

# Difference in Frequency of Aeroallergen Hypersensitivity in Patients from Urban Versus Rural Households

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## ABSTRACT

**Objective:** To indirectly evaluate the hygiene hypothesis in the Pakistani population by assessing the difference in aeroallergen hypersensitivity frequency among patients from urban and rural households.

**Study Design:** Cross-sectional, descriptive study.

**Place and Duration of the Study:** Department of Immunology, The Armed Forces Institute of Pathology (AFIP) / NUMS, Rawalpindi, Pakistan, from January to December 2023.

**Methodology:** Patients advised skin prick test (SPT) by their treating clinicians and meeting the inclusion criteria were included. SPT was performed using six common inhalational allergens, with negative (normal saline) and positive (0.1% histamine hydrochloride) controls. Results were assessed after 15 minutes, and a wheal diameter >3 mm was considered positive. Aeroallergen sensitisation frequencies were compared between urban and rural populations using the Chi-square test. A p-value of <0.05 was considered statistically significant.

**Results:** Out of 312 patients, 106 (34%) tested positive for SPT, comprising 60 (57%) males and 46 (43%) females. Among these, 44 (41%) were polysensitised to two or more than two allergens. The most prevalent allergens were house dust mite mix (59%) and Cannabis (31%), while *Aspergillus* was the least common (8%). A statistically significant difference ( $p = 0.008$ ) was observed in aeroallergen sensitisation prevalence between urban and rural dwellers, with urban patients showing higher sensitisation rates.

**Conclusion:** The findings support hygiene hypothesis, demonstrating a higher frequency of aeroallergen hypersensitivity in urban populations compared to rural counterparts. The role of environmental exposures in immune system modulation underscores the impact of urbanisation on allergic diseases in the Pakistani setting.

**Key Words:** Aeroallergens, Allergy, Hygiene hypothesis, Sensitisation, Skin prick test, Urbanisation.

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## INTRODUCTION

Allergic diseases represent a significant and evolving challenge in modern medicine, with allergic rhinitis affecting an estimated 10–40% of the global population.<sup>1,2</sup> Clinically, allergic rhinitis manifests as sneezing, nasal itching, rhinorrhoea, and nasal congestion and is frequently associated with conditions such as sinusitis, asthma, allergic conjunctivitis, and atopic dermatitis.<sup>3</sup>

Over recent decades, a rising trend in allergic diseases, including allergic rhinitis and asthma, has been observed, initially in Western countries and now increasingly in developing nations.<sup>4</sup> This trend is attributed to a complex interplay of genetic predisposition and environmental influences.

Furthermore, factors such as reduced family size, higher standards of cleanliness, and improved personal hygiene within households have been hypothesised to contribute to this increase in allergic disease prevalence.<sup>5,6</sup> One of the most well-known explanations for this phenomenon is the hygiene hypothesis.

The hygiene hypothesis posits that reduced microbial exposure in early life, particularly in industrialised and urbanised settings, leads to an increased predisposition to allergic diseases.<sup>7</sup> This hypothesis is supported by a growing body of evidence linking a higher incidence of allergic conditions to decreased exposure to infectious agents during childhood.<sup>8</sup> Epidemiological studies have demonstrated that individuals from rural areas exhibit significantly lower rates of allergic diseases compared to those from urban populations.<sup>9</sup>

First proposed by David Strachan in 1989, hygiene hypothesis emerged from observations that individuals raised in larger families with older siblings exhibited a lower prevalence of allergic diseases and eczema.<sup>10</sup> Strachan suggested that shifts in lifestyle, smaller family structures, improved living conditions, and heightened hygiene levels contribute to the increasing burden of allergic disorders. At a physiological

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level, the hypothesis is based on the early-life predominance of T-helper Type 2 (Th2) cells, which mediates allergic inflammation. In the presence of sufficient microbial stimulation, a T-helper Type 1 (Th1) response is activated, promoting a balanced Th1/Th2 immune system that offers protection against allergic sensitisation.<sup>11</sup>

The evaluation of hygiene hypothesis typically involves epidemiological assessments of geographic trends, family size, birth order, environmental exposures, contact with livestock, and immunological testing, including skin prick testing (SPT).<sup>12</sup> Such studies provide substantial evidence supporting the role of early microbial exposure in immune system development and in reducing the risk of allergic diseases.<sup>13</sup>

This study aimed to indirectly assess the validity of the hygiene hypothesis in a local setting by evaluating the difference in the prevalence of aeroallergen hypersensitivity among individuals from urban *versus* rural households. The assumption is that rural environments provide greater exposure to microbial agents, thereby offering a protective effect against allergic sensitisation compared to urban areas. Identifying prevalent aeroallergens and associated environmental factors can inform targeted awareness campaigns, preventive strategies, and treatment protocols, thereby aiding healthcare professionals and policymakers in optimising allergy management and reducing the healthcare burden associated with allergic diseases.

