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Sensitivity to house dust mite allergens and prevalence of allergy-causing house dust mite species in Pothwar, Pakistan

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Abstract This study is the first report on the epidemiological status of house dust mite (HDM) allergy in Pothwar, Pakistan. Allergy data of 2087 symptomatic patients were obtained, of whom 1706 (81.7%) patients were skin-prick-test positive for HDM allergens. This percentage was significantly higher than for pollen and food allergens. In the results of this study *Dermatophagoides farinae* (61%) and *D. pteronyssinus* (29%) were the predominant species in the study area. Besides these pyroglyphids, predatory *Cheyletus* sp. (10%) and an oribatid mite sp. (1%) were also observed. Random and patients' houses showed 87.4 and 87.1% positive mite infestation, respectively. Mean (± SEM) *D. farinae* counts per g of dust in random samples was 235.4 ± 7.93 compared to 274.7 ± 10.78 from patients' homes. Mean *D. pteronyssinus* counts from random houses compared to patients' houses were 115.0 ± 4.57 and 124.6 ± 5.76, respectively. Mite counts depicted seasonal variation, with peaks during monsoon season. ELISA results of dust samples demonstrated that of the dust samples with > 10 µg/g of dust, the threshold value described as a risk factor for developing asthma, 57.6% had Der f1 and 20% Der p1 allergen load. Mean Der f1 burden was significantly higher than Der p1, with maximum levels during monsoon and autumn seasons. This research established a better awareness about the epidemiological status of HDM allergy and prevalence of allergy causing HDM species in Pakistan.

Keywords HDM allergy · Epidemiology · Prevalence of allergy causing HDM species · Der f1 and Der p1 allergen

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Introduction

Allergy is the acquired potential of developing immunologically mediated adverse reactions to otherwise harmless substances (allergens), which may induce tolerance in most people (Johansson et al. 2001). Pyroglyphid mites are among the major sources of allergens in house dust (Voorhorst 1967). Millions of these tiny arachnids (size 0.3–1.2 mm) are found inhabiting bedding, carpets, furniture and mattresses in human dwellings (Yan et al. 2016). Shed human skin scales and detritus constitute their food source, whereas high temperature (ca. 25 °C) and relative humidity (ca. 75% RH) help them to thrive inside houses (Valero and Serrano 2004). Epidemiological studies demonstrate that 10–30% of the human population in regions infested by dust mites suffers from HDM allergy diseases like asthma, allergic rhinitis, dermatitis and others (Beasley et al. 2003).

HDM are distributed worldwide, but prevalence and relative abundance of various species varies from one region to another (Arlian and Platts-Mills 2001). Some mite species are taxonomically and morphologically very similar in the adult stage, and their identification is a challenge (Colloff and Stewart 1997). Therefore, molecular characterization (genomic barcoding) is becoming increasingly important. The nuclear ribosomal DNA region (rDNA) consists of more than 100 copies of three highly conserved ribosomal subunits (18S, 5.8S and 28S rDNA) and two internal transcribed spacers, ITS1 and ITS2. These conserved ribosomal subunits are valuable in distinguishing between taxonomic groups, while variation in ITS1 and ITS2 regions help in molecular characterization of species (Wei et al. 2011) and phylogenetic study (Cruickshank 2002). Polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) of ITS1 and ITS2, has effectively been used for taxonomic characterization of mites (Osakabe et al. 2002), ticks (Poucher et al. 1999) and insects (Kampen et al. 2003). ITS2 sequence has now been recognized as a suitable tool in the characterization of astigmatid species (Yang et al. 2011) and has been successfully used for the identification of six major house dust and storage mite species (Wong et al. 2011).

In Pakistan, an estimated 23 million people are suffering from asthma. Similarly, the prevalence of individuals diagnosed of various kinds of allergies is approximately 34 million and there is a steady rise of about 5% per year. Although HDM sensitization in allergy patients has previously been reported from Rawalpindi and Islamabad (Abbas et al. 2012; Katelaris et al. 2007; Ullah et al. 2005), no work has been carried out to establish species diversity of HDM in this region. Diversity of acarofauna in the neighboring geographical regions is well known. There are reports of *Dermatophagoides pteronyssinus* dominating house dust from many cities in India along with other mite species including *D. farinae*, *Euroglyphus maynei* and *Blomia tropicalis* (Saha 2016). In Iran, humid areas have abundant *D. pteronyssinus* (Amoli and Cunnington 1977; Soleimani-Ahmadi et al. 2017), whereas inland cities with a considerably drier climate are infested with *D. farinae* (Fereidouni et al. 2013).

This study was designed to determine the prevalence of HDM allergy in the Pothwar region, in the north of Pakistan. It further investigated species diversity of HDM and levels of major HDM allergens in dust samples obtained from random and patients' houses.

