Analysis of total immunoglobulin E and specific immunoglobulin E of 3,721 patients with allergic disease

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Abstract. Due to the increase in the prevalence and incidence of allergic diseases, improving the sensitivity and specificity of screening indexes is critical. Total immunoglobulin E (IgE) is a traditional index for judging allergic diseases, while its specificity is relatively poor. Serum-specific IgE (sIgE) is an objective index with high specificity in the diagnosis of allergic diseases. In the present research, the total IgE and sIgE of 3,721 patients with allergic diseases were analyzed to further illuminate the association between them. The data were derived from 3,721 patients. The serum-sIgE to 14 types of common allergens and total IgE were detected. A total of 2,419 cases (65.0%) of 3,721 patients exhibited increasing total IgE and 1,215 patients (32.7%) exhibited positive sIgE. The consistency rate of the two indexes was 60.4%, and the κ -value was 0.28. In 135 patients with normal total IgE, 82.2% exhibited one sIgE positive and 17.8% exhibited two or more sIgE positive. While the number of positive sIgE increased and the detecting level enhanced, the number of positive total IgE markedly increased. Patients (84.1%) with increasing total IgE were associated with positive sIgE, but the increase of total IgE could not be completely explained by the total accumulation of sIgE. Total IgE may play an important role on screening allergic disease while sIgE could be used as crucial evidence for allergy diagnosis. Although the consistency of the two methods was poor, neither total IgE nor sIgE could

replace each other. Combining the two indexes with clinical manifestations together will improve the method.

Introduction

The prevalence of allergic disease has increased in recent years worldwide. The data from the International Study of Asthma and Allergies in Childhood showed that the incidence of asthma in children increased greatly in Europe and Asia (1-3). In America, the incidence of asthma is much higher than that in other countries and it is still increasing (4). The prevalence data from the America Centers for Disease Control showed that >24.6 million people suffered from asthma until 2009, which was nearly twice that in 1993 (5,6).

In addition, the incidence of another allergic disease, anaphylactic rhinitis, also increased greatly. The incidence in Asia was 10-30% from the investigation performed in 11 countries (7). In America, >60 million people suffered from allergic rhinitis with direct and indirect medical costs of 1.5-2.0 billion each year (8). Despite the high expense, the life quality of the patients was affected (9).

In the process of prevention and treatment of allergic diseases, the majority of important steps involve determining the allergens and subsequently avoiding contact or ingestion of them in the environment. Traditionally, total immunoglobulin E (IgE) was used to determine allergic disease, but the increased total IgE was not only associated with allergy, but also with parasitic, as IgE is a type of multiple myeloma, and other diseases. Serum-specific IgE (sIgE) is an objective index with high specificity in the diagnosis of allergic diseases; however, its clinical positive rate was relatively low. In order to analyse the diagnostic value of total IgE and sIgE in northeast Chinese patients and to illuminate the association between them, the present study was followed.

Subjects and methods

Subjects. The study was approved by the Ethics Committee of The Second Affiliated Hospital of Harbin Medical University (Harbin, Heilongjiang, China). Harbin is the capital city of Heilongjiang province in the northeast of China at longitude 125°42'-130°10'E and latitude 44°04'-46°40'N and its annual average temperature is ~38.48°F (3.60°C).

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A total of 5,473 patients with full data were enrolled in the study [Chang *et al* (10)]. Among them, 3,721 cases with clinical suspicion of respiratory and/or food allergies were tested for 14 types of common allergens and their data were analyzed in detail as described below.

Laboratory assay. sIgE and total IgE were assayed by the AllergyScreen® test (Mediawiss Analytic GmbH, Moers, Germany) according to the manufacturer's instructions (11). All the patients (n=3,721) were tested using 14 allergens composed of 7 types of common aeroallergens and 7 types of food allergens. Among them, the aeroallergens included a house dust mite mixture (*Dermatophagoides farinae* and *Dermatophagoides Pteronyssinus*), short ragweed estragon, cat epithelium/dog epithelium, cockroach, mold mixture (*Penicillium notatum*, branch spore mildew, *Aspergillus fumigates* and *Alternaria*), trees (mixture of cypress, elm, phoenix tree, willow and cottonwood) and grass. The food allergens included cow milk, beef-mutton, cashew-peanut-soybean, mango, wheat, egg white/yolk and fish-prawn-crab. According to the results from the AllergyScreen® system, sIgE was divided into 7 levels: Grades 0 (<0.35 IU/ml), 1 (0.35-0.70 IU/ml), 2 (0.70-3.50 IU/ml), 3 (3.5-17.5 IU/ml), 4 (17.5-50 IU/ml), 5 (50-100 IU/ml) and 6 (>100 IU/ml). An sIgE level >0.35 IU/ml was considered to be positive and the significant reference range of serum total IgE was defined as >100 IU/ml.

