Original Article

Allergy-related Evidences in Relation to Serum IgE: Data from the China State Key Laboratory of Respiratory Disease, 2008-2013*

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Abstract

Objective To investigate the serum total IgE (tIgE) and specific IgE (sIgE) to common allergens among allergic patients in Guangzhou, China.

Methods 7 085 patients were examined for tIgE and sIgE to 15 allergens, based on the protocols of reversed enzyme allergosorbent test and the sandwich enzyme-linked immunosorbent assay.

Results 3 758 (53.04%) patients tested positive for tIgE, and 4 640 (65.49%) for sIgE. *Der pteronyssinus*, *Der farinae,* eggs, and cow's milk were the most common allergens leading to higher positive rates of sIgE responses. Several peaks of sensitization were: *Der pteronyssinus*, *Der farinae*, and *Blomia tropicalis* at age 10-12; cow's milk at age below 3; eggs at age 4-6. The mean level and positive rate of tIgE tended to increase in subjects sensitized to more allergens. Sensitization to *Der pteronyssinus* (OR, 1.6; *P*<0.05), *Der farinae* (OR, 1.5; *P*<0.05), *Blomia tropicalis* (OR, 1.4; *P*<0.05), *Blattella germanica* (OR, 1.5; *P*<0.05), cow's milk (OR, 1.3; *P*<0.05), and soy beans (OR, 2.0; *P*<0.05) were independently correlated with allergy-related conditions in preliminary diagnosis.

Conclusion The major allergens in Guangzhou include *Der pteronyssinus*, *Der farinae*, cow's milk, and eggs. Sensitization to these allergens appears to be predictors of allergy-related disorder.

Key words: Allergy; Allergen; Symptoms; Serum IgE

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INTRODUCTION

The increasing epidemic of allergic diseases over the past decades[1] has become a global health concern and a priority for prevention and management. While human allergies are believed to be underlain by intricate mechanisms which largely remain to be fully elucidated, the role of allergen exposure in the development of these conditions has been well defined[2]. However,

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distribution of allergens may vary across geographic areas[3] with different local climates[4], natural environments and lifestyles[5]. Besides, the pattern of sensitization to allergens may differ across age groups[6]. Therefore, local epidemiological data on allergens can be of great value to bolster evidence-based prevention against allergic diseases, especially in a vast populous territory like China. Unfortunately, such data are so far limited for this country.

While skin prick tests (SPT) are the first level of allergy diagnosis, reliability and interpretation of SPT rely on a variety of factors[7], such as the expertise of allergists, technical skills of the manipulators, recent use of anti-histamines and patient compliance (which is hard to achieve in young children). These factors render SPT not a suitable approach for a large-sample study on allergen sensitization. In contrast, measurement of serum total IgE (tIgE) and allergen specific IgE (sIgE) may stably provide standardized and quantitative results. We presented the serum profiles of tIgE and sIgE to 15 common allergens among patients with allergic symptoms during a 6-year observational study in China State Key Laboratory of Respiratory Disease, Guangzhou, southern China.

METHODS

Study Design and Population

Guangzhou is the third largest Chinese city and southern China's largest city, with an area of nearly 20 000 square kilometers. As of the latest census in 2010, the city had a population of 12.78 million. Geographically, Guangzhou spans from 112° 57' to 114° 03' E longitude and 22° 26' to 23° 56' N latitude. Located just south to the Tropic of Cancer, Guangzhou has a humid and warm subtropical climate influenced by the East Asian monsoon. The relative humidity in Guangzhou is about 68%, and the annual rainfall in the metropolitan area exceeds 1 700 mm.

The present study was a review on serum levels of tIgE and allergen sIgE in a large sample-size population in Guangzhou between January 2008 and December 2013. During the study period, 7 085 consecutive patients were referred by physicians from 51 Guangzhou hospitals for a simultaneous test of serum tIgE and sIgE against common allergens in our center, one of China state-certified key laboratories, for confirmatory diagnosis of allergic diseases. The need to undergo both tIgE and sIgE tests were decided by the attending physicians of these patients based on the patients' medical history and/or allergic symptoms at physical checkup which may or may not correlate with the chief complaints of their hospital visit. These allergic symptoms included rashes, wheals, hives, eczema, eye itching, stuffy or runny nose, sneezing, and gastrointestinal discomfort after consuming certain foods. For all the patients, we recorded the physician preliminary diagnosis and demographic data, and measured the serum tIgE as well as sIgEs against a panel of 15 allergens which were most commonly reported in previous local studies[8-9]. These 15 common allergens included 7 aeroallergens (*Der pteronyssinus*, *Der farinae*, *Blomia tropicalis*, dog hair, cat dander, *Blattella germanica*, and mosquito) and 8 food allergens (egg, cow's milk, wheat flour, codfish, peanut, soybean, crab, and shrimp). For each patient, 5 mL peripheral blood was collected by routine phlebotomy and centrifuged at 3 000 r/min for 10 min. The supernatants were decanted, labeled and stored at -20 degrees Celsius until use within the next 3 d.