## METHODOLOGY

This cross-sectional study was conducted at the Department of Immunology, The Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from January to December 2023. Patients referred for SPT for the diagnostic evaluation of rhinitis and asthma symptoms were included. The study protocol was approved by the Institutional Ethical Review Board, and informed consent was obtained from all participants.

The sample size was determined using the OpenEpi online sample size calculator, based on a 21.4% prevalence of positive SPT in suspected allergic cases, a 95% confidence level, and a  $\pm 5\%$  margin of error. However, to enhance statistical power, a total of 312 patients aged 18–60 years were included. Patients presenting with symptoms of sneezing, nasal congestion, rhinorrhoea, and asthma were recruited.

Patients were excluded if they were on antihistamines at the time of testing, had a history of specific immunotherapy, or had dermatographism, which could interfere with SPT interpretation. A structured questionnaire was completed by each participant to record demographic data, family history of allergic conditions, symptom characteristics (seasonal *vs.* perennial), comorbidities (asthma and rhino-conjunctivitis), and pet exposure history.

Six common aeroallergens were selected based on previous epidemiological data and prevalent plant species in the region. SPT was performed using extracts of house dust mite mix,

Aspergillus mix, grass mix, paper mulberry, cannabis, and mixed pollen. Negative (normal saline) and positive (0.1% histamine hydrochloride) controls were included to ensure test validity. SPT results were assessed 15 minutes post-application, and a wheal diameter  $>3$  mm was considered a positive reaction.

Data were entered into SPSS (Statistical Package for Social Sciences) version 23 for processing and analysis, while GraphPad Prism was used for additional statistical evaluations. Qualitative variables were expressed as frequencies and percentages, whereas mean  $\pm$  standard deviation (SD) was calculated for quantitative variables. Stratification was performed based on age, gender, urban/rural residence, and socio-economic status. The Chi-square test was applied to assess associations between categorical variables, and a *p*-value  $<0.05$  was considered statistically significant.

## RESULTS

A total of 312 patients were evaluated for aeroallergen sensitisation using skin prick testing (SPT). The mean age of participants was 23 ( $\pm 5.09$ ) years with 161 (52%) males and 151 (48%) females. Of these, 106 (34%) patients tested positive for at least one aeroallergen, comprising 60 (57%) males and 46 (43%) females. The demographic characteristics of the SPT-positive patients are summarised in Table I.

Among the SPT-positive patients, 44 (41%) patients, comprising 25 males and 19 females, demonstrated polysensitisation (*i.e.*, reactivity to two or more allergens). In males, 22 were sensitised to two allergens, while 3 were reactive to more than two allergens. Similarly, among females, 15 were positive for two allergens, whereas 4 had reactivity to more than two allergens. No statistically significant difference was observed between the male and female polysensitisation rates ( $p=0.45$ ).

Regarding clinical manifestations, the most frequently reported symptom among SPT-positive patients was sneezing (71 patients, 67%), followed by allergic rhinitis (64 patients, 60%). Allergic conjunctivitis and asthma were each reported in 52 (49%) patients. A positive family history of allergic diseases was noted in 46 (43%) patients, while 24 (23%) patients had a history of animal exposure.

**Table I: Characteristics of SPT-positive patients.**

No. of patients	106
Mean age ( $\pm$ SD)	22.95 ( $\pm 5.09$ ) years
Age range	18-60 Years
Gender	
Male	60 (57%)
Female	46 (43%)
Family history	
Male	28 (26%)
Female	18 (17%)
Animal exposure	24 (23%)
Duration of symptoms	
$\leq 6$ months	31 (29%)
$\geq 6$ months	75 (71%)
Median duration of allergic rhinitis	6.0 years (0.3-6.5)
Periodicity	
Seasonal	58 (55%)
Perennial	48 (45%)

SPT: Skin prick test.

