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TISSUE TYPING METHODS TO ASSESS THE COMPATIBILITY AND CLINICAL OUTCOME IN THE LIGHT OF IMMUNE MONITORING CRITERIA FOLLOWING ORGAN TRANSPLANTS

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ABSTRACT

In the present investigation an assessment was made on the potential renal *transplant* subjects involving HLA *typing* of the donor and recipient (Table 5.1 - 5.10) , screening for alloantibody against HLA, and obtaining a history of sensitizing events. HLA *typing* ideally includes A; B; C; DRB1; DRB3,4,5; DQB1; DQA; DPB1; and DPA; but this is not always performed. The single antigen bead (SAB) solid-phase assay is most commonly used as the first line of screening for alloantibody, but multi-antigen screening beads can also be used. In the tables mentioned above, 5.1 - 5.5 imply live donors, while the tables 5.6 - 5.10 are based on cadaveric donors. Furthermore, the criteria such as CDC HLA crossmatch, PRA Class - I and II HLA, PCR-SSOP are pre transplant data, while the remaining such as DSA IgG HLA Class-I and II, SAB-FCXM HLA Class - I and II , NGS - Illumina MiniSeq are post transplant data. Besides in the current pursuit, more importance was given to renal transplant data, than liver, heart and lung. The data were also analysed in the light of advanced statistical and computational tools and expressed as graphics and tables (5.21 - 5.24).

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INTRODUCTION

Worldwide, the kidneys are the most commonly transplanted organs, followed by the liver and then the heart. Organ donors may be living, brain dead, or dead via circulatory death as well. Tissue may be recovered from the above within 24 hours past the cessation of heartbeat. Unlike organs, most tissues (with the exception of corneas) can be preserved and stored for up to five years, meaning they can be "banked". Transplantation raises a number of bioethical issues, including the definition of death, when and how consent should be given for an organ to be transplanted, and payment for organs for transplantation.

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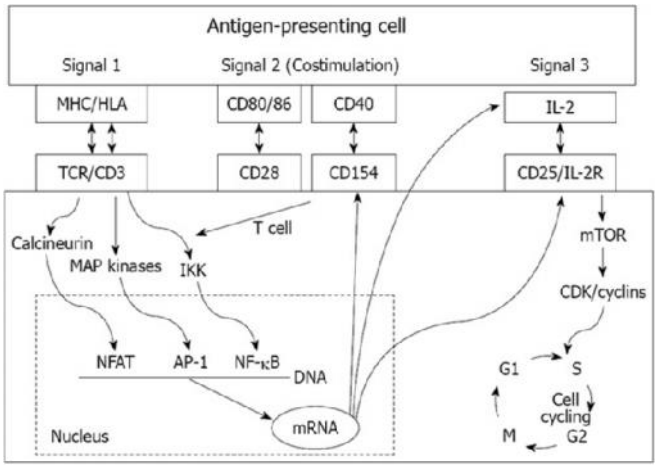
A particular problem is organ trafficking. There is also the ethical issue of not holding out false hope to patients. Some of the key areas for medical management are the problems of transplant rejection, during which the body has an immune response to the transplanted organ, possibly leading to transplant failure and the need to immediately remove the organ from the recipient. When possible, transplant rejection can be reduced through serotyping including HLA typing to determine the most appropriate donor-recipient match and through the use of immunosuppressant drugs.

HLA system and transplantation: HLA-A, HLA-B, and HLA-DR have long been known as major transplantation antigens. Recent clinical data indicate that HLA-C matching also affects the clinical outcomes of hematopoietic stem cell transplantation, but HLA-DQ and HLA-DP(1-2) do not appear critical. Antibodies bound to the graft fix complement and cause damage to the vascular endothelium, resulting in thrombosis, platelet aggregation, and hemorrhage.

