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**Title: House dust mite sensitization, eczema and wheeze increase risk of shellfish sensitization**

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**Running title: Risk factors of shellfish sensitization**

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**To The Editor,**

Shellfish allergy is more prevalent in South-East Asia (~5%1) than in Western populations (e.g. United States ~2-3%.2). Typically commencing in late childhood, it is the leading cause of anaphylaxis in adolescents and adults in Asia.3

Cross-sectional studies have demonstrated a high correlation between shellfish and house dust mite (HDM) sensitization in warm, humid climates and urban environments where HDM are ubiquitous.4,5 The major shellfish allergen, tropomyosin, shares ~80% structural homology with HDM tropomyosin, and primary sensitization to dust mite tropomyosin has been hypothesized to induce cross-sensitization to shellfish.6

However, no prospective studies have investigated the temporal relationship between primary HDM sensitization and the development of shellfish sensitization/allergy. It is also unclear whether the mite-crustacean cross-reactivity syndrome originates from sensitization through the cutaneous or inhalational route.

 We hypothesized that early life HDM sensitization predisposes to the development of shellfish sensitization in later childhood and aimed to evaluate its temporal relationship with early onset atopic dermatitis (AD) and wheezing in the prospective population-based Growing Up in Singapore Towards healthy Outcomes (GUSTO) mother-offspring cohort in Singapore.7 Data were obtained from interviewer-administered questionnaires at birth, 3, 6, 9, 12, 15, 18 months and ages 2, 3, 4, 5, 6, 7 and 8 years. Skin prick testing (SPT) was conducted at 18 months, 3, 5 and 8 years, which included cow’s milk, egg, peanut and HDM [*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* (Greer Laboratories, Lenoir, NC, USA)and *Blomia tropicalis* (developed in-house8)]at 18 months, 3 and 5 years; and all of the above plus crab and shrimp (Greer Laboratories, Lenoir, NC, USA) at 5 and 8 years. Ethical approval was obtained from the Domain Specific Review Board of Singapore National Healthcare Group and the Centralised Institutional Review Board of SingHealth.

Atopic Dermatitis (AD) was defined as a parental-reported doctor’s diagnosis of eczema at any of the above time-points. Wheeze was defined as positive responses to the questions “Has your child ever wheezed” and “Has your child ever been prescribed with nebulizer/inhaler treatment? at any of the above time-points. HDM sensitization was defined as a positive SPT to any of the 3 house dust mites. Shellfish sensitization was defined as a positive SPT to either crab or shrimp allergen.

Data was analyzed using SPSS Version 26.0 (IBM Corp, New York, NY, USA) for Windows. P values <0.05 were considered significant.

In this study, subjects who completed skin prick testing at 18 months, 3, 5 and 8 years and were not sensitized to shellfish at age 5 years were included in the analysis. Table 1 summarizes the demographic characteristics and allergic disease status of the cohort. The prevalence of HDM sensitization was 10.8% at age 18 months, 21.5% at 3 years and 32.3% at 5 years. The prevalence of shellfish sensitization at 8 years was 6.0%.

In multivariate analysis, HDM sensitization at ages 18 months, 3 and 5 years were associated with increased odds of shellfish sensitization at 8 years (Table 2). AD by 6 months in combination with HDM sensitization at age 18 months [AdjOR 10.1 (95% CI 2.0-50.4), p<0.01] and AD by 5 years with HDM sensitization at 5 years [AdjOR 3.4 (95% CI 1.4-8.6), p<0.01] increased the odds of shellfish sensitization at age 8 years. A trend of association between AD by 3 years in combination with HDM sensitization at 3 years with shellfish sensitization at 8 years was observed (p=0.05). Odds ratio of the eczema + HDM sensitization were greater than eczema alone.

