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

Vinayakumar J[1], Jayasimha V L[2], Rajesh B P[3], Rangaswamy B E[4]
[1,4]*Department of Biotechnology & Research Centre, Bapuji Institute of Engineering and Technology, Davangere, Karnataka, India*
[2]*Department of Microbiology, S.S Institute of Medical Science &Research Centre, Davangere, Karnataka, India*
[3]*Department of Pulmonary Medicine, Jagadguru Jayadeva Murugarajendra Medical College, Davanagere, Karanataka, India*

***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 auto immune 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.

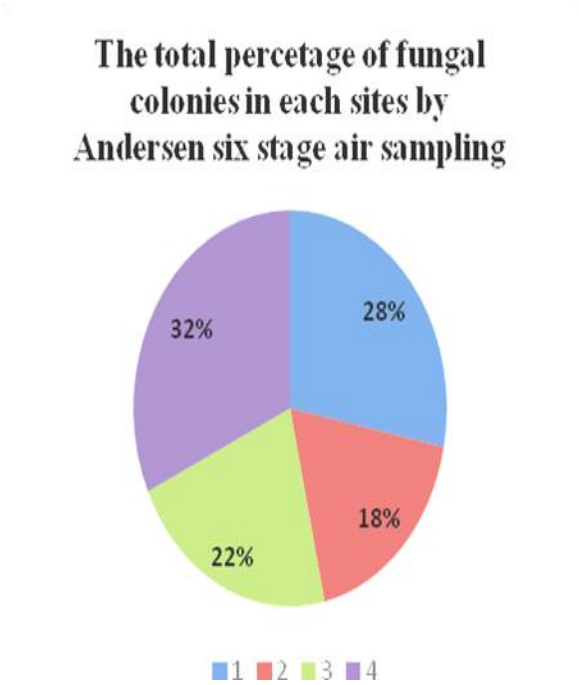
In the present study distribution and diversity of outdoor airborne mycoflora in four occupational sites of Davanger was evaluated during September 2017 to August 2018. For one time sampling we require 6 plates for six stage Andersen sampler so that we

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

Fungus	Site 1		Site 2		Site 3		Site 4		Total	
	No. of colonies	%	No. of colonies	%	No. of colonies	%	No. of colonies	%	No. of colonies	%
A.niger	1594	5.48	811	2.79	1306	4.49	1861	6.40	5572	19.16
A.flavus	1511	5.19	894	3.07	962	3.30	1674	5.75	5041	17.24
A.fumigatus	1613	5.54	764	2.62	918	3.16	1714	5.89	5009	17.23
Alterneria sp.	418	1.43	318	1.09	390	1.34	463	1.59	1589	5.46
Cladosporium sp.	318	1.09	218	0.74	261	0.89	389	1.33	1186	4.08
Curvularia sp.	310	1.06	208	0.71	216	0.74	318	1.09	1052	3.61
Penicillium sp	610	2.09	570	1.96	514	1.76	681	2.34	2375	9.11
Rhizopus sp.	573	1.97	416	1.43	478	1.64	580	1.99	2047	7.042
Mucor sp.	614	2.10	508	1.74	610	2.09	773	2.65	2505	8.61
Fusarium sp.	613	2.10	619	2.12	618	2.12	814	2.80	2664	9.16
Unidentified fungi	08	0.02	6	0.02	5	0.01	9	0.03	28	0.96
Total	8182	28.14	5332	18.34	6278	21.59	9276	31.91	29068	100

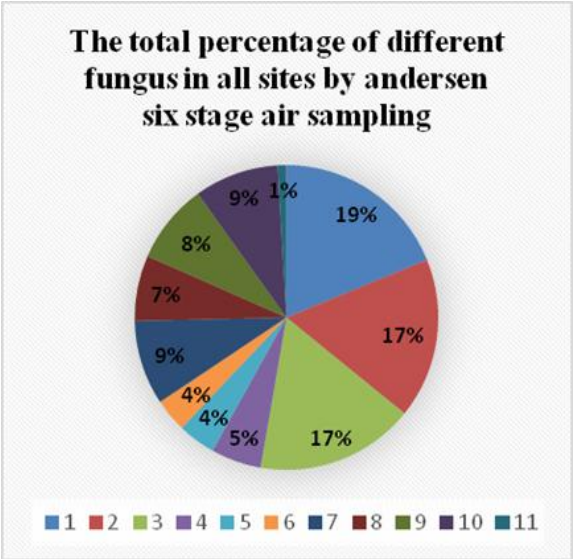
Figure1: Total fungal colonies in all the four occupational sites.



