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RESEARCH PAPER

HLA-DR Association of Anti-dsDNA and Anti-Sm Autoantibodies in Bangladeshi Patients with Systemic Lupus Erythematosus

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Abstract

***Background*:** Rheumatic disorders are one of the largest health problems in the world in both developed and developing countries. Among systemic rheumatic disorders, systemic lupus erythematosus (SLE) is very much common. This debilitating disease most commonly affects females, especially at a young age. Though the exact etiology for the development of SLE still remains vague but genetic factors especially, HLA-DR plays an important role particularly in the development of autoantibodies in SLE cases.

***Objective*:** This study was undertaken to find out the association of HLA-DR with anti-dsDNA and anti-Sm autoantibodies among patients with SLE.

***Methods*:** Buccal swabs for HLA-DR typing and blood samples for detection of anti-dsDNA and anti-Sm were collected from 46 SLE cases. HLA-DR typing was carried out by end point polymerase chain reaction (PCR) with sequence specific primers. Autoantibodies were detected by using ELISA.

***Results*:** Out of 46 cases with SLE 44 (95.65%) were female and 2 (4.35%) were male with female: male ratio was 22: 1. Their mean age at study entry was 27.05 ± 8.17 years (mean ± SD), ranging from 12.5-45 years. Anti-dsDNA was positive in 38 (82.61%) cases and negative in 8 (17.39%) cases. Anti-Sm was positive in 19 (41.30%) cases and negative in 27 (58.70%) cases. The most frequently identified HLA-DR was DR2 (86.96%). When Anti-dsDNA positive cases were compared with Anti-dsDNA negative cases significant association was found with HLA-DR2 (94.73% vs 50%, *p* value = 0.0044, *pc* = 0.044, RR = 18.0000). No positive association of HLA-DR was found with anti-Sm autoantibody in this study. The above data suggest that HLA-DR2 has a role in anti-dsDNA production in Bangladeshi patients with SLE.

***Keywords*:** systemic lupus erythematosus, major histocompatibility complex, human leukocyte antigen, anti-dsDNA, anti-Sm.

Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disorder. It predominantly affects women, especially in their childbearing ages. It is characterized by multisystem organ involvement due to dysregulation of self-reactive B cells leading to autoantibody production against a range of intracellular, cell surface, and serum components. These autoantibodies form immune complexes that deposit in various tissues where they lead to complement activation and cause tissue damage.[1]

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Several autoantibodies are produced in SLE, like anti-dsDNA, anti-Sm, anti-phospholipid antibody, anti-Ro, anti-La. The diversity of these autoantibodies produced in SLE cannot be explained. Among the autoantibodies, anti-dsDNA and anti-Sm autoantibodies are specific for SLE. They present more than 95% of patients. The titer of anti-dsDNA may vary over time and also disease activity but anti-Sm autoantibody titer usually remains constant.[2]

Different studies proved that SLE develops within a complex network of genetic, immunologic, and environmental factors. The role of genetic factors in the development of SLE has been proved by results of studies of family aggregation of the disease, increased concordance of SLE among monozygotic versus dizygotic twins, Gm markers, decreased red

cell CR1 receptors, abnormal T cell suppressor function in healthy relatives of SLE patients and associations with several major histocompatibility complex (MHC) loci.[3-8] The search for genes that predispose a person to develop SLE has been done through association studies of candidate genes and genome-wide linkage analysis, which had measurable success in the past few decades. Eight susceptibility loci have been located for the development of SLE, among them, MHC reached the threshold for significant linkage.

HLA association with SLE has first been described with class I MHC molecule. But later studies found a stronger and consistent association with MHC class II. As an immune response to autoantigens is controlled by MHC, HLA class II alleles are more related to autoantibody subsets than to the disease itself.[9, 10] The association of HLA class II with autoantibody production varies greatly from one ethnic group to another as because HLA is highly polymorphic gene. But in most studies, HLA-DR2 and DR3 were found to be associated with the production of autoantibodies against dsDNA and Sm antigen, respectively.[2, 11] Some studies also found that HLA-DR2 and DR3 predispose autoantibody production in unaffected family members of SLE cases.[9] Although studies regarding the association of HLA with autoantibody production had been performed in different countries but this type of study or any data regarding this association is not available in Bangladesh. This is the first immunogenetics description of SLE for Bangladeshi people. So, for searching the potential associations between autoantibodies production and MHC genes among SLE patients of Bangladesh, we have investigated SLE patients for HLA-DR (DR1 to DR10) antigens and circulating autoantibodies against dsDNA and Sm.

