House dust mite and storage mite IgE reactivity in allergic patients from Guangzhou, China

Chunqing Zhang,[1] Jing Li,[1] Xuxin Lai,[2] Yiwu Zheng,[2] Birgitte Gjesing,[2] Michael D. Spangfort[2] and Nanshan Zhong[1]

Summary

***Background*: In China, house dust mites are important inducers of allergic disease. The importance of allergens from storage mites is less well known.**

***Objective:* The aim of this study is to access the prevalence of house dust mite and storage mite sensitization and investigate the IgE cross-reactivity between house dust mite and storage mite.**

***Method:* The skin prick test (SPT) and specific IgE against the mite species *D. pteronyssinus, D. farinae, B. tropicalis, Lepidoglyphus destructor, Glycyphagus domesticus, Tyrophagus putrescentiae* and *Acarus siro* were measured. Included were 412 patients with asthma and/or rhinitis for SPT, 244 for specific IgE and 29 sera for IgE inhibition studies.**

***Results:* The positive SPT prevalence for *D. pteronyssinus* was 80.3% and for *D. farinae* 83.7%. The specific IgE prevalence for *D. pteronyssinus* was 61.1% and for *D. farinae* 60.2%. The storage mite species, *B. tropicalis* and *T. putrescentiae* had the highest positive SPT prevalence, 66% and 63%, respectively. The specific IgE prevalence was highest for *B. tropicalis* and *G. domesticus*, 41% and 37%, respectively. Both SPT and specific IgE levels were much higher for house dust mites compared to storage mites. Inhibition measurements showed that none of the storage mites could fully inhibit the specific IgE against *D. pteronyssinus.* Only in half of the sera could *D. pteronyssinus* fully inhibit the IgE against *L. destructor* and *G. domesticus* while inhibition of the other storage mites were much lower. Nearly all the specific IgE against storage mites could be inhibited by the other storage mites, though *B. tropicalis* showed a slightly different pattern from the other storage mites.**

Conclusion:* IgE reactivity against storage mites in Chinese patients is due to both storage mite specific IgE and due to IgE mediated cross-reactivity to *D. pteronyssinus.* *(Asian Pac J Allergy Immunol 2012;30:294-300)

Key words: *SPT, serum specific IgE, inhibition experiment, cross-reactivity, house dust mite, storage mite*

From 1. State key Laboratory of Respiratory Disease, The First Affiliated Hospital, Guangzhou Medical University, Guangzhou, China.
2. Research Asia Pacific, ALK A/S, Guangzhou, China
Corresponding author: Jing Li
E-mail: lijing@gird.cn
Submitted date: 19/3/2012
Accepted date: 22/6/2012

Introduction

In China, allergens of the house dust mites *Dermatophagoides pteronyssinus* (DPt) and *D. farinae* (DF) are the most important inducers of allergic asthma and rhinitis. The recently performed Chinese skin prick (SPT) prevalence CARRAD study showed that 59.0% and 57.6% of Chinese patients are sensitized to DPt and DF, respectively.[1] The CARRAD SPT study included the mite *Blomia tropicalis* (BT) and revealed an overall sensitization prevalence of 40.7% making BT the allergen source with the third highest positive SPT prevalence in the CARRAD study. The mite genera *Blomia, Lepidoglyphus,* and *Glycyphagus* belong to the Glycyphagidae family which, together with the Acaridae family (*Tyrophagus* and *Acarus* genera), are collectively called storage mites. Storage mites are present in both rural and urban environments, especially in regions with damp housing conditions[2-4] and house dust mites and storage mites are known to co-exist.[5-6] Sensitization and allergic symptoms towards storage mite have been reported from many

different parts of the world.[3,7-11] However, the prevalence of storage mite sensitization and exposure in China is to a large extent unknown. The major *B. tropicalis* allergen Blo t 5 has been detected in household dust samples from Hong Kong[12] but Blo t 5 could not be detected in dust samples from Guangzhou, a city in close vicinity to Hong Kong.[13] *B. tropicalis* has only been found in the tropical and subtropical regions of the world[14-15] and the relatively high level of BT sensitization in temperate climate regions of China, as detected by the CARRAD, needs further investigation. For effective mite allergen specific immunotherapy it is important to distinguish between house dust and storage mite dual sensitization and, apparent sensitization due to IgE mediated cross-reactivity.

