Original Research

Assessment of Human Leukocyte Antigen (HLA)-DRB1 Alleles Associated with Susceptibility to Rheumatoid Arthritis: A Study on North-Eastern Indian Population

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ABSTRACT

Introduction: Rheumatoid arthritis (RA) is known as a disease of obscure pathophysiology. Immunogenetic factors have always been found to be linked with the susceptibility and severity of Rheumatoid Arthritis (RA). Although polygenic interactions are reported in RA cases, however, strong associations of HLA class II genes with RA have been observed worldwide. Variations in disease progression, pathogenesis and associations with HLA class II alleles have been seen in different ethnic groups. Hence this study was taken up to analyze the frequency of DRB1 alleles in RA patients from the population of North Eastern India attending Gauhati Medical College & Hospital (GMCH).

Aim: To investigate the frequency of DRB1 alleles in RA patients attending GMCH.

Materials and Methods: The study was a case-control study where the case group consists of RA patients attending GMCH and the control group consists of healthy disease-free individuals. Blood samples from 150 cases (following ACR/ EULAR criteria- 2010) and 150 controls were collected in EDTA vials after taking consent from the subjects using standard venipuncture procedure. DNA was extracted from each of the collected blood samples and DRB genotyping was done by the molecular method using SSP kits. Alleles were analyzed using kit specific Hit charts and appropriate software.

Results: HLA allele status was evaluated in RA patients as well as the control group from the same geographical region. The frequency of HLA DRB1*10 was observed higher in the patient group compared to controls. (OR= 5.968, CI= 3.607 to 9.874, P<0.01). The frequency of DRB5 was also found higher in the patient group, suggesting linkage with DRB1*10 and association with RA. The frequencies of DRB1*12 (OR= 0.126, CI= 0.054 to 0.291, P<0.01), DRB1*14 (OR= 0.138, CI= 0.052 to 0.366, P<0.01), DRB1*15 (OR= 0.615, CI= 0.389 to 0.972, P<0.05) along with DRB3 (OR= 0.357, CI= 0.22 to 0.577, P<0.01) were seen higher in controls compared to cases, suggesting a protective effect against RA in the study group. There was no statistical significance in the frequencies of HLA-DRB1 alleles viz. DRB1*01, DRB1*03, DRB1*04, DRB1*07, DRB1*08, DRB1*09 and DRB1*11 as observed in the study.

Conclusion: The study suggests that HLA DRB1*10 allele is associated with the susceptibility to develop RA whereas DRB1*12, DRB1*14, DRB1*15 are having a protective effect on RA in the study population.

Key Words: Rheumatoid arthritis, Pathogenesis, Genotyping, Susceptible, Protection

INTRODUCTION

Rheumatoid arthritis (RA) is a composite, chronic disease-causing inflammation of joints and surrounding tissues. It has a heterogeneous nature, where both genetic and environmental factors have crucial roles in pathogenesis.[1] The prevalence of RA is 1% globally. Several risk factors such as ethnicity, gender, smoking, under-nutrition and host immunogenic factors are found to be associated with susceptibility to RA.[2]

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A genetic contribution to the development of RA is estimated to attribute to about 30% - 50% of the disease risk. Based on the facts on risk rates it has been documented that there is a genetic association of the disease among close relatives such as siblings, offspring and parents. The risk rate is found to be two to three per cent among 1st degree relatives.[3]

The strongest association has been reported with human leucocyte antigen (HLA) alleles, in particular with HLA-DRB1 alleles, Human leukocyte antigen (HLA) molecules play a key role in the immunogenicity by presenting endogenous and exogenous peptides to cd8+ and cd4+ T cells. A specific sequence, present within the peptide-binding cleft of HLA class II molecules (HLA-DR,-DP and –DQ), has been implicated in genetic susceptibility to RA.