Materials and Methods

This case-control study was done within the period of March 2013 to February 2014. In this study, SLE patients who were positive for autoantibodies were considered as cases. Autoantibody-negative patients among SLE were considered the control group. The sampling type was a purposive type of sampling. Samples were collected from the SLE clinic, Department of Rheumatology, BSMMU, Dhaka. Laboratory works were performed in the Department of Microbiology and Immunology, BSMMU, Dhaka. A total of 46 diagnosed cases of SLE were enrolled in this study after taking informed written consent. All patients met at least 4 criteria out of the 11 of the 1997 update of the 1982 American College of Rheumatology revised (ACR) criteria for diagnosis of SLE.[12,13] A complete physical examination was performed and symptoms were noted. History of other associated autoimmune diseases and past medical history were taken from previous records. Patients having other diagnosed autoimmune diseases in association with SLE were not included in the present study.

Sample collection procedure

Buccal swabs were collected for HLA-DR typing. Before the collection of swabs, each person was advised to avoid taking food, tea, coffee, smoking, or betel nut for at least one hour. Then buccal swabs were collected by rubbing the inside of the cheek with a sterile, dry cotton swab for 20 seconds. This procedure was repeated 7 times and swabs were air-dried before placing in a sterile dry tube[14]. After collection, samples were brought immediately to the laboratory for further processing. 3 ml venous blood was collected from each SLE case for detection of anti-dsDNA and anti-Sm autoantibodies, in a plain tube without any anticoagulant and preservatives. Sera were separated as soon as possible, aliquot, and stored at -20ºC temperature until used.

Detection of anti-dsDNA and anti-Sm autoantibodies

These two autoantibodies were detected by the method of ELISA using a commercial ELISA kit (EURO- Diagnostica ELISA kit, Sweden). All components were allowed to reach room temperature prior to use in the assay. The tests were done according to manufacturer instructions. The optical density of reactions at each microtiter well was measured by a microplate reader and was recorded in IU/ml of serum.

HLA-DR typing

Genomic DNA was extracted from buccal swab samples by using Chelex 100 followed by protein digestion in proteinase K solution.[15] HLA-DR typing was done by using polymerase chain reaction sequence specific primer (PCR-SSP) (Morgan™ HLA SSP DRB typing kit) using low resolution typing method. The amplified DNA was examined by agar gel electrophoresis which separates the DNA fragments by size. Specific HLA-DRB type was determined using the worksheet (supplied along with the kit).

Statistical analysis

All data after collection were checked, coded, and entered into a database using online MedCalc software (Version- 12.7.8.0). Descriptive analysis of all relevant variables was done by using proportion, central tendency, and dispersion. Statistical associations of HLA antigens with autoantibodies were determined by chi-square with Yates correction. The strength of association of HLA antigens with autoantibodies was estimated by relative risk (RR) and 95% confidence intervals (95% CI). The relative risk was determined by the odd ratio. *p* corrected (*pc*) was determined by multiplying *p* value by the number of HLA antigens tested (Bonferroni,s correction). *pc* value of less than 0.05 was considered statistically significant.

Results

Out of 46 cases with SLE 44 (95.65%) were female and 2 (4.35%) were male with a female: male ratio was 22: 1. Their mean age at study entry was 27.05 ± 8.17 years (mean ± SD), ranging from 12.5-45 years (Table I).

Table I: Demographic characteristics of study population.

Study population	Sex			Age (year)	
	Female	Male	Female: male	Mean ± SD	Age range
n= 46	44 (95.65)	2 (4.35)	22: 1	27.05 ± 8.17	12.5 - 45

Note: Figure within the parenthesis indicates percentage.

