International Journal of Applied Research 2019; 5(6): 102-105

ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2019; 5(6): 102-105
www.allresearchjournal.com
Received: 18-04-2019
Accepted: 22-05-2019

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Prevalence of *Candida species* in patients with Asthma

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Abstract
Asthma & COPD has worldwide prevalence. Allergic asthma is a respiratory disease that is induced by exposure to environmental antigens that elicit allergic inflammation & intermittent airway obstruction due to which symptoms of cough & dysnea are seen. Inflammation can increase airway hyperresponsiveness which can lead to infections by viruses, fungi & various bacteria including atypical bacteria. Along with other fungi *C. albicans* is a potent allergen in some situations. Both the protein and carbohydrate fraction of *C. albicans* can act as an allergen. Total 150 patients suffering from Asthma were analysed for presence of Candida species in their sputum sample. 12 Patients showed growth of Candida species which were further differentiated as *Candida albicans* (10) & *Candida tropicalis* (02). Antibiotic susceptibility testing was also performed & most of the isolates were found to be sensitive for Amphotericin B followed by Clotrimoxazole & fluconazole. Total IgE was determined for confirmation of atopy & IgE was found to be raised in all patients. *Candida albicans* IgG was elevated in 21 patients. Elevated levels of *C. albicans* IgG along with positive sputum cultures indicates past & current infection by Candida species in asthma patients. Therefore it becomes essential to diagnose & treat these infections with appropriate antibiotics. As it is seen that Antibiotic treatment helps in fast recovery of patient, which can reduce duration for corticosteroid consumption by patients.

Keywords: Environmental antigens, *C. albicans*, Antibiotic susceptibility testing. IgE, *C. albicans* IgG

Introduction
Amongst allergic respiratory diseases allergic rhinitis (hay fever) and asthma are two of the most common allergic diseases. Both diseases appear to have increased significantly in past two centuries [1].

Asthma is a chronic inflammatory disease of the airways which in susceptible individuals causes recurrent episodes of wheezing, breathlessness, chest tightness and cough. Inflammation can increase airway hyper responsiveness which can lead to infections by bacteria including atypical bacteria, fungi and viruses [2].

In asthma condition allergic, toxic, fungal, viral and other initiators of inflammation play a major role [3].

Fungi are known to causative factors that include asthma symptoms. Outdoor airborne fungi including *Cladosporium*, *Alternaria*, *Penicillium* and *Aspergillus* and indoor fungi like *Neurospora*, *Aspergillus* and *Eurotinum* are significant triggers of IgE formation. There is strong fungal/yeast component in the lung and/or gut microflora in individual with asthma. *Candida albicans* may be a prominent allergen for many people with asthma. The cell wall constituent mannan and acid protease an enzyme produced by *C. albicans* are both highly allergic and serum IgE antibodies are often increased in atopic individuals. Animal and *in vivo* studies suggest that if there is an imbalanced Th1/Th2 ratio of immune activity Candida infection is likely to occur [4, 5]. *Candida albicans* has been also identified as a potent allergen in bronchial asthma, rhinitis, chronic urticaria, atopic dermatitis recurrent vaginitis and balanitis [6].

There are variety of risk factors identified to be associated with allergic diseases. They are classified as host factors and environmental factors. Host factors predispose individuals to or protect them developing allergic diseases. Environmental factors influence the susceptibility to the development of allergies in predisposed individuals, precipitate allergic exacerbations and/or cause symptoms to persist [7, 8].

The main environmental factors for the susceptibility to the development of respiratory allergies in the predisposed individuals include exposure to indoor and outdoor allergens

(domestic mites, animals, fungi, moulds, yeasts, pollens etc.) and occupational sensitizers air pollutants, viral and bacterial respiratory infections, diet, tobacco smoke (both active and passive smoke), socioeconomic status and family size [6-8]

Microbial infections associated with allergic respiratory infections increase the severity and duration of the disease as well as may themselves act as an allergen. Therefore their treatment with appropriate antimicrobials is essential. Many studies have suggested that patients with such associated respiratory infections when treated with antibiotics can improve patient's ability to breath. Antibiotic treatment helps in fast recovery of patient, which can reduce corticosteroid consumption by patients [9, 10].

