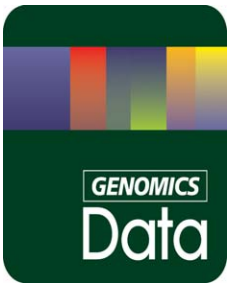

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A genomic study on distribution of human leukocyte antigen (HLA)-A and HLA-B alleles in Lak population of Iran

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Running title: Distribution of HLA class-I alleles in Lak population.

Authors' contribution: F Shahsavar (data analysis and interpretation and the final revision and approve), AM Varzi (executor of the research proposal), SAY Ahmadi (interpretation and writing the article).

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Abstract

Anthropological studies based on the highly polymorphic gene, *Human leukocyte antigen* (*HLA*), provide useful informations for bone marrow donor registry, forensic medicine, disease association studies, as well as infertility treatment, designing peptide vaccines against tumors, and infectious or autoimmune diseases. The aim of this study was to determine *HLA-A* and *HLA-B* allele frequencies in 100 unrelated Lak /l□k/ individuals from Lorestan province of Iran. Finally, we compared the results with that previously described in Iranian population. Commercial HLA-Type kits from BAG (Lich, Germany) company were used for determination of the *HLA-A* and *HLA-B* allele frequencies in genomic DNA, based on polymerase chain reaction with sequence specific primer (PCR-SSP) assay. The differences between the populations in distribution of HLA-A and HLA-B alleles were estimated by chi-squared test with Yate's correction. The most frequent *HLA-A* alleles were *24 (20%), *02 (18%), *03 (12%) and *11 (10%), and the most frequent *HLA-B* alleles were *35 (24%), *51 (16%), *18 (6%) and *38 (6%) in Lak population. *HLA-A**66 (1%), *74(1%) and *HLA-B**48 (1%), *55(1%) were the least observed frequencies in Lak population. Our results based on *HLA-A* and *HLA-B* allele frequencies showed that Lak population possesses the previously reported general features of Iranians but still with unique.

Keywords: HLA class I; Lak population; anthropology; immunology; Iran.

Introduction:

Among the basic medical sciences involving in anthropology, immunology can be considered as a science which deals with the molecular markers playing role in recognition of self and non-self between ethnicities [1]. So biomedicine is not alien to anthropology and offers an ethnographic international classification of ethnicities based on the immunological molecules such as CD markers [2, 3]. Hereby the integration between anthropology and immunology as a kind of integration between social and medical sciences could be a strategic way to have a biological information bank of different cultures in order to reach the aims mentioned in the next such as bone marrow transplantation, infertility prognosis and treatment [4, 5] and finding the identity of persons not grown with their real parents.

In the mankind genome, the *human leukocyte antigen* (*HLA*) also called as major histocompatibility complex (MHC) [6] with the length of 3600 kb is located on chromosome 6 and includes 239 antigenic loci that about 40% of them are immunogenic [7]. Recently, more than 2000 alleles for *HLA* class I are known in humans [8]. There are 2 classes of *HLA* and *HLA* class I in turn falls in two categories of classical (*HLA-A* and *B*) and non-classical (*HLA-C*, *G*, *E* and *F*) [4, 9-13]. The main role of HLA class I is that this biological molecule acts as an identifying card for all nuclear cells of body to be proposed for natural killer cells (CD56CD16 [14]) that different interactions between them results in different outcomes [15]. In addition to the key roles considered by immunologists for *HLA*, such genes have attracted the view point of most developmental biologists because of a high level of allele variety.

Since some alleles of *HLA* are commonplace in specific populations, the alleles are used by anthropologists as markers to determine genetic correlations and interactions in different populations [2]. Clinically, being acquainted with *HLA* distribution is a sine qua non for bone

marrow donating centers [16, 17], forensic medicine [18], studies of HLA related disease such as type 2 diabetes [19-21] or multiple sclerosis [22], designing peptide vaccine and monoclonal antibodies against tumors [23, 24], infectious agents [25-27] and autoimmune disease [20, 28], as well as infertility treatment and assisted reproductive technologies [29-31].

