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House dust mite allergens and nitrated products: Identification and risk assessment in indoor dust

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ABSTRACT

Air pollutants can potentially lead to nitration of allergic proteins, thus promoting sensitization of these allergens. However, little is currently known about the nitration status of house dust mite (HDM) allergens. We identified the occurrence of nitrated products of two major HDM allergens Der f 1 and Der p 1 in dust samples collected from college dormitories in eastern China and assessed their associated health risk. The results showed that both non-nitrated and nitrated forms of the two allergens were detected in the dust in the range of non-detected (ND)-10.6, 1.44-15.4, ND-22.4, ND-7.28 μg/g for non-nitrated Der f 1, nitrated Der f 1, non-nitrated Der p 1 and nitrated Der p 1, respectively. The median rates of nitration were determined as 74.0% for Der f 1 and 20.4% for Der p 1 at consideration of one nitration site. Further analysis reveals that the levels of HDM allergens and their nitrated products were found to be generally higher during winter, in dormitories of lower altitude and with female occupants. Furthermore, the calculated risk indexes were at considerably high levels. Our findings suggest that nitrated HDM allergens have already accumulated in the environment at such significant levels and their associated health risk calls for our immediate attention.

Introduction

House dust mites (HDMs) have been known as a leading source of inhalation allergens globally for several decades, causing respiratory allergic diseases and skin type I allergic reactions (Sharma et al., 2019; Wilson and Platts-Mills, 2018). Moreover, HDMs, such as *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* have been considered as the main source of allergens in indoor dust, whose protein components are believed to be the key contributors to allergies (Zhan et al., 2015). Today, more than 36 groups of HDM allergens have been identified and listed (Pang et al., 2019). Among these, group I allergens, Der f 1 for *D. farinae* and Der p 1 for *D. pteronyssinus*, are further identified as the major sensitizing components of HDMs. Up to 50% of allergic patients have allergic reactions to Der f 1 and Der p 1 (Fernández-Caldas et al., 1997). And previous biochemical studies have showed that Der f 1 and Der p 1 display high binding affinities toward immunoglobulin E (IgE) in the majority (70%-80%) of patients who are allergic to HDMs (Thomas et al., 2004).

Preliminary studies have suggested that the aggravation of air pollution causes the increasing prevalence of allergic diseases, a process thought to be particularly relevant to nitration of allergens induced by atmospheric pollutants (Gruijthuijsen et al., 2006). Protein nitration mainly refers

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to the nitration reaction of tyrosine residues in polypeptide chain to generate 3-nitrotyrosine (Selzle et al., 2013). It has been observed that allergens including pollen are nitrated under urban smog conditions (Franze et al., 2005). Meanwhile, laboratory investigations have found that the rates of nitration for proteins are governed by the abundance of both NO_2 and O_3 in synthetic gas mixtures (Franze et al., 2005; Reinmuth-Selzle et al., 2014; Selzle et al., 2013). Consequently, immune experiments revealed that nitration of pollen allergens enhanced their allergenic activity (Frank and Ernst, 2016; Gruijthuijsen et al., 2006; Karle et al., 2012). The presence of nitrated allergens of pollen has been detected in urban road dust, window dust and air particulate matters (Franze et al., 2005). Nitration of allergens induced by NO_2 and O_3 may be one of molecular rationales for air pollution e.g., traffic-related pollution and summer smog pollution, promoting allergic diseases. So far, no studies have been reported on the nitration of HDM allergens, however, which are highly prevalent in human populations.

Rapid industrialization has caused numerous environmental issues such as air pollution worldwide. Recognizing such negative consequences, a series of environmental protection policies and measures have been put in place in China, which have since led to significant improvement in air quality in the past years. While many atmospheric pollutants indicators, such as PM_{10}, $PM_{2.5}$, and SO_2, are sharply decreasing, the opposite trend has been found of O_3 (Ito et al., 2018). Considering the potential sensitizing effect of O_3 and NO_x on various airborne allergens, therefore, studies that evaluate the nitration of HDM allergens and the associated health risks are in urgent need.

