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HLA Polymorphism in Punjabi Population of Pakistan

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ABSTRACT High polymorphism of the human leukocyte antigen (HLA) alleles is frequently used to determine the origin and migration of human population. Furthermore, HLA-A, -B and -DR alleles specific frequencies are important to distingush the genetic structure between related enthic groups and is helpful in hematopoietic stem cell, organ transplantation and disease association. The data on HLA-A, B and DRBI* loci at a high-resolution level is limited in Punjab Population, Pakistan, therefore, the present research was designed to explore the genetic profile of patients collected from Punjab, Pakistan using HLA-A, HLA-B and HLA-DRBI* genotypes with sequence specific primers. The allelic frequencies, haplotype distribution and genetic distances were investigated to map a genetic relationship. The most common alleles and haplotypes originating in Punjabi population were HLA-02, HLA-B*40, HLA-DRB1*15, A*2602-B*08-DRB*03, and A*02-B*51-DRB1*13. The bootstrap stats was applied with the phylogenetic analysis suggesting a strong evolutionary relationship with 80 percent validation. This shows that Punjabis have heredity with Sindhis as their personal predecessor. Altogether, these findings would be helpful in the selection of transplantation procedure, planning donor registry and for anthropologic studies.

INTRODUCTION

Human leukocyte antigens (HLA) system is a very informative tool for evolution and population based studies due to its high polymorphic nature. HLA genes have vital role in immune system and are involved in graft rejection in bone marrow transplantation. An unrelated hematopoietic stem cells transplantation is suggested for the patients, who don't have HLA genotypically matched sibling. HLA types are genetically inherited, therefore it is important to determine the ethnic background to find a perfect matched donor (Seshasubramanian et al. 2021). HLA is determined by cluster of genes which resided on short arm of chromosome 6 encoded in a ~3,500 kb section on human chromosome. HLA-A located at 6p21.3, HLA-B at 6p22.1 and HLA-D at 6p21.32 on the chromosome 6, which are the foremost variable locale within the genome in the MHC region (Hudson and Allen 2016). Human lymphocytes, T cells, type of leukocytes, plays a crucial role in defining the linkage of cytotoxic and Helper T cells with the HLA- A, B and C (which belongs to MHC class I) and HLA- DR, DQ and DP genotypes (are members of MHC class II) respectively in driving the human immunity (Abbas et al. 2008).

The HLA system located within the MHC is regarded as genetic diverse region due to occurrence of high level of polymorphism (Madden et al. 1993; Stern and Wiley 1994). HLA genes frequency distribution provide valuable information about the diversity, origins and migration between different human populaces (Solberg et al. 2008; Kulski et al. 2019; Mack et al. 2013; Vina et al. 2012). Asian countries like India and Pakistan are known for their vast cultural traits and ethnic diversity, and can be distributed into numerous groups based on different aspects that is, physiographical region (east, west, north, south), religion (Christian, Hindu, Muslim, and Sikh), caste (more than thirty-seven hundred different endogamous groups) and different language (Hindi and Urdu) (Bhasin et al. 1994; Mehra 2000). Previous study

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found Indian populations (Southern region) were closely related to Sri Lankan populations (Seshasubramanian et al. 2021). Hajjej et al. observed linkage between Emirati, Omani and Bahraini with Pakistani populations in their study (Hajjej et al. 2020). HLA allele frequency analysis revealed that Balochi population from Iran are genetically close related to Brahui and Baloch population from Pakistan (Farjadian et al. 2004). Gujjar population of Punjab from Pakistan are found closely related to the Indian Golla tribe from Andhra Pradesh (Raza et al. 2013). Another study found that Parsis of Pakistan and India have the closest ancestry with Italian and Jewish populations (Mohyuddin and Mehdi 2005). The heterogenous matrix of populations among India and Pakistan depicted the reasonable intrusion of Iranians, Greeks, Afghans, Arabs and Caucasians leading to genetic admixture and there has been mass migrations leading to genetic variations (Mahal and Matsoukas 2017; Mehrjoo et al. 2019; Shah and Amjad 2011).

