

Evaluation of Sensitivity Toward Storage Mites and House Dust Mites Among Nasobronchial Allergic Patients of Kolkata, India

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Abstract

House dust mites (HDMs) are the major constituents of house dust (HD). HD and HDM sensitization is well documented worldwide. Storage mite (SM) sensitization is presently lacking from India. The present study evaluated the sensitization of both HDM and SM among 372 allergic rhinitis patients reported to the Allergy and Asthma Research Center of Kolkata metropolitan, India. HD samples were collected from the patients' home and analyzed for the major constituent mites. HD and six constituent mites *Dermatophagoides pteronyssinus* (DP), *Dermatophagoides farina* Hughes (Acari: Pyroglyphidae) (DF), *Blomia tropicalis* (BT) Bronswijk et al., *Acarus siro* Linnaeus (Acari: Acaridae) (AS), *Lepidoglyphus destructor* (Schrack) (LD), and *Tyrophagus putrescentiae* (Schrack) (Acari: Acaridae) (TP) are tested for the allergenic potential through Skin Prick Test (SPT). Three SMs, namely AS, LD, and TP, were newly included in the mite SPT extract for the first time in Kolkata. In total, 330 patients showed significant positive SPT toward any one allergen tested. HD was the major elicitor exhibiting 92.42% response. Individuals of age group 15–40 were the worst sufferers. DF showed the highest sensitization (87.87%) among the dust mites. The SMs also contributed significantly to prove their sensitizing potential. SPT rates for AS, LD, and TP were 33, 25, and 18%, respectively. SPT grades and total Immunoglobulin E (IgE) were positively correlated for each of the allergens. Most of the patients were multi-sensitized (95%) and represented markedly high total IgE levels (>500 IU/ml). Three SMs proved to be significant allergens for the studied population. The sensitization toward these SMs is first time reported from India and can be recommended for inclusion of routine SPT for better outcome in the future.

Key words: house dust, house dust mite, storage mite, Kolkata, skin prick test

There has been a surge of nasobronchial allergic diseases around the world and particularly allergic rhinitis (AR) is reported to affect 40% population worldwide (Croisant 2014, Bhattacharya et al. 2018). Sensitization to house dust (HD) and its constituent mites is known as the independent risk factor for the development of AR (Antonicelli et al. 2013, Yang et al. 2018). House dust mites (HDMs) are significant allergenic components of the heterogeneous mixture of the HD (Podder et al. 2010). Dust mite sensitization is now considered as common among the Asian atopic population (Tham et al. 2016). Data from most of the Asian countries have reported 60–80% rates in sensitized atopic individuals. In contrast, a much

lower prevalence of HDM sensitization (21.7%) is recorded in the European population (Bousquet et al. 2007). A large multicenter study from the United States also described only 20% dust mite sensitization (Salo et al. 2014). *Dermatophagoides pteronyssinus* (DP), *Dermatophagoides farina* Hughes (Acari: Pyroglyphidae) (DF), and *Blomia tropicalis* (BT) are the prevalent HDM species connected to allergic diseases worldwide, whereas *Acarus siro* Linnaeus (Acari: Acaridae) (AS), *Lepidoglyphus destructor* (LD), and *Tyrophagus putrescentiae* (Schrack) (Acari: Acaridae) (TP) are known to be storage mites (SMs) reported from Asian homes (Zhang et al. 2012). Different species of dust mites are reported as significant allergens

from India among which DP dominates (Tham et al. 2016). Overall HDM sensitization rate of South, North, and Western regions of India is 89.7, 7.8, and 47% (Lal et al. 2011, Raj et al. 2013, Doshi and Tripathi 2016). From Eastern India (Kolkata metropolitan) DP, DF, and BT are already documented as significant allergens eliciting 75, 63.7, and 72%, respectively (Podder et al. 2010).

Both HDMs and SMs are known to co-exist (van der Heide et al. 1998). Sensitization toward SMs has been reported by researchers from different parts of the world (Vidal et al. 2004, Yadav et al. 2006). However, data on SM sensitization and exposure in India is scarce. We have aimed to depict a recent scenario of HDM and SM sensitization and its impact on the local population of Kolkata metropolitan. We tried to assess dust mite allergy in patients visiting a local allergy clinic (Allergy and Asthma Research Center) in Kolkata metropolitan, India through routine Skin Prick Test (SPT). The quantitative study of the collected dust samples from bed and floor of the patients' home exposed a total of six mite species: DP, DF, BT, AS, TP, LD. Three SMs namely AS, TP, and LD, in spite of being reported as significant allergens from other parts of the world, were not included in the SPT extracts used in Kolkata, not even in India. Our study delineates the overall pattern of HD and its constituent dust sensitization among the population of Kolkata megacity along with the inclusion of three SMs as offending allergens to the existing mite allergen list.

