



Aero and Food Allergens Sensitization Patterns in a Clinic-Based Sample in Pakistan: A One Year Retrospective Study

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ABSTRACT

Sensitization to different allergens may lead to chronic clinical conditions like asthma and allergic rhinitis. Present study was conducted to investigate the prevalence of food and aero-allergen sensitization patterns. This retrospective study was designed to study allergy patients attending Allergy Centre, National Institute of Health, Islamabad. All enrolled patients were screened for set of allergens using skin prick test (SPT). Based on results of SPT, out of total 50550 allergen sensitized patients, 89.28% had aero-allergen sensitization while remaining 10.72% had food sensitization. Significantly higher prevalence of both aero- and food allergen sensitization was observed among males ($p < 0.05$) within sensitized population. The prevalence of allergen sensitization within sensitized population was highest in 31-40 years age group with significant variation across different age groups ($p < 0.05$). Among sensitized population, allergic rhinitis was most prevalent disease (41.57%) followed by bronchial asthma (25.68%) while allergic rhinitis and bronchial asthma at the same time were 3.9% prevalent. This study revealed a huge number of patients with diverse demographics, suffering from allergic diseases and sensitization to different allergens majorly aero allergens indicating poor indoor and outdoor air quality. This demands more public health facilities, and strict environmental regulations of air quality to manage allergic diseases in Pakistan.

Article Information

Received 17 October 2018

Revised 10 January 2019

Accepted 13 February 2019

Available online 10 May 2019

Authors' Contribution

AH and ZA conceptualized the study. AH and FA collected the data. AH analyzed the data and wrote the article. SD helped in the manuscript review and statistical analysis. SR and SA reviewed the manuscript. ZA supervised the study.

Key words

Allergy, Pollen, Hypersensitivity, Asthma, Rhinitis.

INTRODUCTION

Environmental pollution of both chemical and biological nature produced by natural as well as anthropogenic sources contributes to human health and allergy. Continuously increasing air pollution and climate changes due to urbanization and higher energy consumption increase exposure of human body to many different pollutants and allergens, resulting in the increased prevalence of allergies (Lee *et al.*, 2013; Selzle *et al.*, 2017). Among various biological components, pollens are particularly important aero-allergens because allergic human ailments are increasingly reported worldwide (Schmidt, 2016; Bosch-Cano *et al.*, 2011).

Pollens produced from trees and weeds result largely in the increased prevalence of allergy diseases (Schmidt, 2016; Ghufra *et al.*, 2013). Paper mulberry is one of the most common sources of allergenic pollens in the capital city of Pakistan that further worsens the situation due to its rapid growth and widespread dispersal by shallow root systems (Ghufra *et al.*, 2013). Moreover, "Pakistan is the fourth largest producer of cotton" therefore, general population particularly farmers are at an increased risk of acquiring allergic reactions upon exposure to cotton dust and also to dust produced during various activities of the vegetation and crops cutting (Ahmad *et al.*, 2011).

Allergen sensitizations are produced when susceptible individuals are exposed to particular allergens and result in the increased release of allergen specific immunoglobulin E (IgE) antibodies that lead to inflammatory responses (Saleem *et al.*, 2009). Both environmental factors as well as genetic makeup of the individuals play an important

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0030-9923/2019/0004-1429 \$ 9.00/0
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role in the development of allergic diseases (Ahmad *et al.*, 2011). Aero-allergen sensitization is a risk factor for allergic rhinitis and asthma but not all susceptible individuals develop the disease. Worldwide prevalence of allergic rhinitis ranges from 10 to 25% and asthma from 1 to 6% upon exposure to allergens (Sala-Cunill *et al.*, 2013).

Food allergies include adverse health effects caused by immune reactions in response to the consumption of particular food by the susceptible individuals. Then immune system trigger itself and causes certain responses that are often difficult to diagnose due to lack of simple tests and rapid development of disease along with various clinical forms. Food allergy prevalence is usually reported up to 10% and varies widely depending upon age, geographic areas and food habits of the individuals (Sicherer, 2011).

Pakistan is a developing country facing a very high burden of many different endemic, epidemic infectious and non-communicable diseases (Sultan and Khan, 2013). In this scenario, the main focus is on infectious diseases while allergic diseases are considered to be neglected. Studies based on the prevalence of allergen sensitizations caused by food and aero-allergens among people of Pakistan are very limited. Therefore, the present study was designed to determine the prevalence of food and aero-allergen sensitizations among those attending the country's largest Allergy Centre at National Institute of Health, Islamabad. Furthermore, different demographic parameters were also studied in the sample population to look at variances among allergen sensitized individuals in terms of these attributes.

