Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 04, pp.14476-14480, April, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

INCREASED FREQUENCY OF HLA-DR*B1*12* IN ASTHMA PATIENTS VISITING A TEACHING HOSPITAL LAHORE, PAKISTAN

[1]Khursheed Javed, [*,1]Nadeem Afzal, [1]Faheem Shahzad, [2]Saleem-u-Zaman, [1]Saba Khaliq, [1]Shah Jahan, [3]Waqas Latif, and [4]Shaguftta Khalid

[1]Department of Immunology, University of Health Sciences, Lahore, Pakistan
[2]Department of Medicine Shalamar Hospital Lahore, Pakistan
[3]Quality Enhancement Cell University of Health Sciences, Lahore, Pakistan
[4]Department of Human Genetics and Molecular Biology University of Health Sciences Lahore, Pakistan

ARTICLE INFO

Article History:
Received 09th January, 2015
Received in revised form
15th February, 2015
Accepted 22nd March, 2015
Published online 28th April, 2015

Key words:
Asthma,
HLA,
IgE,
PCR

ABSTRACT

Background: Throughout the world, there are more than 300 million people of asthma and its prevalence in Pakistan is 3.7%. In Pakistan, there are studies on association of HLA with different diseases but data about HLA association with bronchial asthma is limited. An Iranian study described increased frequency of DR*B1*12* allele in asthmatic patients. Therefore, present study was designed to determine frequency of HLA-DRB1*12 allele in patients of bronchial asthma.
Material and Method: Fifty clinically-diagnosed bronchial asthma patients between 18-40 years with the history of allergy and 30 healthy subjects were selected. HLA-DRB1*12 allele and total serum IgE level was determined by PCR and ELISA technique respectively. Student-*t* test was applied to observe group mean difference. A p-value of ≤0.05 was considered statistically significant.
Results: Frequency of HLA-DRB1*12 allele in asthma patients was high (42%) compared to controls (20%) and on comparison there was statistically significant difference (p=0.04). Mean ± SD of total serum IgE level of bronchial asthma patients was high (861.6 ± 559.2 IU/ml) compared to controls (204.7 ± 237 IU/ml) and on comparison there was statistically significant difference (p = <0.001). Mean ±SD of total IgE level in HLA-DR *B1*12* positive asthma patients was high (607.9 IU/ml) compared to HLA-DR *B1*12* negative asthma patients (518.4 IU.ml) and on comparison there was no significant difference.
Conclusion: Frequency of HLA-DR*B1*12* was increased in bronchial asthma patients but this allele did not confer susceptibility to the raised total serum IgE levels.

INTRODUCTION

Bronchial asthma is a chronic inflammatory disorder of airways where increased bronchial hyper-responsiveness causes recurrent episodes of wheezing, breathlessness, chest tightness and cough (Abbas *et al*., 2004). It is the most prevalent disease among children and young adults. Asthma is the main cause of disability, resource utilization and poor quality of life (Moffatt *et al*., 2010). It worsens without treatment which can lead to hospitalization and even death of a patient (Braman 2006). Prevalence of asthma varies around the world (Padmaja *et al*., 2009). About 300 million people around the globe had asthma, and this burden would increase by another 100 million by 2025 (Masoli *et al*., 2004). The global prevalence of asthma in adults was 5.8% while in Pakistan it was 3.75% (Teresa *et al.,* 2012).

Corresponding author: Nadeem Afzal,
Department of Immunology, University of Health Sciences, Lahore, Pakistan.

Asthma is a complex multigenic disease with a wide spectrum of predisposing factors. Asthma is categorized into *extrinsic* i.e. induced by allergen and *intrinsic*i.e. due to drugs, viral infection of respiratory tract, etc (Abbas *et al*., 2004). Dendritic cells present the allergen to $CD4^+$ T cells along with MHC-II molecule which are highly polymorphic genes and their polymorphism plays an important role in the regulation of immune response. MHC haplotype, in part, determines genetic susceptibility of a subject for atopic asthma (Holgate 2012, Huang *et al*., 1991). Association of HLA with bronchial asthma have been documented in various studies e.g. HLA-DRB*1*07* in citrus red mite-sensitive asthmatics (Cho *et al*., 2000), HLA-DRB*1*02* in asthmatics sensitive to grass pollens compared to rhinitis patients (Woszczek *et al*., 2002) and HLA-DR*B1*12* in asthmatic patients (Masoud *et al*., 2008). In Pakistan, different HLA allele with varying frequencies have been detected in healthy and in patients of various diseases e.g. Mohyuddin *et al.* (2002) documented frequency of

