Research Article

Fatma Savran Oguz, Suleyman Rustu Oguz*, Yeliz Ogret, Tanju Sedat Karadeniz, Hayriye Senturk Ciftci, Sule Karatas, Demet Kivanc and Filiz Aydin

Distribution of HLA epitope frequencies in Turkish population

Türk popülasyonunda HLA epitop frekanslarının dağılımı

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Abstract

Objectives: The antibodies interact with the "Human Leukocyte Antigen (HLA) antigens" at specific epitopes. "Epitopes" are present on a single HLA or shared by multiple antigens. In this study, we aim to determine the frequency of prevalent epitopes common in the Turkish population.

Methods: Non-related 644 healthy volunteers were recruited, and The "HLA-A, -B, -C, -DR -DQ's" were typed using the "Next Generation Sequencing". The provisional and confirmed epitopes were identified using the "HLA Epitope Registry databases, HLA Epitopia Maps and Immucor Epitope databases" dated 07.02.2018. Epitope frequencies were calculated by counting the shared epitopes in the total number of shared HLA Class epitopes in our sample database.

Results: Class I HLA's had 298 epitopes that repeated a total of 158,117 times with frequencies ranging between 0.0006 and 2.03%, and the most frequent epitope was 170RY found on 119 different alleles. Class II HLA's had 193 epitopes that repeated a total of 93,082 times with frequencies ranging between 0.002 and 1.36%, and the most frequent epitope was 108P found on 42 different alleles.

Conclusions: Our findings summarize both the provisional, and confirmed epitope frequencies in the Turkish population and may help clinicians and immunogeneticists develop a better understanding of HLA epitope mismatches.

Keywords: allele; epitope; eplet matching; HLA; transplantation.

Öz

Amaç: Antikorlar, spesifik epitoplarda "Human Leukocyte Antigen (HLA) antijenleri" ile etkileşime girer. "Epitoplar" tek bir HLA üzerinde bulunur veya birden fazla antijen tarafından paylaşılır. Bu çalışmada, Türk popülasyonunda yaygın olan epitopların sıklığını belirlemeyi amaçladık.

Gereç ve Yöntemler: Akraba olmıyan 644 sağlıklı gönüllü alındı ve "HLA-A, -B, -C, -DR -DQ'lar" "Yeni Nesil Dizileme" kullanılarak doku tiplemesi yapıldı. Geçici ve teyid edilmiş epitoplar, 07.02.2018 tarihli "HLA Epitope Registry veritabanları, HLA Epitopia Maps ve Immucor Epitope

Fatma Savran Oguz and Suleyman Rustu Oguz contributed equally to this work.

***Corresponding author: Suleyman Rustu Oguz**, Dr. Department of Medical Biology, TC Demiroğlu Bilim University, Istanbul, Turkey, Phone: 00905324935786, E-mail: rusduoguz@gmail.com. https://orcid.org/0000-0002-5854-1163
Fatma Savran Oguz, Yeliz Ogret, Hayriye Senturk Ciftci, Sule Karatas and Demet Kivanc, Department of Medical Biology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey, E-mail: oguzsf@gmail.com (F.S. Oguz), yelizdogret@gmail.com (Y. Ogret), hayriye.senturkciftci@istanbul.edu.tr (H.S. Ciftci), suleorhan007@yahoo.com (S. Karatas), demetkivanc@hotmail.com (D. Kivanc). https://orcid.org/0000-0002-6018-8936 (F.S. Oguz). https://orcid.org/0000-0001-9732-5474 (Y. Ogret). https://orcid.org/0000-0003-3507-482X (H.S. Ciftci). https://orcid.org/0000-0002-8605-4178 (S. Karatas). https://orcid.org/0000-0002-2451-5709 (D. Kivanc)
Tanju Sedat Karadeniz, Department of Bioinformatics and Genetics, Faculty of Medicine, Kadir Has University, Istanbul, Turkey, E-mail: stkaradeniz@gmail.com. https://orcid.org/0000-0002-1947-2484
Filiz Aydin, Department of Medical Biology, Faculty of Medicine, TC Demiroglu Bilim Univesity, Istanbul, Turkey, E-mail: filizkaydin@yahoo.com. https://orcid.org/0000-0001-5984-7538

veritabanları" kullanılarak tanımlanmıştır. Epitop frekansları, örnek veri tabanımızda paylaşılan HLA Sınıfı epitopların toplam sayısındaki paylaşılan epitopları sayarak hesaplandı.