The frequency of autoantibodies, anti-dsDNA, and anti-Sm are shown in Table II.

Table II: Frequency of anti-dsDNA and anti-Sm autoantibodies.

Autoantibody	n = 46 Positive No. (%)	Negative No. (%)
Anti-dsDNA	38 (82.61%)	8 (17.39%)
Anti-Sm	19 (41.30%)	27 (58.70%)

Anti-dsDNA was positive in 38 (82.61%) cases and negative in 8 (17.39%) cases. Anti-Sm was positive in 19 (41.30%) cases and negative in 27 (58.70%) cases.

Among 46 cases, the most frequently identified HLA-DR was DR2 (86.96%) followed by DR7 (43.48%), DR4 (17.39%), DR5 (17.39%), DR6 (13.04%), DR10 (13.04%), DR1 (4.35%) and DR3 (4.35%). HLA-DR8 and DR9 were not expressed in any case (Figure 1).

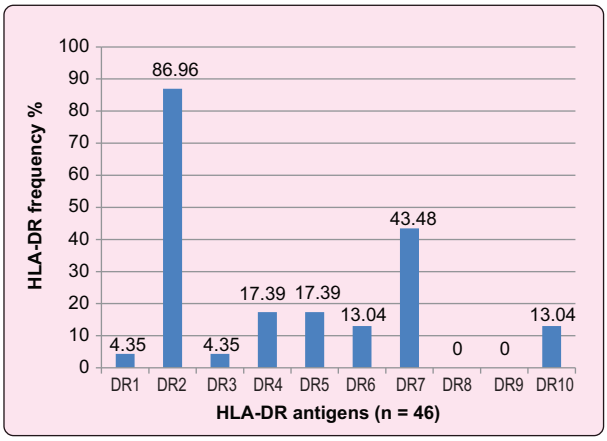

Figure 1: Frequency of HLA-DR antigens.

When the association of HLA-DR antigens and anti-dsDNA was analyzed it was found that, the most frequent HLA-DR in anti-dsDNA positive cases was HLA-DR2 (94.73%) followed by DR7 (39.47%), DR5 (15.79%), DR4 (13.16%), DR6 (13.16%), DR10 (13.16%), DR1 (5.26%) and DR3 (5.26%). When anti-dsDNA positive cases compared with anti-dsDNA negative cases, positive association was found with HLA-DR2 (94.73% vs 50%, *p* value = 0.0044, *pc* = 0.044, RR = 18.0000). HLA-DR4, DR5, and DR7 frequency in anti-dsDNA positive cases decreased compared with anti-dsDNA negative cases. RR of these HLA-DR were less than 1 but at 95% CI level their association with anti-dsDNA production was not significant. The frequency of HLA-DR1, DR3, DR6, and DR10 was slightly increased among cases positive for anti-dsDNA compared to negative cases but statistically not significant (Table III).

The most frequent HLA-DR observed in anti-Sm positive cases was DR2 (89.47%) followed by DR7 (36.84%), DR4 (21.05%), DR5 (15.79%), DR1 (10.53%), DR3 (10.53%), DR6 (10.53%) and DR10 (5.26%). When anti-Sm positive cases were compared with anti-Sm negative cases, no positive association of HLA-DR was found with anti-Sm autoantibody in this study. The frequency of HLA-DR5, DR6, DR7, and DR10 was decreased in anti-Sm positive cases but this decrease was not statistically significant (Table IV).

The study population was divided into 4 groups on the basis of their autoantibody status. Group 1 (n= 17) includes both autoantibody positive cases, group 2 (n= 21) includes anti-dsDNA positive but anti-Sm negative, group 3 (n= 2) includes anti-Sm positive but anti-dsDNA negative and group 4 (n= 6) includes both

anti-dsDNA and anti-Sm negative cases. When a comparison was made, the most frequent HLA-DR observed among cases positive for both anti-dsDNA and anti-Sm autoantibody was HLA-DR2. *p* value was significant but when correction was made it was not statistically significant. No other significant association was found with any other groups (Table v)..

