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Correlation between Fungal Sensitization in Childhood Persistent Asthma and Disease Severity

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ABSTRACT

Background

Fungal sensitization in adults is associated with severe asthma but prevalence and clinical significance of fungal sensitization remains unclear in paediatric population.

Objective

To study association of fungal sensitization with disease severity in children with persistent asthma

Patients and Methods

One hundred children with persistent asthma in age group 7-15 years, symptom duration >2 years and forced expiratory volume in first second >50% of expected were enrolled. Skin prick test (SPT) to 8 fungal antigens and total serum IgE were done. Fungal sensitization was

described as positive SPT (wheel diameter more than 3mm larger than the negative control) to any of the fungal antigens and total serum IgE >200ng/ml.

Results

Seventeen patients showed evidence of fungal sensitization, of which, six demonstrated sensitization to multiple fungi. 17.6% patients with fungal sensitization had severe asthma as compared to 2.4% patients without fungal sensitization (p value 0.032). Significant increase in family history of allergic comorbidities was noted among patients with fungal sensitization (47.1% vs 21.7%, p value 0.03). The most common implicated organism in fungal sensitized patients was *Aspergillus flavus* (47.1%).

Conclusion

The results of this study, a first among Indian children with asthma, suggest that children with fungal sensitization have more severe asthma as compared to children without fungal sensitization.

Keywords: Childhood persistent asthma, Fungal sensitization, *Aspergillus* species, Skin prick test, Serum immunoglobulin E, Severe asthma

Correlation between Fungal Sensitization in Childhood Persistent Asthma and Disease Severity

Introduction

Asthma is characterized by inflammation of airways which causes airway hyper-responsiveness and airflow limitation resulting in cough, wheeze, chest tightness and dyspnoea ⁽¹⁾. An estimated 300 million people suffer from asthma worldwide resulting in

increased burden on governments and healthcare systems ⁽²⁾. Majority of patients with asthma respond to corticosteroids, beta agonists and other anti-inflammatory agents. However, in some patients, asthma may be severe and may not respond to conventional therapy.

There are innumerable factors affecting asthma severity which may vary from patient to patient. Majority of patients with asthma show sensitization to common allergens ⁽³⁻⁵⁾. Exposure to animal dander from pets, house dust mite and environmental fungi can worsen asthma symptoms ^(3, 4). ~~Fungi, as allergens, are important for children with asthma because exposure to fungi can occur in both indoor and outdoor environments.~~ Fungal spores are present in outdoor air throughout the year and damp indoor conditions are associated with hyper-reactivity of bronchi and worsening respiratory symptoms ^(3, 4).

Fungi are responsible for numerous pulmonary diseases including Atopic Asthma, Hypersensitivity Pneumonitis, Invasive Aspergillosis and Allergic Bronchopulmonary Mycosis (ABPM) ⁽³⁾. Epidemiologic studies in various countries have demonstrated a link between fungi and asthma ^(3, 6). Recent observations have shown that patients with severe asthma have high rate of sensitivity to fungal antigens ^(7, 8). Fungal sensitization and prolonged, uncontrolled fungal infection are known to be associated with poorly controlled asthma. The term severe asthma with fungal sensitization (SAFS) illustrates increased fungal sensitization in patients with severe asthma ~~and improvement of symptoms with antifungal therapy~~ ⁽³⁾. However, role of fungi as primary allergens responsible for asthma severity has not been elucidated. Distribution of fungi causing sensitization and infections also varies among different geographical regions ^(3, 6). Sensitization to *Aspergillus* among asthma patients has been reported to vary from 16-38% in different parts of the world ⁽⁹⁾.

Despite various studies suggesting a link between asthma and fungal agents in adult population, the correlation remains unclear in children and sufficient data is not available.

Currently, the prevalence and clinical implications of fungal sensitization in children with persistent asthma is not completely understood. The aim of this study was to screen children with persistent asthma for fungal sensitization and to ascertain the association of fungal sensitization with disease severity.

