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Role of *HLA-DRB1, PTPN22, CTLA4* Gene Polymorphisms in Susceptibility to Rheumatoid Arthritis

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Abstract
Background *and AIM: The genetic and environmental factors are believed to play an important role in the pathogenesis of rheumatoid arthritis (RA), the precise etiology is still uncertain. Regarding its genetic components, the strongest genetic association with RA is that found for certain alleles of HLA-DRB1. Recent studies have revealed that the single-nucleotide polymorphism (SNP) of non-HLA PTPN22 and CTLA4 gene were associated with RA. The associations of HLA, PTPN22 and CTLA4 polymorphisms with RA risk have been less well replicated. Thus, the current study was undertaken to investigate the effects of HLA-DRB1, PTPN22, CTLA4 gene polymorphisms and autoantibodies in susceptibility to RA.*
Methods*: A total of 200 patients fulfilled the criteria for RA and 200 healthy individuals were included in the study. Anti-cyclic citrullinated peptide (anti-CCP) antibodies and rheumatoid factor (RF) were measured by enzyme-linked immunosorbent assay (ELISA). HLA-DRB1 alleles were determined by polymerase chain reaction-sequence specific primer (PCR-SSP) method. Samples were genotyped for PTPN22 1858C/T (rs2476601) and CTLA4 CT60 (rs3087243) variants using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.*
Results: *In RA patients 88% and 82% were positive for anti-CCP and RF autoantibodies, respectively. A significant increase in the frequency of HLA-DRB1*01, HLA-DRB1*04, HLA-DRB1*10, HLA-DRB1*14 shared epitope (SE) alleles were identified in RA patients, whereas in healthy subjects, they were HLA-DRB1*03, HLA-DRB1*07, HLA-DRB1*11, HLA-DRB1*13. There was no association of the PTPN22 1858T variant with RA and it seems to be independent of MHC associations. The frequency of CTLA4 CT60 A allele carriers was significantly higher in RA patients and also it is preferentially in SE-positive RA patients.*
Conclusion: *Our results confirmed the previously reported HLA-DRB1 SE alleles associations with RA. The CTLA4 polymorphism appears to interfere with HLA-DRB1 susceptibility to RA. On the other hand, we failed to provide evidence for the association of the PTPN22 SNP with RA.*
Keywords: *HLA-DRB1; PTPN22; CTLA4; rheumatoid arthritis; polymorphism.*

INTRODUCTION

Rheumatoid arthritis (RA) is the most common chronic, autoimmune and systemic inflammatory joint disease. It affects ~1% of the world population; however, the prevalence differs between 3 and 8% depending on the genetic and other environmental risk factors. [1] Although the genetic and environmental factors are believed to play an important role in its pathogenesis, the precise etiology is still uncertain. The diagnosis of RA especially in the early course is quite impossible, as the clinical criteria are insufficient at the early stage of the disease. In the past 5–6 years, many studies have focused on the value of the diagnostic potential and clinical application of anti-CCP antibody in RA and other rheumatic diseases. Both anti-cyclic citrullinated peptide (anti-CCP) antibody and rheumatoid factor (RF) are regarded as serological markers of RA and showed high specificity (98%) of anti-CCP in patients with RA. [2] The high specificity of the assay remains unproven in patients with other autoimmune and rheumatic diseases. Regarding its genetic components, the human leukocyte antigen (HLA) is found to be the strongest and best-known genetic risk factor for RA, which accounts for 30–50% of overall genetic susceptibility to RA. [3] Increases in the frequencies of *DRB1* alleles, including *DRB1**01, *DRB1**04, *DRB1**10 and *DRB1**14 are associated with structural severity of RA and have been more recently related with production of anti-CCP antibodies were reported in RA patients of different ethnic groups. [4] These allelic products share a conservative amino acid sequence (QKRAA, QRRAA or RRRAA) at positions 70–74 in the third hypervariable region of the DRB1 chain, which is known as the shared epitope (SE). On the other hand, SE negative genotypes, mainly *DRB1**11 and *13 indicating a possible protective effect against RA susceptibility. [5], [6]