Statistical analysis. Data were categorized and analyzed using the IBM SPSS Statistics version 19 (IBM, Corp., Armonk, NY, USA). For categorical values, the χ^2 test or Fisher's exact probability method was used. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics. A total of 3,721 patients aged 0-86 years were divided into 8 groups according to age (Table I). Female patients accounted for 54.1% (n=2,013). The majority of patients (54.9%) were from the Department of Dermatology, followed by Pediatrics (28.6%) (Table II). Clinical diagnosis mainly included allergic rhinitis, allergic asthma, allergic gastroenteritis, urticaria, atopic dermatitis, angioedema and drug anaphylactic shock.

Total IgE and positive sIgE. In 3,721 patients, 2,419 cases (65.0%) exhibited increased total IgE and 1,215 cases (32.7%) exhibited positive sIgE; 1,080 patients (29.0%) exhibited increased total IgE and positive sIgE (Table III). When the test results of sIgE were set as gold standard, the sensitivity of total IgE was 88.9%, specificity was 46.6% and false-positive rate was 53.4%. The consistency rate of the two methods was 60.4% and the κ -value was 0.28.

A total of 135 patients with normal total IgE had positive sIgE and among them 111 cases had 1 positive sIgE allergen, accounting for 82.2%; 16 cases had 2 positive sIgE allergens, accounting for 11.9%; and 8 cases had >2 positive sIgE allergens, accounting for 5.9%. Three patients had 7 positive sIgE allergens (Table IV).

A total of 14 types of allergen sIgE of 135 patients with normal total IgE were calculated and the results were separated

Table I. Gender and age groups for the 3,721 patients.

Age groups, years	Male, no.	Female, no.	Total, no. (%)
0-3	262	154	416 (11.2)
4-6	291	185	476 (12.8)
7-12	287	210	497 (13.4)
13-19	160	144	304 (8.2)
20-29	116	241	357 (9.6)
30-39	167	338	505 (13.6)
40-49	193	419	612 (16.4)
>50	232	322	554 (14.9)
Total	1,708	2,013	3,721 (100.0)

Table II. Distribution of the 3,721 patients in the different hospital departments.

Department	Patients, no. (%)
Skin Venereal Service	2,041 (54.9)
Department of Pediatrics	1,066 (28.6)
Respiratory Medicine	162 (4.4)
Internal Medicine	94 (2.5)
Physical Examination Center	78 (2.1)
Department of Surgical	71 (1.9)
Department of Gerontology	37 (1.0)
Ear, nose and throat	20 (0.5)
Other departments	152 (4.1)
Total	3,721 (100.0)

Table III. Total IgE and sIgE comparison in the 3,721 patients.

Total IgE	sIgE		Total
	Positive	Negative	
Positive	1,080	1,339	2,419
Negative	135	1,167	1,302
Total	1,215	2,506	3,721

$\chi^2=983.4$, $P < 0.05$. IgE, immunoglobulin E; sIgE, specific IgE.

according to 0.35-0.70, 0.70-3.50, 3.50-17.50, 17.50-50.00 and 50.00-100.00 IU/ml, respectively (Table IV). When positive sIgE was only 1, the level of the sIgE of 78 patients (70.3%) was within the scope of 0.35-0.70 IU/ml and 24 patients (21.6%) were within the scope of 0.70-3.50 IU/ml (Table IV). When the positive sIgE number was 2, the value of the sIgE was mostly within the scope of 0.70-3.50 IU/ml. A total of 14 patients accounted for 87.5%. Whether the total IgE was positive or not had no association with the number of positive sIgE or the sIgE level. However, as the number of positive sIgE increased and the detecting level enhanced, the number of positive total IgE increased significantly ($P < 0.001$) (Table IV).

Table IV. Comparison within the different scopes of sIgE and different numbers of positive sIgE in 135 patients with normal IgE levels.

No. of positive sIgE	No. of patients within different scopes of sIgE, IU/ml					Total, IU/ml ^a
	0.35-0.70	0.70-3.50	3.50-17.50	17.50-50.00	50.00-100.00	
1	78	24	8	1	1	111
2	0	14	2	0	0	16
>2	0	2	2	3	1	8
Total	78	40	12	4	2	135

^aTwo-sided P-values. Fisher's exact probability method for the patients in different scopes of sIgE and different number of positive sIgE, P<0.001. IgE, immunoglobulin E; sIgE, specific IgE.

Table V. Comparison of total IgE of 1,215 patients with different levels of sIgE.