Materials and methods

Data of allergy patients were obtained from 2010 to 2013 at Allergy and Asthma Centre Islamabad and Allergy Center-National Institute of Health (AC-NIH), Islamabad, Pakistan. Patient history was recorded in a specially designed questionnaire with informed

consent from the patients. The proposed study was approved by the technical committee on Medical Sciences, of Pakistan Science Foundation (PSF), Islamabad, the Advance Studies and Research Board (ASRB) of Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi (PMAS-AAUR) and the Research Ethics Committee (ERC) of the Institute of Biomedical and Genetic Engineering (IBGE), Islamabad.

Study site

The study site was Pothwar region, at 32°10–34°9N and 71°10–73°55E (Fig. 1). The mean summer temperature is 38.5 °C, in winter it falls to 0–1 °C. Summers include monsoon (rainy season), that spans from early July to September. During this period, there is a high relative humidity (70%) and temperature inside the houses. The study region was grouped into 31 clusters.

Skin prick test (SPT)

Standardized SPT was performed by trained technicians at allergy centers. Allergen extracts for SPT including *D. farinae*, *D. pteronyssinus*, pollen, mold and food were purchased from HollisterStier Allergy (Spokane, WA, USA). Skin of forearm of the patient was thoroughly cleaned with alcohol, sectioned and labelled. To avoid mixing of allergens, a distance of at least 2 cm was maintained. A sterile lancet was used to prick the skin after placing a drop of allergen at the marked area and excess allergen solution was removed. Appearance of a red inflammation/wheel (≥ 3 mm diameter) was considered a positive result (Inam et al. 2016).

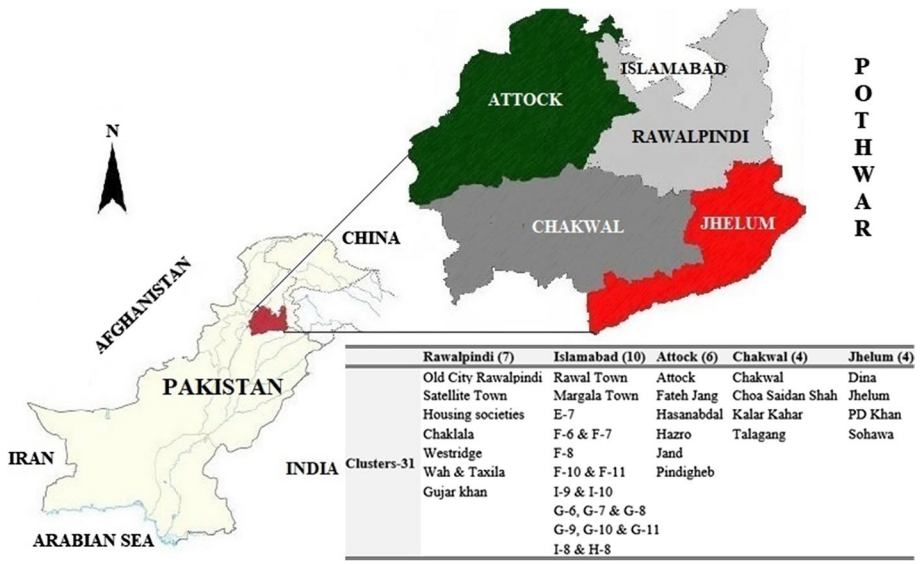

Fig. 1 Map of sampling sites (Pothwar, Pakistan)

House dust sampling and HDM isolation

HDM species isolated from house dust samples were morphologically identified and confirmed through molecular characterization. For estimation of species prevalence in randomly selected houses and in patients' houses, a cross sectional study was designed, which was further proceeded into a longitudinal design to appraise any seasonal variation in mite counts. Dust samples were collected from mattresses of beds and couches in the selected houses, once every month for a period of 2 years, from May 2011 to April 2013. A surface area of 1 m^2 at each sampling site was vacuumed for 1 min (Dautartiene 2001) with a portable vacuum cleaner having a modified metal attachment with silk cloth filter (0.2 mm thick). Dust from the filter was transferred into a zip-lock plastic bag (5 × 8 cm) and temporarily stored at 4 °C to prevent mite proliferation until HDM isolation.

Sodium chloride suspension technique (Colloff 2009; Henszel et al. 2010) was optimized for HDM isolation from household dust. After removing course dust particles and fibrous debris, through a 1.5 mm wire mesh, 50 mg of dust sample was filtered through another sieve (0.3 mm). The fraction obtained on the sieve was used for mite isolation, the finer sieved dust was stored at − 20 °C for allergen extraction. The sieved fraction, was suspended in brine solution (1% dust:brine ratio) with a magnetic stirrer for 1 h and left, over-night, for flotation of mites. On the next day, the supernatant was filtered through Whatman (grade 1 cellulose) filter paper. Filter paper was placed on a Petri dish under a dissecting stereo-microscope, mites on the filter paper were counted and collected with a fine mounted needle into separate Eppendorf tubes and stored at − 20 °C in 70% ethanol, later transferred to 96% ethanol. The number of mites found in a single sample (5 mg) was extrapolated to mites per g dust. The amount of dust collected in a single sample varied from 10 to 500 mg.