In this study, the skin reactivity and prevalence of specific IgE sensitization against seven mite species was investigated in allergic patients from southern China. The mites included were *Acarus siro* (AS), *Lepidoglyphus destructor* (LD), *Tyrophagus putrescentiae (*TP), and *Glycyphagus domesticus* (GD) as well as DPt, DF, and BT. In addition, specific IgE inhibition was used to address IgE mediated cross-reactivity between house dust mite DPt and storage mites and between the different storage mite species.

Methods

Subjects

Patients visiting the outpatient clinic of Guangzhou Institute for Respiratory Diseases, Guangzhou, with a clinical diagnosis of allergic asthma and/or allergic rhinitis were asked to take part in the study. The study included 412 patients. The mean age was 29 years (range 5-72 year), and there were 217 males and 195 females. Of the 412 patients, 244 patients also donated blood samples for the study and the sera were stored at -18ºC until measurements could be carried out.

The study was approved by the local ethical committee and informed consent was obtained from every individual participating in the study and from parents or guardians of pediatric patients.

Skin prick test

All 412 patients were skin prick tested (Soluprick, ALK, Madrid, Spain) with house dust mites DPt and DF and storage mites species BT, AS, TP, LD and GD, in addition to positive and negative controls. The SPT result was considered positive when the diameter of the wheal was 3 mm larger than that of the negative control. The SPT class ranking is according to the value of skin index (SI), the ratio of the allergen wheal size and histamine wheal size. (Class 0: SI = 0, Class 1: SI < 0.5, Class 2: 0.5 ≤ SI < 1, Class 3: 1 ≤ SI < 2, Class 4: SI ≥ 2.)

Specific IgE test

Allergen specific IgE was measured in kU/L in serum on the ADVIA Centaur® immunoassay system (Bayer Healthcare LLC, Tarrytown New York, USA) which is a reverse sandwich immuno-assay using direct chemo-luminescent technology. In brief, the test uses PMP (Paramagnetic particles) bound antihuman IgE (ALK-Abello A/S, Hørsholm, Denmark) as the first layer. IgE in serum is captured and non-IgE washed away. Biotinylated allergen (ALK-Abello A/S, Hørsholm, Denmark) was added in excess and bound to the allergen-specific IgE antibody captured in the solid phase. The conjugate acridinium ester-labeled streptavidin bound to the biotin-labeled allergen and produced a flash when changing pH; the luminescence was then read with a luminometer. A direct relationship exists between the amount of allergen-specific IgE present in the sample and the number of relative light units detected by the system. The concentration of sIgE (kU/L) was calculated by the system. The specific IgE result was considered positive when ≥ 0.35 kU/L. Results measured as > 100 kU/L were for statistical purposes given the value 101 kU/L. The specific IgE allergy class was ranked as: Class 0 < 0.35 kU/L, Class 1 ≥ 0.35 – 0.70 kU/L, Class 2 ≥ 0.70 – 3.5 kU/L, Class 3 ≥ 3.5 – 17.5 kU/L, Class 4 ≥ 17.5 – 50 kU/L, Class 5 ≥ 50 – 100 kU/L, Class 6 ≥ 100 kU/L.

Of the 412 patients included in the study, 244 subjects donated blood for specific IgE measurements. All 244 sera were tested for specific IgE against house dust mites DPt and DF and storage mite species BT, AS, TP, LD and GD (except only 235 sera for BT).

Allergen specific IgE inhibition experiments

Allergen freezed-dried extracts from DPt, BT, AS, TP, LD and GD (provided by ALK-Abello A/S) were used as inhibitors in IgE inhibition experiments. Twenty-nine of the collected sera had sufficient volume and sufficiently high specific IgE levels (> 3 kU/L) to allow inhibition experiments. The specific IgE inhibition was performed using the ADVIA Centaur® immunoassay system. In contrast to specific IgE tests, non-labeled inhibitor extract is added to biotinylated allergen to make the final

inhibitor concentration 1 mg/ml, which is a concentration at which the extract will fully inhibit binding of the same biotinylated extract to serum IgE, allowing the competition between non-labeled inhibitor allergen (or buffer) and biotinylated allergen during the measurement of specific IgE. The IgE response in relative light units (RLU) was used to calculate the Inhibitory capacity (% I). % I = [(specific IgE RLU with buffer) – (specific IgE RLU with inhibitor)] * 100 / [(specific IgE RLU with buffer)-(specific IgE RLU with IgE free serum)]. Results ≤ 9% inhibition were considered to be no inhibition and results ≥ 91% were considered to be total inhibition. Results 10 – 90% were considered to be partial inhibition.