Due to their typically high density of occupants and less-than-ideal ventilation conditions, dormitories represent a major breeding environment for HDMs (Wang et al., 2009). However, up to date, little is known concerning the current status of residue and nitration of HDM allergens in dormitories. In this study, we investigated two major HDM allergens, Der f 1 and Der p 1 and their nitrated products in dust samples collected from college dormitories in Eastern China using liquid chromatography-triple quadrupole tandem mass spectrometry (LC-MS/MS) to reveal the residual levels, nitration status as well as their associated health risks of these allergens.

1. Materials and methods

1.1. *Experimental materials*

Standards of HDM allergen Der f 1 (non-nitrated) and Der p 1 (non-nitrated) (95%) were purchased from MyBioSource (San Diege, CA, USA). Peptide and isotope labeled peptide were commercially synthesized by Qiangyao Biochem Ltd. (Suzhou, China). The information of other reagents or materials is listed in supporting information (**Appendix A Table S1**).

1.2. *HDM allergens digestion and selection of signature peptides*

Standards of Der f 1 and Der p 1 were digested by trypsin according to our previous method (Yang et al., 2015) and analyzed by ultra-high performance liquid chromatography-electrospray ionization-triple quadrupole tandem mass spectrometer (Xevo TQ-S, Waters, Milford, MA, USA) as LC-MS/MS offers high sensitivity, specificity and throughput in protein analysis compared to traditional bioassays (Marx, 2013). The details of digestion and pretreatment for Der f 1 and Der p 1 are described in supporting information (**Appendix A Section S1**). The information of instrumental analysis of HDM allergens for allergen standards or dust is also given in supporting information (**Appendix A Section S2**). The ion peaks of peptides from trypsin digestion of each standard were screened to identify signature peptide for the allergen (**Appendix A Table S2**). Finally, the peptides AFQHYDGR (AR-8) and SYATFEDEEAAR (SR-12) with the most abundant signal were selected as the signature peptide for Der f 1 (non-nitrated) and Der p 1 (non-nitrated), respectively (**Appendix A Fig. S1**). The specificity and representativeness of these signature peptides were checked and affirmed in www.uniprot.org/peptidesearch and www.peptideatlas.org. There is a tyrosine residue in both AR-8 and SR-12. Similarly, the nitrated peptides NO_2-AR-8 and NO_2-SR-12 were selected as the signature peptides for nitrated Der f 1 and nitrated Der p 1, respectively. The signature peptides (AR-8, SR-12, NO_2-AR-8 and NO_2-SR-12) and isotope-labeled signature peptides ($^{13}C_6{}^{14}N_4$-AR-8 and $^{13}C_6{}^{14}N_4$-SR-12) as internal standards were then commercially synthesized.

1.3. *Sample collection and analysis*

120 dust samples were collected from dormitories with similar area in the same building in a college located in eastern China to cover the difference of season, floor and gender. 10 samples for female dormitories and 10 samples for male dormitories were collected at the first, fourth and sixth floor (the top floor) in summer (August) and winter (December), respectively. The dust collected from the same room was pooled. After discharging of coarse grains of sand, paper scraps and hair, the dust samples were wrapped in tin foil and transported to the laboratory for storage at -20°C. The details for dormitories and sampling are described in supporting information (**Appendix A S3**).

About 0.7 g for each sample was weighed and about 7 mL of phosphate buffer containing 1% BSA and 0.05% Tween-20 (1% BSA-PBST) was added at a ratio of 1:100 (W/V). After ultrasonic grinding for 2 hr at room temperature, the sample was oscillated (100 r/min) overnight at 4°C. The sample was then centrifuged at 5600 r/min (~3000 $\times g$) and 4°C for 15 min. The supernatant was filtered through a 0.22 μm hydrophilic membrane for analysis of protein concentration by bicinchoninic acid protein assay kit. Dust sample extract containing 400 μg total protein was then transferred into a 1.5 mL protein LoBind tube and 100 μL of isotope-labeled internal standard solution (dissolved in 5% acetonitrile/water (V/V), 20 ng/mL for each internal standard) was added. Then 200 μL of acetonitrile and 200 μL of 50 mmol/L NH_4HCO_3 buffer were added. The following digestion procedure and instrumental analysis were the same as HDM allergen standards mentioned above but the volume of all adding solvents was fortyfold, except the residue was still re-dissolved in 200 μL of 5% acetonitrile/water (V/V) solution.