Allergens	Patients with grade 1 sIgE ^a			Patients with grade 2 sIgE ^a			Patients with grade 3 sIgE		
	Normal IgE	Positive IgE	Total	Normal IgE	Positive IgE	Total	Normal IgE	Positive IgE	Total
House dust mite mix	27	106	133	1	204	205	2	96	98
Short ragweed estragon	10	35	45	5	88	93	6	59	65
Cat and dog epithelium	4	21	25	2	45	47	1	15	16
Cockroach	8	30	38	14	41	55	3	16	19
Mold mixing	30	74	104	5	161	166	1	48	49
Grass	0	21	21	0	41	41	3	6	9
Cow milk	24	67	91	3	141	144	0	17	17
Beef-mutton	4	29	33	1	71	72	0	5	5
Cashew-peanut-soybean	1	16	17	1	26	27	1	7	8
Mango	1	5	6	0	16	16	0	9	9
Wheat	1	32	33	4	46	50	2	17	19
Trees	4	35	39	3	55	58	2	23	25
Egg white/egg yolk	5	20	25	0	23	23	0	11	11
Fish-prawn-crab	1	15	16	0	26	26	0	9	9
Total	120	506	626	39	984	1,023	21	338	359

^aTwo-sided P-values. χ^2 test or Fisher's exact probability method for comparison of total IgE in different levels of sIgE, P<0.05. IgE, immunoglobulin E; sIgE, specific IgE.

Among the 1,215 sIgE-positive patients, 88.9% (n=1,080) had a higher total IgE, 5.4% of these (n=58) had an accumulated sIgE of >100 IU/ml, the remaining 1,022 patients (94.6%) had an accumulated sIgE of <100 IU/ml, which showed that the increased accumulation of sIgE could not completely explain the increase of total IgE.

Total IgE of 1,215 patients with different sIgE levels were analyzed (Table V). A total of 626 cases of sIgE were grade 1, 19.2% (n=120) had normal total IgE; 1,023 cases were grade 2 and 3.8% (n=39) had normal total IgE; and 359 cases were grade 3 and 5.8% (n=21) had normal total IgE. Statistical results showed that there were no differences of total IgE between patients with grade 2 or 3 sIgE ($\chi^2=2.66$, P>0.05). However, there were significant differences of total IgE levels between patients with grade 1 and 2 sIgE ($\chi^2=33.0$, P<0.05).

A total of 40.2% (n=489) of patients had >2 positive allergens according to sIgE levels and the number of positive allergens, as well as the positive level of sIgE, directly affected the total IgE. Therefore, 105 patients whose house dust mite mixture were positive while the remaining 13 types of allergen were negative were studied, and their sIgE detection levels were grade 2 for analyzing the association of total IgE and sIgE. Only 1 patient had normal total IgE and the others were >100 IU/ml, which was consistent with the above results.

Discussion

Allergic disease is a type of allergic reaction following contact with certain allergens. Total IgE and sIgE are a product of type 1 allergy. The type I hypersensitivity is common in

allergic disease, which can affect the respiratory, gastric and intestinal systems and other systems resulting in anaphylactic rhinitis, asthma, allergic gastroenteritis, sensitization dermatitis, urticaria and even allergic shock.

Exposure to allergens is an important risk factor for the allergic diseases (12). Therefore, the main way for prevention of allergic disease is to identify the allergen, prevent contact with the allergens and improve the patients' tolerance to the allergens by desensitization therapy. Thus far, there are certain types of method for detection of allergens; *in vivo* experiments include the skin-prick test and double-blind placebo-controlled food challenge and *in vitro* studies include the histamine-releasing test and assays of IgE and sIgE. *In vitro* experiments are safer than *in vivo* experiments and meet the ethical requirements, therefore, the detection of IgE and sIgE are widely applied in clinical practice.

IgE is a type of antibody produced by specific B lymphocyte following the intake of allergens. The Fc fragment of IgE combines with the Fc receptors of mast cells and basophile granulocyte to induce sensitization. When the body has contact with the allergen again, the target cells are activated to induce cell degranulation and release medium with bioactivity, which affects different target organs to initiate pathological changes. Normally, the IgE quantity in serum is $\sim 5 \times 10^{-5}$ mg/ml. In 1983, the anti-human IgE monoclonal antibody was produced by Chandler *et al* (13), and since then the total IgE levels can be detected by the method of semiquantitative ELISA. In the present study, the positive rate of total IgE was 65.0% by the method of immunoblotting, which may result from the selection of cases or certain hypersensitivity that were not IgE-mediated (14). The specific ratio of total IgE was only 46.6% as the increased IgE was observed in patients not only with allergic disease but also certain other diseases, such as multiple myeloma, heavy chain disease, liver disease and rheumatoid arthritis. Therefore, if the total IgE results were used as the basis of judging allergic disease, the misdiagnosis rate would be 53.4%. The sensitivity of total IgE was 88.9%, which showed that it is a useful index of screening allergic disease with a low cost compared to sIgE. There is an $\sim 11.1\%$ false-negative rate when total IgE results are the only index of assessment for allergies.