Several studies suggest that polymorphisms in other non-*HLA* genes also influence susceptibility to RA. Among these protein tyrosine phosphatase nonreceptor type 22 (PTPN22) and cytotoxic T lymphocyte antigen-4 (CTLA4) play a major role and known to be associated with increased risk for RA. Both the genes, thought to function as a negative regulator of T-cell activation. [7], [8] The single nucleotide polymorphism (SNP) of the *PTPN22* gene (rs2476601, +1858C→T) and *CTLA4* (CT60, rs3087243) gene has previously been found associated with several autoimmune diseases, for example, RA, diabetes type 1 and Grave's disease, [9], [10], [11] with a stronger association anti-CCP sero-positive RA. [9]

In the light of these findings, the aim of this study was to investigate the effect of the gene polymorphism in *HLA-DRB1* alleles, *PTPN22*, *CTLA4* gene and autoantibodies on the risk of RA in the South Indian population.

PATIENTS AND METHODS

Subjects for this study include 200 RA patients (49 male/151 female) attending the out-patient department at Sri Ramachandra Hospital, between February 2014 and October 2014. The control sample includes 200 healthy subjects matched for age, sex and geographical origin with case subjects (50 male/150 female) (Table 1). All patients were fulfilled the ACR/EULAR 2010 criteria for the classification of RA. [12] The approval for this study was granted by our Institutional Ethics Committee (Ref: IEC-NI/11/FEB/21/06) of Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, and written informed consent was obtained from all participants prior to enrollment in the study. Erythrocyte sedimentation rate (ESR) estimation was measured by using Westergren method, normal range: 0-15 mm/h for men; 0-20 mm/h for female. Disease activity was assessed by using the disease activity score-28 (DAS-28) as: remission: DAS-28 ≤2.6, low disease activity: (DAS-28 2.6 to ≤3.2), moderate disease activity: (DAS-28 3.2 to ≤5.1) and high disease activity: (DAS-28 >5.1). Visual analog scale (VAS) was used for assessment of disease severity. Anti-CCP IgG and RF antibody was detected using a

commercially available *in vitro* quantitative EDRA Genesis CPA and Rheumatoid Factor Screen ELISA kit (Genesis Diagnostics, Cambridgeshire, UK). Serum samples presenting results ≥6.2U/mL and ≥40U/mL were considered to be positive for anti-CCP and RF antibodies, respectively.

Genomic DNA extraction and quantitation

Genomic DNA of patients with RA (*n*=200) and healthy controls (*n*=200) were isolated from 5 ml of peripheral anticoagulated venous blood samples by using the phenol-chloroform-isoamyl alcohol (PCI) method. [13] The quantity of the DNA samples was measured by NanoDrop ND-1000 spectrophotometer. One microlitre of DNA was placed and calculating the absorbance A_{260}/A_{280} and A_{260}/A_{230} ratios is used to assess the purity of DNA. Ratios between 1.8 and 2.0 for A_{260}/A_{280} are accepted as indicating pure DNA. [14] Quantified DNA samples were stored at –20°C until used for genetic analysis.

HLA-DRB1 genotyping

Genotyping of *HLA-DRB1* (*n*=120 RA patients and *n*=120 controls) was performed by polymerase chain reaction with sequence-specific primers (PCR-SSP) using Morgan™ *HLA* SSP DRB Typing Kit (Texas BioGene, Inc., Taiwan) as per manufacturer instruction (Fig. 1).

Detection of the PTPN22 1858C/T polymorphism

The genotyping for *PTPN22* SNP at position 1858C/T in the codon 620 was carried out using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods where standard PCR protocol was followed by restriction digestion of the PCR amplified product with restriction enzyme *Rsa* I (New England Biolabs Inc., USA). Primers used for amplification were forward primer (5'-ACTGATAATGTTGCTTCAA-3') and reverse primer (5'-CACCAGCTTCCTCAACCA-3'), as described by Rani *et al*. [15] Besides the genomic DNA 30ng/µl, each reaction contains of 1× Taq polymerase buffer, 0.1mM of dNTPs, 1.5mM $MgCl_2$, 10pmoles of each primers (Eurofins, Germany), and 0.625U of Taq polymerase in 25µl reaction volume. After initial denaturation at 94°C for 2min, 35 cycles of denaturation at 94°C for 30sec, annealing at 60°C for 30sec and extension at 72°C for 30sec were carried out followed by final extension cycle at 72°C for 7min in gradient thermocycler (Eppendorf, USA). The amplified products were confirmed on 1% agarose gel, 10µl each of the amplified products were digested with 5U of *Rsa*I enzyme (New England Biolabs) at 37°C for 8h. Digested products were electrophoresed on a 2.5% agarose gel in 0.5× TAE buffer along with 100bp ladder. Digested *C* allele yielded fragments of 176 and 42bp and *T* allele yielded 218bp (Fig. 2).