Material and Methods

Approval of Ethics Committee

The study was performed with the approval of the Clinical Research Ethics Committee, Allergy and Asthma Research Center, West Bengal, India (CREC–AARC Ref: 004/17). Written informed consents were taken from the participants.

Study Subjects

In total, 372 patients that visited the Allergy and Asthma Research Center, Kolkata with clinically diagnosed symptoms (namely AR, bronchial asthma (BA), atopic dermatitis (AD), and conjunctivitis) was included in this prospective study (August 2017 to July 2018). Among them, 198 (53.22%) were males and 174 (46.77%) were females. Patients were categorized into three age groups: <15 yr (median age 12.0 yr), 15–40 yr (median age 28.0 yr), and >40 yr (median age 45.5 yr). An initial Questionnaire survey was done that includes the record of the demographical and clinical history of the patients. Pregnant/lactating women and individuals suffering from consistent/infectious diseases were not included in this study. Anti-allergic and steroid drugs were prohibited 72 h prior to the skin testing.

Dust Collection and Processing

Bed dust samples were collected from 43 patients and 15 control houses. Bed dust was collected by dusting the mattresses, bed linens, and pillows, labeled properly and freezing to avoid mite multiplication. Next, the samples were pooled by passing through a set of sieves of decreasing mesh size (2.36 mm, 1.00 mm, 500 µm, 75 µm, 45 µm) in a mechanical sieve shaker for 20 min. The portion of dust that was retained in the sieve 75 µm and 45 µm mesh size were processed following the flotation method of ChannaBasavanna et al. (1984). One gram of each dust sample was mixed with pure kerosene oil and stirred constantly for 10 min in vortex mixture. The mixture was centrifuged at 2,000 r.p.m. for 2 min and the supernatant was filtered using Whatman No.1 filter paper. A mixture of kerosene oil and carbon tetrachloride with specific gravity 1.3 was added to the sediment in the tube and after centrifugation, it was filtered on the

same filter paper. This process was repeated twice with a mixture of kerosene oil and carbon tetrachloride with specific gravity 1.4 and 1.5, respectively. The supernatant was filtered again and the residue collected on the filter paper was washed with a fine jet of 70% alcohol and transferred to a Petridish.

Isolation and Identification of Mites

Mites were picked with a hair brush and mounted in Hoyer's medium. Mounted slides were dried at 40°C for 10–15 d. Taxonomic identification was performed according to Krantz and Walter (2009). The number of mites present in per gram of dust samples was counted using previously described methods (Krzysztof 2011).

Skin Prick Test

SPT was performed (Bousquet et al. 2012) on the patients using six mite allergen and HD extract obtained from MERCK. Briefly, skin prick was done on the flexor side of the forearm of each patient and results were recorded after 15 min. Histamine (10mg/ml of histamine phosphate) and 0.9% sterile saline was used as positive and negative controls, respectively. SPT gradation was done as +1, +2, +3, and +4 categories according to the wheal diameter. +1 category signifies <3 mm wheal diameter in comparison to the negative control and were not considered as significant SPT grade. Positive sensitization was recorded from +2 grades (≥3 mm) and onwards (Dey et al. 2014, Dey et al. 2018a).

Measurement of Total IgE

Total serum IgE estimation was performed by Enzyme Immuno Assay (EIA) using chemicals from Pathozyne Immunoglobulin (Ref. OD 417) supplied by Glaxo Smithkline Pharmaceuticals Ltd, Mumbai. The total IgE concentration is directly proportional to the color intensity of the test and the calibration was done according to the WHO (World Health Organization) standard for different age groups (1–5 yr <60 IU/ml, 6–9 yr <90, 10–16 yr <200 IU/ml, and 16+ yr <100 IU/ml) (Hamilton and Adkinson 1997).