When allergens enter the body, specific B cells are induced selectively to produce sIgE. The level of sIgE depends on the reaction to the allergens of the body. When the positive rank of sIgE is higher, the occurrence of the allergic reaction is more probable. Allergens combined with sIgE induce an allergic reaction, so sIgE is an important objective index for diagnosis of allergic disease. In 1985, Reid *et al* (15) studied an ELISA method for detecting sIgE using the monoclonal allergen sIgE and quantified serum sIgE of perennial ryegrass in 10 patients using the reference curve. In the same year, Haas *et al* (16) detected the sIgE antibody in pollen allergy patients applying the western blot analysis method. At present, these two methods are used for detection of sIgE of numerous allergens.

The consistency rate of total IgE and sIgE was 60.4% and the κ -value was 0.28. It is generally acknowledged that consistency is excellent when the κ -value is ≥ 0.75 and is medium or high when the κ -value is 0.4-0.75. In the present study the κ -value was < 0.40 meaning that the consistency of total IgE

and sIgE was relatively poor. Therefore, the assay of total IgE could not be used instead of the sIgE assay and vice versa.

The sIgE results are exact evidence of allergens, but the data in the present study showed that only 32.7% of sIgE of 3,721 patients suspected to have allergic diseases increased, and the positive rate was low. In 135 patients with normal total IgE, 82.2% had 1 sIgE positive, and 17.8% patients had ≥ 2 sIgE positive. When the number of positive sIgE increased and the detecting level enhanced, the number of positive total IgE markedly increased. This suggests that increased sIgE can lead to increased total IgE, but the total IgE was not completely caused by the accumulation of sIgE. When the quantity of sIgE did not increase enough, the total IgE was still in the normal level.

There are hundreds of allergens that exist in the world. The United States has reported > 170 types of food that caused IgE-mediated allergy (17). Until June 29, 2014, 775 types of allergens had undergone standardized naming by the Association of International Immunology. In the present study, 7 types of common inhaling allergens and 7 types of food allergens were selected as detection targets. Certain allergens were the mixture of several allergens, therefore 29 types of allergens were screened in total. Due to the species property of allergens, a patient who was sensitive to one allergen may also be sensitive to other allergens with similar structures. Therefore, negative results do not signify that the patients had no chance of allergy and sIgE results could also cause certain false-negative rate.

The AllergyScreen[®] system used in the study is a semi-quantitation assay system based on the immunoblotting method for detecting total IgE and sIgE levels (18). Immunoblotting methods have higher sensitivity and specificity. However, this method requires concentrated allergens packaged on a nitro-cellulose membrane, therefore, the component of the allergen, purity and amount of allergen could affect the result of total IgE and sIgE. For example, Haas *et al* (16) reported that the mite protein could be detected from the 43-177 kD mite protein fragment in sodium dodecyl sulfate-polyacrylamide gel electrophoresis, while in the culture of the entire mite, there was no mite protein identified. Furthermore, allergic activity of the mite body immersion was higher than that of the entire mite culture (19). Therefore, the choice of the allergen had a great influence on the test results. Furthermore, whether the allergens packaged in the product were suitable for northern China requires further research.

Clinical symptoms of allergic disease are complex, as certain symptoms are clear whilst others are not; therefore, the allergen detection is extremely important to avoid misdiagnosis and missed diagnosis. In the present study, cases could be divided into three groups: The first group was the patients who had clear symptoms and did not receive any treatment, the second was the patients who received the treatment while their symptoms existed, and the third was the patients whose symptoms completely disappeared following a period of treatment. All the three groups could have positive-sIgE results. The level of sIgE depended on the volume of allergens, the time of sIgE production and the half-life of sIgE. The therapeutic schedule was also a factor that could affect the level of sIgE. For example, the desensitization therapy reduced the response of the body to certain allergens. In general, when total IgE

was elevated, the sIgE test results may be positive or normal; when total IgE is normal, the sIgE results may be positive. In the present study, 11.1% of patients had normal total IgE and positive sIgE.

In conclusion, total IgE that was influenced by numerous factors could not be used as crucial evidence for diagnosis of allergic disease alone, but it played an important role on screening. Testing serum-sIgE is an extremely significant examination that could help identify the cause of allergic disease and also select the effective strategy to prevent allergic disease. However, the negative result of sIgE could not exclude allergic disease completely due to the limitation of allergen selection and the detection method. Furthermore, the positive sIgE and total IgE showed that there was a possibility of allergic reaction. Whether there were clinical manifestations depended on the immune state of the body and medication history. The combination of disease history, clinical manifestation and results of the laboratory test would help to generate the correct diagnosis.

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