Wheeze by age 3 years [AdjOR 2.7 (95% CI 1.1-6.3), p=0.02], HDM sensitized wheeze by 3 years [AdjOR 4.2 (95% CI 1.4-13.1), p=0.01] and HDM sensitized wheeze by 5 years [AdjOR 2.9 (95% CI 1.1-7.6), p=0.03] were associated with shellfish sensitization at age 8 years. A trend of association between wheeze by 5 years and shellfish sensitization at age 8 years was also observed (p=0.06). Odds ratio of the wheeze + HDM sensitization combination were greater than wheeze alone. Rates of sensitization to other food allergens and shellfish allergy were too low for analysis.

This is the first prospective study in a tropical climate demonstrating that early house dust mite sensitization is a risk factor for development of shellfish sensitization, and early onset AD and wheezing disorders are major co-factors which strengthen this risk.

We hypothesized from these findings that an impaired skin or airway epithelial barrier in early life, such as in early onset AD, and wheezing disorders, may predispose to sensitization to ubiquitous environmental allergens such as food allergens or HDM. Further research is needed to study the mechanism of sensitization. This has been well-established in epicutaneous sensitization in AD. A possible mechanism for airway sensitization is through protease allergens such as Der p 1 which possess cysteine proteinase activity that can cleave structural proteins in airway epithelial tight junctions, facilitating allergen sensitization.9 It could be postulated that environmental exposure to HDM from infancy together with a defective epithelial barrier in the presence of eczema and wheeze, predisposes to acquisition of HDM sensitization and cross-reactivity to shellfish tropomyosins. This hypothesis could partially explain the later onset of shellfish allergies in tropical Asian populations, which contrasts with the earlier onset of milk, egg and peanut allergies in most paediatric populations.

We hypothesize that an impaired airway or skin barrier could predispose to allergic sensitization. However, we did not study gut microbiome dysbiosis, a known risk factor for food allergy development. Gut microbiome dysbiosis precedes and affects food allergen sensitization by regulating intestinal barrier integrity, Th2 immune balance, allergen tolerance and basophil production.10 Results from murine models showed that commensal bacteria such as Clostridia can induce IL-22 production in innate lymphoid cells and T cells to prevent the passage of allergens through the intestinal epithelium barrier and protect against food allergen sensitization.11

This study’s strengths lie in its prospective data collection at multiple time-points which enables the analysis of temporal relationships between exposures and outcomes while adjusting for multiple confounders. Limitations include the lack of shellfish oral food challenges at year 8, and a very low prevalence of reported shellfish allergy in our cohort, which precluded further analysis. This is consistent with the known low prevalence of food allergy in our cohort.12 Studies have shown the sensitivity of shrimp SPT to be around 60-70% and thus shrimp sensitization alone may misclassify some subjects’ true clinical reactivity status.13,14 However, our previous data also indicates that the prevalence of shellfish allergy is very low in early life and only increases in adolescence and young adulthood1, thus follow-up studies are needed to evaluate the relationship between HDM sensitization and shellfish allergy later in life. We have observed temporal associations in this study but definitive causation and its mechanisms require further study. Questionnaire-based assessments of eczema and wheeze may also misclassify disease outcomes compared to physician’s diagnosis and is another limitation of our study. The confidence intervals obtained in this study were wide due to modest sample sizes, hence larger studies will be needed to confirm these findings.

Further research is also needed to evaluate the possible role of early interventions such as environmental measures for reduction of HDM sensitization or immunotherapy against the development of shellfish sensitization and subsequent shellfish allergy.