MATERIALS AND METHODS

Study population and duration

In this retrospective study, all patients visiting the Allergy Centre, National Institute of Health (NIH), Islamabad during one year period from July, 2013 to June, 2014 were included to determine the prevalence of food and aero-allergen sensitization patterns.

Ethics statement

This study has been reviewed and approved by the Ethics Committee of the School of Biological Sciences, University of the Punjab, Lahore, Pakistan void reference number: SBS/93/16. Moreover, prior informed and verbal consent was obtained from all human participants of this study. Minimum age of the participants was 10 years so the consent of minors was obtained from their parents or guardians.

Questionnaire

Patients were interviewed individually to gather

information regarding allergy symptoms, duration of disease, family and smoking history. Furthermore, data about age, sex, occupation and geographic location of the individuals was also collected. This data collection is routinely practiced at NIH.

Diagnosis by skin prick test

Skin Prick test (SPT) was carried out to determine the target antigen of the IgE mediated allergic reaction within each patient. Aero-allergens incorporated in this study were pollen (mixed pollens of local allergenic plants), dust (whole house dust), paper mulberry, threshing dust and raw cotton. While beef, mutton, chicken, egg, fish and rice allergens mix was used to test food allergy. Appearance of red itchy wheel of >2mm was interpreted as a positive test result in SPT reaction against particular allergen. Standardized aqueous natural extracts of aero and food allergens (prepared in house by NIH, Islamabad) were applied on the bloodless skin of the forearm of the patients. The skin was pricked with the help of sterile lancet to adsorb allergen extract into the skin and then result was noted within 30 min in the form of red itchy or white bump wheels. Patients below the age of 10 years and above 60 years were not examined by SPT.

Data analysis

Microsoft Excel 2010 and SPSS 20.0 were used to calculate percentages, mean values, prevalence, 95% confidence interval and chi square tests. For statistical significance, p-value of ≤ 0.05 was considered as significant.

RESULTS

Among total study population (n=50550) visiting the NIH, Islamabad, Pakistan, 89.28% (45134) were aero-allergen sensitized while remaining 10.72% (5416) were food allergen sensitized and 1.5% (758) patients exhibited sensitization against both food and aero-allergens. Within the aero-allergen sensitized population, a significantly higher proportion was of males 68.76% (31034) as compared to that of females 31.24% (14100). Similar pattern was also observed in the case of food allergen sensitizations among males 72.83% (3945) and females 27.16% (1471). Age-distribution of patients within the allergen sensitized population, a significantly higher proportion was of 31-40 years age group followed by 21-30 years (25.7%) and 10 to 20 years age group (20.35%) for aero-allergies. However, for food allergies, 31-40 years age group was followed by 41-50 years (22.98%) and 21-30 years age groups (9.97%). Detailed description of these age groups and the respective prevalence are shown in Figure 1.

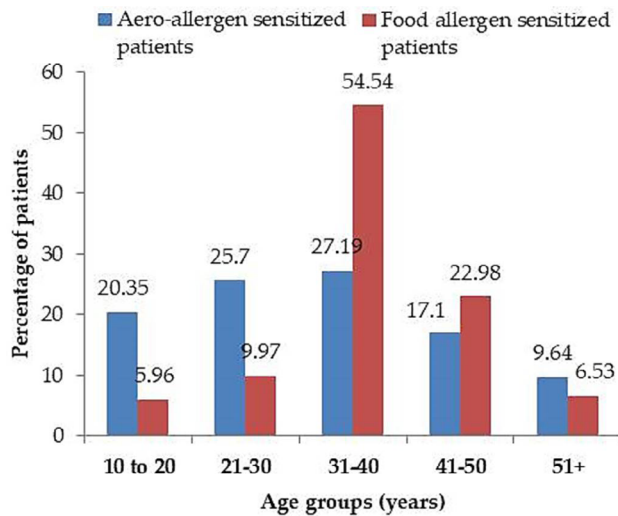


Fig. 1. Prevalence of allergen sensitized patients by age groups among total 45134 aero- and 5416 food-allergen sensitized patients. 31-40 years age group was most prevalent among aero and food-allergen sensitized patients followed by that of 21-30 and 41-50.