Long term use of steroids can give a lot of side effects including suppression of immune system making individual more prone to fungal infections similarly prolonged and irrational use of antibiotic may result in emergence of drug resistance organisms. Unnecessary and random use of medicines without prior diagnosis and also increases total cost of therapy.

Materials & Methods

The present study was carried out at the department of Microbiology, T. N. Medical College and B. Y. L. Nair Charitable Hospital, Mumbai Central, Mumbai, Maharashtra, India.

Total 150 Asthma patients were included in the study. The senior clinician of Medicine department, T. N. Medical College did the selection of subjects on the basis of their clinical and radiological diagnosis.

Patients selected were either hospitalized (Indoor basis) or outdoor patients at B. Y. L. Nair ch. Hospital.

Inclusion criteria

- Patients able to give productive sputum or any other suitable respiratory samples & clinically suspicious of infective etiology.
- Patients who are able to produce brief clinical history.
- Patients in whom antibiotics have not been administered within last 48-72 hours.

Age group and Sex

Patients of all age group and both the sexes

Following exclusion criteria were used while selecting patients for the study

- Patients who are not able to produce adequate sputum or give any other relevant respiratory samples.
- Patients on prolonged antibiotic treatment.
- Patients requiring ICU care.
- Pregnant females.

Collection of respiratory specimens [11, 12].

Sputum samples were collected from selected patients. Minimum 3 consecutive samples were studied for confirmation of results.

All universal safety precautions were taken while collection, transportation, handling and processing of specimens. All specimens were processed within one hour of collection. Fresh morning sputum specimens were collected with aseptic in a clean, sterile, leak-proof container. Each patient was advised to collect on early morning sputum sample after washing the mouth and gargling with tap water.

Processing of sputum specimens [11-13].

All specimens were studied microscopically as well as macroscopically. In microscopic characters like color, appearance, presence or absence of blood in specimens were noted.

Pretreatment: NALC digestion was carried out for mucolysis of all sputum specimens.

II. Culture [11]: A loopful of each clinical specimen was inoculated or streaked on various culture media for isolation of pathogens. All aseptic precautions were taken while processing of samples.

Medium used: Sabouraud's Dextrose agar with tetracycline and chloramphenicol.

After isolation plates were incubated at 37 ºC for 48 hours

Identification of the isolates obtained from the clinical samples [11-15].

Identification of *Candida* species

Demonstration of budding yeast like cells and pseudohyphae in wet mount or Gram's stained smear serves as guidelines for the presence of *Candida* species. The colonies of Candida on Sabourauds dextrose agar slant appear as white, white, raised, soft in consistency and possesses smooth borders.

Confirmation of presumptive colonies

Germ tube test - The yeast like colonies was speciated in to *C. albicans* and other species on the capability of producing a germ tube when inoculated in a serum environment by observing microscopically. (O.5 ml of pooled human serum + one isolated colony → incubate in water bath at 37ºC for 2 hours.)

Fermentation and assimilation of sugars: Speciation of *Candida* was further done by carrying out fermentation and assimilation of sugars. The sugars used for fermentation were sucrose, maltose, dextrose and galactose and for assimilation were sucrose, maltose, dextrose, galactose and xylose.

Antibiotic Susceptibility testing [16-19].

Antibiotic susceptibility testing of the Candida isolates obtained from sputum specimens was carried out by Kirby-Bauer disk diffusion method according to CLSI guidelines [18, 19]. *C. albicans* 10231 were also tested for antibiotic sensitivity for ensuring proper results and to monitor internal quality control.

A few colonies of the isolate to be tested were inoculated in 5 ml of suitable broth and incubated for 3-4 hours at 37 ºC. The turbidity of the broth was matched visually with 0.5 McFarland's standard using sterile saline. A sterile non-toxic cotton swab on a wooden applicator was dipped in to the standardized inoculum. This soaked swab was rotated firmly against the upper inside wall of tube to remove excess fluid. The surface of Mueller Hinton agar plate was uniformly streaked with swab three times by turning the plate at 60° angle between each streaking. The plates were allowed to dry at room temperature for 5 min. Each antibiotic disc was placed aseptically using a pair of sterile forceps on the surface of the culture at an optimum distance on the inoculated plates. The plates were incubated at 37 °C aerobically.