HLA-DR*B1*12* in up to 1.5% in healthy population. Hameed *et al.* (1997) reported increased frequency of HLA *DR1* (HLA-DR*B1*01*) and HLA-*DR 10* in patients of rheumatoid arthritis. Ali *et al.* (2010) described HLA-DQ*R1*04* as protective allele against HCV infection, while HLA-DR*B1*11* and HLA-DQ*B1*0301* with viral clearance after interferon therapy whereas HLA-DR*B1*07* and HLA-DQ*B1*02* with viral persistence (Ali, *et al*., 2010). Tipu *et al.* (2011) reported HLA-DR*B1*13* in non-insulin dependent diabetes mellitus whereas Naqi *et al.* (2011) documented HLA-DR*B1*04* in rheumatoid arthritis and HLA-DR*B1*11* as protective allele. Hussain *et al.* (2011) noted HLA-*A*01, A*03, A*11, A*23, A*26, A*69,* HLA-*B*27, B*40, B*49, B*51, B*52, B*53, B*54, B*95,* HLA-*DRB1*01, DRB1*03, DRB1*11, DRB1*14* in systemic lupus erythmatosis (Hussain *et al*., 2011) whereas Wasay *et al.* (2013) reported HLA-DR*B1*01* and HLA-DR*B1*04* in multiple sclerosis. An Iranian study described HLA-DR*B1*12* in asthma patients (Masoud *et al*., 2008). Despite various studies on HLA association with asthma, the data is not convincing. Therefore present study was designed to determine frequency of HLA-DR*B1*12* allele and level of IgE in patients of bronchial asthma.

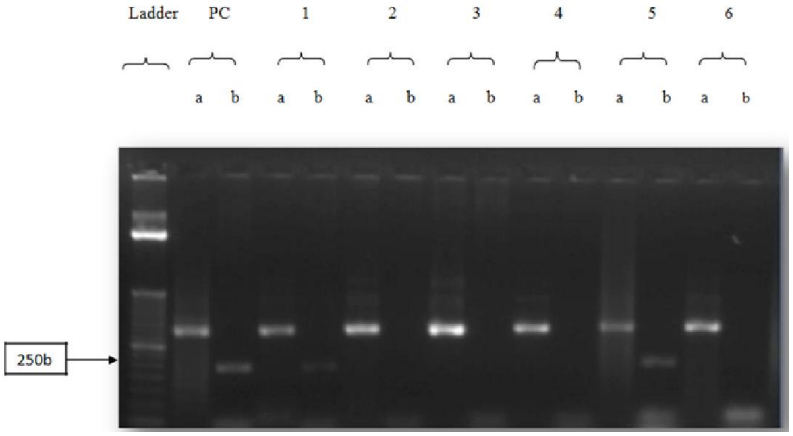
MATERIALS AND METHODS

It was a comparative case-control study that included 50 asthmatic patients and 30 non-asthmatics as controls. The study was performed in the Department of Immunology, University of Health Sciences (UHS) Lahore after the approval of Ethical Review Committee and Advance Studies and Research Board of UHS Lahore and Shalamar Hospital Lahore. Clinically diagnosed asthmatic patients of 18-40 years (Kaplan *et al*., 2009) of either sex with the history of allergy (validated modified questionnaire of European Community Respiratory Health Survey) were recruited from the Department of Medicine, Shalamar Hospital Lahore. Patients of diabetes and autoimmune disorders were excluded. Complete blood count (CBC) was performed on Sysmax 1000 X-I (Japan) and serum total IgE by ELISA technique (KomaBioTech Korea) according to the manufacturer's instructions.

After DNA extraction by standard phenol-chloroform method, HLA typing for DR*B1*12* allele was performed by sequence-specific primers following Olerup *et al.* (1992). Optimization of PCR reaction was performed with samples containing HLA-DR*B1*12* allele. Primers for HLA-DR*B1*12* allele were added in one tube and for *GAPDH* gene (internal quality control) in the other tube (Fig 1). Data was analyzed using SPSS 20.0. Mean ± SD was given for quantitative variables, frequencies and percentages for qualitative variables. Kolmogorov-Smirnov and Shapiro-Wilk test was applied to determine normality of data, student *t*-test and Mann-Whitney test was used to observe group mean differences. Student *t*-test was applied where data was normally distributed and Mann-Whitney test where data was not normally distributed. A *p*-value of ≤ 0.05 was considered as statistically significant.