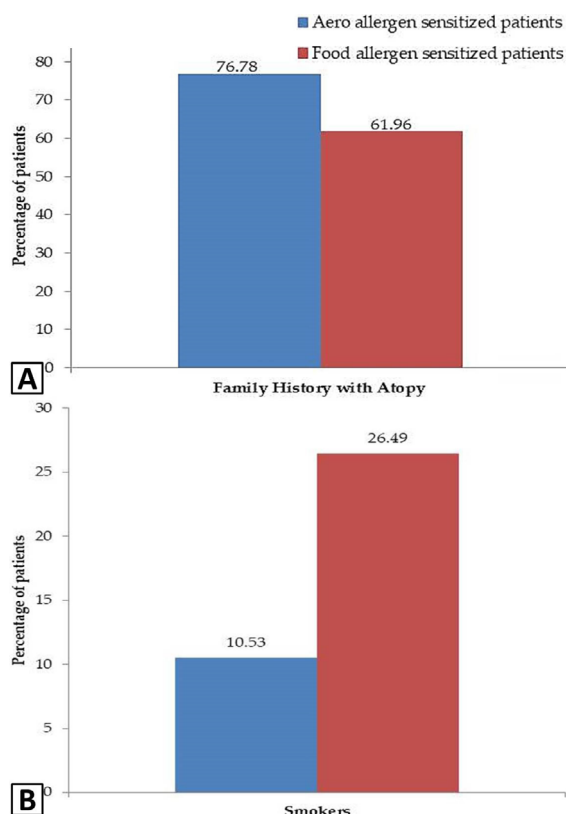


Fig. 2. **A**, pattern of family history with atopy among aero- and food-allergen sensitized patients; **B**, pattern of smoking among study population of aero-allergen and food-allergen sensitized patients.

Within the sensitized population, 76.78% (34656) of the aero-allergen sensitized patients had a family history of atopy while 61.96% (3356) of food-allergen sensitized patients also displayed history of atopy. Within the sensitized population, 26.49% (1435) of food-allergen sensitized patients compared to 10.53% (4753) of aero-allergen sensitized patients were found smokers as shown in Figure 2A and B.

Professional distribution among the allergen sensitized population presented students as the most susceptible group. 34.21% (15439) among total aero-allergen sensitized patients and 36.59% (1982) of food allergen sensitized patients were students as shown in Figure 3. Following students, job holders (doing private jobs of different kinds like computer operators, plumbers, security guards, etc.) were found as the most affected group among allergen sensitized patients in both food and aero-allergies (28.52 and 22.66%, respectively). However, persons with ambiguous answers regarding job type were included in others group that accounted for around 5% in both types of allergen sensitizations.

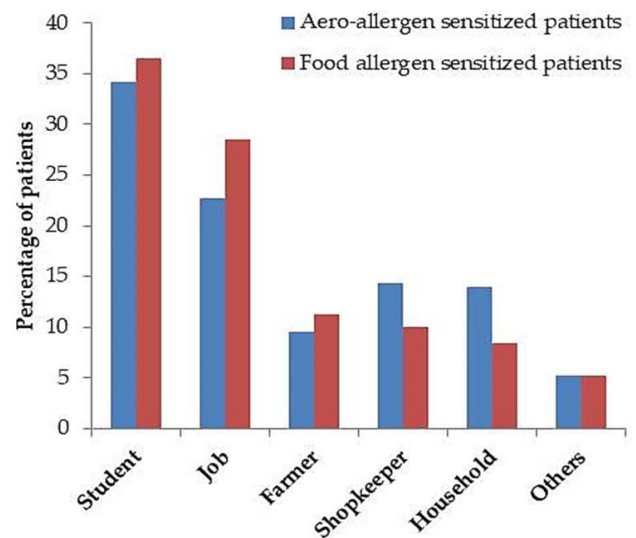


Fig. 3. Distribution of study population by professions among total 45134 aero-allergen sensitized patients (students 15439, job holders 10229, farmers 4320, shopkeepers 6450, household 6320, others 2376) and food allergen sensitized patients (students 1982, job 1545, farmer 610, shopkeeper 542, household 456, others 281).