Results

Patient characteristics

The characteristics of the patients included are shown in Table 1. The three groups: the SPT, the specific IgE and the specific IgE inhibition group are compared in relationship to the distributions of their ages (children and adults), genders (male and female), and symptoms (rhinitis, asthma and asthma + rhinitis). The three groups have a similar distribution for ages and symptoms. The SPT and specific IgE groups also have a similar distribution of gender; while the inhibition group has fewer female child patients.

Table 1. Subject characteristics

	SPT (n=412)	Specific IgE (n=244)	Specific IgE inhibition (n=29)
Children (≤14 years of age), n (%)	121 (29.4)	45 (18.3)	8 (27.6)
Age, median (range)	8 (5-14)	9 (5-14)	10 (6-14)
Male/Female	75/46	26/18	8/0
Adults (>14 years of age), n (%)	291 (70.6)	201 (81.7)	21 (72.4)
Age, median (range)	36 (15-72)	36 (15-66)	32 (15-58)
Male/Female	142/149	100/101	8/13
Number of subjects with Rh[1], n (%)			
Children	41 (33.9)	5 (11.1)	3 (37.5)
Adults	125 (43)	44 (21.9)	7 (33.3)
Number of subjects with As[2], n (%)			
Children	21 (17.4)	13 (28.9)	0 (0)
Adults	52 (17.9)	72 (35.8)	1 (4.8)
Number of subjects with As+Rh[3], n (%)			
Children	59 (48.8)	27 (60.0)	5 (62.5)
Adults	114 (39.2)	85 (42.3)	13 (61.9)

[1]Rh: Patients with rhinitis symptoms.

[2]As: Patients with asthma symptoms.

[3]As+Rh: Patients with both asthma and rhinitis symptoms.

Table 2. Frequencies of positive results for SPT and specific IgE

Group	DPt	DF	BT	AS	LD	TP	GD
SPT (n=412)	80.3%	83.7%	66.3%	46.4%	48.1%	62.6%	39.8%
SPT (n=244)[1]	78.7%	82.0%	66.8%	46.7%	46.7%	61.1%	39.3%
Specific IgE (n=244)[1]	61.1%	60.2%	41.3%	24.2%	25.4%	24.6%	36.9%

[1]Subgroup of 244 patients tested with both SPT and specific IgE.

Positive SPT = the diameter of the wheal was 3 mm larger than the negative control.

Positive specific IgE ≥ 0.35 kU/L

Prevalence of sensitization

Table 2 shows the prevalence of positive mite SPTs and specific IgE among all patients (n=412) and in addition, skin reactivity and positive specific IgE reactivity among the patients donating blood samples for specific IgE testing (n=244).

House dust mites DPt and DF show similar and relatively high prevalence for both SPT sensitizations, DPt 80.3% and DF 83.7%, and for specific IgE sensitization, DPt 61.1% and DF 60.2%.

For the storage mite species, BT and TP had the highest SPT prevalence, 66% and 63%, respectively, whereas the specific IgE prevalence was highest for BT and GD, 41% and 37%, respectively.

There is no difference in the SPT positive rates between the whole group (n = 412) and the subgroup undergoing specific IgE measurements (n = 244).

Table 3 shows SPT and specific IgE sensitization to different combinations of mite species. Patients sensitized to both house dust and storage mites make up the largest group with SPT and specific IgE prevalences of 73.8% and 48%, respectively. The prevalence of patients sensitized to house dust mite without storage mite sensitization was 13% according to SPT and 14% according to specific IgE. Only 1.5%

Table 3. Sensitization to different mite combinations

	HDM+SM+	HDM+SM-	HDM-SM+	HDM-SM-
SPT (n=412)	73.8%	12.6%	1.5%	12.1%
Specific IgE (n=244)	48.4%	14.3%	13.9%	23.4%

HDM+ / SM+ mean number of positive results for House Dust Mites/Storage Mites.