Table III: Frequency of HLA-DR in relation to anti-dsDNA autoantibody.

HLA-DR	Anti-dsDNA					
	Positive(n = 38)	Negative(n = 8)	*P* value	*pc*	RR	95% CI
DR1	2 (5.26)	0 (0)	0.9240	9.240	1.1644	0.051 to 26.549
DR2	36 (94.73)	4 (50)	0.0044	0.044	18.0000	2.467 to 131.289
DR3	2 (5.26)	0 (0)	0.9240	9.240	1.1644	0.051 to 26.549
DR4	5 (13.16)	3 (37.5)	0.1153	1.153	0.2525	0.045 to 1.400
DR5	6 (15.79)	2 (25)	0.5361	5.361	0.5625	0.090 to 3.480
DR6	5 (13.16)	1 (12.5)	0.9600	9.600	1.0606	0.106 to 10.544
DR7	15 (39.47)	5 (62.5)	0.2421	2.421	0.3913	0.0812 to 1.885
DR8	0 (0)	0 (0)	0	0	0	0
DR9	0 (0)	0 (0)	0	0	0	0
DR10	5 (13.16)	1 (12.5	0.9600	9.600	1.0606	0.106 to 10.544

Note:
Figure within parenthesis indicates percentage.
CI = confidence interval.
pc = *p* corrected
pc < 0.05 is considered as significant
RR = relative risk.

Table IV: Frequency of HLA-DR in relation to anti-Sm autoantibody.

HLA-DR	Anti-Sm					
	Positive(n = 19)	Negative(n = 27)	*P* value	*pc*	RR	95%CI
DR1	2 (10.53)	0 (0)	0.1917	1.917	7.8571	0.355 to 173.543
DR2	17 (89.47)	23 (85.19)	0.6720	6.720	1.4783	0.242 to 9.028
DR3	2 (10.53)	0 (0)	0.1917	1.917	7.8571	0.355 to 173.543
DR4	4 (21.05)	4 (14.81)	0.5842	5.842	1.5333	0.331 to 7.088
DR5	3 (15.79)	5 (18.52)	0.8102	8.102	0.8250	0.171 to 3.963
DR6	2 (10.53)	4 (14.81)	0.6720	0.6720	0.6765	0.110 to 4.131
DR7	7 (36.84)	13 (48.15)	0.4475	4.475	0.6282	0.189 to 2.084
DR8	0 (0)	0 (0)	0	0	0	0
DR9	0 (0)	0 (0)	0	0	0	0
DR10	1 (5.26)	5 (18.52)	0.2168	2.168	0.2444	0.026 to 2.286

Note:
Figure within parenthesis indicate percentage.
CI = confidence interval.
pc = *p* corrected
pc < 0.05 is considered as significant
RR = relative risk.

Table V: HLA-DR antigen frequency among cases positive for both autoantibodies and their comparison with anti-dsDNA positive but anti-Sm negative, anti-Sm positive but anti-dsDNA negative and both autoantibodies negative cases.

HLA-DR	Both anti-dsDNA and anti-Sm positive (n= 17)	Anti-dsDNA positive but anti-Sm negative (n= 21)	Anti-Sm positive but anti-dsDNA negative (n= 2)	Both anti-dsDNA and anti-Sm negative (n=6)	*p* value	*pc*
DR1	2 (11.76)	0 (0)	0 (0)	0 (0)	0.3122	3.122
DR2	16 (94.11)	20 (95.23)	1 (50)	3 (50)	0.0086	0.086
DR3	2 (11.76)	0 (0)	0 (0)	0 (0)	0.3122	3.122
DR4	3 (17.65)	2 (9.52)	1 (50)	2 (33.33)	0.3277	3.277
DR5	2 (11.76)	4 (19.05)	1 (50)	1 (16.67)	0.5940	5.940
DR6	2 (11.76)	3 (14.29)	0 (0)	1 (16.67)	0.9356	9.356
DR7	6 (35.29)	9 (42.86)	1 (50)	4 (66.67)	0.6119	6.119
DR8	0 (0)	0 (0)	0 (0)	0 (0)	0	0
DR9	0 (0)	0 (0)	0 (0)	0 (0)	0	0
DR10	1 (5.88)	4 (19.05)	0 (0)	1 (16.67)	0.6137	0.6137