Detection of CTLA4 CT60 polymorphism

The *CTLA4* CT60 polymorphism on located in 3′-untranslated region was genotyped by PCR-RFLP method. CT60 was amplified by PCR from genomic DNA followed by digestion with restriction enzyme *HpyCH4*IV (New England Biolabs Inc., USA). The following primers were used for PCR 5'-GAGGTGAAGAACCTGTGT-GTTAAA-3' (forward) and 5'-ATAATGCTTCATGAGTCAGCTT-3' (reverse) (Eurofins, Germany). [16] Briefly, each 25µl PCR reaction contained 30ng of genomic DNA, 1× PCR buffer, 2.5mM $MgCl_2$, 0.2mM dNTPs, 2.5U Taq DNA polymerase and 10pmoles of each primer (Eurofins, Germany). Reactions were carried out in a gradient thermocycler (Eppendorf, USA) under the following conditions: initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 30sec, annealing at 55.6°C for 30sec, extension at 72°C for 30sec and final extension cycle at 72°C for 5 min. The newly made 178 bp DNA fragment was then digested with *HpyCH4*IV under the following conditions: 15µl of the reaction were incubated with 2U of *HpyCH4*IV (New England Biolabs Inc., USA) at 37°C for 8h along with its corresponding buffer. Digested products were electrophoresed on a 2% agarose gel in 0.5× TAE buffer along with 100bp ladder. Digested *G* allele yielded fragments of 107 and 71bp and *A* allele yielded 178bp (Fig. 3).

Statistical analysis

Data were collected and analyzed by using SPSS, version 20.0 for Windows (SPSS, Chicago, USA). Odds ratio (OR) and 95% confidence interval (95% CI) were calculated to estimate the strengths of the associations. *HLA-DRB1* allelic distribution between patient and control group were made using Chi-square test. Student's *t*-test was used in the statistical analysis. A *P* value *<0.05* was considered significant.

RESULTS

Demographic data and clinical features of 200 RA patients and 200 healthy subjects have been described in Table 1. In RA patients 176 (88%) and 163 (82%) out of 200 were positive for anti-CCP and RF autoantibodies respectively. The *HLA-DRB1* allele frequencies were compared between RA patient group and the control group, In RA patients, the *HLA-DRB1**01, *DRB1**04, *DRB1**10 and *DRB1**14 alleles were significantly more frequently represented in our South Indian RA cohort (OR-3.5; 95% CI-1.63-7.62; *P*=0.0013, OR-5.6; 95% CI-2.5-12.3; *P*<0.0001, OR-3.62; 95% CI-1.92-6.81; *P*=0.0001, OR-6.43; 95% CI-2.64-15.6; *P*<0.0001) whereas, the *HLA-DRB1**03, *07, *11 and *13 alleles were less frequent in RA patients (OR-0.3; 95% CI-0.10-0.82; *P*=0.02, OR-0.28; 95% CI-0.16-0.49; *P*<0.0001, OR-0.24; 95% CI-0.12-0.48; *P*<0.0001, OR-0.35; 95% CI-0.16-0.75; *P*=0.0075). No significant difference was observed in the frequencies of the *HLA-DRB1**08, *09, *12, *15, and *16 alleles (*P*>0.05) between RA patients and controls (Table 2).

According to *PTPN22* gene 1858C/T SNP, allelic distribution for the *T* allele was slightly higher in the RA patients group: 7.5% vs 6.25%. The genotypic frequency of heterozygous CT genotype was found to be increased in RA 10% when compared to controls 8.5%. Nevertheless, the difference did not reach statistical significance (*P* = 0.5312, by Chi square test) (Table 3).