For a clear pattern analysis of allergen distribution and sensitization, we divided the study population into 12 age groups: 0-3 years, 4-6 years, 7-9 years, 10-12 years, 13-15 years, 16-18 years, 19-21 years, 22-30 years, 31-40 years, 41-50 years, 51-60 years, and ≥61 years. Subjects aged below the lower limit of an age group were allocated in the preceding younger age group, such that an age of 3 years and several months old (<4 years old) belonged to the age group 0-3 years, and this was likewise for the other age groups. This study was approved by the Ethics Committee, First Affiliated Hospital of Guangzhou Medical University. Written informed consent was obtained from each adult subject and from the parents or legal guardians of the children who participated in the present study.

Laboratory Measurement Setting and Evaluation of Total and Specific IgE Levels

Serum levels of tIgE and sIgE were determined by using the ALLERG-O-LIQ System (Dr. Fooke Laboratorien GmbH, Neuss, Germany) in a fully automated micro-plate and slide processor (Ap22 Speedy, DAS, Rome, Italy) following the manufacturer's instructions. The sIgE test was based on the protocol of reversed enzyme allergosorbent test (REAST), and tIgE test on the protocol of sandwich enzyme-linked immunosorbent assay (ELISA). Calibrators with defined concentrations of IgE standardized according to the WHO reference preparation for IgE (WHO 75/702) were measured simultaneously with the test samples to generate a calibration curve. Concentrations of tIgE or sIgE in

serum samples were quantified by calculation from the calibration curve in international units per milliliter (IU/mL).

A positive tIgE test was defined when the level of tIgE was ≥20 IU/mL in subjects aged below 3 years old, or ≥35 IU/mL in subjects aged 3 to <6, or ≥50 IU/mL in subjects aged 6 to <20 years old, or ≥100 IU/mL in subjects aged 20 or older[10]. The levels of sIgE were classified quantitatively as[11-12]: class 0, <0.35 IU/mL; class 1, ≥0.35 to <0.7 IU/mL; class 2, ≥0.7 to <3.5 IU/mL; class 3, ≥3.5 to <17.5 IU/mL; class 4, ≥17.5 to <50 IU/mL; class 5, ≥50 to <100 IU/mL; and class 6, ≥100 IU/mL. A positive sIgE test was defined when the level of sIgE was ≥0.35 IU/mL (class 1 or above).

Statistical Analysis

Data were processed and analyzed with the IBM SPSS Statistics for Windows Version 22.0 (IBM Corp, Armonk, NY, USA). Chi-square test or Fisher's exact probability method was used to determine the between-group differences in positive rates of sIgE and tIgE tests according to age group. Multiple logistic regression analysis was performed to obtain the odds ratios (ORs) and 95% confidence intervals (CIs) for the independent effect of sensitization to each allergen on allergic disease, adjusting for the effect of sensitization to other allergens. *P*-values below 0.05 were considered statistically significant.

RESULTS

General Demographics of the Study Population

In the present study, 4 602 (65.0%) subjects were male and 2 483 (35.0%) female, with a mean age of 9.61±14.45 years (range: one month to 85 years old). The study subjects were patients who had visited clinics of respiratory medicine, pediatrics, dermatology or rhinology, and were referred to our laboratory for *in-vitro* tests by their attending physicians from 51 hospitals in Guangzhou. These hospitals were situated in all administrative districts of Guangzhou, including the urban, suburban and rural areas. The preliminary diagnoses included disorders that are highly probable of an allergic nature (allergy-related disorders, such as allergic rhinitis, bronchial asthma, and sensitive skin) in 4 264 (60.2%) subjects and those less likely to be allergic (non allergy-related disorders, such as pneumonia, upper airway infections, tonsillitis, and bronchitis) in 2 821 (39.8%) subjects.

Overall Prevalence of Serum tIgE and sIgE Reactivities to Aeroallergens and Food Allergens in the Study Population

Of the 7 085 subjects in this study, 3 758 (53.0%) tested positive for serum tIgE, and 4 640 (65.5%) tested positive for serum sIgE to at least one of the 15 allergens.

