International Journal of Pharmaceutical and Bio-Medical Science

ISSN(print): 2767-827X, ISSN(online): 2767-830X

Volume 06 Issue 03 March 2026

Page No : 817-821

DOI: https://doi.org/10.47191/ijpbms/v6-i3-06, Impact Factor: 8.428

Function of HLA Typing and Cross Matching in Renal Transplantation

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ABSTRACT

Transplantation is the procedure of transferring the tissue or organ from one person to another person. Solid organ transplantation is quite common nowadays. The transplantation of Kidney, liver, heart, lung ,pancreas are taking place.

Chronic Kidney Disease (CKD) has become very common in our country now. The most suitable treatment of CKD is renal transplantation. Among solid organ transplantation the most common is Kidney or renal transplantation. Transplantation requires two persons, firstly one Donor and Secondly one Recipient. But it is not possible to transplant solid organs from any Donor to any Recipient. There are some procedures which are to be carried out before transplantation. Routine hematological, biochemical, radiological testing are done. The most important test before this procedure is Tissue matching. It is carried out by testing the blood of Recipient and Donor. The tissue matching consists of HLA typing and Cross matching.

Human Leucocyte Antigen system (HLA) is located on the small segment of chromosome number 6. Class I HLA system is known by three loci named as HLA-A, HLA-B, and HLA-C. Class II HLA system consists of HLA-DR, HLA-DQ, and HLA-DP. The HLA system is highly polymorphic . So one set of alleles of one individual is different from the alleles of other individuals. This HLA system plays the main role in regulating T cell mediated immune responses. Here lies the importance of HLA typing of Donor and the Recipient. Otherwise there will be Graft rejection. The other mandatory test is crossmatching. In this test the Donor lymphocytes are tested against the circulating antibodies against the Recipient. These tests are employed before all solid organ transplantation.

ARTICLE DETAILS

Published On:
11 March 2026

Available on:
https://ijpbms.com/

KEY WORDS: Crossmatching, HLA typing, graft rejection, Renal transplantation

INTRODUCTION

Chronic Kidney Disease (CKD) has now become very common in our country. The most suitable treatment of CKD is renal transplantation. Various organ transplantation like heart, lung, liver, skin is now taking place. The renal transplantation is the most frequent among the solid organ transplantation. In most cases CKD is diagnosed in advanced stages. It requires dialysis as both the kidneys are not working. Kidney transplantation is the treatment of choice for CKD (1). The first successful Kidney transplantation was performed in India in 1971 in Cristian Medical College, Vellore by Dr. Mohan Rao (surgeon) and Dr. K. V. Johny (nephrologist), and their team (1). A few cases of CKD can be detected early and planned for renal transplantation before starting of dialysis. Renal transplantation can be Live donor transplantation or Deceased donor (Brain Dead Donor) transplantation. For Live donor transplantation, the donors may be preferably related or sometimes unrelated. Related donors include father, mother and siblings. Apart from these all are unrelated. It is quite difficult to find out live kidney donors, whether related or unrelated.

A battery of tests are required before transplantation. Apart from routine hematological, biochemical, radiological and immunological tests the most important tests are HLA typing and crossmatching(2) . Human Leucocyte Antigen system (HLA) or Major Histocompatibility Complex (MHC) is located on chromosome number 6.There are two types of HLA system, Class I and Class II. Class I HLA includes HLA HLA-A, HLA-B, and HLA-C. Class II HLA system consists of HLA-DR, HLA-DQ, and HLA-DP. The most important HLA system in kidney transplantation are HLA-A, HLA-B, and HLA - DR (A, B, DR) (3). The HLA system is highly

polymorphic . So one set of alleles of one individual is different from the alleles of other individuals. This HLA system plays the main role in regulating T cell mediated immune responses. Here lies the importance of HLA typing or Histocompatability testing of Donor and the Recipient. The more matching of alleles in the loci the better is the graft survival (3,4).

Crossmatching is equally important part for the graft survival. Crossmatching can be carried out by several procedures. Firstly ,Complement Dependent Cytotoxicity Crossmatch (CDCXM). Secondly , Flow Cytometry Crossmtch (FCXM), and thirdly Virtual Crossmatch (1).

HLA typing and CDCXM were introduced by Paul Terasaki in 1967 (5). The HLA typing and Crossmatching are the base of successful kidney transplantation (6,7).

There are several methods of HLA typing. In the beginning the HLA typing was done by serological method. In this process the donor's and recipient's lymphocytes were made to react with different types of sera carrying specific HLA antibodies. Then the typing was done on the basis of antigen and antibody reactions. This method is no longer used now. At present DNA based HLA typing , such as polymerase chain reaction (PCR) (e.g., sequence-specific primers (SSP), sequence-specific oligonucleotides (SSO), next-generation sequencing (NGS), and third-generation sequencing (MinION, a nanopore sequencing platform), are practised to determine the HLA typing (8). Among above techniques PCR - SSP is widely used. Both SSP and SSO techniques have low to intermediate resolution. They are not very expensive method. NGS is expensive and special equipment is required. But it gives high resolution typing (9).