Among the class II HLA genes, the HLA- DRB1 alleles encode a "shared epitope" a five amino acid sequence motif in residues 70-74 of the HLA-DRβ (beta) chain are found to be connected with the severity of RA**.**[4] In some populations the DRB1*01 and DRB1*04 alleles have been reported to have a strong association with RA due to the presence of shared epitope but studies from the northeastern part of India regarding this association have not yet been documented. Ethnic differences both in terms of genetic association and prevalence of RA have been observed worldwide, for instance, about 5 to 6% of people from North American population groups get affected by RA, on the other hand, the rate of effectiveness is quite low in Caribbean people of Africa.[5] The reason behind such inconsistencies may lie in both the genetic as well as on environmental aspects associated with the ethnic groups. Because of these inconsistencies in different geographical areas among different populations, it is necessary to conduct more studies in distinct geographical stretches to examine the factors contributing to disease occurrence and progression.[5,6]

From Northeastern India, only a handful of studies on the association of genetic factors related to RA have been conducted. A recent study states that TNF-α –308 variant *GA* genotype is higher in RA cases (46.03%) than in controls (25%). The presence of TNF-α –308 variant *A* allele is associated with an increased risk of RA susceptibility.[6] Another study was done on the association of HLA-DRB1 snp genotypes with Rheumatoid arthritis in the northeast Indian population. It has been reported that HLA-DRB1 rs 660895 heterozygote AG genotype is associated with a reduced risk of RA compared to controls. Also, a significantly higher distribution of HLA-DRB1 rs 13192471 was observed in RA cases.[7, 8, 9]

Not a single study analyzing the frequency of DRB1 alleles associated with RA in this region has been reported so far, therefore the present study aims at accessing the HLA DRB1 alleles associated with susceptibility to RA among the population of North-eastern India.

MATERIALS AND METHODS

The study had been carried out in Gauhati Medical College & Hospital (GMCH) which is an advanced tertiary care government hospital situated in Guwahati, Assam. This hospital caters for a large number of patients from every nook and corner of the state as well as from almost all other states of North-eastern India. Ethical clearance vide letter no. **MC/108/2012/9** had been obtained from the Institutional Ethical Committee (IEC) of GMCH before conducting the research.

Population Samples

A total of 150 cases, aged between 18 to 65 years, were enrolled. Enrollment was based on the fulfilment of standard American College of Rheumatology (ACR) / European League against Rheumatism (EULAR) 2010 criteria. All the parameters included in the criteria like morning stiffness, RF factor, number of short and long joints involved, ESR, ACCP were checked and documented after thorough clinical examination by a registered medical practitioner.

150 Healthy Controls of same age group free from autoimmune symptoms with normal ESR, CRP levels and no family history of RA were enrolled in the study.

All the RA patients and controls enrolled in the study were from the North-Eastern part of India. Prior Informed consent was taken from all subjects at the time of participation in the study.

DNA extraction and PCR of the HLA-DRB1

Genomic DNA was extracted from peripheral blood leucocytes by salting out a technique using ammonium acetate salts and stored at -20°C for further use in PCR. The purity and concentration of the extracted DNA samples were checked in a spectrophotometer (MultiscanGo) and all DNA samples had been found to have a purity ratio (260/280) between 1.8 to 1.9 and concentration between 300 to 500 ng per microliter. The HLA DRB1 alleles were evaluated in patients and controls by using sequence-specific priming techniques of PCR (SSP-PCR). Inno-Train genotyping kits were used for this purpose.

The PCR program was an initial denaturation at 96°C for 2 minutes, followed by denaturation of 10 cycles at 96°C for 15 seconds and annealing at 65°C for 1 minute, after that another 20 cycles of denaturation at 96°C for 15 seconds, then annealing at 61°C for 50 seconds and a final extension at 72°C for 30 seconds in a Gradient Thermal Cycler. PCR product evaluation was performed by agarose gel electrophoresis. The gel was prepared of 2% Agarose in 0.5x Tris-

acetate EDTA (TAE) buffer. Ethidium bromide (EtBr) was added in the gel which acts as an intercalating agent. The sample loaded agarose gel was run in TAE buffer for 20 minutes in an electrophoretic assembly at 200 V. Documentation of the gel after electrophoresis was done in a Gel Documentation unit (GEL-Doc, Make- Biorad). Hit charts and Helmberg SCORE software were used to assess the HLA DRB1 Alleles.

Statistical Methods

Statistical analysis and results validation were performed by IBM SPSS ver. 25 software using Pearson's Chi-square test formula. The significance was described in terms of the p-value. Results having a p-value < 0.05 was considered statistically significant.

RESULTS

From the demographic profile of the patients as demonstrated in **Table 1** it was observed that the majority of the patients enrolled in the study were women.

