Association of HLA-DRB1 Alleles in Genetic Predisposition of Davangere Population

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Abstract- HLA region encodes several molecules that play key roles in the immune system. Strong association between the HLA region and autoimmune disease has been established for over fifty years. Association of components of the HLA class II encoded HLA-DRB1-DQA1-DQB1 haplotype has been detected with several autoimmune diseases, including allergic asthma, rheumatoid arthritis, type 1 diabetes and Graves’ disease. Molecules encoded by this region play a key role in exogenous antigen presentation to CD4+ T helper cells. These results provide further evidence of a possible role for bacterial, fungal and viral infection and CD8+ T cells in activation of immune responses. The advances being made in determining the primary associations within the HLA region and fungal allergy will not only increase our understanding of the mechanisms behind disease pathogenesis but may also aid in the development of novel therapeutic targets in the future.

Index terms- MHC, HLA, DRB1, T cells

INTRODUCTION

Fungi are ubiquitous airborne allergens and are important causes of human diseases, especially in the upper and lower respiratory tracts1-2. Allergy is one form of human disease which affects about 20% of the population3. The concentration of allergens in the environment varies, depending on various factors including climate, vegetation, and air quality 4. The outdoor allergens are predominantly constituted by plant pollen and fungal spores 5. Fungi from human environment may cause allergic reactions 6. They are associated with number of allergic diseases in humans including allergic rhinitis, conjunctivitis, bronchial asthma and allergic broncho pulmonary mycoses resulting from exposure to spores 7-8. The HLA molecules control the immune response through recognition of “self” and “non-self” belong to a group of molecules known as the Immunoglobulin supergene family8 which includes immunoglobulin, T-cell receptors, CD4, CD8, 9 10.

The main function of the HLA molecules is presenting the antigen (protein chain of antigen) to the T Lymphocytes and initiating the specific immune response11. HLA genes are highly polymorphic, which means that they have many different alleles, allowing them to fine-tune the adaptive immune system12, 13. In the present study predisposition of the selected allergic patients are conducted to know the expression of HLA DRB1 alleles to understand the resistance factor of allergy.

MATERIALS AND METHODS

Outdoor air sampling was carried out by using Six Stage Andersen Sampler at following selected locations are as follows site 1 APMC Market, Site 2 Rice Mill, Site 3 Poultry & Site 4 KR Market. The Andersen sampler is a good option for sampling molds and bacteria. The sampler is a multi-stage, multi-orifice cascade impactor that automatically separates particles into 6 fractions based on size, from 10 μm and above to 0.3 μm in diameter. In the present study distribution and diversity of outdoor airborne mycoflora in four occupational sites of Davanger was evaluated during September 2017 to August 2018. For one time sampling we require 6 plates for six stage Andersen sampler so that we
carried out sampling once in month and we have used total 288 plates for outdoor air samples from four sampling sites in Davangere Table 1 & Figure 1

Table 1: Frequency and distribution of airborne fungi isolated from 4 sampling sites of Davangere city. during September 2017 to August 2018

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
<th>Site 4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of colonies</td>
<td>%</td>
<td>No. of colonies</td>
<td>%</td>
<td>No. of colonies</td>
</tr>
<tr>
<td>A.niger</td>
<td>1594</td>
<td>5.48</td>
<td>811</td>
<td>2.79</td>
<td>1306</td>
</tr>
<tr>
<td>A.flavus</td>
<td>1511</td>
<td>5.19</td>
<td>894</td>
<td>3.07</td>
<td>962</td>
</tr>
<tr>
<td>A.fumigatus</td>
<td>1613</td>
<td>5.54</td>
<td>764</td>
<td>2.62</td>
<td>918</td>
</tr>
<tr>
<td>Alternaria sp.</td>
<td>418</td>
<td>1.43</td>
<td>318</td>
<td>1.09</td>
<td>390</td>
</tr>
<tr>
<td>Cladosporium sp.</td>
<td>318</td>
<td>1.09</td>
<td>218</td>
<td>0.74</td>
<td>261</td>
</tr>
<tr>
<td>Curvularia sp.</td>
<td>310</td>
<td>1.06</td>
<td>208</td>
<td>0.71</td>
<td>216</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>610</td>
<td>2.09</td>
<td>570</td>
<td>1.96</td>
<td>514</td>
</tr>
<tr>
<td>Rhizopus sp.</td>
<td>573</td>
<td>1.97</td>
<td>416</td>
<td>1.43</td>
<td>478</td>
</tr>
<tr>
<td>Mucor sp.</td>
<td>614</td>
<td>2.10</td>
<td>508</td>
<td>1.74</td>
<td>610</td>
</tr>
<tr>
<td>Fusarium sp.</td>
<td>613</td>
<td>2.10</td>
<td>619</td>
<td>2.12</td>
<td>618</td>
</tr>
<tr>
<td>Unidentified fungi</td>
<td>08</td>
<td>0.02</td>
<td>6</td>
<td>0.02</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>8182</td>
<td>28.14</td>
<td>5332</td>
<td>18.34</td>
<td>6278</td>
</tr>
</tbody>
</table>

Figure 1: Total fungal colonies in all the four occupational sites.