Among the 7 tested aeroallergens, *Der pteronyssinus* (34.6%) and *Der farinae* (33.7%) were the first two predominant contributors to positive sIgE tests, followed by *Blomia tropicalis* (9.4%), *Blattella germanica* (5.2%), dog hair (4.7%), cat dander (3.5%), and mosquito (3.2%). By classification of sIgE reactivity, >30% of positive sIgE tests for *Der pteronyssinus* or *Der farinae* were class 3 or above responses (corresponding to a sIgE level ≥3.5 IU/mL); in contrast, >85% of positive sIgE tests for any of the remaining 5 aeroallergens were low-class sIgE responses (classes 1-2, corresponding to a sIgE level ≥0.35 and <3.5 IU/mL) (Figure 1A).

Of the 8 food allergens in this study, cow's milk (36.1%) and egg (23.6%) were the two major ones leading to positive sIgE response, followed by wheat flour (4.9%), peanut (4.5%), shrimp (2.0%), crab (1.9%), soybean (1.6%), and codfish (0.9%). By classification of sIgE reactivity, >70% of positive sIgE tests for any of the tested food allergens were associated with low-class sIgE response, while the sIgE tests with high-class response (≥class 5, corresponding to a sIgE level ≥50 IU/mL) was most likely to be caused by crab and shrimp (Figure 1B).

Prevalence of Positive Serum tIgE and sIgE Response to Aeroallergens and Food Allergens According to Age Groups in the Study Population

Of the 7 085 patients, the positive rates of sIgE test were largely higher for house dust mites (*Der pteronyssinus*, *Der farinae* and *Blomia tropicalis*) than for the other 4 inhalant allergens in all age groups. In any given age group, the positive rates of sIgE to *Der pteronyssinus* and *Der farinae* were higher than that to *Blomia tropicalis*. Sensitization to house dust mites were more common in children aged 10-18 years old, and the prevalence of sensitization to these allergens all peaked in the age group 10-12 (*Der pteronyssinus*, 73.7%; *Der farinae* 73.7%; and *Blomia tropicalis*; 32.3%). Both *Blattella germanica* (18.6%) and mosquito (18.6%) had the peak prevalence of sensitization in the age group 16-18 years old. The positive rates of cat dander and

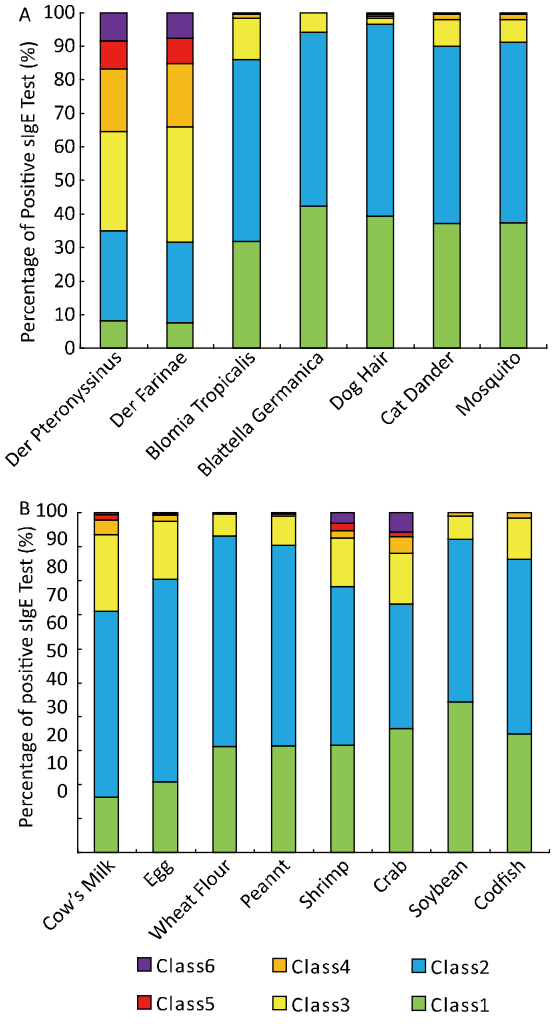

Figure 1. Positive sIgE test against 7 aeroallergens (A) and 8 food allergens (B) according to 6 classes of sIgE reactivity.

dog hair sIgEs were comparable and generally low in all age groups, with a common peak in the age group 19-21 years old (cat dander, 12.2%; dog hair, 6.1%) (Table 1).