Table 1: Gender and age distribution in patients with Rheumatoid Arthritis

	Female	Male	Total
Number (%)	92 (61.33)	58 (38.66)	150 (100)
Age (Mean + SD, years)	42.83 (10.994)	44.29 (10.994)	43.39 (12.059)
Disease progression in months	No. (%)	No. (%)	No. (%)
Less than 6 months	25 (27.2)	18 (31.0)	43 (28.7)
6 to 12 months	13 (14.1)	11 (19.0)	24 (16)
12 to 24 months	29 (31.5)	10 (17.2)	39 (26)
24 to 48 months	16 (17.4)	10 (17.2)	26 (17.3)
More than 48 months	9 (9.8)	9 (15.5)	18 (12)

The allelic distribution of HLA DRB gene among the patients and controls was evaluated and displayed in **Table 2** and **Figure 1**.

Table 2: The frequency of HLA-DRB in Rheumatoid arthritis patients (n = 150) and controls

HLA-DRB alleles	Patients n=150(%)	Controls N=150(%)	OR	95% CI	P value
DRB1*01	32(21.3)	35(23.3)	0.891	0.517 to 1.535	0.782
DRB1*03	17(11.3)	21(14)	0.785	0.396 to 1.556	0.603
DRB1*04	16(10.6)	15(10)	1.075	0.511 to 2.261	1.0
DRB1*07	15(10)	17(11.3)	0.869	0.417 to 1.812	0.852
DRB1*08	14(9.3)	12(8)	1.184	0.528 to 2.652	0.837
DRB1*09	8(5.3)	11(7.3)	0.712	0.278 to 1.823	0.635
DRB1*10	98(65.3)	36(24)	5.968	3.607 to 9.874	< 0.0001*
DRB1*11	15(10)	10(6.6)	1.556	0.675 to 3.583	0.403
DRB1*12	7(4.6)	42(28)	0.126	0.054 to 0.291	< 0.0001*
DRB1*13	5(3.3)	6(4)	0.828	0.247 to 2.773	1.0
DRB1*14	5(3.3)	30(20)	0.138	0.052 to 0.366	0.0001*
DRB1*15	60(40)	78(46.6)	1.615	0.389 to 0.972	0.049*
DRB1*16	4(2.6)	10(6.6)	0.384	0.118 to 1.251	0.171
DRB3	41(27.3)	77(51.3)	0.357	0.22 to 0.577	< 0.0001*
DRB4	36(24)	39(26)	0.899	0.533 to 1.516	0.790
DRB5	117(78)	95(63.3)	2.053	1.233 to 3.416	0.008*
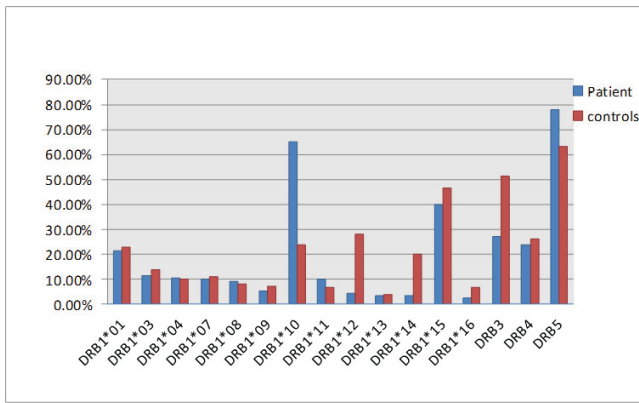
Figure 1: Distribution of HLA DRB alleles.

From the univariate analysis, it had been noticed that the frequency of DRB1*10 was higher in the patient group (65.3%) compared to the control group (24%) (*OR= 5.968, CI= 3.607 to 9.874, P<0.01*). Another substantial observation was a higher frequency of DRB5 in the patients (78%) in contrast to the controls (63.3%) which was likewise statistically significant (*OR=2.053, CI= 1.23 to 3.41, P<0.05*). The higher distributions of HLA DRB1*10 and HLA DRB5 alleles in the patients of RA indicate a linkage association among the two alleles in addition to its association with susceptibility to RA. On the other hand, frequencies of DRB1*12, DRB1*14, DRB1*15 and DRB3 were found higher in the control group (28%, 20%, 46.6% and 51.3%) compared to the patient group (4.6%, 3.3%, 40% and 27.7%). These disparities in the frequencies were also statistically significant, which suggest a possible protective effect is conferred by DRB*12, DRB1*14, DRB1*15 as well as DRB3 against RA to the healthy controls of the study. Differences in frequencies of DRB1*01, DRB1*03, DRB1*04, DRB1*07, DRB1*08, DRB1*09 and DRB1*11 between the patient and control group were not statistically significant.