Statistical Analysis

One-way ANOVA was employed to determine any significant variation in age groups. Paired *t*-test was used to find gender biases of sensitivity, if any. Spearman rank correlation (two-tailed) was performed between SPT grades and total IgE to find any association between these two factors. The differences among the nonsensitized, mono-sensitized, and multi-sensitized group were estimated through one way ANOVA followed by posthoc Tukey's test. Statistical analysis was performed using Graph Pad Prism software ver. 7 (Graph Pad Prism, San Diego, CA).

Results

Patients' Characteristics Obtained From the Primary Questionnaire Survey

In total, 372 patients with symptoms of AR, BA, AD, and conjunctivitis were enlisted for the determination of total IgE and SPT. AR was the most prevalent symptom to be observed in 58.06% population. Most of the patients suffered from multiple symptoms. A perennial pattern of the occurrence of symptoms was recorded in most (90.86%), majority of whom belonged to the urban population (71.77%) (Table 1).

Individuals within 15–40 yr of age showed 43.28% of sensitivity followed by >40 yr of age (39.25%). Less than 15 yr of age was found to be the least sensitive (17.47%) (Table 1). Age group-wise

variation of patients was significant and is presented through one way ANOVA (Fig. 1). Sex distribution of patients was not found to be statistically significant when tested through a paired *t*-test.

Quantitative Analysis of the Dust Samples and Identification of Mites

For this study, the dust samples were collected from 43 patients' and 15 control houses. To investigate the differences in mite density between patients and control houses, unpaired *t*-test was performed.

Table 1. Demographical and clinical characteristics of the patient

Age group	
<15 yr (median: 12 yr)	65 (17.47%)
15–40 yr (median: 28 yr)	161 (43.28%)
>40 yr (median: 45.5 yr)	146 (39.25%)
Gender	
Males	198 (53.22%)
Females	174 (46.77%)
Symptoms	
Airway	
AR	216 (58.06%)
BA	89 (23.92%)
Contact	
AD	68 (18.28%)
Conjunctivitis	63 (16.93%)
Pattern of attack	
Seasonal	34 (9.14%)
Perennial	338 (90.86%)
Residence	
Urban	267 (71.77%)
Semi-urban	74 (19.89%)
Rural	31 (8.33%)

AR (allergic rhinitis), BA (bronchial asthma), AD (atopic dermatitis).

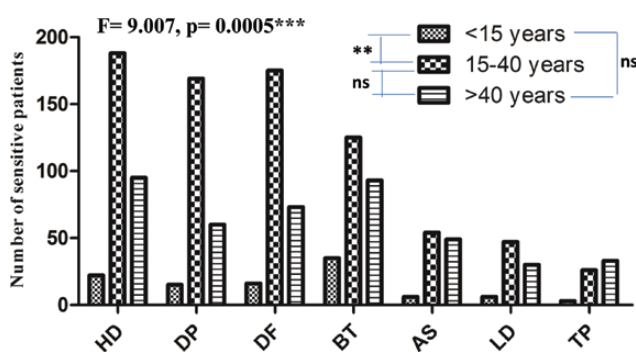


Fig. 1. Age group-wise sensitivity presented through bar diagram. Any significant differences between three age groups are indicated by one-way ANOVA.

Results revealed that almost all the dust samples were positive for the presence of acarine fauna. The allergenic mites namely *Dermatophagoides pteromyssinus*, *D. farinae*, *Blomia tropicalis*, *Acarus siro*, *Lepidoglyphus destructor*, and *Tyrophagus putrescentiae* were isolated from the dusts. The average number of all mites per gram of dusts in patients and control houses were 275.74 ± 37.70 and 44.66 ± 12.89 , respectively. The density of six mite species of interest namely DP, DF, BT, AS, LD, and TP, in both patients and control subjects were identified as significant fractions of the HD samples (Table 2). The mite density between the patient and control houses were significant ($P < 0.0001^{***}$) (Table 2).

Determination of HD and Dust Mite Sensitization Through SPT

Sensitization toward HD and dust mites was noticeably strong. In total, 330 patients (88.70%) showed significant positive SPT response toward either of the HD/dust mite allergens. HD (92.42%) was the highest sensitizer among the tested allergens. Substantial sensitivity was evident from DP (81.21%) and DF (87.87%), respectively, among the tested individuals followed by BT (74.24%). Interestingly, three other mite extracts elicit positive SPT in significant proportions of the study population. Among them, AS triggered 33.03% sensitivity followed by LD (25.15%). TP exhibited comparatively less allergenic response (18.78%) (Fig. 2). Gradation of skin reaction against HD and six dust mite allergens are illustrated in Table 3. +2 grades were found in maximum patients for all the tested allergens.

