KADİR HAS UNIVERSITY

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BIOINFORMATICS AND GENETICS

CORRECTED PRA POSITIVITY RATES FOR HYPERSENSITIZED PATIENTS IN TURKISH POPULATION WITH "CALCULATED PRA-CPRA" SOFTWARE

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MASTER'S THESIS

ISTANBUL, JULY 2017

Sedat Tanju Karadeniz | M.Sc. Thesis | 2017

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MASTER'S THESIS

Submitted to the Graduate School of Science and Engineering of Kadir Has University in partial fulfillment of the requirements for the degree of Master's in the Program of Computational Biology and Bioinformatics.

ISTANBUL, JULY 2017

I, SEDAT TANJU KARADENİZ;

Hereby declare that this Master's Thesis is my own original work and that due references have been appropriately provided on all supporting literature and resources.

SEDAT TANJU KARADENİZ

18 JULY 2017

ACCEPTANCE AND APPROVAL

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ABSTRACT

KARADENİZ, SEDAT TANJU, *CORRECTED PRA POSITIVITY RATES FOR HYPERSENSITIZED PATIENTS IN TURKISH POPULATION WITH "CALCULATED PRA-CPRA" SOFTWARE*, MASTER'S THESIS, ISTANBUL, 2017

High rates of PRA (Panel Reactive Antibody) may decrease the chance of kidney transplant and may result in long-waiting periods before transplantation. The cPRA (Calculated Panel Reactive Antibody) is performed based on unacceptable HLA (Human Leukocyte Antigen) antigens. These antigens are identified by a computer program, which was created based on the antibodies that developed against the HLA antigens circulating in serum, and on the risk of binding of these antibodies to antigens. The antigen profile of the population and antigen frequencies can be measured, and more realistic cPRA positivity rates may be obtained using this method.

We developed a computer program based on the HLA antigens of 494 blood donors in two tissue typing laboratory laboratories in Turkey accredited by EFI (European Federation of Immunogenetics). NGS (Next Generation Sequencing) based tissue typing of the donor samples were performed. PRA screening test was performed to 380 patients who were waiting for organ transplant from a cadaver in Istanbul University, Istanbul Faculty of Medicine. The SAB (Single Antigen Bead Assay) testing was performed to identify the antibody profiles on 48 hypersensitized patients.

The mean of PRA positivity of the sensitized patients using the current methods were found as 44.6%, however, the rate was found as 86.2% using the cPRA software. cPRA software shows the rate of the rejected donors according to all unacceptable antigens. The need for a list of unacceptable antigens, in place of the PRA positivity rate, is a real change in the sensitization dependent calculation as in cPRA positivity rate. In principal, implementation of cPRA will encourage many centers and laboratories to adopt a standard measurement of sensitization in Turkey. It will increase the chances of finding a better donor particularly for hypersensitized patients, by the creation of an unacceptable mismatch program using cPRA software.

Keywords: PRA, HLA, cPRA, Calculated PRA, Next Generation Sequencing

ÖZET

SEDAT TANJU KARADENİZ, *TÜRK POPÜLASYONUNDAKİ HİPERSENSİTİZE HASTALARIN "HESAPLAMALI PRA-CPRA" YAZILIMI İLE DÜZELTİLMİŞ PRA ORANLARI*, MASTER TEZİ, İSTANBUL, 2017

Yüksek PRA (Panel Reaktif Antikor) oranları, böbrek nakli şansını düşürebilir ve nakilden önce uzun süre beklemeye yol açabilir. cPRA (Hesaplamalı Panel Reaktif Antikor), kabul edilemez (uyumsuz) HLA (Human Lökosit Antijen) antijenleri temel alınarak yapılır. Bu antijenler, serumda dolaşan HLA antijenlerine karşı gelişen antikorlara ve bu antikorların antijenlere bağlanma riskine dayanılarak oluşturulmuş bir bilgisayar programı ile tanımlanır. Böylece popülasyonun antijen profili ve antijen frekansları ölçülebilir ve bu yöntem kullanılarak daha gerçekçi cPRA pozitiflik oranları elde edilebilir.

Türkiye'de EFI (European Federation Immunogenetics) tarafından akredite edilmiş iki doku tipleme laboratuvarında 494 kan bağışçısının HLA antijenlerine dayanan bir bilgisayar programı geliştirdik. Verici örneklerinin doku tiplemesi YND (Yeni Nesil Dizileme) tabanlı olarak yapıldı. İstanbul Tıp Fakültesi'nde kadavradan organ nakli yapılmasını bekleyen 380 hastaya PRA tarama testi uygulandı. 48 aşırı duyarlı hastaya antikor profillerini belirlemek için (TAT) Tek Antijen Tanımlama testi yapıldı.

Duyarlılaşmış hastaların mevcut yöntemlerle PRA pozitifliği ortalama %44,6, ancak cPRA yazılımı kullanılarak pozitifliği ortalama %86,2 bulundu. cPRA yazılımı kabul edilemez tüm antijenlere göre reddedilen vericilerin oranını göstermektedir. PRA pozitiflik oranının yerine, kabul edilemez antijenlerin listesi olarak sunulan duyarlılığa bağlı hesaplamalı cPRA pozitiflik oranı ihtiyaç duyulan gerçek bir değişimdir. Esas olarak, cPRA'nın uygulanması Türkiye'de birçok merkezi ve laboratuvarı, duyarlılığın standart bir ölçümü olarak benimsemeye teşvik edecektir. cPRA yazılımını kullanmak, kabul edilemez uyuşmazlık programı oluşturarak, özellikle aşırı duyarlı hastalar için daha iyi bağış yapma şansını artıracaktır.

Anahtar Kelimeler: PRA, HLA, cPRA, Hesaplamalı PRA, Yeni Nesil Dizileme

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1. INTRODUCTION

1.1.The Immune System and the Major Histocompatibility Complex

The immune system protects the body against any foreign intruder such as cancerous cell; viruses and bacteria etc. Defensive mechanisms organized by adaptive immune responses are specific to any of the invading intruder. This adaptive immunity includes both a humoral response produced by antibodies and a cell-mediated response produced by T cells that can destroy other cells. The cell-mediated adaptive immune response is regulated by the MHC (Major Histocompatibility Complex), so named because it is responsible for graft rejection (i.e. transplanted tissue is rejected by the recipient's immune system), or tissue compatibility. Individuals identical for this region can exchange grafts more successfully than those with different MHC combinations. However, this diversity created by combinations in MHC proteins has a protective function, making it more difficult for an invading pathogen to elude the host immune system. Two separate properties of the MHC make it difficult for pathogens to evade immune responses in this way. First, the MHC is polygenic: it contains several different MHC class I and MHC class II genes with different ranges of peptide-binding specificities. Second, the MHC is highly polymorphic; i.e. there are multiple variants of each gene within the population. The MHC genes are the most polymorphic genes known (Figure 1.1) (01). A major role of the MHC is to bind small peptides and to present them to the cell surface where the antigen can be recognized by T cell receptors.

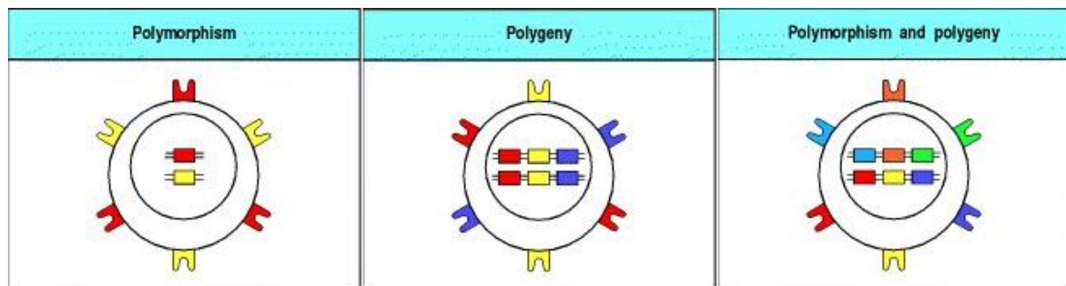

Figure 1.1: Polymorphism and polygene combine to produce the diversity of MHC molecules (01).