DISCUSSION

RA is a complex form of polyarthritis of uncertain aetiology. HLA-DR is considered as the key molecule involved in rendering the risk to RA.[9] Genetic studies conducted worldwide have made it possible to find out the association of Major histocompatibility complex and RA by mapping the genetic regions of the HLA locus which is located in chromosome 6.[10]

This current study was taken up to apprehend the involvement of HLA DRB1 alleles in RA. The study was a case-control study where a comparison had been made between RA patients of Northeastern part of India attending Gauhati Medical College & Hospital and Healthy controls of same age group and from the same geographical location based on the pattern of distributions of HLA-DRB1 alleles. Tribal ethnicity-based analysis was not taken into account because the subjects enrolled were a mixture of different ethnic groups. Purposive sampling was implemented to enrol the cases based on inclusion criteria.[11]

After examining the cases, it had been found that the frequency of occurrence of RA is higher in women (61.33%) compared to men (38.66), the gender predominancy in terms of disease incidence has been reported from other parts of the world in various populations.[11] The higher prevalence in Women is apparently due to the implication of X-linked chromosome and hormonal factors that interplay with several autoimmune diseases.[12, 13]

Upon investigating the distribution pattern of the HLA-DRB1 alleles in cases and controls, it was found that the frequencies of HLA-DRB1*10 and HLA-DRB5 were higher in patients which indicate a strong association of HLA-DRB1*10 with the susceptibility to RA in the patients of the northeastern part of India. Other significant findings were the higher prevalence of DRB1*12 (p < 0.0001), DRB1*14 (p = 0.0001), DRB1*15 (0.049) and DRB3 (p < 0.0001) in the control group of the study suggesting a protective role of these alleles against RA in the people of this region. Studies from different parts of the globe have shown variations in the association of HLA-DRB1 alleles with RA. Along with DRB1*10, DRB1*01 and 04 are found to be associated with RA patients from Italy.[12] Another study from Japan has shown the association of HLA DRB1*04 with both younger age onset RA (YORA) as well as Elder age-onset RA (EORA) susceptibility. However, there was a difference observed at the sub allelic level in both groups.[13] Data obtained from a meta-analysis revealed that HLA- -DRB1*0404 is associated with RA in Latin Americans.[14] A similar kind of Meta-analysis on Asian Mongoloids has displayed that HLA-DRB1*0101, *0401, *0405, *0410 and *1001 have risk associations with RA, whereas HLA-DRB1* 0301, *0403, *0406, *0701, *1301 and *1405 have a protective effect.[15] Previous studies on the Indian population have shown a somewhat peculiar picture of the HLA DRB1 allelic distribution pattern. Since India is considered as a "melting pot" of different races because of the history of invasions made by various races at certain time intervals, its gene pool has become an admixture of different races like Caucasians, Orientals, and Africans. DRB1*0403 is the most prevalent allele of the DR4 family associated with the susceptibility to RA among the north Indian Population,[16] but data presented in this study had shown the statistically insignificant association of HLA- DRB1*04 with RA in the study group. The diversity in the allelic pattern is may be due to diverse population origin, random genetic drift, and intergeneric recombination. In addition to HLA-DRB1*04, frequencies of DRB1*01, DRB1*03, DRB1*07, DRB1*08, DRB1*09 and DRB1*11 were also statistically insignificant in this study. However, to come to a more reliable conclusion on these alleles, surveys on a larger sample

size are obligatory.

The resemblance in findings of this current study has been observed with population from south-east Iran. HLA-DRB1*10 is associated with the susceptibility to RA in 79 RA patients from Zahedan, south-east Iran. In contrast, HLA-DRB1*03 is reported to confer protection against RA in the same population.[17]

CONCLUSION

From the results of the study, it can be concluded that HLA-DRB1*10 and HLA-DRB5 are directly associated with the susceptibility to RA as these alleles are also noted to have risk association with RA in other populations. The veritable mechanism behind this association is suspected to be due to the encoding of a shared epitope at protein level which in turn is directly related to the development of "Anti citrullinated protein Antibodies" (ACPA). The ACPA is known to be the Hallmark of RA and is believed to play a critical role in the progression of the disease. On contrary to the above, it is also concluded that DRB*12, DRB1*14, DRB1*15 as well as DRB3 have an inhibiting effect on RA. Further, to validate the findings confirmations from various independent cohorts are required.

