Role of Sensitization in the Development of Anti-HLA Antibodies in Solid Organ Transplant

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BY

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CANDIDATE'S DECLARATION

I, hereby declare that the work which is being presented in the dissertation entitled, **Role of Sensitization in the development of anti-HLA antibodies in solid organ transplant** in the partial fulfilment of the requirement for the award of degree of Master of Science in Biotechnology, Thapar Institute of Engineering & Technology, Patiala, is an original record of my own research work carried out under the guidance and supervision of **Dr. Aseem Kumar Tiwari**, Director, Department of Transfusion Medicine & Transplant Immunology, Medanta Hospital, Gurugram and **Dr. Rajni Chauhan**, Senior Scientist, Molecular & Transplant Immunology, Medanta Hospital, Gurugram and co-guidance of **Dr. Atul Kumar Upadhay**, Assistant professor, Thapar Institute of Engineering & Technology, Patiala. The content in the dissertation has not been submitted to any other university or institute, for award of any degree.

Date: 17/07/2023

Place: Gurugram

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medanta

CERTIFICATE

TO WHOM IT MAY CONCERN

This is to certify that the dissertation entitled **Role of Sensitization in the development of anti-HLA antibodies in solid organ transplant** comprises research work carried out by **Ms. Rishika Konsal** (Regd. No. 302101019) under my supervision and guidance during the period between January 2023 to July 2023 for the partial fulfillment of the requirement for the award of the degree of Master of Science in Biotechnology, submitted to Thapar Institute of Engineering & Technology, Patiala. The report has not been submitted for award of any other degree or certificate in this or any other university or institute.

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ABBREVIATIONS

ABBREVIATIONS	NAME
HLA	Human Leukocyte Antigen
CDC	Compliment Dependent Cytotoxicity
CDC-XM	Compliment Dependent Cytotoxicity- Crossmatch
FC-XM	Flow Cytometry- Crossmatch
SAB	Single Antigen Bead
DSA	Donor Specific Antibody
CKD	Chronic Kidney Disease
MHC	Major Histocompatibility Complex
CD	Cluster of Differentiation
SE	Sensitization Event
PRA	Panel Reactive Antibody
Ml	Millilitre
NaOH	Sodium Hydroxide
Rcf	Relative Centrifugal Force
AHG	Anti-Human Globulin
RPMI	Roswell Park Memorial Institute
w.r.t	With respect to
IgG	Immunoglobulin G
NC	Negative Control
PC	Positive Control

Ul	Microlitre
PerCP	Peridinin Chlorophyll Protein Complex
PE	Phycoerythrin
Rpm	Revolutions per Minute
MFI	Mean Fluorescence Intensity

ABSTRACT

Human Leukocyte Antigen (HLA) alloimmunization is caused by exposure to HLA antigens by sensitization events such as pregnancy, blood transfusion or any previous transplantation history. Our objective was to evaluate how each sensitization event affects HLA alloimmunization by cell based as well as solid phase assays.

We analysed anti-HLA antibody status of 402 recipients waiting for kidney transplantation on the basis of their sensitization history. Serum from transplant recipients and blood from organ donors was collected to check the probability of survival of transplanted graft in the recipient's body by cell-based assays-complement dependent cytotoxicity crossmatch (CDC-XM) and flow cytometry crossmatch (FC-XM) and solid phase assays- anti-HLA antibody screen assay and single antigen bead (SAB) assay. The test for screening anti-HLA antibodies included CDC-XM, FC-XM and anti-HLA antibody screen. If any of these assays was positive, then confirmation of anti-HLA antibodies was performed by SAB assay. The antibodies detected by SAB assay were then virtually matched with the donor's HLA antigens, to identify DSA.

In this study, HLA-antibody screening tests positive rates (CDC-XM, FC-XM, anti-HLA antibody screen) were higher in patients with previous transplantation followed by previous pregnancy and blood transfusion as compared with patients without any sensitization history. Re-transplant patients had more DSA than pregnancy and blood transfusion.

1.1 Chronic Kidney Diseases (CKD)

In CKD, the kidneys gradually losses its function and hence cannot filter out wastes and excess fluids from the blood via urine. In severe cases, high levels of wastes, fluid and electrolytes builds up in the body. Its end stage is kidney failure. It makes the patient dependent on dialysis.
It majorly arises due to either diabetes or high blood pressure or any family history of kidney failure. The definitive treatment in such cases is organ transplantation.

1.2 Organ Transplantation

In an organ transplantation, the organ is removed from one person's body (donor) and placed into another person's body (recipient). Solid organs that have been successfully transplanted till now are heart, kidneys, liver, lungs, etc. Among them, kidneys are the most transplanted organ across the world. Transplantation medicine is one of the most challenging and complex areas of modern medicine. Some of the major problems faced during transplantation are transplant rejection, during which the body has an immune response to the transplanted organ, possibly leading to transplant failure and the need to immediately remove the organ from the recipient. When possible, transplant rejection can be reduced through pre-transplant compatibility testing to determine the most appropriate donor-recipient match.

1.3 Major Histocompatibility Complex

MHC (major histocompatibility complex) is referred to as the human leukocyte antigen (HLA) complex in humans. It is present on chromosome 6 in humans. HLAs are the cell surface molecules found on all nucleated cells. Each individual has a unique set of these antigens. HLA complex is the locus of gene that encodes for proteins on the surface of cells responsible for regulation of immune system in humans. They continually present peptides to T-cells, hence allowing for tolerance of cells expressing self-antigens and the elimination of cells expressing non-self-antigens. One-half are inherited from the mother and other-half from father, so each brother and sister who shares the same parents, has a 25% chance (1 in 4) of being an identical HLA match. They are polymorphic in nature, i.e., many alternate forms of each gene exist in the population.

They are divided into two classes, i.e., class I and class II. Class I antigens include the HLA-A, -B, and -C antigenic clusters. They are expressed on the surface of all the nucleated cells, platelets, and sometimes on red cells. They present antigenic peptides to CD8+ T cells and help in

identifying self and non-self cells. Conversely, class II antigens include the HLA-DR, -DQ, and -DP antigenic clusters. They are expressed on the surface of antigen presenting cells (APCs) of the immune system, such as dendritic cells and B-Cells. They present antigenic peptides to CD4+ T cells and stimulate immune system.

Antigens and proteins are broken down into peptides, and these peptides form a complex with HLA proteins. The HLA-peptide complex subsequently interacts with effector T-cells causing intracellular signals in both cells, which determines if a specific immune response occurs. Effector T cells differentiate between 'self' and 'non-self' proteins - therefore, if the peptides presented are recognized as 'non-self', an immune response will commence.

1.4 Role of sensitization

The major problem which is faced during transplantation is its rejection which finally leads to transplant failure. One of the reasons for this could be sensitization or the presence of circulating HLA antibodies inside the recipient. Sensitization occurs via prior transplantation, pregnancy, or blood transfusion. Sensitization leads to the formation of anti-HLA antibodies, which could be donor-specific and can limit organ transplantation possibilities.

1.5 Detection of Anti-HLA antibodies

Anti-HLA antibodies can be detected by cell-based assays like complement dependent cytotoxicity crossmatch (CDC-XM) and flow cytometry crossmatch (FC-XM) and solid phase assays like anti-HLA antibody screen assay and single antigen bead (SAB) assay. The test for screening anti-HLA antibodies included CDC-XM, FC-XM and anti-HLA antibody screen. If any of these assays was positive, then confirmation of anti-HLA antibodies was performed by SAB assay. The antibodies detected by SAB assay were then virtually matched with the donor's HLA antigens, to identify DSA.

Renal transplantation is the best treatment for the end stage kidney diseases by far. Though, there has been continuous improvements in immunosuppression therapy, failure of renal allografts still occurs because of cellular or humoral mediated rejection. The presence of circulating anti-HLA antibodies in recipient's blood before transplantation has been associated with allograft rejection, if they are donor specific.

HLA alloimmunization is caused by any of the sensitization events (SE) viz-a-viz transfusion, pregnancy or previous organ transplantation. Patients who are pre-sensitized have increased chances of positive crossmatch, reducing their chances of receiving a compatible graft.

Several recent studies evaluated the prevalence of HLA-specific antibodies and the clinical importance of these antibodies in acute allograft rejection.

Patients who become sensitized to HLA have an increased likelihood of a positive cross-match, reducing their chances of transplantation. Anti-HLA antibodies can be assessed with the use of cell-based assays like CDC-XM or solid phase assays like SAB assay (most sensitive) **(Lopesa, Barraa, Malheiroa et al., 2015).** The study was conducted in 722 kidney transplantation recipients (2007-2014) in whom Panel Reactive Antibodies (PRA) test and anti-HLA antibody screening had been performed. The effect of each SE on HLA allo-immunization was analysed considering only patients exposed to any one of the SE from the total patients exposed to that particular event. Hence 4 groups were made: no SE (control group), previous transfusion only, previous pregnancy only, and previous transplantation only.

They observed prevalence of anti-HLA antibodies against class I or II was significantly higher in patients with previous transplantation than with transfusion. Anti-HLA antibodies strength was similar between patients with previous pregnancy and with previous transplantation history. HLA alloimmunization effect due to pregnancy was found to be inter-mediate between transplantation and transfusion. Transfusion had the least effect on HLA alloimmunization compared with pregnancy and transplantation. They concluded transplantation had the strongest immunization effect in both class I and class II, followed by pregnancy and then transfusion.