Diseases were diagnosed in patients with the appearance of signs and symptoms and clinical findings. In patients with aero-allergen sensitization, allergic rhinitis (AR) was the most frequent disease 41.57% (1876) followed by bronchial asthma (BA) 25.68% (11592), urticaria 10.2% (4604) and the co-existence of both AR

and BA at the same time was found 9.36% (4226) as shown in Figure 4. In the case of food-allergies, 63.97% (3465) patients displayed illness having general symptoms like abdominal cramps, vomiting and itching followed by those having urticaria 24.52% (1328) as shown in Figure 4A. Patients exhibiting different allergic diseases showed different pattern of allergen sensitizations. Patients with

AR showed 83.62% sensitization to pollens followed by dust (79.96%). However, patients with BA also displayed similar pattern of maximum sensitizations to pollens (79.01%) followed by dust (75.29%). In case of patients with both A and B, Urt, AE and AC, maximum patients were sensitized to dust followed by pollens as shown in Figure 4B.

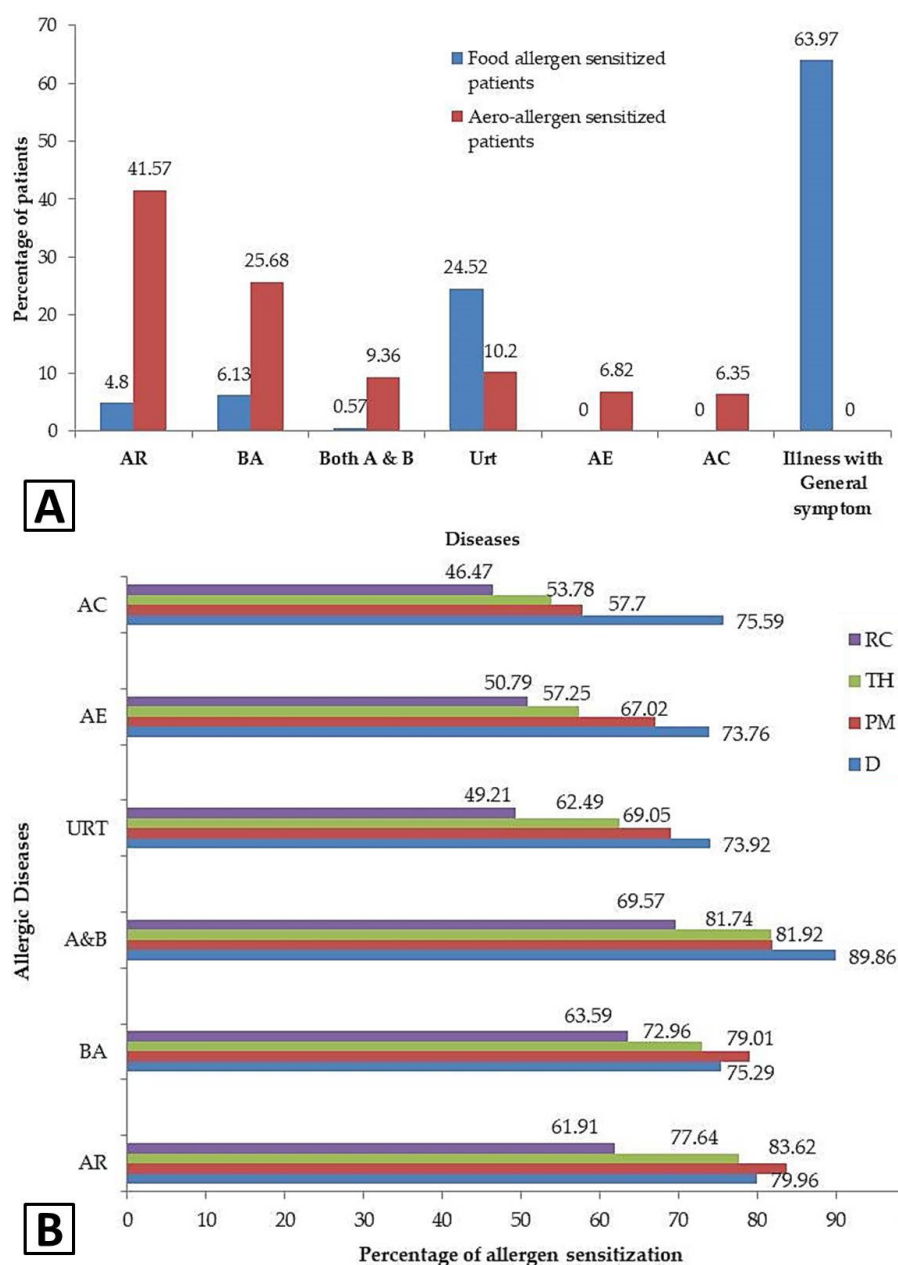


Fig. 4. **A**, prevalence of different diseases acquired by aero-allergen and food-allergen sensitized individuals (AR, allergic rhinitis; BA, bronchial asthma; A&B, allergic rhinitis and bronchial asthma; Urt, urticaria; AE, allergic eczema; AC, allergic conjunctivitis and illness with general symptoms like vomiting and abdominal cramps); **B**, association of aero-allergen sensitization to different allergic diseases (RC, raw cotton; TH, thrashing dust; PM, paper mulberry and other pollens; D, dust).