Morphological characterization

A single mite was kept on a watch glass with a drop of Nesbitt's clearing agent for 24 h, then transferred to a clean microscope slide, mounted in Hoyer's medium and covered by a coverslip. Specimens were stored at 40–45 °C for 2–7 days and after drying sealed with waterproofing paint (Faraji and Bakker 2008). Mite species were identified at 40× using published keys (Colloff 2009; Fain et al. 1990).

Restriction fragment length polymorphism (RFLP)

PCR–RFLP of ITS2 was used to confirm the identification of *D. farinae* and *D. pteronyssinus* (Beroiz et al. 2014; Wong et al. 2011). Digestion was done with Hinf 1 and Taq 1 restriction enzymes. DNA extraction was carried out with QIAamp DNA Micro Kit (Qiagen, Germany). A mite was picked with a mounted needle and placed with a drop of lysis buffer (buffer ATL) in a cavity glass slide. The mite body was teased in buffer to break the chitinous exoskeleton. The broken mite was picked and placed in a 1.5-ml microcentrifuge tube and 180 µl buffer ATL was added. The remaining procedure was carried out as described before (Shafique et al. 2014).

ITS2 primers for mites were designed in Primer3 software. The 5′–3′ sequence of forward and reverse primers is CGACTTTCGAACGCATATTGC (forward) and GCTTAA ATTCAGGGGGTAATCTCG (reverse). In 50-µl reaction volumes, 25 µl Taq master mix

(Qiagen) was taken with 0.25 µl ITS2 forward primer (100 µM), 0.25 µl ITS2 reverse primer (100 µM) and 23.5 µl double-deionized water. A negative control was also setup, where DNA template was replaced with double-deionized water. Thermocycler conditions were 94 °C for 2 min, followed by 35 cycles (denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s and extension at 72 °C for 50 s), finally 72 °C for 3 min (Wong et al., 2011). The PCR product was used for restriction endonuclease reactions, which were performed in a 20 µl mixture containing 8 µl PCR product and 10 U restriction endonuclease Hinf I (incubated overnight at 37 °C). The process was repeated using Taq I restriction endonuclease (20 U) with overnight incubation at 65 °C.

Restriction digests were resolved on 3% agarose gel containing ethidium bromide (10 µl/100 ml dissolved gel). TBE (Tris–Borate–EDTA) was used as buffer. The gel was run at 200 V for 30 min. Bands were visualized in a UV transilluminator. PCR product size of *D. farinae* was 320 bp. Fragments obtained by Hinf I were 180 and 110 bp, whereas incubation with Taq I produced fragments of 150 and 140 bp. Similarly, *D. pteronyssinus* product size was also 320 bp, where only one restriction fragment was obtained after incubation with Taq I (280 bp). Hinf I restriction enzyme did not cut the ITS 2 gene of *D. pteronyssinus.*

Quantification of group 1 allergens in the environment

100 mg sieved dust was agitated in 2 ml of 0.125 M ammonium bicarbonate (pH 8.0), kept overnight at 4 °C. The next day, supernatant was filtered through 0.45 µm acetate filter polypropylene syringe and stored at − 20 °C (Prester et al. 2007; Spertini et al. 2010).

ELISA for Der p1 and Der f1 levels

Der p1 and Der f1 allergen levels were quantified with the help of ELISA kits (Citeq, Groningen, The Netherlands). The working range of the kits was 1–15 ng/ml and the limit of detection (LOD) was 0.5 ng/ml. A standard curve was constructed at 450 nm OD.

Results

Epidemiological status of HDM allergies

Out of 2087 allergy patients, 1706 (82%) were SPT positive for HDM allergens, whereas 1094 (52%) were positive to pollen and 812 (39%) showed sensitivity to food allergens (Table 1). All patients were symptomatic and had been diagnosed with atopic allergies like asthma, allergic rhinitis, conjunctivitis and eczema. The database contained significantly more males than females (all allergens: 915 females, 1171 males; HDM allergens: 741 females, 965 males; both: $P < 0.01$). Approximately 87% of the allergy patients were polysensitized. The percentage of HDM sensitized patients was significantly higher ($P = 0.002$) than the percentage of pollen and food sensitized patients. Pairwise comparison of the three categories also showed that % HDM sensitization was highly significant compared to % pollen sensitization and % food sensitization (Table 2).