Another study evaluated alloimmunization rates in the sensitized patients waiting for kidney transplantation **(Ibrahim Pirim, Mustafa Soyoz et al.,2015)**. The recipients were divided into six groups such as only pregnancy, only blood transfusion, only transplantation, pregnancy and blood transfusion, pregnancy and transplantation and all three sensitization events together. They observed patients with both transplantation and pregnancy history had higher rate of anti-HLA antibodies as compared to other groups. This study concluded that solid organ transplantation had strongest HLA alloimmunization effect followed by pregnancy and blood transfusion. Patients who were sensitized by more than one sensitization event had lower chances to have solid organ transplantation than others.

In 2016**,** a study evaluated around 411 kidney transplantation recipients (**A. Picascia et al.)**. The recipients were categorized into 7 groups according to sensitizing events: no sensitizing event, pregnancy only, transfusion only, transplantation only, pregnancy and transfusion combined, pregnancy and transplantation combined and all together.

The study showed that more than half of the patients had anti-HLA antibodies having pregnancy as the sensitization event. Least anti-HLA antibodies was found in patients with only blood transfusion history. Pregnancies combined with any other sensitizing event than other combinations had maximum anti-HLA antibodies. The concluded pregnancy as strong sensitizing event which remarkably impacts the process of transplantation.

In 2016, a study analyzed all kidney transplants between 1997 and 2014. This study compared highly sensitized recipients with non- sensitized recipients and studied 5,09,196 recipients, out of which more than half of the recipients were non-sensitized **(Robert R. Redfield et al.)**. There is increased risk of graft rejection for highly sensitized patients than non-sensitized patients due to the presence of anti-HLA antibody. The combination of being highly sensitized by either pregnancy or blood transfusion increased the risk of graft loss as compared with being non-sensitized. In case of prior transplant only, the risk of graft loss in the highly sensitized patient was highest.

Another study**,** in 2017 analysed 906 kidney transplantation recipients in whom Panel Reactive Antibody test was positive, hence had anti-HLA antibodies in them **(Akgul, Ciftci, Temurhan et al.)**. This study analysed the effect of each SE on HLA alloimmunization considering only patients exposed to any one of the SE from the total patients exposed to that particular event. Recipients

were divided into 4 groups: no SE (control group), patients with previous transfusion only, patients with previous pregnancy only, and patients with previous transplantation only.

The study concluded that rates of PRA positivity were significant in patients with pregnancy, transfusion or transplantation history compared with patients with no sensitization. They observed, patients having pregnancy as the only sensitizing event had maximum anti-HLA antibodies. Pregnancies combined with any other sensitizing event than other combinations had maximum anti-HLA antibodies. Transfusion had the smallest effect on anti-HLA antibody formation compared with pregnancy or transplantation.

In 2018, a study compared the effects of transfusion, pregnancy, and transplant on HLA alloimmunization in 606 patients waiting for kidney transplantation **(M. Resse, R. Paolillo et al.)**. They studied patients with and without any sensitization history. All samples were studies for the presence of anti-HLA class I and II alloantibodies. The antibody strengths against HLA class I and II came out be higher in patients with previous transplant than in those with transfusion and pregnancy. The sensitizing events were associated with higher prevalence of anti-HLA antibodies compared with patients without sensitization event with transplantation causing greater effect and transfusion and pregnancy showing similar effect on the formation of alloantibodies in the sensitized patients and reducing the chances of graft survival.

In 2022, 1066 patients were analysed to study the effects of a particular sensitization event on HLA immunization **(P. Pandey et al.)**. Patients were divided into 5 groups: no SE (control group), previous transfusion only, previous pregnancy only, and previous transplantation only, pregnancy with transplantation and transfusion.

Study observed that in patients with a single SE, both class I and II positive rates were significantly higher compared with candidates without any identifiable SE. It was found that prevalence of anti-HLA antibodies was significantly higher in patients with previous transplantation than with transfusion. It was similar between patients with previous pregnancy when compared with transplantation. Pregnancy HLA alloimmunization effect was found to be intermediate between transplantation and transfusion. Transplantation had the strongest immunization effect in both classes, followed by pregnancy and then transfusion.

2.1 Objectives of the study

1. Evaluate and compare the role of different sensitizing events like transplantation, pregnancy and blood transfusion on the anti HLA antibody production in the recipients waiting for kidney transplantation.

2. Perform the cell based as well as solid phase assays to detect anti-HLA antibodies.

CHAPTER 3 - MATERIALS AND METHODS

3.1 Patient

Samples of the patients as well as the prospective donors were collected from kidney transplant recipients in the Molecular and Transplant Immunology Laboratory in Medanta Hospital, Gurugram, as follows:

- CDC Crossmatch: 3 Red top plain vacutainers each for the recipient and prospective donor and defibrinated immediately.
- 2 serum separating tubes (SST) for the recipient.
- Flow Cytometry Crossmatch: 3 Heparinized Vacutainer for the prospective donor.

Their sensitization history was evaluated and were divided into five groups consisting of: No sensitization event (control group), only blood transfusion, only pregnancy, re-transplantation, transfusion with pregnancy.

3.2 Settings and compatibility tests

Comprehensive pre-transplant HLA-antibody screening tests viz-a-viz CDC-XM, FC-XM and anti-HLA antibody screen test tests were performed according to the Standard Operating Protocols of the hospital as mentioned the flowchart of figure 3.3.12. If anti-HLA antibody screen test came out to be positive HLA-antibody detection test or SAB test was done to check the Donor Specific Antibodies.

3.3 Tests performed

3.3.1 <u>Complement Dependent Cytotoxicity Crossmatch</u>

CDC-XM testing has been an important test in histocompatibility laboratories to check the compatibility between graft and the host. The CDC crossmatch basically checks if the recipient has alloantibodies against an allograft that may lead to graft rejection. CDC crossmatch results are examined under inverted phase contrast microscope (figure *3.3.1*).

Figure 3.3.1- Inverted Phase Contrast Microscope

Requirements: Equipment- Refrigerated Benchtop Centrifuge, Inverted Microscope, Incubator (37°C)

Materials and Chemicals- Falcons (15ml, 2ml), Hisep, RPMI Media, Pasteur pipette, Terasaki Tray, Micro Centrifuge Tubes, Paraffin oil, AHG, Rabbit Complement, Eosin dye, NaOH, Formaldehyde, Plain Glass Side.

Procedure:

- CDC-XM was performed by layering recipient's and donor's defibrinated blood samples over hisep in the falcons followed by density gradient centrifugation (900 rcf, acceleration=brake=1 for 25-27 minutes).
- Buffy coat was then collected in separate falcons and mixed with RPMI media and again centrifuged (900 rcf, acceleration=brake=9 for 10 minutes) to obtain cells in the form of a pellet.
- The supernatant was discarded, and the pellet was resuspended in the media. Then different dilutions of the patient's serum were made using media and poured in terasaki tray (figure- 3.3.2), followed by pouring of the recipient's as well as donor's cells in respective wells of the tray (according to table 3.3.1) and incubation at 37°C for 75 minutes.
- After incubation, serially diluted AHG (according to table 3.3.2) and rabbit complement was added to the wells and again incubated 37°C for 75 minutes.
- After the second incubation, eosin red dye and formaldehyde were added and incubated at 2-8°C overnight
- Tray was observed under an inverted microscope next day to check if the cells were dead (positive crossmatch) or alive (negative crossmatch) (figure-3.3.3 A & B).

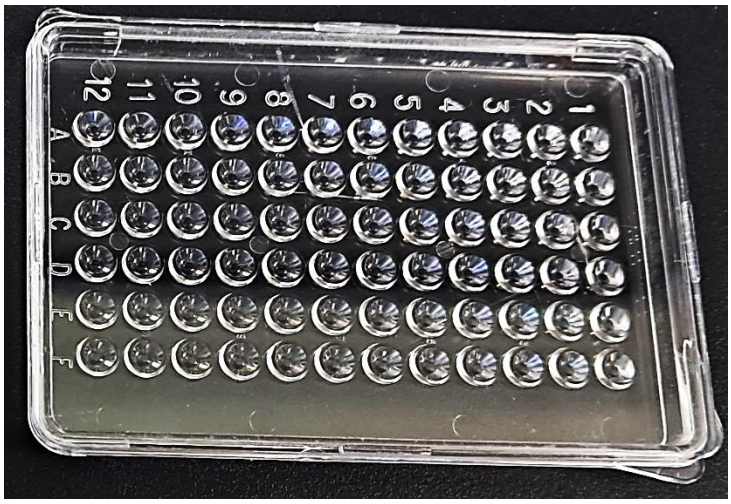


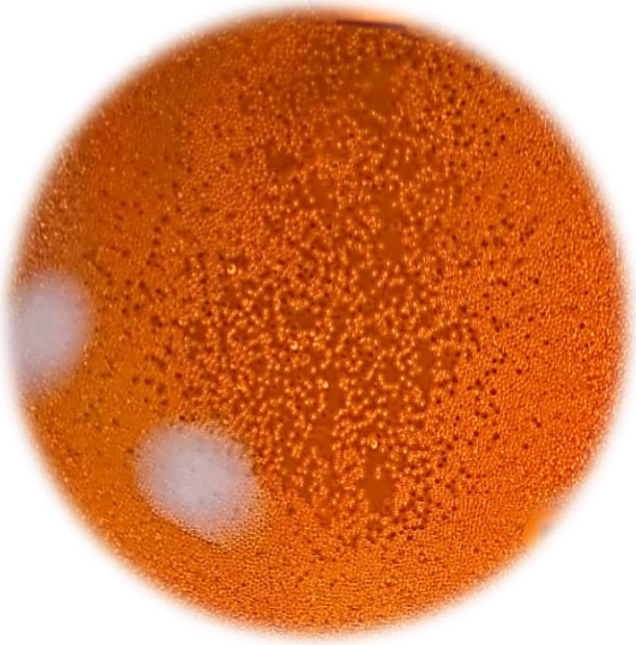
Figure 3.3.2- Terasaki tray

DILUTION OF SERUM	WELLS IN TRAY
Neat (Serum)	Row A and B till 5th column (i.e., 12th-5th)
1/2	Row C and D till 5th column (i.e., 12th-5th)
1/4	Row E and F till 5th column (i.e., 12th-5th)