Fig. 5. Geographical distribution of aero-allergen sensitized patients (Islamabad 4841, Rawalpindi 6595, Punjab 17202, Khyber Pakhtun Khawa KPK 10165, Sindh 785, Balochistan 456, Azad Jammu and Kashmir AJK 1563, Northern Area (NA) 560, FATA 2404, Kabul 563) and food allergen sensitized patients (Islamabad 655, Rawalpindi 1028, Punjab 3489, Khyber Pakhtun Khawa (KPK) 191, Sindh 21, Balochistan 15, Azad Jammu and Kashmir AJK 7, Northern Area (NA) 2, FATA 4, Kabul 4) in Pakistan. F, food allergen sensitized patients; A, aero-allergen sensitized patients.

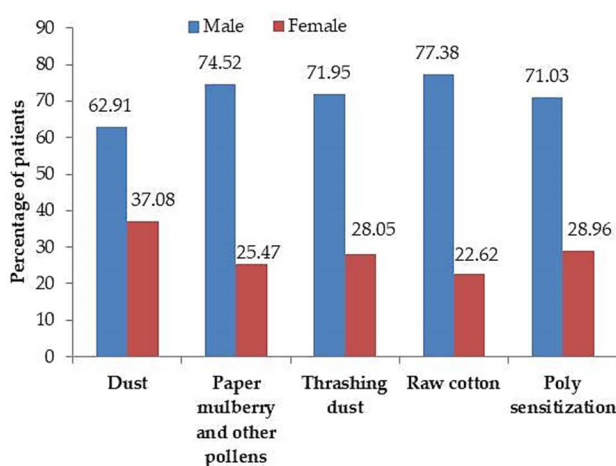


Fig. 6. Pattern of SPT positive aero-allergens among male and female patients. Total counts: house dust, 78.06%; pollens, 73.05%; thrashing dust, 67.64%; raw cotton, 56.90%; poly sensitization, 82.93%.

Majority of aero-allergen sensitization patients came from the province Punjab (17202) followed by Khyber Pakhtun Khawa (KPK, 10165) and Rawalpindi (Rwp, 6595). The patients exhibiting food allergen sensitization were mostly from the province Punjab (3489) followed by twin cities of Rawalpindi and Islamabad (1028 and 655, respectively) as shown in Figure 5.

Among SPT aero-allergens positive patients, poly sensitization (sensitized to between 2 and 4 allergens) was the most frequent (82.93%) followed by house dust (78.06%) and paper mulberry and pollens (73.05%). Among the poly sensitized individuals, males contributed as 71.03% and females as 28.96% while individuals having house dust sensitization included 62.91% males and 37.08% females (Fig. 6).

Association of professions with aero-allergic disease pattern indicated that individuals positive for allergic rhinitis included 31.13% students, 20.19% household, 17.46% shopkeepers, 17.29% job holders and 10.29%

farmers as described in Figure 7.

While individuals with bronchial asthma included 67.84% students, 14.06% farmers, 9.37% job holders and 4.68% shopkeepers. Seasonal distribution of aero-allergen sensitized individuals revealed higher prevalence of 11.46% in the month of September followed by 10.58% in April and 9.59% in June. While, food-allergen sensitizations were prevalent in the month of June (13.34%) followed by that in April (12.98%) and May (12.59%) as shown in Figure 8.

DISCUSSION

Pakistan is an agro-industrial developing country facing critical atmospheric challenges due to several anthropogenic activities (PAQI, 2017). Air quality of Pakistan is an alarming threat to population health and the situation becomes worst with rise in the pollen counts that lead to high prevalence of allergen sensitizations among people (Anonymous, 2018a). In this study, allergen sensitized patients visiting the Allergy Centre, NIH, Islamabad displayed a significantly higher prevalence of sensitization to aero-allergens as compared to food allergens as indicated by the chi square test ($p < 0.05$). There was 1.5% overlap observed among two types of

allergen sensitizations among total patients. Therefore, majority of the patients executed sensitivity reaction towards aero-allergens and another study also reported the higher prevalence of aero-allergen sensitized patients (97.7%) as compared to food-allergen sensitized (2.30%) patients (Ahmad *et al.*, 2011).