The Punjab, has been considered as the most fertile and multiethnic region of the past subcontinent in Pakistan, (Khan et al. 2019). Ethnic diversity of Pakistani people had great influences on the HLA frequencies in different population. Punjab province is of considerable interest because it is the largest province of Pakistan with vast territories of exceptional variety and experienced various invasions. However, previous studies only reported the polymorphism of various ethnic groups of Pakistan as described by (Mohyuddin et al. 2002) with the exception of Punjabi population especially patients based and the role of HLA and its association with different diseases (asthma, aeroallergens, coronavirus disease and diabetes). This shows a significant drawback for unrelated hematopoietic stem cells transplantation indigenously and also for calculating the exact size of a country wise unrelated stem cell library, therefore new donors are still needed and their compatibility need to be addressed (Aslam et al. 2021; Fawwad et al. 2019; Hashmi et al. 2017).

Aim

Main objective of this study, relatedness of Punjabi population and other provinces of Pakistan and the putative haplotypes comparison among major ethnic groups in Pakistan. The researchers investigated the variations in the HLA class I HLA-A, HLA-B and class II HLA-DRB1 gene in the Punjabi population of Pakistan. This study investigated to build understanding of the common HLA polymorphism prevalence in Pakistani Punjabi population by utilizing progressed innovations and selecting characterized masses thus spiking the past studies' impediments of blended ethnicity, test estimate and determination.

This study exhibited the importance of heterogeneity attributes through environmental factors and pathogen pressure which signifies the evolutionary relevance of the HLA polymorphic alleles. Moreover, the findings of prevalent HLA genotypes and polymorphism will also help us to figure out the biological importance and diversity of HLA family which needs to be utilized more at the high resolution, specifically for immune connotations and HLA based therapeutic strategies.

MATERIAL AND METHODS

Study Population

Blood samples of 100 registered unrelated patients from different geographical regions of Punjab were collected with informed consents in 10cc EDTA vacutainers (N=100 with mean age of 58). This research was approved by the ERC (Ethical Review Committee) and ASRB (Advanced Studies Research Board) of Punjab University, Lahore, Punjab, Pakistan. Healthy subjects were participated in study to rule out any biased provision of HLA characterization among population. Patients with diabetes and autoimmune disorders were excluded.

HLA Typing

For HLA typing, nucleic acid was extracted from blood samples of recruited subjects and amplification of DNA was performed using sequence-specific primer (SSP) by polymerase chain reaction (Nguyen et al. 2017), with Collaborative Transplant Study Group under Prof. Opelz G, Institute of Immunology, University of Heidelberg, Germany, HLA plates (ABDR plates). The required quantity of DNA and Taq polymerase was mixed with PCR mix and added to the CTS-PCR-SSP based HLA-ABDR TRAY KITS containing PCR primer mixes in 96 well PCR-Trays. The PCR-Tray was loaded in the thermocycler (Applied Biosystems) and per-

formed the standard temperature accelerated (heating-cooling cycles) protocol following CTS guideline. Gel electrophoresis of amplified DNA was performed, stained with ethidium bromide and images were captured with the Kodak documentation system for results record.

Data Analysis

Genetic Distance and Phylogenetic Analysis (DISPAN) were used to make the phylogenetic tree by using the neighbor-joining method (http://iubio.bio.indiana.edu/soft/molbio/ibmpc/). The tree was constructed after calculation of DA genetic distances, allele frequencies of HLA -A, -B and -DRB loci (with 1000 bootstrap replications) using GNKDST and TREEVIEW (Rey et al. 2013). HLA subtypes frequency and loci positions were elucidated using data mining websites http://www.allelefrequencies.net/hla.asp and HLA database http://hla.alleles.org/nomenclature/index.html. Principle component analysis for correlation milieu was studied by using ViSta v 6.4 (http://www.mdp.edu.ar/psicologia/vista/vista.htm) in order to develop a two-dimensional (2D) and three-dimensional (3D) plots (Habel et al. 2015). Other statistical analysis (correlation, quartiles, contingency tables and normal distribution) were performed by XLSTAT2009 (http://www.xlstat.com/en/home/).

Phylogenetic and principal component analysis were performed to determine the correlation between different populations based on HLA allele frequencies.

Furthermore, the statistical significance was assessed using SPSS software version 21, following descriptive analysis for correlations and one-way ANOVA along with Tukey's Honestly significance difference (HSD) using Bonferroni correction, statistical differences among groups were designated by $^{*}p < 0.05$, $^{**}p < 0.05$ (with increased and decreased significance level), and original significance level (that is, your alpha) of 0.05 respectively.