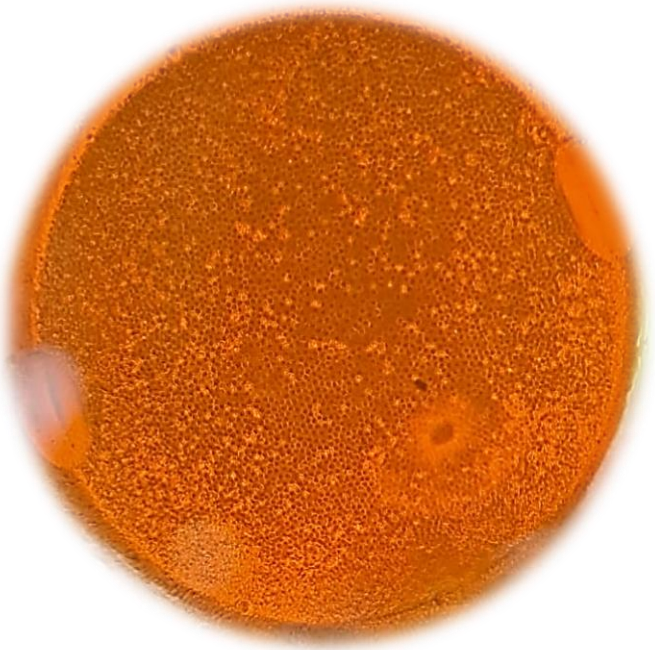
Table 3.3.1- Different dilutions of serum w.r.t. the wells of the Terasaki tray

AHG DILUTIONS	WELLS IN TRAY
1/32	Column 12,11,4,3,1
1/64	Column 10,9
1/128	Column 8,7
1/256	Column 6,5

Table 3.3.2- Different dilutions of AHG w.r.t. the wells of the Terasaki tray



A



B

Figure 3.3.3- A) Negative CDC crossmatch. Live cells don't take up die and hence they are bright.

B) Positive CDC crossmatch. Dead cells take up die and hence are darkly stained.

3.3.2 Flow Cytometry Crossmatch

Flow cytometry is a technique which studies the properties of a cell. In flow cytometry cross-matching (FC-XM), donor lymphocytes were mixed with recipient's immune serum, and fluorescent labelled antibodies. The antibodies used are specific to T-cell and B-cells (CD3 for T-cells, and CD22 for B-cells). During flow cytometry, the sample is suspended in sheath fluid and injected into the flow cytometer. Usually, one cell at a time is passed through a laser beam for analysis purposes. The scattering of light caused by this gives information on the characteristics of the cells of the sample. If there are donor-specific HLA antibodies in the serum, they will bind to the donor lymphocytes, which allows the fluorescently labelled antibodies to bind, giving in turn a positive cross-match. A positive FC-XM is associated with an increased chance for a transplant rejection. FC-XM is important, as it allows for donor antigens and host antibodies to be checked prior to a transplant to avoid a host immune response. This reduces the chances of acute or chronic allograft rejection.

Figure 3.3.4- Flow Cytometer Instrument (BD FACSVerse)

Requirements: Equipment- Refrigerated Benchtop Centrifuge, Incubator (37°C), Flow Cytometer

Materials and Chemicals- Falcons (15ml, 2ml, 5ml), Hisep, RPMI Media, Pasteur pipette, Sheath fluid, Vortex, IgG, CD-3, CD-22

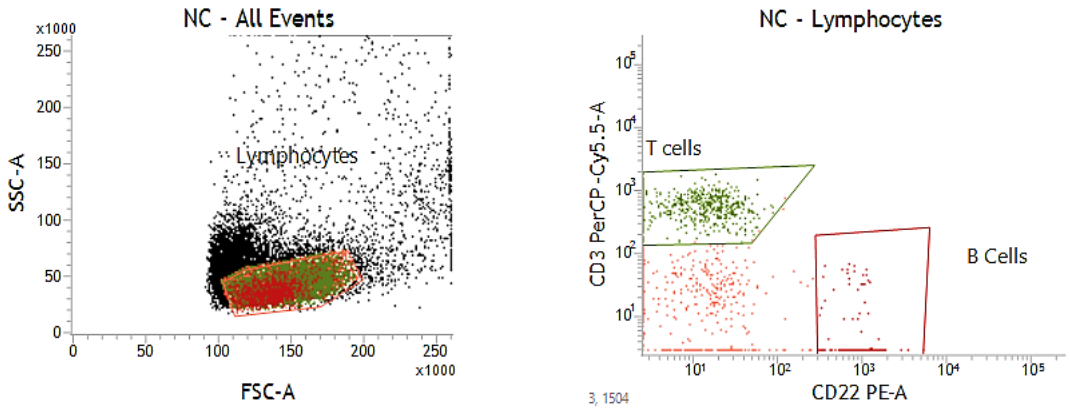
Procedure:

- In flow crossmatch, the donor's defibrinated blood samples were layered over hisep in the falcons, followed by density gradient centrifugation (900 rcf, acceleration=brake=1 for 25-27 minutes).

- Buffy coat was collected in separate falcons and mixed with RPMI media, and incubated at 37°C for 1.5 hrs.
- After incubation, the cells were washed thrice using sheath fluid (centrifugation at 900 rcf, acceleration=brake=9 for 5 minutes at 37°C).
- The supernatant was then discarded, and the pellet was resuspended in the sheath fluid.
- Six falcon tubes (5ml) were taken and labelled as NC, PC, Test 1, Test 2, 1:2 and 1:4. (1:2 and 1:4 serial dilutions were only made in case of any sensitization history of the patient)
- Differential count for 1 ml of cell suspension was done to check the amount of lymphocytes (**X* 10^3 cells/ul**) according to formula: **250/X+20 = Y ul**, and the cells were accordingly added into BD tubes (**Y ul**) already having the recipient's serum and incubated at 4°C for 30 minutes.
- After second incubation, sheath fluid was added to each tube and washed thrice by centrifugation (3300 rpm for 3 minutes at 4°C).
- Then 50ul anti-IgG, 5ul CD3 PerCP, and 5ul CD22 PE was added in each tube and again incubated at 4°C for 30 minutes.
- After the incubation, the mixture was again washed thrice using the sheath fluid (3300 rpm for 2 minutes at 4°C). After washing, the pellet was resuspended in 200ul sheath fluid and finally acquired on the flow cytometer.

	NEGATIVE	**WEAK POSITIVE**	**POSITIVE**
T-CELL	**≤ 25**	**26-49**	**≥ 50**
B-CELL	**≤ 109**	**110-199**	**≥ 200**

Table 3.3.3 - Flow cytometry crossmatch cut off values.



Statistics

Name	Anti IgG FITC-A Median
NC:T cells	28
PC:T cells	286
T1:T cells	26
T2:T cells	26
1:2:T cells	26
1:4:T cells	31

Statistics

Name	Anti IgG FITC-A Median
NC:B Cells	158
PC:B Cells	2,190
T1:B Cells	171
T2:B Cells	168
1:2:B Cells	168
1:4:B Cells	198

A)

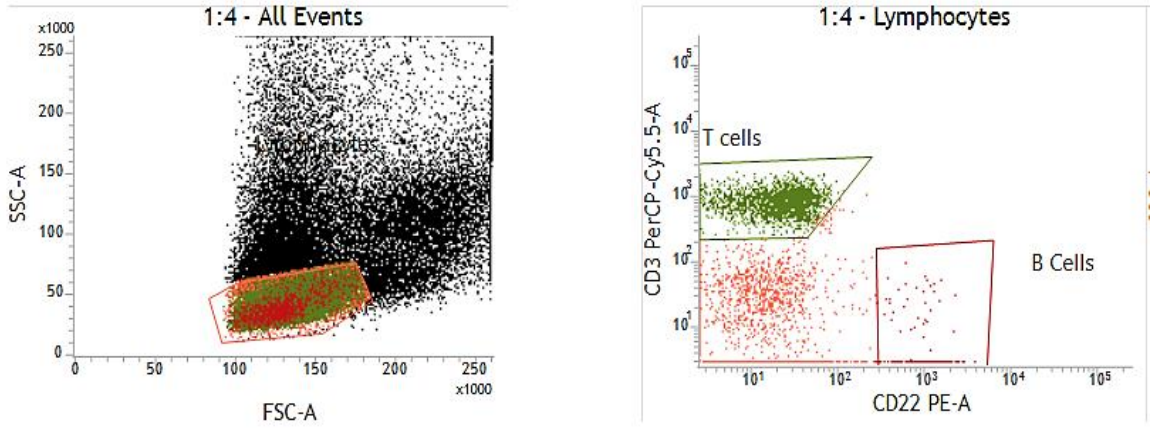
NAME	TEST-NC (T CELLS)	TEST-NC (B CELLS)
TEST 1	26-28= -2	171-158= 13
TEST 2	26-26= 0	168-158= 10
1:2	26-26= 0	168-158= 10
1:4	31-26= 5	198-158= 40

B)

Figure 3.3.5- A) A case of Flow Cytometry crossmatch

B) Results obtained by subtracting test 1&2 and dilutions 1:2&1:4 values with the negative control values as obtained in statistics table of figure 3.3.5 A.