For *Candida species*: Sabourauds Dextrose agar Amphotericin B (100 mcg), Fluconazole (10 mcg), Clotrimoxazole (10 mcg)

Interpretation of results

After overnight incubation at 37 °C, diameter of each zone were measured and recorded in mm. The results were interpreted according to CLSI guidelines, by comparing with the results stated with standard ATCC strain. The pattern obtained was documented as Sensitive, Resistant or Intermediate.

Serological Tests [11].

3-5 cc of whole blood was collected by venipuncture using a disposable 5.0 ml syringe and 21 gauze hypodermic needle in a sterile plain test tube taking all aseptic precautions. The sterile plain tube was incubated at 37 ºC in a slanting position for 1-1.5 hours and later held at 4 ºC for 1 hour. This method facilitates clotting of blood. The supernatant serum layer was separated and centrifuged at 2000 rpm to remove the cellular debris. The clear serum was preserved in the absence of preservatives in plastic storage vials after labeling it properly with patient's registration numbers and date at -20 ºC until utilization.

The stored serum was used for detecting *C. albicans* IgG, and total IgE by Solid phase ELISA method.

The patients were selected at random and were studied for estimation of immunoglobulin levels.

Total IgE estimation: Total IgE estimation was done by Solid phase Enzyme Linked Immunoassay by IBL ELISA (Quantitative).

ELISA for determination of specific IgG

Candida albicans IgG antibodies were estimated by Solid phase Enzyme Linked Immunoassay by IBL ELISA. (Quantitative)

Results & Discussion

Table 1: Incidence of Candida species in samples tested

Total number of samples tested	Samples showing growth of *Candida species*
150	12
(100)	(8)

Candida species were obtained in 8% cases

Amongst Candida species *C. albicans* was the predominant isolates obtained in of cases followed by *C. tropicalis*.

Table 2: Differentiation of Candida isolates

Total number of samples tested	Total no. of Candida isolates	Isolates	
		C. albicans	*C. tropicalis*
150	12	10	02
(100)	(8)	(83.33)	(16.66)

Table 3: Antibiotic susceptibility pattern of Candida isolates

Candida species tested	Number of isolates	Antibiotic Disk used					
		Amphotericin B (100 units)		Clotrimoxazole (10 mcg)		Fluconazole (10 mcg)	
		S	R	S	R	S	R
C. albicans	10	8	2	6	4	5	5
C. tropicalis	02	2	0	1	1	1	1

Table 3 summarizes the antibiotic sensitivity pattern of fungal isolates isolated in the study. Most of the *Candida albicans* were sensitive to Amphotericin B. Resistance was observed more for Fluconazole followed by Clotrimoxazole in case of *C. albicans.* All *Candida tropicalis* isolates were sensitive to Amphotericin B & few were resistant to Fluconazole & Clotrimoxazole.

Determination of total IgE

Total serum IgE levels were found to be elevated in case of all patients. In the present study total serum IgE was estimated in selected asthma patients by ELISA, as total IgE levels provide the evidence in support of atopy.

All of asthma patients showed IgE levels were above 1000 IU/ml, which correlated with the respiratory symptoms and history of atopy.

Chowdhary and Vinaykumar *et al.* reported elevated IgE levels in 90% allergic rhinitis cases. In their study of allergic rhinitis associated with bronchial asthma cases, IgE values were more than 1000 IU/ ml. They also proved that 90% patients with allergic rhinitis with peripheral eosinophil counts in normal ranges. When rhinitis was associated with bronchial asthma, the eosinophil values showed an increase above the normal [20].