Note:
Figure within parenthesis indicates percentage.
pc = *p* corrected
pc < 0.05 is considered as significant

Discussion

The hallmark of SLE is the production of autoantibodies. Among the genetic factors associated with the development of SLE, the most important is MHC. In this study among the 46 SLE cases, the majorities were female (95.65%) and the female-male ratio was 22: 1. The mean age at study entry was 27.05 ± 8.17(mean ± SD) years. This is almost similar to the findings of other studies.[1,15-21] This finding further confirmed that females are more affected than males and it predominantly occurs in the 3rd and 4th decade of life. The younger mean age of females may be due to the direct effect of sex chromosomes or the indirect effects of chromosomes mediated by sex hormones.[22]

Anti-dsDNA was positive in 38 cases (82.61%). A similar and slight variation was reported by some other studies, 91.7% to 58% where they used ELISA.[21,23-28] In other studies reported positivity of anti-dsDNA was 71.12% and 59%.[20,29,30] Treatment status, disease activity, and sample size may be responsible for the variation in this study.

The association of particular HLA and autoantibodies production has been reported before with inconsistent results. The most frequent HLA-DR observed in anti-dsDNA positive cases in this study is DR2 (94.73%). When a comparison was made with negative cases, DR2 was found to be positively associated with anti-dsDNA. A similar association was reported in studies of European, Thai, Baltimore, and Japanese populations.[15,27-29,31] Haplotype analysis found that genotype DR2/DR3 heterozygotes were strongly associated with the production of anti-dsDNA.[9] But other studies among Malay, Japanese, Jamaican, Northern Italian, and southern Spain (Caucasian and Gypsy people) did not find any positive association of HLA-DR with anti-dsDNA.[19,21,23,25,32] Again increase of HLA-DR7 in anti-dsDNA positive patients was reported by some studies.[21] Although in the present study DR7 was second highest in anti-dsDNA positive cases it was not statistically significant. In the Jamaican population negative association of DR6 with anti-dsDNA was found.[32] But in the present study association of DR6 with anti-dsDNA was not significant,

neither positive nor negative. A positive association of HLA-DR3 with anti-dsDNA was reported by some studies, but in this study, no association was found.[33] No other HLA-DR was found to be associated with anti-dsDNA in this study.

The autoantibody Anti-Sm, was positive in 19 (41.30%) cases in this study. A similar and slight variation was reported by other investigators, where the detection rate of anti-Sm varies from 42% to 26% as detected by EIA.[21,23-25,27-29,31,35]

In this study, no positive association between HLA-DR with anti-Sm was seen. Similarly, others also did not find any association of HLA-DR with anti-Sm among Malay, Japanese, Baltimore, Norwegian, southern Spain (Caucasian and Gypsy people), and European patients with SLE.[20,21,25,27,28,31,34] But the positive association of HLA-DR4, DR2, and DR3 with anti-Sm was reported among USA, Japanese, and UK patients with SLE, which is contradictory to the findings of this study.[11,23,24,35] Again haplotype analysis in the USA reported that genotypes DR2 (heterozygote and homozygote) and DR3 (homozygote) are strongly associated with the production of anti-Sm autoantibody.[9] But in the present study HLA-DR1, DR2, DR3, and DR4 were slightly increased in anti-Sm positive cases compared to anti-Sm negative cases but this increase was not statistically significant. Moreover, the frequency of HLA-DR5, DR6, DR7, and DR10 decreased in anti-Sm positive cases compared to negative cases and their RR was also less than 1 but not significant at 95% CI. The study population was divided into 4 groups on the basis of their autoantibody status. When a comparison was made among these 4 groups, a positive association of HLA-DR2 was found in cases positive for both antibodies but when *p* value was corrected it was no more statistically significant.