According to cut off values from table 3.3.3, the FC-XM is negative.



Statistics

Name	Anti IgG FITC-A Median
NC:T cells	28
PC:T cells	412
T1:T cells	2,134
T2:T cells	2,950
1:2:T cells	2,970
1:4:T cells	2,051

Statistics

Name	Anti IgG FITC-A Median
NC:B Cells	242
PC:B Cells	4,865
T1:B Cells	1,799
T2:B Cells	2,924
1:2:B Cells	2,486
1:4:B Cells	2,063

A)

NAME	TEST-NC (T CELLS)	TEST-NC (B CELLS)
TEST 1	2134-28= 2106	1799-242=1557
TEST 2	2950-28=2922	2924-242=2682
1:2	2970-28=2942	2486-242=2244
1:4	2051-28=2023	2063-242=1821

B)

Figure 3.3.6- A) A case of Flow Cytometry crossmatch

B) Results obtained by subtracting test 1&2 and dilutions 1:2&1:4 values with the negative control values as obtained in statistics table of figure-3.3.6 A.

According to cut off values from the table 3.3.3, the FCX-M is positive

3.3.3 Anti-HLA Antibody Screen test

Anti-HLA Antibody Screen test is a group of antibodies in the recipient's serum that are reactive against any of the known specific antigens in donor's lymphocytes. Usually, serum is exposed an aliquot of the beads to which affinity purified Class I & 11 specific HLA glycoproteins from different individuals are conjugated separately. anti-HLA antibody screen test score is expressed as a percentage representing the proportion of the population to which the person being tested will react via pre-existing antibodies against human cell surface antigens. It is a test of the degree of allo-immunity in a graft recipient and thus tells if the transplantation will be successful. Individuals with a high anti-HLA antibody screen test value are often termed "sensitized", which indicates that they have been exposed to "foreign" (or "non-self") proteins in the past and have developed antibodies to them. Hence the individual will react immunologically against a large proportion of the population.

Requirements: Equipment- Immucor Luminex, Shaker, Vaccum pump, Immucor Lifecodes LifeScreen kit

Materials and Chemicals- Falcons (2ml), Sterile water, Vortex, Multi-pore Plate

Procedure:

- The anti-HLA antibody screen test was performed by adding 2-3 drops of sterile water in the wells of the multi-pore plate.
- 45ul wash buffer +12.5ul patient's serum + 5ul beads having all the possible antigenic alleles (for both classes, class I and II) commonly found in the people were mixed in a separate micro centrifuge tube and vortexed.
- The mixture was then poured into wells of the plate, followed by incubation for 30 minutes on a shaker.
- It was then washed 3 times with 150ul wash buffer. Then 45ul wash buffer + 5ul conjugate was added followed by incubating it for 30 minutes on a shaker and adding 150ul wash buffer.
- Finally, it was Acquired on Luminex machine.

		Class I Results								Class II Results						CONs
		CI-01	CI-02	CI-03	CI-04	CI-05	CI-06	CI-07	Assignment	CII-01	CII-02	CII-03	CII-04	CII-05	Assignment	Pos Ctrl
Sample ID: R[redacted]	Raw	220	137	163	91	178	140	130		135	71	80	120	2979		11879
Patient Name: R[redacted]	Adj 1	-3.67	-2.34	-2.07	-2.73	-2.15	-2.84	-2.55		-2.91	-2.99	-3.20	-2.93	15.36		157
Accession:	Adj 2	-2.80	-2.11	-1.88	-2.39	-1.85	-2.50	-2.11		-2.60	-2.61	-2.95	-2.67	15.59		159
Draw Date:	Adj 3	-2.25	-1.74	-1.47	-1.97	-1.52	-1.96	-1.76		-2.16	-1.99	-2.14	-2.14	9.03		255
	Score	0	0	0	0	0	0	0	Negative	0	0	0	0	3	Positive	

Comments:

Figure 3.3.7 - Anti-HLA antibody screen test *test showing class I as negative and class II as positive. Hence, SAB test is performed to check the presence of Donor Specific Antibodies.*

3.3.4 Single Antigen Bead assay

Single Antigen Bead (SAB) assays are the most sensitive diagnostic method for HLA antibody detection and identification. In this assay, a population of around 100 unique beads that are distinguished by a unique colour, created by 2 fluorescent dyes are used. Each bead is coated with multiple copies of a single recombinant HLA antigen. The assay detects the relative level of recipient's antibody bound to each bead coated with the target HLA antigen using indirect immunofluorescence. Unbound antibody is removed, and a secondary fluorochrome-conjugated anti-human IgG antibody is added. The beads are analyzed on the Luminex machine, which measures the intensity of the fluorescent signal from the secondary antibody. This signal is represented as the MFI (Mean Fluorescence Intensity) value. It measures relative fluorescence intensity corresponding to the amount of HLA antigen specific IgG antibody bound to each bead.

Figure 3.3.8- Immucor Luminex Instrument

Requirements: Equipment- Immucor Luminex, Shaker, Vaccum pump, Immucor Lifecodes LSA class I and class II kit

Materials and Chemicals- Falcons (2ml), Sterile water, Vortex, Multi-pore Plate

Procedure:

- In SAB test, 2-3 drops of sterile water were added in the wells of the multi-pore plate.
- 10ul patient's serum + 45ul respective beads of class I and II, having all the possible antigenic alleles commonly found in the people, was mixed in separate micro centrifuge tubes and vortexed.
- The mixture was then poured into wells of the plate, followed by incubation for 30 minutes on a shaker.
- It was then washed 3 times with 150ul wash buffer and adding 45ul wash buffer + 5ul conjugate.
- It was then again incubated for 30 minutes on a shaker and 150ul wash buffer is added.
- Finally, it was acquired on Luminex machine.

HLA Typing Report

Lab Number : 236/23

HLA Gene	Allele:Protein:Coding Region	Allele:Protein:Coding Region
HLA-A*	11:01:01	24:02:01
HLA-B*	40:06:01	51:06 :01
HLA-C*	14:02:01	15:02:01
HLA-DRB1*	15:01:01	-
HLA-DQB1*	05:02:01	06:01:01

Figure 3.3.9- HLA typing report of the donor, of the recipient whose SAB report has been shown in figure 3.3.10 (class I) and figure 3.3.11 (class II). This figure shows class I and II alleles (A, B, C, DR, DQ alleles respectively)

Positive CON MFI: 19476 **Negative CON MFI:** 241 **Analysis Mode:** Manual

Antigen ID	Cut-off	Raw Value	MFI/LRA	Assignment	BG Adjusted	AD-MFI	AD-BG Adjusted	A	B	C	Bw
103	3.74	8130	34.01	Positive	7951	6626	6480	A*01:01			
145	4.21	4129	19.57	Positive	3950	2885	2760		B*15:16		Bw4
125	4.03	3438	14.38	Positive	3236	2622	2468	A*36:01			
143	3.76	1564	7.41	Positive	1396	1091	974		B*15:12		Bw6
133	3.58	1014	4.24	Positive	857	842	712	A*80:01			
108	3.84	901	3.77	Negative	717	747	595	A*02:05			
118	3.91	801	3.35	Negative	621	652	505	A*29:02			
107	3.76	796	3.33	Negative	610	664	509	A*02:03			
106	3.82	715	2.99	Negative	542	575	436	A*02:02			
104	3.80	695	2.91	Negative	524	570	430	A*02:01			
174	3.73	659	3.12	Negative	509	467	361		B*57:01		Bw4
131	4.31	658	2.75	Negative	440	740	495	A*69:01			
175	4.05	649	3.07	Negative	474	519	379		B*58:01		Bw4
117	4.12	635	2.65	Negative	431	600	407	A*29:01			
163	5.14	560	2.65	Negative	291	566	294		B*46:01		
130	3.73	540	2.26	Negative	366	457	310	A*68:02			
162	4.10	536	2.54	Negative	339	449	284		B*45:01		Bw6
129	3.59	521	2.18	Negative	352	433	293	A*68:01			
160	4.00	504	2.39	Negative	330	395	259		B*44:02		Bw4
181	3.97	502	2.38	Negative	322	402	258		B*82:02		Bw6
161	4.46	497	2.35	Negative	285	378	216		B*44:03		Bw4
186	3.89	479	1.56	Negative	201	881	369			C*04:01	
188	3.65	473	1.55	Negative	232	547	269			C*05:01	
159	4.19	465	2.20	Negative	266	360	206		B*42:01		Bw6
139	5.08	456	2.16	Negative	187	371	152		B*14:02		Bw6
198	3.76	455	1.49	Negative	200	596	262			C*17:01	
128	3.65	454	1.90	Negative	280	352	217	A*66:02			
197	3.51	450	1.47	Negative	234	497	258			C*16:01	
187	3.52	436	1.42	Negative	215	453	223			C*04:03	
147	3.81	435	2.06	Negative	268	312	192		B*18:01		Bw6
158	4.25	432	2.05	Negative	228	352	186		B*41:01		Bw6
185	3.56	427	1.40	Negative	192	395	177			C*03:04	
176	3.78	423	2.00	Negative	262	303	188		B*59:01		Bw4
193	3.43	415	1.35	Negative	198	433	206			C*08:02	
178	4.15	410	1.94	Negative	214	329	172		B*73:01		
183	3.50	409	1.34	Negative	189	540	250			C*02:02	
169	4.20	407	1.93	Negative	215	318	168		B*52:01		Bw4
182	3.45	404	1.32	Negative	191	326	154			C*01:02	
135	3.93	403	1.91	Negative	222	306	168		B*07:03		Bw6
134	4.45	402	1.91	Negative	182	312	141		B*07:02		Bw6
196	3.41	399	1.30	Negative	189	388	183			C*15:02	
113	3.96	398	1.67	Negative	194	310	151	A*24:02			Bw4
191	3.53	398	1.30	Negative	165	484	201			C*07:02	
142	3.76	393	1.86	Negative	232	290	171		B*15:03		Bw6
177	3.61	392	1.86	Negative	253	264	170		B*67:01		Bw6
199	3.50	392	1.28	Negative	166	416	176			C*18:01	
151	4.22	391	1.85	Negative	189	320	155		B*35:01		Bw6

Figure3.3.10- SAB class I report showing the positive and negative antibodies of the recipient. Positive alleles of the recipient are matched with HLA alleles of the donor, if they match, this means recipient has Donor Specific Antibodies.