HDM-/SM- mean number of negative results for House Dust Mites/Storage Mites .

Positive SPT = the diameter of the wheal was 3 mm larger than the negative control.

Positive specific IgE ≥ 0.35 kU/L

of the patients were sensitized to storage mites without house dust mite sensitization, as judged by SPT, and 14% according to specific IgE.

Comparing the results by Spearman ranking showed a significant correlation among all the mites with P <0.0001 both for SPT and for specific IgE.

Levels of sensitization

Figure 1 and Figure 2, show the levels of mite SPT and specific IgE sensitizations, respectively. For the patients with positive SPT results, the house dust mite SPT results are mainly of class 3 and 4, while the SPT results for storage mites are mainly of class 2. Similarly, for the patients with positive specific IgE sensitization, the distribution for house dust mite IgE levels was mainly of class 4, 5 and 6, while storage mite specific IgE levels were mainly of class 2 and 3 with no or very few sera of class 4 and above.

Specific IgE inhibition

The levels of specific IgE for the 29 sera used in IgE inhibition experiments are shown in Table 4. For almost all subjects the levels of DPt kU/L are much higher compared to the storage mite species.

Figure 3 shows the percentage inhibition of IgE against the tested mite species after inhibition with the other five mite species, respectively. Inhibition is the result of serum IgE recognizing allergens present in both the tested mite species and in the inhibiting mite species (cross-reactivity). No inhibition indicates lack of cross-reactive serum IgE.

From Figure 3, panel A, it can be seen that none of the storage mites species could fully inhibit serum IgE against DPt, whereas DPt could fully or partially inhibit serum IgE against storage mite species LD and GD in all the sera tested (Figure 3, panel E and F). However, for almost all sera tested, Dptcould only partially inhibit IgE against BT, AS and TP (Figure 3, panels B-D). The storage mite species, AS, TP, LD and GD, could to a very high degree fully inhibit each other, indicating the presence of cross-reacting serum IgE (Figure 3, panels C-F). However, BT shows a somewhat different inhibition pattern compared to the other storage mites. BT as inhibitor could fully inhibit serum IgE against AS and TP in all sera tested (Figure 3, panel C and D), whereas full inhibition of GD and LD occurred to a lesser extent (Figure 3, panel E and F). When AS, TP, LD or GD were used as inhibitors for measuring BT specific IgE, only AS and TP showed full inhibition in 60% of the tested sera (Figure 3, panel B). In contrast, only a very small fraction of sera showed full inhibition of IgE against BT when LD and GD were used as inhibitor (Figure 3, panel B).

Discussion

In this study, the prevalence of SPT and specific IgE sensitization against house dust and storage mites were tested in 422 allergic patients originating from southern China. In agreement with Li et al.[1] relatively high levels of house dust mite DPt and DF SPT sensitizations (>80%) were detected. In addition, there were significant levels of SPT sensitizations against storage mite, as high as 66% and 63% for BT and TP, respectively. A high prevalence of mite specific IgE sensitization was also evident among the subjects; about 60% for house dust mites

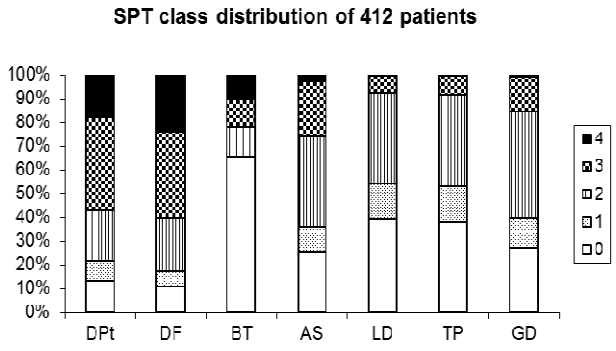

Figure 1. Classes of SPT for 412 patients. For each species the percentage included in each class 0 – 4 of SPT results is given.