REFERENCES

1. Klareskog L. Genes, environment and immunity in the development of rheumatoid arthritis. Eur J Clin Investig. May 2007;37:29-30.
2. Song Li, Yangsheng Yu, Yinshi Yue, Zhixin Zhang, Kaihong Su. Microbial Infection and Rheumatoid Arthritis. J Clin Cell Immunol. 2013. Dec;4(6):174.
3. Muazzam AG, Mansoor A, Ali L, Siddiqi S, Hameed A, et al. Association of HLA-DRB1 and -DQB1 alleles and haplotypes with rheumatoid arthritis in a Pakistani population. Arthritis Res Ther. 2013;15:R95.
4. Joseph Holoshitz. The Rheumatoid Arthritis HLA-DRB1 Shared Epitope. Curr Opin Rheumatol. 2010 May;22(3):293-298.
5. Peschken CA, Hitchon C, Robinson DB, Smolik I, Barnabe CR, et al. Rheumatoid Arthritis in a North American Native Population: Longitudinal Followup and Comparison with a White Population. J Rheumatol. 2010 Aug 1;37(8):1589-1595.
6. Das S, Baruah C, Saikia AK, Tiwari D, Bose S. Genetic and expression changes in TNF-α as a risk factor for rheumatoid arthritis pathogenesis in Northeast India. J Genet. 2019 Mar;98:3
7. Das S, Baruah C, Saikia AK, Bose S. Associative role of HLA-DRB1 SNP genotypes as risk factors for susceptibility and severity of rheumatoid arthritis: a North-east Indian population-based study. Int J Immunogenet. 2018 Feb;45(1):1-7.
8. Woude D, Duistermaat J, Toes RE, Huizinga TW, Thomson W, et al. Quantitative heritability of anti-citrullinated protein antibody-positive and anti-citrullinated protein antibody-negative rheumatoid arthritis. Arthritis Rheum 2009;60:916-923.
9. Drongelen V, Holoshitz J. HLA-disease associations in Rheumatoid Arthritis. Rheum Dis Clin North Am. 2017 Aug;43(3):363-376.
10. Matzaraki V, Kumar V, Wijmenga C, Zhernakova A. The MHC locus and genetic susceptibility to autoimmune and infectious diseases. Genome Biol. 2017;18:76.
11. Alamanos Y, Drosos AA. Epidemiology of adult rheumatoid arthritis. Autoimmun Rev. 2005;4(3):130-136.
12. Bongi SM, Porfirio B, Rombola G, Palasciano A, Beneforti E, Bianucci G. Shared-epitope HLA-DRB1 alleles and sex ratio in Italian patients with rheumatoid arthritis. Joint Bone Spine. 2004;71:24-28.
13. Oka S, Furukawa H, Shimada K, Hashimoto A, Komiya A, et al. Association of HLA-DRB1 genotype with younger age onset and elder age-onset rheumatoid arthritis in Japanese populations. J Med. 2019;98:48.
14. Delgado AM, Anaya JM. Meta-analysis of HLA-DRB1 polymorphism in Latin American patients with rheumatoid arthritis. Autoimmun Rev. 2007;6:402-408.
15. Jun KR, Choi SE, Cha CH, Oh HB, Heo YS, et al. Meta-analysis of the association between HLA-DRB1 allele and rheumatoid arthritis susceptibility in Asian populations. J Korean Med Sci. 2007;22:973-980.
16. Jaini R, Kaur G, Mehra NK. Heterogeneity of HLA-DRB1*04 and its associated haplotypes in the North Indian population. Hum Immunol. 2002 Jan;63(1):24-29.
17. Sandoughi M, Fazaeli A, Bardestani G, Hashemi M. Frequency of HLA-DRB1 alleles in rheumatoid arthritis patients in Zahedan, Southeast Iran. Ann Saudi Med 2011;31(2):171-73.