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HLA-A, HLA-B and-HLA-DRB1 Alleles Diversity among Sudanese Renal & Bone Marrow Donors

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Abstract

***Background:** Human Leukocyte Antigen (HLA) is cell surface glycoproteins encoded by Major Histocompatibility Complex (MHC) gene of human genome. It plays an important role in solid organ transplant, bone morrow transplant, and auto immune diseases.This study was carried out to determine the distribution and frequencies of the HLA-A, HLA-B, and HLA-DRB1 alleles among healthy unrelated renal and bone morrow donors, and compare them with that of geographically African and Arab related populations, and also to provide a frequency data to assist in further investigations in anthropological and HLA-associated disease studies.*

***Materials and Method:** A cross-sectional study was performed of a group of 351 unrelated renal and bone morrow donors, they were typed for HLA-A, HLA-B, and HLA-DRB1 by low resolution Polymerase Chain Reaction-Sequence Specific Primer (PCR-SSP) techniques at tissue typing laboratory, Ibn Sina Center for Kidney Diseases and Renal Transplantation (Tissue typing Laboratory)in the period from April to October 2017. The frequency for HLA-A, HLA -B, and -DRB1 alleles were defined as the percentage of the population possessing the antigen[15]. Samples containing one allele were considered homozygous and that allele was counted twice in the analysis.*

***Results:** In this study considerable allele diversity was revealed at each locus. The most frequent HLA-A* alleles were A*02(54.4%), A*30(39.8%), A*68(16.8%), A*03(13.1%), A*01(11.4%) and A*24(11.4). In the HLA-B* locus were B*51(23%), B*41 (12.8%), B*39 (12.0%), B*50(12.0%), B*15:03(11.0%) and B*35(10.8%). In the HLA-DRB1, all alleles were observed. HLA-DRB1*13 (49.0%) was the most frequent allele, followed by DRB1*301(22.8%), DRB1*15(21.0%), DRB1*07(19.9%), DRB1*08(19.9%) and DRB1*11(19.4%).*

***Conclusions:** The present study confirmed the observations on other Sudanese population studies[7], with slight difference but largely consistent in allele frequencies values,that may explain the possible differences from one area to another within one country. The present results of allele distribution and frequencies demonstrated that Sudanese population shares HLA patterns with Arab related populations specially North African and some of the neighboring African populations. Furthermore, HLA phenotype frequencies and distribution among Sudan population, if well documented, will serve many purposes including solid organ transplant, bone marrow transplant, studying HLA antigens associated diseases. It is also a valuable resource for individuals who are interested in population genetics and be helpful in the anthropologic studies.*

Keywords: *HLA-A, -B and DRB1, Allele frequency, Population, Sudan, Transplant.*

Introduction

The Human Major Histocompatibility Complex (MHC), known as human leukocyte antigen (HLA) complex, constitutes a specific group of molecules expressed on the cell surface that is crucial for the recognition of non-self-molecules by the acquired immune system [1].Human Leukocyte Antigens are divided into three classes (I, II, and III), all encoded by a gene complex located on the short arm of chromosome 6 (6p21.3), HLA class I molecules are expressed on the surface of almost all nucleated cells while MHC class II antigens are expressed on antigen presenting cells (APC) such as B lymphocytes, activated T lymphocytes, monocytes, macrophages, Langerhans cells, dendritic cells, endothelium, and epithelial cells, class III region, contains genes for complement (C2, C4, factor B), tumor necrosis factors (TNF-ά-and TNF-β) and heat shock Proteins [2]. HLA genes are closely linked and the entire MHC is inherited in bloc as an HLA haplotype in a Mendelian fashion from each parent, (One from father, one from mother).

The HLA molecules are the main antigens that differ between individuals of the same species and are related to rejection in solid organ transplantation and graft-versus-host disease in hematopoietic stem cell transplantation, the study of gene frequency of HLA alleles is important in both related and unrelated bone marrow transplantation programs and is based on HLA compatibility to prevent graft rejection and in the search for suitable donors [3]. Investigations into the HLA genes and proteins have been useful tool for transplantation, anthropological and disease association studies [4].

Identification of the frequencies of HLA alleles within nations was found to be important because it eases the burden of searching compatible donors, decreases the wait for transplants and therefore increases the chances of survival of the recipients [5].