Positive CON MFI: 19960 **Negative CON MFI:** 306 **Analysis Mode:** Manual

Antigen ID	Cut-off	Raw Value	MFI/LRA	Assignment	BG Adjusted	AD-MFI	AD-BG Adjusted	DR/DRSx	Epitopes	DQA	DQB	DPA	DPB
251	4.64	5506	23.84	Positive	5314	5914	5708		18SI	DQA1*02:01	DQB1*03:02		
215	3.70	5180	28.46	Positive	5053	5340	5209	DRB1*07:01	96H				
252	4.57	5151	22.30	Positive	4956	5474	5267		18SI	DQA1*03:01	DQB1*03:02		
218	3.72	4789	26.31	Positive	4652	4942	4704	DRB1*09:01	96H				
253	4.14	4386	18.99	Positive	4218	5018	4826		18SI	DQA1*03:02	DQB1*03:02		
254	4.38	4164	18.03	Positive	3974	4663	4450		18SI	DQA1*03:02	DQB1*03:03		
257	4.13	3956	17.13	Positive	3798	4386	4211		18SI	DQA1*02:01	DQB1*04:01		
255	4.13	3602	15.59	Positive	3438	4263	4069		18SI	DQA1*04:01	DQB1*03:03		
261	4.30	3337	14.44	Positive	3168	4140	3930		18SI	DQA1*04:01	DQB1*04:02		
260	4.25	3260	14.11	Positive	3075	4245	4004		18SI	DQA1*03:01	DQB1*04:02		
238	4.40	3085	16.95	Positive	2929	3278	3113	DRB3*03:01	96H				
236	4.52	2941	16.16	Positive	2749	2946	2754	DRB3*01:01	96H				
262	4.07	2803	12.13	Positive	2642	3698	3485		18SI	DQA1*06:01	DQB1*04:02		
256	3.88	2754	11.92	Positive	2598	3586	3383		18SI	DQA1*06:01	DQB1*03:03		
258	4.17	2576	11.15	Positive	2410	3354	3138		18SI	DQA1*04:01	DQB1*04:01		
259	4.16	2404	10.40	Positive	2232	3252	3020		18SI	DQA1*05:01	DQB1*04:01		
223	3.98	2328	12.79	Positive	2184	2664	2499	DRB1*12:01	96H				
237	4.29	2323	12.76	Positive	2160	2445	2274	DRB3*02:02	96H				
280	3.99	2070	10.67	Positive	1924	1907	1773		8L			DPA1*02:01	DPB1*04:01
299	4.67	1810	9.33	Positive	1629	2050	1845		8L, 56EE			DPA1*02:02	DPB1*28:01
297	3.97	1791	9.23	Positive	1632	1811	1650		56EE			DPA1*01:03	DPB1*18:01
224	4.24	1770	9.73	Positive	1604	4005	3629	DRB1*12:02	96H				
283	4.13	1644	8.47	Positive	1484	1572	1419		8L			DPA1*04:01	DPB1*04:01
279	4.41	1546	7.97	Positive	1385	1464	1312		8L			DPA1*01:03	DPB1*04:01
284	4.29	1533	7.90	Positive	1359	1466	1299		8L, 56EE			DPA1*01:03	DPB1*04:02
281	4.13	1373	7.07	Positive	1214	1348	1192		8L			DPA1*02:02	DPB1*04:01
286	4.30	1363	7.02	Positive	1207	1365	1209		8L			DPA1*02:01	DPB1*05:01
277	4.19	1362	7.02	Positive	1185	1351	1175		8L, 56EE			DPA1*01:03	DPB1*02:01
222	4.41	1190	6.54	Positive	1014	1441	1228	DRB1*11:04	96H				
287	4.56	1131	5.83	Positive	948	1133	950		8L			DPA1*02:02	DPB1*05:01
282	4.14	1125	5.80	Positive	978	1095	952		8L			DPA1*03:01	DPB1*04:01
228	4.09	1115	6.13	Positive	965	1161	1005	DRB1*14:01	96H				
288	3.98	1100	5.67	Positive	954	1134	984		8L			DPA1*03:01	DPB1*05:01
285	3.86	984	5.07	Positive	840	995	849		8L, 56EE			DPA1*03:01	DPB1*04:02
230	4.10	969	5.32	Positive	826	1009	860	DRB1*14:04	96H				
226	4.11	960	5.27	Positive	812	935	791	DRB1*13:03	96H				
221	4.92	934	5.13	Positive	710	1081	822	DRB1*11:03	96H				
216	4.44	873	4.79	Positive	672	1045	804	DRB1*08:01	96H				
220	3.99	852	4.68	Positive	710	1408	1174	DRB1*11:01	96H				
209	4.20	828	4.55	Positive	678	854	699	DRB1*03:03	96H				
229	4.14	808	4.44	Positive	654	851	688	DRB1*14:03	96H				
217	3.97	789	4.34	Positive	644	913	745	DRB1*08:02	96H				
248	4.35	854	3.70	Negative	676	967	766			DQA1*03:02	DQB1*03:01		
225	4.82	782	4.30	Negative	587	848	637	DRB1*13:01	96H				
207	4.67	772	4.24	Negative	591	856	655	DRB1*03:01	96H				
247	4.39	765	3.31	Negative	582	875	666			DQA1*03:01	DQB1*03:01		
227	4.27	761	4.18	Negative	594	1016	793	DRB1*13:05	96H				
208	4.28	721	3.96	Negative	550	844	643	DRB1*03:02	96H				
249	3.95	685	2.97	Negative	530	728	563			DQA1*05:01	DQB1*03:01		
250	4.05	562	2.43	Negative	398	741	525			DQA1*06:01	DQB1*03:01		
294	4.70	521	2.69	Negative	341	583	382					DPA1*02:01	DPB1*14:01
272	6.99	484	2.10	Negative	115	560	133			DQA1*01:02	DQB1*06:04		
231	4.10	425	2.33	Negative	275	413	267	DRB1*15:01					
211	3.85	404	2.22	Negative	270	413	276	DRB1*04:02					

Figure3.3.11- SAB class II report showing the positive and negative antibodies of the recipient. Positive alleles of the recipient are matched with HLA alleles of the donor, if they match, this means recipient has Donor Specific Antibodies.