In this study, the most frequent HLA-DR observed in cases was HLA-DR2. Similar findings were reported among Thais, Japanese, Malay, South Africans, Taiwanese and Kuwaiti populations with SLE.[2,17,20,23,28,29,31,36]

The difference in the pattern of production of autoantibodies stated in different studies has been stated in international collaboration studies (Eleventh International Histocompatibility Workshop and Conference).[37] Thus ethnic differences among study populations, the sample size of different studies, and heterogenecity of the HLA could explain the contradictory results found in different studies mentioned above. This correlation between autoantibody responses and HLA alleles may indicate that these autoimmune reactions are mediated by genetically restricted antigen-specific T- helper cells interacting with specific HLA molecules.[34]

This study included only one locus. Examination of other loci of HLA complex at allele level is also required because HLA-DR is in linkage disequilibrium with other HLA. Also, a study regarding the association of HLA with clinical heterogenicity among SLE patients could provide useful information and thus can affect treatment modalities.

Conclusion

The results of this study further confirm the findings of previous studies indicating that HLA-DR2 is associated with the production of autoantibodies, particularly anti-dsDNA in Bangladeshi SLE cases.

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References

1. Castano- Rodriguez N, Diazo- Gallo LM, Pineda- Tamayo R, Rojas-Villarraga A, Anaya JM. Meta-analysis of HLA-DRB1 and HLA-DQB1 polymorphism in Latin American patients with systemic lupus erythematosus. Autoimmunity Reviews. 2008; 7:322-30.
DOI: 10.1016/j.autrev.2007.12.002

2. Mok CC and Lau CS. Pathogenesis of systemic lupus erythematosus. Journal of Clinical Pathology. 2003; 56: 481-90.
DOI: 10.1136/jcp.56.7.481

3. Arnett FC and Shulman LE. Studies in familial systemic lupus erythematosus. Medicine (Baltimore). 1976; 55:313-22. DOI: 10.1097/00005792-197607000-00003

4. Deapen D, Escalante A, Weinrib L, Horwitz D, Bachman B, Roy-Burman P, et al. A revised estimate of twin concordance in SLE. Arthritis and Rheumatism. 1992; 35:311-DOI: 10.1002/art.1780350310

5. Wilson JG, Wong WW, Schur PH, Fearon DT. Mode of inheritance of decreased C3b receptor on erythrocytes of patients with systemic lupus erythematosus. The New England Journal of Medicine. 1982; 307:981 – 6. DOI: 10.1056/NEJM198210143071604

6. Miller KB, Schwartz RS. Familial abnormalities of suppressor cell function in systemic lupus erythematosus. The New England Journal of Medicine.1979; 301:803 – 09. DOI: 10.1056/NEJM197910113011502

7. Fronek Z, Timmerman LA, Alper CA, Hahn BH, Kalunian K, Peterlin BM, et al. Major histocompatibility complex genes and susceptibility to systemic lupus erythematosus. Arthritis and Rheumatism. 1990; 33:1542 – 53. DOI: 10.1002/art.1780331012

8. Walport MJ, Black CM, Batchelor JR. The immunogenetics of SLE. Clinical Rheumatology Disease.1982; 8:3 – 21. PMID: 6811190

9. Graham RR, Ortmann W, Rodine P, Espe K, Langefeld C, Lange E, et al. Specific combinations of HLA-DR and DR3 class 11 haplotypes contribute graded risk for disease susceptibility and autoantibodies in human SLE. European journal of human genetics. 2007; 15:823-30. DOI: 10.1038/sj.ejhg.5201827

10. Reveille JD, Macleod MJ, Whittington K, Arnett FC. Specific amino acid residues in the second hypervariable region of HLA DQA1 and DQB1 chain genes promote the Ro (SS-A)/La (SS-B) autoantibody responses. The Journal of Immunology. 1991; 146: 3871 – PMID: 2033256

11. Smolen JS, Klippel JH, Penner E, Reichlin M, Steinberg AD, Chused TM, et al. HLA-DR antigens in systemic lupus erythematosus: association with specificity of autoantibody response to nuclear antigens. Annals of the Rheumatic Diseases. 1987; 46:457-62. DOI: 10.1136/ard.46.6.457