1.2.The Human Leukocyte Antigen (HLA) System

HLA (Human Leukocyte Antigen) is the human version of the Major Histocompatibility Complex (MHC). In humans, the MHC complex consists of more than 200 genes located closely on chromosome 6. Genes in this complex are categorized into three basic groups: class I, class II, and class III (Figure 1.2).

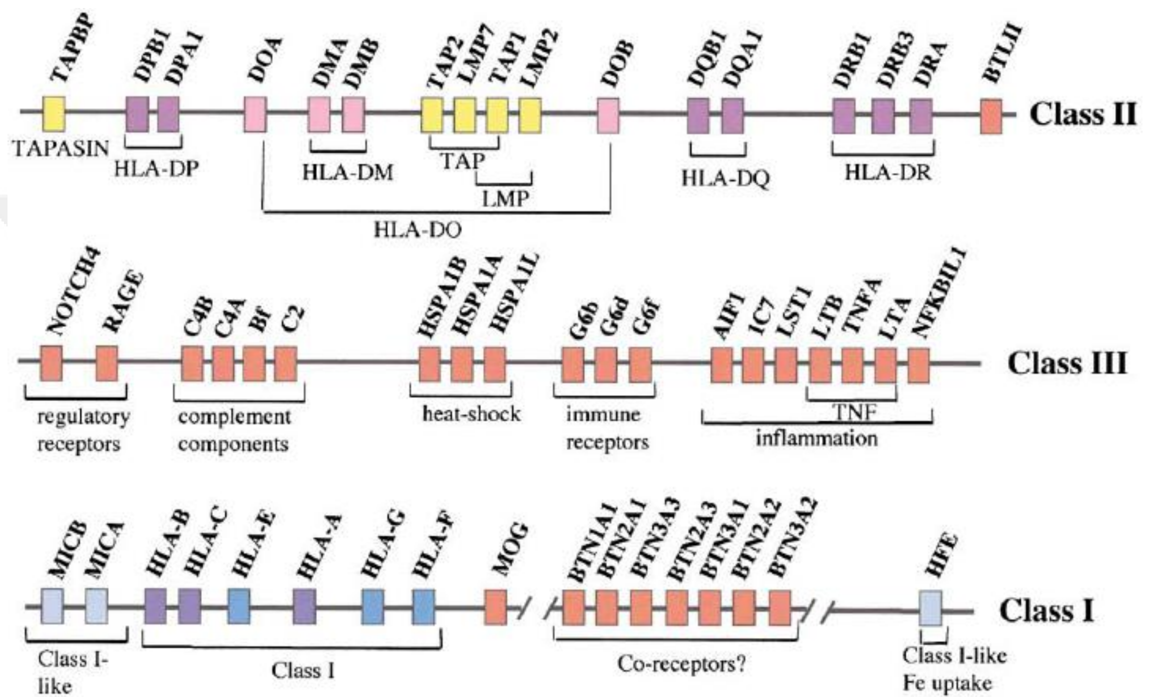

Figure 1.2. Some of the Main Immune Genes in the MHC (02) (03)

Humans have three main MHC class I genes, known as HLA-A, HLA-B, and HLA-C. The proteins produced from these genes are present on the surface of almost all cells. On the cell surface, these proteins are bound to protein fragments (peptides) that have been exported from within the cell. MHC class I proteins display these peptides to the immune system. If the immune system recognizes the peptides as foreign, it responds by triggering the infected cell to self-destruct. There are six main MHC class II genes in humans: HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRA, and HLA-DRB1. MHC class II genes provide instructions for making proteins that are present almost exclusively on the surface of certain immune system cells. Like MHC class I proteins, these proteins also display peptides to the immune system. The proteins

produced from MHC class III genes have somewhat different functions; they are involved in inflammation and other immune system activities. The functions of some MHC genes are unknown (02) (03).

HLA genes have many possible variations, allowing each person's immune system to react to a wide range of foreign invaders. Some HLA genes have hundreds of identified versions (alleles), each of which is given a particular number (such as HLA-B27). Closely related alleles are categorized together (The HUGO Gene Nomenclature Committee (HGNC) provides an index of gene families and their member genes.); for example, at least 40 very similar alleles are subtypes of HLA-B27. These subtypes are designated as HLA-B*27:01 to HLA-B*27:43 (02) (03).

More than 100 diseases have been associated with different alleles of HLA genes. For example, the HLA-B27 allele increases the risk of developing an inflammatory joint disease called ankylosing spondylitis. Many other disorders involving abnormal immune function and some forms of cancer have also been associated with specific HLA alleles. However, it is often unclear what role HLA genes play in the risk of developing these diseases (04).

As explained above, the human MHC is called the HLA system because these antigens were identified and characterized using alloantibodies against leukocytes (05). Leukocyte-agglutinating antibodies (that are directed against neutrophilic and other leukocytes) were observed in sera from multiparous women (having borne more than one child) and previously transfused patients. Graft rejection (i.e. transplanted tissue is rejected by the recipient's immune system, which destroys the transplanted tissue) was found to be associated with the development of antibodies against allogeneic leukocytes. The HLA system has been well known as transplantation antigens, but the primary biological role of HLA molecules is in the regulation of immune response (06).

1.3.Antigen

Antigen is any substance capable, under appropriate conditions of inducing a specific immune response and reacting with the products of that response; that is, with a specific antibody or specifically sensitized T lymphocytes, or both. Antigens may be soluble substances (such as toxins and foreign proteins) or particulates (such as bacteria and tissue cells); however, only the portion of the protein or polysaccharide molecule known as the antigenic determinant combines with antibody or a specific receptor on a lymphocyte (07).

1.4.Antibody

Antibodies are glycoproteins that contain two identical light chains, each of them which is about 200 amino acids long and two identical heavy chains, twice as long as the light chains. About first 100 amino acids at the N-terminal of both heavy and light chains vary from antibody to antibody. The amino acid sequence variability is located in 3 hypervariable regions within these first ~100 amino acids and they construct the antigen binding site against which the epitope fits. Because of this, the hypervariable regions are also called complementarity determining regions. Only a few different amino acid sequences are found in the C-terminals of heavy and light chains and they construct the constant regions (Figure 1.3) (08).

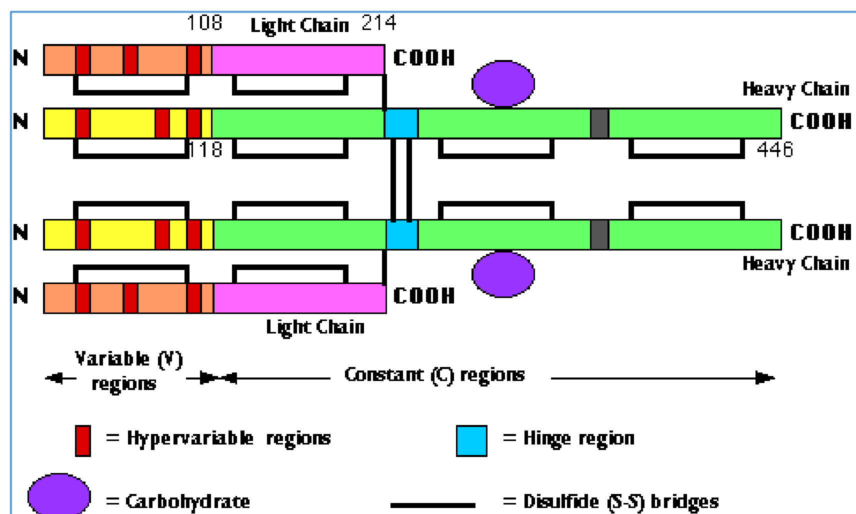

Figure 1.3: Polypeptide chain of an antibody (08).