RESULTS

The researchers analyzed the allelic frequencies of HLA- A, B and DRB* in Punjabi population by using primer specific sequencing and recorded the percentage of each haplotype presented in Tables 1-3 and Figure 1. The researchers identified 18, 31 and 21 alleles in HLA- A, B and DRB* loci, respectively in the selected population. The most frequently observed HLA-A allele were A*02 (45%) and A*01 (24%) (Table 1). While 5 alleles (A*25, A*3601, A*6601, A*6901 and A*7401) were not observed in the Punjab population after compared with neighboring populations in Pakistan. Out of

Table 1: List and percentage of HLA-A alleles frequencies in population of Pakistani

HLA-A	*Punjabi N=100*	*Baloch n=66*	*Brahui n=104*	*Burusho n=99*	*Kalash n=69*	*Pathan n=100*	*Sindhi n=101*
HLA-A*01	*24	**3.8	5.3	5.6	**0.0	*12.5	*13.4
02	*45	**9.1	20.7	13.1	*39.9	16.0	17.3
03	*13	7.6	**4.8	13.1	9.4	9.5	6.9
11	20	*23.5	*22.6	**12.1	**1.4	16.0	18.3
2301	*6	**0.8	0.0	0.0	0.0	1.5	**1.0
24	*17	9.1	**6.7	14.1	**8.0	11.0	12.9
25	0.0	0.0	0.5	0.5	0.0	0.0	0.0
2601	*15	8.3	8.2	10.1	**1.4	**6.0	8.4
2901	*7	0.8	1.9	**0.5	-	1.5	**0.5
30	8	6.8	3.4	**1.5	*8.7	5.5	**0.5
31012	2	1.5	**4.3	2.5	**5.1	**1.5	4.0
3201	5	*9.1	8.7	**2.5	8.7	5.0	4.5
33	12	*16.7	8.7	*16.2	8.0	8.0	**7.4
3601	0.0	0.0	0.0	0.5	0.0	0.0	0.0
6601	0.0	0.0	0.5	0.0	0.0	0.0	0.0
68	*13	**2.3	**2.4	7.1	9.4	6.0	4.5
6901	0.0	0.8	*1.0	**0.5	0.0	0.0	**0.5
7401	0.0	0.0	0.5	0.0	0.0	0.0	0.0

*frequency significantly increased (one-way parametric aov, P<0.05: Tukey HSD, $\alpha = 0.05$)
** frequency significantly decreased (one-way parametric aov, P<0.05: Tukey HSD, $\alpha = 0.05$)

thirty-one alleles, HLA-B alleles were found to be statistically significant (Table 2). HLA-B alleles were the most dominant in the studied population among which HLA-B*40 (26%) and B*51 (25%) and were found with high frequencies while HLA-B*45 and HLA-B*49 were least frequent alleles Among the identified HLA-DRB alleles, HLA-DRB3*01 (69%), DRB1*15 (48%) and DRB1*03 (45%) were the most frequent in the studied population (Table 3). We observed 43 HLA haplotypes in the Punjabi Population among which 9 percent were HLA-A*2602-B*08-DRB*03, 7 percent HLA-A*02-B*51-DRB1*07 and HLA-A*02-B*51-DRB1*13 each were observed to be the most common haplotypes as shown in Figure 1.

The conduction of bootstrap stats with the phylogenetic analysis (Fig. S1) of each of the seven populations suggested the presence of strong evolutionary relationship evidence based on allelic frequencies at HLA-A, -B and –DRB1. This indicated that the Punjab's population have connection with Sindh's population as their intimate forefather. Further, possible genetic relationship was investigated among these populations. The major analysis presented in Figure 2 showed a strong link among the Punjab and Sindh's population and this has ensured that both of the groups share an equal proportion of the HLA allele diversity and includes the chief population of Pakistan.

The biplot (Fig. S2) showed that the most of the HLA-A, HLA-B and HLA-DRB* alleles are confined to Sindh's, Punjab's, Burushu's and Pathan's population and in the same direction. Whereas the Kalash's population is long vector representing due to unique set of HLA alleles. A relationship observed among the population of Brahui and Baloch. However, allelic based biplot (Fig. S3) showed that the HLA-A*11, HLA-A*02, HLA-B*35, DRB5*01/02, HLA-DRB*15, HLA-B*51, and DRB4*01 are closely distributed among the Punjab, Barushu, Sindh and Pathan populations.