Figure 2: Percentage distribution of fungal colonies in different occupational sites.


Present investigation is an institutional based prospective study. In order to investigate the role of Aspergillus, Alternaria, Mucor, Cladosporium, Penicillium, Rhizopus, Fusarium species and unidentified fungi in respiratory allergies. A total of 60 patients have been subjected for sample collection who are working in occupational sites for more than one year and who have been clinically diagnosed with common symptoms like sneezing,
wheezing and breathlessness are included in the study. Patients with history of Tuberculosis, recent nasal surgery, immunocompromised conditions will be excluded.

Ethical Committee Clearance has been obtained from Institutional Ethical committee of SS Institute of Medical Sciences and Research Center, (SSIMS&RC) and samples were collected under the supervision of clinicians and paramedical staff. Nasal samples were collected from patients with allergic respiratory infections from SSIM&RC, Davangere by taking necessary consent. After collection of nasal sample, the sample was carried to laboratory for further tests.

Isolation and Identification of fungi from clinical samples:
Nasal samples were taken twice from a single patient to confirm the Aspergillus species. The nasal samples were streaked on Sabouraud Dextrose Agar and incubated at 25°C for up to 5 days. A wet mount preparation of each fungal colony was prepared by using lacto-phenol cotton blue solution and examined microscopically. Identification of fungi was based mainly on growth, colonial appearance microscopic examination of the spore and hyphal characteristics of the stained preparations.

RESULTS

A total of 29068 colonies belonging to 8 fungal genera were isolated. The number of fungal colonies in all occupational sites are represented in figure 1. Site 4 KR Market was the most contaminated region with 9276 followed by site 3 Poultry (8182 colonies), site 1 APMC Market (6278 colonies), and site 2 Rice Mill (5332 colonies) as the least contaminated area. Aspergillus was the most prominent fungal genera isolated followed by Fusarium sp, Mucor sp, Pencilium sp, Rhizopus sp, Alternaria sp, Cladosporium sp. and Curvularia . The members of these genera were the only fungi which were isolated from outdoor air of the entire site from 1- 4. Along with these few unidentified fungi which has less then 1% were also recorded. Clinical samples were collected among different age groups from both male and female patients from SS Institute of Medical Science and Research Center, Davangere. Out of 60 patients 21 patients were diagnosed with Tracheo Bronchial Aspergillosis (TBA) 11 patients were diagnosed with AR-Allergic Rhinitis, and 08 patients were diagnosed with (AFS)-Allergic Fungal Sinusitis. Table 2

Table 2: Number of individuals sensitized to different type of fungi.

<table>
<thead>
<tr>
<th>Type of fungi</th>
<th>TBA</th>
<th>AR with asthma</th>
<th>AFS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.niger</td>
<td>19</td>
<td>6</td>
<td>4</td>
<td>29</td>
</tr>
<tr>
<td>A.fumigates</td>
<td>12</td>
<td>3</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>A.flavus</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>11</td>
<td>08</td>
<td>60</td>
</tr>
</tbody>
</table>

Where TBA- Trachea Bronchial Aspergillosis, AR-Allergic Rhinitis, AFS-Allergic Fungal Sinusitis

DISCUSSION

Current diagnostic and therapeutic approaches fall short in addressing the problem of respiratory infection 14

The clinical findings of 60 patients are presented in table 2. The subjects consisted of 38 men and 22 women with a range, 16-85 years). All patients with trachea bronchial Aspergillosis had mild to moderate fever (38-39°C), cough, chest pain while 41 patients had wheezing even though these were not specific symptoms but may be useful indicators of early stage of Tracheo Bronchial Aspergillosis.15 11 patients diagnosed with allergic rhinitis with asthma and 08 patients diagnosed with allergic fungal sinusitis . The role of environmental factors in relation to asthma and allergy has become increasing from 1990’s, there has been wide spread public concern that changing patterns of outdoor air pollution underlie the rising burden of asthma, but professional are not sure.16

Genetic Predisposition Test (HLADR-B1)
The human leukocyte antigen (HLA) system, the major histocompatibility complex (MHC) in humans, is controlled by genes located on chromosome 617. It encodes cell surface molecules specialized to present antigenic peptides to the T- cell receptor (TCR) on T cells18. HLA genes are highly polymorphic, which means that they have many different alleles19, allowing them to fine-tune the adaptive immune system. In the present study predisposition of the selected allergic patients is conducted to know the expression of HLA DRB1 alleles to understand the resistance factor of allergy.
The manifestations of Aspergillosis are diverse and are dependent on the site and severity of infection. Our preliminary investigations showed most of the cases are Trachea Bronchial Aspergillosis with common predominant symptoms as wheezing, fever, and cough and chest pain. Among 60 patient’s 33 patients genetic predisposition result shown positive (HLA DRB1 positive)

CONCLUSION

Genotyping was performed by Luminex PCR-SSOP methodology at LalPath labs New Delhi. We have conducted clinical and predisposition study for 60 patients who are suffering from respiratory allergy in selected occupational sampling sites. Aspergillus species still present as prime fungal pathogen in our area. Pathogenicity of it and the genetic predisposition of the population have to be evaluated.

REFERENCES