

Distribution of HLA – Antigens Class I and II in Iraqi Arab population

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Abstract:

Human leukocyte antigen (HLA) is the most polymorphic genetic system in man, various populations display various frequencies of HLA alleles. The aim of this work is to establish a database for HLA-profile in Iraqi Arab population which form most of Iraqi people. The antigen and gene frequencies of HLA for Iraqi Arab, apparently healthy, unrelated individuals were evaluated. The work involves testing 2500 persons for class I and 400 for class II by using microlymphocytotoxicity assay. The antigens with highest frequencies were A₂ (38.48%) A₁ (22.92%) , B₅₁ (20%) , B₃₅(19.8%), C W₄ (23.4%) , C W₇ (19.28%) , D R₂ (27.75%) , D R₃(27.25%) , D Q₁ (23.25%) and D Q₃ (23.25%) . These findings may be considered as a data base for HLA profile of Iraqi Arab population .

Introduction:

Human leukocyte antigen system contains a number of closely linked genes located on the short arm of chromosome 6 (1) , and represent about 1% of the whole genome (2), the HLA region has been subdivided into class I, class II and class III region. Each region contains numerous gene loci and each locus may encode a large number of polymorphic alleles. The classical class I HLA-Ags include HLA-A, B and C antigens which are expressed on almost all cells of body.

The classical class II antigens are further divided into DR, DP and DQ antigens and constitutively expressed on B-cells and monocytes as well as can be induced during inflammation on many other cell types which normally have little or no expression (3). The class III region contains several components of the complement system, C4, C2 and BF (4).

HLA genes are segregate en bloc to the offspring, furthermore, those linked genes which reside on one of the pair of homologous chromosomes, and that segregate to the offspring is called haplotype. Each individual inherits two HLA haplotypes- one from each parent. Although the HLA genes are closely linked, across over has been reported in a number of families (5).

Many HLA alleles are found in all ethnic groups although some alleles differ in frequency between different ethnic groups(6,7) for example , the HLA - A1 allele occurs predominantly in Caucasians at frequency of (15%) while in African black (3.3%) and its virtually absent in Japanese people(0.5%) (8).

Iraqi people of over 25 million is composed of many ethnic groups , Arab forms the major part of these groups . They are not uniformly distributed in all over Iraqi geographical area , therefore , this work was performed to establish standard line for HLA class I and II in Iraq among Arab nation both Muslims and Christians .

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Subjects and Methods:

Subjects:

Unrelated , apparently healthy Iraqi Arab individuals (2500 for class I and 400 for class II) were randomly chosen . They were refereed to immunology and tissue typing center in Al-Karamah teaching hospital and to Baghdad medico – legal institute for paternity test. All cases were registered in the above medical centers .

Methods:

HLA – Typing:

Microlymphocytotoxicity assay has been applied for HLA – typing as described by (Terasaki and McClelland, 1964) (9) and modified by(Dick et al.,1979 and Bender, 1984) (10, 11).

Principle of Test:

Ten ml of the healthy individuals blood is heparinized. The lymphocyte cells are purified by ficoll hypaque gradient centrifugation. HLA-antisera react with the correspondent membrane- bound antigens on lymphocytes. The addition of rabbit complement results in a structural changes of the cell membrane which leads to a penetration of an indicator dye. Stained lymphocyte = positive reaction, unstained cells = negative reaction, The lysed and vital lymphocytes are assessed using an inverse phase contrast microscope.

Statistical Analysis:

The gene frequency can be calculated from the following formula (12):

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

S= gene frequency

AG= frequency of certain allele

Results:

The distribution of HLA class I and class II antigen and gene frequencies for Iraqi population (2500 for class I and 400 for class II) are shown in tables (1,2,3,4 and 5) .

The antigens with the highest frequencies were: A₂ (38.48%) , A1 (22.92%) , B₅₁ (20%) B₃₅ (19.8%),Cw₄ (23.4%) , Cw₇ (19.28%) , DR₂(27.75%) , DR₃ (27.25%) , DQ₁ (23.25%) and DQ₃ (23.25%).

