DISTRIBUTION OF HLA POLYMORPHISM IN A SAMPLE OF IRAQI ARABS IN COMPARISON WITH THREE ARAB GULF POPULATIONS

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Abstract

The HLA-class I (A and B) and -class II (DR and DQ) alleles were investigated in 145 (72 males and 73 females) unrelated Iraqi Arabs, and their percentage frequencies were compared with the corresponding frequencies in three populations of the Arabian Gulf region (Kuwaitis, Saudis and Omanis). At HLA-A locus, the distribution of the alleles showed no significant difference, while alleles of HLA-B, -DR and -DQ loci showed a significant different distribution in the four populations, especially, B and DR loci. These findings point to differences in the origins of these four distinct Arabic-speaking 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.

توزيع تعدد الأشكال الوراثي لمستضدات خلايا الدم البيض البشرية (HLA) في عينة من العراقيين العرب وبالمقارنة مع ثلاث مجاميع سكانية خليجية

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الخلاصة

درست أليلات نظام مستضدات خلايا الدم البيض البشرية من الصنف الأول (A و B) والصنف الثاني (DR و DQ) في 145 (72 ذكور و 75 إناث) من العراقيين العرب، وقورنت نسبها المئوية مع نظائرها في ثلاث مجاميع سكانية خليجية (كويتيين وسعوديين وعمانيين). لم تظهر تكرارات الأليلات في الموقع A فروقا معنوية بين المجاميع السكانية المدروسة، إلا أن أليلات المواقع B و DR و DQ قد اختلفت معنويا بين هذه المجاميع وبالأخص B و DR. تبين هذه النتائج الاختلافات الأصولية لهذه المجاميع التي تتكلم اللغة العربية والناتجة من مزج تطوري حديث للسكان الأصليين مع مجاميع سكانية مجاورة وأخرى بعيدة، بالرغم من وجود أصل مشترك ومن ثم حصل انحراف خلال التطور.

Introduction

Human genetic diversity has long been a subject of interest, and has important implications for human evolution and distribution of genetic diseases in populations, and in this regard, polymorphism provides the genetic markers for linkage analyses of many hereditary diseases, and leads to the prospect of a high resolution map of the human genome (1). Furthermore, the analysis of diversity of marker polymorphisms in various populations has been applied to important questions about the natural history of human origin and migration, and has provided the foundation for the proposed human

diversity project, which promises to describe the extent and character of molecular genetic variation in divergent human populations (2). In agreement with such theme, the highly polymorphic major histocompatibility complex (MHC), which is called HLA system in human, has been validated as useful for distinguishing and/or relating populations (and individuals) in many papers and in all the subsequent international workshops since the First International HLA Anthropology Workshop (3).

The HLA genes encode cell surface proteins endowed with a peptide-binding region that captures short protein fragments and presents them to thymus-derived lymphocytes. The HLA peptide complex is recognized by the T-cell receptor, and the recognition of foreign peptides sets a train of events into motion that leads to a clonal immune response against the pathogen from which the peptides are derived (4). The hallmark of functional HLA genes is their polymorphism, which is characterized by long persistence of allelic lineages and large number of nucleotide differences between alleles (3, 4). Some of the allelic lineages now found in the human population may have been established as long ago as 85 million years, before the divergence of prosimians and anthropoid primates, while other lineages may be younger, but they all predate the emergence of *Homo sapiens* (5). The number of nucleotide differences between alleles varies depending on the particular pair of alleles compared but may be as high as 50-60 nucleotides. The extent of genetic polymorphism is still being determined but currently at least 59,126 and 36 different alleles are known for HLA-A, -B and -C loci, respectively (HLA-class I region). Similarly, 135, 25, and 65 different alleles have been recognized for the respective HLA class II genes; HLA-DR, -DQ and -DP (6). In most populations, a few alleles are frequent (gene frequency >10%) but most of them occur at low frequency (less than10%) and a number of the latter may be rare (gene frequency <1%).

As is the case for other genetic polymorphisms, the frequency of HLA alleles differs among populations around the globe, and 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 (7). Accordingly, the present study was aimed to investigate the polymorphism of HLA system in a sample of Iraqi Arabs in regard to other three Arab Gulf populations.

Subjects, Materials and Methods

One hundred and forty five (72 males and 73 females) unrelated Iraqi Arabs were investigated during the period 2003-2006. Their age range was 20-40 years (30 ± 5 years), and they comprised blood and potential kidney donors from all parts of Iraq. Venous blood (10 ml) was drawn in a heparinized tube, and then it was subjected to a density gradient centrifugation using lymphoprep as a separating medium to collect lymphocytes. The collected cells were further separated into T and B lymphocytes using the nylon wool method. T cells were phenotyped for HLA-class I alleles (A and B), while B cells were employed in the phenotyping of HLA-class II alleles (DR and DQ) in the microlymphocytotoxicity test (8), using a panel of monoclonal antibodies (Biotest Company, Germany) that were able to recognize 8 HLA-A (A*1, A*2, A*3, A*9, A*10, A*11, A*19 and A*28), 22 HLA-B (B*5, B*7, B*8, B*12, B*13, B*14, B*15, B*16, B*17, B*18, B*21, B*22, B*27, B*35, B*37, B*40, B*41, B*42, B*48, B*53, B*59 and B*73), 9 HLA-DR (DR*1, DR*2, DR*3, DR*4, DR*5, DR*6, DR*7, DR*8 and DR*10) and 4 HLA-DQ (DQ*1, DQ*2, DQ*3 and DQ*4) alleles on the surface of the tested lymphocytes. The laboratory manipulations were carried out at the Tissue Typing Laboratory of Al-Karama Teaching Hospital.

