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Molecular Analysis of HLA-DR and Their Association with Systemic Lupus Erythematosus

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Abstract

Systemic lupus erythematosus (SLE) is an autoimmune disease that develops within a complex network of genetic and immunologic factors. Both genetic and environmental factors strongly influence the development of SLE. But genetic factors are more important both in determining the overall susceptibility to SLE and in influencing immunologic heterogenecity in affected subjects. Now it is accepted that major histocompatibility complex (MHC) genes particularly HLA (Human leukocyte antigen) class II constitute a part of the genetic factor for susceptibility to develop SLE. To determine the association of HLA-DR antigens with SLE, this case-control study was conducted over a period of twelve months from March 2013 in Dhaka. Buccal swabs for HLA-DR typing were collected from 46 SLE cases and 46 age and sex matched unrelated healthy controls. HLA-DR typing was carried out by polymerase chain reaction (PCR) with sequence specific primers. Among 46 cases, female versus male ration was 22: 1 and mean age at study entry was 27.05 ± 8.17 years, ranging from 12.5 – 45 years. A total of 10 (HLA-DR1 to DR10) HLA antigens were determined in both cases and controls. The most frequent HLA-DR observed among cases was DR2 (86.96%) followed by DR7 (41.30%). When compared with healthy controls, the HLA-DR2 was significantly associated with SLE (p <0.05, RR: 4.6914, 95% CI: 1.658 to 13.267). No other HLA-DR had significant association with SLE. No association of HLA-DR was observed with age of onset of disease among SLE cases. Results of the study reveal that HLA-DR2 gene is a risk factor for development of SLE in Bangladeshi population.

Key words: Systemic lupus erythematosus, Major histocompatibility complex, Human leukocyte antigen, Polymerase chain reaction.

Introduction

Health problem due to rheumatic disorders are increasing day by day. Now-a-days they are the commonest cause of morbidity. Among the multisystem rheumatic disorders systemic lupus erythematosus (SLE) is the commonest autoimmune disorder.[1] this systemic autoimmune disease affects multiple organs, which is characterized by production of autoantibodies against a variety of self antigens such as double stranded DNA (dsDNA), intracellular ribonucleoproteins and membrane phospholipids. Severity, acquisition risk and clinical manifestations of this disease may vary by ethnicity, geography and sex. It primarily affects female (female: male-9:1), especially during their childbearing age.[2] Some non-European populations such as African Americans, Hispanics and Asians are at increased risk for acquiring the disease. Although the exact aetiology of SLE remains vague, genetic predisposition, environmental and hormonal factors play important role in its pathogenesis. The high concordance rate for SLE in monozygotic twins than in dizygotic twins or siblings (24-56% versus 2-5%) and the high sibling recurrence risk ratio of patients with SLE (between 8-fold and 29-fold higher than general population) proved a strong genetic contribution for development of the disease.[3, 4]

SLE is a polygenic disorder. Effects of multiple genes are required for development of SLE. Although in rare cases SLE may be associated with deficiency of a single gene. The search for gene that predisposes a person to develop SLE has been going on through association studies of candidate genes and genome wide linkage analysis. A large number of genetic regions have been identified that may contain susceptibility gene. Among them, eight susceptibility loci have been confirmed: 1q23, 1q31, 1q41-42, 2q37, 4p16, 6p21, 12q24 and 16q12-13.[5, 6] But region 6p21.1-q15 and 20q11- q13.13 have reached the threshold level for significant linkage.[6] Of the genetic elements, the genes of the major histocompatibility complex (MHC) have been most extensively studied for their contribution to development of SLE. MHC, located on chromosome 6p21.3, harbours a gene reach and transcriptionally active segment that encodes for immunologically important gene, including highly polymorphic human leukocyte antigen (HLA) class I and class II genes. Class I region contains HLA-A, -B, -C genes, which present antigenic peptides to CD8+ T cells. Class II region contains highly polymorphic HLA-DR, -DQ and -DP genes. They present antigenic peptides to CD4+ T cells. The class III region lies between class I and class II that contains many immune related genes, including cytokines, tumor necrosis factor-α, lymphotoxin- α, the complement components C2, C4 and Factor B.[7]