1.5.Antibody Antigen Interaction

As described above, antibodies are proteins that are synthesized and secreted by B cells and bind to antigens. The interaction between antibody and antigen occurs by noncovalent forces between the antigen-combining site on the antibody and a portion of the antigen called epitope (the part of an antigen molecule to which an antibody binds). Generally, antibodies have two functions; recognizing and binding to an epitope on an antigen and acting in response to the antigen (09).

1.6.Antigen Receptors

There are two kinds of cells; B cells and T cells, they have thousands of surface receptors of a single specificity that bind to a particular epitope.

- T-cell receptors which enable the cell to bind to an epitope presented by another cell called the antigen-presenting cell.
- B-cell receptors (also called as antibodies) which enable the cell to bind to an epitope on molecules of a soluble antigen.

1.7.Genetic Organization of the HLA System

The genetic loci involved in the rejection of foreign organs are known as the major histocompatibility complex (MHC), and highly polymorphic cell surface molecules are encoded by the MHC. The human MHC located on the short arm of chromosome 6 (6p21) and the total size of genes approximately 3,600 kilobases. The human MHC is divided into three regions (Figure. 1.4) (10).

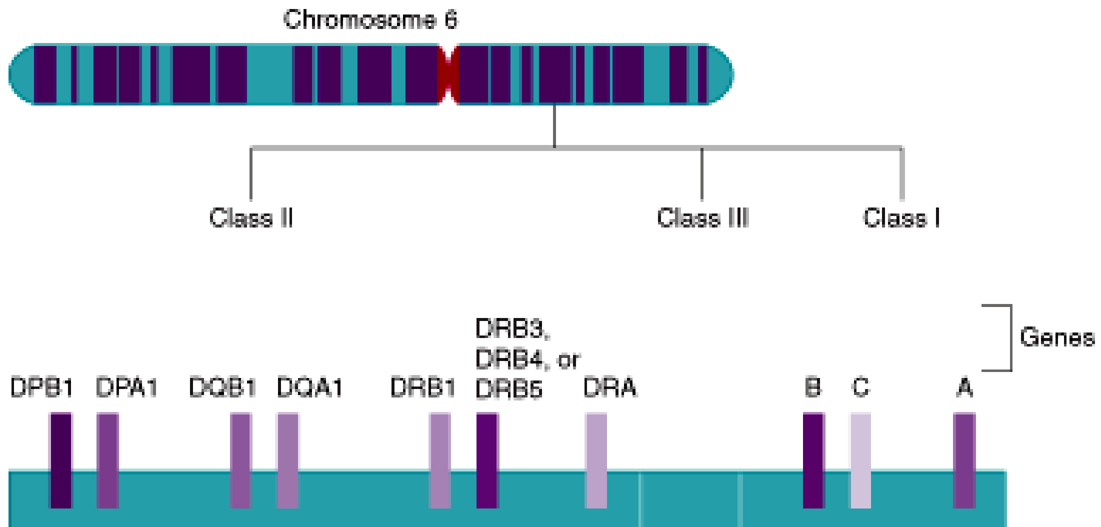

Figure 1.4: Location of Major histocompatibility complex (MHC) (10)

The class I region consists the HLA-A, HLA-B, and HLA-C genes that encode the heavy chains of class I molecules. The class II region consists of a series of sub regions that contain A and B genes which encode α and β chains, respectively. The DR gene group consists of a single DRA gene and DRB genes from DRB1 to DRB9 and HLA-DR antigen is determined by the polymorphic DRβ1 chains with DRB1 alleles. The DQA1 and DQB1 gene products form DQ molecules and the DPA1 and DPB1 form DP molecules. There is also another region between class I and class II region named class III region that does not encode any HLA molecules, but they are related with the genes for complement components and tumor necrosis factors (TNFs) (11).

1.8.Clinical HLA Testing

Clinical HLA laboratories perform various tests to support transplant programs; including HLA typing of the recipient and the potential donor in low resolution or high resolution, screening and identification of HLA antibodies in the recipient and the detection of antibodies in the recipient that are reactive with lymphocytes of a prospective donor (Figure 1.5) (03).

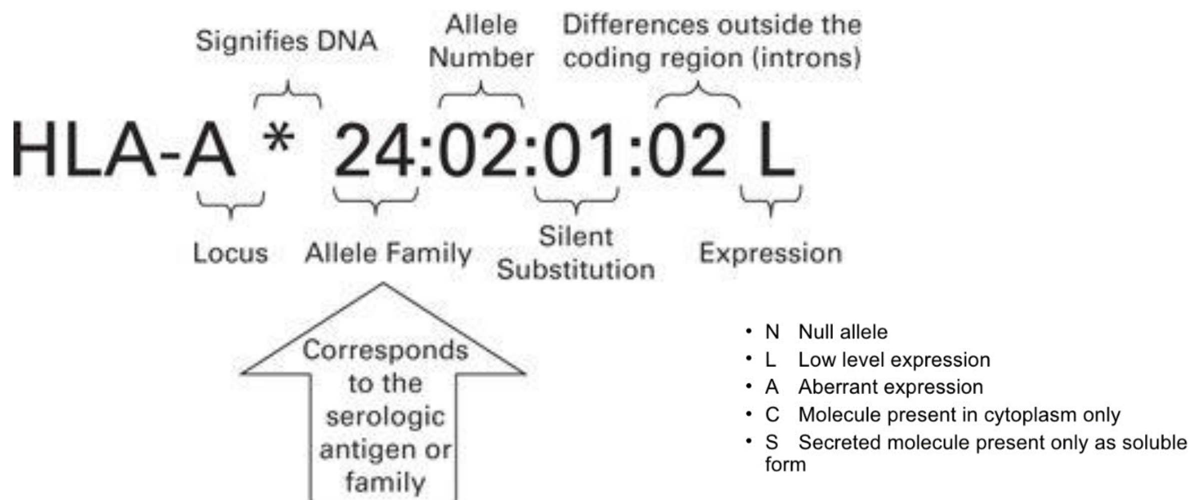

Figure 1.5: HLA nomenclature and levels of resolution (03).

1.9.Serologic Typing of HLA

The complement-dependent cytotoxicity (CDC) testing is accepted as the standard for serologic typing of HLA class I and class II antigens (12) (13). Peripheral blood lymphocytes express HLA class I antigens and are used for the serologic typing of HLA-A, HLA-B, and HLA-C. For HLA class II typing, B lymphocytes are isolated from peripheral blood because these cells express class II molecules.

1.10. Molecular Typing of HLA Alleles

The polymerase chain reaction (PCR)-based technology is used for molecular HLA typing (14). The first method using PCR, described as using sequence-specific oligonucleotide probes (SSOP) within a panel of synthetic oligonucleotide sequences matching to variable regions of the gene are designed and used as SSOP in hybridization with the amplified PCR products. The second method using PCR utilizes polymorphic DNA sequences as amplification primers, and amplifying only alleles containing sequences complementary to these primers called the sequence-specific primer (SSP) method. Another method as sequence based typing (SBT) of amplified products of multiple HLA loci is being most sensitive and specific HLA typing method especially for unrelated donor hematopoietic stem cell transplantation (15).

1.11. HLA Haplotypes

HLA genes and entire MHC are inherited as an HLA haplotype in a Mendelian fashion from each parent (Figure 1.6).

