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DISTRIBUTION OF HLA ALLELES AND HAPLOTYPES IN SELECTED POPULATION GROUPS FROM TAMIL NADU, SOUTH INDIA

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ABSTRACT

The identity of India is in its heritage and ethnically diversified endogamous populations characterized by unique custom, marriage system and occupation. It contributed a great genetic diversity or polymorphism in HLA system leading to linkage disequilibrium resulting in ethnically isolated sympatric populations. Hence, the genetic diversity of human populations can be traced by analyzing the highly polymorphic HLA antigenic profiles as markers. The present study aims at finding out the distribution of such HLA alleles and their haplotypes in the selected populations of Tamil Nadu state (South India). In this study, 222 healthy unrelated individuals from three endogamous groups such as Agamudayar (n = 74), Veerakudi Vellalar (n = 73), and Paraiyar (n = 75) inhabiting across southern region of Tamil Nadu were genotyped for HLA-A/-B, DRB1* and DQB1* alleles by the low-resolution-PCR-SSP method, and their Two-locus haplotypes were identified. Based on frequency distribution, alleles HLA-DRB1*15, DRB1*07, and DRB1*10 were identified as the predominant ones and are linked with the corresponding predominant DQ alleles to form predominant tow-locus HLA-DRB1*-

DQB1* haplotypes. The most predominant two-locus haplotypes were DRB1*15-DQB1*06, DRB1*07-DQB1*02, and DRB1*10-DQB1*05. They revealed a positive linkage disequilibrium contributing unique ethnicity, novel, and evolutionarily conserved alleles and their haplotypes in all the three south Indian endogamous population groups studied.

Keywords: HLA, HLA Antigens; Haplotype; Diversity; Linkage disequilibrium

INTRODUCTION

India is known for its diversity with genetically stratified populations into various caste groups. In the historical point of view, four different waves of migration to India resulted in social, behavioral and linguistic diversity which isolated the population sympatrically and led to caste system **[1]** which is characterized by caste endogamy, clan exogamy, inbreeding and specialized occupation. The caste system in India is the "grandest genetic experiment of nature ever done on humans" **[2]**. Each endogamous community is a breeding isolate **[2, 3]**. The Dravidian communities are the original inhabitants of India, now restricted to southern region of the country **[4]**. Tamil Nadu, the southern - most part of India, is inhabited by Tamil – the ancient Dravidian dialect, speaking communities, having own literary tradition **[5]**. In Tamil Nadu, 16 different populations were analyzed by a team of Indian social anthropologists and geneticists for their genetic, anthropometric and anthropological characters, and a model was proposed based on the genetic diversity for their migration **[1, 6]**. Various investigators have identified vast differences among HLA alleles in different castes of Tamil Nadu by serological studies on the distribution of HLA-A, B, and DR alleles **[7, 8]**. An extended multi - locus haplotype, A33-Cw7-B44 - Bf*S-C4A*3-C4B*1-DR7-DQw2 was identified in the Iyers (a Brahmin population) of Madurai, Tamil Nadu **[9]**. HLA-DRB1*15 was found to be more frequent allele in South Indian castes and tribal groups, and in contrast, HLA-DRB1*16, a rare allele was reported in Kani tribes, Pallars, Iyers and Kallars only moderately **[10]**. In the present study, we analyzed the distribution of HLA-DRB1* and DQB1* alleles of HLA genes, and HLA-DRB1*-DQB1*two-locus haplotypes, in the three selected endogamous population groups of South India.

MATERIALS AND METHODS

Two hundred and twenty-two healthy unrelated individuals belonging to three different Dravidian, Tamil speaking populations from southern region of Tamil Nadu were selected for this study. The populations selected were Agamudayar, Veerakudi Vellalar, and Paraiyar. They inhabit in restricted areas of Tamil Nadu state. Local village leaders from these areas

were approached and were explained the purpose of the study. The volunteers were consented through an explanatory questionnaire. EDTA blood samples (2 ml) from those volunteers who were willing were drawn and the genomic DNA was extracted from mononuclear cells, adopting standard salting out method **[11]**. Molecular HLA typing was done adopting Polymerase Chain Reaction – Sequence Specific Primer (PCR – SSP) method.