**Table II: Prevalence of positive SPT to selected allergens.**

Allergens	Positive %	No. of patients (n = 106)		p-value (Male vs. Female)
		Male (n = 60)	Female (n = 46)	
Aspergillus mix	9 (8 %)	7 (11.6 %)	2 (4.3 %)	0.18
Cannabis	33 (31%)	19 (31.6 %)	14 (30.4%)	0.89
House dust mite	63 (59%)	39 (65 %)	24 (52.1%)	0.18
Mixed pollen	28 (26%)	10 (16.6%)	18 (39.1%)	0.009
Paper mulberry	16 (15%)	6 (10 %)	10 (21.7%)	0.09
Grass mix	24 (23%)	13 (21.6 %)	11 (23.9 %)	0.78

\*Chi-square test, SPT: Skin prick test.

Among the SPT-positive patients, 48 (45%) had perennial symptoms, whereas 58 (55%) exhibited a seasonal allergic pattern. Wheal induration sizes ranged from 3 to 8 mm, with 91 (86%) patients showing 3–5 mm, 13 (12%) patients showing >5 mm, and two (2%) patients exhibiting a reaction >8 mm.

A comparison of sensitisation patterns between urban and rural dwellers revealed a statistically significant difference ( $p = 0.008$ ). Among SPT-positive patients, 63 (60%) were from urban areas, while 43 (40%) resided in rural settings. However, no statistically significant difference in allergen sensitisation rates was observed between male and female patients ( $p = 0.204$ ).

The prevalence of positive-SPT results for specific allergens is presented in Table II. The most frequently identified allergen was house dust mite mix (63 patients, 59%), followed by Cannabis (31%), mixed pollen (26%), grass mix (23%), paper mulberry (15%), and Aspergillus mix (8%). A significant difference was noted in mixed pollen sensitisation between males (16.6%) and females (39.1%,  $p = 0.009$ ), while sensitisation rates for other allergens showed no significant gender-based differences.

The distribution of aeroallergen sensitisation between urban and rural populations aligns with the hygiene hypothesis, suggesting that reduced microbial exposure in urban environments may contribute to a higher prevalence of allergic sensitisation. Among the 106 SPT-positive patients, house dust mite sensitisation, the most common allergen, was observed in 39 (62%) urban patients compared to 24 (55%) rural patients, supporting the premise that urban living conditions may favour allergen susceptibility. Mixed pollen sensitisation was similarly more prevalent in urban dwellers (28%; 18 patients) than in rural dwellers (24%; 10 patients), further reinforcing this trend. Notably, cannabis and paper mulberry sensitisation, both allergens associated with outdoor and agricultural exposures, were higher in the rural group (34% and 18%, respectively) compared to urban individuals (29% and 9%, respectively), possibly reflecting greater environmental exposure to natural allergens. Sensitisation to Aspergillus mix and grass mix remained comparable between the two populations, with minor variations. These findings suggest that greater microbial diversity and environmental exposures in rural settings may have a protective effect against allergic sensitisation, lending further support to the hygiene hypothesis.

## DISCUSSION

Identifying the most prevalent allergens in a given region is essential for developing effective prevention and management strategies for allergic diseases. Numerous studies across different geographic locations have sought to determine local allergen prevalence, facilitating the selection of region-specific allergen panels for SPT and specific immunotherapy. Such research plays a crucial role in guiding clinicians and policymakers to optimise allergy management based on local environmental and epidemiological factors.

The findings of this study demonstrated a higher prevalence of allergen sensitisation among urban dwellers (60%) compared to rural residents (40%), supporting the hygiene hypothesis, which postulates that reduced microbial exposure in urban settings contributes to increased allergic sensitisation. Similar observations have been reported in other studies. Tizek *et al.*, in Germany found a comparable trend,<sup>14</sup> while a study from Southern Vietnam documented aeroallergen sensitisation in 259 (70%) urban patients, which was significantly higher than in 128 (30%) rural patients.<sup>15</sup>

Among all the aeroallergens tested, house dust mite (HDM) was the most prevalent, affecting 59% of sensitised patients, underscoring its dominant role in indoor allergen exposure. This finding aligns with previous studies, such as one conducted by Dey *et al.*, in 2019 in India, where HDM elicited 80% positivity among allergic patients.<sup>16</sup> Similarly, Qatar-based research reported HDM allergy prevalence at 49%, while a 2018 study from Thailand documented a 50% positivity rate for HDM allergy.<sup>17,18</sup> The high prevalence of HDM allergy in Pakistan and neighbouring regions may be attributed to climatic factors such as high temperature and humidity, as well as lifestyle modifications, including increased use of curtains, carpets, and mattresses, and prolonged indoor exposure.