1.4. Nitration rates of HDM allergens

The nitration fraction of Der f 1 or Der p 1 was indicated by nitration rate (NR). In the present study, NR refers to the nitration degree of the Tyr residues in the signature peptide, which can be defined as the amount of the nitrated peptide divided by the sum of the amount of the nitrated and non-nitrated peptides, and calculated as follows:

For Der f 1 (AR-8) according to Eq. (1):

$$NR_{AR-8} = \frac{\frac{A_{nitrated\ AR-8}}{MW_{nitrated\ AR-8}}}{\frac{A_{nitrated\ AR-8}}{MW_{nitrated\ AR-8}} + \frac{A_{non-nitrated\ AR-8}}{MW_{non-nitrated\ AR-8}}} \quad (1)$$

For Der p 1 (SR-12) according to Eq. (2):

$$NR_{SR-12} = \frac{\frac{A_{nitrated\ SR-12}}{MW_{nitrated\ SR-12}}}{\frac{A_{nitrated\ SR-12}}{MW_{nitrated\ SR-12}} + \frac{A_{non-nitrated\ SR-12}}{MW_{non-nitrated\ SR-12}}} \quad (2)$$

where, NR_{AR-8} is the nitration rate of peptide AR-8; $A_{nitrated\ AR-8}$ (μg) is the amount of nitrated AR-8; $MW_{nitrated\ AR-8}$ is the molecular weight of nitrated AR-8; $A_{non-nitrated\ AR-8}$ (μg) is the amount of non-nitrated AR-8; $MW_{non-nitrated\ AR-8}$ is the molecular weight of non-nitrated AR-8. NR_{SR-12} is the nitration rate of peptide SR-12; $A_{nitrated\ SR-12}$ (μg) is the amount of nitrated SR-12; $MW_{nitrated\ SR-12}$ is the molecular weight of nitrated SR-12; $A_{non-nitrated\ SR-12}$ (μg) is the amount of non-nitrated SR-12; $MW_{non-nitrated\ SR-12}$ is the molecular weight of non-nitrated SR-12.

1.5. Risk assessment

The risks of the HDM allergens and their nitrated products in the dust samples were assessed based on an interim criterion established at the International Conference on Dust Mites and Asthma convened by World Health Organization (WHO, 1988). More than 2 μg Der p 1 (non-nitrated)/g dust is enough to cause allergic symptoms and identified as a risk threshold for sensitization and asthma, while more than 10 μg Der p 1 (non-nitrated)/g dust is enough to induce acute or more serious clinical symptoms of asthma for people who are allergic to dust mites. There was no threshold raised for Der f 1 yet. Alternatively in practice, the threshold of Der p 1 was often employed to assess the risk of Der f 1 due to the homology of these two allergens (Winn et al., 2016). However, the risk of nitrated HDM allergens was not considered and included in the criteria as the nitrated HDM allergens were not reported and studied then. The criterion of Der p 1 was also employed to assess the risks of nitrated Der f 1 and nitrated Der p 1 in the present study. To better understand the risk of the allergens, a risk index (RI) was defined and calculated based on the criteria (2 and 10 μg Der p 1 (non-nitrated)/g dust) recommended by WHO according to Eq. (3):

$$RI = \sum C_{given\ allergen(s)}/2 \quad (3)$$

where, $C_{given\ allergen(s)}$ (μg/g dust) is the concentration of given allergen(s).

When RI < 1, it means that the concentration of allergen/s is less than 2 μg/g dust, and the risk is at low level; when $1 \leq RI < 5$, it means that the concentration of allergen/s is between 2 and 10 μg/g dust, and the risk is at medium level; when $RI \geq 5$, it means that the concentration of allergen/s is higher than 10 μg/g dust, and the risk is at high level.

1.6. Statistics

The statistical software of SPSS 20.0 was used for statistical analysis. Non-parametric statistical methods were chosen to analyze the data since the concentrations of HDM allergens are not normally distributed in the environment. The allergen levels between different groups were compared by Kruskal-Wallis test and the paired comparisons were performed by Mann-Whitney U test.