RESULTS

HLA allele frequencies

HLA-DRB1* alleles

The frequencies of HLA-DRB1* and DQB1* alleles in the study populations are presented **(Table 1 and Table 2 respectively)**. In Agamudayar population, the highly frequent alleles were DRB1*07 (31.79%), DRB1*15 (22%), DRB1*10 (12.23%); moderately frequent alleles were DRB1*12 (11%) and DRB1*14 (8.55%); while the alleles DRB1*13 (6.1%) and DRB1*04 (4.88%) were least frequent **(Table 1, Figure 1 and 1.1)**. In Veerakudi Vellalar population, the highly frequent alleles were DRB1*15 (43.8%), DRB1*07 (23.77%), DRB1*10 (16.26%); the moderately frequent alleles were DRB1*12 and DRB1*14 (6.24% each); alleles DRB1*04 and DRB1*13(5% each) were least frequent **(Table 1, Figure 1 and Figure 1.2)**. In Paraiyar population, the alleles DRB1*15 (38.26%), DRB1*07 (23.9%) and DRB1*10 (17.93%) showed higher frequencies; alleles DRB1*12 and DRB1*13 (11.94% each) revealed moderate frequencies; while, the alleles DRB1*04 (7.16%) and DRB1*14 (5.97%) revealed lower frequencies **(Table 1, Figure 1 and 1.3)**.

HLA-DQB1* alleles

The HLA-DQB1* allele frequencies are presented in Table 2. In Agamudayar population, the alleles DQB1*06 (53.06%) and DQB1*0301,0304 (DQB1*07) showed higher frequencies; the alleles DQB1*05 (20.97%) was moderately frequent, while the allele DQB1*02 (13.57%) was less frequent **(Table 2, Figures 2 and 2.1)**. In Veerakudi Vellalar population, the alleles DQB1*06 (48.81%) and DQB1*05 (36.86) were highly frequent; the allele DQB1*02 (16.38%) showed moderate frequency, while the allele DQB1*0301,0304 (DQB1*07) was less frequent **(Table 2, Figures 2 and Figure 2.2)**. In Parayar population, the alleles DQB1*06 (62.16%), and DQB1*02 (28.68%) were most frequent and the alleles DQB1*0301,0304 (DQB1*07, 26.30%), and DQB1*05 (17.93%) showed moderate frequency **(Table 2, Figures 2, and 2.3)**.

HLA-DRB1*-DQB1* Two-locus haplotypes

Two-locus HLA haplotype analysis has revealed, a total of 6 two-locus haplotypes in all the three populations

studied. However, the haplotype DRB1*14-DQB1*0301,0304 was absent in Veerakudi Vellalar population. Of these, three most predominant haplotypes in the descending order were DRB1*15-DQB1*06, DRB1*07-DQB1*02, and DRB1*10-DQB1*05 invariably in all the three populations studied. The observed Haplotype Frequencies (HF) of DRB1*15-DQB1*06 were 6.53% in Agamudayars, 15.26% in Veerakudi Vellalars, and 14.25% in Paraiyars; haplotype DRB1*07-DQB1*02 showed 4.97% in Agamudayars, 4.09% in Veerakudi Vellalars, and 7.12% in Paraiyars; haplotype DRB1*10-DQB1*05 revealed 4.67% in Agamudayars, 5.58% in Veerakudi Vellalars, and 2.78% in Paraiyars. The frequencies of two-locus haplotype DRB1*14-DQB1*0301,0304 were 4.35% in Agamudayar and 1.23% in Paraiyar and was not detected in Veerakudi Vellalar population **(Table 3, and Figure 3)**.