Table 1 Sensitization to house dust mites (HDM), pollen and food allergens in allergy patients at the Allergy and Asthma Centre Islamabad and Allergy Center-National Institute of Health (AC-NIH), Islamabad, Pakistan

Year (n)	% Sensitization		
	HDM (n)	Pollen (n)	Food (n)
2010 (260)	73.8 (192)	60.8 (158)	28.1 (73)
2011 (695)	82.9 (576)	60.0 (417)	29.9 (208)
2012 (701)	85.9 (602)	43.9 (308)	46.9 (329)
2013 (431)	78.0 (336)	49.0 (211)	46.9 (202)
Mean % (n)	81.7 (1706)	52.4 (1094)	38.9 (812)

Table 2 Pairwise comparison of sensitization (%) to house dust mite (HDM), pollen and food allergens in allergy patients at the Allergy and Asthma Centre Islamabad and Allergy Center-National Institute of Health (AC-NIH), Islamabad, Pakistan

Comparison	Mean diff.	P^a	95% CI
HDM versus pollen	26.75	< 0.001	10.37–43.13
HDM versus food	42.25	< 0.0001	25.87–58.63
Pollen sensitization versus food	15.5	ns	− 0.8849 to 31.88

ns not significant

[a]Tukey's multiple comparison test

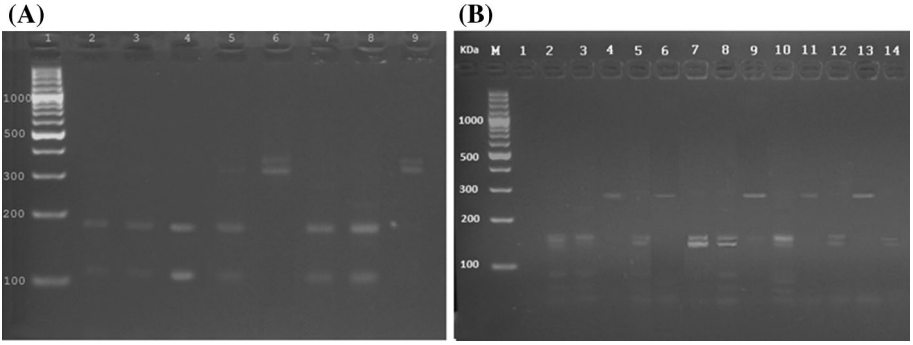

Fig. 2 Restriction fragments of ITS 2 rDNA from *Dermatophagoides farinae* and *D. pteronyssinus.* **a** Hinf 1 digests; lane 1: DNA marker; lanes 2, 3, 4, 5, 7 and 8: *D. farinae* 180 and 110 bp; lanes 6 and 9: *D. pteronyssinus* 320 bp. **b** Taq 1 digests; lane M: DNA marker; lane 1: negative control; lanes 2, 3, 5, 7, 8, 10, 12 and 14: *D. farinae* fragments 150 and 140 bp; lanes 4, 6, 9, 11 and 13: *D. pteronyssinus* 280 bp

Species diversity

A total of 9216 mites were examined and morphologically identified. 75 single-mite DNA samples were selected randomly for PCR of the ITS2 gene. All bands appeared on gel at expected product sizes for *D. farinae* and *D.* pteronyssinus. Digests of both Hinf 1 and Taq 1 confirmed morphologically identified *D. farinae* and *D. pteronyssinus* (Fig. 2). Accuracy of PCR–RFLP was 99.9%. *Dermatophagoides farinae* was the most prevalent species, with 61% abundance, followed by *D. pteronyssinus* showing 29% infestation. Besides these

pyroglyphids, a predatory *Cheyletus* sp. and an unidentified oribatid mite were also seen (in 10 and 1% of the total mite counts, respectively; Table 3).

Comparison of mite counts between random and patient houses

Dust samples from 419 randomly selected houses were examined along with 59 samples obtained from HDM allergy patients visiting clinics. *D. farinae* and *D. pteronyssinus* were isolated from 87.4 to 87.1% dust samples, respectively. *D. farinae* counts in random samples ranged between 0 and 666/g dust, with (mean ± SEM =) 235.36 ± 7.93 compared to 75–441 mites/g dust (274.74 ± 10.78) taken from patients' homes. Similarly, mean *D. pteronyssinus* counts from random houses were 115.04 ± 4.57 (0–435 mites/g dust) and 124.58 ± 5.76 from patients' houses (38–265 mites/g dust). Mean total Acari counts were greater from patients' homes (423.05 ± 12.63) compared to random houses (384.24 ± 11.33). Mite species counts in random and patients' houses did not differ significantly (Wilcoxon matched pair test: $P = 0.27$). 100% of the dust samples from patients' dwellings were infested with pyroglyphid mites.

In the Pothwar region, mite counts were found to fluctuate around the year. Mean counts of both *D. farinae* and *D. pteronyssinus* were significantly higher in monsoon (July–September), i.e., (mean ± SEM =) 273.1 ± 37.69 and 127.5 ± 12.41, respectively, than at any other time of the year (counts never exceeding 18.71 ± 5.19). Comparison of 2-year *D. farinae* and *D. pteronyssinus* counts with average temperature and humidity showed that highest mite counts coincided with 37–40 °C and 70–75% RH (Fig. 3).