Association of Human leukocyte antigen (HLA) with SLE was first reported in 1971 and it was HLA class I B8.[8] But later studies showed that association of HLA class II genes are more strong and consistent with SLE than HLA class I genes. Among the HLA class II, HLA-DR2 and DR3 alleles are proved to be associated with a two to five fold risk for the development of SLE.[9] However this association varies considerably in different races and ethnic groups due to genetic haeterogenecity, which is much more common among populations of SLE.[10,11] Amongst Europeans most studies showed association of SLE with HLA-DR3 and in Asian countries most studies found association with DR2. But data regarding association study of SLE with HLA among Bangladeshi is not available. In addition, several studies also suggested that the contribution of HLA class II genes in SLE is also involved in specific autoantibody production like HLA-DR2 and DR3 are associated with development of anti-dsDNA and anti-Sm autoantibodies.[12] It has also been found that DR2/DR3 genotype may predispose to development of autoantibodies in unaffected family members of SLE cases.[7] So, identification of candidate genes and understanding of genetic influences is necessary to comprehend the pathophysiology of this autoimmune disease and to predict high risk patients who carry genetic susceptibility factors in general population or within families. Therefore, the present study was undertaken to determine the association of HLA-DR (DR1 to DR10) with development of SLE in SLE cases.

Materials and Methods

This case-control study was done during the period of March 2013 to February 2014. Samples were collected from SLE clinic, Department of Rheumatology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. Laboratory works were performed in the Department of Microbiology and Immunology, BSMMU, Dhaka. A total of 46 diagnosed case 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 criteria for diagnosis of SLE.[13] A complete physical examination was performed and symptoms were noted. History of other associated autoimmune disease and past medical history were taken from previous records. Patients having other diagnosed autoimmune diseases in association with SLE were excluded for the present study. A total 46 healthy volunteer age and sex matched unrelated healthy controls were enrolled in this study after taking informed written consent. The control population were medical students and nurses. Control population included in this study were negative for anti-dsDNA, anti-Sm and did not have history of rheumatic disorders. Persons having family history of SLE and other rheumatic disorders were not included as control population. Buccal swabs were collected from both cases and controls for HLA-DR typing.

HLA typing: Genomic DNA was extracted from buccal swab samples by using Chelex® 100 followed by protein digestion in proteinase K solution.[14] 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 that separates the DNA fragments by size. Specific HLA-DRB type was determined using the worksheet (supplied along with the kit).

Statistical analysis: The allelic frequencies of HLA-DR in cases and controls were compared using chi-square with Yates correction (by using online available GraphPad QuickCalcs calculator). The strength of association between HLA-DR antigens and SLE was estimated by relative risk (RR) and 95% confidence intervals (95% CI) using online MedCalc software (Version-12.7.8.0). The relative risk was determined by the odd ratio (OR). p value of less than 0.05 was taken to be significant. p corrected (p corr) was determined by multiplying p value with the number of HLA alleles tested (Bonferroni's correction).

Results

A total of 46 cases with SLE and 46 healthy controls were enrolled in this study. There were 44 (95.65%) female and 2 (4.35%) male in both cases and controls. Their mean age was 27.05 ± 8.17 years (mean ± SD), ranging from 12.5 – 45 years. In control group, there were 44 (95.65%) female and 2 (4.35%) male with a mean age of 26.91 ± 5.96 years (mean ± SD), ranging from 16 – 45 years. In both case and control group female: male ratio was 22: 1 (table I)

Table I: Distribution of study population according to their sex and age.

Study population	Sex			Age (year)	
	Female	Male	Female: male	Mean ± SD	Age range
Case (n= 46)	44 (95.65)	2 (4.35)	22: 1	27.05 ± 8.17	12.5 - 45
Control (n= 46)	44 (95.65)	2 (4.35)	22: 1	26.91 ± 5.96	16 - 45

Note: Figure within the parenthesis indicates percentage.

Forty six SLE cases were divided into two groups: first group less than 30 years and second group ≥30 years on the basis of their age of onset of disease. There were 36 (78%) cases in first group and 10 (22%) in second group (figure 1)

Out of 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 by any cases.

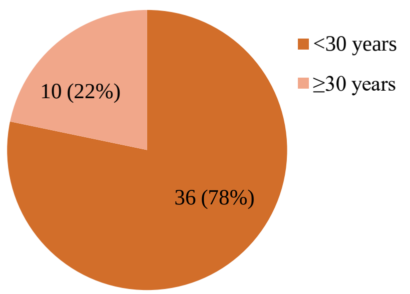

Figure 1: Distribution of study population according to their age of onset of disease (n = 46)

Out of 46 controls, the most frequently identified HLA-DR was DR2 (58.70%) followed by DR7 (41.30%), DR4 (21.72%), DR6 (19.57%), DR10 (19.57%), DR5 (10.87%), DR1 (8.70%), DR3 (8.70%), DR8 (8.70%) and DR9 (2.17%) (figure 2).