RESULTS

Among 50 bronchial asthma patients there were 17 (34%) males and 33 (66%) females while 30 healthy controls comprised of 16 (53%) males and 14 (47%) females. Mean ± SD of age of the patients was high (28.4 ± 10.5 years) as compared to controls (26.3 ± 3.0 years) (Fig 2). Family history of asthma was present in 23 (46%) of asthma patients (Fig. 3) while none of the healthy controls had family history of asthma. Among 50 asthmatic patients, 21 (42%) had HLA-DR*B1*12* allele while out of 30 controls, 6 (20%) had HLA-DR*B1*12* allele. On comparison the difference between two groups was statistically significant (p=0.044). Mean ± SD of total serum IgE concentration was high in bronchial asthma patients (861.6 ± 559.2 IU/ml) compared to controls (204.7 ± 237 IU/ml). On comparison the difference was statistically significant (p=0.000) (Table 2). Total serum IgE concentration of HLA-DR*B1*12* positive patients was high (951 ± 607.9 IU/ml) compared to patients who were negative for his allele (768 ± 518.4 IU/ml). On comparison the difference between two groups was not statistically significant. Mean ± SD of total leukocyte count (TLC), eosinophil percentage and eosinophil absolute count of asthma patients was high compared to control.



Ladder: DNA size marker (50-800 base pairs), **PC:** Positive control (sample known for HLA-DR*B1*12*)
1-6: Samples numbers, **a:** Internal control (*GAPDH*),
b: HLA-DR*B1*12*, ⟶ :Allele of interest (HLA-DR*B1*12*),
HLA-DR*B1*12*: Human leukocyte antigen-DR gene beta one allele 12,
GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

Figure 1. Gel Electrophoresis showing Results for HLA-DR*B1*12* and *GAPDH* as internal control

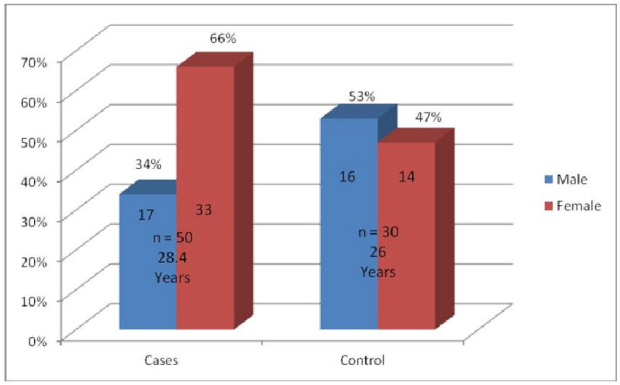


Figure 2. Age and gender distribution of studied subjects

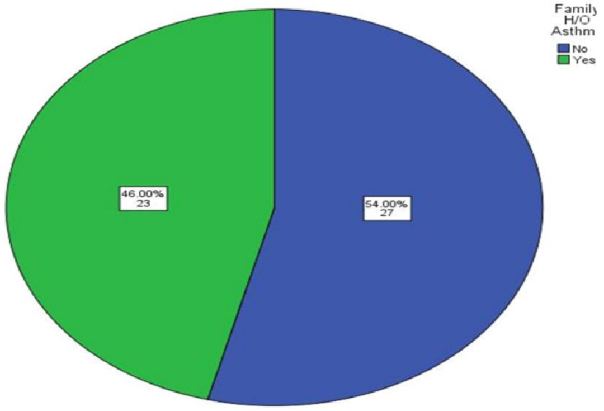


Figure 3. Frequency of family history of asthma in asthma patients

On comparison the difference was statistically significant (p=0.014, and 0.000). The difference of Hb, percentage of neutrophil, lymphocyte, monocyte and basophil absolute count between asthma patients and healthy controls was not significant (Table 1).