Group 1 allergen levels

Sixty-seven randomly selected allergen extracts were tested with ELISA to determine Der f1 and Der p1 levels. Allergen levels in 59 (81.2%) dust samples tested for Der f1 were above 0.5 ng/ml (LOD). Five out of 59 (8.5%) dust samples were below sensitization threshold of 2 µg/g dust, whereas 34 (57.6%) had more than 10 µg/g dust allergen load, described as a risk factor for developing asthma. Similarly, dust tested for Der p1 indicated 50 (69.4%) samples above LOD, four (8%) samples with Der p1 levels below 2 µg/g dust and 10 (20%) samples with Der p1 levels higher than 10 µg/g dust. Mean (±SEM) Der f1 burden was higher than that of Der p1 (12.03 ± 0.86 [range 0.21–29.8] vs. 6.06 ± 0.73 [0–16.6] µg/g; $P < 0.0001$).

Table 3 Mite species and their counts (% in parentheses)

Pothwar districts	Mite species				
	D. farinae	*D. pteronyssinus*	*Cheyletus* sp.	Oribatid mite sp.	Total mites
Rawalpindi	1304 (60)	601 (28)	235 (11)	18 (1)	2158
Islamabad	2476 (62)	1198 (30)	273 (7)	23 (1)	3970
Attock	808 (60)	354 (26)	174 (13)	14 (1)	1350
Chakwal	339 (60)	112 (20)	105 (18)	12 (2)	568
Jhelum	678 (58)	376 (32)	89 (8)	27 (2)	1170
Total specimens	5605 (61)	2641 (29)	876 (10)	94 (1)	9216

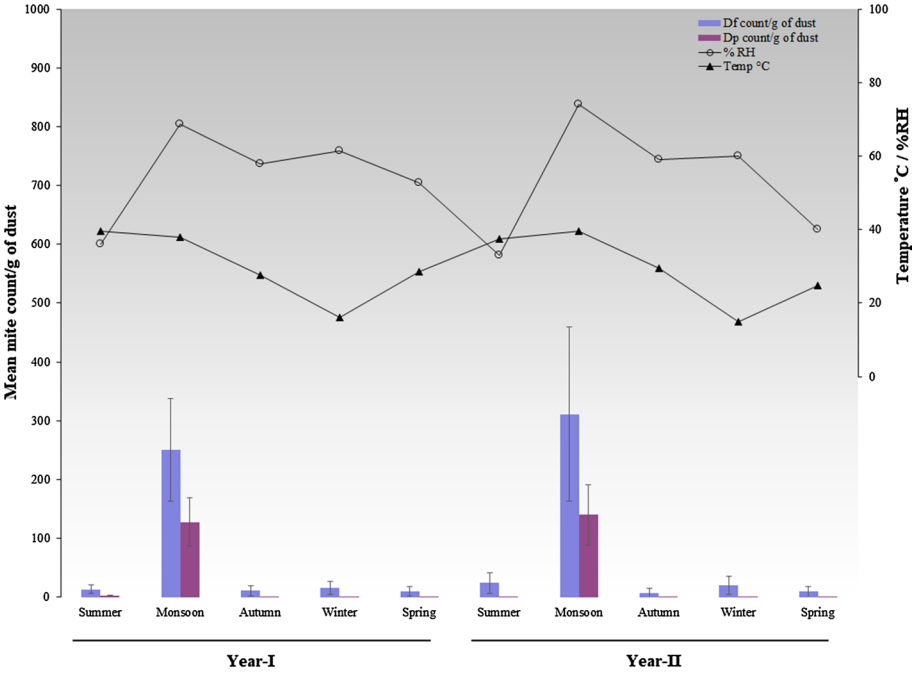

Fig. 3 Fluctuation in *Dermatophagoides farinae* (Df) and *D. pteronyssinus* (Dp) counts throughout 2 years of sampling in the Pothwar region, Pakistan

All samples collected during the rainy monsoon and autumn carried an allergen load above the LOD (0.5 ng/ml). Both allergens displayed seasonal fluctuations. Mean Der f1 levels in dust samples obtained during monsoon and autumn were 15.96 and 7.59 µg/g, respectively. These levels were much higher than those during the remaining seasons of the year ($P < 0.0001$). Also the mean Der p1 levels during monsoon and autumn (10.88 and 4.31 µg/g, respectively) were higher than those during the other seasons of the year (Fig. 4).

Discussion

We found that 82% of patients visiting allergy centers in the study location were sensitized to HDM allergens. Previous reports from various parts of the world indicated 45–85% of atopic allergy and asthmatic patients were SPT positive to HDM allergens (Assarehzadegan et al. 2013). Only one study in the region is known, reporting 64% (n = 200) of allergic rhinitis patients—visiting the ENT Department at the Combined Military Hospital (CMH) in Rawalpindi—have HDM sensitization, where 72.6% gave positive SPT to house dust (Ullah et al. 2005). Out of a total of 702 patients from Islamabad, 50% were SPT positive to house dust extract (Katelaris et al. 2007). In Islamabad 87.5% individuals visiting an allergy clinic were SPT positive against aeroallergens, most of which (44.6%) showed HDM sensitization (Abbas et al. 2012). Prevalence of HDM sensitized allergic rhinitis patients in a study from Lahore was 50.9% (Jalil and Bajwa 2014). As in the present study,