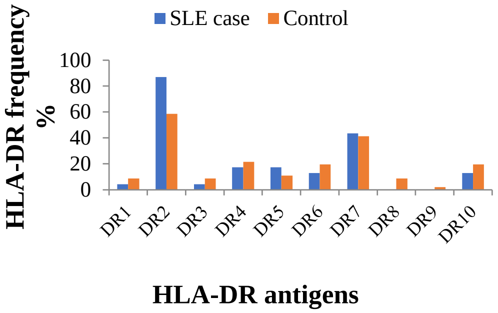

Figure 2: HLA-DR antigens in cases and controls

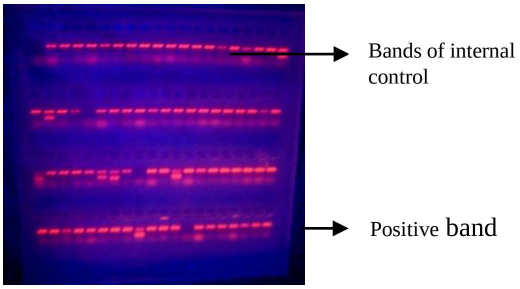

Figure 3: Gel electrophoresis of PCR reaction products showing bands of internal controls and positive alleles.

Positive association with SLE was observed for HLA-DR2 (86.96% Vs. 58.70%, p = 0.0036, pc = 0.036, RR = 4.6914, 95% CI = 1.658 to 13.267) when compared with healthy controls. There were a weak increased of HLA-DR7 and HLA-DR5 in cases Vs. controls but it was not statistically significant at 5% significance level. HLA-DR1, DR3, DR4, DR6, DR8, DR9 and DR10 were found to be slightly decreased in cases as compared to controls. RR of these antigens was less than 1 but at 95% CI their association was not significant (table II)

HLA-DR frequencies in cases that had disease onset less than 30 years of age were DR2 (86.11%), DR7 (44.44%), DR5 (19.44%), DR10 (13.89%), DR4 (16.67%), DR3 (2.78%), DR1 (5.56%) and DR6 (11.11%). In second group HLA-DR frequencies were DR2 (90%), DR7 (40%), DR4 (20%), DR6 (20%), DR3 (10%), DR5 (10%), and DR10 (10%). HLA-DR1 was not expressed by any cases in this group. In both groups no cases express DR8 and DR9 (table III)

Table II: Association of HLA-DR antigens in cases compared to controls.

HLA-DR	Cases n = 46 No.	Control n = 46 No.	*p* value	*pc*	RR	95% CI
DR1	2 (4.35)	4 (8.70)	0.4072	4.072	0.4773	0.083 to 2.744
DR2	40 (86.96)	27 (58.70)	0.0036*	0.036*	4.6914*	1.658 to 13.267
DR3	2 (4.35)	4 (8.70)	0.4072	4.072	0.4773	0.083 to 2.744
DR4	8 (17.39)	10 (21.72)	0.5998	5.998	0.7579	0.269 to 2.134
DR5	8 (17.39)	5 (10.87)	0.3731	3.731	1.7263	0.519 to 5.739
DR6	6 (13.04)	9 (19.57)	0.3999	3.999	0.6167	0.200 to 1.900
DR7	20 (43.48)	19 (41.30)	0.8329	8.329	1.0931	0.478 to 2.499
DR8	0 (0)	4 (8.70)	0.1288	1.288	0.1016	0.005 to 1.942
DR9	0 (0)	1 (2.17)	0.4962	4.962	0.3262	0.012 to 8.217
DR10	6 (13.04)	9 (19.57)	0.3999	3.999	0.6167	0.200 to 1.900

Note: Figure within parenthesis indicates percentage.
CI = confidence interval, *pc* = *p* corrected, RR = relative risk. *Statistically significant.

Table III: Frequency of HLA-DR in cases divided according to their age of onset of disease.