Table 1: Shows frequencies of the HLA-DRB1*alleles identified in study the populations

HLA – DRB1* Alleles	Allele frequencies in the study populations		
	Agamudayar (n = 74) %	Veerakudi Vellalar (n = 73) %	Paraiyar (n = 75) %
DRB1*15	22	43.8	38.26
DRB1*07	31.79	23.77	23.90
DRB1*10	12.23	16.26	17.93
DRB1*12	11	6.24	11.94
DRB1*14	8.55	6.24	5.97
DRB1*04	4.88	5.00	7.16
DRB1*13	6.10	5.00	11.94

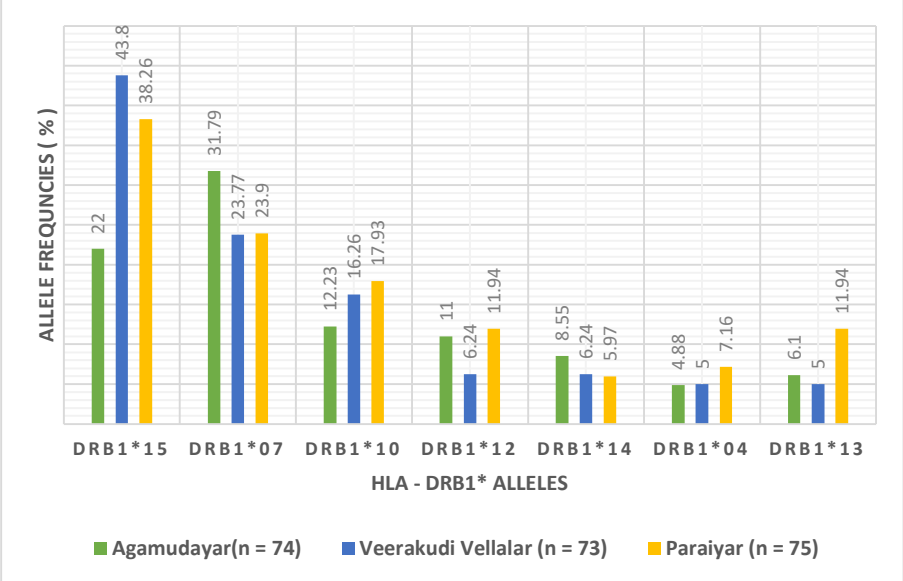

Figure 1: Shows the diagrammatic representation of HLA-DRB1* alleles identified in the study populations

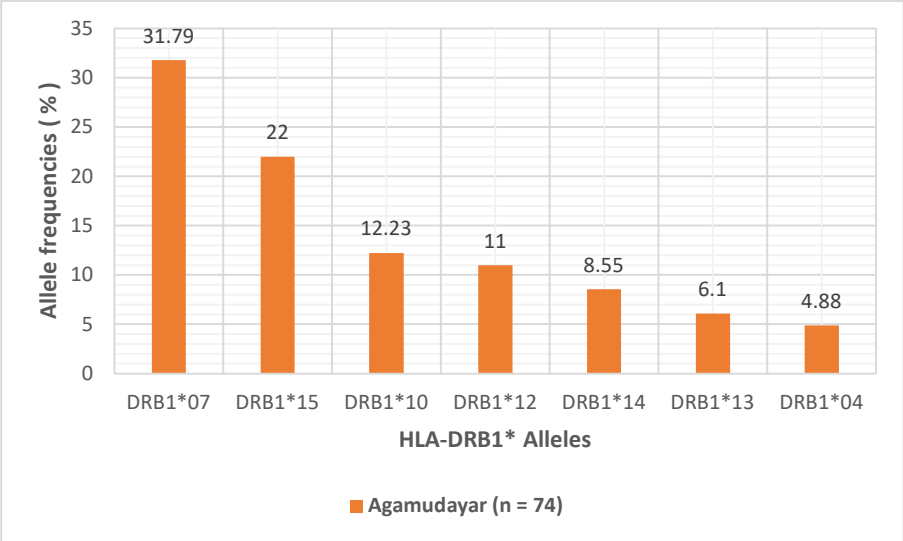

Figure 1.1: Shows diagrammatic representation of HLA-DRB1* Alleles identified in Agamudayar population