The data in the scatter plot (Fig. 3) showed variation of about 74.05 percent in 1st component of HLA allele's data account, which can be explained as of the high HLA allelic frequency with the populations (right side) in competition with those populations with less HLA allelic frequency (left side). The projection study of the points onto the later dimension represents less HLA frequency depicting populaces of Kalash, Baloch and Brahui and at the top with high allelic frequency, corresponding Punjabi, Sindhi, Burushu and

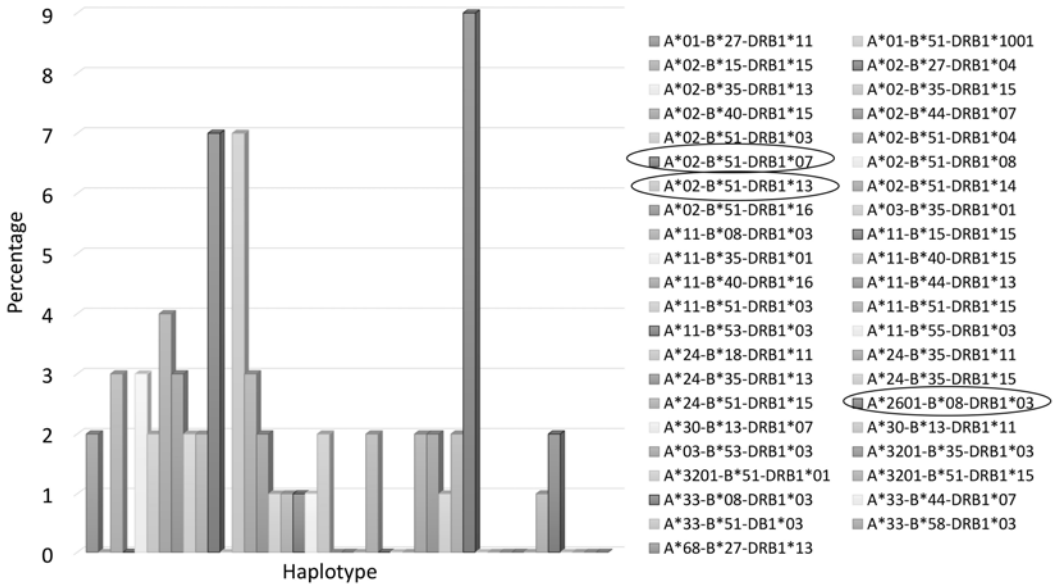

Fig. 1. Percent haplotypes of HLA -A, -B and -DRB1 in the Punjabi population of Pakistan (N=100)

Table 2: List and percentage of HLA-B frequencies alleles in population of Pakistan

HLA-B	*Punjabi N=100*	*Baloch n=66*	*Brahui n=104*	*Burusho n=99*	*Kalash n=69*	*Pathan n=100*	*Sindhi n=101*
HLA-B *07	5	**3.1	3.8	*5.1	**0.7	4.0	4.0
08	*22	10.8	10.6	15.3	**4.3	6.6	11.9
13	5	1.5	**0.5	1.5	*7.2	6.1	**0.5
1401	*8	2.3	1.4	0.0	0.0	**0.5	2.0
1402		0.0	0.0	0.0	0.0	0.0	0.5
15	*9	1.5	2.4	7.1	6.5	6.6	**4.0
18	4	*4.6	2.4	**0.0	0.7	0.5	4.5
27	4	1.5	1.9	2.0	*9.4	4.0	**0.5
35	*21	**13.8	15.9	20.4	18.1	16.7	10.4
37	3	*3.1	**0.5	1.5	5.8	1.0	2.0
38	3	0.8	2.4	**0.5	*5.8	0.5	0.0
39	*2	1.5	1.9	**0.5	0.0	0.0	1.0
40	*9/17=26	*16.2	15.4	**1.0	0.0	6.1	13.4
41		*3.1	1.9	*4.1	0.0	1.5	**0.5
42		*1.5	1.4	0.0	0.0	1.0	**0.5
44	*15	0.0	**1.9	4.6	5.8	4.5	4.0
45	1	0.0	0.0	0.0	0.0	0.0	*2.5
46	0	0.0	0.5	0.0	0.0	0.0	0.0
47	0	0.8	0.0	**0.5	0.0	0.0	0.0
4801		0.0	0.0	**1.0	0.0	0.0	0.5
4802	0	0.0	0.0	0.0	0.0	0.0	1.0
49	1	0.8	**0.5	1.0	0.0	1.0	*1.5
50	*7	**0.8	1.9	**0.5	0.0	3.5	3.5
51	25	**10.8	11.5	17.9	*27.5	20.7	15.3
52	*9	2.3	1.4	7.1	**0.7	3.0	8.4
53	2	4.6	*7.7	0.0	**0.7	0.0	1.0
55	0	0.0	*4.3	**1.0	1.4	**1.0	0.0
57	*14	0.0	1.4	1.5	**0.7	2.5	2.5
58	5	*15.6	6.3	4.6	**4.3	6.6	4.5
73	0	0.0	0.0	0.0	0.0	0.5	0.0
78	0	0.0	0.0	**0.5	0.0	1.0	0.0