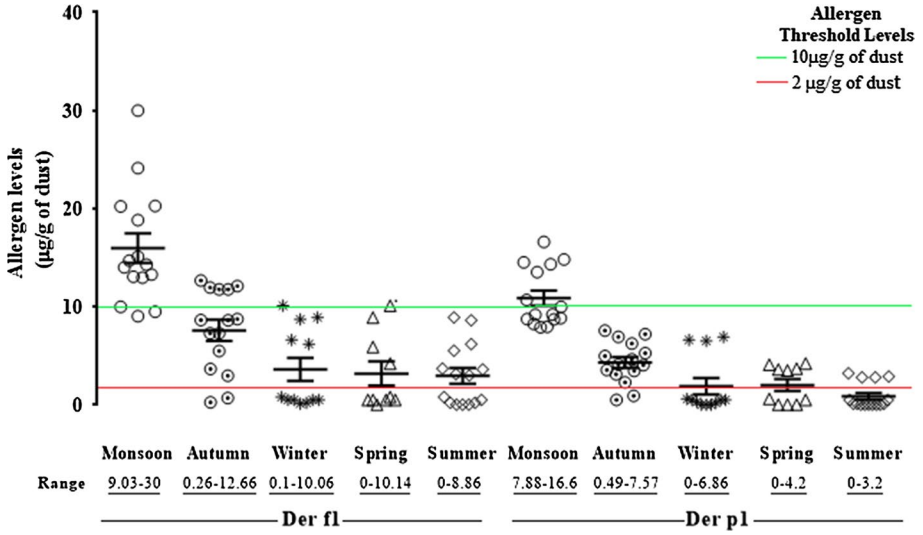

Fig. 4 Seasonal variation in Der p1 and Der f1 levels based on 2 years of sampling in the Pothwar region, Pakistan

Jalil and Bajwa (2014) also reported a bias in the ratio of male to female patients (2.6:1; in the HDM sensitized group: 3.4:1). A gender bias towards male allergy patients has also been reported from Chile (Calvo et al. 2005), Philippines (Yap et al. 2014) and Iran (Assarehzadegan et al. 2013).

This study found that 87% patients were sensitive to multiple allergens. Similar data were reported from neighboring Iran, showing high sensitization (91%) to multiple allergens, compared to only 8.9% patients sensitive to a single allergen (Nabavizadeh and Al-Yasin 2007). Another study from Iran reported a total of 84% patients to be polysensitized to various allergens when tested through SPT (Assarehzadegan et al. 2013).

Species diversity and seasonal variation

Our results revealed *D. farinae* as the most abundant species, followed by *D. pteronyssinus.* Dust mite species diversity in given human habitats is usually low (Feng et al. 2009). Our study found no significant difference in mite species counts in random versus patient houses; however, all dust samples from patient dwellings were infested with HDM. Similar to our findings, inland Iranian cities, with a considerably drier climate, are heavily infested with *D. farinae* (Fereidouni et al. 2013), whereas humid areas have abundant *D. pteronyssinus* (Sepasgosarian and Mumcuoglu 1979). Few studies report that *D. pteronyssinus* and *D. farinae* are equally abundant (Boquete et al. 2006). Many cities in India are reported to have *D. pteronyssinus* dominating house dust, along with other HDM species like *D. farinae*, *E. maynei* and *B. tropicalis* (Saha 2016). In China and Turkey, a predominance of *D. pteronyssinus* has been reported (Feng et al. 2009; Zeytun et al. 2017).

In Pothwar region, mean counts of both *D. farinae* and *D. pteronyssinus* were significantly higher in monsoon (July–September). A clear association of increase in relative indoor humidity with increase in mite counts has been established before (Sinclair et al.

2010; Aykut et al. 2016). Worldwide, seasonal variation of HDM species shows diverse patterns, based on varying temperature, humidity, and food (Feng et al. 2009).

Der f1 and Der p1 allergen levels

58% dust samples had Der f1, whereas 20% had Der p1 allergen exceeding the threshold described as a risk factor for developing asthma (10 µg/g dust). Der f1 and Der p1 levels displayed seasonal fluctuations, associated to %RH. In Quito (Ecuador), high annual mean RH (75%) was assumed to be the major contributing factor for higher counts of mites and their allergen levels throughout the year (Valdivieso et al. 2010). In Zagreb (Croatia), with a less humid environment, Der f1 allergen levels ranged between 0.1 and 31.2 µg/g house dust and Der p1 between 0.1 and 12.5 µg/g (Prester et al. 2007). HDM allergens have been reported to show fluctuations with changing seasons in many studies (Feng et al. 2009; Nascimento et al. 2017).