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**Table 1. Demographic Characteristics and allergic disease status**

|  |  |
| --- | --- |
| **Demographics**  | **N (%)** **N=536**  |
| Male | 272 (50.7) |
| Ethnicity |  |
| Chinese | 307 (57.3) |
| Malay | 144 (26.9) |
| Indian | 85 (15.9) |
| Caesarean delivery | 163 (30.4) |
| Maternal tertiary education  | 378 (71.1) |
| Maternal history of allergy | 204 (38.9) |
|  |  |
| **House Dust Mite (HDM) sensitization status** |  |
| Any HDM sensitization @ month 18 | 58 (10.8) |
| *D. Pteronyssinus* | 44 (8.2) |
| *D. Farinae* | 34 (6.3) |
| *B. Tropicalis* | 5 (0.9) |
| Any HDM sensitization @ month 36 | 115 (21.5) |
| *D. Pteronyssinus* | 101 (18.8) |
| *D. Farinae* | 78 (14.6) |
| *B. Tropicalis* | 12 (2.2) |
| Any HDM sensitization @ year 5 | 173 (32.3) |
| *D. Pteronyssinus* | 157 (29.3) |
| *D. Farinae* | 139 (25.9) |
| *B. Tropicalis* | 36 (6.7) |
|  |  |
| **Shellfish sensitization at 8 years** | 32 (6.0) |
|  |  |
| **Atopic dermatitis (AD) status** |  |
| AD by 6 months of age | 35 (7.2) |
| AD by 6 months + HDM sensitized at 18 months | 7 (1.4) |
| AD by 3 years of age | 106 (23.7) |
| AD by 3 years + HDM sensitized at 3 years | 35 (7.8) |
| AD by 5 years of age | 116 (26.9) |
| AD by 5 years + HDM sensitized at 5 years | 58 (13.4) |
|  |  |
| **Wheeze status** |  |
| Wheeze by 18 months | 56 (12.5) |
| Wheeze + HDM sensitized at 18 months | 9 (2.0) |
| Wheeze by 3 years | 94 (20.9) |
| Wheeze + HDM sensitized at 3 years | 31 (6.9) |
| Wheeze by 5 years | 113 (26.4) |
| Wheeze + HDM sensitized at 5 years | 52 (12.1) |

HDM = House dust mite

**Table 2. Multivariate analysis for the associations between early onset atopic dermatitis, wheeze, house dust mite sensitization and shellfish sensitization at 8 years**

|  |  |
| --- | --- |
|  | **Shellfish sensitization at 8 years** |
| **AdjOR (95% CI)** | **p value** |
| HDM sensitized at 18 months | **2.6 (1.03-6.3)** | **0.04** |
| HDM sensitized at 3 years | **2.5 (1.1-5.3)** | **0.02** |
| HDM sensitized at 5 years | **3.6 (1.7-7.7)** | **<0.01** |
|  |  |  |
| Atopic dermatitis by 6 months | 2.3 (0.7-7.3) | 0.16 |
| Atopic dermatitis by 6 months + HDM sensitized at 18 months | **10.1 (2.0-50.4)** | **<0.01** |
| Atopic dermatitis by 3 years | 1.6 (0.7-3.8) | 0.27 |
| Atopic dermatitis by 3 years + HDM sensitized at 3 years | 2.9 (1.0-8.4) | 0.05 |
| Atopic dermatitis by 5 years | 1.4 (0.6-3.4) | 0.42 |
| Atopic dermatitis by 5 years + HDM sensitized at 5 years | **3.4 (1.4-8.6)** | **<0.01** |
|  |  |  |
| Wheeze by 18 months | 2.3 (0.8-6.1) | 0.10 |
| Wheeze + HDM sensitized at 18 months | 2.4 (0.3-21.0) | 0.44 |
| Wheeze by 3 years | **2.7 (1.1-6.3)** | **0.02** |
| Wheeze + HDM sensitized at 3 years | **4.2 (1.4-13.1)** | **0.01** |
| Wheeze by 5 years | 2.2 (1.0-5.1) | 0.06 |
| Wheeze + HDM sensitized at 5 years | **2.9 (1.1-7.6)** | **0.03** |

Adjusted for gender, ethnicity, mode of delivery, maternal education levels and maternal history of allergy
Bold text indicates statistical significance
HDM=house dust mite