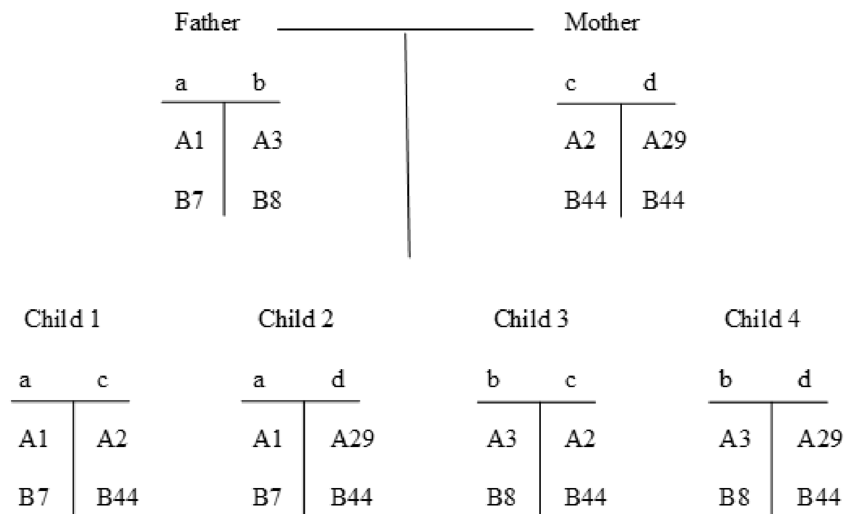

Figure 1.6: Mendelian inheritance of HLA haplotypes demonstrated in a family only for HLA-A and HLA-B locus.

With this Mendelian explanation, two siblings have a 25% chance of being genotypically HLA identical, a 50% chance of being HLA haploidentical (sharing one haplotype) and a 25% chance that they share no HLA haplotypes.

1.12. Screening and Identifying the Antibodies

HLA antibodies can be detected using the patient's serum against a panel of lymphocytes with known HLA types. This test is called HLA antibody screening and the results are expressed as the percentage of the panel cells that are reactive; this is called the % Panel Reactive Antibody (% PRA). For instance, if 10 of 40 panel cells are reactive with a serum, the PRA is 25%.

Several methods as enzyme-linked immunosorbent assay (ELISA) and fluorescence-based flow cytometry or fluoresced bead array technologies are used for HLA antibody screening and antibody specificity identification (16).

1.13. The HLA System and Transplantation

In solid-organ transplantation, HLA-matching means absence of HLA antigens in the donor that could be the target of anti-HLA antibodies present in the recipient. The matching is not between the antigens in the recipient and those in the donor, like in bone-marrow transplantation. This does not mean that antigen matching is not beneficial, but it limits organ allocation in a way that is not acceptable in the current clinical setting. In fact, there is evidence that antigen matching, like in HLA-identical siblings, significantly improves long-term survival. The desperate clinical condition of patients and the limitations in the availability of donated organs gives priority to the problem of allocation and dictates the practice of matching recipient antibodies against donor antigens, instead of doing recipient-donor phenotypic matching (12).

The success of bone-marrow transplantation greatly depends on matching the recipient’s HLA phenotype against the donor’s. There has been a long debate about which HLA loci are clinically relevant. As clinical outcome data accumulates, it becomes evident that all HLA loci are relevant, including A, B, C, DRB1, DRB3/4/5, DQB1, and DPB1. In addition, DQA1 and DPA1 are polymorphic and form different DQ and DP heterodimers with the same DQB1 or DPB1, and it is important to take that into account. Although all HLA loci may be relevant, not all are relevant to the same extent. It is well known that some HLA molecules are expressed on the cell surface more extensively than others. HLA-A, HLA-B, HLA-C, and HLA-DRB1 are expressed at a higher level, whereas HLA-DRB3/4/5, HLA-DQB1, and DPB1 are expressed at a lower level. Those loci corresponding to high-expression molecules play a major role in the immune interactions between donors and recipients, and those corresponding to low-expression molecules play a lesser role, with mismatches in these loci being more tolerable. Nevertheless, it is the cumulative effect of multiple mismatches that seems to

be critical, and clinical outcome studies should not isolate the effect of one HLA locus, but analyze the cumulative effect of mismatches at all loci (12).

Generally, HLA-A, HLA-B and HLA-DR have known as major transplantation antigens. Recent clinical data indicate that HLA-C, HLA-DQ and HLA-DP matching also affects both solid organ and hematopoietic stem cell transplantation.

Even though many factors are considered in the clinical practice of both bone-marrow transplantation and solid-organ transplantation, sometimes relegating HLA typing and matching to a secondary position, graft rejection and graft-versus-host disease (GVHD is a complication that can occur after certain stem cell or bone marrow transplants.) are fundamentally dependent on the identification and characterization of the HLA phenotype in both donor and recipient, and the assessment of the degree of matching between them (12).

In so far as the genetic diversity is bound to increase due to the mixing of populations of different ethnic origins, it may be anticipated that in the long term the proportion of difficult HLA matches will increase. In these cases, the problem of selecting the optimal mismatched donor will require (17):

- detailed knowledge of population genetics,
- a functional assessment of mismatches between specific pairs of alleles, and
- knowledge of the clinical implications of the cumulative effect of mismatches in multiple loci.

1.14. Calculated Panel Reactive Antibody (cPRA)

Antibodies, that develop against HLA after blood transfusions, pregnancies and previous transplants are generally described as Panel Reactive Antibody (PRA). High rates of PRA may decrease the chance of kidney transplant and may result in long-waiting periods before transplantation (18-19).

The PRA positivity rate is identified considering the number of antigen groups and the total counts of antigens in the panel by current methods. The exact PRA positivity rate cannot be identified because the antigen counts in the panel does not reflect the antigen profile of the population.

The Calculated Panel Reactive Antibody (cPRA) is performed based on unacceptable HLA antigens. These antigens are identified by a software, which was created based on the antibodies that developed against the HLA antigens circulating in serum, and on the risk of binding of these antibodies to antigens (20).

The antigen profile of the population and antigen frequencies can be measured, and more realistic cPRA positivity rates may be obtained using this method. In 2009, the Organ Procurement and Transplantation Network (OPTN) that is governed by the United Network for Organ Sharing (UNOS) established the calculated panel reactive antibody first time (21-23).

2. MATERIALS AND METHODS

We developed a software based on the HLA antigens of 494 blood donors in two EFI (European Federation for Immunogenetics) accredited TTL (Tissue Typing Laboratories) in Turkey. NGS (Next Generation Sequencing) based tissue typing were performed for HLA-A, HLA-B, HLA-C, HLA-DRB1and HLA-DQB1 loci as high resolution (4 or more digits) on Illumina (San Diego, California, United States) MiSeq instrument by using Omixon Holotype HLA kit (Omixon, H-1117 Budapest, Fehérvári Ave. 50-52, Hungary).

Panel Reactive Antibody (PRA) screening test was performed to 380 patients who were waiting for organ transplant from a cadaver in Istanbul Faculty of Medicine. To have detailed antibody identification, the Single Antigen Bead named assay (SAB) test (One Lambda - Thermo Fisher Scientific Inc. Los Angeles, California) was performed to identify the antibody profiles on 48 hypersensitized patients selected according to results of the PRA screening test.

The developed software created a database by using the results obtained from the NGS based HLA typing results for all 494 blood donors. Normally the resolution of NGS based HLA typing is more than 4 digits as *01:01:01 or *01:01:01:01. But, by using the definition, the results can be shown as 4 digits by adding the G letter as *01:01G. All observed results added to the database (Table 2.1).

Table 2.1: NGS based HLA typing results shown as 4 digits with G letter

Locus	**HLA-A***		**HLA-B***		**HLA-C***		**HLA-DRB1***		**HLA-DQB1***	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
Donor 1	01:01G	02:01G	07:02G	35:02G	04:01G	06:01G	01:01G	11:01G	02:02G	03:02G
Donor 2	02:01G	33:01G	14:01G	51:01G	02:02G	03:02G	04:01G	16:01G	03:02G	05:02G

3. RESULTS

According to the calculation performed by the developed software, the 4 digits allele frequencies were significantly different than the 2 digits allele frequencies for 494 blood donors (Table 3.1, Table 3.2).