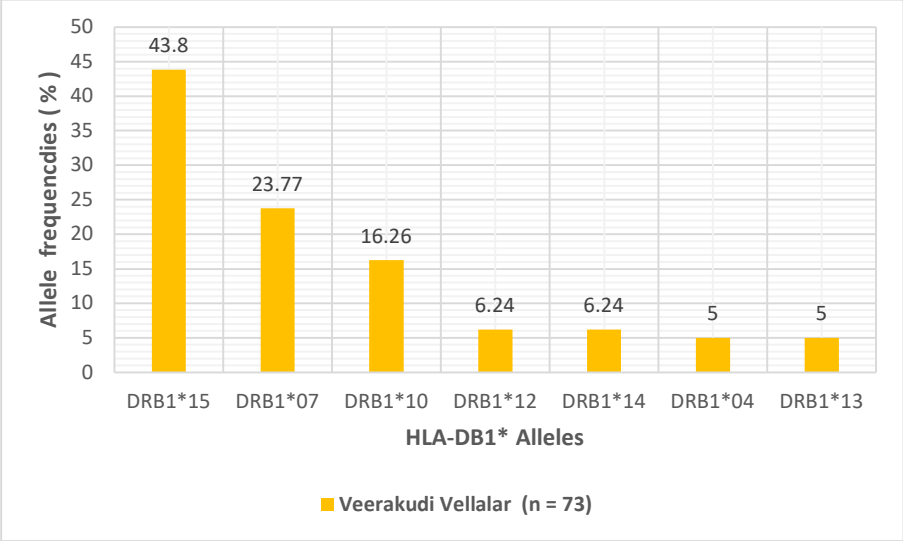

Figure 1.2: Shows diagrammatic representation of HLA-DRB1* Alleles identified in the Veerakudi Vellalar population

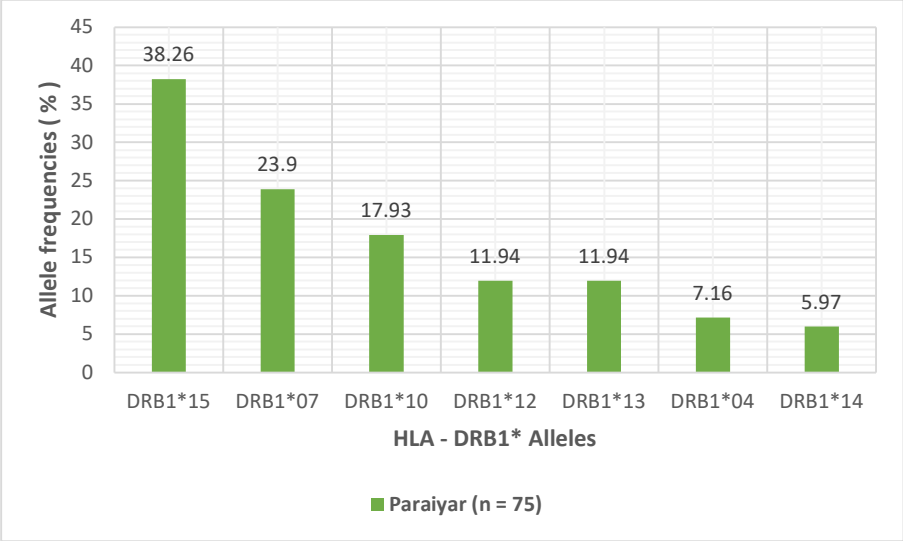

Figure 1. 3: Shows diagrammatic representation of HLA-DRB1* Alleles identified in the Paraiyar population

Table 2: Shows frequencies of HLA-DQB1* alleles identified in the study populations

HLA – DQB1* Alleles	Allele frequencies in the study populations		
	Agamudayar (n = 74) %	Veerakudi Vellalar (n= 73) %	Paraiyar (n = 75) %
DQB1*02	13.57	16.38	28.68
DQB1*05	20.97	36.86	17.93
DQB1*06	53.06	48.81	62.16
DQB1*0301,0304 (DQB1*07)	46.90	12.96	26.30