*frequency significantly increased (one-way parametric aov, P<0.05: Tukey HSD, α = 0.05)
** frequency significantly decreased (one-way parametric aov, P<0.05: Tukey HSD, α = 0.05)

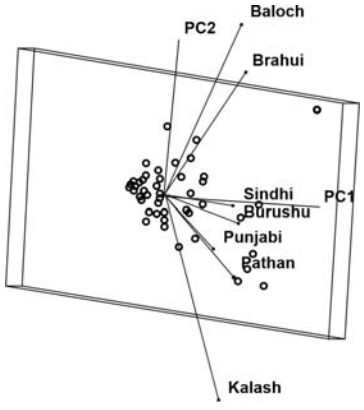

Fig. 2. Principle component analysis. Scatter Plot showing the observation of the distribution of HLA -A, -B and -DRB* alleles in seven ethnic groups of Pakistan in three dimensions

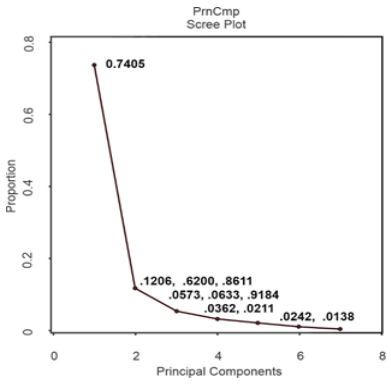

Fig. 3. Principle component analysis of variance in the first component of the high HLA allelic frequency with populations (right side of the plot) in competition with those populations with less HLA allelic frequency (left side)

Table 3: List HLA-DRB alleles in Pakistani populations and their frequencies percentage

HLA-DRB	*Punjabi N=100*	*Baloch n=66*	*Brahui n=104*	*Burusho n=99*	*Kalash n=69*	*Pathan n=100*	*Sindhi n=101*
HLA-B *07	5	**3.1	3.8	*5.1	**0.7	4.0	4.0
HLA-DRB1*01	5	*15.1	13.5	11.8	5.8	6.5	**2.5
03	*45	39.6	34.5	23.7	**9.4	17.0	24.7
04	0	**0.9	0.0	4.8	*13.0	0.0	0.0
04/1122	*13	4.7	5.0	4.8	0.0	**3.0	4.0
07	*22	**1.9	**2.0	3.2	13.8	13.0	10.1
08	3	**0.9	**0.5	*7.2	1.4	1.5	2.0
09	1	0.0	0.0	0.0	0.0	1.0	0.0
1001	6	**1.9	2.5	*6.5	5.1	5.0	3.0
11	*20	7.5	6.0	**2.7	9.4	13.0	11.6
12	*8	0.0	0.0	0.0	0.0	**0.5	1.5
13	*20	**2.8	**3.5	14.0	13.0	8.5	*15.2
1302		0.0	0.0	0.0	0.0	0.5	0.0
14	*19	**1.9	**2.0	4.3	9.4	8.5	4.0
15	*48	**8.5	13.5	17.2	19.6	19.0	19.7
16	2	13.2	*17.0	0.0	0.0	2.0	**1.5
17	0	0.9	0.0	0.0	0.0	0.0	0.0
DRB3*01	*69	11.3	**2.5	5.9	0.0	5.5	7.6
02/03		0.0	**0.5	1.6	*3.6	2.5	3.0
DRB4*01	*36	8.5	**7.0	14.5	*28.2	18.5	16.3
0101102		1.8	**0.5	1.6	2.1	5.5	*4.6
DRB5*01/02	*39	14.1	26.0	**13.4	18.8	16.0	17.8