2. Results and discussion

2.1. Confirmation of HDM allergens and their nitrated products in dust

The HDM allergens and their nitrated products were detected in most of the dust samples, with detection rates ranging from 88.3% for non-nitrated Der f 1 to 100% for nitrated Der f 1. The concentrations of non-nitrated Der f 1, nitrated Der f 1, non-nitrated Der p 1 and nitrated Der p 1 were detected in the range of non-detected (ND)-10.6, 1.44-15.4, ND-22.4, ND-7.28 μg/g (in dust) (Fig. 1). The level of non-nitrated Der f 1 was significantly lower than that of nitrated protein ($p < 0.001$), while a reverse trend was observed for Der p 1 ($p < 0.001$). The medians of non-nitrated Der f 1, nitrated Der f 1, non-nitrated Der p 1 and nitrated Der p 1 were determined as 1.08, 4.11, 7.90 and 1.88 μg/g, respectively (Fig. 1). For non-nitrated allergen, the level of Der p 1 was higher than that of Der f 1 ($p < 0.001$). For nitrated allergen, however, nitrated Der f 1 was higher than nitrated Der p 1 ($p < 0.001$).

A previous survey on HDM allergens in indoor dust in different cities in China showed that the concentrations of non-nitrated Der f 1 and non-nitrated Der p 1 ranged from < 0.006 to more than 85 μg/g (Zheng et al., 2015). Moreover, it is reported that *D. pteronyssinus* is the dominant species in Eastern China (Chen et al., 2019), suggesting that the content of Der p 1 should be higher than that of Der f 1 in indoor environment, which is confirmed in our study. The occurrences of nitrated products of HDM allergens have not been reported in previous studies. The detection of nitrated Der f 1 and nitrated Der p 1 indicates that the nitrated HDM allergens may be also ubiquitous in indoor environment like their parent proteins; the level of nitrated Der f 1 was even higher than that of non-nitrated allergen. Evidently, the health risks of nitrated HDM allergens should not be neglected.

The accumulation of HDM allergens and their nitrated products were further compared in terms of gender, season and floor among the dormitories (Fig. 2). For gender-specific accumulation, the median concentrations of non-nitrated Der f 1, nitrated Der f 1, total Der f 1, non-nitrated Der p 1, nitrated Der p 1 and total Der p 1 were 1.22, 4.11, 5.96, 8.92, 1.98 and 10.8 μg/g in the female dormitories, and 0.86, 4.17, 5.48, 7.66, 1.77 and 9.18 μg/g in the male dormitories. On the whole, the

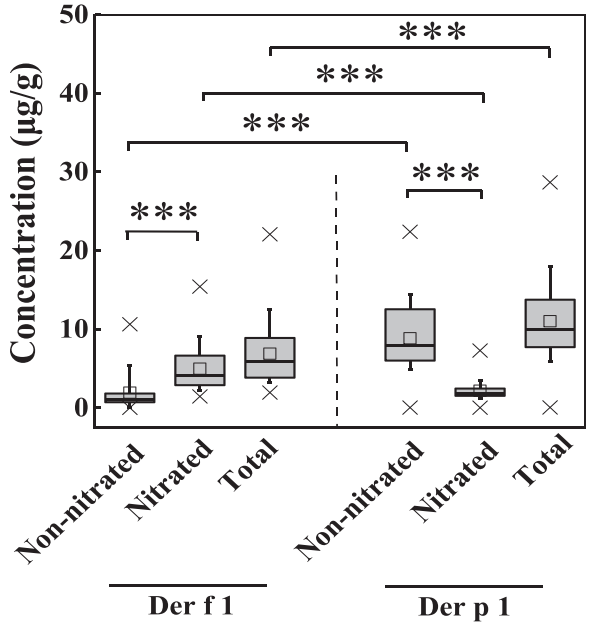

Fig. 1 – Levels of house dust mite HDM allergens and their nitrated products in dust samples. The data are reported as a box/whiskers plot, where the upper, middle, and lower levels of the box represent the 75th, 50th, and 25th percentiles and the whiskers represent the 90th and 10th percentile. □ stands for mean value and × stands for maximum and minimum value. * indicates a significant difference with $p < 0.001$. The number of dust sample is 120.**

accumulation of all types of allergens were higher in the female dormitories. Furthermore, significantly gender-specific differences were found for non-nitrated Der f 1 ($p < 0.05$), non-nitrated Der p 1 ($p < 0.05$) and total Der p 1 ($p < 0.05$) among the six types of allergens.