Sudan was the largest country in Africa until 2011, when South Sudan separated into an independent country, officially called the Republic of South Sudan. The Sudanese populationis highly diverse, consisting of about 19 different ethnic groups and almost 600 subgroups[5].The present study was aimed to observe the HLA-A, HLA-B and-HLA-DRB1 alleles distribution and frequencies of Sudan population and compare them with that of geographically African and Arab related populations and to provide frequency data to assist in further investigations in anthropological and HLA-associated disease studies. Very few studies regarding such works are done in Sudan. This work is attempted to participate in bridging major deficiencies in this types of studies.

Materials and Methods

The study was conducted at Al-Neelain University, Faculty of Medical Laboratory Sciences, and IbnSina Center for Kidney Diseases and Transplantation (Tissue typing Laboratory) from April to October 2017. Labeled Stored buffy coat separated from blood samples collected in Acid Citrate Dextrose (ACD). 351 randomly, unrelated donors for renal and bone marrow transplant was enrolled in this study,these samples bearing no donors specific information (no names). Age average varies between 3 years for the bone marrow donation and above 18 years for both bone marrow and kidney donation, included both gender. These donors were from different states of Sudan. The study was descriptive cross sectional study.

DNA Extraction and HLA Typing

As per Tissue Typing Laboratory procedure and information provided, blood was collected in Acid Citrate Dextrose (ACD) vacutainer tubes; the buffy coat was separated and collected into a microcentrifuge tube and kept at -20°C.DNA was extracted from the buffy coat using the Qiagen Blood Mini Kit, (QIAgene Blood Mini Kit.Germany) according to the manufacturer's instructions, and was quantified by standard UV spectrophotometric analysis.The DNA was amplified for HLA-A, HLA-B, and HLA-DRB1, using HISTO TYPE SSP kit as described in the manual of the manufacturer (BAG Health Care GmbH, 35423 Lich / Germany). After the PCR-SSP process, the amplified DNA fragments were separated by agarose gel electrophoresis. The gel was viewed and photographed on the UV transilluminator located in the photo gel system with ethidium bromide filter and connected to the computer. An amplification reaction is considered if bright bands being visible. The results were analyzed by the assistance of the HLA software provided by the manufacture, (BAG Health Care GmbH, (2017).

Statistical Analysis

The frequency for HLA-A, HLA -B, and HLA -DRB1 alleles were defined as the percentage of the population possessing the antigen by dividing the total number of occurrences of that allele by the total

number of individuals (n/N) x100, where n is the number of particular allele, and N the total number of individuals. Samples containing one allele were considered homozygous and that allele was counted twice in the analysis.

Results

The frequencies and distribution of HLA-A,HLA- B, and HLA -DRB1 allele for three hundred fifty one unrelated Sudanese renal and bone morrow donors comprising 142 (40.5%) females and 209 (59.25%) males were observed in this study. A total of 18 HLA-A alleles, 32 HLA-B alleles, and 14 HLA-DRB1 alleles were identified.

Distribution of HLA-A* Alleles (Table1)

A total of 18 HLA-A alleles were identified in this study. The percentage of heterozygous was estimated to 79.8%. The most frequent HLA-A alleles included: A*02(54.4%) and A*30(39.8%) followed by A*68(16.8%), A*03(13.1%), A*24and A*01(11.4%). HLA-A*34, -A*36, -A*66 and -A*69 were present at low frequencies (<2%). A*25, A*43and A*80 were absent. Samples containing one allele were considered homozygous and that allele was counted twice in the analysis. The highest frequencies of homozygosity for HLA-A antigens were A*02 (8.0%), and A*30 (6.3%).

Table1. *HLA-A* Allele Frequencies (N=351)*

HLA-A*Alleles	Alleles Frequency	Alleles Frequency (%)
A*01	40	11.4
A*02	191	54.4
A*03	46	13.1
A*11	15	4.3
A*23	36	10.3
A*24	40	11. 4
A*26	14	3.98
A*29	10	2.8
A*30	140	39.8
A*31	26	7.4
A*32	23	6.5
A*33	24	6. 8
A*34	6	1.7
A*36	3	0.8
A*66	5	1.4
A*68	59	16.8
A*69	3	0.8
A*74	21	6.0

Distribution of HLA-B* Alleles (Tables 2)

For HLA-B antigens, thirty three alleles were detected. The estimated percentage of heterozygous is 88.3%. The highest frequency (23%) was detected for the B*51, followed by B*41(12.8%), B*50(12.0%), B*39(12.0%), B*15:03(11.0%) and B*35(10.8%). The least frequent alleles were B*55(0.28%) and B*73(0.28%). B*46, B*48, B*54, B*56, B*59, B*67, B*78, and B*81 were absent.Samples containing one allele were considered homozygous and that allele was counted twice in the analysis. Some homozygote's, such as, B51 (3.1%), B41 (1.7%), and B07 (1.4%) were also detected.