*frequency significantly increased (one-way parametric aov, P<0.05: Tukey HSD, α = 0.05)
** frequency significantly decreased (one-way parametric aov, P<0.05: Tukey HSD, α = 0.05)

Pathan. The diversity of the normal distribution of the HLA alleles is presented in spread plot (Fig. 4), scatter plot (Fig. 2) and box plot (Fig. 5). Statistical analysis of allele's population demonstrates the strong relation among Sindh's and Punjab's population as shown in Principal component analysis of variable correlation Matrix Table 4.

DISCUSSION

The human leukocyte antigen (HLA) loci are the leading candidates for infectious disease susceptibility (Thorsbv 1974). HLA system is a most widely accepted approach used for differentiation and association among different populations. Additionally, HLA alleles frequencies and genetic distances data is useful tool for tracing the presence or absence of gene flow between neighboring populations (Moatter et al. 2010). HLA polymorphism in was studied in the six populations of the Pakistan three from the provinces in south of the Pakistan (that is, Sindhi, Baloch and Brahui) and the other three from the Northern Pakistan (Pathan, Burusho, and Kalash) by Mohyuddin and the coworkers (Mohyuddin et al. 2002). However, no data is found on the population of Punjab. Fre-

Table 4: Principal components analysis of variable correlation matrix

Variables	*Punjabi*	*Baloch*	*Brahui*	*Burushu*	*Kalash*	*Pathan*	*Sindhi*
Punjabi	1.0000	0.5843	0.6097	0.6676	0.5819	0.7668	**0.8098***
Baloch	0.5843	1.0000	0.8960	0.6999	0.3243	0.6471	0.7182
Brahui	0.6097	0.8960	1.0000	0.6964	0.4792	0.7055	0.7815
Burushu	0.6676	0.6999	0.6964	1.0000	0.6571	0.8254	0.8032
Kalash	0.5819	0.3243	0.4792	0.6571	1.0000	0.7727	0.6164
Pathan	0.7668	0.6471	0.7055	0.8254	0.7727	1.0000	0.8904
Sindhi	**0.8098***	0.7182	0.7815	0.8032	0.6164	0.8904	1.0000

*shows strong correlation between Punjabi and Sindhi populations of Pakistan

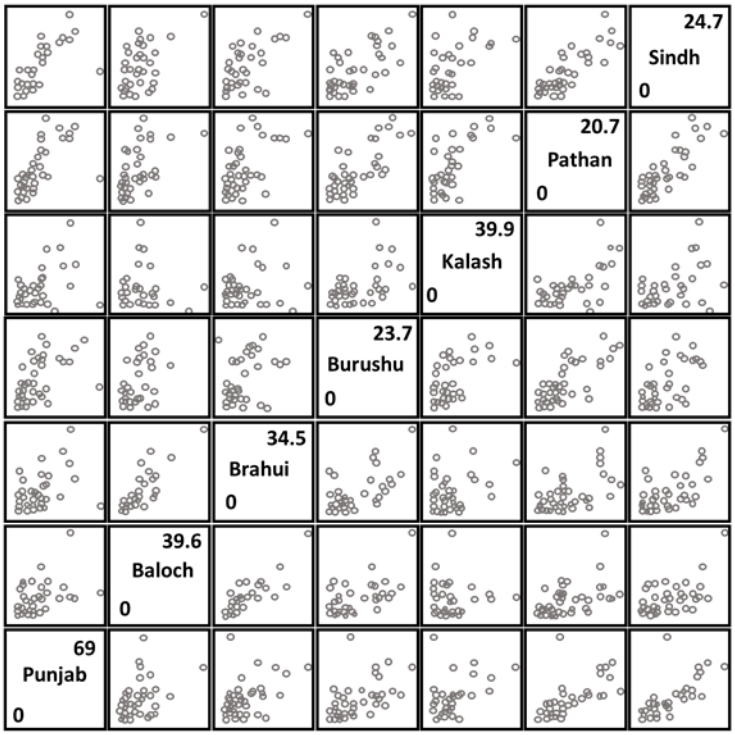

Fig. 4. Ordinary dissemination investigation. The multivariant spread plot lattice is utilized to show the connection between all sets of a few factors. The HLA allelic recurrence information is addressed by focuses in little dissipate plot and each point addresses the qualities for one perception on two factors. Typically dispersed factors should dissipate plots that have the best thickness in the center and are generally elliptical