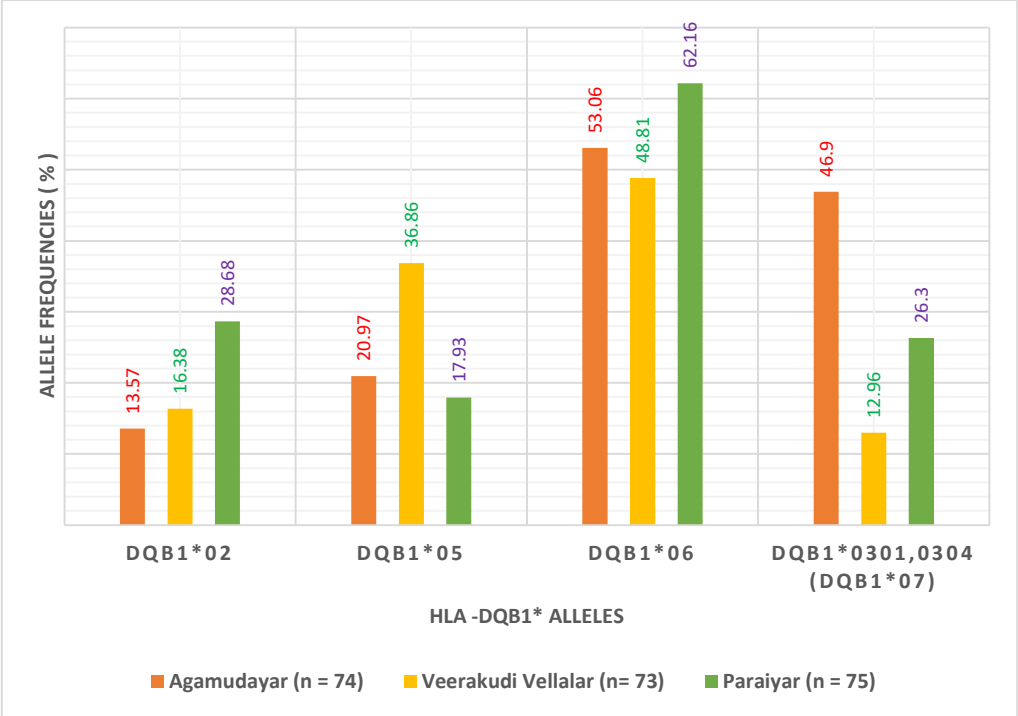

Figure 2: Shows the diagrammatic representation of HLA-DQB1* alleles identified in the study populations

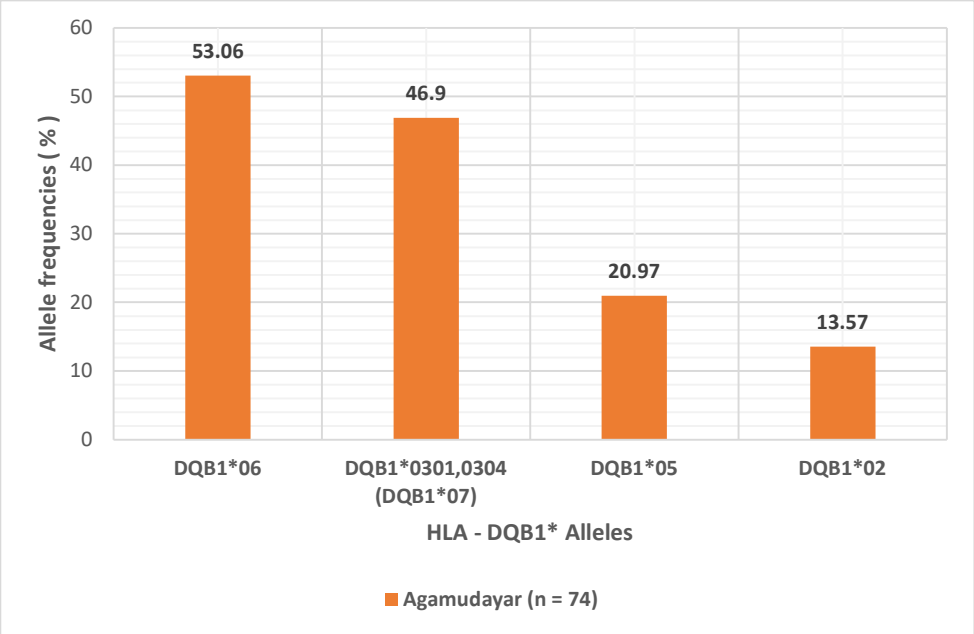

Figure 2.1: Shows diagrammatic representation of HLA-DQB1* Alleles identified in the Agamudayar population