The recorded alleles were presented in terms of percentage frequencies, together with the corresponding frequencies in three Arab Gulf populations (Kuwaitis, Saudis and Omanis) that have been reviewed by White and co-workers (9). These frequencies were compared with Chi-square (X^2) test to assess significant differences between populations, in which the probability was corrected (Pc) for the number of alleles tested at each locus. The analysis was carried out using the computer programme Smith's Statistical Package (SSP) version 2.80.

Results

HLA-A alleles showed a non-significant distribution in the four populations, although some variation was observed (Table 1). The

most frequent allele in Iraqi population was A*19 (42%), while it was a less frequent in Kuwaitis (28%), Saudis (36%) and Omanis (36%). However, the latter three populations were more characterized with the allele A*2 (36, 38 and 40%, respectively), which showed a lower frequency in Iraqis (31%), but it was interesting to note that A*2 showed a gradual increased frequencies as you move from Iraq to Oman. The allele A*28 was the less frequent one in Iraqis (8%), and such picture was shared with the Omanis (10%), while it was almost doubled in Kuwaitis and Saudis (18 and 19%, respectively).

Table 1: Percentage frequencies of HLA-A alleles in Iraqis, Kuwaitis, Saudis and Omanis.

HLA-A Alleles	Percentage Frequency			
	Iraqis (No.= 145)	Kuwaitis (No.= 100)	Saudis (No.= 1661)	Omanis (No.= 321)
A*1	15	26	16	15
A*2	31	36	38	40
A*3	16	14	14	10
A*9	22	23	29	14
A*10	15	10	12	18
A*11	19	11	7	20
A*19	42	28	36	36
A*28	8	18	19	10

X2 = 32.978; D.F. = 21; P = 0.046; Pc = Not significant

In contrast, HLA-B alleles were more divergent in the four populations, and their distribution was observed with a highly significant difference ($Pc = 2.2 \times 10^{-6}$), although a common theme between them was noticed in relation to B*5, which was the most frequent allele and showed approximated frequencies with minor differences (32, 27, 26 and 39% respectively), however, other alleles contrasted such theme. The allele B*12 shared a similar frequency in Iraqis and Kuwaitis (12%), while its frequency in Saudis and Omanis was much lower and similar (6 and 5%, respectively). The other allele was B*17, which was an exceptional to note that its frequency was 2% in Iraqis, while it shared a similar frequency in Kuwaitis and Saudis (11%), and a much higher frequency was observed in Omanis (21%). The profile of B*17 was applicable for B*40, which was a rare allele in Iraqis (0.7%), but its frequency in Kuwaitis, Saudis and Omanis was 8, 4 and 15%, respectively (Table 2).

Table 2: Percentage frequencies of HLA-B alleles in Iraqis, Kuwaitis, Saudis and Omanis.

HLA-B Alleles	Percentage Frequency			
	Iraqis (No.= 145)	Kuwaitis (No.= 100)	Saudis (No.= 1661)	Omanis (No.= 321)
B*5	32	27	36	39
B*7	8	8	13	4
B*8	9	6	14	18
B*12	12	12	6	5
B*13¶	3	3	3	3
B*14¶	6	6	5	4
B*15¶	10	8	8	6
B*16¶	4	NT	6	4
B*17	2	11	11	21
B*18¶	6	1	3	6
B*21	3	12	33	8
B*22¶	0.7	4	3	5
B*27¶	6	4	2	0.3
B*35	19	19	18	30
B*37¶	1	NT	2	2
B*40	0.7	8	4	15
B*41¶	9	NT	6	1
B*42¶	0.7	NT	2	1
B*48¶	0.7	NT	NT	0.3
B*53¶	9	NT	5	3
B*59¶	0.7	NT	NT	NT
B*73¶	0.7	NT	NT	NT

$X^2 = 73.241$; D.F. = 21; $P = 1 \times 10^{-7}$; $Pc = 2.2 \times 10^{-6}$ (Significant)

¶: Not included in Chi-square analysis; NT: Not tested

The picture of divergence was much clearer when the alleles of DR locus were considered, and almost each population was characterized with a single allele as a predominant one. The most frequent allele in Iraqis was DR*2 (25%), while the correspondent most frequent alleles in Kuwaitis, Saudis and Omanis were DR*5 (29%), D*R7 (41%) and DR*2 (66%), respectively. Such differences contributed to the significant distribution (Pc = 1.52 x 10^{-12}) of DR alleles in these populations (Table 3).