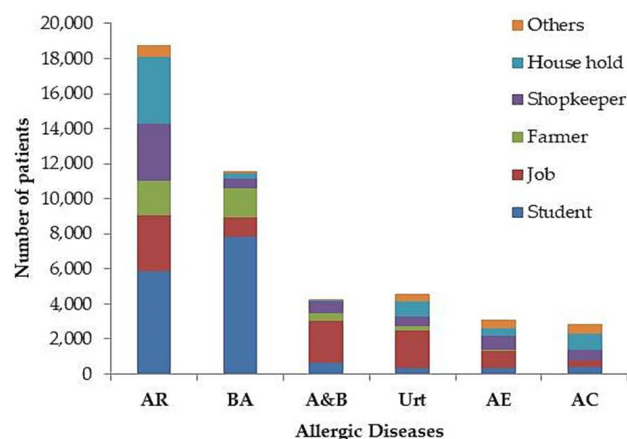


Fig. 7. Association of professions with aero-allergic disease pattern. AR, allergic rhinitis; BA, bronchial asthma; A&B, allergic rhinitis and bronchial asthma; Urt, urticaria; AE, allergic eczema; AC, allergic conjunctivitis.

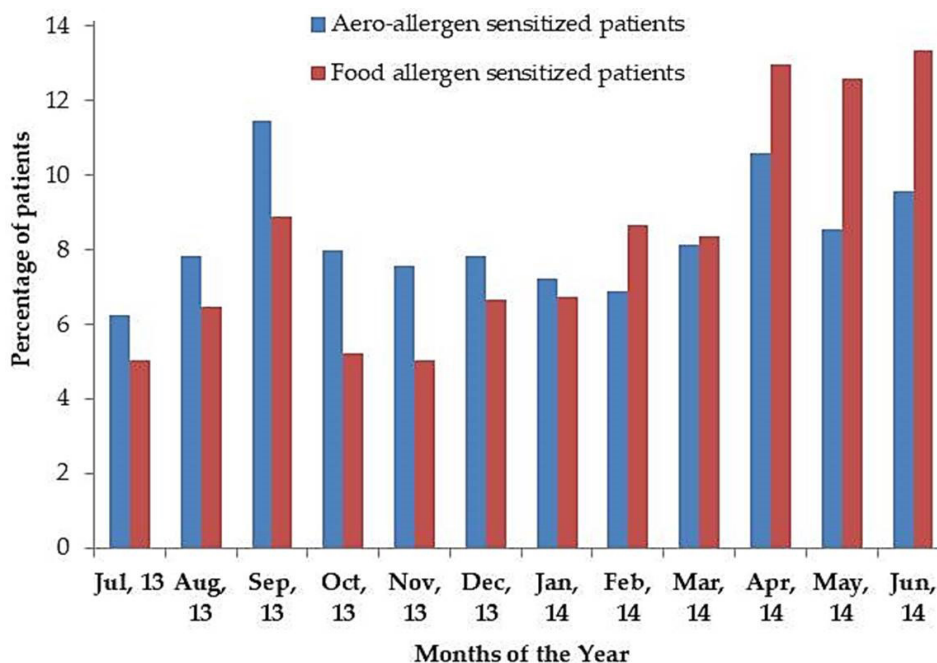


Fig. 8. Seasonal (month wise) distribution of aero and food allergen sensitized patients during the study period. Percentages of aero-allergen sensitized patients: Jul-13, 6.26%; Aug-13, 7.83%; Sep-13, 11.46%; Oct-13, 7.98%; Nov-13, 7.59%; Dec-13, 7.82%; Jan-14, 7.24%; Feb-14, 6.89%; Mar-14, 8.14%; Apr-14, 10.58%; May-14, 8.56%; Jun-14, 9.59%. Percentages of food-allergen sensitized patients: Jul-13, 5.04%; Aug-13, 6.49%; Sep-13, 8.89%; Oct-13, 5.21%; Nov-13, 5.04%; Dec-13, 6.65%; Jan-14, 6.75%; Feb-14, 8.66%; Mar-14, 8.35%; Apr-14, 12.98%; May-14, 12.59%; Jun-14, 13.35%.