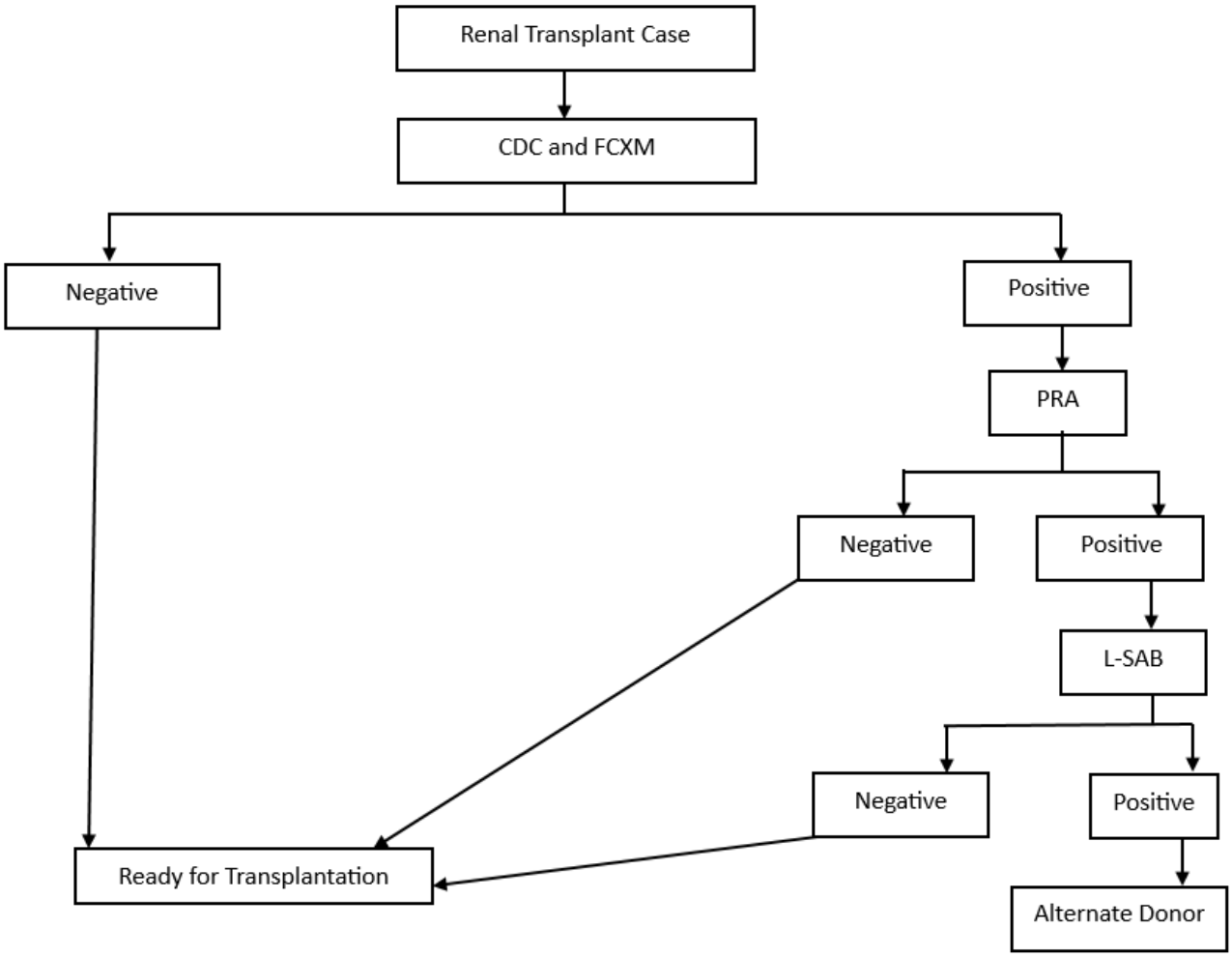


Figure 3.3.12- Pretransplant compatibility flowchart according to the SOP set by the Molecular & Transplant Immunology Laboratory at Medanta Hospital, Gurugram.

CHAPTER 4 – RESULTS AND DISCUISSION

4.1 *Data Analysis*

Data for 402 patients waiting for kidney transplant was collected along with their history of any sensitization event(s) if present. HLA positivity rates and antibody strengths in positive cases were studied according to different sensitization events. The effect of each SE on HLA alloantibody was studied examining only the patients who were exposed to any kind of SE out of total patients. Therefore, 5 groups were made: No sensitization event (control group; n=196), only blood transfusion (n=134), only pregnancy (n=22), re-transplantation (n=24), transfusion with pregnancy (n=26).

4.2 Statistical Analysis

After compiling all the data in the MS excel, numerical values, averages and percentages was calculated. All the data of the positivity rates of CDC-XM, FC-XM, anti-HLA antibody screen test, SAB assay was analysed and graphs were prepared.

Out of 402 patients under study, 83.08% were males and 16.91% were females. Average age of the patient was 43.68 and average donor age was 48.25. 206 (51.24%) patients had sensitization history. Out of sensitized patients, maximum had blood transfusion history (64.08%), followed by pre-transplant history (11.65%) and pregnancy history (10.68%). 12.62% patients had a history of blood transfusion combined with pregnancy (Table- 4.1).

	Occurance (%)	**CDC-XM (%)**	**FC-XM T-cells (%)**	**FC-XM B-cells (%)**	**Anti-HLA antibody screen test class I (%)**	**Anti-HLA antibody screen test class II (%)**	**SAB Class I (%)**	**SAB Class II (%)**
Non-Sensitization	48.76	0	8.11	19.28	0	15.63	0	0
Blood transfusion Only	64.08	7.46	8.96	18.66	10.45	8.20	3.73	2.24
Pregnancy only	10.68	13.64	22.73	59.09	13.64	22.73	0	9.09
Re-transplant	11.65	16.67	41.67	79.17	20.83	33.33	12.5	12.5
Transfusion + Pregnancy	12.62	30.77	26.92	38.46	15.38	11.54	0	3.85

Table 4.1- Positivity rate of anti-HLA antibodies via different screening and identification assays due to different sensitization events.

After performing screening tests, i.e., CDC-XM, FC-XM, anti-HLA antibody screen test, for all the 402 patients, it was found that 23.13% transfused patients, 68.18% patients with previous pregnancy, 83.34% with re-transplant patients, 53.84% patients with a history of blood transfusion as well as pregnancy and 10.71% non-sensitized patients were found to be positive in anti-HLA antibody test.

4.2.1 Blood transfusion and HLA-Ab screening tests positivity rates

In this study, 64.08% patients had a history of blood transfusion. Among them, 61.94% had <5 units of blood transfused, 6.71% had 5-10 units of blood transfused and 8.21% patients had >10 units of blood transfused.

It was seen that, as the units of blood transfused increased, positivity rates of HLA-Ab screening tests also increased. Patients with <5 units of blood transfused showed 13.25% positivity rates,

while patients with 5-10 units of transfused blood showed 44.45% positivity rates and patients with >10 units of blood transfused showed 72.73% positivity rates (Figure-4.2.1). Patients showed 7.46%, 8.95%, 18.66%, 10.44%, 8.2% positivity rate in CDC-XM, FC-XM T-cells and B-cells, anti-HLA antibody screen test class I and II respectively (Figure- 4.2.3).

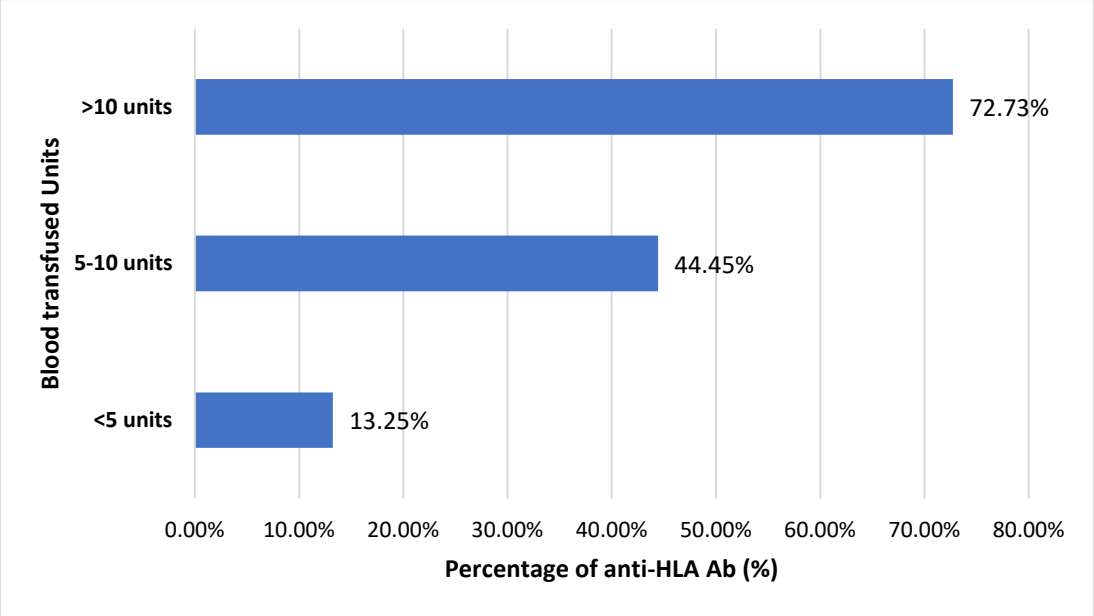


Figure 4.2.1- Comparison of units of blood transfused with positivity rates of HLA-Ab

4.2.2 Pregnancy and HLA-Ab screening tests positivity rates

In this study, 10.68% patients had the history of only pregnancy while 12.62% patients had the history of pregnancy along with blood transfusion. Pregnancies without blood transfusion history was found to have high HLA-Ab screening tests positivity rates, i.e., 68.18% as compared with Pregnancies with blood transfusion history, i.e., 53.85% (Figure- 4.2.2). Patients with history of pregnancy without blood transfusion showed 13.64%, 22.73%, 59.09%, 13.64%, 22.73% positivity rate in CDC-XM, FC-XM T-cells and B-cells, anti-HLA antibody screen test class I and II respectively while Patients with history of pregnancy with blood transfusion showed 30.77%, 26.92%, 38.46%, 15.38%, 11.53% positivity rate in CDC-XM, FC-XM T-cells and B-cells, anti-HLA antibody screen test class I and II respectively (Figure- 4.2.3).

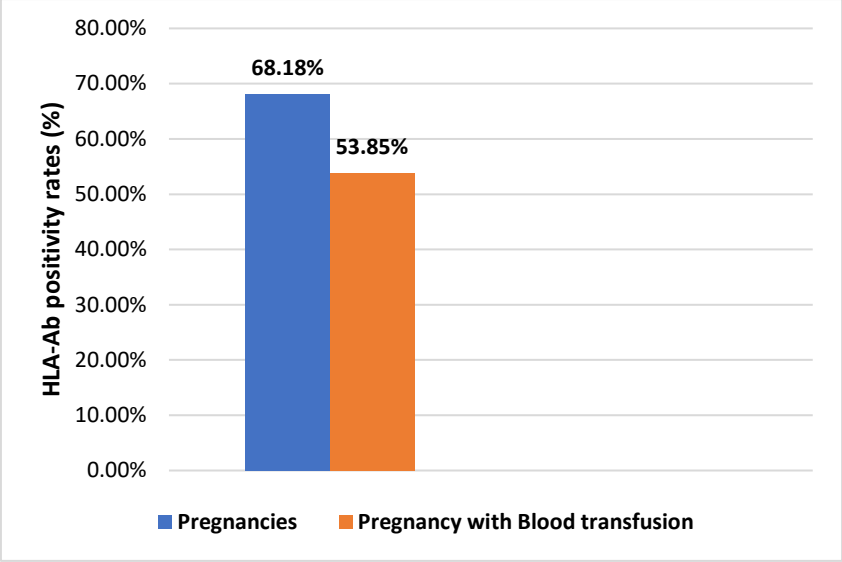


Figure 4.2.2- Comparison of pregnancies with and without blood transfusion with positivity rates of HLA-Ab

4.2.3 Retransplant and HLA-Ab screening tests positivity rates

Among the kidney transplant candidates, 11.65% patients had the history of previous transplant out of which, 83.34 patients were found to be positive for HLA-Ab screening tests. Patients showed 16.67%, 41.67%, 79.17%, 20.84%, 33.34% positivity rate in CDC-XM, FC-XM T-cells and B-cells, anti-HLA antibody screen test class I and II respectively (Figure-4.2.3).