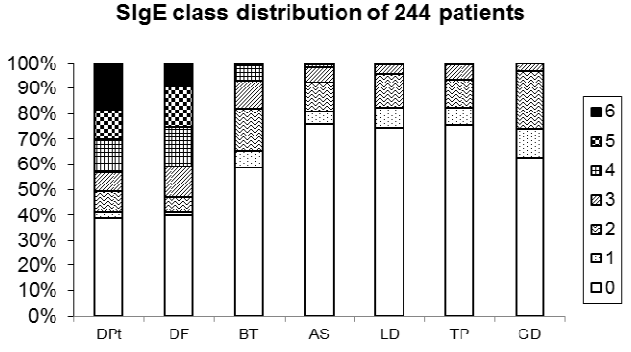

Figure 2 Classes of specific IgE for 244 patients. For each species the percentage included in each class 0 – 6 of specific IgE results is given.

in

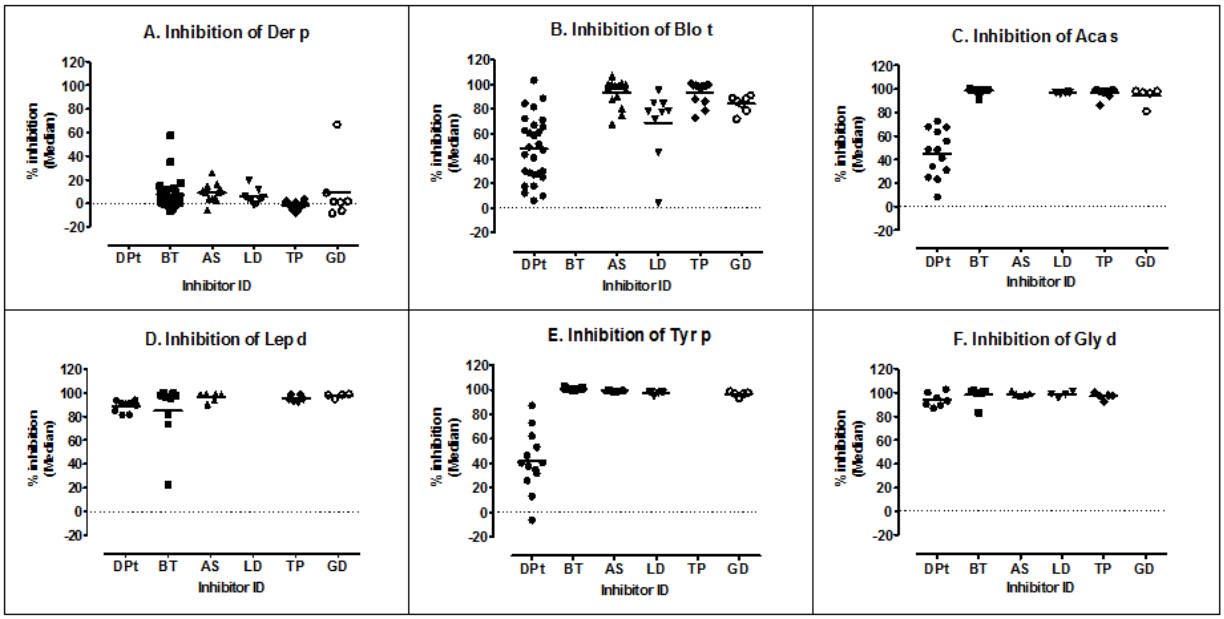

Figure 3. Inhibition pattern between *D. pteronyssinus* (Dpt), *D. farina* (DF), *B. tropicalis* (BT), *A. siro* (AS), *L. destructor* (LD), *T. putrescentiae* (TP) and *G. domesticus* (GD).
Panels A-F show the % Inhibition of DPt, BT, AS, LD, TP and GD respectively, after inhibition by the other mite species. Each symbol represents one serum. The median level of % Inhibition is shown as a line for each group.

and between 24 - 42% for the different storage mite species BT, AS, TP, LD, GD. In general, the mite specific IgE prevalence was lower compared to the SPT prevalence, however, such differences between SPT and specific IgE prevalence results are not unusual.[16-18]

Most of the patients were apparently sensitized to both house dust mites and storage mites; 73.8% as judged by SPT and 48.4% according to specific IgE. However all patients had much higher SPT skin index values and higher kU/L specific IgE values for house dust mite compared to storage mites. This relatively lower level of storage mite sensitization could be due to low exposure to storage mite allergens and/or IgE mediated cross-reactivity towards house dust mite allergens. The content and composition of storage mite allergens in house hold dust from southern China is at present not known. However it is not likely that storage mites will be among the most abundant species since house dust mites always seems to dominate the indoor environment[19] and very high levels of house dust mite group allergens have been detected in house hold dust from Guangzhou.[13]

To address the influence of IgE mediated cross-reactivity, we conducted IgE inhibition experiments different species-specific mite extracts was inhibited after pre-incubation of sera with a single mite allergen extract.