Analysis of *CTLA4* CT60 SNP, the *A* allele is significantly increased in patients with RA as compared to controls (OR-3.07; 95% CI-2.28-4.12; *P*<0.0001). While homozygous genotype GG was significantly reduced (OR-0.038; 95% CI-0.01-0.10; *P*<0.0001), heterozygous *GA* and homozygous *AA* were significantly increased in RA patients compared to controls (for GA: OR-1.66; 95% CI-1.11-2.47; *P*=0.0121, for AA: OR-2.38; 95% CI-1.57-3.61; *P*<0.0001) (Table 4). All genotype frequencies in patients as well as controls were in Hardy Weinberg equilibrium. As expected, the genotypic frequencies of heterozygous *GA* and homozygous *AA* of *CTLA4* gene variant were significantly associated with 2 copies of *HLA-DRB1* SE alleles (for GA: OR-9.64; 95% CI-1.20-77.39; *P*=0.0329, for AA: OR: 74.25; 95% CI: 4.47-1232.3; *P*=0.0027) (Table 4). We found that the presence of *CTLA4* CT60 SNP and *HLA-DRB1* alleles was not independent from each other in South Indian RA patients.

Table 1: Clinical characteristics and demographics of patients

Parameters	RA patients (*n*=200)	Control group (*n*=200)	*P*
Age (Mean ± SD), years	39.2 ± 6.9	32.6 ± 9.4	<0.0001
Female: male ratio	3:1	3:1	
RA disease duration, (Mean ± SD), years	5.6 ± 3.5	-	
Visual analog scale (Mean ± SD)	29.85±19.88		
DAS28, (mean ± SD)	5.8 ± 1.3	-	
Remission n(%)	1 (0.5)	-	
Low disease activity n(%)	2 (1)	-	
Moderate disease activity n(%)	62 (31)	-	
High disease activity n(%)	135 (67.5)	-	
ESR, (mean ± SD) (mm/hr)	56.66 ± 24.85	17.22±6.21	<0.0001
Anti-CCP, (Mean ± SD), U/ml	68.02 ±100.85	2.83±5.58	<0.0001
Rheumatoid factor (RF), (Mean ± SD), U/ml	205.66±234.11	25.98±40.67	<0.0001

n: number of RA patients; DAS-28: disease activity score 28; ESR: erythrocyte sedimentation rate; SD: standard deviation; Anti-CCP: anti-citrullinated peptide antibodies; RF: rheumatoid factor. Data are reported as means ± SD or number (%). Student's *t*-test were used and differences were considered significant at *P<0.05*.

Table 2: *HLA-DRB1* allele frequencies in rheumatoid arthritis patients and controls.

***HLA-DRB1* genotype**	**RA patients (*n*=120) No of alleles 2*n*=240**		**Control group (*n*=120) No of alleles 2*n*=240**		**Statistical analysis**	
	Number	***AF (%)***	***Number***	***AF (%)***	***OR (95% CI)***	***P***
***HLA-DRB1**01**	**29**	**12**	**9**	**4**	**3.5 (1.63-7.62)**	**0.0013**
*HLA-DRB1**03	5	2	16	7	0.3 (0.10-0.82)	0.02
***HLA-DRB1**04**	**39**	**16**	**8**	**3**	**5.6 (2.5-12.3)**	**<0.0001**
*HLA-DRB1**07	20	8	58	24	0.28 (0.16-0.49)	<0.0001
*HLA-DRB1**08	7	3	16	7	0.42 (0.16-1.04)	0.06
*HLA-DRB1**09	3	1	1	0.41	3.02 (0.31-29.2)	0.33
***HLA-DRB1**10**	**44**	**18**	**14**	**6**	**3.62 (1.92-6.81)**	**0.0001**
*HLA-DRB1**11	12	5	42	17	0.24 (0.12-0.48)	<0.0001
*HLA-DRB1**12	6	2	3	1	2.02 (0.50-8.19)	0.32
*HLA-DRB1**13	10	4	26	11	0.35 (0.16-0.75)	0.0075
***HLA-DRB1**14**	**34**	**14**	**6**	**2**	**6.43 (2.64-15.6)**	**<0.0001**
*HLA-DRB1**15	31	13	41	17	0.71 (0.43-1.19)	0.20
*HLA-DRB1**16	0	0	0	0	0	0
SE Positive	**146**	**73**	**37**	**18.5**	**11.91 (7.41-19.1)**	**<0.0001**

AF: allele frequency; OR: odds Ratio; 95% CI: confidence interval at 95%. SE positive (indicated in bold) = the sum of *DRB1* *01, *04, *10 and *14. *HLA-DRB1* allele frequencies observed in patients and controls were compared using the Chi-square test. Differences were considered significant at $P<0.05$.

