Molecular Analysis of Class II HLA-DRB Polymorphism

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ABSTRACT:

BACKGROUND:

Major Histocompatibility Complex (MHC) system is a polymorphic system and the extensive polymorphism of MHC gene in human and the allelic distribution in different ethnic population become a key component in investigating the genetic relations between populations. Geographical variety is one of the factors that affect HLA polymorphism.

OBJECTIVE:

This study is designed to study class II HLA-DRB polymorphism in Iraqi populations by molecular method.

METHODS:

Seventy four healthy Arab Iraqi populations were enrolled in this study
during the period between November 2012 till April 2013. Direct interview was done with the populations and HLA typing was done by molecular method using Sequence Specific Primer (PCR-SSP) method using One Lambda Kit-USA.

RESULTS:

The most frequent alleles are DRB1*03,*11,*07 with a frequency of (0.47, 0.41, and 0.26) respectively. There are no significant difference between male and female regarding non-DRB1 alleles.

CONCLUSION:

1-The most frequent alleles in Arabic Iraqis populations are DRB1*03,*11, and *07.

2-Gender is not a significant factors affecting the frequencies and hetro- or homozygosity for the nonDRB1 alleles in adults.

KEY WORDS: HLA-DRB1, HLA polymorphism .

INTRODUCTION:

Major Histocompatibility Complex (MHC) system encode for HLA-A, B, C, DR, DP, and DQ and are present on the surface of many cells[1] .It is a polymorphic system and the extensive polymorphism of MHC gene in human and the allelic distribution in different ethnic population become a key component in investigating the genetic relations between populations[2,3]. Geographical variety is one of the factors that affect HLA polymorphism[1]. An allele that is common in one population may be rare in another, while, some alleles are limited to particular ethnic populations and others are widely shared among ethnically distinct populations. For example, the allele HLA-A36 is found only among individuals having African ancestry, on the other hand, the serologically defined HLA-A2 allele occurs rather frequently in most populations studied around the world[4].

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Based on this polymorphism, different studies are relevant for both anthropology and for clinical medicine. HLA class II haplotypes often contain a second expressed HLA-DRB locus tightly linked to the classical HLA-DRB1 locus on the haplotype, which can be either HLA-DRB3, -DRB4 or -DRB5. These encode the HLA-DR51, -DR52 or -DR53 supertypic specificities and mark the ancestral lineages[5,6]. It is generally believed that this allelic polymorphism is maintained by selection pressures for inbreeding avoidance and/or maximal immune system diversity. Immune system diversity could be quantitatively estimated by calculating a Supertype Diversity Index (SDI) which is the number of different MHC supertypes possessed by an individual. This hypothesis generates a number of testable predictions. First, it predicts that a reduced inherited diversity of MHC allele supertypes may predispose to the development of malignancies because of a decreased native ability to present

different tumor-associated antigens. Furthermore, specific autoimmune diseases may be associated with the presence or absence of a particular MHC supertype rather than a particular MHC haplotype.The natural selection should favor maximization of the heterozygosity of allele supertypes instead of the heterozygosity of individual alleles [7,8].

In Iraq, there are several published studies for HLA-typing and disease association done by molecular method [9, 0].

Serological HLA typing especially for class II is a long procedure with many difficulties and gives an ambiguous results, so molecular methods is more accurate with less time consuming and more reliable results. Because of these causes, this study is designed to study HLA-DR polymorphism in Iraqi populations by molecular method.

PATIENTS AND METHODS:

Seventy four healthy Arab Iraqi populations from Baghdad governorate were enrolled in this study. They are admitted to HLA-typing research unit at AL-kindy College of Medicine between November 2012- April 2013.

This study was approved by the scientific and ethical committee of Al-Kindy College of Medicine. All the patients and controls were assigned a confirmed consent about the study.

Two and a half ml of blood were withdrawn from each subject and collected into EDTA tube, kept at -20C. The DNA was extracted by using Reliaprep spin column kit (Promega, USA). All DNA were stored at -20C until tested. HLA Class II-DRB typing were performed by PCR-Sequence Specific Primer (SSP) according to (Olerup and Zetterquist,1993) using Micro SSP generic Class II DNA typing tray –DRB only with lot number of SSP2LB-004 from One Lambda company (USA) . Each patient requires one plate composed of 24 small PCR tubes containing mixture of primers (24 primers) in aliquot dried form as supplied by the company. The SSP method is based on the fact that primer extension and hence successful PCR relies on an exact match at the 3´-end of both primers. The Master Mix (D-Mix®) that is provided by the company with the kit contain the followings:

Water
Buffer
dNTP´S
MgCl2
Dye

Taq polymerase is provided from Amplicon® (Denmark).

The PCR machine is MyGenie™ 96 Gradient Thermal Block from Bioneer Company (Korea), Horizontal Gel electrophoresis system from One Lambda and Gel documentation system for detection of DNA bands also used.

The following shows the program for PCR cycling as recommended by the company and used in this article:

# of cycles	Step	Temp. C°	Time (sec.)
1	1	96	130
	2	63	60
9	1	96	10
	2	63	60
20	1	96	10
	2	54	50
	3	72	30
Final	1	4	----------

- Genotype frequency is calculated according to the following formula:

$PR = 1 - \sqrt{1 - FR}$

(PR = gene frequency of R allele).