Table 3.1: The 5 of the most frequent alleles are listed in the table with 2-digit resolution for each locus

Sorted Allele Frequency for 2 digits	HLA-A*	HLA-B*	HLA-C*	HLA-DRB1*	HLA-DQB1*
Allele and Frequency #1	02 (23.7%)	35 (18.0%)	07 (19.4%)	11 (20.6%)	03 (40.0%)
Allele and Frequency #2	24 (14.1%)	51 (11.8%)	04 (18.3%)	04 (14.8%)	05 (24.0%)
Allele and Frequency #3	01 (12.9%)	44 (8.2%)	12 (14.7%)	15 (10.5%)	06 (17.0%)
Allele and Frequency #4	03 (12.0%)	18 (7.0%)	06 (9.7%)	13 (9.8%)	02 (16.1%)
Allele and Frequency #5	11 (7.6%)	38 (4.6%)	03 (7.1%)	07 (9.5%)	04 (2.8%)

Table 3.2: The 5 of the most frequent alleles are listed in the table with 4-digit resolution for each locus

Sorted Allele Frequency for 4 digits	HLA-A*	HLA-B*	HLA-C*	HLA-DRB1*	HLA-DQB1*
Allele and Frequency #1	02:01G (20.0%)	51:01G (11.3%)	04:01G (18.1%)	07:01G (9.5%)	03:01G (26.1%)
Allele and Frequency #2	24:02G (13.8%)	35:01G (8.2%)	07:01G (11.5%)	11:04G (9.3%)	03:02G (9.2%)
Allele and Frequency #3	01:01G (12.7%)	18:01G (6.8%)	12:03G (11.4%)	11:01G (9.2%)	05:02G (8.8%)
Allele and Frequency #4	03:01G (9.2%)	35:03G (5.0%)	06:02G (9.4%)	03:01G (8.3%)	02:01G (8.3%)
Allele and Frequency #5	11:01G (7.6%)	44:02G (4.9%)	07:02G (6.1%)	15:01G (6.5%)	02:02G (7.8%)

The developed software calculated and sorted allele frequencies for each locus by counting two alleles separately (for the most 5 frequent) (Table 3.3, Table 3.4, Table 3.5, Table 3.6, Table 3.7):

Table 3.3: HLA-A Locus Allele Frequency

Order	HLA-A* (4 digits)	Allele Frequency
1	02:01G	20.04%
2	24:02G	13.77%
3	01:01G	12.65%
4	03:01G	9.21%
5	11:01G	7.59%

Table 3.4: HLA-B Locus Allele Frequency

Order	HLA-B* (4 digits)	Allele Frequency
1	51:01G	11.34%
2	35:01G	8.20%
3	18:01G	6.78%
4	35:03G	4.96%
5	44:02G	4.86%

Table 3.5: HLA-C Locus Allele Frequency

Order	HLA-C* (4 digits)	Allele Frequency
1	04:01G	18.12%
2	07:01G	11.54%
3	12:03G	11.44%
4	06:02G	9.41%
5	07:02G	6.07%

Table 3.6: HLA-DRB1 Locus Allele Frequency

Order	HLA-DRB1* (4 digits)	Allele Frequency
1	07:01G	9.51%
2	11:04G	9.31%
3	11:01G	9.21%
4	03:01G	8.30%
5	15:01G	6.48%

Table 3.7: HLA-DQB1 Locus Allele Frequency

Order	HLA-DQB1* (4 digits)	Allele Frequency
1	03:01G	26.11%
2	03:02G	9.21%
3	05:02G	8.81%
4	02:01G	8.30%
5	02:02G	7.79%

The developed software calculated and sorted allele frequencies for combination of two loci by counting two alleles separately (for the most 5 frequent) (Table 3.8, Table 3.9, Table 3.10, Table 3.11, Table 3.12, Table 3.13, Table 3.14, Table 3.15, Table 3.16, Table 3.17):

Table 3.8: Allele Frequency for HLA-A and -B loci combinations

Order	HLA-A* and -B* (4 digits)		Allele Frequency
1	02:01G	51:01G	2.48%
2	02:01G	18:01G	1.97%
3	24:02G	51:01G	1.82%
4	01:01G	51:01G	1.47%
5	02:01G	35:01G	1.37%

Table 3.9: Allele Frequency for HLA-A and -C loci combinations

Order	HLA-A* and -C* (4 digits)		Allele Frequency
1	02:01G	04:01G	3.29%
2	24:02G	04:01G	3.09%
3	02:01G	07:01G	2.83%
4	02:01G	12:03G	2.23%
5	11:01G	04:01G	1.97%

Table 3.10: Allele Frequency for HLA-A and -DRB1 loci combinations

Order	HLA-A* and -DRB1* (4 digits)		Allele Frequency
1	02:01G	16:01G	2.13%
2	02:01G	07:01G	2.13%
3	24:02G	11:04G	2.07%
4	02:01G	11:01G	1.72%
5	01:01G	07:01G	1.47%

Table 3.11: Allele Frequency for HLA-A and -DQB1 loci combinations

Order	HLA-A* and -DQB1* (4 digits)		Allele Frequency
1	02:01G	03:01G	4.40%
2	24:02G	03:01G	4.30%
3	01:01G	03:01G	3.39%
4	03:01G	03:01G	2.78%
5	02:01G	05:02G	2.63%

Table 3.12: Allele Frequency for HLA-B and -C loci combinations

Order	HLA-B* and -C* (4 digits)		Allele Frequency
1	35:01G	04:01G	4.30%
2	49:01G	07:01G	2.33%
3	38:01G	12:03G	2.18%
4	35:03G	04:01G	2.07%
5	13:02G	06:02G	1.87%

Table 3.13: Allele Frequency for HLA-B and -DRB1 loci combinations

Order	HLA-B* and -DRB1* (4 digits)		Allele Frequency
1	08:01G	03:01G	1.77%
2	18:01G	11:04G	1.42%
3	13:02G	07:01G	1.42%
4	51:01G	11:01G	1.27%
5	52:01G	15:02G	1.21%

Table 3.14: Allele Frequency for HLA-B and -DQB1 loci combinations

Order	HLA-B* and -DQB1* (4 digits)		Allele Frequency
1	51:01G	03:01G	3.49%
2	18:01G	03:01G	2.68%
3	35:01G	03:01G	2.43%
4	08:01G	02:01G	1.77%
5	44:02G	03:01G	1.72%

Table 3.15: Allele Frequency for HLA-C and -DRB1 loci combinations

Order	HLA-C* and -DRB1* (4 digits)		Allele Frequency
1	06:02G	07:01G	2.58%
2	04:01G	11:04G	1.92%
3	04:01G	07:01G	1.67%
4	04:01G	11:01G	1.67%
5	04:01G	14:54G	1.47%

Table 3.16: Allele Frequency for HLA-C and -DQB1 loci combinations

Order	HLA-C* and -DQB1* (4 digits)		Allele Frequency
1	04:01G	03:01G	5.21%
2	12:03G	03:01G	3.34%
3	07:01G	03:01G	3.29%
4	06:02G	03:01G	2.53%
5	04:01G	05:03G	2.18%

Table 3.17: Allele Frequency for HLA-DRB1 and -DQB1 loci combinations

Order	HLA-DRB1* and -DQB1* (4 digits)		Allele Frequency
1	11:04G	03:01G	5.87%
2	11:01G	03:01G	5.47%
3	07:01G	02:02G	4.25%
4	03:01G	02:01G	4.20%
5	16:01G	05:02G	3.54%

The developed software calculated and sorted allele frequencies for combination of three loci by counting two alleles separately (for the most 5 frequent) (Table 3.18, Table 3.19, Table 3.20, Table 3.21, Table 3.22, Table 3.23, Table 3.24, Table 3.25):

