VI: Study on the impact of aeromycoflora on human health - Human allergy through Protein, Amino acid and Carbohydrate Analysis and recommendation for health care

Introduction

Air carries a large number of bioparticles (biopollutants) and chemicals, which posses burdens for the respiratory tract of human beings. The bioparticles includes pollen grains, fungal spores, insect debris, plant parts, animal’s dander and mites, etc. These materials of biological origin are known to be causative agents of respiratory disorders like asthma, allergic rhinitis and atopic dermatitis. Many scientists throughout the world carried out aerial surveys using different samplers to identify different fungal types and studied fungal sensitivity in hypersensitive individuals (Singh & Shahi, 2006). Since late 1950s the incidence of allergy in developed and developing countries is arising. Recent estimates suggest about one third of the world population will develop symptoms due to allergy at some point or the other in their lives (Singh, 2008).

Allergy plays a role in various disorders and allergic reactions that can be acute, chronic, mild or severe. Aeroallergens play a major role in the pathogenesis of respiratory allergic diseases, particularly asthma and rhinitis. Pollen, fungi, animal danders, house dust mites, domestic pets, and insects are of particular importance as triggering factors (Verma et al., 2013; Verma and Sonway, 2014). Fungal spores are of great interest in aerobiology and allergy due to their high incidence in both outdoor and indoor environments (Docampo et al., 2010). Fungi are among the earliest recognized allergens (Vandana et al., 2014). Charles Blackley was the first to show by direct
experiment on himself that grass pollen was the cause of hay fever, a very rare disease at that time.

Prevalence of fungi in a particular area depends upon various factors. The relative abundance of fungal species is influenced by the plant ecology of that area. Corcadian periodicity, seasons, geographical locations also affect the concentration of particular fungal spore types in the air. The fungi have been implicated in inflammatory reactions such as hypersensitivity pneumonitis, allergic bronchopulmonary mycoses etc. Knowledge about diurnal, seasonal and annual fluctuation in airborne fungus in any geographical area is essential for effective diagnosis and treatment of fungal allergy. The release of pollen and fungal spores is greatly influenced by location, local vegetation, season, sunlight, temperature, rainfall, humidity, wind speed direction and turbulence to mention only the major factors.

Fungi possess highly evolved mechanism of spore liberation due to which the spores remain suspended in the air for varying duration. With the studies establishing the role of fungal spores as a major causative agent for respiratory allergic disorders, the seasonal and annual variations in the bioaerosols have been extensively studied in different parts of the world including India (Singh, 2006). Fungi implicated in respiratory allergy represent all major groups viz. Phycomycotina, Ascomycotina, Basidiomycotina and Deuteromycotina. Fungal allergenic extracts are complex mixtures of proteins, glycoproteins, polysaccharides, and other substances.

Fungal bioaerosol and pollen grain allergy is a clinical disorder affecting the human health in India and other countries of the world. It appears to be one of the
The common most major health problem which cause allergenic reactions (Shivpuri, 1982). The prevalence of respiratory allergy to fungi is estimates to be 20 to 30% among the atopic individuals and upto 6% in the general population. The major allergic manifestations induced by fungi are asthma, rhinitis, allergic bronchopulmonary mycoses, and hypersensitivity pneumonitis. These diseases can result from exposure to spores, vegetative cells and fungal metabolites (Singh and Shahi, 2006).

The fungal spore counts in outdoor and indoor air vary considerably depending upon the various environmental and other factors. The prevalent weather conditions such as rain, humidity, wind speed and direction, temperature or the amount of sunshine may have direct and indirect effects on bioaerosols, the effect of which may be immediate or cumulative (Singh et al., 2006).

Fungal extract are complex mixture of allergenic proteins that show variation in their content, even when prepared under optimized conditions (Homer et al., 1995). The allergy diagnosis and therapy suffers due to variations in extract. Therefore, use of purified allergenic proteins has been recommended for diagnosis and therapy (Bisht et al., 2003). The majority of people who suffer from IgE- mediated allergy are said to be “atopic”. The European Academy of Allergology and clinical Immunology (EAACI) defines atopy as “a personal or families tendency to produce IgE antibodies in response to low doses of allergens. Usually proteins, and, as a consequence, to develop typical symptoms such as asthma, rhinoconjunctivities or the atopic eczema/dermatitis syndrome (AEDS)” . Fungi imperfecti are the most important class of fungi to cause IgE mediated allergy in man.
Keeping the above in view some observations were made on the protein, carbohydrate and amino acid analysis of the fungal spores. So that, their probable impact on the human health can highlighted and discussed.