Table 2. *HLA-B* Allele Frequencies (N =351)*

HLA-B*Alleles	Alleles Frequency	Alleles Frequency (%)
B*07	36	10.2
B*08	23	6.5
B*13	15	4.2
B*18	6	1.7
B*14:01	3	0.86
B*14:02	15	4.2
B*15:01	8	2.2
B*15:03	39	11.0
B*15:10	4	1.1
B*15:17	18	5.2
B*15:18	10	2.8
B*27	10	2.8
B*35	38	10.8
B*37	6	1.7
B*38	29	8.2
B*39	42	12.0
B*40:01	7	1.9
B*40:02	4	1.1
B*41	45	12.8
B*42	21	5.9
B*44	17	4.8
B*45	17	4.8
B*47	14	3.9
B*49	33	9.4
B*50	42	12.0
B*51	81	23.0
B*52	33	9.4
B*53	27	7. 7
B*55	1	0.28
B*57	20	5.7
B*58	34	9.7
B*73	1	0.28
B*82	3	0.86

Distribution of HLA-DRB1* Alleles (Table 3)

For HLA-DRB1, all alleles were observed. Heterozygous percentage is 94.2%. HLA-DRB1*13 (49.0%) was the most frequent allele, followed by DRB1*301(22.8%), DRB1*15(21.0%), DRB1*07(19.9%), DRB1*08 (19.9%) and DRB1*11(19.4%), the alleles with minimal frequencies were DRB1*302 (1.4%) and DRB1*09(0.8%). Samples containing one allele were considered homozygous and that allele was counted twice in the analysis. The highest frequencies of homozygosity for DRB1 locus were 31%, 19%, 16.7%, and 11.9% for DRB1*13, DRB1*07, DRB!*15, and DRB1*301, respectively.

Table 3. *HLA-DRB1* AlleleFrequencies (N =351)*

HLA-DRB1*Alleles	Alleles Frequency	Alleles Frequency %
DRB1*01	43	12.2
DRB1*0301	80	22.8
DRB1*0302	5	1.4
DRB1*04	49	13.9
DRB1*07	70	19.9
DRB1*08	70	19.9
DRB1*09	3	0.8
DRB1*10	39	5.4
DRB1*11	70	19.4
DRB1*12	7	1.9
DRB1*13	172	49.0
DRB1*14	11	3.1
DRB1*15	74	21.0
DRB1*16	9	2.6

Discussion

The study of HLA antigens distribution is necessary for the estimation of the likelihood of obtaining matched donors for individuals requiring transplants. Based on the previous studies which have been done on Sudanese populations, the Sudanese population is highly diverse, consisting of about 19 different ethnic groups and almost 600 subgroups[5]. Sudanese are close to sub-Saharan Africans (Nigerians, Congolese, and Senegalese), and North Africans, in particular Egyptians, suggesting that the genetic profile of Sudanese is the admixture between North Africans (especially Egyptians) and sub-Saharan Africans throughout history[6]. In this study, the objective was to determine the predominant HLA-A, HLA-B and HLA-DRB1 allele frequencies in unrelated Sudanese renal and bone morrow donors .The HLA antigen data was compared with previous studies of various populations.