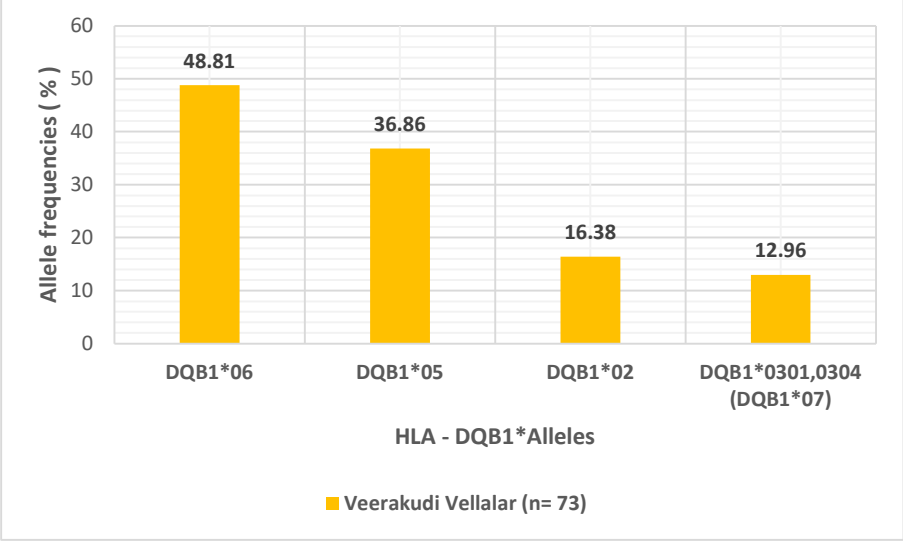

Figure 2.2: Shows diagrammatic representation of HLA-DQB1* Alleles identified in the Veerakudi Vellalar population

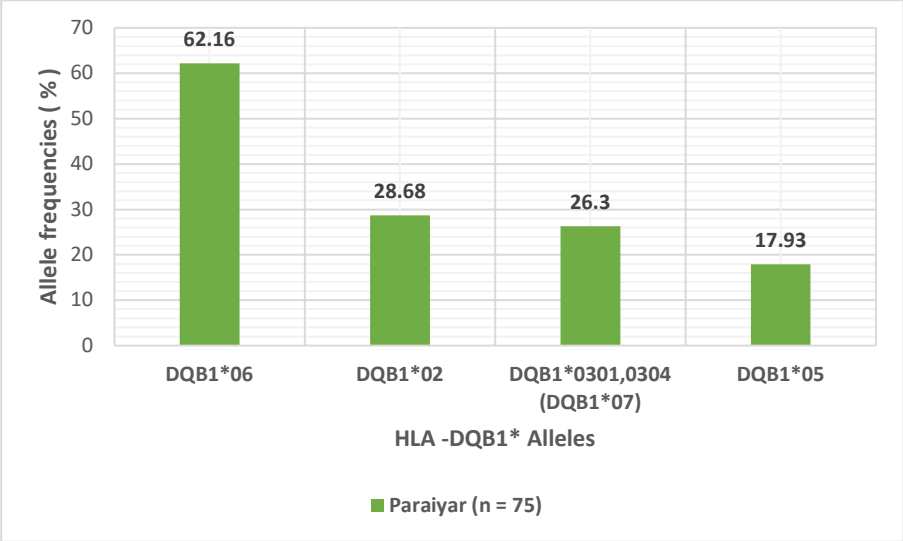

Figure 2.3: Shows diagrammatic representation of HLA-DQB1* Alleles identified in the Paraiyar population

Table 3: Shows frequencies HLA-DRB1* - DQB1* Two-Locus Haplotypes identified in study populations

HLA-DRB1*- DQB1* Two-Locus Haplotypes	Haplotype frequencies in the study populations		
	Agamudayar (n = 74) %	Veerakudi Vellalar (n = 73) %	Paraiyar (n = 75) %
DRB1*15-DQB1*06	6.53	15.26	14.25
DRB1*07-DQB1*02	4.97	4.09	7.12
DRB1*10-DQB1*05	4.67	5.58	2.78
DRB1*14-DQB1*0301,0304	4.35	Not detected	1.23
DRB1*07-DQB1*06	4.04	4.39	2.78
DRB1*10-DQB1*06	5.60	2.60	3.09