Materials and Methods

Chemicals used

The following chemicals were used for protein estimation:

a) BSA solution
b) Sodium carbonate (Na\(_2\)CO\(_3\))
c) Copper Sulphate (CuSO\(_4\))
d) Potassium sodium tartarate
e) Sodium Hydroxide
f) Folin’s reagent
g) Fungal extract (unknown)

Lowrey’s methods (Lowry et al., 1951), Preparation of reagents for protein estimation:

1) BSA stock solution
   - 200 µg of BSA in 1ml of distilled water.
   
   Working solution: 2000 µg (2mg) in 10 ml of distilled water.

2) Solution A
   - 10 gm of Na\(_2\)CO\(_3\)+ 2gm of NaOH in 500ml of distilled water.
Working solution: 2gm of Na$_2$Co$_3$ + 0.4 gm of NaOH in 100 ml of distilled water.

3) Solution B
   - a) 5.5% CuSO4 in 100 ml of distilled water.
   - b) 1.1% Potassium sodium tartarate in 100ml of water.

   Working solution B : 5ml of (b) + 0.5 ml of (a)

4) Solution C
   - 98 ml of solution (A) + 2ml of solution (B)

5) Folin’s reagent (1:1)
   - Working solution : 5ml of Folin’s reagent + 5ml of distilled water

The following chemicals were used for estimation of carbohydrate:

i) Anthrone reagent
ii) Fungal extract (unknown)
iii) Distilled water.
iv) Standard glucose sample

Total free amino acids estimation were done using ninhydrin methods (Moore and Stein, 1948): 0.2 M Citric acid buffer, Ph5.0, Dissolve 21 g of citric acid in 200 ml of N NaOH in a 500ml volumetric flask and make up the volume to 500 ml with glass distilled water.

Ninhydrin reagent: Dissolve separately 800 mg of hydrated stannous chloride in 500 ml of the citrate buffer at Ph 5.0 and 20 g of recrystallized ninhydrin in 500 ml of methyl
cellosolve (ethylene glycol monomethyl ether). Mix the two solution prepared fresh on the day of used.

**Diluent solution:** Mix equal volumes of glass distilled water and n-propanol.

**Quantitative estimations:**

**Lowrey’s methods were used for estimation of protein:**

- 0.0, 0.2, 0.4, 0.8 and 1.0 of BSA solution were taken in different tubes.
- Then made up the total volume to 1ml by adding distilled water in all the tubes.
- Again added 5ml of solution C and kept at room temperature for 10 min.
- Finally 0.5 ml of Folin’s reagent was added and allowed it to stand for 30 min. at room temperature.
- The absorbance was taken at 750nm.
- Similarly, the absorbance’s of the unknown i.e., fungal powder extract (0.1 & 0.05 ml) samples were also taken.

**Estimation of carbohydrate:**

Anthrone method (Dubois et al., 1958) was used for the estimation of carbohydrate:

- 0.0, 0.2, 0.4, 0.8, 1.0 ml concentration of standard glucose were taken in different test tubes.
- Then it was made up to the total volume to 1ml by adding distilled water to each of the tubes
- Then 3 ml of anthrone reagent was added to all the tubes and kept at room temperature for 10-15 min.
- The absorbance was taken 620 nm
- Similarly for the unknown solution (0.1 & 0.05 ml) absorbance’s was taken.

**Estimation of amino acids:**

Ninhydrin method were used for estimating amino acid. The samples were extracted in 80% ethanol to estimate the total free amino acids. Pipette 1 ml of the sample into a test tube. Add 1 ml of ninhydrin reagent, mix thoroughly and place a glass marble on top of each tube. The contents was heated in the tubes for 20 min. in a boiling water bath. 5 ml of the diluents solution was added to the mixture while still on the water bath. A blank was maintained with 1 ml of distilled water instead of the sample. It was removed and the tubes were cooled under a running tap and was mixed the contents thoroughly. The purple colour is stable. Reading was taken at 570 nm using Spectrophotometer. The amount of amino acids present were calculated using a standard curved prepared from leucine.
Result and Discussion

Many epidemiological studies have noted that residential exposure to molds and chronic dampness can increase asthma/wheezing incidence or morbidity in both children and adults (Ostro et al., 2001 and Lee et al., 2003). Of the total fungal types trapped and identified from Moreh area, Manipur, 10 fungal species which are well known to be associated with allergic disease were selected on the basis of their dominance. The fungal types selected for biochemical analysis are Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Aspergillus ochraceous, Penicillium spinulose, Curvularia lunata, Alternaria alternata, Penicillium citrinum, Rhizopus, Mucor (Table :6.1).

