ATYPICAL BACTERIA & CANDIDA: THREATS FOR ALLERGIC RESPIRATORY DISEASES

TARFE K.S, DESHPANDE S. D

Abstract: Amongst human respiratory diseases currently asthma & allergic rhinitis are found to be most common allergic diseases. Atypical bacteria like *Mycoplasma pneumoniae* & *Chlamydia pneumoniae* are present in lower airways of approximately 50% of asthmatics. Various microorganisms including atypical bacteria and fungi play a major role in exacerbations of asthmatic conditions. *Candida albicans* has been identified as a potent allergen in bronchial asthma. A total of 300 patients with allergic respiratory conditions were screened for presence of *Mycoplasma pneumoniae, Chlamydia pneumoniae* IgM antibodies & *Candida albicans* IgG antibodies in their serum samples. Out of 100 patients tested for *Mycoplasma pneumoniae* IgM antibodies, 29 patients showed presence of elevated levels whereas out of 100 patients tested for *Chlamydia pneumoniae* IgM antibodies 34 patients showed elevated levels. Out of 100 patients tested for presence of *Candida albicans* in respiratory specimens 5 patients showed positive culture findings & 17 showed elevated levels of serum *Candida albicans* IgG antibodies.

Bacterial & fungal antigens have a major role in inflammation& bronchospastic response in etiopathogenesis of asthma. Therefore it becomes essential to treat these infections with appropriate antibiotics. Antibiotic treatment helps in fast recovery of patients which can reduce duration for corticosteroid consumption by patients.

Keywords: Atypical bacteria, *Candida albicans,* asthma.

Introduction: Amongst allergic respiratory diseases allergic rhinitis & asthma are the most common allergic diseases. Both diseases appear to have increased significantly in past few years [1].

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

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

There are varieties of triggering factors identified for asthma. Amongst these respiratory tract infections mainly by fungi, viruses & bacteria including atypical bacteria like *Mycoplasma pneumoniae* & *Chlamydia pneumoniae* are important [4]. *Mycoplasma pneumoniae* is an extracellular pathogen that attaches to & destroys ciliated epithelial cells of the respiratory tract mucosa. In asthmatic patients, infection due to *Mycoplasma pneumoniae* can cause worsening of conditions with significant & specific immune responses [4].

Chlamydia pneumoniae is a ubiquitous obligate intracellular bacterium which is entirely dependent on energy produced by the host for its replication within the host cell cytoplasm where the bacteria form characteristic inclusions. *Chlamydia pneumoniae* appears to have propensity to cause chronic infections & is associated with ciliary dysfunction & epithelial damage in bronchial cells [4].

Fungi are known to be causative factors for asthma symptoms. Outdoor fungi including *Cladosporium*, *Alternaria*, *Penicillium* & *Aspergillus* & indoor fungi like *Neurospora*, *Aspergillus* & *Erotinum* are significant triggers of IgE formation. There is strong fungal/yeast component in the lung and/or gut microflora in individuals with asthma. The cell wall

component mannan & acid protease -an enzyme produced by *C. albicans* are both highly allergic & serum IgE antibodies are often highly increased in atopic individuals [5-6].

Microbial infections associated with allergic respiratory infections increase in severity & duration of the disease as well as they 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 patients ability to breathe. Antibiotic treatment helps in fast recovery of patients which can reduce corticosteroid consumption by them [7-8].

Therefore a study was performed to detect presence of elevated levels of serum *Mycoplasma pneumoniae* & *Chlamydia pneumoniae* IgM antibodies & *C. albicans* IgG antibodies in patients with allergic respiratory diseases. Along with this respiratory specimens were also cultured to detect presence of *C. albicans* in their respiratory tract.

Materials & Methods:

A total of 300 patients were included in the study.

These patients were enrolled for treatment of various allergic respiratory diseases & were visiting Medicine Department of T. N. Medical College & B. Y. L. Nair charitable Hospital, Mumbai Central, Mumbai, Maharashtra, India.

These patients were located in different areas in Mumbai as well as outside Mumbai.

100 patients were screened for elevated levels of *Mycoplasma pneumoniae* IgM antibodies & 100 were screened for *Chlamydia pneumoniae* IgM antibodies.

100 patients were screened for elevated levels of serum *C. albicans* IgG antibodies. Respiratory specimens such as sputum, tracheal secretions, Bronchoalveolar lavage (BAL), were collected & cultured to detect presence of *C. albicans*.

To confirm their allergic status total serum IgE estimation was also done.