Bulgular: Sınıf I HLA'lar, % 0.0006–2.03 arasında değişen frekanslarda, toplam 158.117 kez tekrarlanan 298 epitopa sahipti ve en sık görülen epitop, 119 farklı alelde bulunan 170RY idi. Sınıf II HLA, % 0.002–1.36 arasında değişen frekanslarda toplam 93.082 kez tekrarlanan 193 epitopa sahipti ve en sık görülen epitop 42 farklı alelde bulunan 108P idi.

Sonuç: Bulgularımız, Türk popülasyonundaki hem geçici, hem de doğrulanmış epitop frekanslarını özetlemektedir. Ve bu bulguların gerek klinisyenlerin gerekse immünogenetikçilerin HLA epitop uyumsuzluklarını daha iyi anlamalarına yardımcı olabileceği düşüncesindeyiz.

Anahtar Kelimeler: HLA; epitop; transplantasyon; eplet eşleştirme; alel.

Introduction

The human leukocyte antigen (HLA) system is considered as the most polymorphic region in the human genome. More than 100 genes were suggested to have immunological functions [1, 2]. The HLA system is recognized with its associations in infectious diseases [3], autoimmune diseases [4], and studies of diversity in populations [5] and its importance in transplantation [6]. Recently, there is also a growing field of study identifying associations between particular HLA polymorphisms and increased risk for adverse drug reactions [7, 8].

From a clinical point of view, the primary goal of detecting HLA antibodies in recipient blood is to find potential donors with non-perfect but acceptable HLA matching. The presence of donor specific anti-HLA antibodies (DSA) is a strong predictor for tissue and graft rejection. The antibodies specifically target certain epitopes found on the surface of the HLA molecule.

HLA epitopes are the specialized portions of HLA molecules that bind with antibodies or paratopes of T-cell receptors (TCR). The paratope consists of three light chains and three heavy chain (CDR: Complementarity Determining Regions) regions (CDR-L1, -L2, -L3, -H1, -H2, and -H3). The structural epitope consists of 12–22 amino acids and contains the binding site for antibodies. The functional epitope in the middle of these amino acid residues is 2–5 amino acids long. The "functional epitope" regions interact with CDR-H3, which has several residues localized in the center and play a dominant role in epitope specificity. Functional epitope specificity is responsible for structural epitope avidity and increases the stability of the antigen-antibody complex [9].

Current guidelines for kidney transplantation recommend matching based on the allele sequencing. However, recent evidence suggests that an epitope-based approach may be more efficient [10, 11].

Studies have shown that analyzing the epitope specificities may be useful in desensitization protocols [12].

Although such protocols are not totaly effective, in some cases, it may be possible to eliminate an antibody specific to an epitope altogether through sequestration. HLA matching at the epitope level has been shown to be tightly linked to graft survival, and antibody development in several studies [12–24].

As the mismatched eplets can be determined with great ease using the free "HLA Eplet Matching" excel sheet available at "epitopes.net", these findings may soon have practice changing implications. The mismatched epitope load may be considered as a risk factor for antibody-mediated rejection. This is also considered useful information for clinical protocols that aim immune tolerance [25].

In this study, we aimed to determine the epitope frequencies in the Turkish population to form a basis for future studies.

Materials and methods

HLA typing was performed at EFI-accredited HLA Laboratory in Istanbul University Istanbul Faculty of Medicine using Illumina MiSeq Sequencing System. The following loci: HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1, were typed by "Next Generation Sequencing (NGS)" methods using commercially available kits: Omixon Holotype HLA™ assay and Omixon HLA Twin™ software.

Sample population

Six hundred and fourty four healthy volunteer Turkish citizens aged between 18 and 55 years without known first- or second-degree family ties to one another were enrolled in the study.