Patients and Methods

This prospective cross-sectional study was conducted in our institute over a period of one year, between July 2013 and June 2014. Institute's ethics committee approved the study; and informed written consent was obtained from the subjects or their guardians before enrolment in the study.

Patients

For the purpose of study, Bronchial Asthma was defined by presence of two of the following three criteria ⁽¹⁰⁾: (1) recurrent episodes of cough (more so at night), dyspnoea and chest tightness; (2) clinical examination revealing presence of wheeze; (3) spirometry showing evidence of obstructive defect – ratio of forced expiratory volume in first second to forced vital capacity ($FEV_1:FVC$) < 70%, with or without significant bronchodilator reversibility (increase of $FEV_1 \geq 12\%$ or 200 ml after giving inhaled salbutamol at a dose of 400 μ g). Reversibility in FEV_1 alone was not included as a criterion for diagnosis of asthma because some children were diagnosed with asthma at other health care facilities and were on treatment at the time of first presentation to our institute. These children, especially with controlled asthma, may not exhibit significant reversibility in FEV_1 which, thus, lacks sensitivity ⁽¹⁰⁾. For the same reason, obstructive defect on spirometry was not an essential criterion for such children, provided history and clinical examination were consistent with a diagnosis of asthma and previous medical records revealed evidence of obstructive defect on spirometry. ~~Reversibility in FEV_1 (increase of $FEV_1 \geq 12\%$ or ≥ 200 ml after giving inhaled~~

salbutamol at a dose of 400µg) alone was not included as a criterion for diagnosis of asthma because it lacks sensitivity⁽¹⁰⁾.

Hundred (100) children were enrolled who fulfilled the following inclusion criteria: (1) a diagnosed case of persistent asthma in age group 7 – 15 years, with duration of symptoms more than two years, in follow-up in Asthma and Allergy Clinic of the department (2) FEV₁ value >50% of expected; (3) Mild, Moderate and Severe Persistent Asthma defined according to GINA classification⁽¹¹⁾; (4) patient on optimal treatment for the given severity of asthma. Children presenting in acute exacerbation, and those with intermittent asthma and heart disease were excluded from the study. Patients with FEV₁ < 50% of expected were assumed to be in acute exacerbation and thus, were not subjected to tests for fungal sensitization.

Methods

Children were screened with Skin Prick Test (SPT) to eight available fungal antigens along with total serum immunoglobulin E (IgE) levels. Fungal sensitization was described based upon the following criteria: (1) positive fungal SPT; and (2) total serum IgE levels >200ng/ml. Two groups of patients were described depending upon the result of SPT and total serum IgE - patients of persistent asthma with 1) fungal sensitization (group I), and with 2) no fungal sensitization (group II).

Skin Prick test (SPT)⁽⁸⁾

All patients with persistent asthma underwent SPT using 1:10 concentration of the following antigens: *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus versicolor*, *Candida albicans*, *Cladosporium herbarium*, *Helminthosporium*, *Mucor mucedo*.

Before carrying out the test, it was ensured that patients have stopped taking any form of anti histaminic drugs at least 24 hours before the test. Also, antihistaminic drug (syrup/tablet) along with hydrocortisone ointment or calamine lotion was kept ready before the procedure.

Test was performed on the skin of forearm after ensuring that no emollient or other topical medications were applied on the skin surface. No antiseptic was used to clean skin prick site.

A drop of reagent (solution with a fungal allergen) was put on the forearm and was scratched using a lancet. Each allergen was tested in same way along with a negative control. Separate lancet was used for each allergen. Test was read after 20 minutes for Type 1 Hypersensitivity reaction manifesting as a wheal and a flare.

Largest diameter of the wheal (not including flare) and the diameter 90 degrees to first measurement were obtained. Reaction was considered positive if longest wheal diameter was at least 3mm larger than negative control.

Total Serum IgE level

5ml blood was withdrawn aseptically from peripheral vein after informed consent. Serum IgE level was assayed with commercial kits using quantitative enzyme linked immunosorbent assay (Calbiotech Inc. IgE ELISA kit). For study purpose, total serum IgE level >200ng/ml was considered significant for inclusion as one of the criteria for fungal sensitization, along with positive skin prick test.