Race wise, Iran is a variable country that has different ethnicities such as Kurd, Lur, Torkaman, Azeri, Arab and Balouch. Majority of Iranians are Muslims, but Zoroastrians, Christians and Jewish also live in this country [32]. History has it that the majority of Iranians are Aryan but during the history they were attacked by different foreigners such as Alexander and Macedonians, Arabs, Turks and Moguls [32]. As described by a thesis under the supervision of Dr. Maziar Ashrafian Bonab [33], Aryans are believed to be one of the early Proto-Indo-European speaking ethnicities migrated toward Iran. They arrived and settled in the north of Afghanistan around 2,000-1,500 BC and kept on migrating and headed west settling in Iran and others south into India and Pakistan. It seems that as a result of their vast distribution, the Aryans were the main advocates of the Indo-European languages and promoting their proliferation via cultural and demic diffusion and therefore displacing the indigenous languages. Also Iran has played a key role in connection of different populations via the Silk Road [34, 35]. Thus the living populations in this country might be mixed because of migrations and mentioned relations.

Lak (or Laki) population is an ethnicity living in southwest of Iran and southeast of Iraq that there is still a controversy whether they are Kurd or Lur; because their culture and language are a complex of Kurdish and Luri populations. Kurds, Lurs and Persians are accounted as Indo-Iranian group of Aryans. The geographical condition of Laks (figure 1) shows that the majority of Iranian Laks live in the north of Lorestan province and some of them lives in Kermanshah, Ilam and Hamadan [36].

Figure 1 *Geographical status of Laks (the red color) (adapted from the reference [36])*

In previous studies, allele frequency of *HLA* class-II were determined in majority of different Iranian populations and compared with each other [18, 37]. In the present study, we intend to find allele frequency of *HLA* class-I in Lak /l□k/ population of Lorestan province (west of Iran) regarding to the lack of this study (on the class-I) in Lak population. Then based on the *HLA-I* profile the genetic relations between this population and total Iranian population [38] is investigated.

Material and methods:

For the present study, 100 healthy and unrelated Lak individuals living in Lorestan province were randomly chosen by convenient sampling based on the including criteria in 2015. Our including criteria was having the same race (the two recent generations of each sample should be Laks) and having 20-40 years of age, and the excluding criteria was having history of some specific diseases. Complete blood samples were obtained with informed and written consents from the participating individuals. The study was approved by the ethic committee of Lorestan University of Medical Sciences.

The genomic DNA of individuals were extracted by using kit BAG (Germany). HLA-typing kit for polymerase chain reaction with sequence specific primer (PCR-SSP) – a method which has longer and more specific primers for each allele of polymorphisms instead of using restriction enzymes [39, 40] – with low resolution (BAG Germany) were used to determine *HLA-A* and *B* alleles via genomic DNA. PCR products got visible in 2% agarose gel including 0.5 mg/ml Ethidium Bromide electrophoresis under an ultra violet light. Since there is a specific primer for each allele in SSP method (and *HLA* is highly polymorphic), we cannot write their sequences in the article and also it's a patent for the company.

Allele frequencies of *HLA-A* and *B* were determined through the direct counting method. The differences between the populations in allele frequencies of *HLA-A* and *B* were estimated by Chi-squared 2 multiplied by 2 test with degree of freedom 1. According to being multiple of the compares, we used Yate's correction to correct the randomized significance. After the correction $p<0.05$ considered as the level of significance.

Results:

Allele frequency of *HLA-A* and *B* in Iranian Lak population are given in table 1. Fifteen allele for *HLA-A* and 23 allele for *HLA-B* were identified. In Lak population, the most frequent *HLA-A* alleles were respectively A*24 (20%), A*02 (18%), A*3 (12%) and A*11 (10%). The most frequent *HLA-B* alleles were respectively B*35 (24%), B*51 (16%), B*18 (6%) and B*38 (6%). The least frequent *HLA-A* alleles were A*66 (1%) and A*74 (1%) and the least frequent *HLA-B* alleles were B*48 (1%) and B*55 (1%).

Discussion:

In the present study the most frequent *HLA-A* and *HLA-B* alleles in 100 healthy and unrelated Lak individuals were determined with method PCR-SSP. Allele frequency of *HLA-A* and *B* in Lak and total Iranian population [38] is shown in table 1.

As it is shown in table 1, the frequency of the alleles *HLA*-A*24, *HLA*-B*35 and *HLA*-B*51 in Lak population were significantly more than the frequency of these alleles in Iranian population.

In addition, comparing our findings with the findings of total Iranian population study shows that allele frequency of *HLA-A* and *B* in Lak population has a high similarity to total Iranian population. Recently, *HLA* class I typing has been performed also in the Lur and Kurd populations of western Iran [41]. Based on this study, A*24 and B*51 was high frequent in both Lur and Kurd populations of Iran. Because their PCR kit was high resolution, we could not statistically compare this study with the study of ours.