12. Gill JM, Quisel AM, Rocca PV, Walters DT. Diagnosis of Systemic Lupus Erythematosus. American Family Physician. 2003; 68 :2179-86. PMID: 14677663

13. Gillespie KM, Valovin SJ, Saunby J, Hunter KM, Savage DA, Middleton D, et al. HLA class II typing of whole genome amplified mouth swab. Tissue Antigens. 2000; 56:530- DOI: 10.1034/j.1399-0039.2000.560607.x

14. Suenaga E and Nakamura H. Evaluation of three methods for effective extraction of DNA from human hair. Journal of Chromatography B. 2005; 820:137- 41. DOI: 10.1016/j.jchromb.2004.11.028

15. Sirikong M, Tsuchiya N, Chandanayingyong D, Bejrachandra S, Suthipinittharm P, Luangtrakool K, et al. Association of HLA-DRB1*1502-DQB1*0501 haplotype with susceptibility to systemic lupus erythematosus in Thais. Tissue antigen. 2002; 59:113-17 DOI: 10.1034/j.1399-0039.2002.590206.x

16. Hussain N, Jaffery G, Sabri AN, Hasnain S. HLA Association in SLE patients from Lahore-Pakistan. Bosnian journal of basic medical sciences. 2011; 11:20-DOI: 10.17305/bjbms.2011.2618

17. Fouad F, Johny K, Kaaba S, Alkarmi TO, Sharma P & Al-Harbi S. MHC in systemic lupus erythematosus: A study on a Kuwaiti population. European Journal of Immunogenetics. 1994; 21:11-4. DOI: 10.1111/j.1744-313x.1994.tb00171.x

18. Ling- Ying LU, Ding WZ, Fici D, Deulofeut R, Cheng HH, Cheu CC, et al. Molecular analysis of major histocompatibility complex allelic associations with systemic lupus erythematosus in Taiwan". Arthritis and Rheumatism. 1997; 40:1138-45. DOI: 10.1002/art.1780400619

19. Savi M, Ferraccioli GF, Neri TM, Zanelli P, Dall'Aglio PP, Tincani A, et al. HLA-DR antigens and anticardiolipin antibodies in Northern Italian systemic lupus erythematosus patients. Arthritis and Rheumatism. 1988; 31:1568 – 70. DOI: 10.1002/art.1780311216

20. Rudwaleit M, Tikly M, Gibson K, Pile K and Wordsworth P. HLA class II antigens associated with systemic lupus erythematosus in black South Africans. Annals of Rheumatic Diseases. 1995; 54:678-80. DOI: 10.1136/ard.54.8.678

21. Azizah MR, Ainol SS, Kong NC, Normaznah Y, Rahim MN. HLA antigens in Malay patients with systemic lupus erythematosus: association with clinical and autoantibody expression. The Korean Journal of Internal Medicine. 2001; 16:123-31. DOI: 10.3904/kjim.2001.16.2.123

22. Moser KL, Kelly JA, Lessard CJ and Harley JB. Recent insights into the genetic basis of systemic lupus erythematosus. Genes and Immunity. 2009; 10:373- 9. DOI: 10.1038/gene.2009.39

23. Shimane K, Kochi Y, Suzuki A, Okada Y, Ishii T, Horita T, et al. An association analysis of HLA-DRB1 with systemic lupus erythematosus and rheumatoid arthritis in a Japanese population: effects of *09:01 allele on disease phenotypes. Rheumatology. 2013; 52:1172-82. DOI: 10.1093/rheumatology/kes427

24. McHugh NJ, Owen P, Cox B, Dunphy J, Welsh K. MHC class II, tumor necrosis factor alpha, and lymphotoxin alpha gene haplotype associations with serological subsets of systemic lupus erythematosus. Annals of Rheumatic Diseases. 2006; 65:488-94. DOI: 10.1136/ard.2005.039842