	< 30 years (n= 36)	***p* value**	***p* corr**	**RR**	**≥30 years (n= 10)**	***p* value**	***p* corr**	**RR**
DR1	2 (5.56)	0.4460	4.460	1.5217	0 (0)	0.44	4.4	0.657
DR2	31 (86.11)	0.746	7.46	0.6889	9 (90)	0.746	7.46	1.451
DR3	1 (2.78)	0.909	9.09	0.2571	1 (10)	0.909	9.09	3.888
DR4	6 (16.67)	0.805	8.05	0.800	2 (20)	0.805	8.05	1.250
DR5	7 (19.44)	0.8216	8.216	2.1724	1 (10)	0.821	8.21	0.460
DR6	4 (11.11)	0.8355	8.355	0.500	2 (20)	0.729	7.29	2.000
DR7	16 (44.44)	0.8020	8.020	1.200	4 (40)	0.802	8.02	0.833
DR8	0 (0)	0	0	0	0 (0)	0	0	0
DR9	0 (0)	0	0	0	0 (0)	0	0	0
DR10	5 (13.89)	0.7467	7.467	1.451	1 (10)	0.746	7.46	0.688

Note: Figure within parenthesis indicates percentage.
p corr = *p* corrected, RR = relative risk.

When comparison was made in these 2 groups, no positive association of HLA-DR with age of disease onset was found.

Discussion

Systemic lupus erythematosus (SLE) is a genetically complex disease. The course of this chronic multisystem inflammatory disease may range from benign to fatal like organ failure (e.g., kidney) or malignancy. In spite of lots of study, it is difficult to say the exact etiological factors causing this disease. The importance of genetic factors for development of disease has been confirmed. But it is still controversial the contribution of immunogenetic factors causing the disease. A lot of work has been done to find out the degree and nature of association of human leukocyte antigen (HLA) and SLE. Genome wide association studies and haplotype study persuasively demonstrated the presence of SLE susceptibility factors in HLA-DRB1 and DQB1 alleles. Due to high degree of polymorphism within the genes of HLA, the distribution of HLA antigen is different according to race. Due to this variation, degree of association between SLE and specific genes of the major histocompatibility complex (MHC) also varies from one population group to another. This is the first description of immunogenetics of SLE in Bangladesh.

A total 92 study population (46 cases & 46 controls) were enrolled in this study. Mean age of cases at study entry was 27.05 ± 8.17 years which is almost similar to the findings of other studies. Study by Sirikong *et al.*, Hussain *et al.*, Fouad *et al.* and Castano-Rodriguez *et al.* average age was stated as 30.1 ± 10.5, 30.35 ± 1.687, 28 years and 34.7 ± 12.9 years in their studies, respectively.[5,15-17] These findings indicate that SLE develops predominantly at 3rd and 4th decade of life. In this study, majority of the cases were female that was in agreement with other studies. Study among Pakistani population, Taiwan population, Northern Italian population, black South Africans and Malay people reported almost similar findings.[16,18-21] All these studies indicate that SLE is more prevalent among female. Higher prevalence of SLE in female could be due to direct effects of sex chromosomes or indirect effects of chromosomes such as those mediated by sex hormones.[22] A recent study showed a 14 times higher incidence of SLE among Klinefelter's, defined by 47, XXY karyotypes, than normal men. This risk (1:900) is closer to the female risk of SLE (1: 1400 in European ancestry).[23] The recent identification of IRAK1 gene supports direct effects of chromosome X, possibly through gene dosage effects. In addition to IRAK1, a risk haplotype in the methyl-CpG-binding protein 2 gene (MECP2) has been associated with SLE and suggests a potential role for DNA methylation in the pathogenesis of SLE. As with IRAK1, mapping of MECP2 on chromosome X raises the possibility of a gene dosage effect that may contribute to increased prevalence of SLE among women.[22] Abnormal aestrogen metabolism has also been demonstrated in patients with SLE of both sexes. The concentrations of androgens correlate inversely with disease activity. Excessive aestrogen causes prolonged survival of autoimmune cells and increase Th2 cytokine production which in turn stimulates B cells to produce autoantibodies.[12]

In this study, the most frequent HLA-DR observed in cases was HLA-DR2. A positive association of DR2 with SLE was observed when compared with controls. Similarly among Thais, Japanese, Malay, South Africans, Taiwanese and Kuwaiti populations with SLE, the frequency of expression of HLA-DR2 was more. In these studies, positive association of HLA-DRB1*1502 (DR2) and DQB1*0501 among Thai population; HLA-DRB1*1501 (DR2), DRB5*0101 and DQB1*0602 among Japanese; HLA-DR2, DQB1*0501 and DQB1*0601 in Malay people; HLA-DR2 in black South Africans; HLA-DR2 among Kuwaiti population and Baltimore people; and HLA-DR2 among Chinese population have been reported.[15,17,20,21, 24-28] Haplotype analysis showed that haplotype containing DRB1*1501 and DRB1*0801 showed risk allele for SLE susceptibility and similar association was also showed in another study.[7, 29] Haplotype study in Saudis also showed association of HLA-DRB1*15 and DQB1*06 with SLE.[30] Several other studies also showed association of HLA-DR2 with SLE in other populations such as study in California and Latin American populations HLA-DR2 and DR3 showed the strongest association for susceptibility to SLE.[5, 31]