4.2.4 Non sensitizing event and HLA-Ab screening tests positivity rates

No sensitizing events or control group were found in 196, i.e., 48.76% patients. Among them, 8.16% patients were found positive in HLA-Ab screening tests. Patients showed 8.1%, 19.27%, 15.62% positivity rate in FC-XM T-cells and B-cells, anti-HLA antibody screen test class II respectively (Figure-4.2.3).

4.2.5 Group comparison study for HLA-Ab screening tests positivity rates

In this study, it was observed that CDC-XM positive rates were highest in patients with the history of pregnancies with blood transfusion (30.77%), followed by retransplant patients (16.67%), patients with the history of pregnancies without blood transfusion (13.64%), and blood transfused patients (7.46%). Patients without any sensitization history had no positive CDC-XM.

FC-XM positive rates were highest in retransplant patients (T cells: 41.67%; B cells: 79.17%), patients with the history of pregnancies with blood transfusion had 26.92% positive FC-XM for T cells and 38.46% positive FC-XM for B cells, followed by patients with the history of

pregnancies without blood transfusion (T cells: 22.73%; B cells: 59.09%), and blood transfused patients (T cells: 8.96%; B cells: 18.66%). Patients without any sensitization history were least positive for FC-XM, i.e., 8.10% and 19.277% positive for T and B cells FC-XM respectively.

Anti-HLA antibody screen test positivity rates were highest in re-transplant patients (class I: 20.84%; class II: 33.34%), followed by patients with the history of pregnancies with blood transfusion (class I: 15.39%; class II: 11.54%), patients with the history of pregnancies without blood transfusion (class I: 13.64%; class II: 22.73%), blood transfused patients (class I: 10.45%; class II: 8.21%). Patients without any sensitization history had no positive class I anti-HLA antibody screen test, while anti-HLA antibody screen test class II had positivity rate of 15.62%.

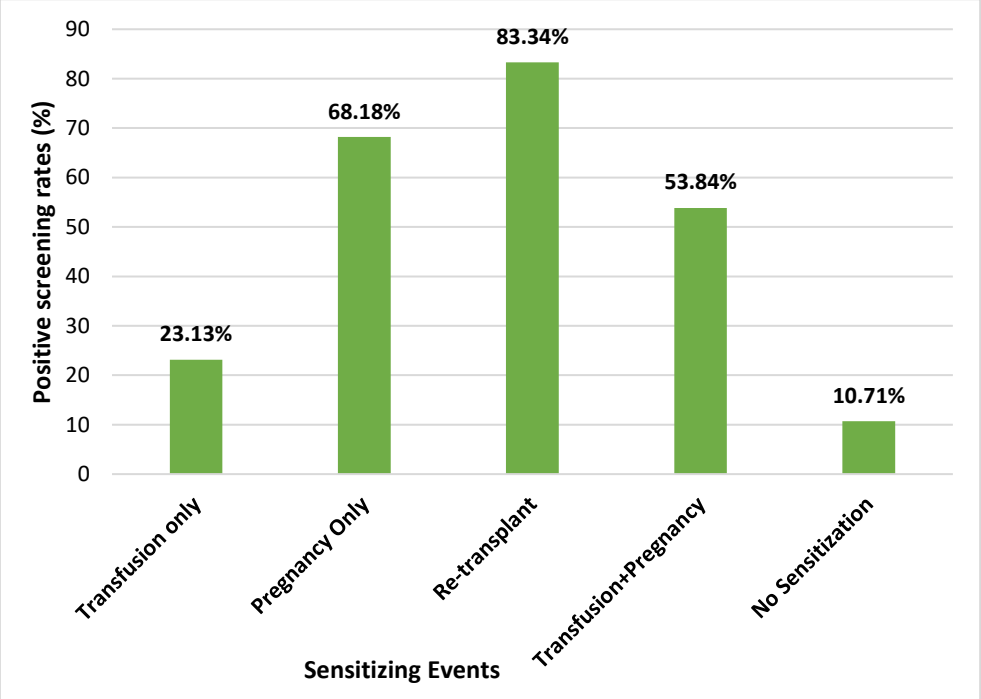


Figure 4.2.3 – Positive screening tests w.r.t. different sensitization events.

4.2.6 DSA identification

After performing screening tests, antibody identification test, SAB assay, was performed for positive anti-HLA antibody screen test cases. In this study, a total of 46 patients had positive anti-HLA antibody screen test, out of them 11 patients had DSA, i.e., 23.91%. Of which, 12.5% patients had class I as well as class II positive SAB assay for re-transplant patients, 9.09% patients with the history of pregnancy had positive SAB assay class II, 3.73% and 2.24% blood transfused patients had DSA for class I and II respectively, 3.85% patients with the history of pregnancy with

blood transfusion had positive SAB assay class II. None of the non-sensitized patients had positive SAB assay.

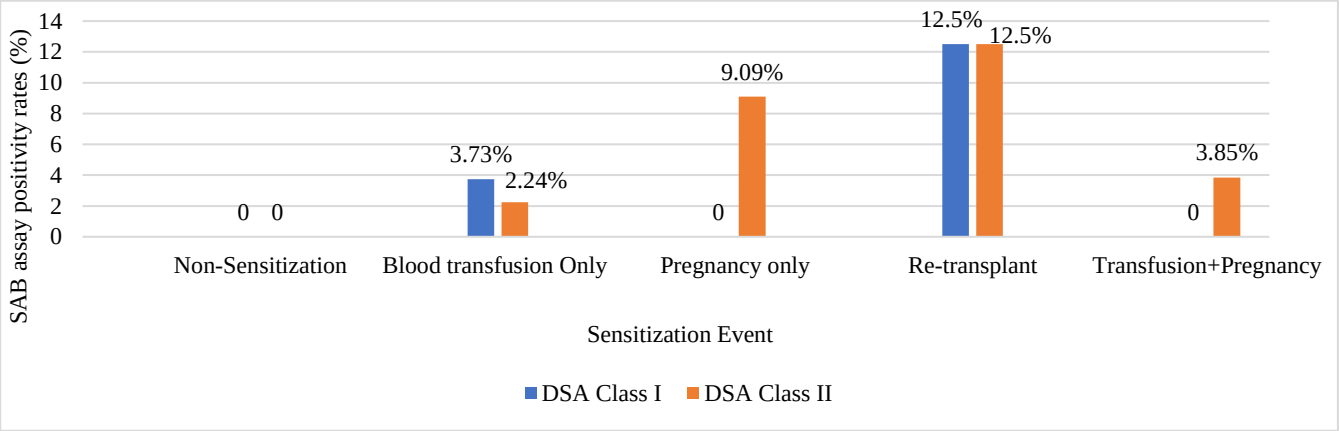


Figure 4.2.4 – Percentage of occurrence DSA in recipient due to different sensitizing events.