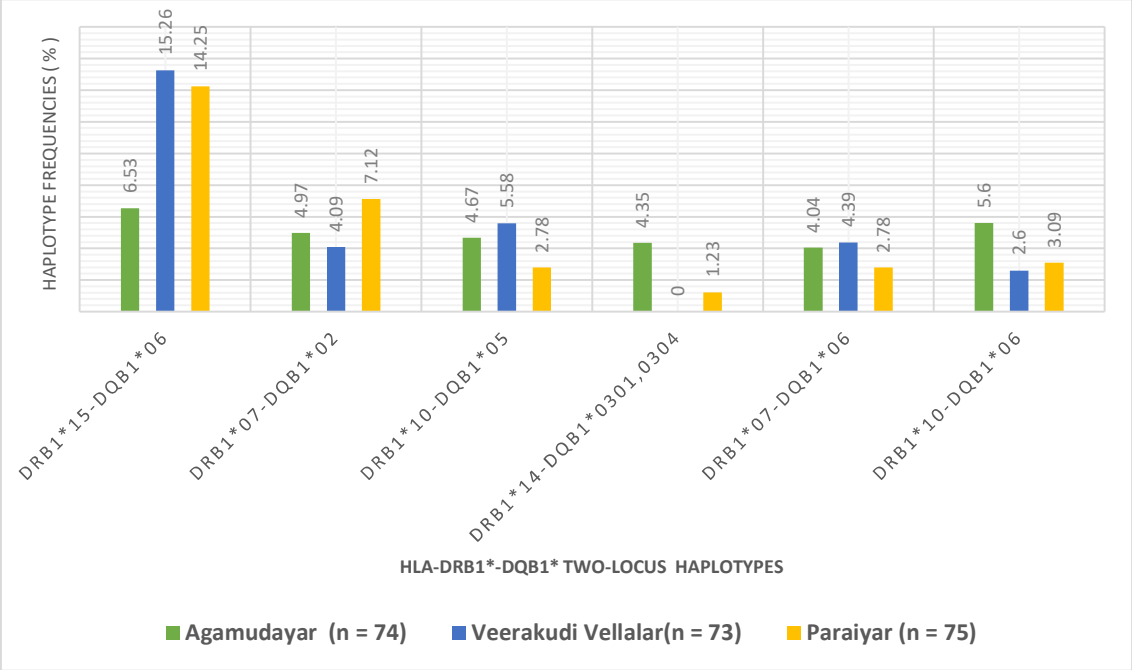

Figure 3: Shows diagrammatic representation of HLA-DRB1* - DQB1* Two-Locus Haplotype in study populations

DISCUSSION

The present gene pool of the Indian subcontinent population is largely constituted by its native population groups and immigrants with different ethnic stocks, cultures, and languages from Middle-East, Central Asia and from Mongolia **[12]**. The genetic composition and social structure of Indian population is predominantly governed by the customs of various ethnic and religious groups. These socio-biological factors influence the diversity of HLA alleles and design their ethnic specific pattern of gene flow leading to population stratification with differences in adaptive immunity and predisposition to various diseases. The non-genetic lifestyle factors and environmental factors also have an influence on the genetic properties of HLA system. The influence of ethnicity on the association of HLA alleles with various infections and diseases have been extensively studied. The investigation found that the lifestyle and environmental pathogenic factors like Epstein-Barr Viral (EBV) infection influence the association of HLA alleles with multiple sclerosis **[13]**. The association of HLA-DRB1*11 allele with HCV clearance and protective association of HLA-DQB1*04 with hepatitis C viral (HCV) infection was reported in the white population of Brazil **[14]**.

