International Journal of Biotechnology and Biomedical Sciences
p-ISSN 2454-4582, e-ISSN 2454-7808, Volume 3, Issue 2; July-December, 2017 pp. 88-91
© Krishi Sanskriti Publications
http://www.krishisanskriti.org/Publication.html

Association of Human Leucocyte Antigen (HLA) Class II with Systemic Lupus Erythematosis (SLE) Patients from Western India

Leenam Dedhia[1,3], Vandana Pradhan[1], Kanjaksha Ghosh[1], Milind Nadkar[2] and Sunil Parekh[3]

[1]National Institute of Immunoheamotology, 13th floor, New Multistoreyed Building, KEM Hospital Campus, Parel, Mumbai, Maharashtra 400012, India
[2]King Edward Memorial Hospital and Seth G.S. Medical College, Acharya Donde Marg, Parel, Mumbai, Maharashtra 400012, India
[3]Marrow Donor Registry India (MDRI), Mumbai, India, S.L. Raheja/Fortis Hospital, Old Wing, 2nd Floor, Raheja Rugnalaya Marg, Mahim (W), Mumbai, Maharashtra 400016, India
E-mail: leenammota@gmail.com

Abstract

Background:
Systemic Lupus Erythematosus (SLE) is a multisystem generalized chronic autoimmune disorder characterized by humoral autoimmunity. The etiology of SLE is thought to be multifactorial involving an interplay of environmental, humoral, and genetic factors. There is a strong association of the human leukocyte antigen (HLA) with SLE, however, the association is likely to be heterogeneous among different ethnic groups. The aim of this study was to determine the association of HLA-DRB1, HLA-DQA1 and HLA-DQB1 with SLE susceptibility and clinical manifestations in the western Indian population.

Methods:
A total of 250 SLE patients fulfilling the ACR criteria were recruited and an equal number of age sex and ethnicallymatched normal healthy controls were recruited for this study.
HLA types were determined by the polymerase chain reaction on a Luminex platform with sequence-specific oligonucleotide primers (PCR-SSOP) method in 250 patients and 250 control subjects.

Results:
*The following HLA alleles were found to be positively associated with SLE: HLA-DRB1*04 (P=.00380), HLA DRB1*11 (P=0.0001), HLA-DQB1*03 (P=.0008), HLA DQB1*05(P=0.040)and DQA1*01(P=0.0018)*

Conclusions:
*Our data suggest an association between HLA class II with susceptibility to SLE in the Indian population. DQB1*05 and DQA1*01 are strongly positively associated with SLE in western Indian SLE patients. DRB1*04, DRB1*11, DQB1*03 and DQA1*03 are negatively associated with SLE in western India*

Keywords*: Systemic Lupus Erythematosis (SLE), Human Leukocyte Antigen (HLA), clinical manifestations, arthritis, lupus nephritis, HLA class II, HLA DRB1, HLA DQB1, HLA DQA1*

1. INTRODUCTION

Systemic Lupus Erythematosis (SLE) is a multifactorial autoimmune disease mostly influenced by genetic and environmental factors. Although the exact etiology of the disease is not known, it is highly hypothesized that the accelerated rate ofapoptosis and/or impaired clearance of apoptotic bodies are the most likely causes of SLE development. SLE comprises of an inflammatory response that occurs due to the immune system attacking self-cells and tissues leading to damage. In most cases of SLE there is damage to the renal system, skin and musculoskeletal system, cardiovascular system, nervous system and respiratory system; in fact SLE can affect any tissue in the body and shows remissions and exacerbations.[1]

The first genetic factor to be identified as important in the pathogenesis of SLE was the Major Histocompatibility Complex (MHC). The human MHC is also called the Human Leukocyte Antigen (HLA) complex (often just the HLA). Defects in certain HLA genes lead to autoimmune disorders due to failure of self-tolerance eg. Systemic Lupus Erythematosus (SLE) where the recognition of a self-antigen fails and the immune system reacts to it causing damage to various organs. It is now widely accepted that HLA genes constitute a part of the genetic susceptibility to SLE. Human Leukocyte Antigen (HLA) class II and SLE has been shown to be associated since 1971 by Grumet et al. 1971[2.] Our previous study showed positive linkage with HLA-DRB1*03 and DQB1*0302 in a subset of severely affected patient from India[3]. However number of patient studied was small and robust molecular typing of HLA antigen was not available in this laboratory at that time.Hence the current study was

undertaken with more robust HLA detection technique involving large number of unselected SLE patients.