$FR = \frac{NR}{N}$

FR = frequency of R alleles

NR = Number of individuals with R allele

N = Total number of individuals

The results of HLA DR typing were in agreement with Hardy-Weinberg equilibrium.

Statistical analysis:

Data were analyzed using Mini Tab version 16. Descriptive statistics were used as frequencies and percentages. Chi sequare test used to determine the P-value for the significance of association. P-value of less than 0.05 is considered significance.\

RESULTS:

This study was done on 74 Iraqi population, 40 (54%) individuals are Male and 34(46%) are female as shown in table I.

Table I: Gender distribution in the studied populations.

Total populations	Male	Female
74	40 (54%)	34 (46%)

Table II shows allele and genotype frequency in Iraqi population. The most frequent alleles are DRB1*03,*11,*07 with a frequency of (0.47, 0.41, and 0.26) respectively.

Table II: Alleles frequency and genotypes in Iraqi populations.

Alleles genotype	Number	Allele frequency	Genotype frequency
DRB1* 03	32	0.47	0.27
DRB1*04	10	0.14	0.07
DRB1*07	18	0.26	0.13
DRB1*08	10	0.14	0.07
DRB1*11	28	0.41	0.23
DRB1*12	8	0.11	0.05
DRB1*13	12	0.17	0.08
DRB1*14	8	0.11	0.05
DRB1*15	8	0.11	0.05
DRB1*16	2	0.02	0.01
DRB1*17	0	0	-
DRB1*18	0	0	-

Table III shows allele haplotype frequency (hetro- or homozygosity) of non DRB1 alleles in male and female. There are no significant difference between male and female.

Table III: Non DRB1* alleles haplotype frequency in both male and female.

Haplotype	Male no. And frequency	Female no. And frequency	Haplotype frequency in both male and female	p-value
DRB3-DRB3	24(60%)	14(41.1%)	38(51.3%)	0.918
DRB3-DRB4	8 (20%)	6 (17.7%)	14 (19%)	0.797
DRB3-DRB5	0	0	0 (0%)	-
DRB4-DRB4	4 (10%)	6 (17.7%)	10(13.5%)	0.497
DRB4-DRB5	2 (5%)	6 (17.7%)	8 (10.8%)	0.132
DRB5-DRB5	2 (5%)	2 (5.8%)	4 (5.4%)	1
	40	34	74	

DISCUSSION:

For the DRB1* alleles, this study showed that the most frequent alleles is DRB1*03 followed by DRB1*11, and *07.

For comparison of this study with other studies, there are little published Iraqi studies for the HLA class II DRB1polymorphism. In a study done by Ad'hiah[11] in which he compared allele polymorphism in arab Iraqis with saudian,kuwaitain and Omanian groups, he found that DR2 is most common (25%) followed by DR4,DR3 and DR7.Another Iraqi study published at 2008[12] in which they compare the allele frequencies in patient with irritable bowel syndrome (IBS) and healthy control, (DR1,DR2,DR3,DR7) are the most frequent alleles in healthy control group with frequency of

(20%) while DR11 was (4.3%), in addition DR3 and DR8 play a protective role in IBS. The significance behind allele frequency is the disease association as for example; DR3 is highly associated with Type I D.M [13] and celiac disease is associated with DRB*03, and *07[9].

Regarding Arabian studies, in a Tunisian study [14], three more common different alleles were identified, DRB1*07,*11,*13, and *03 with (36%, 28%, 28%, 23%) respectively. In a Lebanese study, DRB1*11, DRB1*04 and DRB1*03 were the three most common DRB1* alleles observed[15].

For studies other than arabian one, an Armenian study [16] found that DRB1*1104 and DRB1*1501 were observed, followed by DRB1*1101 and DRB1*1401. An Iranian study [17] found that the most frequent allele is DRB1*07,*15 .Another study, [18] they found that the most frequent allele is DRB1*11,*03, and *07. There is a study reveals a close genetic relationship between Iraqis, Kurds, Caspian Iranians and Svani Georgians[19,20].

For the second expressed DRB alleles (DRB3,4,and 5) as shown in table III , Whether there is an age-related change from newborns toward adults has not been well studied because it was done on adult population, there are no significant difference between male and female adult regarding haplotype alleles frequency or hetro- and homozygosity. This is in agreement with a Korean study [21] that shows no significant difference in the HLA-DRB3/B4/B5 homozygosity and heterozygosity rates between male and females in both newborns and adults. In the comparison between newborns and adults, homozygosity rate was significantly higher in newborn females than in adult females (31.0% vs. 25.0%, p = 0.01). On the other hand, a study done on welsh male newborns for HLA-DRB3/B4/B5 heterozygote, they suggest a possibility of male-specific major histocompatibility complex (MHC)-mediated prenatal selection[22]. However, these controversial results may indicate that there might be some ethnic differences in the gender-specific prenatal selection events.

CONCLUSION:

1-The study showed that the most frequent alleles are DRB1*03,*11,*07.

2-There are no significant difference between male and female allele frequency of nonDRB1* alleles.

3-There are no significant difference between male and female for non DRB1* hetro or homozygosity.

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