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ORIGINAL ARTICLE

HLA DR/DQ type in a Malay population in Kelantan, Malaysia

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Abstract

The human leucocyte antigen (HLA) has been documented to be involved in various disease susceptibilities or in resistance against certain diseases. An important element in susceptibility and resistance to disease is ethnic genetic constitution. Cognizant of this, the present study aimed at studying the prevalence of particular HLA class II in a normal healthy Malay population which may serve as a guide for further genetic and immunological studies related to the Malay Malaysian population. The study involved 40 normal healthy Malay persons in Kelantan. HLA typing was conducted on venous blood samples through a polymerase chain reaction-sequence specific primer method (low resolution Olerup SSP® HLA Typing Kits). The study found HLA DR12 and HLA DQ8 to be the most frequent HLA class II type. HLA DQ5 was significantly associated with female subjects.

Keywords: HLA DR/DQ typing, Malay, Malaysian.

INTRODUCTION

The human leucocyte antigens (HLA) describe a region of genes located on chromosome 6 in humans and comprise a series of highly polymorphic genes based on multiple alleles.[1] Among HLA class II genes, DRB1 coding for DR chains has the highest degree of polymorphism, and appears to be responsible for variations in the immune response of different individuals to different antigens.[1] There is evidence supporting the influence of genetic variability in susceptibility to several diseases.[2]

A wide range of diseases have been linked with different HLAs, including ankylosing spondylitis, rheumatoid arthritis, coeliac disease, insulin-dependent diabetes mellitus, multiple sclerosis, Goodpastures' syndrome, narcolepsy, Chagas disease, alopecia, and tuberculosis.[3] Some HLA types protect against certain diseases or are host factors in determining disease progression. For example, HLA molecules have been found to be involved and associated with either delayed or accelerated disease progression in human immunodeficiency (HIV) patients .[4] In the Malay population, a study pertaining to the role of HLAs in dengue fever, showed that HLA-B*13 and B*18 are associated with disease susceptibility and protection, respectively.[5]

In a study on leprosy,[6] the classic HLA class I loci which include HLA-A, -B, and –C; binds peptides of intra-cellular origin and present them to CD8+T cells. This leads to the death of cells infected by *Mycobacterium leprae*. The classic class II loci, which refer to HLA-DR, -DQ, and –DP, primarily bind peptides of extra-cellular origin and present them to CD4+T cells, subsequently resulting in cytokine and antibody production. In conditions where the HLAs are involved in protection against disease, two behaviours are likely. The HLA genes eliminate the agent with inflammatory cytokine production or damage the infected cells.[6]

This opposing nature of HLA is of particular interest in research on the role of genetic composition and variability in disease susceptibility and resistance. Cognizant of this and as a means to enrich understanding of genetic variability in medical interventions, the purpose of this study was to determine the class II HLA type among individuals from a normal

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healthy population. This particular study was *apriori* to determine the possible association between specific diseases and particular HLA types in a subsequent study. It may also serve as a fundamental guide for further genetic and immunological studies particularly in the Malay population.

MATERIALS AND METHODS

Forty healthy normal Malay subjects were included in this study. They were above 18 years old with no history of underlying illness and currently healthy with no specific medical complaints. Malay subjects were selected based on the subjects' first and last names as well as their identification cards. Although all patients were Malay, we did not further define the study population as it is was not our intention to relate and ascertain the ancestry of the study population.

Written informed consent was obtained from each subject prior to their participation. A venous blood sample of 2mls in EDTA tube was obtained. Initially, genomic DNA from whole blood taken was obtained using QIAmp DNA Blood Mini Kit (QIAGEN, Germany). HLA-DR/DQ typing determination was performed in blood obtained from subjects through polymerase chain reaction-sequence specific primer method (low resolution Olerup SSP® HLA Typing Kits) according to the manufacturer's instructions. This kit is a qualitative *in vitro* diagnostic kit for the DNA typing of HLA Class II antigens.