Reportedly, the HLA allele-DRB1*11:01 confers protectivity to pulmonary interstitial fibrosis in North American white people in comparison with North American black (with DRB1*11:04 allele) and Japanese people (with DRB1*15:02 allele) which are negatively associated **[15]**. The sub-type of HLA-B27 allele including - B*27:04, B*27:05, B*27:07, B*27:08 and B*27:14 alleles identified in Maharashtra state, differ in population and tribal group specific distribution **[16]**. The HLA-A*02 and A*33 alleles revealed greater diversity along with many sub-type alleles in Indian population. Notably, the allele A*02:11 is found in Indian population alone and almost absent in Caucasoid and Oriental ethnic groups **[17]**. The A*33:03 is found in Indian population of Mongoloid ethnic origin. Moreover, the haplotypes HLA-A33-B44-DR7, HLA-A33-B58-DR3 and HLA-A2-B50-DR3 are common in North Indian population. Of these, the haplotype HLA-A33-B44-DR7 is the common haplotype in Asian countries (more particularly in South East Asian countries) and not reported in rest of the word **[18]**.

The alleles, DRB1*07, DRB1*15, DQB1*02, DQB1*05 and DQB1*06 are reported to be the most common and highly frequent among South Indian endogamous groups **[10]**. In the Indian caste groups, the alleles DRB1*03, DRB1*05, DRB1*06,

DRB1*07, DRB1*10, DRB1*14, and DRB1*15 were the most frequent **[9, 19-22]**. The allele DRB1*15 is the predominant allele in Caucasian and Asian ethnic groups **[23]**; most frequent allele in South Indian caste and tribal groups **[11]** revealed predisposition to pulmonary tuberculosis in South Indian population **[24]**. Similarly, the allele DRB1*15 is predominant in all the South Indian castes and tribes **[10]**. Reportedly, the allele DRB1*07 was predominant in Kallars, Sourashtrans, Iyers, and Narikuravars of Tamil Nadu, and Namboothiris of Kerala.

The present study examined the profiles of HLA-DRB1* and DQB1*alleles, and DRB1*-DQB1* two-locus haplotypes. The study revealed that the alleles DRB1*15, DRB1*07, DRB1*10, DRB1*12, DRB1*14, DRB1*04, and DRB1*13 are the more common in all the populations studied. However, the study revealed the differential frequency distribution of these HLA alleles in the three study populations. Remarkably, the DRB1*07 allele showed highest frequency followed by the allele DRB1*15 in Agamudayar population. Whereas it is reverse in the remaining two populations namely Veerakudi Vellalar and Paraiyar populations. In both populations, the HLA-DRB1*15 is the highly frequent allele followed by DRB1*07. In the overview, the HLA-DRB1*15, and HLA-DRB1*07 alleles are dominant HLA-DRB1* alleles, the alleles DRB1*10 and DRB1*12 are moderately frequent, and the alleles DRB1*14, DRB1*04 and DRB1*13 are least frequent in all the three study populations.

Among DQB1* loci alleles, the allele DQB1*06 is predominant in all the three study populations indicating its significant conservation in the evolutionary course. Other DQB1* alleles are differentially distributed in these populations and it could be attributed to the ethnicity-based selection of these alleles to stabilize these endogamous populations.

In the analysis of HLA-DRB1*-DQB1* two-locus haplotypes in the study populations, six two-locus haplotypes have been identified. Of these, three haplotypes namely, DRB1*15-DQB1*06, DRB1*07-DQB1*02, and DRB1*10-DQB1*05 are the most predominant ones and in confirmation with the previous studies on other populations from South India. The absence of the two-locus haplotype DRB1*14-DQB1*0301,0304 in Veerakudi Vellalar population signifies its negative selection attributing to the influence of ethnicity in the course of natural selection. The population specific gradations in the distribution of HLA alleles and their haplotypes obviously contributing to the stability of respective populations and ethnic based specific

pattern of gene flow. It resulted in ethnically isolated sympatric endogamous populations.

CONCLUTION

The predominance and differential distribution of DRB1* 15, DRB1*07 and DQB1*06 alleles and the two-locus haplotypes DRB1*15-DQB1*06, DRB1*07-DQB1*02 and DRB1*10-DQB1*05 are influenced by the unique ethnicity contributed by positive linkage disequilibrium and are highly conserved in the populations by the nature operating in favour of the study populations. They contribute the homogenous gene pool in each endogamous population and thus the heterogenous Indian population. However, it remains to be resolved in a cohort of large size in these populations.

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