The least frequent antigens were A₃₆ (0.48%) , B₆₄ (0.04%) , Cw₈ (0.76%) , DR₈ (0.25%) and DQ₄ (10%) , so these antigens may be considered as rare variants in Iraqi Arab population .

Current results were tabulated in relation to studies from other countries . Tables (6&7) represent HLA antigen frequencies among normal Iraqi Arab population and few neighborhood and other nations .

Table – 1: Antigen and gene Frequencies of HLA – A alleles in Iraqi Arab population .

| HLA – A Alleles | Positive panel | | Antigen Frequency | Gene Frequency |
|--------------------|----------------|-------|----------------------|-------------------|
| | No | % | | |
| 1 | 573 | 22.92 | 0.2292 | 0.12 |
| 2 | 962 | 38.48 | 0.3848 | 0.21 |
| 3 | 490 | 19.6 | 0.196 | 0.103 |
| 11 | 297 | 11.88 | 0.1188 | 0.06 |
| 23 (9) | 164 | 6.56 | 0.0656 | 0.03 |
| 24 (9) | 457 | 18.28 | 0.1828 | 0.09 |
| 25 (10) | 38 | 3.32 | 0.0332 | 0.016 |
| 26 (10) | 278 | 11.12 | 0.1112 | 0.057 |
| 28 | 300 | 12 | 0.12 | 0.06 |
| 29 (19) | 119 | 4.76 | 0.0476 | 0.02 |
| 30 (19) | 320 | 12.8 | 0.128 | 0.06 |
| 31 (19) | 70 | 2.8 | 0.028 | 0.014 |
| 32 (19) | 100 | 4 | 0.04 | 0.02 |
| 33 (19) | 248 | 9.92 | 0.0992 | 0.05 |
| 36 | 12 | 0.48 | 0.0048 | 0.002 |
| Blank | 572 | 22.88 | ----- | ----- |
| Total | 5000 | ----- | ----- | ----- |

Table – 2 : Antigen and gene Frequencies of HLA – B alleles in Iraqi Arab population .

| HLA – B Alleles | Positive panel | | Antigen Frequency | Gene Frequency |
|--------------------|----------------|-------|----------------------|-------------------|
| | No | % | | |
| 7 | 256 | 10.2 | 0.102 | 0.05 |
| 8 | 238 | 9.52 | 0.0952 | 0.04 |
| 13 | 167 | 6.68 | 0.0668 | 0.03 |
| 12 | 14 | 5.6 | 0.0056 | 0.002 |
| 14 | 123 | 4.92 | 0.0492 | 0.02 |
| 17 | 154 | 6.16 | 0.0616 | 0.03 |
| 18 | 160 | 6.4 | 0.064 | 0.03 |
| 27 | 86 | 3.44 | 0.0344 | 0.017 |
| 35 | 495 | 19.8 | 0.198 | 0.10 |
| 37 | 33 | 1.32 | 0.0132 | 0.006 |
| 38 (16) | 177 | 7.08 | 0.0708 | 0.03 |
| 39 (16) | 49 | 1.96 | 0.0196 | 0.009 |
| 40 | 68 | 2.72 | 0.0272 | 0.013 |
| 41 | 299 | 11.96 | 0.1196 | 0.06 |
| 42 | 13 | 0.52 | 0.0052 | 0.002 |
| 44 (12) | 224 | 8.96 | 0.0896 | 0.04 |
| 45 (12) | 47 | 1.88 | 0.0188 | 0.009 |
| 47 | 23 | 0.92 | 0.0092 | 0.004 |
| 49 (21) | 201 | 8.04 | 0.0804 | 0.04 |
| 50 (21) | 319 | 12.76 | 0.1276 | 0.06 |
| 51 (5) | 500 | 20 | 0.2 | 0.105 |
| 52 (5) | 127 | 5.08 | 0.0508 | 0.02 |
| 53 | 96 | 3.84 | 0.0384 | 0.01 |
| 55 (22) | 90 | 3.6 | 0.036 | 0.01 |
| 57 (17) | 28 | 1.12 | 0.0112 | 0.005 |
| 60 (40) | 14 | 0.56 | 0.0056 | 0.002 |
| 62 (15) | 52 | 2.08 | 0.0208 | 0.01 |
| 63 (15) | 58 | 2.32 | 0.0232 | 0.01 |
| 64 (14) | 1 | 0.04 | 0.0004 | 0.0002 |
| 65 (14) | 14 | 0.56 | 0.0056 | 0.002 |
| 70 | 33 | 1.32 | 0.0132 | 0.006 |
| 73 | 8 | 0.32 | 0.0032 | 0.001 |
| Blank | 833 | 33.32 | ----- | ----- |
| Total | 5000 | ----- | ----- | ----- |