MATERIALS AND METHODS

This study was carried out in the Pathology department in a Tertiary care hospital for a period 7 years from August 2016 to August 2023. We used the method of DNA based HLA typing PCR SSP.

Previously, HLA typing was done by the serological method by using predropped plates where corresponding antibodies were coated inside the micro wells in microplates. By using the antibody dependent lymphocytotoxicity method, the typing was done. Now this method has become obsolete. It has been replaced by DNA-based typing, PCR SSP method.

For HLA typing of Recipient and Donor EDTA blood was collected in a purple topped vial. DNA was extracted manually by using DNA extraction kit. Then DNA was added to primer and master mix. This master mix contained dNTP, taq polymerase, primer, nuclease free water. Master mix was added to DNA and amplification of DNA done in Thermal Cycler using Polymerase Chain Reaction (PCR). Next step was separation of DNA fragments by Agarose Gel Electrophoresis (AGE) using 2% agarose in electrophoresis apparatus. The amplified DNA was placed in the wells made by comb on the agarose and electrophoresis was done. After AGE, DNA fragments were separated. DNA bands were stained by ethidium bromide and compared with DNA ladder . The reading of these DNA fragments was taken in presence of UV light by Gel Documentation System (GELDOC). The DNA fragments were seen in a banding pattern. This banding pattern was displayed on the connected computer screen. The final step was evaluaion of the result of HLA typing online by comparing the bands by the help of HLA kit based software .

For cross-matching, the serum of the Recipient and the cells of the Donor were needed . We used the most commonly used method, the Complement Dependent Cytotoxicity (CDCXM) . During cross-matching, the Donor's T cells and B cells were made to react with the Recipient's serum in the presence of Rabbit complement. Eosin dye was added at the end. The recipient's serum, donor's lymphocytes and rabbit complement were added to the microwells of the Terasaki plate. Positive Control and negative control were also set up in the Terasaki plate. When corresponding antibody was present in patient's serum there was reaction occurred between antigen and antibody. As a result there was cell lysis. The Plates were observed under inverted phase contrast microscope . Eosin dye could enter the lysed cell membrane and the cells were stained pink. Eosin was added to see the lysis of cells. These cells were stained pink, whenever there was lysis of cell membrane . The result was given as negative or positive. If there was lysis the result was positive and the transplantation could not be done.

HLA A, HLA B and HLA DR are the most important antigens for renal transplantation and these are assessed for renal transplantation..

Cross-matching can be done by several methods. The other methods are Flow Cytometric Crossmatch (FCXM) and virtual crossmatch (SAB).

Flow Cytometric crossmatch is more accurate. In a virtual crossmatch, no physical crossmatch is done. The antigens of HLA typing of the donor and recipient are matched. Panel reactive antibody (PRA) and Donorspecific antibody (DSA) are also assessed. Luminex is used for this. This equipment is not available in every centre. Panel reactive antibody (PRA) is assessed in some centres. Donor-specific antibody is also important for pretransplant workup. These are required for the prognosis of the graft.

In 12 patients, only the disease was detected early. The transplantation was done before any need for dialysis. These are called preemptive transplantation. Mostly they were the related donor. 55 cases were unrelated donors.

RESULT AND ANALYSIS

The report of HLA typing was done online using the HLA kit-based software on the basis of banding pattern after AGE seen by Gel Documentation System (Image -1) We did HLA typing in 341 patients. The majority of the recipients were males (255 of 341), whereas the majority of the donors were females (296). The age of most of the kidney transplantation recipients were less than 60 years. Predominantly, they were

within 21 to 35 years of age (250 of 341). The Donors were mostly mothers. In some cases, father and siblings also donated the kidneys. We did not come across any identical twins in this study. We used HLA ABDR typing. This is widely used in our country for HLA typing before Renal Transplantation. 6/6 matching of Donor and Recipient alleles is not possible except for identical twins. Most commonly, 3/6 matches occur. The more the match occurs, the better is the outcome of the transplant. We came across 142 Recipients who had 3 alleles matched (3/6) with the Donors. 51 Donors and Recipients had only one allele match. There was no 6/6 match. There were 5 alleles matched (5/6) in 22 Donor and Recipient (Table 1).

CDC crossmatch with B cell and T cell is commonly used. If the CDC Crossmatch is negative, the Renal transplantation can be carried out. If the CDC Crossmatch demonstrates a positive result, then the Flow Cytometry Crossmatch is tried. If it is also positive the transplntation can not be done.