But there are several studies that did not have similar findings with present study. Study among Pakistani population, western Indian, Egypt people showed different types of association other than HLA-DR2.[16,32,33] Other studies among Norwegians, Spanish, Oklahoma, Toronto, UK, Southern Sweden and Netherland patients with SLE also showed positive association of DR3 with SLE.[11,34-39] Study in Canada reported that among SLE patients with French origin HLA-DQ6 was positively associated and among non French Canadian SLE cases HLA-B8, DR3, Dw24 and DQ2 were associated.[40] In all these studies, predominant HLA-DR among SLE patients was DR3 where as in the present study association of HLA-DR3 with SLE was not significant. A large series study among European SLE patients both HLA-DR2 and DR3 were found to be positively associated with SLE.[41]

A wide variation of negative association of HLA-DR with SLE was reported in many studies. A study among Toronto people reported a negative association of HLA-DR1, DR6 and DR7 with SLE.[37] Negative association of HLA-DR7 with SLE in UK and HLA-DRB1*04, DRB1*07, DRB1*08 and DRB1*15 in Pakistani patients have also been reported.[16, 38] Some other studies also reported that HLA-DR9 and DR13 (split antigen of DR6) alleles may be protective alleles for Portuguese, Gypsies and Japanese people against SLE.[24, 42, 43] A large series study of HLA class II alleles among European SLE patients showed negative association of some HLA class II antigens with SLE.[41] Similarly negative association of HLA-DR5 with SLE among Latin American people has also been found.[5] But in this study no HLA-DR was found to be negatively associated with SLE. Although, frequency of HLA-DR1, DR3, DR4, DR6, DR8, DR9 and DR10 decreased in cases compared to controls and their RR is less than 1 but at 95% CI their association was not significant (table II). In future, if another study will do with large sample size these alleles may turn into negatively associated with SLE.

Some studies did not find any HLA-DR association with SLE. Association of HLA-DR with SLE among Jamaican, Iceland, Northern Italian, American Blacks, Chinese and Bulgarians SLE patients could not.[19, 44 - 48] These variations in the expression of HLA-DR among SLE patients reported in different studies and present study may be due to differences in ethnic, environmental exposure among study populations and confounding factors in the extended MHC (xMHC) due to strong linkage disequilibrium.[49] Although few studies showed an independent association of HLA class I with SLE, but the association was not consistent with the findings of other studies. Moreover, increase frequency of HLA-DR2 (58.70%) among control population compared to other DR in this study also indicate that these people may be at risk of development of SLE in future life if they exposed to appropriate environmental stimuli.

So, from the findings of above studies it has been revealed that some HLA alleles are protective and some are associated with increased risk for development of SLE. Several studies have been carried out to understand the mechanisms underlying MHC association with SLE, but yet not clearly understood. But it has been hypothesized that physiochemical differences in the nature of critical amino acid side chains that shape the peptide-binding groove in the DRβ chain might be related to the risk or protection conferred by HLA-DRB1 alleles associated with SLE.[5]

In this study, it was already reported that the cases were divided into 2 groups according to their age of onset of disease (figure 1). When frequency of HLA-DR was compared among 2 groups, both groups have almost same frequency of HLA-DR irrespective of their age of onset of disease. Although frequency of HLA-DR2 was increased in second group but it was not statistically significant. Similarly, study in Malay people any HLA-DR association with age of onset of disease could not confirmed.[21] But another study reported increased frequency of DR3 bearing haplotype in patients with disease onset before 30 years of age compared to those above 30 years of age.[50] This could be explained by the difference in race/ ethnic of study populations and small sample size. To get a better picture regarding association of HLA with age of onset of disease large sample size from multicenter analysis is needed.

Thus, the ethnic difference among study populations, sample size of different study and heterogenecity of the HLA could explain the contradictory results found in different studies mentioned above.

Conclusion

Results of this study further confirmed that HLA-DR particularly DR2 has role in development of SLE, may be by influencing production of specific autoantibodies. But it also has been observed that onset of SLE is more likely the consequence of cooperation of many other genes including HLA-DR, interacting with appropriate external stimuli. Because of small sample size, the study population in this study may not represent the entire SLE cases in Bangladesh. This study only included certain serological types of HLA. As

HLA-DR is in linkage disequilibrium with other HLA, study of other loci of HLA at allele level, also required for proper identification of HLA association with SLE.

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