DISSCUSSION

In this study an increased percentage (42%) of bronchial asthma patients had HLA-DR*B1*12* allele compared to healthy subjects (20%). On comparison, the difference between two groups was statistically significant (p value 0.037). Masoud *et al.* (2008) also reported significantly increased frequency of HLA-DR*B1*12* in pediatric allergic asthma patients (4.5%) compared to controls (0%). The difference in the reported frequencies of two studies could be due to larger sample size (n = 112) of Masoud *et al.* (2008) compared to the current study (n = 50). Different studies have reported increased frequencies of other HLA alleles such as HLA-DR*B1*07* (17.6%) in asthmatics sensitive to citrus red mite and house dust mite (Cho *et al*., 2000), HLA-DR*B1*02* (47.5%) and HLA-DR*B5* (24%) in grass pollen-sensitive asthmatic (Woszczek *et al*., 2002), birch-sensitization association with HLA-DR*B1*13* and mugwort sensitization with HLA-DR*B1*03* (Munthe *et al*., 2007). Hanchard *et al.* (2010) described positive association between HLA-DR*B1*03* and childhood asthma. Mishra *et al.* (2014) reported association of HLA-DR*B1*04* and Paediatric bronchial asthma in Asian Indian population (odd ratio 3.61 and 95% confidence interval 1.75-7.43).

In the above mentioned studies asthma patients were sensitive to specific allergens whereas in the current study bronchial asthma patients were selected on the history of allergy and frequency of only one allele was determined i.e. HLA-DR*B1*12*. Genetic differences among our population, Americans, Europeans and Australians might also be the cause for this disparity. In the current study frequency of HLA-DR*B1*12* in healthy subjects was 20%, which is not in agreement with Mohyuddin *et al.* (2002) who reported its frequency in healthy subjects as 1.6% in six ethnic groups of Pakistan but they excluded Punjabi population.

Table 1. Mean ± SD and comparison of laboratory parameters of asthma patients (n = 50) and controls (n = 30)

Parameter	Asthma Patients (n = 50)	Controls (n = 30)	p value
Hb[1] (g/dl)[2]			
Male	14.8 ± 1.2	16.0 ± 1.0	
Female	13.3 ± 1.2	12.6 ± 1.4	0.112
TLC[3] × 10^9/L	9.3 ± 3.1	7.6 ± 1.5	0.014*
Neutrophil %[4]	52.8 ± 13.6	57.3 ± 6.5	0.083
Lymphocyte %[4]	30.5 ± 10.7	30.8 ± 5.7	0.904
Monocyte %[4]	8.2 ± 3.6	8.6 ± 1.8	0.383
Eosinophil %[4]	7.9 ± 4.7	2.8 ± 2.1	0.000*
Eosinophil Abs.[5] (× 10^9/L)	0.7 ± 0.4	0.2 ± 0.1	0.000*
Basophil Abs.[5] (× 10^9/L)	0.03 ± 0.02	0.04 ± 0.01	0.325

[1]: Hemoglobin, [2]: Gram per deciliter, [3]: Total leukocyte count, [4]: Percentage, [5]: Absolute Count*: p value ≤0.05 was considered as statistically significant

Table 2. Frequency and comparison of HLA-DR*B1*12*[1] and Mean ± SD of total serum IgE[2] concentration of asthma patients and controls

Parameter	Asthma Patients (n = 50)	Controls (n = 30)	p value
HLA-DRB1*12	21 (42.0 %[3])	06 (20.0 %[3])	0.037*
Mean Total IgE Conc.[4] (IU/ml)[5]	861.8 ± 559.2	204.7 ± 237	0.000*

[1]: Human leukocyte antigen-DR gene beta one allele 12, [2]: Immunoglobulin E
[3]: Percentage, [4] : Concentration, [5]: International Units per milliliter
*: p value ≤0.05 was considered as statistically significant

The discrepancy of two studies could be due to genetic disparity between Punjabi populations from others as in the current study only Punjabi population was included. Further, sample size was small in the current study i.e. 30 compared to 513 in Mohyuddin *et al.* (2002). Mean ± SD of total serum IgE concentration in asthma patients was high compared to controls. Increased IgE levels have been documented in a number of conditions e.g. atopic diseases (Stone *et al*., 2010), parasitic and non-parasitic infections (Smith *et al*., 2009), inflammatory diseases, hematologic malignancies, primary immunodeficiency, etc (Pien *et al*., 2008). Total serum IgE concentration is also affected by genetic makeup, race and immune status of the person (Smith *et al*., 2009). Demirjian *et al*., (2012) concluded increased total serum IgE level in atopic asthma patients. Masoud *et al*. (2008) documented raised mean total IgE levels in positive skin prick test pediatric asthma patients. Similarly, Mishra *et al*. (2014) reported significantly increased total serum IgE in pediatric bronchial asthma. The current study confirms the findings of the above mentioned studies. In the current study, although mean ± SD of total serum IgE concentration in HLA-DR*B1*12* positive was high compared to patients who were negative for this allele but on comparison it was not significantly different. Odd ratio was 1.02 with 95% confidence interval of 0.37 – 2.82 which means that HLA-DR*B1*12* allele was not a risk factor for high total IgE concentration in these patients. This finding is in agreement with Masoud *et al*. (2008) who also documented non-significant difference of total serum IgE concentration in bronchial asthma patients who were positive or negative for HLA-DR*B1*12* allele. If allergen-specific IgE, skin prick test or provocation test was performed and other alleles of HLA-DRB1 region were studied, there could have been association of HLA-DRB1allele(s) with high total IgE levels.

