Original Article

HLA Diversity in Iraqi Population: Molecular Typing

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Abstract:

Background: Different populations show various human leukocyte antigens (HLA). Data of HLA distribution is important in field of vaccines, therapy, Anthropology and for future studies of disease association with HLA.

Objective: To highlight on frequency of HLA alleles in Iraqi population by using molecular technique.

Patients and methods: Two hundred individuals were genotyped for HLA class I and II alleles by polymerase chain reaction sequence-specific oligonucleotides (PCR-SSO).

Results: This study observed that the alleles with highest frequency were: [A*02(27.75%,A*01(10.75%,A*03(8%),B*51(17.75%),B*35(9%),B*07(6%),C*04(26.75%),C*07(20.25%),C6*0(9.75%),DRB1*02(17.5%,DRB1*07(17%),DRB1*04(14.75%)DQB1*01(25.5%),DQB1*03(21.75%),DQB1*02(19%)].

Conclusion: Current study give additional information about HLA distribution in Iraqi Arab society and could be provide standard control for future studies about HLA association with disease in Iraq.

Key words:; HLA; Genotyping; PCR.

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Introduction:

Human leukocyte antigen (HLA) is human immune-genetic system characterized by polymorphism. The primary function of HLA is the regulation of the immune system by present processed peptide to T-cell. It is associated with certain diseases with unknown specific mechanisms (1,2).

The HLA system is consisted of three regions, the class I region contains the classical HLA-A, B, and-C genes, while the class II region contains the classical HLA-DR, -DP,-DQ genes. The class III region include genes for complement components (C2, C4, factor B), 21-hydroxylase, tumor necrosis factors (TNFs), and some others (3,4).

Alleles of HLA are inherited as an HLA haplotype in a Mendelian law from each parent. Certain HLA haplotypes are present more frequently in some populations than other, for example, HLA-A1, B8, DR17 is the most presence HLA haplotype in Caucasians (2).

Certain HLA-alleles are common while other alleles vary in frequency among various ethnic groups. For example, HLA-A1 antigen occurs in 15 % of Caucasians, 3.3 % of African Black, 0.5 % of Japanese (5), while in Iraqi people occur in 22.92 % (6).

Differences in the frequency of HLA alleles may participate in geographic differences of the susceptibility to different diseases, such as infectious, metabolic and autoimmune diseases (7).

This study was conducted to highlight on frequency of HLA alleles in Iraqi Arab population by using molecular technique.

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Patients and methods:

Two hundred unrelated apparently healthy Iraqi Arab persons were referred to tissue typing laboratory in Al-Karamah Teaching Hospital for the purpose of organ donation from 2011 till 2015 were included in this study. Two ml of blood sample was collected from each individual put in EDTA tube for DNA extraction.

The DNA was extracted for HLA genotyping. The HLA genotyping done by PCR-SSO method according to the manufacturer's instructions in the HLA-typing laboratory of Al-Karama teaching hospital, Baghdad (PCR-SSO kit: Innogenetics-Line Probe Assay, INNO—LiPA; Belgium). INNO-LiPA method depends on reverse hybridization. Biotinylated DNA was amplified, chemically denatured, and separated strands were hybridized with specific oligonucleotide probes immobilized on membrane-based strips as parallel lines. Streptavidin conjugated with alkaline phosphatase has been added and bound to any biotinylated hybrid that previously formed. After that the substrate solution containing a chromogen will be added results in a purple/brown precipitate and the reactivity pattern of the probes was recorded.

Statistical analysis: The results were presented in terms of percentage frequencies and gene frequency. The gene frequency was calculated as following (8):

$S=1-\sqrt{1-AG}$

S=gene frequency

AG=frequency of certain allele

Results:

The frequency distribution of various class I and class II HLA-alleles for unrelated Iraqi Arab individuals are presented

in tables (1,2.3,4,5).This study showed that the highest frequencies were for the following alleles: [A*02 (27.75 %,A* 01(10.75%,A*03(8%),B*51(17.75%),B*35(9%),B*07(6%),C *04(26.75%),C*07(20.25%),C6*0(9.75%),DRB1*02(17.5%, DRB1*07(17%),DRB1*04(14.75%)DQB1*01(25.5%),DQB 1*03(21.75%),DQB1*02(19%)].