2. METHODS

This prospective study was conducted in 250 consecutive SLE patients from Mumbai, western India, collected over a span of three years (June 2014- June 2017). These patients were referred from Department of Medicine, KEM hospital, Mumbai, Maharashtra, India, to National Institute of Immunohaematology, (NIIH), Mumbai. All these patients were diagnosed according to the American College of Rheumatology (ACR) criteria[4]. Disease activity was assessed at the time of evaluation using Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)[5]. Pregnant and post-menopausal women, smokers, patients with diabetes and patients with significant hyperlipidemia were excluded. The source of normal controls (n=250) were voluntarystem cell donors from Marrow Donor Registry India (MDRI). Blood samples (5 ml) were collected after obtaining written informed consent from all the individuals. The study protocol was approved by the Institutional Ethical Committee (IEC) of KEM hospital and NIIH. The patients were categorized into three groups based on presence of clinical manifestations of arthritis and Lupus nephritis for more than 3 years. First group was of 157 patients who had arthritis based on the clinicians diagnosis, second group of 97 patients had renal damage assessed by renal biopsy and were classified as lupus nephritis based on WHO criteria[6] and the third group had all the SLE patients diagnosed on the basis of ACR criteria.

Molecular analysis: DNA extraction was done by standard salting out technique (Bag Healthcare, Lich, Germany) followed by HLA typing using the Luminex Xmap technology (Gen-probe, CT, USA). Ambiguities were resolved using the Sequence Specific primer (SSP) technique (Bag Healthcare, Lich, Germany).

Statistical analysis: Continuous variables were expressed as mean+SD. Statistical analysis was carried out using fisher exact test in 2x2 contingency table using Graph Pad In Stat 2 software (Graph Pad Software Inc., USA). P values below 0.05 were considered statistically significant.

3. RESULTS

A total of 230 female patients and 20 male patients were included in the study. Their age ranged from 5-53 yr (mean ± SD; 28.14 ± 9.99) in females and 7-45 yr (mean ± SD; 25.75 ± 13.76) in males with about 78% patients in the age group of 11-30 years.(see figure 1).

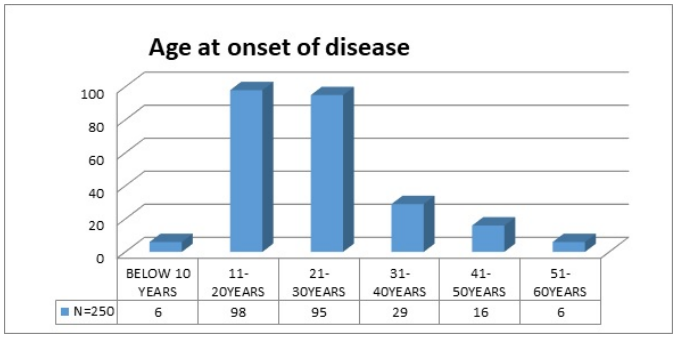

Figure 1: Age at onset of disease in 250 SLE patients.

Clinical manifestation included arthritis (63.6%), alopecia (51%), mucosal ulcers (49.8%), hematological manifestations (44.5%), Lupus Nephritis (38.5%) etc as shown in figure 2.

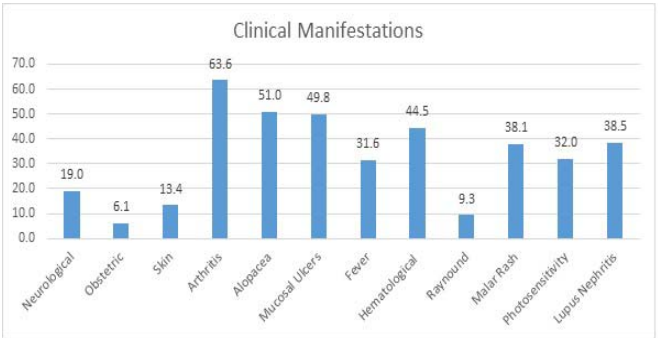

Figure 2: Clinical manifestations in SLE patients

We included 157 SLE patients who showed the clinical symptoms of arthritis, 97 patients with LN and 250 SLE patients as a whole group. The allele prevalence reported in percentage was compared to 250 healthy normal individuals.