TLC, eosinophil percentage and eosinophil absolute count of asthma patients was high compared to controls and on comparison the difference was statistically significant (p=0.014, and 0.000). Gupta *et al*. (1975) reported statistically significant difference of TLC and eosinophil count between asthma patients and controls (p= 0.005). Luksza *et al.* (1982) also documented statistically significant difference of eosinophil count between acute and chronic asthma patients (p=0.001) Mehta *et al.* (2008) concluded elevated percentage of eosinophils in the blood and sputum of asthma patients (p=0.001) Demirjian *et al*. (2012) concluded non-significant difference of eosinophil percentages between atopic patients (diagnosed by skin prick test) and asthma patients, whereas in the current study only bronchial asthma patients with history of allergy were compared with the healthy controls. Probably the discrepancy could be due to difference in the selection of study subjects.

Conclusion

An increased frequency of HLA-DR*B1*12* allele was detected in asthma patients but this allele did not confer susceptibility to raised total serum IgE levels.

Acknowledgements

The study was supported by the grants of Higher Education Commission Pakistan and University of Health Sciences (UHS) Lahore Pakistan. We are also thankful to the Departments of Biochemistry and Hematology UHS for allowing us to use thermal cycler and Sysmex equipment at their laboratories respectively.

REFERENCES

Abbas, A.K., Kumar, V. and Fausto, N. 2004. The lung In. Robins andCotran Pathologic basis of disease. Philadelphia: Saunders, 7thEd. 723-730.

Ali, L., Manssor, A., Ahmad, N., Siddiqi, S., Mazhar, K., Muazzam, A.G. and Qamar, R. 2010. Patient HLA-DRB1* and -DQB1* allele and haplotype association with hepatitis C viruspersistence and clearance. The Journal of general virology, 91(Pt 8): 1931–1938.

Braman, S.S. 2006. The global burden of asthma. Chest, 130(1 Suppl):4S-12S.

Cho, S.H., Kim Y.K., Oh, H.B., Jung, J.W., Son, J.W. and Lee, M. H. *et al*. 2000. Association of HLA-DRB1*07 and DRB1*04 to citrus red mite (Panonychus citri) and house dust mite sensitive asthma. Clinical and Experimental Allergy, 30: 1568-1575.

Demirjian, M., Rumbyrt, J.S, Gowda, V.C., Klaustermeyer, W.B. 2012. Serum IgE and eosinophil count in allergic rhinitis-Analysis using a modified Bayes' theorem. AllergolImmunopathol (Madr), 40(5): 281-287.

Gupta, S., Frenkel, R., Rosentein, M. and Grieco, M.H.1975. Lymphocyte subpopulations, Serum IgE and total eosinophil counts in patients with bronchial asthma. Clin Exp Immunol, 22: 438-445.

Hameed, K., Bowman, S., Kondeatis, E., Vaughan, R. and Gibson, T. 1997. The association of HLA-DRB genes and the shared epitope with rheumatoid arthritis in Pakistan. *British Journal of Rheumatology*, 36(11): 1184–8.

Hanchard, N., Jacobson, R.M., Poland, G.A. and Juhn, Y.J. 2010. An assessment of the association between childhood asthma and HLA DRB1*03 using extended haplotype analysis. *Tissue antigens,* 76(6): 491–494.

Holgate, S.T.2012. Innate and adaptive immune responses in asthma. Nature Med, 18(5): 673-683.

Huang, S.K., Zwollo, P. and Marsh, D.G. 1991. Class II major histocompatibility complex restriction of human T cell responses to short ragweed allergen Amb a V. *Eur J Immunol,* 21(6): 1469-1473.

Hussain, N., Jaffery, G., Sabri, A.N. and Hasnain, S. 2011. HLA Associations in SLE patients from Lahore-Pakistan. BJBMS, 11(1): 20-26.