Statistical analysis

Data was analysed using IBM SPSS Statistics 20 and Microsoft Excel (Microsoft Corporation) software. Frequency analysis for allele frequency was checked by Microsoft Excel. Fisher exact test was used to determine the association between two categorical variables and each of HLA type whether present or absent. P-value of less than 0.05 was determined as significant.

RESULTS

Tables 1 to 3 provide descriptive results of the healthy Malay adult subjects who participated in this Malaysian study. Table 1 depicts the demographic data which includes gender, male:female ratio and mean age. The majority of subjects were males with a male:female ratio of 3.4:1. Table 2 provides descriptive results of HLA-DR. For the 40 subjects, 16 (40%) were of HLA DR12. HLA DR1 and HLA DR18 were not detected in these subjects. Table 3 shows the frequency of HLA-DQ antigens extracted from venous blood samples of all subjects. HLA DQ8 was the most frequent [23 (57.5%)] of HLA class II type. HLA DQ4 was the least frequent [1(2.5%)] HLA DQ type (Table 3).

Tables 4 and 5 show the HLA DR and HLA DQ profiles according to gender. There was no gender association observed for HLA DR. All male and female subjects did not have HLA DR1 and HLA DR18. HLA DQ5 [7(77.8%)] was the most common HLA DQ allele in females and showed significant association with female subjects.

DISCUSSION

Gene products from the major histocompatibility complex (MHC) play key roles in the regulation of both humoral and cell-mediated immune responses. HLAs are the products of MHC which are involved in genetic variability. Genetic variability in turn has been found to be associated with either various diseases or with

TABLE 1: Demographic data of healthy Malay adult subjects (n=40)

Variable	Frequency n (%)
Gender:	
Male	31 (77.5)
Female	9 (22.5)
Male: female ratio	3.4:1
Mean Age (+/-SD)	33.00
Standard Deviation for Age	10.9

TABLE 2: Frequency of HLA-DR antigens in healthy Malay subjects (n=40)

	Frequency n (%)
HLA DR1	0 (0)
HLA DR4	6 (15)
HLA DR7	8 (20)
HLA DR8	2 (5)
HLA DR9	9 (22.5)
HLA DR10	1 (2.5)
HLA DR11	6 (15)
HLA DR12	16 (40)
HLA DR13	6 (15)
HLA DR14	6 (15)
HLA DR15	15 (37.5)
HLA DR16	2 (5)
HLA DR17	4 (10)
HLA DR18	0 (0)

TABLE 3: Frequency of HLA-DQ antigens in healthy Malay subjects (n=40)

	Frequency n (%)
HLA DQ2	9 (22.5)
HLA DQ4	1 (2.5)
HLA DQ5	18 (45)
HLA DQ6	12 (30)
HLA DQ7	21 (52.5)
HLA DQ8	23 (57.5)
HLA DQ9	2 (5)

protective roles against certain diseases. For instance, inheritance of the HLA-DRB1*0301 is significantly associated with susceptibilty to Type I diabetes mellitus while HLA-DQB1*0601 has been described as a protective allele.[7]

The high frequency of HLA DR12 and HLA DQ8 in our Malay samples most probably reflects the predominant genetic inheritance of HLA DR serotype in the Malaysian population. This finding is supported by a previous local study that found DRB1*12 to be the most frequent HLA type, accounting for 15–36% of Malays in the Malaysian population. It also has been included as among the most common HLA alleles in all Malay subethnic groups in Malaysia including the Kelantan population.[8] This finding was also supported by Dhaliwal *et al.,* 2007, who reported HLA DR12 as one of the most common HLA DR allele.[9]

No HLA serotype DR1 and DR18 were detected in our samples. These lack of findings could possibly reflect the absence or low frequency of HLA DR1 and HLA DR18 in the Malay population. As all the subjects were Malays residing in the Kelantan state, the assertion of absence or low frequency may possibly reflect the HLA type of subethnic Malay Kelantanese. This assertion is further supported by a study on HLA polymorphism of various subethnic groups in Malaysia. In that particular study, the Kelantan population showed a low frequency of HLA DRB1*01 and DRB1*03 alleles of 4% respectively.[8]

TABLE 4: Frequency of HLA DR antigens between gender of healthy Malay subjects (n=40)