1. APMC market, 2.Rice Mill, 3.Poultry, 4.KR Market

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

1-A.niger, 2-A.flavus, 3- A.fumigatus, 4- Alterneria sp, 5-Cladosporium sp., 6-Curvularia sp. 7-Penicillium sp, 8-Rhizopus sp., 9- Mucor sp, 10-Fusarium sp. 11-Unidentified fungi



CLINICAL SAMPLES

Present investigation is an institutional based prospective study. In order to investigate the role of Aspergillus, Alterneria, 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, Alterneria 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.

Type of fungi	TBA	AR with asthma	AFS	Total
A.niger	19	6	4	29
A.fumigates	12	3	2	17
A.flavus	10	2	2	14
Total	41	11	08	60

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.1511 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 infection20. Our preliminary investigations showed most of the cases are Trachea Bronchial Aspergillosis with common predominant symptoms as wheezeing, 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

[1] Blumenthal MN, Rosenberg A. 1999 Allergens and Allergen Immunotherapy, in: Lockey R.F., Bukantz S.C. (Eds.), Marcel Dekker, Inc, New York, pp. 39– 51.

[2] Wuethrich B. Epidemiology of allergic diseases: Are they really on the increase. Int Arch Allergy Appl Immunol 1989; 90: 3–10.

[3] Rangaswamy B.E, Prakash K. k, Francis.F & amp; Jayasimha V.L.2012.Distribution Pattern of Air borne Fungi and Bacteria in the school Environment. Indian journal of Aerobilogy,25 (2):53-58.

[4] Burge HA. 1989 Airborne-allergenic fungi. Immunol Allergy Clin North Am; 9: 307–319.

[5] Gravesen S. 1985 Indoor airborne mould spores. Allergy; 40: 21–23.

[6] Crameri R, Weichel M, Fluckiger S, 2006 Fungal allergies: yet unsolved problem. Chem Immunol Allergy; 91: 121-33.

[7] Latge JP, Paris S. 1991 The fungal spore and disease initiation in plants and animals, in: Cole G.T., Moch H.C. (Eds.), Plenum Press, New York, pp. 379–401.

[8] Portnoy J, Chapman J, Burge H, Muieleberg M, Solomon W. Epicoccum 1987 allergy. Skin reaction patterns and spore/ mycelium disparities recognized by IgG and IgE ELISA inhibition. Ann Allergy; 59: 39–43.

[9] Tiwari JL,Terasaki 1985. PI.HLA and disease association Newyark:Springer-Verlag,

[10] Schwartz RH. 1985 T-lympocyte recognition of antigen in association with gene products of the major histocompatibility comples. Ann Rev Immunology; 3:237-361

[11] Schwartz RH. 1985The interaction of antigen and the histocompatibility molecule during T-cell activation.Prog clin Biol Res; 172B; 333-341

[12] Morris MJ,Vaughan H, Lane DJ, Morris PJ. 1977 HLA in Asthma. Mongr Allergy: 11:30

[13] Morris MJ, Faus JA, Ting A, Morris PJ, Lane DJ.HLA-A, Band C1980 HLA-DR antigens in intrinsic and allergic asthma. Clin Allergy; 10:173-9.

[14] Denning D.W., A. Marinas, J.cohen.1998. An EORTC multicenter prospective survey of invasive aspergillosis in haematological patients: diagnosis and therapeutic outcome .EROTC Invasive Fungal Infections cooperative Group .J. Infect 37: 173-180

[15] Bousquet J,Burney PGJ. 1993 Evidence foran increase in atopic disease and possible cause .clin Exp Allergy,23; 484-92

[16] Jones DH,May AG, Candemi JJ. 1984 HLA-DR Typing of Aspirin sensitiveve asthmatics .Ann Allergy; 25: 87-89

[17] Burge HA. 1989 Airborne-allergic fungi.immunol Allergy Clin North Am; 9:307-319

[18] Amirzargar, A., Mytilineos, J., Farjadian, S., Doroudchi, M., Scherer, S., Opelz, G., Ghaderi, A.Human leukocyte antigen class II allele frequencies and haplotype association in Iranian normal population.Hum.Immunol.2001; 62:1234–1238.

[19] Baggi, F., Antozzi, C., Andreetta, F., Confalonieri, P., Ciusani, E., Begovich, A.B., Erlich, H.A., Cornelio, F., Mantegazza, R. 1998 Identification of a novel HLA class II association with DQB1* 0502 in an Italian myasthenic population.Ann. N. Y. Acad. Sci.; 841:355–359.

[20] Saruhan-Direskeneli, G., Kiliç, A., Parman, Y.i., Serdaroğlu, P., Deymeer, F. 2006 HLA-DQ polymorphism in Turkish patients with myasthenia gravis. Hum. Immunol.; 67:352–358.