Table 3.18: Allele Frequency for HLA-A and -B and -C loci combinations

Order	HLA-A* and -B* and -C* (4 digits)			Allele Frequency
1	02:01G	18:01G	07:01G	0.73%
2	23:01G	49:01G	07:01G	0.66%
3	02:01G	35:01G	04:01G	0.63%
4	03:01G	35:01G	04:01G	0.63%
5	24:02G	35:02G	04:01G	0.58%

Table 3.19: Allele Frequency for HLA-A and -B and -DRB1 loci combinations

Order	HLA-A* and -B* and -DRB1* (4 digits)			Allele Frequency
1	01:01G	18:01G	12:01G	0.40%
2	02:01G	18:01G	11:04G	0.38%
3	01:01G	08:01G	03:01G	0.38%
4	02:01G	51:01G	16:01G	0.38%
5	02:01G	51:01G	11:01G	0.38%

Table 3.20: Allele Frequency for HLA-A and -B and -DQB1 loci combinations

Order	HLA-A* and -B* and -DQB1* (4 digits)			Allele Frequency
1	02:01G	51:01G	03:01G	0.78%
2	02:01G	18:01G	03:01G	0.63%
3	01:01G	18:01G	03:01G	0.63%
4	23:01G	49:01G	03:01G	0.53%
5	24:02G	35:02G	03:01G	0.51%

Table 3.21: Allele Frequency for HLA-A and -C and -DRB1 loci combinations

Order	HLA-A* and -C* and -DRB1* (4 digits)			Allele Frequency
1	24:02G	04:01G	11:04G	0.56%
2	02:01G	06:02G	07:01G	0.56%
3	02:01G	04:01G	07:01G	0.43%
4	01:01G	01:02G	12:01G	0.40%
5	02:01G	12:03G	16:01G	0.38%

Table 3.22: Allele Frequency for HLA-A and -C and -DQB1 loci combinations

Order	HLA-A* and -C* and -DQB1* (4 digits)			Allele Frequency
1	24:02G	04:01G	03:01G	1.19%
2	02:01G	07:01G	03:01G	0.73%
3	01:01G	06:02G	03:01G	0.68%
4	02:01G	04:01G	03:01G	0.61%
5	02:01G	02:02G	05:02G	0.58%

Table 3.23: Allele Frequency for HLA-B and -C and -DRB1 loci combinations

Order	HLA-B* and -C* and -DRB1* (4 digits)			Allele Frequency
1	13:02G	06:02G	07:01G	0.78%
2	52:01G	12:02G	15:02G	0.61%
3	35:01G	04:01G	01:01G	0.56%
4	44:02G	16:04G	11:04G	0.56%
5	5:0:01G	06:02G	07:01G	0.51%

Table 3.24: Allele Frequency for HLA-B and -C and -DQB1 loci combinations

Order	HLA-B* and -C* and -DQB1* (4 digits)			Allele Frequency
1	35:01G	04:01G	03:01G	1.27%
2	35:02G	04:01G	03:01G	0.78%
3	49:01G	07:01G	03:01G	0.73%
4	13:02G	06:02G	02:02G	0.73%
5	44:02G	16:04G	03:01G	0.71%

Table 3.25: Allele Frequency for HLA-C and -DRB1 and -DQB1 loci combinations

Order	HLA-C* and -DRB1* and -DQB1* (4 digits)			Allele Frequency
1	06:02G	07:01G	02:02G	1.34%
2	04:01G	11:04G	03:01G	1.29%
3	04:01G	11:01G	03:01G	0.99%
4	04:01G	14:54G	05:03G	0.96%
5	12:03G	11:04G	03:01G	0.86%

The developed software calculated and sorted allele frequencies for combination of four loci by counting two alleles separately (for the most 5 frequent) (Table 3.26, Table 3.27, Table 3.28, Table 3.29, Table 3.30):

Table 3.26: Allele Frequency for HLA-A and -B and -C and -DRB1 loci combinations

Order	HLA-A* and -B* and -C* and -DRB1* (4 digits)				Allele Frequency
1	01:01G	18:01G	01:02G	12:01G	0.40%
2	24:02G	44:02G	16:04G	11:04G	0.22%
3	29:02G	44:02G	16:04G	11:04G	0.20%
4	24:02G	35:02G	04:01G	11:04G	0.19%
5	3:0:01G	13:02G	06:02G	07:01G	0.19%

Table 3.27: Allele Frequency for HLA-A and -B and -C and -DQB1 loci combinations

Order	HLA-A* and -B* and -C* and -DQB1* (4 digits)				Allele Frequency
1	01:01G	18:01G	01:02G	03:01G	0.40%
2	24:02G	35:02G	04:01G	03:01G	0.38%
3	24:02G	35:01G	04:01G	03:01G	0.29%
4	23:01G	49:01G	07:01G	03:01G	0.28%
5	24:02G	44:02G	16:04G	03:01G	0.27%

Table 3.28: Allele Frequency for HLA-A and -B and -DRB1 and -DQB1 loci combinations

Order	HLA-A* and -B* and -DRB1* and -DQB1* (4 digits)				Allele Frequency
1	01:01G	18:01G	12:01G	03:01G	0.40%
2	24:02G	44:02G	11:04G	03:01G	0.29%
3	02:01G	51:01G	16:01G	05:02G	0.27%
4	02:01G	51:01G	11:01G	03:01G	0.25%
5	02:01G	18:01G	11:04G	03:01G	0.24%

Table 3.29: Allele Frequency for HLA-A and -C and -DRB1 and -DQB1 loci combinations

Order	HLA-A* and -C* and -DRB1* and -DQB1* (4 digits)				Allele Frequency
1	24:02G	04:01G	11:04G	03:01G	0.44%
2	01:01G	01:02G	12:01G	03:01G	0.40%
3	02:01G	06:02G	07:01G	02:02G	0.29%
4	02:01G	04:01G	14:54G	05:03G	0.28%
5	23:01G	07:01G	11:01G	03:01G	0.24%

Table 3.30: Allele Frequency for HLA-B and -C and -DRB1 and -DQB1 loci combinations

Order	HLA-B* and -C* and -DRB1* and -DQB1* (4 digits)				Allele Frequency
1	13:02G	06:02G	07:01G	02:02G	0.52%
2	44:02G	16:04G	11:04G	03:01G	0.49%
3	18:01G	01:02G	12:01G	03:01G	0.40%
4	50:01G	06:02G	07:01G	02:02G	0.37%
5	35:01G	04:01G	11:04G	03:01G	0.32%

The developed software calculated and sorted allele frequencies for combination of five loci by counting two alleles separately (for the most 5 frequent) (Table 3.31):

Table 3.31: Allele Frequency for HLA-A and -B and -C and -DRB1 and -DQB1 loci combinations

Order	**HLA-A and -B* and -C* and -DRB1* and -DQB1* (4 digits)**					**Allele Frequency**
1	01:01G	18:01G	01:02G	12:01G	03:01G	0.40%
2	24:02G	44:02G	16:04G	11:04G	03:01G	0.22%
3	29:02G	44:02G	16:04G	11:04G	03:01G	0.20%
4	24:02G	35:02G	04:01G	11:04G	03:01G	0.15%
5	3:0:01G	13:02G	06:02G	07:01G	02:02G	0.14%

Then, the software calculates all possible summed allele frequencies for unacceptable antigen combinations according to the identified antibody profile of the patient as below:

- Sum of each locus's allele frequencies that are calculated (F as frequency):
  S1 = F(HLA-A) + F(HLA-B) + F(HLA-C) + F(HLA-DRB1) + F(HLA-DQB1)
- Sum of combinations of two loci's allele frequency that are calculated:
  S2 = F(HLA-A and HLA-B) + F(HLA-A and HLA-C) + … + F(HLA-DRB1 and HLA-DQB1)
- Sum of combinations of three loci's allele frequencies that are calculated:
  S3 = F(HLA-A and HLA-B and HLA-C) + … + F(HLA-C and HLA-DRB1 and HLA-DQB1)
- Sum of combinations of four loci's allele frequencies that are calculated:
  S4 = F(HLA-A and HLA-B and HLA-C and HLA-DRB1) + … + F(HLA-B and HLA-C and HLA-DRB1 and HLA-DQB1)
- And sum five loci's allele frequencies that are calculated:
  S5 = F(HLA-A and HLA-B and HLA-C and HLA-DRB1 and HLA-DQB1)

Then the software calculates the cPRA by using the all summed allele frequencies according to the modified probability equilibrium $(1- (1-\Sigma pi)^2)$ based on population genetics as below:

$$cPRA = 1 - (1 - (S1 - S2 + S3 - S4 + S5)^2)$$

According to the results of PRA screening test performed to 380 patients who were waiting for organ transplant from a cadaver in Istanbul Faculty of Medicine, we have made detailed antibody identification with the SAB test for 48 hypersensitized (high positive HLA antibody rated) patients (Table 3.32).

Table 3.32: The Calculated PRA (cPRA) for all 48 hypersensitized patients by using this software and by using the PRA positivity rates with SAB test.

Patient No	PRA %		Results of SAB antibodies
1	Class I	63	B47, A26, B59, B78, B63, A68, A69, B38, A80, B77, B18, B13, A2, B27, B44, B35, B49, B73, B45, B37, A1, B76, A33, A66, B52, B65, B64, B51, A43, B53, B75, B67, B42, B81, A34, B58, B57, A11, B54, B62, A24, B7, A32, A25, A23
	Class II	57	DR9, DR12, DR8, DQ5
2	Class I	54	B81, B7, B27, B60, B42, B55, B61, B67, B48, B13, B56, B73, A66, A25, CW12, A26, B47, B82, A23, A34, A43, CW9, B49, B41, CW8, B54, B46, B50, B71, B62, CW10, CW16, CW7, CW1, CW15, A69, A68, B72, A31, CW14, A33, A32
	Class II	71	DR51, DR16, DR103, DR15, DR1, DR7, DR9, DR4, DR8, DR12, DR17, DR18, DR10, DR14, DR13, DR52, DQ8, DQ9, DQ2, DQ5, DQ6, DQ4
3	Class I	53	A29, B67, B55, B42, A11, B76, B54, A80, B56, B7, B71, A2, B39, A23, B81, A69, B75, B82, A24, B8, B35, B62, B41, CW14, B78, B64, CW18, B45, B50, B61, B72, A68, A32, CW6, B18, CW15, B53, A33, CW2, CW9, CW4, B65, B60, B73
	Class II	54	DR17, DR13, DR8, DR18, DR11, DR14, DR11, DR12, DP9, DP20, DP14, DP6, DQ7, DP1, DP11, DP17, DP19, DQ9, DP13, DQ8, DP10, DR52, DR4
4	Class I	38	A68, A69, B57, B58, A2, CW7, B7, A31, A66, A29, A32, A43, A33, A26, A74, A25, B73, B42, A24, B81, B67, B55, B56, A23, CW16, B82, CW17, B27
	Class II	18	DR51, DR15, DR11, DR16, DR8, DQ2, DQ7, DQ8, DQ9
5	Class I	18	A25, A26, A1, A80, A36, B73, A66, A43, A34, A11, A3, A24, B76, B45, A32, A23, B44
	Class II	20	DQ7, DQ8, DQ9, DR4, DR5

Now, we can try to calculate with the developed software for the patient ordered 5 by selecting the listed antibodies as unacceptable antigens on the main screen of software (Table 3.33) (Figure 3.7) (Figure 3.8).

Table 3.33: Patient ordered 5 from the hypersensitized patients list.

Patient No	PRA %		Results of SAB antibodies
5	Class I	18	A25, A26, A1, A80, A36, B73, A66, A43, A34, A11, A3, A24, B76, B45, A32, A23, B44
	Class II	20	DQ7, DQ8, DQ9, DR4, DR5

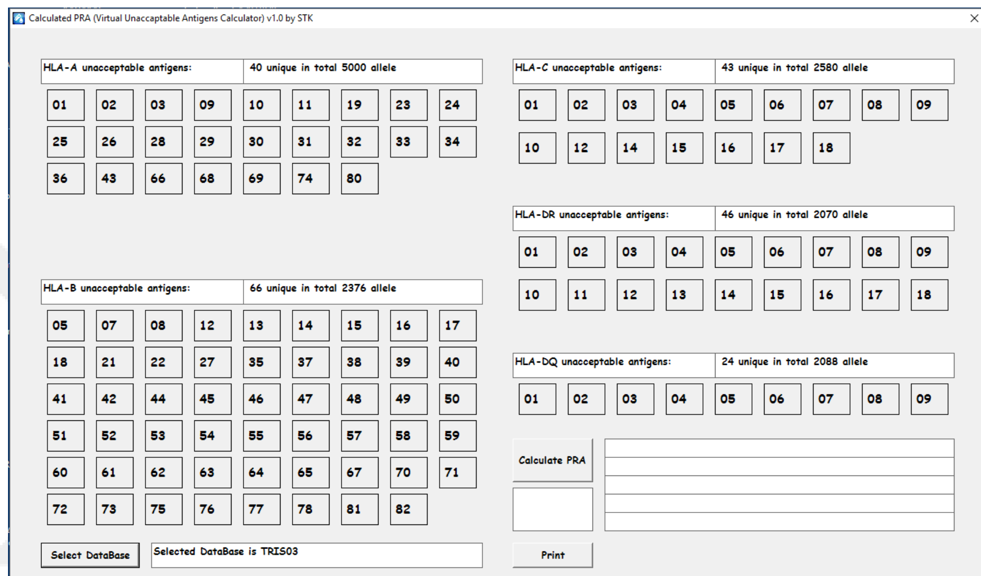

Figure 3.7: Main screen of the calculated PRA software.

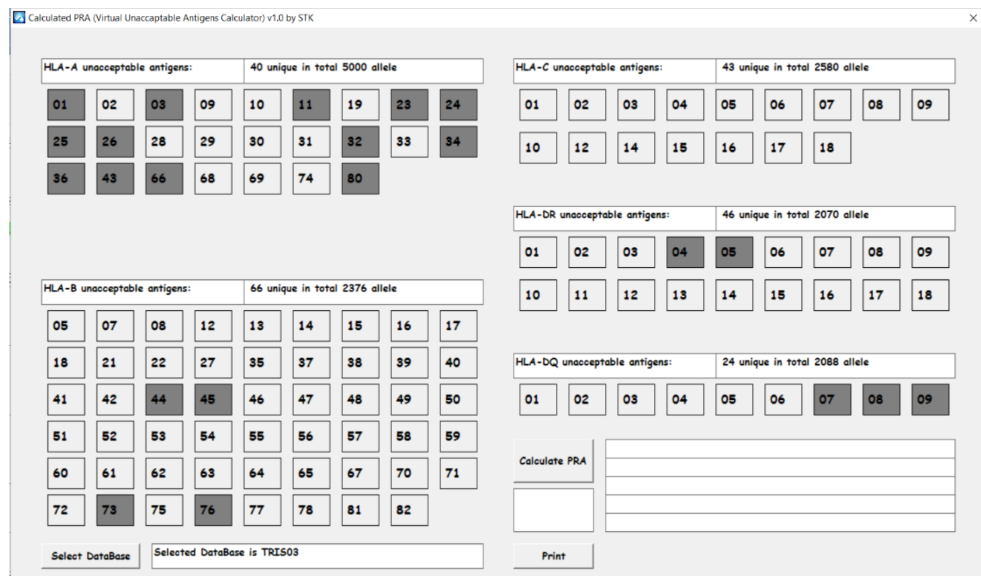

Figure 3.8: Selecting the listed antibodies as unacceptable antigens on the main screen of the calculated PRA software.