As far as the season of sampling is concerned, the accumulations of the HDM allergens and their nitrated products were generally higher in the dust samples in winter. The concentrations of non-nitrated Der f 1, nitrated Der f 1, total Der f 1, non-nitrated Der p 1, nitrated Der p 1 and total Der p 1 were detected in the range of 0.40-10.6, 1.58-15.4, 2.35-22.1, 5.32-22.4, ND-7.28 and 7.01-28.7 μg/g with a median of 1.81, 3.56, 6.53, 12.0, 2.22 and 13.8 μg/g in winter. Meanwhile the corresponding concentration ranges were ND-1.72, 1.44-14.5, 1.93-15.4, ND-12.8, ND-3.04, ND-12.9 μg/g with a median of 0.72, 4.73, 5.30, 6.12, 1.72 and 8.01 μg/g in summer, respectively.

It was also observed that the accumulation of HDM allergens and their nitrated products decreased as the floor level increases. Taking non-nitrated Der f 1 for example, the concentration range was detected as ND-10.6, ND-8.46, ND-2.99 μg/g in the first, fourth and sixth floor with a median of 1.36, 1.09 and 0.88 μg/g. As high as a median of 5.79, 7.60, 10.7, 1.77 and 12.6 μg/g was detected for nitrated Der f 1, total Der f 1, non-nitrated Der p 1, nitrated Der p 1 and total Der p 1 at the first floor. When reaching to the sixth floor, the medians decreased to 3.12, 4.05, 7.68, 2.13 and 9.32 μg/g, respectively.

As small arthropods, dust mites prefer to live in warm and humid environment (Acevedo et al., 2019). The mean indoor temperatures in dormitories were detected at 26.9°C in winter and 27.3°C in summer (**Appendix A Fig. S3**). No significant difference in the indoor temperature was observed between the two seasons due to the applications of heating devices which actually increased the indoor temperature in the dormitories in winter. The relative humidity was also recorded in the dormitories when sampling and found to be in the range of 60%-70%. Similarly, there was also no significant difference between the two seasons (**Appendix A Fig. S3**). Therefore, the temperature and relative humidity are not considered as the main causes to the difference in concentration of these aller-