For the 8 food allergens, the positive sIgE response tended to occur in children and teenagers below 18 years, and was less frequent along with older age (P<0.05). The positive rates of sIgE test were largely higher for cow's milk and eggs than for the other 6 food allergens in all age groups. A peak of sensitization to cow's milk (50.8%) was seen among young children aged 0-3 years, and to eggs (33.1%) in the group aged 4-6, supervened by increasing tolerance to the both later in life. The prevalence of sensitization to crab and shrimp was comparable and both peaked in the age group 10-12 (crab, 6.1%; shrimp, 5.7%). In all age groups, the positive rates of sIgE to wheat flour, codfish, peanut, soybean, crab and shrimp were <10% (Table 1).

Overall, the rates of positive tIgE test were higher in subjects aged ≤18 years than in those aged ≥19. Children aged 7-18 were more likely to be positive for serum tIgE, particularly for the age groups 10-12 (72.2%) and 13-15 (70.9%).

Correlation between Serum tIgE and sIgE according to Poly-sensitization in the Study Population

Of the 7 085 subjects in this study, 2 445 were sIgE-negative to all 15 allergens; among them, 759 (31.0%) were tIgE-positive with a mean level of 49.9±100.3 IU/mL. Interestingly, there was an increasing trend in the mean level of serum tIgE (from 63.7±109.2 to 347.6±355.8 IU/mL) and in positive rate of tIgE test (from 46.3% to 100.0%) in those who were sensitized to increasing number of allergens (from 1 to >10) (Table 2).

A total of 724 subjects were highly positive for serum tIgE (tIgE >300 IU/mL), accounting for 10.2% of the study population and nearly one-fifth (19.3%) of all patients with positive tIgE. In this subgroup, only 55 (0.76%) patients were negative for sIgE to any of the 15 allergens; the remaining 669 patients showed varied rates of positive sIgE depending on individual allergens. Still, *Der pteronyssinus* (82.2%), *Der farinae* (80.9%) and *Blomia tropicalis* (29.6%) contributed to the first three highest rates of sIgE positivity to aeroallergens, as did the cow's milk (30.8%) and eggs (24.2%) to the first two to food allergens. For the remaining allergens, the rates of positive sIgE tests were <13% (5.5%-12.4%) for aeroallergens and <8% (1.2%-7.7%) for food allergens (Table 3).

Sensitization to Certain Allergens as Risk Factors Associated with the Preliminary Diagnoses being Allergy-related Disorders in the Study Population

As described above, there were 4 264 patients in our study population for whom their physicians' preliminary diagnoses included allergic rhinitis, bronchial asthma, and sensitive skin which appeared to be allergy-related disorders, in contrast to the other 2 821 subjects whose preliminary diagnoses were non allergy-related disorders such as pneumonia, upper airway infections, tonsillitis, and bronchitis. Conceivably, not all of these 4 264 subjects diagnosed with allergy-related disorders were sIgE-positive to each of the 15 common allergens tested in this study (Table 4).

Table 1. Positive Rates of Aeroallergens and Food Allergens in Patients Stratified by Age Groups