Table 4: Determination of prevalence of *Candida albicans* IgG antibodies in patients included in the study

Total number of samples tested	No. of patients with *Candida albicans* IgG positive
100	21

Table 4 shows the incidence of *Candida albicans* IgG antibodies in the cases studied. Amongst all the cases studied 21% cases were positive for *Candida albicans* IgG.

Several species and genera have been reported to cause fungal allergy. Epidemiological, environmental and clinical research was focused on relevant species like *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium*. Some studies reported the clinical relevance of *Candida*, *Trichiphyton* and *Malssezia* in either respiratory or skin allergic diseases. Allergy to spores of *Basidiomycetes* (e.g. *Boletus*, *Coprinus*, *Pleorotus*, *Psilocybes*) has been reported and the relevance of their causative role in respiratory allergy has been documented [21].

Sensitivity to fungal allergens has also been found to be a risk factor for severe life-threatening asthma. A New Zealand study of patients admitted to Hospital intensive care unit revealed that patients admitted to the ICU had a significantly greater incidence of reactivity to *Alternaria tenuis*, *Cladosporium cladosporoides*, *Helminthosporium maydis* or *Epicoccum nigrum*. Fungal cultures were performed from bronchial secretions of 13 asthma patients and from the skin of 91 patients with atopic dermatitis. The predominant yeast species present on the skin were *Candida* and *Rhodotorula* species, while *Candida species* were most predominant species isolated from bronchial secretions [22].

There is strong literature evidence which suggest that environmental fungi and / or colonization with *Candida* or other organisms probably contribute in asthma severity [22].

Some authors reported the finding of respiratory allergies associated with recurrent candidiasis. *Candida albicans* and pollen specific IgE was seen in the vaginal swab from patients with recurrent vaginal candidiasis by Witkin *et al.* [4]

It is suggested that the yeast is an important causative allergen in bronchial asthma, rhinitis, chronic urticaria, atopic dermatitis, recurrent vaginitis and balanitis. In 1951

Keeny first reported asthma due to the yeast form of *Candida albicans*. [4]

Conclusion

In this study12 patients out of 150 showed growth of Candida species, 10 of which were *Candida albicans* & 2 were sensitive to Amphotericin B followed by Clotrimoxazole & Flucoazole. All patients with Candida culture positive showed raised IgE which confirmed their allergic status. Also they showed elevated IgG for *C. albicans* which indicates current & past infection by *C. albicans*. Total 12 patients showed positive culture, positive *C. albicans* IgG and raised IgE levels. They also gave history of atopy. In this case it is possible that *C. albicans* can itself act as an allergen.

Therefore it is recommended that while treating allergic patients presence of *Candida albicans* in respiratory tract should be considered and focused treatment to irradicate *C. albicans* should be given so that *C. albicans* is cleared from respiratory tract otherwise as it is a well known fact that steroid therapy suppresses the immune system and fungal emergence as most severe in such cases.

Most isolates of *Candida albicans* were sensitive to amphotericin B and clotrimoxazole whereas were resistant to fluconazole. *Candida tropicalis* were found to be resistant to amphotericin B, clotrimoxazole and fluconazole.

The emergence of antimicrobial resistant strains of pathogenic bacteria has become a great threat to the public health.

Extensive use of cotrimoxazole, erythromycin and other antimicrobials in restricted areas has led to emergence of strains resistant to these antibiotics [23].

The frequency of serious fungal infections is also rising and this might be due to factors such as the increasing use of cytotoxic and immunosuppressive drugs to treat both malignant and non-malignant diseases. the increasing prevalence of infection due to human immunodeficiency type I and the widespread use of newer and more powerful antibacterial agents [24].

In order to control the rise in antibiotic resistance and conserve the activity of current agents, the volume of antibiotics to which bacteria are exposed should be reduced [25].

It is seen that the emergence of microbial strains with multiple patterns of antimicrobial resistance has reduced the efficiency of conventional therapies.

Acknowledgements

Authors are thankful to Dr. S. T. Nabar, Former Head, Department of Medicine, & Dr. S. N. Oak, Former Dean T. N. Medical College & B. Y. L. Nair Charitable Hospital, Mumbai Central, Mumbai, Maharashtra India

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