***Serological Tests* [9]:**

3-5 cc of whole blood was collected by venipuncture using a disposable 5.0 ml syringe & 21 gauge hypodermic needle in a sterile plain test tube taking all aseptic precautions. The sterile plain tube was incubated at 37°C in a slanting position for 1-1.5 hours & later held at 4°C for 1 hour. This method facilitates clotting of blood. (This was a routine practice followed at T. N. Medical College & B. Y. L. Nair charitable Hospital, Mumbai Central, Mumbai, Maharashtra, India where the current study was carried out).

he supernatant serum layer was separated & centrifuged at 2000 rpm to remove cell debris.(centrifuge REMI, Temperature 28+/-2° C) The clear serum sample was preserved in absence of preservatives in plastic storage vials after labeling properly with patient's registration number & date at -20°C until utilization. (Maximum capacity of storage vials was 5.0ml & were obtained from Himedia Laboratories, Pvt. Ltd., Ghatkopar, Mumbai, Maharashtra, India).

The stored serum was used for detecting *IgM antibodies against *Mycoplasma pneumoniae* & *Chlamydia pneumoniae* by solid phase ELISA.

IgG antibodies against *C. albicans* by solid phase ELISA.

Total IgE by solid phase ELISA

(Details are mentioned below)

Mycoplasma pneumoniae IgM antibodies were estimated by solid phase Enzyme Linked Immunoassay by NovaTec IgM. ELISA. (Quantitative) (Manufacturer NovaTec). This kit contained Negative control, positive control & cut-off control.

Samples are considered positive if absorbance value is higher than 10% over the cut-off value.

Samples are considered negative if absorbance value is lower than 10% below the cut-off value. But should be confirmed after collecting & testing repeat specimen.

Chlamydia pneumoniae IgM antibodies were estimated by solid phase .Enzyme Linked Immunoassay by VIRCELL IgM ELISA. (Quantitative) (Manufacturer VIRCELL)

This kit contained Negative control (O.D. < 0.55) , positive control (O.D. >0.9) & cut-off control (<0.7 x O. D. positive control & >1.5 x O. D. negative control).

O. D. refers to Optical density.

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

This kit contained standards A-D (1, 10,80, 150 U/ml)

Standard A – Negative control

Standard B – Cut-off control

Standard C – Weakly positive control

Standard D – Positive control

U/ml Interpretation –

<8 negative, 8-12 equivocal, >12 positive

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

This is IBL manufactured kit. Kit contained 6 standard samples which had IgE concentrations from 0 IU/ml to 1000 IU/ml.

On a semi-logarithmic graph paper the concentrations of the standards (abscissa logarithmic) were plotted against their corresponding optical density (ordinate linear) The concentration of the samples can be read directly from this standard curve by using their average optical density. Any sample reading greater than the highest standard should be diluted appropriately. The result has to be multiplied with corresponding dilution factor.

As all the above ELISA used were quantitative titers were determined & then interpretation was done.

Processing of Respiratory specimens [10-12]:

Respiratory specimens such as sputum, Bronchoalveolar lavage (BAL) , tracheal aspirate were used.

Out of total 100 specimens collected 95 were sputum, 3 specimens were BAL & 2 specimens were Tracheal aspirate.

Pretreatments-

-NALC (N-Acetyl L-cysteine) digestion was carried out for mucolysis of all specimens.

-BAL specimens were first centrifuged at 3000 rpm for 15 mins & sediments were processed. (Centrifuge REMI, temperature 28+/-2°C.

Tracheal aspirates were processed directly.

Culture [9] [10] [13]

A loopful of each clinical specimen was inoculated or streaked on sterile Sabouraud's dextrose agar plate & incubated at 37°C for 48 hours.

Identification of Candida species [10] [14-15]:

Colonies of Candida on Sabouraud's dextrose agar appear as white, raised, soft in consistency & possess smooth borders.

Confirmation of presumptive colonies was done by -

a. Germ tube test:

The yeast-like colonies were differentiated into *Candida albicans* & other species on the basis of capacity to produce a germ tube when inoculated in human serum. The germ tube was detected as a tube like structure originating from the yeast cell. (One isolated colony in 0.5 ml human serum – incubate at 37°C for 2 hours)

b. Fermentation & assimilation of sugars [15]:

The yeast-like colonies were further identified by performing sugar fermentation & sugar assimilation tests. Candida species differ in capacity to ferment or assimilate various sugars. The sugars used for testing fermentation were sucrose, maltose, dextrose & galactose.

The sugars used for testing assimilation were sucrose, maltose, galactose & xylose.

Results & Discussion:

Mycoplasma pneumonia & *Chlamydia pneumoniae* act as a cofactor in severe respiratory tract infections. It can lead to secondary bacterial lower respiratory tract infections. This may lead to subsequent bronchiectatsis or other pulmonary abnormalities.

There is a strong literature evidence which suggests that there is colonization of the airways in a significant numbers of asthmatics by *Mycoplasma pneumoniae* resulting in pathogenic sequence of events of varied clinical significance.[16-17]

*Chlamydia pneumonia*e appears to have a

propensity to cause chronic infections & is associated with ciliary dysfunction & epithelial damage. High titres of antibodies to *Chlamydia pneumoniae* appeared to be associated with several markers of asthma severity [17].