Table 3: Genotype and allele frequencies of *PTPN22* C1858T and *CTLA4* CT60 polymorphism in rheumatoid arthritis patients and controls

	RA Patients (*n*=200) (%)	**Control Group (*n*=200) (%)**	**OR (95% CI)**	***P***
***PTPN22* C1858T**				
Genotype frequency				
CC	175 (87.5)	179 (89.5)	0.82 (0.44-1.52)	0.5312
CT	20 (10)	17 (8.5)	1.19 (0.60-2.35)	0.6050
TT	5 (2.5)	4 (2)	1.25 (0.33-4.74)	0.7365
CT+TT	25 (12.5)	21 (10.5)	1.21 (0.65-2.25)	0.5312
Allele frequency				
C allele	0.92 (92.5)	0.93 (93.75)	0.82 (0.47-1.42)	0.4853
T allele	0.08 (7.5)	0.07 (6.25)	1.21 (0.70-2.10)	0.4853
***CTLA4* CT60**				
Genotype frequency				
GG	4 (2)	69 (34.5)	0.038 (0.01-0.10)	<0.0001
GA	101 (50.5)	76 (38)	1.66 (1.11-2.47)	0.0121
AA	95 (47.5)	55 (27.5)	2.38 (1.57-3.61)	<0.0001
GA+AA	196 (98)	131 (65.5)	25.8 (9.19-72.4)	<0.0001
Allele frequency				
G allele	0.27 (27.2)	0.54 (53.5)	0.32 (0.24-0.43)	<0.0001
A allele	0.73 (72.75)	0.46 (46.5)	3.07 (2.28-4.12)	<0.0001

PTPN22 and *CTLA4* allele frequencies observed in patients and controls were compared using the Chi-square test. Differences were considered significant at $P<0.05$. OR: Odds Ratio; 95% CI: Confidence Interval at 95%.

Table 4: Distribution of the *CTLA4* CT60 SNP stratified by the presence of *HLA-DRB1* shared epitope alleles

***CTLA4* + *HLA-DRB1* SE**	**RA patients (*n*=120) (%)**	**Control group (*n*=120) (%)**	**Statistical analysis**	
			OR (95% CI)	***P***
CTLA4 GG + 0 Copy SE	2 (1.6)	21 (17.5)	0.07 (0.018-0.34)	0.0008
CTLA4 GG + 1 Copies SE	1 (0.8)	13 (10.8)	0.06 (0.0089-0.53)	0.0107
CTLA4 GG + 2 Copies SE	1 (0.8)	0	3.02 (0.12-75.0)	0.4992
CTLA4 GA + 0 Copy SE	5 (4.1)	31 (25.8)	0.12 (0.046-0.334)	<0.0001
CTLA4 GA + 1 Copies SE	32 (26.6)	9 (7.5)	4.48 (2.03-9.88)	0.0002
CTLA4 GA + 2 Copies SE	9 (7.5)	1 (0.8)	9.64 (1.20-77.39)	0.0329
CTLA4 AA + 0 Copy SE	6 (5)	32 (26.6)	0.14 (0.05-0.36)	<0.0001
CTLA4 AA + 1 Copies SE	36 (30)	13 (10.8)	3.52 (1.75-7.07)	0.0004
CTLA4 AA + 2 Copies SE	28 (23.3)	0	74.25 (4.47-1232.3)	0.0027

CTLA4 and *HLA-DRB1* SE allele frequencies observed in patients and controls were compared using the Chi-square test. Differences were considered significant at $P<0.05$. OR: odds ratio; 95% CI: confidence interval at 95%.

Fig. 1 Caption: *HLA-DRB1* alleles genotyping results by PCR-SSP.