Protein estimation

Many studies have revealed the presence of various cross-reactive proteins in fungi (Breitenbach et al., 1999; Shen et al., 1998). In the present investigation, Aspergillus fumigatus was found to have the highest protein content (92.4mg/ml), followed by Aspergillus flavus (73.0mg/ml) and Aspergillus niger (64.8 mg/ml) and the lowest protein content was found in Penicillium spinulose (28.2 mg/ml). Aspergillus fumigatus has been extensively studied associated with several forms of allergy (Bisht et al., 2008). Fungal extracts contain primarily proteins and glycoproteins with molecular weight of 5-70 KD. The extract may contain polysaccharides such as mannans and galactomannans, which are essentially the cell wall components. The number and strength of antigenic components depend on nutritional composition of growth medium and period of incubation. Different components are released at different stages of
incubation. A correlation between the extent of fungal aerial bio-contamination and cases of invasive aspergillosis has been reported (Alberti et al., 2001; Nolard, 1994).

**Free amino acid contents**

The total free amino acid contents of the ten selected fungal species are given. Among the total 10 fungal species tested for free amino acid contents, *Alternaria alternata* has been found to have the highest amino acid content (141.1 mg/ml) followed by *Penicillium citrinum* (132.0 mg/ml) and *Penicillium spinulose* (138.0 mg/ml). *Cladosporium cladosporoid* (37.2 mg/ml) was found to have the lowest amino acid content. *Alternaria* is a potential source of allergic disorders in human beings (Kothari et al., 1993).

**Carbohydrate contents**

Of the ten fungal species tested for total carbohydrate content, the highest carbohydrate content was found in *Aspergillus niger* (34.2 mg/ml), followed by *Aspergillus flavus* (26.16 mg/ml) and *Penicillium citrinum* (15.1 mg/ml) and the lowest carbohydrate content was found in *Cladosporium cladosporoids* (5.04 mg/ml). Self protein, and partial inhibition using extracts from common allergenic fungi including; *Alternaria alternata, Aspergillus fumigatus, Cladosporium herbarium, Candida albicans, Epicoccum purpurascens*, and *Penicillium notatum* showed complete loss of IGE binding (Westwood et al., 2005).

Improvement in the safety, efficacy, and accuracy of fungal allergy diagnosis and immunotherapy will not be achieved without a coordinated effort by manufacturers, regulatory authorities, clinicians, and researchers (Robert, 2004).
Table 6.1: Level of total soluble protein, free amino acids and Total carbohydrate in 10 selected well known allergenic airborne fungal species trapped from the residential area of Moreh, Manipur

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Total soluble protein (mg/ml)</th>
<th>Free amino acids (mg/ml)</th>
<th>Total carbohydrate (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alternaria alternate</em></td>
<td>65.7</td>
<td>141.1</td>
<td>11.16</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>73.0</td>
<td>127.6</td>
<td>26.16</td>
</tr>
<tr>
<td><em>Aspergillus fumigatus</em></td>
<td>95.4</td>
<td>78.2</td>
<td>8.94</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>64.8</td>
<td>74.00</td>
<td>34.2</td>
</tr>
<tr>
<td><em>Aspergillus ochraceous</em></td>
<td>39.1</td>
<td>129.0</td>
<td>11.8</td>
</tr>
<tr>
<td><em>Cladosporium cladosporoides</em></td>
<td>59.2</td>
<td>37.2</td>
<td>5.04</td>
</tr>
<tr>
<td><em>Curvularia lunata</em></td>
<td>46.2</td>
<td>46.7</td>
<td>7.86</td>
</tr>
<tr>
<td><em>Mucor haemilis</em></td>
<td>57.4</td>
<td>73.4</td>
<td>11.6</td>
</tr>
<tr>
<td><em>Penicillium citrinum</em></td>
<td>32.5</td>
<td>132.0</td>
<td>15.1</td>
</tr>
<tr>
<td><em>Penicillium spinulose</em></td>
<td>28.2</td>
<td>138.0</td>
<td>8.2</td>
</tr>
</tbody>
</table>
Conclusion

There is a rich fungal biodiversity in the environment of Moreh, Manipur. However, there is an urgent need to organize all the information available in the form of seasonal calendars and all allergenic fungal types. The present study made an attempt for airborne allergenic fungal spore types readily available for the use of common man and physicians as a diagnostic tool. Further work may be taken up on the above.