Conclusion

Our study has shown that HDM sensitization in Pothwar region is significantly higher compared to pollen and food. The presence of *D. farinae* and *D. pteronyssinus* is reported for the first time, as is the greater abundance of *D. farinae* in this region. This may help shed light on a hitherto ignored cause of allergy in the Pakistani population. The use of molecular characterization of species that are morphologically similar is a contemporary approach, that reduces chances of error in identification. The mite counts in Pothwar showed seasonal variation, with highest peaks during monsoon (July–September), when average temperature and relative humidity are highest. Exposure to mite allergens does not appear to be associated to an individual's home environment, as there was no difference in HDM allergen levels between random houses and patient houses. This emphasizes a need to further investigate the role of genetic predisposition in allergen sensitization.

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References

Abbas S, Katelaris CH, Singh AB et al (2012) World allergy organization study on aerobiology for creating first pollen and mold calendar with clinical significance in Islamabad, Pakistan: a project of world allergy organization and Pakistan allergy, asthma and clinical immunology Centre of Islamabad. World Allergy Organ J 5:103–110. https://doi.org/10.1097/WOX.0b013e31826421c8

Amoli K, Cunnington AM (1977) House dust mites in Iran. Clin Allergy 7:93–101

Arlian LG, Platts-Mills TA (2001) The biology of dust mites and the remediation of mite allergens in allergic disease. J Allergy Clin Immunol 107:S406–S413

Assarehzadegan MA, Shakurnia A, Amini A (2013) The most common aeroallergens in a tropical region in Southwestern Iran. World Allergy Organ J 6:7. https://doi.org/10.1186/1939-4551-6-7

Aykut M, Erman OK, Doğan S (2016) Variability in population density of house dust mites of Bitlis and Muş, Turkey. J Med Entomol 53:513–518. https://doi.org/10.1093/jme/tjw009

Beasley R, Ellwood P, Asher I (2003) International patterns of the prevalence of pediatric asthma the ISAAC program. Pediatr Clin North Am 50:539–553

Beroiz B, Couso-Ferrer F, Ortego F et al (2014) Mite species identification in the production of allergenic extracts for clinical use and in environmental samples by ribosomal DNA amplification. Med Vet Entomol 28:287–296. https://doi.org/10.1111/mve.12052
Boquete M, Iraola V, Fernandez-Caldas E et al (2006) House dust mite species and allergen levels in Galicia, Spain: a cross-sectional, multicenter, comparative study. J Investig Allergol Clin Immunol 16:169–176
Calvo M, Fernandez-Caldas E, Arellano P, Marin F, Carnes J, Hormaechea A (2005) Mite allergen exposure, sensitisation and clinical symptoms in Valdivia, Chile. J Investig Allergol Clin Immunol 15:189–196
Colloff MJ (2009) Dust mites. Springer, Dordrecht
Colloff MJ, Stewart GA (1997) House dust mites. In: Barnes P, Grunstein MM, Leff AR, Woolcock AJ (eds) Asthma. Lippincott-Raven, Philadelphia, pp 1089–1103
Cruickshank RH (2002) Molecular markers for the phylogenetics of mites and ticks. Syst Appl Acarol 7:3–14. https://doi.org/10.11158/saa.7.1.1
Dautartiene A (2001) Seasonal changes in house dust mites. Ecology 2:1–5
Fain A, Guerin B, Hart B (1990) Mites and allergic disease. Allerbio, Varennes en Argonne
Faraji F, Bakker F (2008) A modified method for clearing, staining and mounting plant-inhabiting mites. Eur J Entomol 105:793–795
Feng M, Sun W, Cheng X (2009) Seasonal dynamics and distribution of house dust mites in China. Biosci Trends 3:210–215
Fereidouni M, Fereidouni F, Hadian M, Nourani Hasankiadeh S, Mazandarani M, Ziaee M (2013) Evaluation of the level of house dust mite allergens, Der p 1 and Der f 1 in Iranian homes, a nationwide study. Allergol Immunopathol (Madr) 41:381–386. https://doi.org/10.1016/j.aller.2012.10.013
Henszel L, Kalisinska E, Kosik-Bogacka D, Kuzna-Grygiel W (2010) Mites in dust samples collected from sleeping places in apartments. Pol J Environ Stud 19:723–730
Inam M, Shafique RH, Roohi N, Irfan M, Abbas S, Ismail M (2016) Prevalence of sensitization to food allergens and challenge proven food allergy in patients visiting allergy centers in Rawalpindi and Islamabad, Pakistan. Springerplus 5:1330. https://doi.org/10.1186/s40064-016-2980-0
Jalil S, Bajwa RA (2014) Allergic rhinitis pattern of allergen sensitivity. Pak J Med Health Sci 8:922–925
Johansson SG, Hourihane JO, Bousquet J et al (2001) A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. Allergy 56:813–824
Kampen H, Sternberg A, Proft J, Bastian S, Schaffner F, Maier WA, Seitz HM (2003) Polymerase chain reaction-based differentiation of the mosquito sibling species *Anopheles claviger* s.s. and *Anopheles petragnani* (Diptera: Culicidae). Am J Trop Med Hyg 69:195–199
Nabavizadeh SH, Al-Yasin S (2007) Distribution of allergens among allergic rhinitis patients living Shiraz (Iran) region. World Allergy Organ J S258
Nascimento JM, Reis-Avila G, Dutra MS, Silva DE, Castro LCd, Ferla NJ (2017) Seasonal and environmental variations in community structure of house dust mites (Acari) in subtropical southern Brazil. Int J Acarology 43:86–90. https://doi.org/10.1080/01647954.2016.1232309
Osakabe M, Hirose T, Sato M (2002) Discrimination of four Japanese Tetranychus species (Acari: Tetranychidae) using PCR-RFLP of the inter-transcribed spacer region of nuclear ribosomal DNA. Appl Entomol Zool (Jpn) 37:399–407. https://doi.org/10.1303/aez.2002.399
Poucher KL, Hutcheson HJ, Keirans JE, Durden LA, Black WC (1999) Molecular genetic key for the identification of 17 Ixodes species of the United States (Acari: Ixodidae): a methods model. J Parasitol 85:623–629
Prester L, Brcic Karaconji I, Macan J (2007) Determination of mite allergens in house dust using the enzyme immunoassay. Arh Hig Rada Toksikol 58:413–419. https://doi.org/10.2478/v10004-007-0034-2
Saha GK (2016) House dust mite allergy—indian perspective. In Dust Allergy: Cause and Concern (pp. 53–63). Springer, Singapore. https://doi.org/10.1007/978-981-10-1825-1_8
Sepasgosarian H, Mumcuoglu Y (1979) Faunistische und ökologische studien der hausstaubmilben in Iran. Int J Acarology 5:131–138
Shafique RH, Klimov PB, Inam M, Chaudhary FR, OConnor BM (2014) Group 1 allergen genes in two species of house dust mites, *Dermatophagoides farinae* and *D. pteronyssinus* (Acari: Pyroglyphidae): direct sequencing, characterization and polymorphism. PLoS One 9:e114636. https://doi.org/10.1371/journal.pone.0114636
Sinclair W, Coetzee L, Joubert G (2010) House-dust mite species in Bloemfontein, South Africa. S Afr Med J 100:164–167
Soleimani-Ahmadi M, Zare M, Abtahi SM, Khazeni A (2017) Species identification and prevalence of house dust mites as respiratory allergen in kindergartens of the Bandar Abbas City. Iran J Allergy Asthma Immunol 2017:7