In addition the alleles with minimal frequencies were [A*36(0.25%, A*69(0.25%), B*81(0.25%),B*82(0.25%),C* 17(1.25%),C*18(1.25%),DRB1*17(0.50%),DQB1*8(0.50%) and DQB1*9(0.50%).

Table-1: Distribution of HLA-A alleles in Iraqi Arab population.

HLA-A alleles	No. of Positive cases	%	Gene frequency	HLA-A alleles	No. of Positive cases	%	Gene frequency
A*01	43	10.75	0.05	A*31(19)	18	4.5	0.023
A*02	111	27.75	0.149	A*32(19)	9	2.25	0.012
A*03	32	8	0.04	A*33(19)	13	3.25	0.017
A*9	2	0.50	0.003	A*34(10)	4	1	0.006
A*24(9)	31	7.75	0.04	A*36	1	0.25	0.002
A*23(9)	13	3.25	0.017	A*66(10)	11	2.75	0.014
A*30(19)	30	7.50	0.039	A*68(28)	12	3	0.016
A*25(10)	3	0.75	0.004	A*69(28)	1	0.25	0.002
A*26(10)	19	4.75	0.024	A*74(19)	13	3.25	0.017
A*29(19)	9	2.25	0.012	A*80	2	0.50	0.003
A*11	23	5.75	0.029				
TOTAL					400		

Table-2: Distribution of HLA-B alleles in Iraqi Arab population.

HLA-B alleles	No. of Positive cases	%	Gene frequency	HLA-B alleles	No. of Positive cases	%	Gene frequency
B*07	24	6	0.031	B*48	2	0.50	0.003
B*08	29	7.25	0.073	B*49(21)	16	4	0.021
B*13	15	3.75	0.019	B*50(21)	22	5.5	0.028
B*14	4	1	0.006	B*51(5)	71	17.75	0.089
B*15	24	6	0.031	B*52(5)	13	3.25	0.017
B*17	2	0.50	0.003	B*53	4	1	0.006
B*18	16	4	0.021	B*54(22)	3	0.75	0.004
B*27	12	3	0.016	B*55(22)	8	2	0.011
B*35	36	9	0.047	B*56(22)	4	1	0.006
B*37	10	2.5	0.013	B*57(17)	3	0.75	0.004
B*38(16)	11	2.75	0.014	B*58(17)	9	2.25	0.012
B39(16)	9	2.25	0.012	B*59	2	0.50	0.003
B*40	16	4	0.021	B*60(40)	2	0.50	0.003
B*41	10	2.5	0.013	B*78	3	0.75	0.004
B*44(12)	14	3.50	0.018	B*81	1	0.25	0.002
B*45(12)	2	0.50	0.003	B*82	1	0.25	0.002
B*46	2	0.50	0.003				
TOTAL					400		

Table-3: Distribution of HLA-C alleles in Iraqi Arab population.

HLA-C alleles	No. of Positive cases	%	Gene frequency
C*01	12	3	0.016
C*02	25	6.25	0.032
C*03	21	5.25	0.027
C*04	107	26.75	0.145
C*05	25	6.25	0.032
C*06	39	9.75	0.05
C*07	81	20.25	0.107
C*08	18	4.50	0.023
C*12	38	9.50	0.049
C*14	8	2	0.011
C*15	8	2	0.011
C*16	8	2	0.011
C*17	5	1.25	0.007
C*18	5	1.25	0.007
TOTAL	400 (NO. of individuals=200)		

Table-4: Distribution of HLA-DR alleles in Iraqi Arab population.

HLA-DR-B1 alleles	No. of Positive cases	%	Gene frequency
DR*01	40	10	0.052
DR*02	70	17.5	0.089
DR*03	45	11.25	0.057
DR*04	59	14.75	0.073
DR*07	68	17	0.089
DR*08	13	3.25	0.017
DR*9	3	0.75	0.004
DR*10	7	1.75	0.009
DR*11 (5)	15	3.75	0.019
DR*12 (5)	6	1.50	0.008
DR*13(6)	15	3.75	0.019
DR*14(6)	18	4.50	0.023
DR*15(2)	21	5.25	0.027
DR*16(2)	18	4.50	0.023
DR*17 (3)	2	0.50	0.003
TOTAL	400 (NO. of individuals=200)		

Table-5: Distribution of HLA-DQ alleles in Iraqi Arab population.