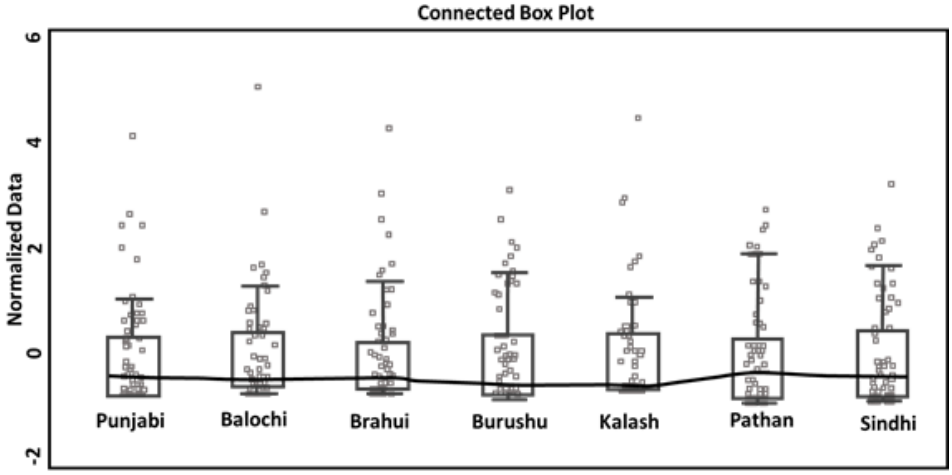

Fig. 5. Normal distribution analysis. BoxPlot showing normalized data of HLA allelic frequencies across the seven populations

quently occuring allele frequencies in the HLA-A found in Punjab's population are the HLA-A*02, with frequency of 45 percent followed by A*01 with 24 percent frequency rate. A*02 found at high frequency in the population of the Kalash with frequency of 39 percent, than other of the Pakistan's populations supporting the interpretation of a common lineage or the gene admixture. A*11 is more frequent allele than A*02 in Brahui and Baloch population of Pakistan (Moatter et al. 2010). Most interesting, HLA-A*02, -A*01 and A*11 were also found in Iran, and due to the close boundaries with Pakistan, thousands of travelers and pilgrims visit yearly across the boarders (Mehrjoo et al. 2019). At locus HLA-B the most shared allele frequencies were observed in the Punjab's population, that is, HLA-B*40, with frequency of 26 percent followed by B*51 (25%). B*40 is also most common allele in Baloch population and second most frequent allele in Sindhi and Brahui (Moatter et al. 2010). While B*51 has highest frequency in Kalash, Pathan and Sindhi population (Raza et al. 2013). This demonstrates that Punjabi and Sindhi are very closed to each other and similar finding were also observed in phylogenetic tree analysis. Recently, Fatmah et al., found the association between HLA-B*51 with COVID-19 infected patients, which is very important to study the severity in other linkage populations (Naemi et al. 2021). At the HLA-DRB locus, we found 16 alleles in the Punjabi population while 10 in Kalash, 11 in Brahui and Bursh u, 12 in Sindhi, 13 in Baloch, and 14 in Pathans were previously reported (Mohyuddin et al. 2002). In Punjab's population, the alleles DRB1*15 (48%), DRB1*03 (45%) and HLA-DRB3*01 (69%) are the commonly occurring alleles. HLA-DRB1*07 is associated with recurrent pregnancy loss was also found in Punjab population which could be also correlate with other populations (Thomsen et al. 2021).