Kaplan, A.G., Balter, M.S., Bell, A.D., Kim, H., Mclvor, 2009. A. Diagnosis of asthma in adults. CMAJ, 181(10): 210-220.

Luksza, A.R., Jones, D.K., 1982. Comparison of whole blood counts in extrinsic asthmatics with acute and chronic asthma. MBJ, 285(10): 1229-1231.

Masoli, M., Fabian, D. and Holt, S.2004. Global burden of asthma Global Initiative for Asthma (GINA). Available:www.ginasthma.com/ReportItem.asp?l1=2andl2=2&intId=94

Masoud, M.I., Mostafa, M.I., Mohammad, G., Asghar, A., Saied, D., Batoul, M. *et al*. 2008. Association of HLA class II Alleles with Childhood Asthma and Total IgE Levels. *Iran J. Allergy Asthma Immunol,* 7(4): 215-220.

Mehta, V., Campeau, N.G., Kita. H., Hagan, J.B. 2008. Blood and sputum eosinophil levels in asthma and their relation to sinus computed tomographic findings. Mayo Clin Proc, 83(6): 671-678.

Mishra, M.N., Dudeja, P. and Gupta, R.K. 2014. Association of HLA class II and IgE serum levels in pediatric asthma. Iran J Immunol, 11(1): 21-28.

Moffatt, M.F., Gut, I.G., Demenias, F., Strachan, D.P., Bouzigon, E. and Heath S. *et al*., 2010. A large-scale, consortium-based genomewide association study of asthma. NEJM**,** 363(13): 1211–1221.

Mohyuddin, A., Ayub, Q., Khaliq, S, Mansoor, A., Mazhar, K. and Rehman, S. *et al*. 2002. HLA polymorphism in six ethnic groups from Pakistan. Tissue Antigens, 59(6): 492-501.

Munthe, M.C., Carlsen, K.L., Carlsen, K.H., Egeland, T., Haland, G., Devulapalli, C.S *et al*., 2007. HLA Dr-Dq haplotypes and the TNFA-308 polymorphism: associations with asthma and allergy, Allergy, 62: 991–998.

Naqi, N., Ahmed, T.A., Malik, J.M., Ahmed, M. and Bashir, M.M. 2011. HLA DRß1 Alleles in PakistaniPatients with Rheumatoid Arthritis. Jcpsp, 21(12): 727-730.

Olerup, O. and Zetterquist, H. 1992. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. Tissue Antigens, 39(5): 225-35.

Padmaja, S., Piush, J.M. and Malcolm, R.S. 2009. Asthma: epidemiology, etiology and risk factors CMAJ, 181(9): 181-190.

Pien, G.C. and Orange, J.S. 2008. Evaluation and clinical interpretation of hypergammaglobulinemia E: differentiation atopy from immunodeficiency. Annals of Allergy, Asthma &Immunology, 100(4): 392-95.

Smith, P. and Ownby, D.R. 2009. Clinical significance of IgE. In: Adkinson, N., Bochner, B.S., Busse,W.W., Holgate, S.T, Lemanske, R.F. and Simons, FER eds. Middleton's allergy: principles and practice. St Louis: Mosby Elsevier

Stone, K.D, Prussin, C. and Metcalfe, D.D. 2010. IgE, mast cells, basophils and eosinophils. J AllergyClin Immunol, 125: S73-80.

Teresa**,** T., Stanojevic, S., Moores, G. and Gershon, A.S. 2012. Bateman ED, Cruz AA and Boulet L-P. Global asthma prevalence in adults: findings from the cross-sectional world health survey. BMC Public Health, 12: 204-12.

Tipu, H.N., Ahmed, T.A. and Bashir, M.M. 2011. Human Leukocyte Antigen Class II Susceptibility Conferring Alleles among Non-Insulin Dependent Diabetes Mellitus Patients. Jcpsp, 21(1): 26–29.

Wasay, M., Moatter, T., Mehmmod, K., Subhan, M., Ahmed, A., Tariq, M. *et al*. 2013.Multiple sclerosis in Pakistan: histocompatibility antigen composition and disability. Mult Scler, 19(2): 254–255.

Woszczek, G., Kowalski, M.L. and Borowiec, M. 2002. Association of asthma and total IgE levels with human leucocyte antigen-DR in patients with grass allergy. *European Resp J*, 20(1):79–85.

****