HLA-DQ –B1 alleles	No. of Positive cases	%	Gene frequency
DQ*01	102	25.5	0.134
DQ*02	76	19	0.10
DQ*03	87	21.75	0.11
DQ*04	35	8.75	0.045
DQ*05(1)	18	4.50	0.023
DQ*06(1)	69	17.25	0.089
DQ*07(3)	9	2.25	0.012
DQ*08(3)	2	0.50	0.003
DQ*9(3)	2	0.50	0.003
TOTAL	400 (NO. of individuals=200)		

Discussion

It is well known that there are many studies regarding HLA association with different diseases in Iraq, but few studies were available regarding the distribution of HLA in Iraqi population. Current findings found that there are high frequencies of some alleles of class I and II of HLA among Iraqi population [A*02 (27.75 %,A*01(10.75%,A*03(8%),B*51(17.75%),B*35(%), B*07(6%),C*04(26.75%),C*07(20.25%),C6*0(9.75%),DR B1*02(17.5%,DRB1*07(17%),DRB1*04(14.75%)DQB1* 01(25.5%),DQB1*03(21.75%),DQB1*02(19%)]. and these results confirm our previous study in 2005 done by serotyping method (Microlymphocytotoxicity assay) which found that the highest frequencies of HLA-A alleles were: HLA-A2 (38.48%, A1 (22.92%), and A3 (19.6%), in addition there were the same tabulation regarding the highest frequencies for HLA-B, HLA-C HLA-DR and HLA-DQ alleles as following: B51(20%), B35 (19.8%), CW4(23.4%),CW7(19.28%), DR2 (27.75%), DR3 (27.25%), DQ1(23.25%) and DQ3 (23.25%) (6). Moreover, other Iraqi study conducted by Ad (hiah in2009 was investigate the HLA- (A, B, DR and DQ) antigens by serotyping method (not molecular typing method as present study) in 145 non related Iraqi individuals revealed that the highest frequencies were: A19,A2, A9, B5,B35,B12 regarding HLA-class I antigens while for HLA class II antigens were DR2,DR1,DR4,DQ2,DQ3,DQ1 (9). The results of above study in comparison with this study showed some variation concerning HLA-A alleles, the most frequent allele in Iraq was A2 in present study while in Ad (hiah)s study was A19. On the other hand the HLA-B and HLA-DR-alleles of both studies showed approximated results regarding the first two alleles which have the highest frequency while other alleles

showed minor differences. The picture of HLA-DQ of both studies revealed some differences and this may be due to the disadvantages of serotyping method of HLA-typing especially with low size of samples as in Ad (hiah)s study there are more than half of alleles give blank (no results). Also current result approximate to other local study (HLA typing done for HLA- class I-A and B only) about the distribution of A2 and B 51 antigen with frequency: (38.7 %) ,(20.5%) respectively (10). As mentioned in reference no.9, the frequencies of HLA-antigens in 145 Iraqi individuals were compared with the HLA frequencies in three populations of Kuwaitis, Saudis and Omanis. Antigens of HLA-A present no significant difference, while the antigens of HLA-B, DR and DQ showed a significant difference in distribution in these populations, especially B and DR antigens.These findings point to differences in the origins of these four communities, brought about possibly by an evolutionary recent admixture of the original inhabitants with neighboring and distant populations, although a common ancestor is clear and a later divergence had occurred during evolution (9). The present study show clear variation when compared with Saudis (11), Turkish (12), Indian (13) and other communities. The reason of race variation may be in partial due to gene drift or by gene flow (14). Current work confirm the concept of race variation, some alleles occur at higher or lower distribution in Iraqis as compared with other population. in Conclusion Current study give additional information about HLA distribution in Iraqi Arab society and could be provide standard control for future studies about HLA association with disease in Iraq.

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