Spertini F, Berney M, Foradini F, Roulet CA (2010) Major mite allergen Der f 1 concentration is reduced in buildings with improved energy performance. Allergy 65:623–629. https://doi.org/10.1111/j.1398-9995.2009.02230.x

Ullah S, Niazi SA, Akhter MR (2005) Allergens in allergic rhinitis. Pak Armed Forces Med J 55:126–128

Valdivieso R, Estupinan M, Iraola V (2010) Exposure to risky concentrations of *Dermatophagoides* allergens in a high-altitude population (Quito, 2800 m above sea level in the Andean Mountains). J Investig Allergol Clin Immunol 20:362–363

Valero A, Serrano C (2004) Are environmental controls effective for house-dust-mite allergies? Arch Bronconeumol 40:389–391

Voorhorst R (1967) Correspondence: of mites and men. Allergy 40:357–358

Wei DD, Yuan ML, Wang ZY, Wang D, Wang BJ, Dou W, Wang JJ (2011) Sequence analysis of the ribosomal internal transcribed spacers region in psocids (Psocoptera: Liposcelididae) for phylogenetic inference and species discrimination. J Econ Entomol 104:1720–1729

Wong SF, Chong AL, Mak JW, Tan J, Ling SJ, Ho TM (2011) Molecular identification of house dust mites and storage mites. Exp Appl Acarol 55:123–133. https://doi.org/10.1007/s10493-011-9460-6

Katelaris CH, Mir AK, Raza MS, Abbas S, Singh AB, Syed MH (2007) Aerobiology of Islamabad. World Allergy Organ J S258–S259

Yan DC, Chung FF, Lin SJ, Wan GH (2016) The relationships among Dermatophagoides pteronyssinus exposure, exhaled nitric oxide, and exhaled breath condensate pH levels in atopic asthmatic children. Medicine (Baltimore) 95:e4825. https://doi.org/10.1097/md.0000000000004825

Yang B, Cai J, Cheng X (2011) Identification of astigmatid mites using ITS2 and COI regions. Parasitol Res 108:497–503. https://doi.org/10.1007/s00436-010-2153-y

Yap J, Ching M, Cabanilla C, Ramos J (2014) Multiple house dust mite allergen-sensitization profiles in children with allergic asthma. J Allergy Ther 5:2

Zeytun E, Doğan S, Özçiçek F, Ünver E (2017) Sensitivity to house dust mites allergens in patients with allergic asthma in Erzincan Province, Turkey. Turkiye Parazitol Derg 41:34–41. https://doi.org/10.5152/tpd.2017.5059