Results

Out of 100 patients, 17 showed evidence of fungal sensitization (Group I) while remaining 83 did not fulfil the criteria (Group II). Six patients (35.3%) demonstrated sensitization to multiple fungal antigens.

General characteristics

Mean age was comparable between two groups, but patients with fungal sensitization developed symptoms of asthma at a younger age than those without fungal sensitization. Female gender, rural residence, average body mass index (BMI), dampness, exposure to cigarette smoking and family history of asthma was higher among patients with fungal sensitization; however, difference was not significant. Significant increase in family history of allergic comorbidities was noted among patients with fungal sensitization (47.1% vs 21.7%). Demographic details of the two groups are summarized in Table I.

Most common presenting complaint was cough followed by shortness of breath, wheeze and expectoration. Higher percentage of fungal sensitized patients presented with expectoration as compared to patients without fungal sensitization.

Severity of asthma and fungal sensitization

Group without fungal sensitization had higher percentage of patients with mild and moderate asthma. 17.6% of patients with fungal sensitization had severe asthma as compared to only 2.4% of patients without fungal sensitization (p value 0.032). Of the 5 patients with severe persistent asthma, 60% had a positive SPT. Outcomes in two study groups are summarized in Table II.

Patients with fungal sensitization had a higher mean total serum IgE level (3541.76ng/ml) as compared to patients without fungal sensitization (2260.14ng/ml). Percentage of patients with total serum IgE <1200ng/ml was higher in the group without fungal sensitization; however, higher percentage of patients with fungal sensitization had total serum IgE >2000ng/ml. Mean FEV1 was comparable in both the groups. 47.1% of patients with fungal sensitization experienced more than 2 exacerbations of asthma symptoms in preceding 1 year in

comparison to only 14.4% of patients without fungal sensitization (p value 0.005). Patients requiring prolonged duration of inhaled corticosteroids (>12 months) for control of asthma symptoms were more in SPT positive group as compared to SPT negative group (70.6% vs 50%).

Aspergillus antigen and fungal sensitization

The most common implicated organism in fungal sensitized patients was *Aspergillus flavus* (47.1%) followed by *Mucor mucedo* (29.4%). Frequency of various fungal antigens among SPT positive patients is shown in Table III. Six patients showed positive SPT for multiple fungal antigens while 11 patients had positive SPT for a single antigen.

In SPT positive group, higher numbers of patients were positive for *Aspergillus* species (59%) as compared to non *Aspergillus* species (41%). Among *Aspergillus* species, higher percentage of patients was positive for *Aspergillus flavus* as against *Aspergillus fumigatus* which has been described as the causative agent for most of the known clinical entities including Allergic Bronchopulmonary Aspergillosis (ABPA). Asthma severity was either moderate or severe for all fungal antigens except *Aspergillus flavus*, *Mucor mucedo* and *Cladosporium herbarium* (figure I). Episodes of exacerbations were highest for *Aspergillus flavus* followed by *Mucor mucedo* (figure II).

Discussion

Majority of children with asthma respond to conventional therapy with inhaled corticosteroids, beta agonists and other anti inflammatory agents, but some patients have uncontrolled asthma not responding adequately to these agents. Why some patients respond to conventional medications while some do not is not exactly known, with innumerable factors affecting asthma control. Recent studies in adults have shown a link between fungal

sensitization and severity of asthma ⁽⁸⁾ but the same has not been studied in paediatric age group.

This prospective cross-sectional study was designed to study the association of fungal sensitization with persistent asthma and whether disease severity could be correlated to fungal sensitization. We found that out of 100 pediatric patients with persistent asthma, 17 showed evidence of fungal sensitization. In study by Agarwal et al ⁽⁸⁾ and Vicencio et al ⁽⁷⁾, 52.5% and 39% of patients respectively had fungal sensitization. Difference in rates of fungal sensitization between different studies can be explained by differences in methods used to standardise the extracts of mould allergens or due to differences in techniques of skin testing ⁽⁸⁾. We used SPT with commercially available antigens.