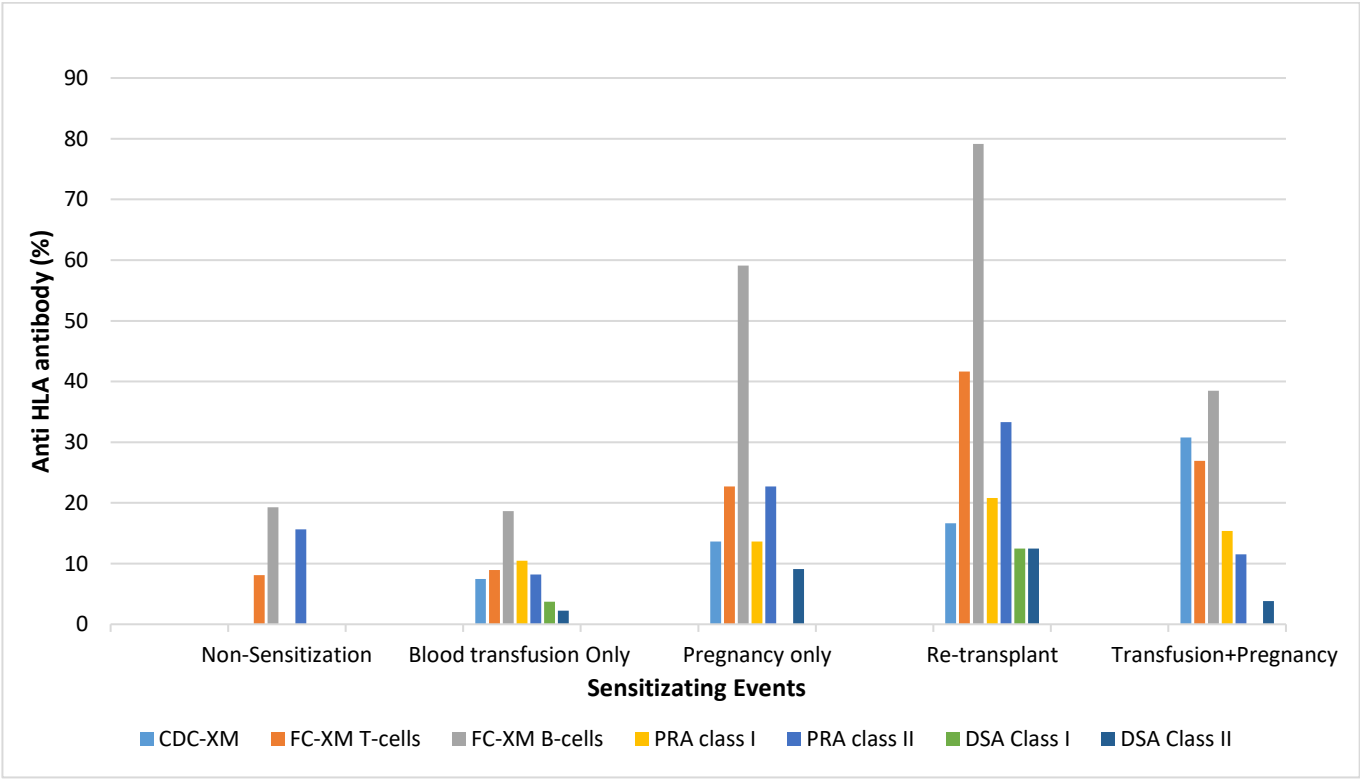


Figure 4.2.5- Effect of different sensitization events on anti-HLA antibody.

Kidney transplantation is a life-saving option for patients with end-stage renal disease. The role of HLA antibodies in the pre-transplant period remains major issue in renal transplantation. Previous transplants, pregnancies and blood transfusions are the major sensitizing events that causes anti-HLA antibodies. In this study, we compared the effects of sensitizing events on HLA

alloimmunization using CDC-XM, FC-XM and in positive cases antibody screening test too in kidney transplant patients. As expected, sensitizing events were indeed linked with higher occurrence of anti-HLA antibodies compared with patients without any sensitizing events.

It was seen that, both antibody screening test and FC-XM were more sensitive in the detection of HLA antibody as compared with the CDC-XM similar to **P. Pandey et al.**

However, in our study, sensitized patients showed higher FC-XM positivity rate (40.29%) and CDC-XM positivity rate (12.13%) than **P. Pandey et al.** which showed 27.76% positive FC-XM and 6.7% positive CDC-XM. Antibody screening test were almost similar to our study which showed 21.84% positivity and **Pandey et al.** which showed 19% positivity.

Akgul, Ciftci, Temurhan et al. concluded pregnancy as the only sensitizing event to have maximum anti HLA-antibodies followed by transplantation and blood transfusion only, but in our study, positivity rates of anti HLA-antibodies were higher in re-transplant patients, followed by previous pregnancy only, pregnancy with blood transfusion and blood transfusion only as compared to patients without any sensitization events.

Our study also observed, as the units of transfused blood increased, risk of developing anti-HLA antibody also increased similar to **P. Pandey et al**.

In this study, it was observed that pregnancy related HLA alloimmunization effect was intermediate between transplantation and blood transfusion. These results matched the study **Ibrahim Pirim, Mustafa Soyoz et al.**

The presence of anti-HLA antibodies detected by screening methods- CDC-XM, FC-XM and anti-HLA antibody screen test in patients with pregnancy only was 68.18% in comparison to 83.34% in re-transplant only and 23.13% in blood transfusion only.

In our study, among kidney transplantation patients, re-transplantation groups showed stronger SAB assay than other groups. 12.5% patients had DSA in class I as well as class II respectively followed by pregnancy only (9.09% in class II), Pregnancy with blood transfusion (3.85% in class II) and blood transfusion only (3.73% in class I and 2.24% in class II) (Figure- 4.2.5). In another study, **Akgul, Ciftci, Temurhan et al.,** SAB assay strength was highest in patients with the history of pregnancy (35.5%, 29.0% for class I and II respectively), followed by re-transplantation (15.6%, 34.4% for class I and II respectively), and transfusion only (13.1%, 6.3% for class I and II respectively). Even non-sensitized patients were positive for SAB assay (3.3%, 5.5% for class I and II respectively).The present study concluded that re-transplantation showed maximum prevalence of alloantibodies which was predicted through screening tests. It also had maximum possibility of presence of DSA. Hence, it had a stronger HLA- alloimmunization effect and had maximum chances of post-transplant graft rejection.

CONCLUDING REMARKS

- In kidney transplant, presence of HLA antibodies is responsible for graft loss and hence its rejection. Hence it is important to know the importance of HLA alloimmunization.
- We studied the role of sensitization in the development of anti-HLA antibodies in solid organ transplant.
- Our results matched with those reported in several studies so far.
- We observed that re-transplantation showed maximum prevalence of alloantibodies which was predicted through screening tests. It also had maximum possibility of presence of DSA. Hence, it had a stronger HLA- alloimmunization effect and had maximum chances of post-transplant graft rejection.
- Effect of re-transplantation on graft loss was followed by pregnancy only, pregnancy with blood transfusion and then blood transfusion only.
- The prevalence of HLA antibodies class I and II was significantly higher in patients exposed with any of the sensitization as compared with the patients who were non-sensitised.
- To conclude, our study revealed the vital role of each sensitization event on post-transplantation graft survival. Hence, thesis will be useful in the field of transplant immunology.

Document Information

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REFERENCES

- Akgul, S. U., Ciftci, H. S., Temurhan, S., Caliskan, Y., Bayraktar, A., Tefik, T., ... & Oguz, F. S. (2017, April). Association between HLA antibodies and different sensitization events in renal transplant candidates. In Transplantation proceedings (Vol. 49, No. 3, pp. 425-429). Elsevier.
- Anwar, I. J., Jackson, A. M., Locke, J. E., & Kwun, J. (2021). Sensitization and Desensitization in Organ Transplantation. Frontiers in Immunology, 12, 784472.
- Cook, D. J., Terasaki, P. I., Iwaki, Y., Terashita, G., Takeda, A., Fujikawa, J., ... & Fine, R. (1987). The flow cytometry crossmatch in kidney transplantation. Clinical transplants, 409-414.
- DiMartini, A. F., Dew, M. A., & Trzepacz, P. T. (2005). Organ transplantation. Focus, 3(2), 280-303.
- Duensing, T. D., & Watson, S. R. (2018). Complement-dependent cytotoxicity assay. Cold Spring Harbor Protocols, 2018(2), pdb-prot093799.
- Ellis, T. M. (2013). Interpretation of HLA single antigen bead assays. Transplantation reviews, 27(4), 108-111.
- Ellis, T. M. (2013). Interpretation of HLA single antigen bead assays. Transplantation reviews, 27(4), 108-111.
- Lopes, D., Barra, T., Malheiro, J., Tafulo, S., Martins, L., Almeida, M., ... & Cabrita, A. (2015, May). Effect of different sensitization events on HLA alloimmunization in kidney transplantation candidates. In Transplantation proceedings (Vol. 47, No. 4, pp. 894-897). Elsevier.
- Mahowald, G. K. (2021). The CDC crossmatch in the era of flow cytometric cross-match and single antigen beads. Brazilian Journal of Nephrology, 43, 299-300.
- Mishra, M. N., & Baliga, K. V. (2013). Significance of panel reactive antibodies in patients requiring kidney transplantation. Saudi Journal of Kidney Diseases and Transplantation, 24(3), 495.
- Pandey, P., Pande, A., Mandal, S., Devra, A. K., Sinha, V. K., Bhatt, A. P., & Mishra, S. (2022). Effects of different sensitization events on HLA alloimmunization in renal transplant cases; a retrospective observation in 1066 cases. Transplant Immunology, 75, 101680.
- Picascia, A., Grimaldi, V., Sabia, C., & Napoli, C. (2016). Comprehensive assessment of sensitizing events and anti-HLA antibody development in women awaiting kidney transplantation. Transplant immunology, 36, 14-19.
- Pirim, I., Soyoz, M., Ayna, T. K., Kocyigit, A. O., Gurbuz, B. C., Tugmen, C., ... & Ozyilmaz, B. (2015). De novo produced anti-human leukocyte antigen antibodies relation to alloimmunity in patients with chronic renal failure. Genetic testing and molecular biomarkers, 19(6), 335-338.

- Resse, M., Paolillo, R., Pellegrino Minucci, B., Costa, D., Fiorito, C., & Santangelo, M. (2018). Effect of single sensitization event on human leukocyte antigen alloimmunization in kidney transplant candidates: A single-center experience. Exp Clin Transplant, 16(1), 44-49.
- Schinstock, C. A., Gandhi, M. J., & Stegall, M. D. (2016). Interpreting anti-HLA antibody testing data: a practical guide for physicians. Transplantation, 100(8), 1619.
- Shankarkumar, U. (2004). The human leukocyte antigen (HLA) system. International Journal of Human Genetics, 4(2), 91-103
- Wisse, B. W., Kamburova, E. G., Joosten, I., Allebes, W. A., van der Meer, A., Hilbrands, L. B., ... & Otten, H. G. (2019). Toward a sensible single-antigen bead cutoff based on kidney graft survival. Transplantation, 103(4), 789.Ellis, T. M. (2013). Interpretation of HLA single antigen bead assays. Transplantation reviews, 27(4), 108-111.
- Zhang, R. (2018). Donor-specific antibodies in kidney transplant recipients. Clinical journal of the American Society of Nephrology: CJASN, 13(1), 182.