The IgE inhibition experiments showed that there is a very high degree of IgE mediated cross-reactivity among the different storage mite species, including BT. Cross-reactivity among storage mites has been reported before[8,16,20-21] and can in parts explain the high prevalence of BT skin reactivity observed in regions of China with unfavorable growth conditions for BT mites as reported by Li et al.[1]

In addition, the IgE inhibition experiments also showed limited cross-reactivity between BT and DPt, since although 50% of sera tested had IgE specific for BT not cross-reacting with DPt allergens, the other 50% of sera contained BT IgE cross-reacting with DPt. Thus, the high prevalence of SPT BT observed in the in CARRAD SPT study[1] can be explained by a combination of reactivity to another storage mite species and cross-reactivity towards DPt. Skin test reactivity to BT in DPt patients not exposed to BT has also been reported previously[22] and a limited amount of IgE mediated cross-reactivity between house dust mites and BT has been reported.[14,23-27]

Table 4. Specific IgE (kU/L) values for 29 sera used in IgE inhibition experiments

Sample	DPt	BT	AS	LD	TP	GD
Sam 1	<0.35	19.5	5.1	<0.35	2.0	<0.35
Sam 2	27.0	34.7	19.6	6.6	14.6	2.5
Sam 3	124.3	11.1	4.7	1.3	4.8	1.7
Sam 4	174.3	28.3	17.8	4.6	12.5	1.9
Sam 5	551.8	21.4	3.3	2.0	2.0	1.2
Sam 6	163.8	24.9	10.0	3.6	7.7	1.5
Sam 7	437.0	19.9	5.6	0.58	4.6	4.5
Sam 8	316.5	24.4	<0.35	3.5	<0.35	<0.35
Sam 9	54.2	42.6	21.1	10.7	16.6	3.1
Sam 10	5.1	12.3	3.3	1.0	3.4	0.89
Sam 11	185.7	25.3	<0.35	5.4	<0.35	1.6
Sam 12	457.7	5.6	1.1	3.0	2.3	1.0
Sam 13	<0.35	7.0	5.7	1.6	3.3	1.2
Sam 14	289.9	17.3	3.4	2.5	2.4	<0.35
Sam 15	185.9	9.5	<0.35	<0.35	<0.35	0.37
Sam 16	41.1	23.0	12.6	2.9	8.9	1.2
Sam 17	309.1	86.1	40.8	20.5	26.4	11.1
Sam 18	87.8	9.2	6.9	1.5	5.4	0.44
Sam 19	69.6	13.5	9.5	2.5	7.1	1.0
Sam 20	427.1	27.7	0.46	0.55	<0.35	<0.35
Sam 21	462.5	14.3	7.3	5.7	7.1	3.6
Sam 22	226.9	11.8	1.11	0.55	0.68	<0.35
Sam 23	25.8	91.6	1.4	1.3	<0.35	9.5
Sam 24	149.1	12.8	3.7	1.3	2.2	0.53
Sam 25	99.0	4.5	<0.35	0.64	0.59	4.0
Sam 26	49.1	5.0	1.3	<0.35	0.74	<0.35
Sam 27	137.7	40.2	0.97	1.3	0.83	0.91
Sam 28	51.0	47.6	6.9	0.64	6.1	1.7
Sam 29	76.1	28.6	9.3	2.9	6.8	0.82

The IgE inhibition experiments also showed limited cross-reactivity between DPt and the storage mite species AS, TP, LD and GD which could, in parts, explain the relatively low levels of AS, TP, LD and GD specific IgE kU/L detected among the patients. However, the existence of a genuine storage mite sensitization is indicated by the fact that 50% of sera contain IgE specific for storage mites AS and TP not cross-reacting with DPt. In the absence of any indoor exposure data for storage mites and the observed large degree of storage mite IgE cross-reactivity it is at present not possible to identify or even verify the presence of a primary sensitizing storage mite species.

