-100%) and a relatively high specificity (ranged 35-100%) were also reported in other studies using different methods such as Enzyme-Linked Immunosorbent Assay (ELISA), monoclonal antibody cocktail-based ELISA, high affinity-IgE receptor-based ELISA, Fc-epsilon receptor alpha-chain protein-based assayand immunodot assay, etc. (Hammerling and De Weck, 1998; Zunic, 1998; Mueller *et al*., 1999; DeBoer and Hillier, 2001b; Foster *et al*., 2003; Saevik *et al*., 2003). The source of allergens, allergen extraction process, reacting phase of allergens, IgE-specific detection reagentand signal molecule can also affect results of tests (Meyer *et al*., 1994; Patel *et al*., 1995; Hammerling and De Weck, 1998; Mari *et al*., 1999; Stedman *et al*., 2001; Olivry *et al*., 2005; Lee *et al*., 2009).

In the past, IDT has been the favored method for immediate demonstrating the presence of IgE for diagnosis of atopic dermatitis in dogs. Subsequent selection of offending allergens for immunotherapy is also completely based on the results of the test (Park *et al*., 2000). Nevertheless, availability of skin surface area in small breeds of dogs for number of tested allergensand lacking familiarity of techniques and procedures limits the clinical application of intradermal allergen test in general practices. Identification of offending allergens in small breed dogs or dogs with limited skin surface for IDT and subsequent immunotherapy based on reliable methods *in vitro* is highly expected. Improvement of arrayed protein chip in identification of offending allergens is warranted.

CONCLUSION

Total serum IgE concentrations could be a screening test and determine severity of atopic dermatitis in dogs with discrete clinical evaluation. However, Arrayed Protein Chip Method might not be suitable for screening functional allergen-specific IgE.

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