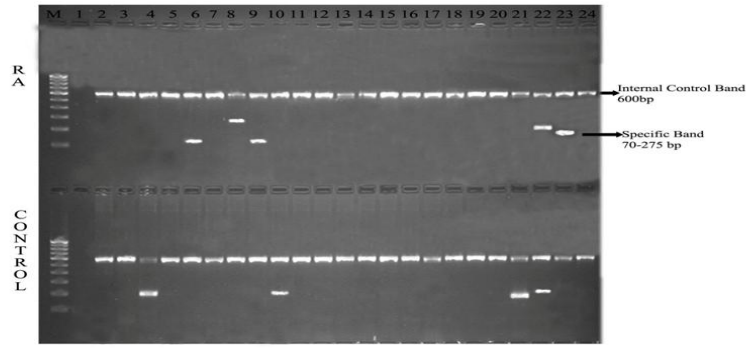

Fig. 1 Legend:
M: 100 bp Ladder
RA sample: Positive band appeared in Lanes 6, 8, 9, 22 & 23 representing the allelotypes of *HLA-DRB1* were *HLA-DRB1**03 & *HLA-DRB1**04.
Control sample: Positive band appeared in Lanes 4, 10, 21 & 22 representing the allelotypes of *HLA-DRB1* were *HLA-DRB1**11 & *HLA-DRB1**15.

Fig. 2 Caption: PCR-RFLP of *PTPN22* C1858T polymorphism on agarose gel

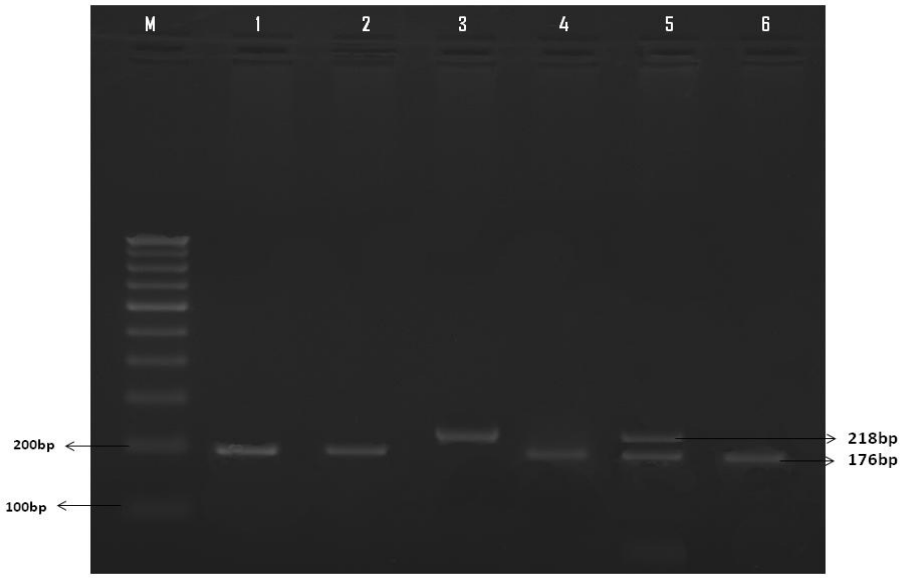

Fig. 2 Legend:
M: 100 bp Ladder
Lanes 1,3 & 5 contains RA Sample, Lanes 2, 4 & 6 contains control Sample
Lanes 1, 2, 4 & 6: Shows homozygous 1858*CC* genotype with 176 bp and 42 bp bands
Lane 3: Shows homozygous 1858*TT* genotype with 218 bp band.
Lane 5: Shows heterozygous 1858*CT* genotype with 218 bp, 176 bp and 42 bp bands

Fig. 3 Caption: PCR-RFLP of *CTLA4* CT60 polymorphism on agarose gel

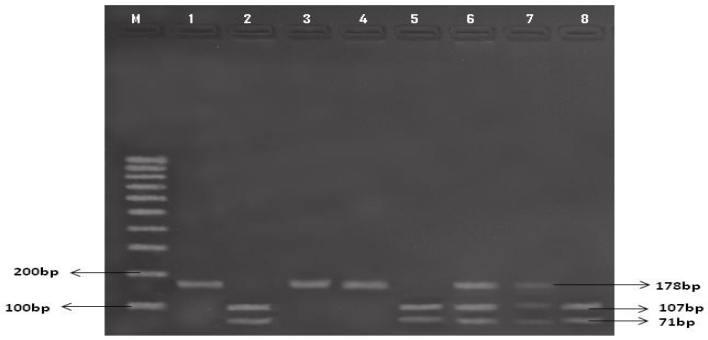

Fig. 3 Legend:
M: 100 bp Ladder
Lanes 1,3, 5 & 7 contains RA Sample, Lanes 2, 4, 6 & 8 contains control Sample
Lanes 1, 3, & 4: Shows homozygous *AA* genotype with 178 bp bands
Lanes 2, 5, & 8: Shows homozygous *GG* genotype with 107 bp and 71 bp bands
Lanes 6 & 7: Shows heterozygous *GA* genotype with 178 bp, 107 bp and 71 bp bands