Table 1 Prevalence of *Mycoplasma pneumoniae* & *Chlamydia pneumonia* IgM antibodies in patients with allergic respiratory diseases

Parameter Analyzed	Number of patients tested	Number of patients with elevated antibodies level
Serum *Mycoplasma pneumonia* IgM	100	29
Serum *Chlamydia pneumonia* IgM	100	34

In present study 29 patients with allergic respiratory infections were positive for *Mycoplasma pneumoniae*& 34 were positive for *Chlamydia pneumoniae* IgM antibodies when tested by ELISA**. (As indicated in table number 1)**

Acharya & Sahoo et al in 2005 reported association between *Mycoplasma pneumoniae*& asthma with 18% asthmatics showing presence of the organisms in sputum by culture & 16% were culture positive for *Mycoplasma pneumona* Kraft et al detected *Mycoplasma pneumoniae*by PCR in respiratory secretions of 10 of 18 stable asthmatics (56%) & I only 1 of 11 healthy controls [16].Arora & Daga et al reported 20% cases with *Mycoplasma pneumoniae*in acute exacerbations of COPD [17].Number of studies has supported an association between *Chlamydia pneumonia*e infection & asthma. A population based study conducted by Ferrari & Polietal in Italy found a significant association between *Chlamydia pneumonia*e seropositivity & atopy in young children [17].

Candida species were obtained in 5% cases which were confirmed as *Candida albicans*. 17% cases showed elevated serum C. albicans IgG levels. All 5 cases which showed *C. albicans* in culture had elevated levels of *C. albicans* IgG & increased serum IgE levels. **(As indicated in table number 2)**

Table 2 Prevalence of *Candida albicans* in patients with allergic respiratory diseases

Parameter analyzed	Number of patients tested	Number of patients with positive findings
Elevated serum *Candida albicans* IgG antibodies	100	17
Candida albicans growth on culture of respiratory speciemens	100	05

Several species & genera have been reported to cause fungal allergy. Epidemiological, environmental & clinical research was focused on relevant species like *Alternaria*, *Aspergillus*, *Cladosporium* & *Penicillium*. Some studies reported the clinical relevance of *Candida*, *Trichophyton* & *Malssezia* in respiratory or skin allergic diseases [18]. A. Mari & P. Schneider et al reported 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 & from skin of 91 patients with atopic dermititis. Predominant yeast species on skin were Candida & Rhodoturula species while Candida species were the most predominant isolates isolated from bronchial secretions [19].

There is a strong evidence which suggests that environmental fungi and/or colonization with *Candida* or other organisms probably contributes in asthma severity [19].

Some authors reported the finding of respiratory allergies associated with recurrent Candidiasis. *Candida albicans* & pollen specific IgE was seen in the vaginal swabs from patients with recurrent vaginal candidiasis by Witkin et al [5].

It is suggested that yeast is an important causitive allergen in bronchial asthma, rhinitis, chronic urticaria, atopic dermititis, recurrent vaginitis & balanitis. In 1951 Keeny first reported asthma due to the yeast form of *Candida albicans* [5].

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

In the present study total serum IgE levels were estimated by ELISA as total IgE levels provide the evidence in support of atopy. [21] All of allergic patients showed IgE levels above 1000IU/ml, which correlated with respiratory symptoms, history & atopy.

Chowdhary & 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 [20-21].

Treatment of asthma patients with macrolides & other antibiotics results in improvement in Pulmonary Function Tests. Therefore it is recommended that while treating patients with asthma or any other respiratory allergies, presence of *Mycoplasma pneumoniae* & *Chlamydia pneumonia*e & *Candida albicans* in respiratory tract should be considered & focused treatment considering these pathogens should be given which will result in early improvement of patients condition [17-18].

Conclusion:

There is a need to study the role of microorganisms in allergic conditions which will help in identifying the exact cause of the exacerbations & symptoms of allergic

conditions. Focused treatment considering these infectious agents is important in minimizing the severity of allergic conditions [22]. As *M. pneumonia*e & *C. pneumonia*e & *Candida albicans* play a major role in exacerbations & overall outcome of allergic respiratory diseases their presence should be detected either by culture or by serological tests so that treatment with appropriate antibiotics can be started to which they respond quite well. This will help in early recovery of the patient.

Note- As *Mycoplasma pneumoniae* & *Chlamydia pneumonia*e are difficult to culture antibody titers are used for preliminary diagnosis. Further to confirm cultures for the same should be done.

Antibody status indicates exposure to pathogen. Does not necessarily mean presence of pathogen in patient. Only by culture or a molecular mean one can be sure of presence of pathogen justifying antibiotic use.

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Tarfe K.S. / Deshpande S. D. /Department of Biotechnology, Smt. C.H.M. College, Ulhasnagar, Dist Thane, Maharashtra,India

Former Professor/ T. N. Medical College & B. Y. L. Nair charitable hospital/ Mumbai/Maharashtra/India