Allergens	Number of Positive Patients (%)												
	0-3 y	4-6 y	7-9 y	10-12 y	13-15 y	16-18 y	19-21 y	22-30 y	31-40 y	41-50 y	51-60 y	≥61 y	Total
Number of Patients	2761	2244	603	297	110	43	49	203	270	238	166	101	7085
Aeroallergens													
Der pteronyssinus	437(15.8)	971(43.3)	385(63.8)	219(73.7)*	81(73.6)*	29(67.4)*	25(51.0)	88(43.3)	92(34.1)	79(33.2)	28(16.9)	19(18.8)	2453(34.6)
Der farinae	378(13.7)	979(43.6)	385(63.8)	219(73.7)*	79(71.8)*	27(62.8)*	25(51.0)	86(42.4)	84(31.1)	78(32.8)	31(18.7)	18(17.8)	2389(33.7)
Blomia tropicalis	83(3.0)	185(8.2)	95(15.8)	96(32.3)*	32(29.1)*	13(30.2)*	14(28.6)*	52(25.6)*	45(16.7)	34(14.3)	11(6.6)	9(8.9)	669(9.4)
Blattella germanica	101(3.7)	120(5.3)	36(6.0)	26(8.8)	6(5.5)	8(18.6)*	3(6.1)	22(10.8)	23(8.5)	16(6.7)	6(3.6)	3(3.0)	370(5.2)
Dog hair	167(6.0)*	118(5.3)*	14(2.3)	9(3.0)	6(5.5)*	2(4.7)*	3(6.1)*	3(1.5)	4(1.5)	8(3.4)	0(0.0)	0(0.0)	334(4.7)
Cat dander	63(2.3)	84(3.7)	28(4.6)	25(8.4)	12(10.9)	0(0.0)	6(12.2)*	8(3.9)	10(3.7)	12(5.0)	0(0.0)	2(2.0)	250(3.5)
Mosquito	33(1.2)	64(2.9)	21(3.5)	24(8.1)	8(7.3)	8(18.6)*	4(8.2)	29(14.3)	15(5.6)	15(6.3)	5(3.0)	2(2.0)	228(3.2)
Food allergens													
Cow's milk	1402(50.8)*	901(40.2)	136(22.6)	51(17.2)	14(12.7)	6(14.0)	5(10.2)	11(5.4)	11(4.1)	10(4.2)	11(6.6)	3(3.0)	2561(36.1)
Egg	731(26.5)	771(33.4)*	117(19.4)	27(9.1)	8(7.3)	4(9.3)	1(2.0)	2(1.0)	4(1.5)	2(0.8)	2(1.2)	1(1.0)	1670(23.6)
Wheat flour	116(4.2)	174(7.8)	29(4.8)	11(3.7)	4(3.6)	4(9.3)*	0(0.0)	2(1.0)	2(0.7)	3(1.3)	1(0.6)	2(2.0)	348(4.9)
Peanut	100(3.6)	137(6.1)*	31(5.1)	9(3.0)	5(4.5)	1(2.3)	3(6.1)*	6(3.0)	16(5.9)*	5(2.1)	6(3.6)	2(2.0)	321(4.5)
Crab	23(0.8)	53(2.4)	16(2.7)	18(6.1)*	4(3.6)	0(0.0)	1(2.0)	1(0.5)	5(1.9)	8(3.4)	3(1.8)	1(1.0)	133(1.9)
Shrimp	32(1.2)	54(2.4)	19(3.2)	17(5.7)*	4(3.6)	2(4.7)	1(2.0)	0(0.0)	4(1.5)	7(2.9)	3(1.8)	0(0.0)	143(2.0)
Soybean	57(2.1)	39(1.7)	6(1.0)	2(0.7)	0(0.0)	0(0.0)	0(0.0)	2(1.0)	2(0.7)	3(1.3)	2(1.2)	0(0.0)	113(1.6)
Codfish	31(1.1)*	30(1.3)*	0(0.0)	1(0.3)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	3(1.3)	0(0.0)	0(0.0)	66(0.9)
TIgE	1534(55.6)	1230(54.8)	389(64.5)*	216(72.2)*	78(70.9)*	25(58.1)*	24(49.0)	66(32.5)	66(24.4)	67(28.2)	34(20.5)	29(28.7)	3758(53.0)

Note. Two-sided *P*-values: chi-square test or Fisher's exact probability method for specific IgE positive rates to allergens among 12 age groups. *$P<0.05$.

Table 2. Correlation between Serum tIgE and sIgE Stratified by Number of Allergens Detectable in a Subject

Number of Detectable Allergens	Subjects Sensitized to Allergens [n (%)]*		Sensitized Subjects with Positive tIgE [n (%)]**		Mean Level of Serum tIgE (IU/mL)
0	2445	(34.5)	759	(31.0)	49.9±100.3
1	1263	(17.8)	585	(46.3)	63.7±109.2
2	1435	(20.3)	922	(64.3)	145.6±193.6
3	872	(12.3)	633	(72.6)	186.6±219.4
4	539	(7.6)	428	(79.4)	204.1±223.3
5	273	(3.9)	221	(81.0)	224.0±215.2
6	144	(2.0)	108	(75.0)	242.6±312.9
7	59	(0.8)	54	(91.5)	264.59±225.9
8	30	(0.4)	24	(80.0)	281.5±272.5
9	14	(0.2)	13	(92.9)	242.7±194.2
10	4	(0.1)	4	(100.0)	340.7±337.6
>10	7	(0.1)	7	(100.0)	347.6±355.8
Total	7085	(65.49)	3758	(53.04)	115.1±179.83

Note. *Calculated as the percentage of the study population; **Calculated as the percentage of subjects sensitized to allergens.