25. Ramal LM, Lopez-Nevot MA, Sabio JM, Jaimez L, Paco L, Sanchez J, et al. Systemic lupus erythematosus in southern Spain: a comparative clinical and genetic study between Caucasian and Gypsy patients. Lupus. 2004; 13:934 – 40. DOI: 10.1191/0961203304lu2036oa

26. Shankarkumar U, Ghosh K, Badakere SS, Mohanty D. HLA-DRB1*03 and DQB1*0302 associations in a subset of patients severely affected with systemic lupus erythematosus from western India. Annals of Rheumatic Diseases. 2003; 62:92–3.
DOI: 10.1136/ard.62.1.92

27. Galeazzi M, Sebastiani GD, Morozzi G, Carcassi C, Ferrara GB, Scorza R, et al. HLA class II DNA typing in a large series of European patients with systemic lupus erythematosus. Medicine. 2002; 81:169-78.
DOI: 10.1097/00005792-200205000-00001

28. Hochberg MC, Boyd RE, Ahearn JM, Arnett FC, Bias WB, Provost TT, et al. Systemic lupus erythematosus: A review of clinic-laboratory features and immunogenetic markers in 150 patients with emphasis on demographic subsets. Medicine. 1985; 64:285 – 95.
PMID: 2412088

29. Furukawa H, Kawasaki A, Oka S, Ito I, Shimade K, Sugii S, et al. Human leukocyte antigens and systemic lupus erythematosus: A protective role for the HLA-DR6 alleles DRB1*13:02 and *14:03. Plos One. 2014; 9(:e87792.
DOI: 10.1371/journal.pone.0087792

30. Slater NG, Cameron JS, Lessof MH. The Crithidia luciliae kinetoplast immunofluorescence test in systemic lupus erythematosus. Clinical and. Experimental Immunology. 1976; 25:480–86.
PMID: 786521

31. Hashimoto H, Nishimura Y, Dong RP, Kimura A, Sasazuki T, Yamanaka K, et al. HIA Antigens in Japanese Patients with Systemic Lupus Erythematosus. Scandinavian Journal of Rheumatology. 1994; 23:191-6.
DOI: 10.3109/03009749409103059

32. Christian N, Smikle NF, DeCeulaer K, Daniels L, Walravens MJ and Barton EN. Antinuclear antibodies and HLA class II alleles in Jamaican patients with systemic lupus erythematosus. West Indian Medical Journal. 2007; 56:130-3.
DOI: 10.1590/s0043-31442007000200005

33. Chung SA, Taylor KE, Graham RR, Nititham J, Lee AT, Ortmann WA, et al. Differential genetic associations for systemic lupus erythematosus based on anti-dsDNA autoantibody production. PLoS Genetic. 2011; 7:e1001323.
DOI: 10.1371/journal.pgen.1001323

34. Skarasvag S, Hansen KE, Moen T and Eggen BM. Distribution of HLA class II alleles in autoantibody subsets among Norwegain patients with systemic lupus erythematosus. Scandinavian Journal of Immunology. 1995; 42:564-71.
DOI: 10.1111/j.1365-3083.1995.tb03697.x

35. Olsen ML, Arnett FC and Reveille JD. Contrasting molecular patterns of MHC class II alleles associated with the anti-Sm and anti-RNP precipitin autoantibodies in systemic lupus erythematosus. Arthritis and Rheumatism. 1993; 36:94-104.

DOI: 10.1002/art.1780360117

36. Mohd-Yusuf Y, Phipps ME, Chow SK and Yeap SS. HLA-A*11 and novel associations in Malays and Chinese with systemic lupus erythematosus. Immunology 2011; 139:68-72.
DOI: 10.1016/j.imlet.2011.05.001

37. Martin-Villa JM, Martinez-Laso J, Moreno-Pelayo MA, Castro-Panete MJ, Martinez-Quiles N, Alvarez M, et al. Differential contribution of HLA-DR, DQ and TAP2 alleles to systemic lupus erythematosus susceptibility in Spanish patients: role of TAP2*01 alleles in Ro autoantibody production. Annals of the Rheumatic Diseases. 1998; 57:214-9.
DOI: 10.1136/ard.57.4.214