Other than different Iranian populations and ethnicities, these findings are also similar to other studies on different white populations (table 2). In contrast, these findings are not similar to the findings of Shen et al in Han population of China [42] and Yao et al in total Chinese population [43] (except A*24, about 12% in the Chinese and 20% in Laks that in the both populations was higher than 10% as the par of high frequency). Of course this fact is reasonable through genetic similarity of Iranians and others Caucasians and genetic difference between the whites and yellow-skinned.

In a Brazilian population the most frequent alleles were A*02, A*03, B*15, B*35, B*51 [44]. Alleles B*35 and B*51 high frequency was like Lak population, whereas B*15 is low frequent in Lak population. The allele frequency of B*27 – which is associated with Ankylosing spondylitis [45] – is low frequent in Lak population as other populations so. In Africans, alleles B*35 and B*53 were high frequent [46] as the Laks respectively are and are not so. These findings suggest

that some alleles like A*24 are widespread in human race. As it's shown in table 2 the high frequent alleles (>10%) in different ethnicities [7] are compared with Laks. Allele A*02 seems the most high frequent in different populations (table 2).

Analyzing frequency of *HLA-A* and *B* alleles enable us to assay genetic relations among various populations in our anthropological studies. As well, determining allele frequency of *HLA-A* and *B* in Lak population could be a good source for other studies in future such as correlations between the alleles and genetic disorders.

Conclusions:

At the end of the study, our results based on the allele frequencies showed that the population has general features of the Lurs, the Kurds and the total Iranian population reported before. Of course it's obvious that the main conclusion is that establishing immunology-based laboratories is necessary to diagnose and treat diseases through molecular methods. It could be useful for transplantation, assisted reproductive technologies and HLA-related disease.

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Table 1 *Allele frequency of HLA-A and B in Lak population (our study) in comparison to total Iranian population [38]. *(p<0.05)*

Allele A	Frequency in Lak population	Frequency in total Iranian population	Allele B	Frequency in Lak population	Frequency in total Iranian population
A*01	8	9.25	B*07	4	4.75
A*02	18	18.16	B*08	4	4.25
A*03	12	12.08	B*13	4	3.91
A*11	10	10.41	B*14	2	3.00
A*23	2	2.25	B*15	2	3.16
A*24	20*	16.41	B*18	6	4.33
A*26	8	6.83	B*27	2	2.58
A*29	2	2.5	B*35	24*	21.66
A*30	6	4.66	B*37	2	1.00
A*31	6	6.16	B*38	6	4.33
A*32	2	5.66	B*39	2	0.91
A*33	2	3.66	B*40	4	3.58
A*43	-	0.16	B*41	3	2.75
A*66	1	0.25	B*42	-	0.165
A*68	2	4.16	B*44	2	4.165
A*69	-	0.16	B*45	-	0.165
A*74	1	0.41	B*47	-	0.165
A*80	-	0.16	B*48	1	0.50
			B*49	2	2.50
			B*50	3	3.58
			B*51	16*	13.33
			B*52	3	3.50
			B*53	-	0.25
			B*54	-	0.165
			B*55	1	0.35
			B*56	2	0.50
			B*57	2	1.00
			B*58	3	1.915

Table 2 *High frequent alleles in different populations. X= not mentioned in reference [7] or the frequency was <10% in spite of their high frequency.*

Ethnicity	**High frequent alleles (>10%)**	
Laks	A*02,03,11,24	B*35,51
Iranians	A*02,03,11,24	B*35,51
Caucasians (the whites) of the USA	A*01,02,03	B*04,44
The Lebanese	A*01,02,24	B*35
Jordanians	A*02	X
The Irish	A*01,02	B*04,44
The Japanese	A*02,24	B*52
Moroccans	A*01,02	X
Koreans	A*02,11,24	B*15
Africans	A*23	X [B*35,53 [46]]
Bulgarians	A*02,24	B*18,51
The Chinese	A*11,24	B*46
Brazilians	A*02,03,24	X [B*15,35,51 [44]]
Omanis	A*02,11,26,32,68	B*51
The Polish	A*01,02,03,24	X
The Spanish	A*02	X
Armenians [47]	A*01,02,03,24	B*35,51

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Conflict of interest

Meanwhile we don’t have conflict of interest.