Table 3. SIgE Positivity to Allergens among 724 Subjects with a High Level of serum tIgE (>300 IU/mL) in this Study

Allergens	Positive sIgE to Allergens	
	Number of Subjects	%
Der pteronyssinus	595	82.2
Der farinae	586	80.9
Blomia tropicalis	214	29.6
Blattella germanica	90	12.4
Dog hair	40	5.5
Cat dander	51	7.0
Mosquito	72	9.9
Cow's milk	223	30.8
Egg	175	24.2
Wheat flour	56	7.7
Peanut	44	6.1
Shrimp	45	6.2
Crab	49	6.8
Soybean	20	2.8
Codfish	9	1.2

We examined whether sensitization to certain allergens could predict the probable allergic nature of physicians' preliminary diagnosis in the subjects. As shown by the results of Logistic regression analysis, sensitization to *Der pteronyssinus* [odds ratio (OR), 1.6; 95% confidence interval (CI), 1.3-1.9; $P<0.05$], *Der farinae* (OR, 1.5; 95% CI, 1.2-1.8; $P<0.05$), *Blomia tropicalis* (OR, 1.4; 95% CI, 1.1-1.7; $P<0.05$) and *Blattella germanica* (OR, 1.5; 95% CI, 1.2-2.0; $P<0.05$) in the aeroallergens, and to cow's milk (OR, 1.3; 95% CI, 1.1-1.4; $P<0.05$) and soy beans (OR, 2.0; 95% CI, 1.2-3.3; $P<0.05$) in the food allergens, were independent risk factors associated with probable allergic conditions as a physician's preliminary diagnosis in the study population, adjusting for the effect of sensitization to other allergens. A combination of sIgE response against these six allergens could predict a preliminary diagnosis being allergy-related disorder in nearly 98% of the patients.

DISCUSSION

Exposure and sensitization to allergens, especially in the early life[13], has been recognized as a risk factor for allergic diseases which are increasingly prevalent in the world during the recent years[14]. Because the spectrum of these sensitizing allergens may vary with geographical regions[3] and age groups[6], local data regarding these aspects should thus be indispensable for supporting evidence-based prevention and management of allergies, particularly in China, a vast territory in the

Table 4. Correlation between Allergen Sensitization and Physician's Preliminary Diagnosis being an Allergy-related Disease by Logistic Regression Analysis in 4 264 Subjects

Allergen sIgE Test	Patients Preliminarily Diagnosed with Allergy-related Disorders			
	Number of patients	Predictive rate (%)[‡]	Odds Ratio[†]	95% CI
Der pteronyssinus				
Negative	2 456	53.0	1.0	
Positive	1 808	73.7	1.6	1.3-1.9[*]
Der farinae				
Negative	2 503	53.3	1.0	
Positive	1 761	73.7	1.5	1.2-1.8[*]
Blomia tropicalis				
Negative	3 739	58.3	1.0	
Positive	525	78.5	1.4	1.1-1.7[*]
Blattella germanica				
Negative	1 979	29.5	1.0	
Positive	285	77.0	1.5	1.2-2.0[*]
Dog hair				
Negative	4 055	60.1	1.0	
Positive	209	62.6	0.7	0.6-1.0
Cat dander				
Negative	4 079	59.7	1.0	
Positive	184	73.6	1.3	0.9-1.8
Mosquito				
Negative	4 095	59.7	1.0	
Positive	169	74.1	0.9	0.7-1.2
Cow's milk				
Negative	2 629	58.1	1.0	
Positive	1 635	63.8	1.3	1.1-1.4[*]
Egg				
Negative	3 176	58.7	1.0	
Positive	1 088	65.1	1.0	0.9-1.2
Wheat flour				
Negative	4 027	59.8	1.0	
Positive	237	68.5	1.0	0.7-1.3
Peanut				
Negative	4 037	59.7	1.0	
Positive	227	70.7	1.2	0.9-1.5
Crab				
Negative	4 161	59.9	1.0	
Positive	103	77.4	0.9	0.5-1.6
Shrimp				
Negative	4 152	59.9	1.0	
Positive	112	78.9	1.5	0.8-2.5
Soybean				
Negative	4 173	59.9	1.0	
Positive	91	80.5	2.0	1.2-3.3[*]
Codfish				
Negative	4 214	60.0	1.0	
Positive	50	75.8	1.4	0.7-2.5

Note. [*]$P<0.05$; [†]Adjusted for the effect of sensitization to other allergens; [‡]Positive predictive rate is calculated as (number of sensitized patients preliminarily diagnosed with allergy-related disease)/(total number of patients tested positive for a given allergen)×100%; negative predictive rate is calculated as (number of non-sensitized patients preliminarily diagnosed with allergy-related disease)/[7085-(total number of patients tested positive for a given allergen)] ×100%; 95% CI = 95% confidential interval.

eastern hemisphere with a wide variation in natural environment, local climate and regional culture. The present study was the first attempt during the recent years to investigate the sensitization to common allergens by simultaneous use of REAST and sandwich ELISA protocols for *in-vitro* detection of serum sIgE and tIgE in Guangzhou, the largest city in southern China. The study population consisted of 7 085 subjects from all parts of this city, thereby showing a wide geographical coverage of the region.