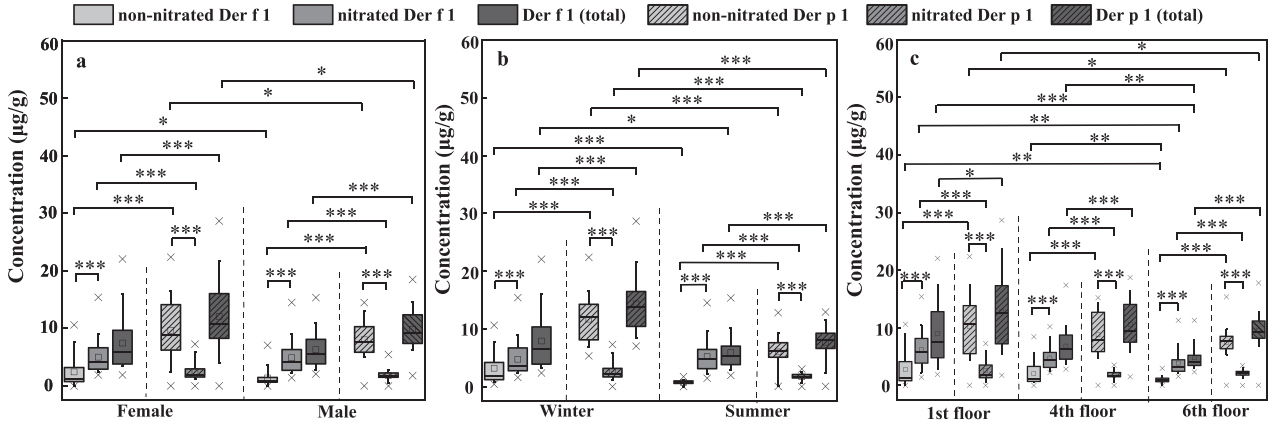

Fig. 2 – Levels of HDM allergens and their nitrated products in dust samples among dormitories in terms of gender (a), season (b) and floor (c). The data are reported as a box/whiskers plot, where the upper, middle, and lower levels of the box represent the 75th, 50th, and 25th percentiles and the whiskers represent the 90th and 10th percentile. □ stands for mean value and × stands for maximum and minimum value. * indicates a significant difference with $p < 0.05$, ** indicates a significant difference with $p < 0.01$ and * indicates a significant difference with $p < 0.001$. $n = 60$ for each gender; $n = 60$ for each season; $n = 40$ for each floor.**

gens between winter and summer. On the other hand, more frequent ventilation and cleaning of clothes and bedding undoubtedly lead to the decrease of dust in indoor environment and are speculated to consequently reduce the accumulation of HDM allergens and their nitrated products in dust in summer. Similar season-specific occurrence of HDM allergens was also reported by Xiang et al. (2013) who found Der f 1 and Der p 1 at highest levels in winter among four seasons.

As for gender-specific occurrence of HDM allergens and their nitrated products in the dust, it was found that these allergens are inclined to accumulate in the female dormitories. It is well known that hair is one of the major feed for HDMs. Therefore, HDMs may incline to live in female dormitories and excrete more allergens. Another possible cause is that more plush toys and other items found in the female dormitories provided good places for the accumulation of dander and other substances which are the main food sources of dust mite.

Floor-specific occurrence of HDM allergens and their nitrated products might be related to the humidity and ventilation conditions in dormitories. In the present study, the means of relative humidity in the dormitories were detected as 68.4% at the first floor, 67.0% at the fourth floor and 64.3% at the sixth floor, respectively (**Appendix A Fig. S3**). Although the relative humidity was close among the floors, obviously, it actually decreased with the increase of floor and was closer to the most suitable humidity for dust mite at the first floor (Acevedo et al., 2019). Previous study on the distribution of dust mites in student dormitories also showed that the detection rates of dust mite was negative with the floors, which decreased from 78.9% at the first floor to 56.5% at the sixth floor (Cui and Ruan, 2014). In our study, the daily ventilation mostly depends on doors and windows since no additional ventilation systems were used in the dormitories. But, the natural air flow is better in higher floor as the building of dormitories is close to other buildings with similar height.

2.2. *Nitration of HDM allergens in dust*

In the present study, the nitration status of Der f 1 and Der p 1 was indicated by nitration of tyrosine residue in AR-8 and SR-12, respectively. The median rates of nitration in all dust samples were determined as 74.0% for Der f 1 (AR-8) and 20.4% for Der p 1 (SR-12) (**Fig. 3**). The nitration rate of Der f 1 (AR-8) was significantly higher than that of Der p 1 (SR-12) ($p < 0.001$). Moreover, the nitration rates of Der f 1 (AR-8) were significantly higher than those of Der p 1 (SR-12) at gender- ($p < 0.05$), season- ($p < 0.001$) and floor-specific ($p < 0.01$) levels. The results also showed that the nitration degree of Der f 1 (AR-8) was higher in the male dormitories compared to in the female dormitories and in summer compared to in winter, although such significant difference was not observed for Der p 1 (SR-12).