Table 1: Prevalence of DRB1 genes (% prevalence) compared to SLE patients and SLE patients with arthritis and Lupus nephritis manifestations

DRB1*	Normal Controls N=250	SLE With Arthritis N=157	SLE With Ln N=97	SLE N=250
DRB1*01	1	2	2	2
DRB1*03	6	6	6	7
DRB1*04	9	4(P=0.0129) ↓	4(P=0.0436) ↓	4(P=0.0380) ↓
DRB1*07	17	12	16	15
DRB1*08	0	2	0	0
DRB1*10	5	6	7	7
DRB1*11	10	6	3(0.0006) ↓	4(P=0.001) ↓
DRB1*12	2	2	2	1
DRB1*13	7	9	8	8
DRB1*14	10	12	12	13
DRB1*15	34	40	37	37
DRB1*16	0	2	2	1

We recorded a statistically significant decrease in the DRB1*04 allele in all three groups and decrease in the

DRB1*11 alleles in the SLE with LN and SLE groups but not in the SLE with arthritis group as compared to the normal (refer table 1).

Table 2: Prevalence of DQB1 genes (% prevalence) compared to SLE patients and SLE patients with arthritis and Lupus nephritis manifestations.

	Normal Controls N=250	SLE With Arthritis N=157	SLE With Ln N=97	SLE N=250
DQB1*01	1	0	0	0
DQB1*02	17	18	17	17
DQB1*03	26	17(P=0.0349) ↓	14(P=0.0006) ↓	14(P=0.0008) ↓
DQB1*04	1	1	1	1
DQB1*05	20	25	28(P=0.0382) ↑	28(0.040) ↑
DQB1*06	35	39	40	40

There was a significant decrease in the prevalence of DQB1*03 alleles in all three SLE groups as compared to the normal. We recorded significant increase in the DQB1*05 in the SLE with LN and SLE groups but not in the SLE with arthritis group as compared to normal.(refer Table 2)

Table 3: Prevalence of DQA1 genes (% prevalence) compared to SLE patients and SLE patients with arthritis and Lupus nephritis manifestations.

	Normal Controls N=250	SLE With Arthritis N=157	SLE With Ln N=97	SLE N=250
DQA1*01	54	65	67(P=0.0235)↑	68(P=0.0018)↑
DQA1*02	16	13	14	13
DQA1*03	14	5(p=0.0162) ↓	5 (P=0.0033) ↓	4 (P=0.0016) ↓
DQA1*04	1	0	0	0
DQA1*05	14	15	13	13
DQA1*06	2	2	2	2

DQA1*01 was increased in all the three groups but the increase was not significant in the SLE with arthritis group. DQA1*03 was significantly decreased in the three groups.

DISCUSSION

The cause of SLE is widely unknown but several studies have presumed that it affects persons with a genetic disposition or susceptibility affected by unknown environmental triggers with defects in their immune system. The main theory that is thought to explain SLE by Lisnevskaia et al, 2014[7] is defects in apoptotic destruction of cells.

SLE has a familial association and multiple genes such as class I, class II, and class III HLA genes appear to be responsible for a person developing lupus, this according to Martens et al, 2009[8]The HLA-DRB1*03 allele has been associated with SLE in Caucasian populations while the HLA-DRB1*02 allele and the HLA-DRB1*15 and HLA-DRB1*16 subtypes have been associated with SLE in black and Asian ethnic groups [9-10]Although our study did not reproduce these results owing to the different genetic and ethnic backgrounds. The prevalence of DRB1*15 is very common allele in Indian population[11-12]and this could also be a contributing factor for high prevalence of autoimmunity in Indian population as compared to the western world.HLA DRB1*04 alleles are associated with early Rheumatoid Arthritis, due to an elevated inflammatory activity and an increase of joint affections. [13] To the best of our knowledge we report the first scenario where DRB1*04 and DRB1*11 prove be protective against SLE.