The comparative analysis of the haplotype frequencies found in this study with a previous study shows significant variations in HLA allele frequencies (Mohyuddin et al. 2002, 2003). In Punjab's population, HLA-A*2601-B*08-DRB1*03 haplotypes were the commonly present and were also found previously reported in other populations of Pakistan except Kalash (Mohyuddin et al. 2002). No common haplotype was observed in this study population and the other populations of Pakistan. HLA-A*11-B*40-DRB1*15 and HLA-A*11-B*40-DRB1*16 are the two haplotypes found in the population of the Punjab whereas, the haplotype HLA-A*11-B*40-DRB1*15 was previously reported in the Sindh's and Baloch's population and the haplotype HLA-A*11-B*40-DRB1*16 was observed in the Brahui's and Baloch's population. Furthermore, HLA-A*02-B*40-DRB1*15 a 3 locus haplotype was found in Pathan's and Sindh's population (Rehman et al. 2009; Shah and Amjad 2011). Presence of common haplotype which is not found in any other populations shows that although the origin and the language varies but they must have experienced the inter-tribe marriages and genetic admixture. Timor and Brushu confirmed the presence of haplotype HLA-A*24-B*35-DRB1*15. Timor and Brushu populations shared haplotype HLA-A*24-B*35-DRB1*15 with Punjabi's populations (Rehman et al. 2009). Haplotype HLA-A*33-B*58-DRB1*03 was found in each of the studied populations, except in Punjab's and Brahui and was also present in many different Oriental populations (Muazzam et al. 2013). HLA-A*33-B*58-DRB1*03 is a typical oriental haplotype, found in almost all groups of Pakistani population with the exception of Brahui and Punjab. Specific haplotypes found in different ethnic groups of Pakistan's population, HLA-A*11-B*40-DRB1*15 is specific to Baloch, Sindh and Punjab, HLA-A*33-B*08-DRB1*03 in Brushu and Punjab whereas the haplotype HLA-A*11-B*40-DRB1*16 in Brauhi and Baloch (Mohyuddin et al. 2002). This maybe the result of the adaptive immune response to different pathogens or by gene transfers.

The A*11-B*35-DRB1*01 haplotype was detected previously in population of Europe as well as in Balochi, Brushu, Brahui population of Pakistan and has now also found in the Punjab population in this study. While HLA-A*02-B*51-DRB1*16 haplotype also present in the Punjab population and previously reported in Brahui only (Sanchez-Mazas et al. 2013). Punjabi population also share haplotypes HLA-A*02-B*51-DRB1*04 HLA-A*02-B*44-DRB1*07 and HLA-A*02-B*35-DRB1*13 with European population (Sanchez-Mazas et al. 2013). The haplotype HLA-A*33-B*44-DRB1*07 observed in this study was also previously reported in neighbour countries like India, Bangladesh, China, USA and Brazil (Dedhia et al. 2015). Furthermore, most of the allele frequency distribution results of Punjab population, closely correlate with

Sindhis suggesting near evolutionary lineage inside Pakistan. Heterogeneous behaviour of HLA and its genotypic diversity among population is associated with autoimmune, haematological and other disorders. Finding of HLA alleles frequencies based study can be useful in selecting donor for the bone marrow/solid organ transplantation and also helpful in understanding of drugs reaction (antiretroviral and antiepileptic). And the overall biological significance with the drug and vaccine development and adaptive immunity, reveals that it is the need of the time to generate widespread HLA data from the Asian continent including Pakistan.

CONCLUSION

This study reveals that in the Punjab population, most frequent HLA antigens are HLA -A*02, -B*40, and -DRB3*01 while most common haplotype was HLA-A*2602-B*08-DRB*03 in Punjab population. This study provides an estimate of HLA allelic distribution among the Punjabi population and comparison with other ethnic groups showed a significant similarity with the Sindhi population. The analysis of this study can be helpful to develop a better understanding of transplantation selection procedure, investigation of associated disease marks and anthropological relations based on Pakistan population as well as neighbour countries.

RECOMMENDATIONS

Future studies based on large scale sampling, evaluation of HLA polymorphisms and its association with different disease in this region should be analysed.

ABBREVIATIONS

HLA	Human Leukocyte Antigen
HLA-DRB1	HLA class II histocompatibility antigen, DRB1 beta chain
EDTA	Eethylenediamine Tetraacetic Acid
SSP	Sequence-Specific Primer
DISPAN	Genetic Distance and Phylogenetic Analysis
DA	Nei's standard genetic distance

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