Statistical analyses

HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 allele frequencies (in the meaning of how common an allele is in a population) were calculated by counting the repeated alleles in the total number of alleles for each HLA loci. The Hardy–Weinberg Equilibrium (HWE) were performed by Arlequin software package, version 3.5.2.2 (Excoffier and Lischer, 2010), and the p-values are considered significant at the 0.05 level. Principal Component Analysis (PCA) was performed based on HLA-A, HLA-B, HLA-C, HLA-DRB1 and HLA-DQB1 allele frequencies using SPSS v21.0 software.

$$\text{Frequency of an allele} = \frac{\text{Number of copies of an allele}}{\text{Total number of copies of alleles in population}}$$

Both provisional and confirmed epitopes were identified using the HLA Epitope Registry databases from "epregistry.com.br", HLA Epitopia Maps from "epitopes.net" and 07.02.2018 dated Immucor Epitope databases dated 07.02.2018. Epitope frequencies (in the meaning of how common a shared epitope is in a population) were calculated by counting the shared epitopes in the total number of shared HLA Class epitopes in our sample database.

$$\text{Frequency of an epitope} = \frac{\text{Number of copies of a shared epitope}}{\text{Total number of copies of shared epitopes in population}}$$

Results

Population genetics analyses were done on Arlequin v.3.5.2.2. Genotype frequencies at all loci HLA-A, -B, -C, -DRB1 were in Hardy-Weinberg Equilibrium (HWE) ($p \geq 0.25$) except the HLA-DQB1 genes. Also, when assessed by the inbreeding coefficient (*Fis*), observed and expected heterozygosity did not differ at any loci ($p \geq 0.40$). Ewens-Watterson tests of selective neutrality tests did not indicate any statistically significant selection ($p=0.99$)

The analysis of the HLA class I, and II allele frequencies of a total of 644 volunteers showed that the first three most frequent alleles were HLA-A*02:01, *24:02, *01:01, HLA-B*51:01, *35:01, *18:01, HLA-C*04:01, *07:01, *12:03, HLA-DRB1*07:01, *11:01, *11:04, HLA-DQB1*03:01, *03:02, *05:02 (Table 1).

For Class I, a total of 298 unique epitopes were observed in our population database corresponding to a total number of 158,117 counted epitopes. The frequency of any given epitope ranged between 0.0006 and 2.03%. The top 10 most frequent epitopes listed were 170RY, 99Y, 142ITQ, 163T, 102DV, 163TEW, 11SV, 156L, 97R, 194V, and are shown in the table (Table 2). We also observed that the first three of the most shared Class I epitopes (170RY, 99Y, 142ITQ) and the first three of the most frequent epitopes in our population database were same as shown in the table (Table 3).

For HLA class II, a total of 193 unique epitopes were observed in our population database, corresponding to a total number of 93,082 counted epitopes. The frequency of any given epitope ranged between 0.002 and 1.36%. The top 10 most frequent epitopes listed were 108P, 23R, 189R, 112H, 85V, 40F, 56P, 130R, 16H, 3S, and were shown in the

Table 2: Class I epitope frequencies.

Class I epitope	Count	Freq
170RY	3,217	2.03%
99Y	2,879	1.82%
142ITQ	2,581	1.63%
163T	2,180	1.38%
102DV	2,087	1.32%
163TEW	1,947	1.23%
11SV	1,870	1.18%
156L	1,862	1.18%
97R	1,837	1.16%
194V	1,831	1.16%

Table 3: Most shared epitope list by class I alleles.

Class I epitope	Total alleles	HLA-A	HLA-B	HLA-C
170RY	119	37	57	25
99Y	114	34	61	19
142ITQ	108	20	63	25
102DV	87	37	47	3
9Y(ABC)	79	15	49	15
156L	78	18	49	11
193PI	72	15	57	0
156LA	71	17	43	11
97R	69	14	34	21
72QTD	67	29	25	13

Table 1: HLA class I and II allele frequencies (first 10 most frequent alleles shown here).

HLA-A	Freq	HLA-B	Freq	HLA-C	Freq	HLA-DRB1	Freq	HLA-DQB1	Freq
*02:01	20.19%	*51:01	10.64%	*04:01	17.70%	*07:01	9.47%	*03:01	25.47%
*24:02	13.82%	*35:01	7.84%	*07:01	11.72%	*11:01	9.39%	*03:02	9.32%
*01:01	13.04%	*18:01	7.14%	*12:03	11.41%	*11:04	8.93%	*05:02	9.24%
*03:01	9.47%	*35:03	4.89%	*06:02	9.70%	*03:01	8.15%	*02:01	8.23%
*11:01	7.07%	*44:02	4.66%	*02:02	6.37%	*16:01	6.60%	*05:01	8.00%
*26:01	6.29%	*38:01	4.43%	*07:02	6.37%	*15:01	6.37%	*02:02	7.69%
*23:01	4.27%	*49:01	4.35%	*01:02	4.43%	*04:03	5.51%	*05:03	7.69%
*68:01	4.11%	*08:01	4.11%	*15:02	3.42%	*01:01	4.81%	*06:03	5.12%
*32:01	3.96%	*07:02	4.11%	*03:04	2.87%	*13:01	4.43%	*06:02	4.89%
*03:02	2.64%	*13:02	3.49%	*12:02	2.87%	*14:54	4.43%	*06:01	3.18%