Table – 3 : Antigen and gene Frequencies of HLA – Cw alleles in Iraqi Arab population .

| HLA – Cw Alleles | Positive panel | | Antigen Frequency | Gene Frequency |
|---------------------|----------------|--------|----------------------|-------------------|
| | No | % | | |
| 1 | 82 | 3.28 | 0.0328 | 0.016 |
| 2 | 168 | 6.72 | 0.0672 | 0.02 |
| 3 | 133 | 5.32 | 0.0532 | 0.02 |
| 4 | 585 | 23.4 | 0.234 | 0.12 |
| 5 | 57 | 2.28 | 0.0228 | 0.01 |
| 6 | 316 | 12.64 | 0.1264 | 0.06 |
| 7 | 482 | 19.28 | 0.1928 | 0.10 |
| 8 | 19 | 0.76 | 0.0076 | 0.003 |
| Blank | 3158 | 126.32 | ----- | ----- |
| Total | 5000 | ----- | ----- | ----- |

Table -4 : Antigen and gene Frequencies of HLA – DR alleles in Iraqi Arab population .

| HLA – DR Alleles | Positive panel | | Antigen Frequency | Gene Frequency |
|---------------------|----------------|-------|----------------------|-------------------|
| | No | % | | |
| 1 | 76 | 19 | 0.19 | 0.1 |
| 2 | 111 | 27.25 | 0.2775 | 0.149 |
| 3 | 109 | 27.25 | 0.2725 | 0.14 |
| 4 | 85 | 21.75 | 0.2125 | 0.11 |
| 5 | 31 | 7.75 | 0.0775 | 0.03 |
| 6 | 35 | 8.75 | 0.0875 | 0.04 |
| 7 | 88 | 22 | 0.22 | 0.11 |
| 8 | 51 | 12.75 | 0.1275 | 0.062 |
| 10 | 22 | 5.5 | 0.055 | 0.02 |
| 11 | 22 | 5.5 | 0.055 | 0.02 |
| 12 | 4 | 1 | 0.01 | 0.005 |
| 14 | 12 | 3 | 0.03 | 0.01 |
| Blank | 154 | 38.5 | ----- | ----- |
| Total | 800 | ----- | ----- | ----- |

Table -5 : Antigen and gene Frequencies of HLA – DQ alleles in Iraqi Arab population .

| HLA – DQ Alleles | Positive panel | | Antigen Frequency | Gene Frequency |
|------------------|----------------|-------|-------------------|----------------|
| | No | % | | |
| 1 | 93 | 23.25 | 0.2325 | 0.12 |
| 2 | 82 | 20.5 | 0.205 | 0.1 |
| 3 | 93 | 23.25 | 0.2325 | 0.23 |
| 4 | 40 | 10 | 0.1 | 0.05 |
| Blank | 492 | 123 | ----- | ----- |
| Total | 800 | ----- | ----- | ----- |

Table – 6 : The highest HLA- A,B and Cw antigen Frequencies among normal Iraqi Arab population and Some Iraqi neighborhood nations .