Aero-allergen sensitization pattern among patients suggest poor indoor and outdoor air quality in Pakistan because of house dust, pollens and agricultural dust (thrashing and raw cotton). Due to the lack of awareness and poor socio-economic lifestyle, frequency of allergen sensitization caused by house dust is significantly high. Moreover, the pattern of aero-allergen sensitizations varied significantly among study population, χ^2 (1, $N = 5$). However, gender based distribution of aero-allergens showed a higher prevalence among male population as compared to that in females. House dust may be major contributing factor for aero-allergies among females because of their household activities followed by working women with their exposure to outdoor allergens. This study reported a higher prevalence of aero- and food allergen sensitization among males than in females as supported by the statistically significant difference of the disease prevalence among two genders (Chi square test, $p < 0.05$). Findings of another study were in harmony with the present study that showed 60% male population among SPT positive patients while females were 30% (Ahmad *et al.*, 2011). The frequency of male patients was high probably due to their frequent and prolonged exposure to pollen, thrashing and raw cotton dust in the outer environment followed by exposure to house dust. Moreover, male population in Pakistan has easy access to medical centers and clinic as compared to females that may be an important factor in higher male prevalence observed in this study.

Individuals belonging to age groups 31-40 and 21-30 were found most susceptible to execute aero-allergen sensitivity reactions in this study. While food allergens sensitization was frequently observed among individuals of 31-40 years followed by those of 41-50 years. Likely to the present study, highest prevalence of hypersensitivity reaction was observed among individuals of the age group 31-40 followed by 41-50 and 21-30 in another study (Saleem *et al.*, 2009). However, statistical analysis pointed out a significant difference among individuals of different age groups being contracted by the allergen sensitizations ($p < 0.05$). Higher prevalence of food allergen sensitization among adults may be because in certain cases, children outgrow allergies with age and they carry these allergies throughout the life and such adults are the major part of adults having food allergies. Moreover, individuals of this active lifestyle group (31-40 years) often can't avoid such foods because of busy schedules, travelling and dinner meetings. Whereas, children have most of the time in homes and their dietary habits are well balanced and checked by parents so they have characteristically less frequency of food allergen sensitizations. Similarly, females spending most of their time at home in Pakistan are less prone to food

sensitizations as they can have specific foods every time to avoid allergen sensitizations. Moreover, food allergens that NIH still uses (beef, mutton, chicken, egg, fish and rice) are according to the initial allergy manifestations but they should also use other potential food allergens like nuts, peanut, soy and wheat. This may further alter the pattern of food allergen sensitizations in Pakistan.

Family history of allergic diseases is one of the most predisposing factors in the development of allergen sensitizations. Another study also reported a higher prevalence of allergy among people having family history (76.3%) (Saleem *et al.*, 2009). Genetic factors along with environmental factors lead to atopy (Blumenthal, 2005). Therefore, it is suggested that individuals with family history of atopy must avoid exposure to such allergens as they are more susceptible to develop hypersensitivity reactions. Statistical analysis also suggested significant difference in the prevalence of allergen sensitization among atopic and non-atopic individuals by chi square test ($p < 0.05$).

Significantly higher prevalence of sensitization was observed among non-smokers compared to smokers ($p < 0.05$). This may be due to overall pattern of smoking in Pakistan is low (15.2%) as described in another study conducted in 2013 (Gilani and Leon, 2013). However, according to a recent study, overall prevalence of smoking in Pakistan was reported as 21.6% with females as major contributors (Shah and Siddiqui, 2015). Therefore, smoking may not be a prominent factor in causing allergen sensitizations. Regardless of their non-smoking habits, genetic and other environmental factors are main factors responsible for causing the disease. The present study revealed that profession characteristically influenced the occurrence of disease among study subjects as students were the most prevalent group among patients. The results of a study were somewhat different from the present study in this regard that maximum cases of allergic rhinitis were found in people doing jobs (49.1%) followed by those doing nothing (30.2%) (Saleem *et al.*, 2009). Present study showed students as the most affected group of population possibly due to their residence in most populated and polluted urban cities like Lahore, Faisalabad, Islamabad and Rawalpindi that make them prone to hypersensitivity reactions.