Table 4: Class II epitope frequencies.

Class II epitope	Count	Freq
108P	1,262	1.36%
23R	1,254	1.35%
189R	1,246	1.34%
112H	1,241	1.33%
85V	1,232	1.32%
40F	1,230	1.32%
56P	1,224	1.31%
130R	1,216	1.31%
16H	1,215	1.31%
3S	1,215	1.31%

Table 5: Most shared epitope list by class II alleles.

Class II epitope	Total	HLA-DRB1	HLA-DQB1	Other class II
112H	50	41	0	9
25R	49	41	0	8
40F	49	41	0	8
78Y	49	39	0	10
108P	48	42	0	6
58A	47	37	0	10
85V	47	38	0	9
4R	45	38	0	7
16H	45	35	0	10
33N	45	35	0	10

table (Table 4). We also observed that the first three of the most shared Class II epitopes (112H, 25R, 40F) and the first three of the most frequent epitopes in our population database were different but the most frequent epitope 108P was the most shared epitope between the HLA-DRB1 alleles (Table 5).

Discussion

Epitope matching has become an important issue in organ transplants. Recent studies discuss whether epitope matching can replace allele matching. Currently, HLA laboratories receive epitope matching requests, and there are even recommendations based on epitope matching in the UNOS organ allocation algorithm. Continuous advances in the identification and mapping of functional HLA epitopes or eplets make the use of epitope matching almost indispensable in assessing immunological risk. While determining the number of eplet mismatches for each donor recipient pair is not a practical approach, it should be considered for highly sensitized patients and young transplant candidates who may need repeat transplantation. Thus, maximum survival rate can be achieved in the first kidney transplant, and the risk of allosensitivity after transplantation can be minimized [26].

HLA mismatches predispose to the development of DSA that are strongly associated with antibody mediated rejection (AMR) and late allograft loss [27, 28]. The presence of DSAs is often a barrier to transplantation, and highly sensitized patients experience a longer waiting time for a suitable donor kidney than non-sensitized patients. In kidney transplantation, graft survival rates were similar for transplants from fully HLA matched donors, and low HLA matched donors, and the researchers focused on areas where HLA epitopes interact with antibody (eplet regions) [29].

Experimentally defining of eplets and epitopes:
Step 1 – The monoclonal antibody or alloantibody isolated from the prepared serum is tested with single antigen beads (SAB Test) for each possible single allele.
Step 2 – Positive results show that the related antibody targets a unique epitope on the positive allele bead and negative results show that no antigen-antibody binding for current related antibody.
Step 3 – Alignment of the amino acid sequences of all positive and negative alleles shows that all positive alleles share the same amino acid changes at the same positions.
Step 4 – Amino acid positions of positive antibodies can be visualized using molecular structure visualization software to evaluate and understand the binding site specifications for the antigen-antibody binding relation. If more than one amino acid defines an epitope, the distance between any two amino acids stays within the binding range of the antibody [30].

Studies have shown that in cases of equal numbers of HLA antigen mismatches, lower eplet mismatch numbers are associated with a lower incidence of DSA formation, and a lower incidence of AMR compared to higher eplet mismatch numbers [31, 32].

However, not all eplet mismatches are equally immunogenic and can result in different antibody responses. Therefore, not all epitope or eplet mismatches can provoke anti-HLA antibody homogeneously [9]. Therefore, considering immunogenic eplet mismatches instead of the total number of eplet mismatches can provide more effective results when assessing risk during the transplant procedure.

The level of eplet mismatch of a donor is determined by the eplet repertoire of the recipient's HLA allele phenotype.