DISCUSSION

The most interesting findings of this study are, the positivity of the autoantibodies gave a high specificity for the disease. This study has revealed an association of SE alleles *HLA-DRB1**01, *04, *10, and *14 with patients suffering from RA. We have confirmed that the *CTLA4* gene CT60 polymorphism is associated with increased risk for RA and it is associated with *HLA-DRB1* SE alleles. The *PTPN22* 1858C/T polymorphism was not associated with increased risk for RA. The present study provides evidence for a statistically significant association between South Indian RA patients with RA and *HLA-DRB1**01, *04, *10, as well as *14. These findings are in accordance with the results of previous research regarding the *HLA-DRB1* associations of RA of different ethnicity. This finding is similar to Turkish, Italian, Finnish, Syria and Kurd population. [6], [17-20] *HLA-DRB1**04 was significantly associated in RA patients in Pakistan, North India and Syria, [1], [6], [21] but no significant correlation between *DRB1**04 and RA susceptibility in Peruvian and Mexican American populations. [22], [23] In this study, the *HLA-DRB1**10 frequency was significantly expressed in patients as compared to control group. This finding is similar to the Asian, Syrian and Brazilian studies. [4], [6], [24] *DRB1**14 in Mexican American, Peruvian, Ecuadorian and North Indian RA patients, [1], [22], [23] on the other hand, Pakistan and Syrian studies showed no significant correlation between *HLA-DRB1**14 and RA susceptibility. [6], [21]

The protective effect of certain *HLA-DRB1* alleles against RA has been reported in several studies and revealed in various populations. In this study, *HLA-DRB1**03, *07, *11 and *13 alleles were found to be significantly increased in control group as compared to patients indicating a possible protective effect. *HLA-DRB1**03 was informed to be protective against RA in Asian populations, [24] and *DRB1**07 in Finnish, and Slovakians. [19], [25] *DRB1**11 in Syrians and Peruvians, [6], [22] whereas *DRB1**13 in Asian, Turkish, Slovakian, Syrian and Finnish population. [6], [17], [19], [24], [25]

The association between the *PTPN22* gene +1858C/T SNP and RA has been documented in several studies. Numerous studies revealed the correlation between 1858C/T SNP with RA [26], [27] but, the *PTPN22* 1858T/T genotype as well as the *PTPN22* 1858T allele were not found to be

associated with RA diagnosis in our population, which is consistence with the results obtained in Japanese, Iraqi and Black South African population. [28], [29], [30] No significant association was found between *PTPN22* 1858C/T and RA, this may be explained by the variation in *PTPN22* allele frequencies in different ethnic groups, as described by Mori *et al*. [31]

Our study shows that the *A* allele of the *CTLA4* CT60 polymorphism is more prevalent in RA patients than in controls. This result would support the view that this SNP may constitute a factor influencing the RA disease. The present study were agree with the meta-analysis carried out by Li et al., who analysed that CT60 *A* allele may be a risk factor of RA in Asians, but not in Europeans. [32] *CTLA4* gene variant were significantly increased with *HLA-DRB1* SE alleles. Thus, the present study provides evidence for a statistically significant association between *CTLA4* CT60 and *DRB1* shared epitope alleles in RA patients. Extended analyses with larger sample size should be carried out from different ethnic origins to further verify this association.

CONCLUSION

Our result show significant association of *HLA* class II alleles *DRB1**01, *04, *10 and *14 as well as autoantibodies with increased risk of RA. A significant disease protection was also found with alleles *DRB1**03, *07, *11 and *13. The frequency of *CTLA4* CT60 *A* allele carriers was significantly higher in RA patients and also it is preferentially in a SE-positive RA patients. On the other hand, we failed to provide evidence for the association of the *PTPN22* 1858 C/T SNP with RA and it does not seem to influence the course or severity of RA in our population.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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