In this study, the prevalence of aeroallergens was observed as follows: House dust mite (59%), cannabis (31%), mixed pollens (26%), grass mix (23%), paper mulberry (15%), and Aspergillus (8%). These results were compared to an earlier study by Khan *et al.*, conducted at the same institution during 2014–2016, which reported positivity rates of house dust mites (33%), cannabis (17%), mixed pollens (19%), grass mix (27%), paper mulberry (31%), and Aspergillus (7%).<sup>19</sup>

The increase in house dust mite sensitisation in the present study could be attributed to greater indoor exposure during the COVID-19 pandemic and post-pandemic period.

In the 1990s, the National Institutes of Health (NIH) data identified paper mulberry pollen as the leading cause of allergy symptoms in Islamabad and Rawalpindi.<sup>20</sup> The study by Khan *et al.*, reported 31% positivity for paper mulberry, whereas the present study found that only 15% of patients were sensitive to this allergen in the same geographic region. This decreasing trend may reflect greater public awareness, increased adoption of protective measures such as mask-wearing, and the implementation of desensitisation strategies for paper mulberry allergy.

A positive family history of allergic diseases was observed in 43% of patients in this study, whereas a study from Iran reported a higher frequency (64%) and another study from Thailand found a 67% prevalence of family history.<sup>21,22</sup> The relatively lower family history positivity in the present study's population could be attributed to genetic variability in allergic predisposition.

Regarding symptom patterns, perennial symptoms were noted in 45% of patients, while 55% exhibited seasonal symptoms. This contrasts with an Iranian study, which documented 22% perennial and 78% seasonal patterns, suggesting regional variations in allergen exposure.<sup>21</sup> In terms of clinical symptoms, sneezing was the most frequent (67%), followed by allergic rhinitis (60%), allergic conjunctivitis (49%), and asthma (49%), whereas an Iranian population-based study reported higher frequencies of sneezing (79%), allergic rhinitis (85%), and allergic conjunctivitis (47%).<sup>21</sup>

This study also found polysensitisation in 41% of patients, which is lower than reported rates in other studies. A study conducted by De Bot *et al.*, in United Kingdom in 2013, reported 69% polysensitisation,<sup>23</sup> while a study conducted by Thalappil *et al.*, in Qatar, showed polysensitisation to 62% of allergic patients.<sup>17</sup> Another study conducted by Ndjindji *et al.*, showed 60% polysensitisation for aeroallergens.<sup>24</sup> The lower polysensitisation rate in Pakistani population may be attributable to genetic and environmental factors, as well as the relatively small sample size of this study.

The findings of this study should be interpreted with consideration of its limitations. The sample size was relatively small, which may limit generalisability to a larger population. Additionally, only six aeroallergens were tested, which may not comprehensively capture the full spectrum of allergens present in the environment. Furthermore, as this study was conducted in northern Pakistan, regional variations in climatic conditions and vegetation may influence allergen distribution, limiting the applicability of these findings to other parts of Pakistan.

These findings underscore the importance of early-life microbial exposure in immune system development and empha-

size the need for region-specific allergen screening panels, targeted prevention strategies, and public health interventions. Future research should focus on larger, multi-centre studies across different regions of Pakistan to provide a comprehensive understanding of allergen distribution and environmental influences on allergic diseases.

## CONCLUSION

This study provides compelling evidence supporting hygiene hypothesis, demonstrating a higher prevalence of aeroallergen sensitisation in urban populations compared to rural dwellers. The most frequently identified allergen was house dust mites, which aligns with global trends and highlights the impact of indoor environmental factors on allergic disease prevalence. The decreasing sensitisation to paper mulberry over time suggests greater awareness and protective interventions, while the relatively lower rates of polysensitisation in this population may reflect genetic and environmental differences.

### ETHICAL APPROVAL:

This study was approved by the Ethical Review Committee of the Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, and ethical approval was obtained prior to the initiation of the research work.

### PATIENTS' CONSENT:

Informed consents were taken from all patients about the study and the detailed history was recorded.

### COMPETING INTEREST:

The authors declared no conflict of interest.

### AUTHORS' CONTRIBUTION:

MH: Concept, design, and drafting of the work.

MOR: Critical revision of the manuscript.

MA: Interpretation of data and intellectual content.

MAH: Data collection.

MZA: Analysis.

All authors approved the final version of the manuscript to be published.

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