Given the large sample size and long-term observation in our study, ALLERG-O-LIQ was employed for detection of the serum tIgE and sIgE, which was decided based on a balance between the cost and outcome of the testing methods. The consistency of ALLERG-O-LIQ with the most commonly used method for allergy diagnosis, ImmunoCAP, in quantitative or semi-quantitative detection of various allergens has been verified by several well-designed studies[15-17], although correlation between the two was closer for inhalant than for food allergens.

Of the 15 common allergens tested, *Der pteronyssinus* (34.6%) and *Der farinae* (33.7%) were the most common among aeroallergens, as were cow's milk (36.1%) and eggs (23.6%) among food allergens, contributing to higher rates of sIgE positivity in the study population. Except for *Der pteronyssinus* and *Der farinae*, over 70% of the positive sIgE tests to any of the remaining allergens showed low-class (class 1 or 2) responses. Given that 60.2% of all subjects were preliminarily diagnosed by physicians with disorders highly probable to be of an allergic nature (allergy-related diseases) before referral to our laboratory for sIgE measurement, we speculated that the diagnosis of allergic disease in a subject should not be ruled out simply according to a low-class sIgE response, but should be considered in the context of clinical presentations.

The levels of serum tIgE per se are deemed less clinically meaningful than sIgE data[18-19] because they may overlap between non-atopic and atopic subjects or between different allergic diseases, and may also be affected by genetic and environmental factors[18]. However, nearly 1 in 2 (53.0%) of the subjects in our study were positive for serum tIgE, and the level of tIgE was apt to increase along with positive sIgE against more allergens detected in these subjects. These findings, together with the results of sIgE reactivity in our study, suggested that a higher level of tIgE may be predictive of poly-sensitization to several allergens despite of the low sIgE levels in the serum. Moreover, in a subgroup of 724 subjects (19.3% of all tIgE-positive patients) with a high level of tIgE (>300 IU/mL), the prevalence of sensitization to five allergens (*Der pteronyssinus*, *Der farinae*, *Blomia tropicalis,* cow's milk, and eggs) was significantly higher than those to the other ones. Salo and colleagues[20] clarified the role of sIgEs in relation to total IgE by modeling them together. They found that increases in total IgE had a small but independent effect on the primary allergic outcomes after adjusting for most of the specific IgEs and IgE clusters. These observations may have important clinical implications in that, although total IgE levels alone may be diagnostically less informative than sIgEs, simultaneous measurement of serum tIgE and sIgE can be more useful for allergy diagnosis, especially if sIgEs alone cannot be accounted for in the study.

Der pteronyssinus, *Der farinae*, and *Blomia tropicalis* have been shown to be closely associated with human allergies, among all species of mites[21-22]. In Guangzhou, a subtropical southern China city with humid and warm environments, mites or mite allergens have been reported to be widely present, both outdoors and indoors[23]. Compared with northern China cities, the special geographical features of Guangzhou may have promoted optimal growth of house dust mites; and the much more days in a year when daily temperature necessitates indoor air-conditioning may have facilitated regular exposure to mite allergens. Using skin prick test, Wong[24] revealed that atopy and allergic sensitization to *Der pteronyssinus* s and *Der farinae* were more common in Guangzhou than in Beijing, and that the differences in the prevalence of atopy and allergic sensitization could not be explained by the differences in the age and sex distribution of their study populations. In our tests for aeroallergens, higher positive rates were noted for *Der pteronyssinus* and *Der farinae*, followed by *Blomia tropicalis*. The prevalence of sensitization to *Der pteronyssinus Der farinae*, or *Blomia tropicalis* was highest in the age group 10-12. This echoed the data from a large-sample Japanese study on sIgE reactivities to common allergens, which demonstrated high positive rates to indoor house dusts and mites in the study population and a peak of sensitization in children aged 10[25].

In a study of 85 asthmatic children who underwent skin prick tests in Atlanta of the United States, 48% of the subjects were diagnosed as allergy to cockroach, suggesting that cockroach was also a

major indoor allergens immediately following dust mites[26]. While this was true according to the ranking of positive rates in the present study, only 5.2% of patients tested positive to *Blattella germanica*. Such a low prevalence of sensitization may be explained by the fact that nearly 85% of the subjects in the present study aged below 16 while the peak for sensitization to *Blattella germanica* was identified at the age group 16-18.