	Male **n=31**	Female **n=9**	*p-value
HLA DR1	0 (0%)	0 (0%)	-
HLA DR4	4 (12.9%)	2 (22.2%)	0.41
HLA DR7	6 (19.4%)	2 (22.2%)	0.59
HLA DR8	2 (6.5%)	0 (0%)	0.60
HLA DR9	6 (19.4%)	3 (33.3%)	0.32
HLA DR10	1 (3.2%)	0 (0%)	0.76
HLA DR11	4 (12.9%)	2 (22.2%)	0.41
HLA DR12	13 (41.9%)	3 (33.3%)	0.48
HLA DR13	5 (16.1%)	1 (11.1%)	0.59
HLA DR14	5 (16.1%)	1 (11.1%)	0.59
HLA DR15	12 (38.7%)	3 (33.3%)	0.55
HLA DR16	1 (3.2%)	1 (11.1%)	0.40
HLA DR17	3 (9.7%)	1 (11.1%)	0.66
HLA DR18	0 (0%)	0 (0%)	-

*Fisher exact test

TABLE 5: Frequency of HLA DQ antigens between gender of healthy Malay subjects (n=40)

	Male n=31	Female n=9	*p-value
HLA DQ2	8 (25.8%)	1 (11.1%)	0.33
HLA DQ4	1 (3.2%)	0 (0%)	0.78
HLA DQ5	11 (35.5%)	7 (77.8%)	0.03
HLA DQ6	10 (32.3%)	2 (22.2%)	0.45
HLA DQ7	17 (54.8%)	4 (44.4%)	0.43
HLA DQ8	19 (61.3%)	4 (44.4%)	0.30
HLA DQ9	2 (6.5%)	0 (0%)	0.60

*Fisher exact test

The increased frequency of HLA DQ8 probably reflects the predominant genetic inheritance of HLA in the Malaysian population. HLA DQB1*03 alleles are gene products of HLA DQ3. HLA DQ3 is a broad antigen of HLA DQ7, DQ8 and DQ9. As HLA DQ8 is the antigen split for HLA DQ3, a high frequency of DQB1*03 alleles (25–51% among all Malay subethic groups) [8] could possibly explain the increased frequency of HLA DQ8 in our present study. HLA DQ8 has been previously described to be associated with good prognostic in certain diseases such as clearance of hepatitis B.[10]

To the best of our knowledge, the HLA allele has not been reported to be significantly associated with gender, now further supported by this study. The exception was HLA DQ5. Our study provides some evidence that HLA DQ5 was more frequent in females than males. It may be a preponderant allele in the female Malay Kelantanese.

Several limitations are acknowledged in this study. As the study was conducted in Kelantan, the racial subethnic distribution may narrow the HLA patterns found. Generally, the dominant subethnic group in Kelantan is the Malay Kelantanese. This dominance may not be a true representation of the Malay ethnic group in Malaysia. It is likely that the HLA patterns reflect a subethnic group who may be a minor subethnic group in the country.

In addition, mixed marriages between individuals of Thai descent and Malay descent or other ancestry may have impact on the generalisation of these research findings. Ancestry of at least three generations should have to be included as the inclusion criteria. This was previously suggested in a local study that sought to determine the precision of HLA type in genetic inheritance.[8]

Due to cost limitation, the present study was conducted using only Class II low resolution HLA typing. This may have limited the scope of genetic composition identification. A subsequent study into Class I HLA (HLA A, B and C) typing may be helpful in clarifying the actual distribution of HLA types associated with the healthy Malay, Kelantanese population in Malaysia.

The current results are also limited in sample size and may not represent the HLA profile of the actual Malay Kelantanese population. Future studies involving larger numbers of healthy subjects could address this limitation. Ancestry of at least three generations should be included using an interview session as being suggested in a previous local study may add to the precision of findings of genetic inheritance of HLA type.[8]

The main advantage and added value of this study compared to previous studies is that a larger number of subjects from 'Kelantan' and broader HLA class II subtypes which is the HLA DQ subtypes have been included.

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