Then we can calculate the S1 below by using the formula used in the software:

S1 = F(HLA-A) + F(HLA-B) + F(HLA-C) + F(HLA-DRB1) + F(HLA-DQB1)

To calculate the S1 we must calculate the F(HLA-A), F(HLA-B), F(HLA-C), F(HLA-DRB1) and F(HLA-DQB1) separately then put to the formula above for our patient ordered 5 as below.

F(HLA-A) = F(HLA-A*01) + F(HLA-A*03) + F(HLA-A*11) + F(HLA-A*23) + F(HLA-A*24) + F(HLA-A*25) + F(HLA-A*26) + F(HLA-A*32) + F(HLA-A*34) + F(HLA-A*36) + F(HLA-A*43) + F(HLA-A*66) + F(HLA-A*80)

F(HLA-A) = 0.1285 + 0.1204 + 0.0759 + 0.0435 + 0.1406 + 0.0070 + 0.0607 + 0.0384 + 0.0001 + 0.0001 + 0.0001 + 0.0071 + 0.0001 = 0.6224

F(HLA-B) = F(HLA-B*44) + F(HLA-B*45) + F(HLA-B*73) + F(HLA-B*76)

F(HLA-B) = 0.0820 + 0.0010 + 0.0010 + 0.0001 = 0.0840

F(HLA-C) = 0

F(HLA-DRB1) = F(HLA-DRB1*04) + F(HLA-DRB1*05)

F(HLA-DRB1) = 0.1477 + 0 = 0.1477

F(HLA-DQB1) = F(HLA-DQB1*07) + F(HLA-DQB1*09) + F(HLA-DQB1*09)

F(HLA-DQB1) = 0.3997

S1 = F(HLA-A) + F(HLA-B) + F(HLA-C) + F(HLA-DRB1) + F(HLA-DQB1)

S1 = 0.6224 + 0.0840 + 0 + 0.1477 + 0.3997 = 1.2540

S2, S3, S4 and S5 can be calculated by using the same calculation principle.

The developed software can use the cPRA formula below to calculate cPRA positivity (or unacceptable antigen ratio) by pressing the “Calculate PRA” button (Figure 3.9).

$$cPRA = 1 - (1 - (S1 - S2 + S3 - S4 + S5)^{2}) = 94\%$$

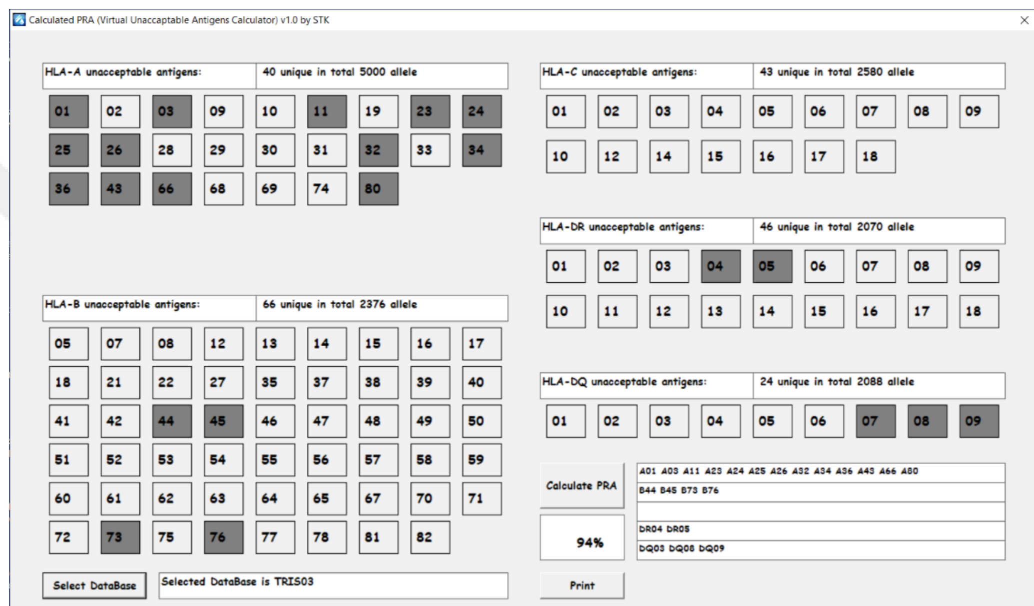

Figure 3.9: Calculated PRA result for the patient ordered 5

This cPRA value shows the calculated PRA positivity for unacceptable antigens in the Turkish population for counted 494 donors in our work.

According to the PRA testing positivity rate using the current methods were found as 44.6%±18.5 but the cPRA positivity rate was 86.2%±5.1 for all 48 hypersensitized patients in our work.

The frequencies of HLA-A, -B, -C, -DR and -DQ loci performed by Pingel et al with donor parentage from 17 different countries using DKMS (Deutsche Knochenmarkspenderdatei / German Marrow Donor Program) donors were compatible with the results of our study (23).

We also calculated the cPRA for the patient ordered 5 with the cPRA calculator on the Organ Procurement and Transplantation Network (OPTN) site and the result was highly compatible with our calculated PRA software (Figure 3.10).

The actual CPRA provided to a candidate is calculated by UNet based solely on the unacceptable antigens that are entered by the transplant center for that candidate. The value produced by the CPRA Calculator on this Web site is for your informational use only.

A:	1, 3, 23, 24, 25, 26, 32, 34, 43, 66, 80
B:	44, 45, 73, 76
BW:	
C:	
DR:	4, 5
DRW:	
DQB1:	7, 8, 9

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CPRA value used for allocation per OPTN policy: 94
Detailed CPRA value: 94.18 %

Figure 3.10: OPTN cPRA calculator result for patient ordered 5.

4. DISCUSSION

Traditional PRA test positivity result can be measured greater as 50% and even more, but, this does not reflect about the frequency of positive matched antigens in the donor population. Even the antibody positivity strength can be much higher but the frequency of that positive matched antigen does not affect negatively the chance of finding the donor. Traditional measure of PRA is not therefore a good reflection of the chances of the patient finding a compatible donor for patients on the waiting list and may not reflect the antigen frequencies in the donor population.

The calculated PRA (cPRA) was introduced to overcome this problem and developed first in the USA and adopted by UNOS (United Network for Organ Sharing). The cPRA is based on a calculation of the reaction frequency of a listed set of unacceptable mismatches for a patient, against a panel of recently added deceased donors in the population. Because of this, the cPRA therefore gives a measure of the chances of a patient finding a compatible donor in the donor pool.

The cPRA also removes some of the variability between laboratories using different panels and allows a PRA value to be assigned which reflects the patients transplantability and provides a significant change in measurement of the sensitization. Transplant centers and laboratories report PRA of one of the tests that has wide ranges of sensitivity and specificity before the implementation of cPRA.

A kidney patient with a PRA > 80% is considered highly sensitized in the Turkey and could potentially benefit from the system of kidney allocation which prioritizes highly sensitized patients for matched organs.

The CPRA provides consistent definition of sensitization and a more efficient means of organ allocation. A significant increase in transplantation of the sensitized patients has been observed but, overall graft survival remains unchanged. Thus, the utilization of cPRA has allowed for a more efficient means of organ allocation and increased access to transplant for the sensitized patients (24).

The PRA positivity rate will be identified more accurately in the future after this method is transferred to electronic environment, and with the frequent use of transplant units and tissue typing laboratories. In addition, this will have a significant role in identification of the genetic structures of HLA antigens in the population. It will increase the chances of better donor particularly for hypersensitized patients by the creation of an unacceptable mismatch program using cPRA software.

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