Determination of Total IgE and Correlation With SPT Grades

Total IgE values of all the patients are categorized into three categories: Low (<50 IU/ml), Moderate (50–500 IU/ml), and High (>500 IU/ml) and presented in Table 4. High level of total IgE was found in most of the patients (84.67%). A strong positive correlation was found between SPT grades and tIgE values for each allergen (Table 3). Level of significance (*P*-value, two-tailed) and Spearman *r* values are presented in Table 3.

Association of Sensitization Rate with Total IgE Values

Among the 330 sensitive patients, 315 showed multi-sensitization toward HD and dust mite allergens. Mono-sensitization appeared only in 4.5% of patients and most of the 4.5% patients were sensitive toward HD allergen. When the association of patients' sensitivity through SPT is investigated with total IgE values, the number of nonsensitized (SPT negative/+1SPT), mono-sensitized (>+2 SPT for single allergen), and multi-sensitized (>+2 SPT for more than one allergen) patients varied significantly when tested with one-way ANOVA ($F = 57.50$, $P < 0.0001^{***}$). Distribution of patients is shown based on the total IgE values in Fig. 3.

Table 2. Distribution and density of TMC, DP, DF, BT, TP, LD, and AS mites in patients and control houses (no. of mites per gram of dust)

Patient (N = 43)	TMC	DP	DF	BT	TP	LD	AS
	275.74 ± 37.70	62.53 ± 20.8	45.93 ± 26.36	47.11 ± 25.2	31.37 ± 21.58	23.88 ± 15.97	23.76 ± 16.25
Control (N = 15)	44.66 ± 12.89	15.86 ± 5.96	7.86 ± 5.23	5.73 ± 4.47	4.46 ± 3.83	3.33 ± 2.71	4.46 ± 2.92
Significance	$P < 0.0001^{***}$	$P < 0.0001^{***}$	$P < 0.0001^{***}$	$P < 0.0001^{***}$	$P < 0.0001^{***}$	$P < 0.0001^{***}$	$P < 0.0001^{***}$

TMC (total mite count).

*** denotes *P*-value of <0.0001 and values are significant.

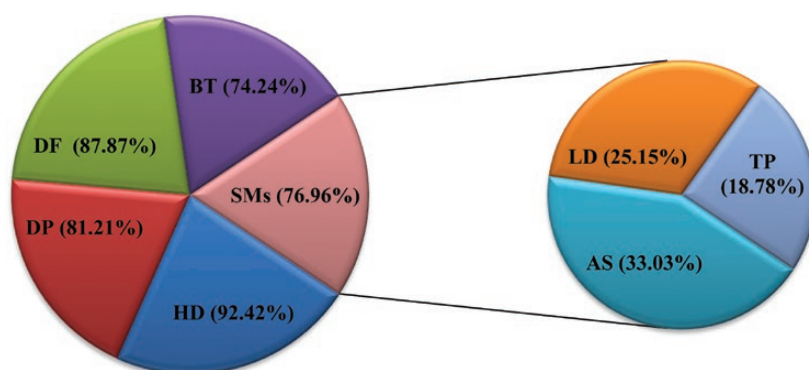


Fig. 2. Percentages of SPT response shown by the patients toward HD, HDMs, and SMs.

Table 3. Gradation of SPT positivity among the patients and correlation with total IgE

Allergens	SPT grades			Total number of sensitive patients	Correlation with tIgE	
	+2	+3	+4		Significance	Spearman <i>r</i>
HD	220 (72.13%)	68 (22.3%)	17 (5.57%)	305	$P < 0.0001^{***}$	0.4101
DP	163 (60.82%)	65 (24.25%)	40 (14.92%)	268	$P < 0.0001^{***}$	0.6051
DF	208 (71.72%)	51 (17.58%)	31 (10.7%)	290	$P < 0.0001^{***}$	0.4962
BT	137 (55.9%)	61 (24.9%)	47 (19.2%)	239	$P < 0.0001^{***}$	0.6502
AS	94 (86.24%)	15 (13.76%)	0	109	$P = 0.0003^{***}$	0.3436
LD	76 (91.56%)	7 (8.43%)	0	83	$P = 0.0089^{**}$	0.2855
TP	54 (87.1%)	8 (12.9%)	0	62	$P < 0.0001^{***}$	0.0528