In this study HLA-A*02, was the most prevalent allele, and its frequency was 54.4% followed by HLA-A*30(39.8), HLA-A*68(16.8), A*03(13.1) and A*24(11.4) HLA-A*34, HLA-A*36, HLA-A*66 and HLA-A*69 were present at low frequencies (<2%). HLA-A*25, HLA-A*43 and HLA -A*80 were absent. The highest frequency (23%) was detected for the B*51, followed by B*41 (12.8%), B*50 (12.0%), B*39(12.0%), B*15:03(11.0%) and B*35(10.8%).The least frequent alleles were B*55(0.28%) and B*73(0.28%). B*46, B*48, B*54, B*56, B*59, B*78, and B*81 were absent. For HLA-DRB1, all alleles have been observed, the most frequent allele is HLA- DRB1*13(49.0%) followed by DRB1*301(21.0%), DRB1*15(21.0%), DRB1*07(19.9%), DRB1*08(19.9%) and DRB1*11 (19.4%). The alleles with minimal frequencies were DRB1*302 (5.0%) and DRB1*09(3.0%).The outcome of the present study was compared to the earlier reports of Sudanese renal donors, some Arabs and African Countries. The study that have been carried out by Dafalla AM, et al.[7] shows similarity to the present study and have revealed that the most frequent HLA-A* locus alleles were A*02 (22.20%), A*30 (13.30%), A*24 (7.16%) and A*68 (7.06%), in the HLA-B* locus B*51 (10.92%), B*15 (8.72%), B*35 (6.33%) and B*39 (6.15%), and in the DRβ1* locus DRβ1*13 (19.54%) and DRβ1*15 (13.85%) the B*15 allele frequency was observed for the broad antigen, while in the present study was observed for the splits. Some alleles occurred at higher or lower frequency in the present study but largely consistent with the results of Dafalla AM, et al. In the DRβ1* locus the results of the present study slightly different from the results of Dafalla AM, et al,which showed that the second frequent HLA-DRB1*allele was DRβ1*15 while in the present study is DRB1*301, some variation was indicated between the present study and the previous study report that may explain the possible differences from one area to another within one country. In comparing the distribution of HLA antigens in this

study with that of other previous studies from Arabs and African Countries, the HLA-A*02 was the most common allele in Saudi Arabia (28.9%) [8], (24%) in Omani population[9], (18%) in Iraqi population [10], (21.3%) in Syrian population[11],(16.76%) in Tunisian population [12], (18.4%) in Moroccan population [13], and (10.90%) among East African population such as Kenya, eastern Tanzania and southern Uganda around Lake Victoria[14]. B*51(23%) which is the most frequent allele in the present study is similar to that reported for Saudis (19.3%), Omanis (17.5%), and (15.6%) in Arab Emirates population [15]. B*50 was also a frequent B* allele in most Arabs, including Saudis (18.8%) with clear differences when compared with Syrian, Iraqi and Jordanian population where as the most common alleles were HLA-B*35, for Tunisian HLA-B*44:02/03 and for East African population HLA-B*58:02. In conclusion the most frequent alleles in Arabs are A*01, A*02, B*35, B*51, DRB1*03:01, DRB1*07:01[13].

In this study, the most frequent alleles DRB1*13, DRB1*301(21.0%) and DRB1*15(21.0%) were consistent with the observation reported in the United Arab Emirates population (UAE) study, where the DRB1*13 and DRB1*15(21.0%) found with higher frequency in the North African Arabs,DRB1*301(21.0%) has been reported as the second most frequent antigen in the UAE Emiratis and as a common allele among Bahrainis, while the first frequent antigens was DRB1*16[15]. The DRB1*11 allele was the six most common in this study but is the first most frequent allele among the Jordanian population[16], Iraqi and East African population. Several HLA alleles such as A*02, A*30; B*1503, DRB1*08 and DRB1*13 were African-specific alleles found in similar frequencies in Sudanese population, as well as some other alleles that were found highly distributed in Arabs and North African[12] .Comparatively, the allele distribution and frequencies demonstrated that Sudanese population shares HLA patterns with some Arab related countries, North African and some of the other African populations.

Conclusion

Sudan population is characterized by being clear diversity of their roots and origin although the areas at which different ethnic groups lives are known for long times .The population movement and the different population dynamics has profound impact on the frequencies of HLA antigens. HLA antigens frequency among Sudan ethnic groups are not unique for each group, rather, it reflects the Sudan community admixture but not Sudan ethnic groups. In this study the results revealed a considerable diversity in the frequencies and distribution of HLA alleles in the study population and demonstrated that Sudanese population shares HLA patterns with Arabs, North African and some of the neighboring African populations. Furthermore, HLA phenotype frequencies and distribution among Sudan population, if well documented, will serve many purposes including solid organ transplant, bone marrow transplant, studying HLA antigens associated diseases. It is also a valuable resource for individuals who are interested in population genetics and be helpful in the anthropologic studies.

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