Patients with fungal sensitization had more severe asthma than patients without fungal sensitization. This is in accordance with study by Vicencio et al ⁽⁷⁾ and Hui Zou et al ⁽¹²⁾. Agarwal et al ⁽⁸⁾ in their study suggested a weak association between disease severity and *Aspergillus* sensitization. However, fungi other than *Aspergillus* were not included in their study. We noted more episodes of exacerbation of asthma symptoms and longer duration of inhaled corticosteroids for control of asthma symptoms in patients with fungal sensitization. These patients also had a higher mean total serum IgE level. Apart from fungal antigens, sensitization to other aero-allergens could have probably contributed to elevated total IgE level. Measurement of fungus specific IgE level, thus, would have been more informative. Mean FEV1 was comparable between two groups and there was no significant difference in distribution of PEF percentage based on two groups of fungal sensitization. These findings show that most of the patients were well controlled at the time of inclusion into the study.

Various mould species have been described to cause allergies of the respiratory tract. Evidence of connection between *Aspergillus* related fungal sensitization and asthma (the first

paper to suggest a link between fungal sensitization and asthma severity) was published in 1978 ⁽¹³⁾. Since then, several mould species have been associated with asthma severity, among which, non *Aspergillus* moulds have been studied extensively. *Alternaria* sensitivity has been shown to be associated with increased bronchial hyperreactivity, higher risk of respiratory arrest ⁽¹⁴⁾, and severe asthma ⁽¹⁵⁾. *Helminthosporium*, *Epicoccum* and *Cladosporium* have been implicated in difficult to control asthma ⁽¹⁵⁾, need for recurrent hospital admissions ⁽¹⁶⁾ and need for intensive care for acute asthma ⁽¹⁷⁾. However, most studies have not studied sensitivity to *Aspergillus* species despite it being associated with two important immunological conditions, the *Aspergillus* sensitive asthma and ABPA.

Aspergillus flavus was the most common organism responsible for fungal sensitization in our study. Higher percentage of patients positive for *Aspergillus flavus* as compared to *Aspergillus fumigatus* assumes significance because *Aspergillus fumigatus* has been described as the causative agent for most of the known clinical entities including ABPA, and not *Aspergillus flavus* ^(3, 8). *Aspergillus flavus* was also associated with higher number of episodes of exacerbation of asthma symptoms. Association between number of episodes of asthma exacerbations and fungal sensitization has not been studied previously, nor the common fungal sensitizers responsible for asthma exacerbations.

Studies have shown that patients residing in rural area have less incidence of fungal sensitization than patients living in urban area ⁽⁸⁾. However, same could not be established in present study as area of residence, whether rural or urban, did not affect sensitization to fungal antigens in our cohort. Exposure to microbial agents in children living on farmlands can explain protection against the development of asthma in these children as compared to those who are not living on farms ⁽¹⁸⁾.

Patients with fungal sensitization had significantly higher family history of allergic comorbidities as compared to SPT negative group. It highlights the importance of eliciting detailed history in children with asthma. Children with positive family history should be closely monitored and screened for development of fungal sensitization in case of persistence of symptoms and non improvement with conventional therapy.

Several questions need to be answered with respect to fungal sensitization in children with persistent asthma. First, clinical significance of fungal sensitization in children with asthma needs to be elucidated. There are two aspects to it – these patients need to be worked up for ABPA, and these patients could be potential candidates for antifungal therapy to reduce risk of progression to ABPA. Second, whether fungal sensitization itself can progress to result in ABPA or merely acts as a trigger is not known. Also, it is unclear whether persistent infection of airways is responsible for fungal sensitization or it results from transient exposure to environmental fungal antigens. Third, difference in clinical implications of fungal sensitization in comparison to fungal colonization and/or infection is not known. Longitudinal studies may be required to answer these questions.