Many research groups have, during the last 25 years, investigated the similarity or dissimilarity between house dust and storage mite allergens using mite extracts, purified allergens or recombinant allergens. Griffin et al.[5] showed the occurrence of dual sensitization to house dust mites and storage mite, as well as allergenic cross-reactivity in different allergic subjects. Van der Heide *et al*[6] reported that co-sensitization to storage mites is frequently found in patients sensitized to DPt and Johansson et al.[8] showed by immunoblotting and immunoblot inhibition that IgE against different allergens in DPt, AS, TP and LD has different abilities to be inhibited by extract from different species. Arias-Irigoyen et al.[16] showed limited IgE mediated cross-reactivity in RAST inhibition experiments between DPt and GD in patients exposed to both species. GD and LD could inhibit each other to a very high degree, while TP could only partially inhibit both GD and LD. The general finding is that there is some IgE cross-reactivity between house dust and storage mites mainly in the major allergen group 2.[16,21] This is consistent with our data showing a high degree of IgE cross-reactivity among the different storage mites but also the existence of storage mite specific IgE indicating independent sensitization towards storage mites in patients also sensitized to house dust mites.

The main conclusion from this study is that the measured skin and specific IgE reactivity against storage mites in patients from southern China is due to both storage mite specific IgE and due to IgE mediated cross-reactivity to DPt. Some patients appear to be independently sensitized to both house dust and storage mite, requiring storage mite specific reagents for proper clinical diagnosis. The level of storage mite exposure and clinical relevance in China needs to be further investigated.

Acknowledgements

The authors wish to thank Tiantian Liu for assisting with specific IgE measurements and Suiying Li and Xiaoting Xu for performing the SPT.

References

1. Li J, Sun B, Huang Y, Lin X, Zhao D, Tan G, et al. A multicentre study assessing the prevalence of sensitizations in patients with asthma and/or rhinitis in China. Allergy. 2009;64:1083-92.
2. Iversen M, Dahl R. Allergy to storage mites in asthmatic patients and its relation to damp housing conditions. Allergy. 1990;45:81-85.
3. Iversen M, Korsgaard J, Hallas T, Dahl R. Mite allergy and exposure to storage mites and house dust mites in farmers. Clin Exp Allergy. 1990;20:211-9.
4. Ebner C, Feldner H, Ebner H, Kraft D. Sensitization to storage mites in house dust mite (Dermatophagoides pteronyssinus) allergic patients. Comparison of a rural and an urban population. Clin Exp Allergy. 1994;24:347-52.