*** denotes *P*-value of <0.0001 and values are significant.

Table 4. Total IgE levels found in the patients

Total IgE	Mean	Number of patients
Low (<50 IU/ml)	38 IU/ml	42 (11.29%)
Moderate (50–500 IU/ml)	310 IU/ml	15 (4.03%)
High (>500 IU/ml)	1,580 IU/ml	315 (84.67%)

Discussion

Pyroglyphid mites (*Dermatophagoides* spp.) have been well documented to elicit allergic response (Gafvelin et al. 2001, Zeal et al. 2005). The nonpyroglyphid mites, or SMs, are also clinically significant (van Hage-Hamsten et al. 1998). Since the nonpyroglyphid mite species are present in both temperate and subtropical regions, a large number of individuals are exposed. Studies from different countries have shown that IgE-mediated allergy in rural populations is prominent and SMs are reported to be major allergens (Bostrom et al. 1997). SMs like TP, AS, and LD have been reported to cause occupational allergy among farmers, bakers, and grain workers (Blainey et al. 1989, Revsbech and Dueholm 1990) and also affect individuals from an urban environment without occupational exposure (Zhang et al. 2012). Identification and characterization of several SM allergens have been reported and limited allergenic cross-reactivity between storage and HDMs is also depicted (van Hage-Hamsten et al. 1998). To add some new insights on HDM and SM allergy, we started our investigation with a cohort of patients that visited an allergy clinic with major complaints of AR. The scheme of work we employed was to evaluate the mite diversity in the HD of the patients' home and the allergenic potential of the dominant mites was tested through SPT. This study was first of its kind to depict the rate of HD, HDMs, and SMs allergenicity through clinical techniques followed by their quantitative estimation.

The highest densities of dust mites are generally found in beds and floors, rather than elsewhere in houses, where shed skin scales are mostly accessible as a food source (Miller 2018). Following this fact, we tried to analyze the bed and floor dust from patients' home. The collected dust samples were probed for isolation and identification of mites. The dominance of DP, DF, and BT in the HD was not surprising as these mites were already reported to be a considerable fraction of HD from different countries throughout the world including India. But we found three SMs (TP, AS, and LD) from the dust samples that are not previously reported from India. Hence, we designed our next line of work to assess the allergenic potential of all the six mites in the studied population that would represent the current HD and dust mite sensitivity of Kolkata population. We used SPT as the key technique to evaluate the rate of HD and dust mite sensitization. The highest sensitization rate showed by HD extract was inevitable as HD is reported to be a heterogeneous mixture of substances of both plant and animal origin and a similar rate of sensitivity was depicted previously from Kolkata population (Podder et al. 2009, 2010). When we looked into the SPT reactivity of the six dust mites, DF and DP appeared to dominate and this result is comparable with previous reports (Dutta et al. 2017, Ghosh et al. 2018, Dey et al. 2018b). For BT, a single report from Kolkata metropolitan is available with 72% skin prick reactivity (Podder et al. 2010). We found 74.24% BT sensitivity that supports the previous observation. Next, we focussed on the fact whether the SMs elicit significant SPT response in the tested individuals. AS, LD, and TP caused 33, 25, and 18% SPT response, respectively, that were reported for the first time in Kolkata metropolitan. Elevated skin sensitization was found in HDMs than the SMs probably due to the dominance of HDMs in the indoor environment. This result was comparable with a previous study on a Chinese population (Zhang et al. 2012).