The strength of our study was the large sample size. Although other aero-allergens could have affected the development of sensitization, present study was done to screen patients with persistent asthma for fungal sensitization and to ascertain the association of fungal sensitization with disease severity. Hence, sensitization to other allergic agents in the environment was not measured. Fungal sensitization can be described based on positive skin prick test or fungus specific IgE level in serum. Use of both skin testing and specific serum IgE level is likely to identify more patients with fungal sensitization because not all sensitivities will be identified with the use of one alone. Specific IgE level were not carried out in present study because facilities were not available at our institute. This could have arguably resulted in underestimation of fungal sensitization.

We demonstrated that patients with fungal sensitization have more severe asthma. Thus, children with persistent asthma who remain symptomatic on optimal therapy with conventional medications may be considered for evaluation of fungal sensitization. Further studies are needed to study long term implications of fungal sensitization as it may have important therapeutic implications while treating pediatric patients with persistent asthma. Targeted intervention in the form of anti-fungal therapy needs to be explored so as to reduce the number of exacerbations of asthma symptoms and to ensure a better quality of life to children with persistent asthma and fungal sensitization.

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References

1. Pocket Guide For Asthma Management And Prevention (Internet). Global Initiative for Asthma (GINA).2012. Available from: <http://www.ginasthma.org/documents/1>.
2. Global Burden of Asthma (Internet). Global Initiative for Asthma (GINA). Available from: [http://www.ginasthma.org/local/uploads/files/GINABurdenReport 1.pdf](http://www.ginasthma.org/local/uploads/files/GINABurdenReport%201.pdf).
3. Knutsen AP, Bush RK, Demain JG, et al. Fungi and allergic lower respiratory tract diseases. The Journal of allergy and clinical immunology. 2012;129(2):280-91.
4. Langley SJ, Goldthorpe S, Craven M, Morris J, Woodcock A, Custovic A. Exposure and sensitization to indoor allergens: association with lung function, bronchial reactivity, and exhaled nitric oxide measures in asthma. The Journal of allergy and clinical immunology. 2003;112(2):362-8.

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5. Kalliel JN, Goldstein BM, Braman SS, Settipane GA. High frequency of atopic asthma in a pulmonary clinic population. *Chest*. 1989;96:1336-40.
 6. Agarwal R, Gupta D. Severe asthma and fungi: current evidence. *Medical mycology*. 2011;49(Suppl.1):S150-7.
 7. Vicencio AG, Santiago MT, Tsirilakis K, et al. Fungal sensitization in childhood persistent asthma is associated with disease severity. *Pediatric pulmonology*. 2014;49(1):8-14.
 8. Agarwal R, Noel V, Aggarwal AN, Gupta D, Chakrabarti A. Clinical significance of *Aspergillus* sensitisation in bronchial asthma. *Mycoses*. 2011;54:e531-38.
 9. Shah A. Asthma and aspergillus. *The Indian journal of chest diseases & allied sciences*. 2004;46(3):167-70.
 10. Bateman ED, Hurd SS, Barnes PJ, et al. Global strategy for asthma management and prevention: GINA executive summary. *The European respiratory journal*. 2008;31(1):143-78.
 11. Koshak EA. Classification of asthma according to revised 2006 GINA: evolution from severity to control. *Annals of thoracic medicine*. 2007;2(2):45-6.
 12. Zou H, Su L, Fang QH, Ma YM. Correlation between fungal sIgE and bronchial asthma severity. *Experimental and therapeutic medicine*. 2013;6:537-41.
 13. Schwartz HJ, Citron KM, Chester EH, et al. A comparison of the prevalence of sensitization to *Aspergillus* antigens among asthmatics in Cleveland and London. *J Allergy Clin Immunol*. 1978;62:9-14.
 14. O'Hollaren MT, Yunginger JW, Offord KP, et al. Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients with asthma. *N Engl J Med*. 1991;324(6):359-63.