Of the 8 common food allergens in this study, cow's milk and eggs were associated with first two highest rates of positive sIgE responses. Cow's milk protein allergy represents one of the leading causes of food allergy in infants and young children. The immune reaction may be IgE mediated, non-IgE mediated, or mixed. IgE-mediated cow's milk protein allergy is revealed by immediate and acute symptoms which can be severe. The immature digestive and immune functions in the newborns and young children, hence the vulnerability of their guts to food allergens, may have accounted for the high positive rate of sIgE response to cow's milk which is usually among the earliest foods given to babies. The present study indicated a peak of sensitization to cow's milk among young children aged 3 years or below, and another peak to eggs at the age group 4-6 years, followed thereafter by decreasing rate of positive sIgE tests to these food allergens along with older age. These findings reflected a tendency of diminishing food allergy as children grow up, which was consistent with the studies by García-Ara[27] and Payot[28], where 68% and 75.8%, respectively, of the infants grew tolerant to certain food allergen. The sooner tolerance is detected, the earlier the substitute diets can be suspended and the quicker family emotional hardship is alleviated. Since sensitization to cow's milk and eggs occur mostly during early years of life, infants and young children should be monitored closely for food allergy in order to avoid these allergens. Furthermore, monitorization of specific IgE concentration for cow's milk and eggs in sensitized children would enable us to predict, to a high degree of probability, the clinical reactivity.

In our study, the highly prevalent sensitization to cow's milk and eggs during early years of life was preceded by increasing sensitization to *Der pteronyssinus* and *Der farinae* at older age groups. Although this was noted from a cross-sectional observation on age-group-specific sensitization rather than from a longitudinal study in individuals, a tendency of major sensitizing allergens switching from certain foods to aeroallergens (especially the house dust mites) along with older age groups could nevertheless be obvious. Such a sequence of events may be a result of allergy march[29]. Knowledge on the prevalence of allergen sensitization in different age groups would help early diagnosis and intervention of allergies.

Relationship between allergen sensitization and the development of allergy-related disorder is intricate. Although atopy is generally believed to be a major risk factor for allergic diseases, how much atopy accounts for the allergies remains inconclusive. Simple IgE-mediated disorders, such as hay fever, are clearly ascribable to pollen sensitization. However, the scenario in certain disorders, such as asthma and perennial rhinitis, may be more complicated. Serum total IgE as an important marker for allergic airway diseases[30] has been questioned. On the other hand, normal levels of total IgE are frequently recorded in subjects with allergic symptoms. Even in allergic eczema, non-IgE-mediated inflammatory mechanisms may be a key player. Using positive sIgE test as a predictor, we examined whether sensitization to certain allergens could be more correlated than others with the physicians' preliminary diagnosis as an allergy-related disorder. As shown by Logistic regression analysis, sensitization to *Der pteronyssinus*, *Der farinae*, *Blomia tropicalis*, *Blattella germanica*, cow's milk, and soy beans was independently associated with physician's preliminary diagnosis being a probable allergic disorder in the study population. In a whole population birth cohort study[31], Arshad and colleagues found that 50% of children sensitized to house dust mite had asthma, and 68.4% of children sensitized to house dust mite had asthma, eczema, and/or rhinitis, whereas the family history of allergic disease and environmental risk factors were comparable between sensitized and un-sensitized children. Lau et al.[32] noted a robust correlation between dust mite sensitization and respiratory symptoms during the early childhood. Several other studies also confirmed that sensitization to house dust mites is a strong independent risk factor for asthma[33-35]. Our data showed that sensitization to various inhalant and food allergens has a significant albeit differential effect on the preliminary diagnosis of allergy-related diseases in all subjects. A combination of sIgE responses against these six allergens could preliminarily predict allergy-related disorders in nearly 98% of the patients. This can be a useful guide in screening patients for atopy in

Guangzhou, although further studies are required to validate our findings and etiological analyses are warranted to reveal the possible determinants for allergic diseases in Chinese.

In conclusion, the present study indicated that *Der pteronyssinus*, *Der farinae*, cow's milk and eggs were the major allergens responsible for allergic diseases in Guangzhou, southern China. Sensitization to these allergens appears to be risk factor predictive of allergy-related disorder. Along with older age, there is a switch in the major sensitizing allergens from certain foods to house dust mites, which may be a result of allergy march. Peaks of sensitization to individual allergens in different age groups suggest the need for close monitoring to facilitate early diagnosis and intervention of allergic diseases in this large geographical region.

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CONFLICTS OF INTEREST

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