This type of analysis can easily be done using special software. The HLA Matchmaker program (http://www.epitopes.net) is a computer algorithm that determines the HLA compatibility between donors and recipients by evaluating the 3D molecular modeling of the epitope-paratope interfaces of antigen-antibody complexes [33]. That is, by evaluating foreign and shared eplets between donors and recipients, it can calculate the number of eplet mismatches for each donor-recipient pair and significantly expand the potential donation pool for highly sensitive recipients.

HLA Matchmaker is not the only method available for predicting epitope matching. Another method is the **P**redicted **I**ndirectly **R**e-**C**ognizable **H**LA **E**pitopes PIRCHE score. It probably explains the recognition of HLA polymorphisms corresponding to epitopes by the recipient CD4 + T cell and has often been studied in the context of class II molecules [34]. HLA Matchmaker and PIRCHE are examples of complex computational algorithms designed to predict epitopes found in HLA, but these algorithms cannot determine which epitopes are truly antigenic (capable of binding to antibody) or immunogenic (capable of inducing antibody production in response to antigen-antibody interaction) [35].

For the clinical purposes targeted in the transplantation process, it is important that the transplantation program focuses on alleles in the relevant population. Due to the global increase of racial and ethnic heterogeneity in all world populations, such alleles cause more mismatches independent of rare alleles.

High resolution HLA typing of new HLA alleles with high prevalence from different populations and their incorporation into the HLA Matchmaker will enable more accurate identification and calculation of immunogenic mismatched eplets.

The http://www.hlamatchmaker.net program was used to define the discrepancies between the donors and recipients, and for estimating the donor specific *de novo* antibody risk in the study of Udeme et al. Acute rejection, allograft fibrosis, and antibody mediated rejection were retrospectively investigated in this study [36].

DSA were detected in 20 out of 42 (48%). The antibody against HLA-DQB1 * 02 was associated with acute rejection, and DQ epitope mismatch was found higher in recipients with class II DAS. DSA were detected to have developed in recipients in case when the DQ epitope mismatch was higher than >5 or>6. The eplet mismatches of 4Q, 45GE, 52PQ, and 52PL were suggested to be the immunodominant epitopes in many recipients. The data of the epitope discrepancies were suggested to be possibly helpful for transplant physicians in developing immunosuppression strategies between recipients, and donors [36].

Our current study shows that "HLA-A*02:01, *24:02, *01:01, HLA-B*51:01, *35:01 and *18:01, HLA-C*04:01, *07:01, *12:03, HLA-DRB1*07:01, *11:01, *11:04, HLA-DQB1*03:01, *03:02, *05:02" allele frequencies, revealed similarities with previous studies of the Turkish population [37, 38]. Uyar and Kaya et al. [37, 38]. It is of interest that the DQB1, showed the greatest deviation from HWE, which is consistently less polymorphic than the other loci. Certainly, other explanations for the deviation from HWE, including nonrandom mating patterns with regard to admixed individuals within the ethnic group, must also be considered, as significant deviations from HWE are also seen in our population [39].

This is the first study demonstrating the most frequently detected HLA antigens in our population. The first 10 epitopes detected for Class I, and Class II HLA were 170RY, 99Y, 142ITQ, 163T, 102DV, 163TEW, 97R, 194V, 156L, 156LA as HLA Class I epitopes, and 108P, 189R, 23R, 112H, 85V, 40F, 16H, 56P, 38V, 3S, respectively as HLA Class II epitopes

We suggest that the first comprehensive HLA epitope data on the Turkish population will help clinicians and immunogenetics researchers in determining the HLA-epitope frequencies and mismatches in their population of interest and will be useful for further antibody related transplant studies.

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Author contributions: Fatma Savran Oguz provided the concept, design, interpretation of the data, drafting of the paper, and gave final approval. Suleyman Rustu Oguz contributed to the concept and design, assembled the data, and helped with the data analysis. Tanju Sedat Karadeniz analysis of data and statistical input. Yeliz Ogret provided technical support and materials. Hayriye Senturk Ciftci help with assembly of data with technical support. Sule Karatas and Demet Kivanc provided technical support, assembled data. Filiz Aydın contributed to the study design and gave final approval.

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Informed consent: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval: This study was approved by the Ethics Committee of the Istanbul University Faculty of Medicine (Ethics no: 470) and in accordance with the standards of the Declaration of Helsinki.

Data availability: The data that support the findings of this study are available on request from the corresponding author, [Oguz FS].

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