Previous work has identified the dependence of tyrosine nitration degree on their locations in protein. Walcher et al. found that nitration degrees of tyrosine residues in bovine serum albumin were site-specific when exposure to gaseous NO_2 and O_3 (Walcher et al., 2003). It is generally considered that the tyrosine residues located the surface of protein are more susceptible to nitration by NO_x and O_3 due to their easy

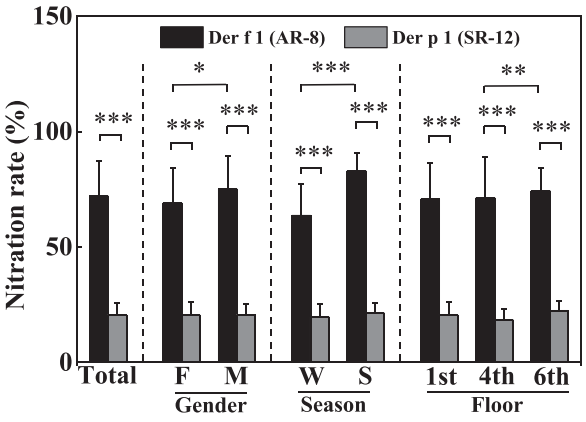

Fig. 3 – Nitration rates of Der f 1 (AR-8) and Der p 1 (SR-12) in dust samples among dormitories in terms of gender, season and floor. * indicates a significant difference with $p < 0.05$, ** indicates a significant difference with $p < 0.01$ and * indicates a significant difference with $p < 0.001$. $n = 60$ for each gender; $n = 60$ for each season; $n = 40$ for each floor. F: female; M: male; W: winter; S: summer.**

accessibility (Bayden et al., 2011). Since the tyrosine residues in both AR-8 and SR-12 appear to locate near the surface of the corresponding protein (**Appendix A Fig. S4**), it is uncertain whether the location of tyrosine is the major cause for the difference of nitration degree or not for the two allergens in the present study.

The production of nitrated allergens in natural environment is related to NO_x and O_3. Between the two species, O_3 is considered to play a more significant role during the nitration of protein (Franze et al., 2005). In our study, the nitration degree was found at higher level for both Der f 1 and Der p 1 in summer compared to in winter. The concentrations of NO_x and O_3 were not continuously monitored in the dormitories although the dust accumulated for at least 2-3 months. According to the records of airborne pollutants released by local environmental department, the outdoor concentration of O_3 in summer was higher than that in winter (**Appendix A Fig. S5**), while the concentration of NO_2 is just the opposite (**Appendix A Fig. S6**). Although the outdoor concentrations of NO_x and O_3 are not equal to their levels in the dormitories, the indoor concentrations of NO_x and O_3 should depend on the outdoor levels due to the air exchange between outdoor and indoor environment during daily ventilation. Besides, almost none of electronic devices and disinfectants which can produce O_3 was used in the dormitories. So, it also indicates that nitration of Der f 1 and Der p 1 is more dependent on the concentration of O_3 other than NO_2. Moreover, the findings suggest that the means involving O_3 in indoor disinfection may promote production of nitrated allergens, such as ultraviolet light.

Note that, it is known from the amino acid sequences of Der f 1 and Der p 1 that there are 20 tyrosine residues in both proteins. However, only one of them was chosen to indicate the nitration status for each allergen as the only tyrosine in AR-8 or SR-12 was investigated for the two allergens in the study. While ideally this should be done for each tyrosine

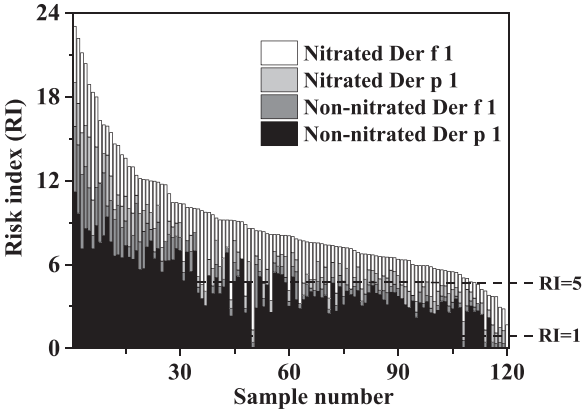

Fig. 4 – Risk index of HDM allergens and their nitrated products in dust samples.