Allergic rhinitis was found the most common allergy ailment among the aero-allergen sensitized patients visiting NIH, Islamabad followed by bronchial asthma. One of the study reported that allergic rhinitis (24.62%) was most frequent disease observed among population of Pakistan followed by urticaria (24.04%), co-existence of AR and BA in the same individual (19.41%) and bronchial asthma (19.36%) (Ahmad *et al.*, 2011). Whereas, illness

with general symptoms of vomiting and abdominal cramps was found the most frequent among food-sensitized patients followed by urticaria. Variance of different diseases from one another indicated that frequency of all diseases is significantly different from one another as statistically tested by two factor ANOVA test $F(9, 4) = 6.92$. Association of profession with disease pattern revealed that the prevalence of allergic diseases was influenced strongly by professional attitudes.

Punjab, Khyber Pakhtunkhwa and twin cities were found as the most affected geographical localities for the prevalence of aero-allergies in this study. Just like the results of present study, one other study reported highest prevalence of allergy patients in the province Punjab followed by that of KPK, Rawalpindi and Islamabad (Ahmad *et al.*, 2011). While, other regions including Sindh, Balochistan, Azad and Jammu Kashmir (AJK), and Kabul reported less frequency of patients that may be due to distance from the Allergy Centre and less socio-economic factors of those areas. Therefore, Punjab is the most sensitive zone for aero-allergen patients because of its environmental conditions and enrichment with the flora and vegetation. Furthermore, activities in the urban areas such as increased traffic, pollution and human actions lead to an increased frequency of aero-allergens in such populated areas. The prevalence of allergen sensitizations varied widely in geographic localities $\chi^2(1, N = 10) = 90.00$ and likewise, the pattern of each disease was significantly different in all of the areas being studied, $F(4, 9) = 4.67$.

Prevalence of allergy diseases significantly varied across the year $\chi^2(1, N = 12) = 132.00$. In contrast to the present study, the results of another study described that most of the asthma patients in Karachi were reported during March and the cold months (December, January, February) (Ahmed *et al.*, 2013). Though, minimum number of patients was observed during May and November. These results exhibited varying pattern from our study due to climatic differences between Karachi (coastal area, average temperature 30°C) and the province Punjab (24°C), Islamabad, Rawalpindi (21°C) and KPK (23°C) that covers most of our study participants (Anonymous, 2018b). The results of study on aerobiology conducted revealed that during the flowering seasons prolonged from March to April and July to September, various pollen counts such as paper mulberry were at peak that accounted for allergic reactions among susceptible individuals (Abbas *et al.*, 2012). Therefore, flowering seasons, floral species and climatic conditions of various geographical locations being included in the study strongly influenced the prevalence of allergies over the year. However, in the case of food allergies, higher prevalence was observed

in the months of April, May, June and September that varied according to different eating habits and varied food availability depending on the seasons and local festivals.

Recombinant allergens have been known to play significantly important roles in allergen specific immunotherapy that also limits the possible allergenic reactions posed by natural extracts used for traditional allergy vaccines preparations (Valenta *et al.*, 2011). In Pakistan, only natural extract based therapy is used for the treatment of allergies. As diagnostics feed into clinical decision making, therefore local allergens should be identified and move towards recombinant allergens for diagnosis through allergen specific immunotherapy. This would also make possible non-invasive tests like BAT, ELISA immunocap *etc.*

CONCLUSION

This study was aimed to highlight the current trends of allergic patients in Pakistan due to its consideration as neglected diseases. This study enlightened different allergy scenarios among people visiting one of the major public allergy centers in Pakistan. It highlighted that within a year, a huge number ($n=50,550$) of individuals with diverse demographics attending the allergy center were sensitized to allergens and suffered from different allergic diseases that represented burden on allergy center. Therefore, this condition demands setting of more allergy centers in the country. There are very limited studies in Pakistan that bring this consistent issue on frontline which should be addressed promptly at public health level. There is also need to improve the indoor and outdoor air quality which is playing a major role in exacerbating the allergic diseases upon exposure. While it is also revealed here that local in-house whole allergen and dust extracts are used routinely to diagnose and treat the sensitizations. However, detailed research is required to determine the nature and structural elucidation of local allergens and purified recombinant allergens so that allergy should effectively be managed with advanced strategies. Besides all this, this study also demands a detailed research on general population to analyze the allergy patterns, complications and real trends related to allergy.

ACKNOWLEDGMENTS

University of the Punjab, Lahore, Pakistan is highly acknowledged to support this research work.

Statement of conflict of interest

The authors declared no conflict of interest regarding this study.

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