Table 1. Number of HLA ALLELES matched & number of patients

No of matched HLA alleles	No of patients
1/6	51
2/6	64
3/6	142
4/6	62
5/6	22
6/6	0

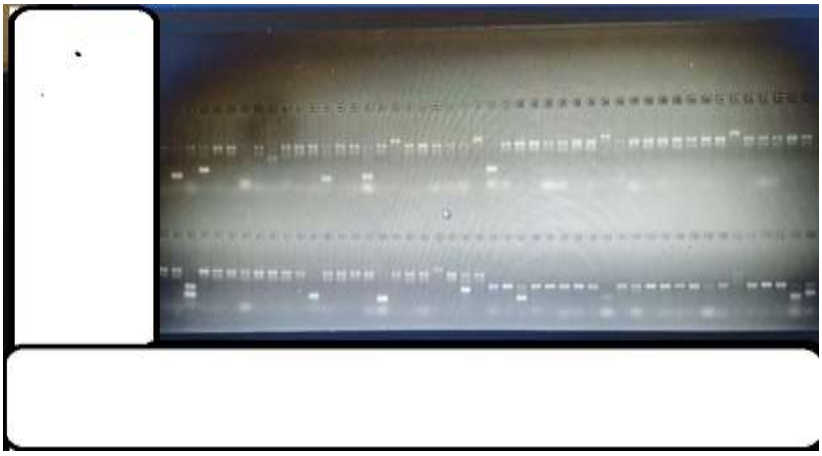

Image 1. Image of bands AGE by Gel Documentation System

DISCUSSION

Among solid orgsn transplantation the Kidney transplantation is the most common (10). It is the treatment of choice for End Stage Renal Disease (ESRD) (10). The Chronic Kidney disease is incresing day by day , particularly from 2018 to 2023 (12). It has been noticed more frequently in the individuals more than 15 years of age. This is more common in rural areas than urban areas (13). There are various etiologies for ESRD. Most common is Diabetic Nephropathy. In a number of cases no particular disaease can be found out (14). In our study also we had the same findings. Majority of the patients were diabetic . In some cases no obvious cause was noted.

On an average 13,000 Renal transplantations take place in India per year(12). HLA typing and Crossmatching are the essential part of the pretransplant workup. HLA ABDR typing is the most important workup for Renal transplantation (3). We also did HLA AB DR for our Renal Transplantation. The better the HLA matching the better will be the graft survival (1,3). We have also noticed the same findings. Our 5 year follow up graft survival was 75-80%, which is almost similar to other studies (1). In case of DNA based HLA typing we have used PCR- SSP , 2 digit typing. It is of low to intermediate grade resolution. Bilkay Baştürk et al found that for class I, HLA-A*02,HLA-B*35, and HLA-A*24 and, for class II, DRB*11, DRB*01, and DRB*04 were the most frequent antigens (15). We found that HLA-*11 A*02, HLA-B*15, B*35 for Class I and HLA DR*04, DR*15 and DR*10 for Class II are the most common alleles in recipients of kidney transplantation. This was quite similar to above study. Assessment of Panel Reactive Antibody (PRA) and Donor Specific Antibody (DSA) are also necessary prior to renal transplantation. But all centres do not have the facility for such assessment. We could not assess such antibodies. Indirectly these cases can be assessed by crossmatch. Some antibodies give positive crossmatch result during CDCXM. Such positive cases are not cosidered for transplantation. Those cases are assessed for PRA and DSA prior to transplantation.

In our study the donors were predominantly females and they are the first degree relatives, mostly mothers. 296 cases out

of 341 donors were females. In some cases donors are fathers and siblings.

The majority of the recipients were males and the donors were frequently females. 255 Recipients out of 341 were males. In this respect our study has the similarity with the study of Map et al (16). All the recipients and donors were less than 60 years of age. Usually the recipients were in the age group of 21- 35 years. A few were elderly.

The graft becomes functional after the transplantation. It has been seen that in elderly patients the graft function is delayed. But there is minimal difference of graft survival.The kidney from older Donors to Young Recipients show same graft survival. The presence of PRA decreases the graft survival (17). Graft survival depends on various factors, e.g the age of the recipient, presence of comorbity, relation between the Donor and Recipient, degree of HLA matching, presence of proteinuria after transplantation and association with cardiovascular disease. Management of cardivascular disease and maintainance of the renal function are also necessary for longterm graft survival (18 -19).

CONCLUSION

Renal Transplantation is the most suitable treatment for endstage renal disease. HLA typing and crossmatching are the essential investigation for the pre transplant work up. Graft survival depends on various factors like age of the reciient, HLA matcing, relation between the Donor and the Recipient, presene of co morbities , presence of cardiovascular disease, proteinuria and infection. Live Donor transplantation shows better graft survival than Deceased donor transplantation

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