| Iraq * | | Saudi | | Khorasan province (Iran) | | Turkey | |
|----------------|-------|---------|--------|---------------------------|------|---------|-------|
| (This Study) | | (13) | | (14) | | (15) | |
| Antigen | % | Antigen | % | Antigen | % | Antigen | % |
| A2 | 38.48 | A2 | 43.60 | A2 | 33 | A2 | 56 |
| A1 | 22.92 | A29 | 27.50 | A9 | 31 | A9 | 36.42 |
| A3 | 19.6 | A24 | 20.20 | All | 30.5 | A19 | 26.78 |
| A24 | 18.28 | A1 | 19.90 | A3 | 30 | A10 | 22.54 |
| A30 | 12.8 | A3 | 17.10 | A1 | 21.8 | A1 | 21.22 |
| A28 | 12 | A28 | 15.10 | A10 | 21 | A3 | 17.28 |
| B51 | 20 | B 50 | 37-60 | B5 | 42 | B5 | 33.63 |
| B35 | 19.8 | B 51 | 25. 70 | B21 | 18.5 | B35 | 32.71 |
| B50 | 12.76 | B 35 | 19.30 | B 13 | 14.3 | B 21 | 21 |
| B41 | 11.96 | B8 | 14.70 | B7 | 14 | B12 | 16.20 |
| B7 | 10.2 | B7 | 11.40 | B27 | 13.8 | B 40 | 10.04 |
| Cw4 | 23 | Cw4 | 48.70 | Cw4 | 15.3 | ----- | ----- |
| Cw7 | 19.28 | Cw2 | 19.90 | Cw1 | 14.7 | ----- | ----- |

* *This Study* : (2500 person) .

(13) *Ollier et al . (1985)* : (1145 Person) .

(14) *Farid et al . (1989)* : (1000 Person) .

(15) *Sheth et al . (1985)* : (117 Person)

Table – 7 : The highest HLA- DR and DQ antigen frequencies among normal Iraqi Arab population and some other nations.

| Iraq * (This Study) | | Saudi (13) | | Tunis (16) | | Indian Gurkha (17) | |
|--------------------------|-------|-----------------|-------|-----------------|-------|-------------------------|-------|
| Antigen | % | Antigen | % | Antigen | % | Antigen | % |
| DR2 | 27.75 | DR7 | 35.20 | DR11 | 29.40 | DR4 | 34 |
| DR3 | 27.25 | DR4 | 30.90 | DR3 | 28.40 | DR7 | 34 |
| DR4 | 21.25 | DR3 | 25.20 | DR7 | 25.70 | DR1 | 32 |
| DR1 | 19 | DR2 | 17.80 | DR4 | 20.20 | DR3 | 30 |
| DQ1 | 23.25 | DQ1 | 51.40 | DQ1 | 51.30 | ----- | ----- |
| DQ3 | 23.25 | DQ3 | 45.79 | DQ3 | 39.40 | ----- | ----- |
| DQ2 | 20.5 | ----- | ----- | ----- | ----- | ----- | ----- |

* This Study : (400 person) .

(13) Ollier et al . (1985) : (1145 Person) .

(16) Ayed et al . (1987) : (109 Person) .

(17) Chaudhuri et al . (1995) : (50 Person) .

Discussion :

Several workers have shown great variation in the frequency of HLA - antigens among different racial groups. Our results of HLA polymorphism of healthy Iraqi Arab individuals confirm the concept of race variation. Some antigens occur at higher or lower frequency in Iraqi Arab population as compared with other countries .

The present study show clear differences when compared with Saudi (13), Turkish (15), Indian (17) and other nations (Tables 6 & 7) . The reason for this race variation is still mysterious , it may be due to gene drift , when some genes associated together by chance or by gene flow which is the result of admixture between different population (18) .

The present work is nearly compatible with local study about the prevalence of class I in Iraqi population where A₂ & B₅₁ were (38.7%) and (20.5%) respectively(19) .

Concerning class II (DR & DQ) , no previous studies on these antigens are available in Iraq for comparison .

On the other hand careful look to (Table-6) shows that in both Turkey and Iran there is similarity in distribution of class I antigens , where A₂ , A₉ and B₅₁ antigens show the highest frequencies in both . This observation could be attributed to mixing of these two neighborhood nations due to invation and emigration .

Further molecular study using polymerase chain reaction (PCR) and other advanced genetic techniques is recommended for further understanding of HLA complex of Iraqi population .

Lastly the current results may be considered as a database of HLA profile for Iraqi Arab population so it will be useful to anthropologist to predict the progenitor of them and for studies of disease association (as healthy control) , moreover , to explain why some disease occur with high frequency in this group of Iraqi population .

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