15. Torres-Rodríguez JM, Pulido-Marrero Z, Vera-Garcia Y. Respiratory allergy to fungi in Barcelona, Spain: Clinical aspects, diagnosis and specific treatment in a general allergy unit. *Allergologia et immunopathologia*. 2012;40(5):295-300.
16. Roux P, Smit M, Weinberg EG. Seasonal and recurrent intensive care unit admissions for acute severe asthma in children. *S African Medical Journal*. 1993;83:177-9.
17. Black PN, Udy AA, Brodie SM. Sensitivity to fungal allergens is a risk factor for life threatening asthma. *Allergy*. 2000;55:501-4.
18. Ege MJ, Mayer M, Normand AC, et al. Exposure to Environmental Microorganisms and Childhood Asthma. *N Engl J Med*. 2011;364(8):701-9.

Table I. Demographic characteristics of the two study groups

(Group I: with fungal sensitization, Group II: without fungal sensitization)

Characteristics	Group I (n=17)	Group II (n=83)	p-value
Mean age (years)	10.23	10.42	0.716
Mean age at symptom onset (years)	3.5	4.7	-
Female gender	4 (23.5%)	17 (20.5%)	0.779
Rural residence	9 (52.9%)	42 (50.6%)	0.86
Average BMI (Kg/m ²)	15.77	15.4	0.66
Dampness	6 (35.3%)	25 (30.1%)	0.674
Overcrowding	1 (5.9%)	8 (9.6%)	0.622
Exposure to cigarette smoking	5 (29.4%)	21 (25.3%)	0.725
Family history of asthma	3 (17.6%)	10 (12%)	0.532

Family history of allergic comorbidities	8 (47.1%)	18(21.7%)	0.03
Symptoms of asthma			
-Cough	17 (100%)	82 (99%)	0.649
-Shortness of breath	14 (82.4%)	72 (86.7%)	0.634
-Wheeze	13 (76.5%)	66 (79.5)	0.779
-Expectoration	2 (11.8%)	3 (3.6%)	0.16

- BMI: Body mass index
- Significant p values have been highlighted in bold

Table II. Outcomes in the study groups

Variable	Group I (n=17)	Group II (n=83)	p-value
Asthma severity			
Mild	6 (35.3%)	36 (43.4%)	0.032
Moderate	8 (47.1%)	45 (54.2%)	
Severe	3 (17.6%)	2 (2.4%)	
Total serum IgE (ng/ml)			
<1200	6 (35.3%)	45 (54.2%)	0.363
1200-2000	3 (17.6%)	10 (12.1%)	
>2000	8 (47.1%)	28 (33.7%)	
Mean total serum IgE (ng/ml)	3541.76	2260.14	0.231

PEFR			
50-60%	1 (5.9%)	3 (3.6%)	0.408
>60-80%	4 (23.5%)	11 (13.3%)	
>80%	12 (70.6%)	69 (83.1%)	
Mean FEV1	95.69	93.6	0.51
No of exacerbations in preceding one year			
<=2	9 (52.9%)	71 (85.5%)	0.005
>2	8 (47.1%)	12 (14.4%)	
Duration of inhaled corticosteroids			
Total patients	17	36	
<6 month	3 (17.6%)	14 (38.9%)	0.287
6-12 month	2 (11.8%)	4 (11.1%)	
>12 month	12 (70.6%)	18 (50%)	

- IgE: Immunoglobulin E, PEFR: Peak expiratory flow rate, FEV1: Forced expiratory volume in first second
- Significant p values have been highlighted in bold

Table III. Frequency of fungal antigens among patients with positive Skin Prick Test

Fungal antigen	Number of patients with positive SPT (n=17)
Aspergillus flavus	8 (47.1%)
Aspergillus fumigatus	3 (17.6%)

Aspergillus niger	4 (23.5%)
Aspergillus versicolor	2 (11.8%)
Candida albicans	2 (11.8%)
Cladosporium herbarium	4 (23.5%)
Helminthosporium	2 (11.8%)
Mucor mucedo	5 (29.4%)

- SPT: Skin Prick Test

Figure legends:

Legend to figure I. Association of fungal antigens to degree of asthma severity

Legend to figure II. Association of fungal antigens to episodes of exacerbation of asthma symptoms