Our previous study with much less number of samples and using a much restricted technology did show positive association of the disease with HLA-DRB1*03 and DQB1*0302 [3] which with unselected consecutive patients with SLE could not be sustained .

We also report an individual association of increased frequency of DQB1*05 and DQA1*01 alleles in SLE patients and SLE patients with LN but not with SLE patients having arthritis.

Significantly decreased frequency of DQB1*03 and DQA1*03 genes are seen in all three groups showing negative associations.

Conclusion: DQB1*05 and DQA1*01 are strongly positively associated with SLE in western Indian SLE patients. DRB1*04, DRB1*11, DQB1*03 and DQA1*03 are negatively associated with SLE in western India. Finally, the association supports the importance of ethnic background and indicates that the relative importance of different genes may vary indifferent ethnic populations around the world.

Conflict of interest statement: authors have no conflict of Interest.

4. ACKNOWLEDGMENTS

We would like to thank MDRI for the collaboration and giving access to the HLA typing instrument and kits for this study.

REFERENCES

[1] Rahman A. and Isenberg A "Review Article: Systemic Lupus Erythematosus" N Engl J Med 2008; 358 (9): 929-939

[2] Grumet, A. Coukell, A.J. Bodmer, W.F. Bodmer, H.O. McDevitt Histocompatibility (HL-A) antigens associated with systemic lupus erythematosus, a possible genetic predisposition to disease N Engl J Med, 285 (1971), pp. 193-196

[3] 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.Ann Rheum Dis. 2003 Jan;62(1):92-3.

[4] Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997;40:1725.

[5] Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of SLEDAI: a disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthritis Rheum. 1992;35:630–40.

[6] Weening JJ, D'Agati VD, Schwartz MM, Seshan SV, Alpers CE, Appel GB, Balow JE, Bruijn JA, Cook T, Ferrario F, Fogo AB, Ginzler EM, Hebert L, Hill G, Hill P, Jennette JC, Kong NC, Lesavre P, Lockshin M, Looi LM, Makino H, Moura LA, Nagata M: The classification of glomerulonephritis in systemic lupus erythematosus revisited. J Am Soc Nephrol 2004; 15: 241–250.

[7] Lisnevskaia L, Murphy G, Isenberg D "Seminar: Systemic lupus erythematosus". The Lancet.2008; 384 (9957): 1878-1888

[8] Martens HA, Nolte IM, van der Steege G "An extensive screen of the HLA region reveals an independent association of HLA class I and class II with susceptibility for systemic lupus erythematosus". Scand. J. Rheumatol. 2009 Mar. 38 (4): 1-7.

[9] Arnett FC. Genetic aspects of human lupus. Clin Immunol Immunopathol 1992; 63: 4-6

[10] Doherty DG, Ireland R, Demaine AG, Wang F, Veerapan K, Welsh KI, et al. Major histocompatibility complex genes and susceptibility to systemic lupus erythematosus in southern Chinese. Arthritis Rheum 1992; 35: 641-646.

[11] Dedhia L, Gadekar S, Mehta P, Parekh S. HLA haplotype diversity in the South Indian population and its relevance. Indian Journal of Transplantation Vol 9, Iss 4, Pgs 127-170.

[12] Dedhia L, Vijapukar-Valluri M, Gadekar S, Mehta P, Parekh S, Ghosh K. Distribution of HLA genes and disease predisposition in Bengali speaking people from India. Indian Journal of Transplantation, Volume 9, Issue 1, January–March 2015, Pages 2-6

[13] Seidl C, Koch U, Buhleier T, Frank R, Möller B, Markert E, Koller-Wagner G, Seifried E, Kaltwasser JP.HLA-DRB1*04 subtypes are associated with increased inflammatory activity in early rheumatoid arthritis.Br J Rheumatol. 1997;36(9):941-4.