5. Griffin P, Ford AW, Alterman L, Thompson J, Parkinson C, Blainey AD, et al. Allergenic and antigenic relationship between three species of storage mites and the house dust mite *Dermatophagoides pteronyssinus*. J Allergy Clin Immunol. 1989;84:108-17.
6. van der Heide S, Niemeijer NR, Hovenga H, de Monchy JGR, Dubois AEJ, Kauffman HF. Prevalence of sensitization to the storage mites *Acarus siro*, *Tyrophagus putrecentiae* and *Lepidoglyphus destructor* in allergic patients with different degrees of sensitizations to the house-dust mite *Dermatophagoides pteronyssinus*. Allergy. 1998;53:426-30.
7. Luczynska CM, Griffin P, Davies RJ, Topping MD. Prevalence of specific IgE to storage mites (A. siro, L. destructor and T. longior) in an urban population and cross reactivity with the house dust mite (D. pteronyssinus). Clin Exp Allergy. 1990;20:403-6.
8. Johansson E, Johansson SGO, van Hage-Hamsten M. Allergenic characterization of *Acarus siro* and *Tyrophagus putrescentiae* and their cross reactivity with *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus*. Clin Exp Allergy. 1994;24:743-51.
9. Van-Hage-Hamsten M, Johansson E. Clinical and immunologic aspects of storage mite allergy. Allergy. 1998;53 (suppl):49-53.
10. Vidal C, Boquete O, Gude F, Rey J, Meijide LM, Fernández-Merino MC, et al. High prevalence of storage mite sensitization in a general adult population. Allergy. 2004;59:401-5.
11. Yadav A, Elder BL, Morgan MS, Vyszenski-Moher DL, Arlian LG. Prevalence of serum IgE to storage mites in a southwestern Ohio population. Ann Allergy Asthma Immunol. 2006;96:356-62.
12. Sun B, Wu A, Chan A, Chik S, Wong D, Zhong N. House dust mite allergen (Der p 1 and Blo t 5) levels in asthmatics' home in Hongkong. Chin. Med. Sci. J. 2004;19:185-8.
13. Zhang C, Gjesing B, Lai X, Li J, Spangfort MD, Zhong N. Indoor allergen levels in Guangzhou city, southern China. Allergy. 2011;66:186-91.
14. Arlian LG., Vyszenski-Moher DL, Fernandez-Caldas E. Allergenicity of the mite, Blomia tropicalis. J Allergy Clin Immunol. 1993;91:1042-50.
15. Chua YC, Cheong N, Kuo IC, Lee BW, Yi FC, Huang CH, Liew LN. The *Blomia tropicalis* allergens. Protein and peptide letters. 2007;14:325-33.
16. Arias-Irigoyen J, Lombardero M, Arteage C, Carpizo JA, Barber D. Limited IgE cross-reactivity between Dermatophagoides pteronyssinus and Glycyphagus domesticus in patients naturally exposed to both mite species. J Allergy Clin Immunol. 2007;120:98-104.
17. Ricci G, Capelli M, Miniero R, Menna G, Zannarini L, Dillon P, et al. A comparison of different allergometric tests, skin prick test, Pharmacia UniCAP and Advia centaur, for diagnosis of allergic diseases in children. Allergy. 2003;58:38-45.
18. Haahtels T, Jaakonmäki I. Relationship of allergen-specific IgE antibodies, skin prick tests and allergic disorders in unselected adolescents. Allergy. 2007;36:251-6.
19. Arlian LG, Morgan MS, Neal JS. Dust mite allergens: Ecology and distribution. Curr Allergy Asthma Reports. 2002;2:401-11.
20. Puerta L, Fernández-Caldas E, Lockey RF, Caraballo LR. Sensitization to *Chortoglyphus arcuatus* and *Aleuroglyphus ovatus* in *Dermatophagoides spp*. allergic individuals. Clin Exp Allergy. 1993;23:117-23.
21. Gafvelin G, Johansson E, Lundin A, Smith AM, Chapman MD, Benjamin DC, et al. Cross-reactivity studies of a new group 2 allergen from the dust mite *Glycyphagus domesticus*, Gly d 2, and group 2 allergens from *Dermatophagoides pteronyssinus, Lepidoglyphus destructor* and *Tyrophagus putrescentiae* with recombinant allergens. J Allergy Clin Immunol. 2001;1907:511-18.
22. Simpson A, Green R, Custovic A, Woodcock A, Arruda LK, Chapman MD. Skin test reactivity to natural and recombinant *Blomia* and *Dermatophagoides spp*. Allergens among mite allergic patients in the UK. Allergy. 2003;58:53-6.
23. Stanaland BE, Fernandez-Caldas E, Jacinto CM, Trudeau WL, Lockley RF. Sensitization to Blomia tropicalis: skin test and cross-reactivity studies. J Allergy Clin Immunol. 1994;94:452-7.
24. Tsai JJ, Wu HH, Shen HD, Hsu EL, Wang SR. Sensitization to Blomia tropicalis among asthmatic patients in Taiwan. Intl Arch Allergy Immunol. 1998;115:144-9.
25. Chew FT, Yi FC, Chua KY, Fernandez-Caldas E, Arruda LK, Chapman MD, et al. Allergenic differences between the domestic mites Blomia tropicalis and Dermatophagoides pteronyssinus. Clin Exp Allergy. 1999;29:982-8.
26. Garcia Robaina JC, Sanchez Machin I, Fernandez-Caldas E, Iraola Calvo V, Vazquez Moncholi C, Bonnet Moreno C, et al. Skin tests and conjunctival and bronchial challenges with extracts of *Blomia tropicalis* and *Dermatophagoides pteronyssinus* in patients with allergic asthma and/or rhinoconjunctivitis. Int Arch of Allergy Immunol. 2003;131:182-8.
27. Kuo IC, Cheong N, Trakultivakorn M, Lee BW, Chua KY. An extensive study of human IgE cross-reactivity of Blo t 5 and Der p 5. J Allergy Clin Immunol. 2003;111:603-9.