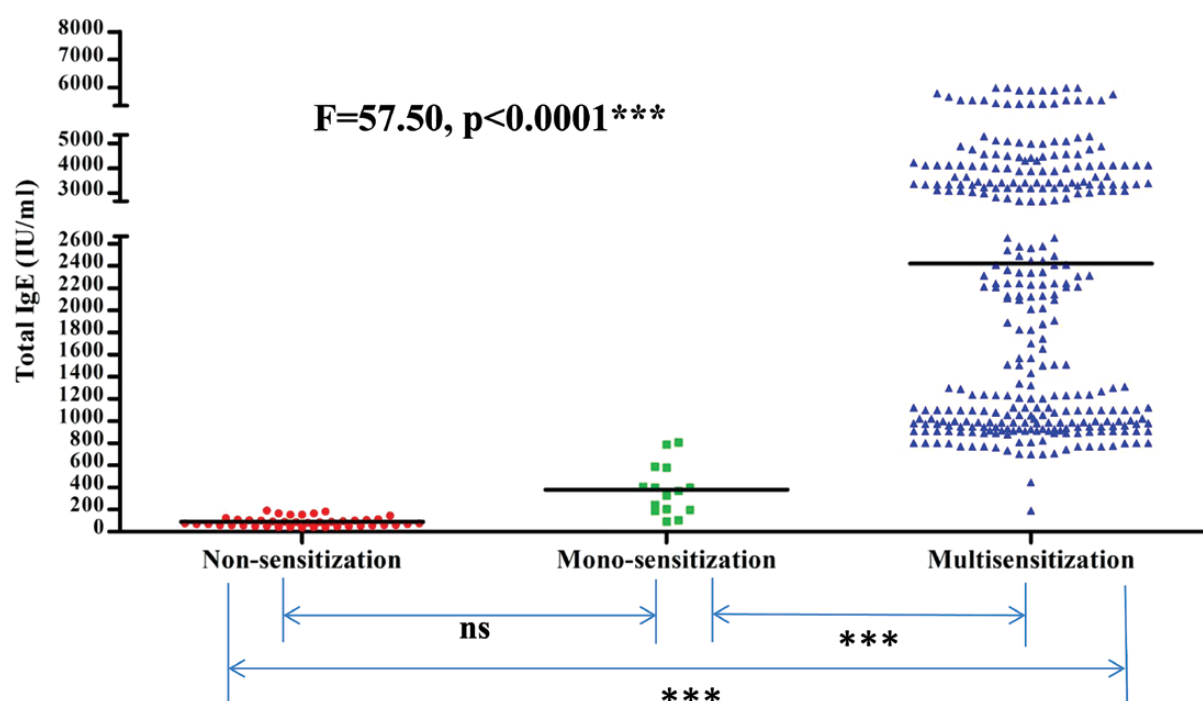


Fig. 3. Influence of total serum IgE with the sensitization pattern (whether nonsensitized, mono/multi-sensitized) toward HD and dust mites.

Perennial AR was found to be mostly common than is usual for HDM allergy patients from other populations studied before (Sheikh et al. 2010, Ghosh et al. 2018). The suffering of the young population is not uncommon in comparison with others findings (Dey et al. 2014, Dey et al. 2016). Patients of Kolkata metropolitan mostly represents a tropical urban population that is expected to show a high percentage of HDM allergy due to environmental effect. High HDM sensitivity is reported from tropical countries such as Hong Kong, Malaysia, Thailand, Indonesia, and Vietnam (Tham et al. 2016). Even migrated human population is reported to reinforce the effect of geographical determinants of HDM sensitization (Han et al. 2009). A group of migrated population from China to Singapore gradually become affected with increasing sensitization toward HDM allergens after 8 yr of residing in Singapore and eventually the sensitization rate became similar to Singapore-born individuals (Andiappan et al. 2014). As predominant indoor allergens, HD and HDMs are expected to cause pronounce effect in population adapting to urbanization that is associated with the altered lifestyle pattern toward the indoor (Han et al. 2009). This could probably explain the high rate of HD and HDM sensitization in our studied population. We found multi-sensitization in most of the patients rather than mono-sensitization. Multi-sensitization being common to many atopic populations (Andiappan et al. 2014) was predictable for this study.

A high level of serum total IgE is often considered as the general tendency to develop allergic diseases, whereas SPT detects sensitization toward a particular allergen. When we tried to explore any correlation between total IgE and SPT grade, a strong positive correlation was found in HD and dust mite-sensitized individuals.

In conclusion, the occurrence of sensitization to SMs along with HDMs seems to be soaring in the present study. This may be due to high indoor humidity and the presence of grain storage in the bed-room of patients' home seem to play a most pivotal role for SM sensitization. However, further studies are warranted to find out whether this phenomenon is due to multiple sensitization or to cross-reactivity with the other three HDMs and it is strongly recommended

for the inclusion of three SMs (AS, LD, and TP) in the SPT catalogue of Kolkata city for better outcome.

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