At HLA-DQ locus, the analysis was limited to DQ*1 and DQ*3 alleles, because the data of DQ*2 allele was not given for the Saudi sample, while DQ*4 was not tested in Kuwaitis, Saudis and Omanis. The distribution of DQ*1 and DQ*3 alleles was significantly (Pc = 0.012) different in the four populations (Table 4). The lowest frequency of DQ*1 was observed in Iraqis (17%), while the highest frequency was recorded in Omanis (76%), and between these two ranges, the Kuwaitis and Saudis shared approximated frequencies (57 and 51%, respectively). For DQ*3, the two extreme frequencies (lowest and highest) were observed in Iraqis (18%) and Saudis (46%), while Kuwaitis and Omanis shared a similar frequency (27%).

Table 3: Percentage frequencies of HLA-DR alleles in Iraqis, Kuwaitis, Saudis and Omanis.

HLA-DR Alleles	Percentage Frequency			
	Iraqis (No.= 145)	Kuwaitis (No.= 100)	Saudis (No.= 425)	Omanis (No.= 283)
DR*1	21	13	14	7
DR*2	25	26	19	66
DR*3	16	28	26	29
DR*4	18	13	31	13
DR*5	10	29	14	18
DR*6	3	3	18	8
DR*7	16	28	41	11
DR*8¶	9	3	3	1
DR*10¶	2	6	1	4

X^2 = 100.393; D.F. = 18; P = 1.9 x 10^{-13}; Pc = 1.52 x 10^{-12} (Significant)
¶: Not included in Chi-square analysis.

Table 4: Percentage frequencies of HLA-DQ alleles in Iraqis, Kuwaitis, Saudis and Omanis.

HLA-DQ Alleles	Percentage Frequency			
	Iraqis (No.= 145)	Kuwaitis (No.= 100)	Saudis (No.= 425)	Omanis (No.= 283)
DQ*1	17	57	51	76
DQ*2¶	21	12	NT	36
DQ*3	18	27	46	27
DQ*4¶	10	NT	NT	NT

X2 = 13.639; D.F. = 3; P = 0.003; Pc = 0.012 (Significant)
¶: Not included in Chi-square analysis; NT: Not tested

Discussion

To shed light on the genetic variation of HLA-class I and -class II alleles in Iraqi Arabs, the scope was presented in comparison with the frequencies of these alleles in three regional related Arab populations (9), which represent an ascending line starting from the north of Arabian Gulf (Iraq) down to the south passing through Kuwait, Saudi Arabia and finally Oman. Comparative analysis of the HLA-A, -B, -DR and -DQ alleles revealed differences in the

distribution of these alleles among the four populations, and such findings suggest ancient and recent admixtures between these populations (2, 3, 5). In this regard, B, DR and DQ alleles were more informative, and some of these alleles showed shared frequencies, while others may characterize each of the investigated populations. At HLA-B locus, the populations of Iraq and Kuwait shared approximated frequencies of five alleles (B*7, B*12, B*13, B*14 and B*15), while in Saudis and Omanis, the number of alleles was much higher (B*5, B*12, B*13, B*14, B*16, B*37 and B*42), but it is interesting to note that some alleles shared approximated frequency in the four populations; for instance B*5. These findings suggest the common ancestor of these neighbored populations, while alleles of HLA-DR locus may reflect the divergent of the populations due to evolution and internal admixture, and these alleles were DR*2 (Iraqis and Omanis), DR*5 (Kuwaitis) and DR*7 (Saudis).

These observations have significant implications for analyzing human migration trends throughout history, especially if we consider that the four populations share an open land with no regional barrier between them. Furthermore, the deviated alleles, as well as, other HLA alleles have shown different associations with diseases, especially autoimmune disorders, and therefore the implication of their frequencies may have an important impact on the predisposition to these diseases (8).

Studies of the HLA origin of Arabs are not overwhelming, but studies of related eastern and western Mediterranean groups including Turks, Kurds and others suggest a common ancestry (10). Variation in allelic distribution of HLA among these populations can be explained by the admixture of the ancient population with invading and migratory societies, which may have given rise to present-day racial and ethnic groups (3, 5).

In view of the heterogeneity of the Arab populations, which comprise people of distinct ethnic backgrounds and whose origins can be classified according to their area of habitation (North Africa, Arabian peninsula, and eastern Mediterranean), the present study elucidated the diversity in HLA allele distribution among four distinct Arab communities located in the Arabian Gulf, and therefore this study provided basic information for further studies of the MHC differences between Arabs of distinct origins and may serve as a reference for further anthropological studies, as well as, for studies of associations between HLA and diseases. Again, the data presented here in defining the HLA profile among Arabian Peninsula Arabs, point to differences in the origins of these four distinct Arabic-speaking 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. Therefore, these results provide information that can be used for future anthropological studies and also in the analysis of disease susceptibility and organ transplantations (11).

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