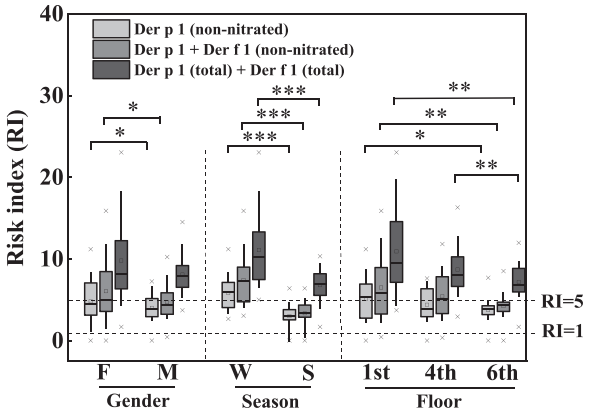

Fig. 5 – Comparison of risk index in dust samples in terms of gender, season and floor. * indicates a significant difference with $p < 0.05$, ** indicates a significant difference with $p < 0.01$ and * indicates a significant difference with $p < 0.001$. $n = 60$ for each gender; $n = 60$ for each season; $n = 40$ for each floor.**

present, such operation is impractical because not all of the peptides containing tyrosine from the allergen could be successfully detected by LC-MS/MS. Despite all this, it is expected that nitration should also occur in other tyrosine residues besides the tyrosine in AR-8 or SR-12. The actual nitration rates of the two allergens are believed to be higher than those reported in the study.

2.3. *Risk assessments of HDM allergens*

The RI values of non-nitrated Der p 1 in the dust samples were calculated in the range of ND-11.2 with a median of 3.95 (Fig. 4). The RIs in 112 out of 120 samples were higher than 1, indicating the risk of non-nitrated Der p 1 was at medium or high level in 93.3% of the dust samples. When non-nitrated Der f 1 was also considered, the RI values ranged at ND-15.9 with a median of 4.55 in the dust samples. Only 8 out of 120 samples were at low level of risk if the non-nitrated Der p 1 and non-nitrated Der f 1 were assessed together, and 41.7% of the dust samples have a RI over than 5. The RI values were calculated in the range of 1.69-23.0 with a median of 8.03 if the four types of HDM allergens were all included. At least medium level of risk was observed in all of the dust samples. Moreover, more than 90% of the samples have a RI value over than 5.

The risks of HDM allergens and their nitrated products were also investigated according to gender, season and floor-specific levels. Higher risks were found in the female dormitories, in winter, and lower floors (Fig. 5). For example, the medians of RI value for the total HDM allergens (non-nitrated and nitrated) were detected as 9.45, 8.07, and 6.81 from the low to high floors.

Previous studies have suggested that nitration of allergens may promote their sensitization (Reinmuth-Selzle et al., 2014), leading to great increase of health risk. Although the risk criteria are not provided for nitrated HDM allergens up to date, the risks of nitrated allergens are believed to be higher than their corresponding non-nitrated allergens at the same level of exposure. Risk assessment has already revealed a high risk for all of the dust (RI higher than 1) at present. Higher risks are expected when the real sensitization of nitrated Der f 1 and nitrated Der p 1 is considered. Moreover, the nitration status of Der f 1 and Der p 1 was analyzed for only one tyrosine in the present study. The other tyrosine in the allergens is believed to be more or less nitrated at the same time. Therefore, the actual nitration degrees of Der f 1 and Der p 1 should be higher. Their risks are thus believed to be underestimated in the study. The criteria for nitrated HDM allergens are urgent to be develop for precise assessment of health risk of nitrated HDM allergens.

3. Conclusions

HDM allergens and their nitrated products were detected in most of the dust samples in the dormitories, indicating the occurrence of nitrated products of HDM allergens in the dust. For Der f 1, the level of nitrated protein is even higher than that of its non-nitrated allergen. The findings suggest nitrated HDM allergens have already accumulated in dust around us at significant levels. The distribution of HDM allergens including non-nitrated and nitrated proteins is gender, season, and floor dependent. In general, the accumulation of HDM allergens is higher in the female dormitories compared to in the male dormitories and in winter compared to in summer. A negative relationship was found between the levels of all types of HDM allergens and the floor. Risk assessment revealed an at least medium level of risk for most of the dust samples based on the criteria raised for non-nitrated Der p 1. The actual risks are expected higher as the sensitization of nitrated HMD allergen is stronger than the corresponding non-nitrated allergen at the same level of exposure.

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Appendix A Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jes.2021.11.004.

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