Hyperacute rejection occurs in patients who already have antibodies specific to a graft. Natural antibodies against ABO blood group and preformed HLA antibodies induce hyperacute rejection. Natural anti-A and anti-B antibodies cause hyperacute rejection because AB antigens are expressed on endothelial cells of grafts. HLA alloimmunization can be induced by blood transfusions, pregnancies, or transplants. Hyperacute rejection can be avoided in most cases by ABO-identical or ABO-major compatible transplantation and by confirming negative lymphocyte crossmatching. Acute rejection is primarily the result of T cell-mediated response. Chronic rejection may be due to antibody and cell-mediated responses(3,4).

REVIEW OF LITERATURE

HLA Typing: The HLA system includes a complex array of genes located on chromosome number 6 and their molecular products that are involved in immune regulation and cellular differentiation. Human leukocyte antigen (HLA) molecules are expressed on almost all nucleated cells, and they are the major molecules that initiate graft rejection. There are three classical loci at HLA class I: HLA-A, -B, and -Cw, and five loci at class II: HLA-DR, -DQ, -DP, -DM, and -DO. The system is highly polymorphic. The contribution of the allelic diversity of class I and II genes to immune recognition and alloreactivity can be analyzed by serological methods and molecular methods at the DNA level by different methods like sequence specific primer (SSP) and oligotyping with locus- and allele-specific oligonucleotide probes (SSOP)(5-9).



Molecular Typing: HLA compatibility in terms of RT-PCR. The development and extensive usage of molecular methods soon substituted serologic techniques, for determination of individuals HLA type using realtime PCR. High-resolution typing at the four-digit level for all HLA loci is an unrealistic goal with these techniques. Molecular methods mainly focus on identifying polymorphisms in exons 2 and 3 of the class I locus and exon 2 of the class II locus, which are crucial for HSCT, as mentioned before (10-11).

Next Generation Sequencing NGS: Over the past decade, next generation sequencing (NGS) has advanced remarkably, allowing its widespread use in clinical settings. Until recently, a genetic test aimed at answering a question that arose from a specific clinical suspicion, pointing toward a selected genetic target. This gene-centered approach, although very reliable to detect single mutations, was inefficient and expensive because it often required several attempts to make a diagnosis. In recent years, the introduction of NGS has accomplished the simultaneous analysis of a large number of genes, up to whole exome sequencing (WES) or even whole genome sequencing (WGS).

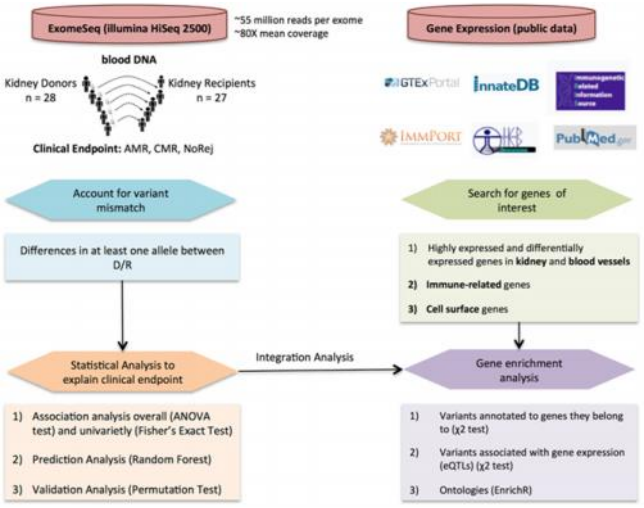
AIM AND OBJECTIVES

Aim: To tissue type organ transplants in the light of immune monitoring criteria towards compatibility and clinical outcome

Objectives: 3.2.1 To tissue type donor and recipient patients of organ (kidney, liver, heart and lung) Transplants using HLA typing; 3.2.2 To assess the Efficiency of immune system in dealing with transplant-inection. To identify and catalogue the genetic polymorphism in the backdrop of histocompatibility and immunogenetics. To decipher the influence of HLA compatibility in organ transplantation subjects in view of RT-PCR, SSP and SSOP. To assess the HLA compatibility in terms of SSO and SSOP; 3.2.6 To correlate the tissues matching with clinical outcome and its significance; 3.2.7 To analyse the above outcome / output data in the light of modern statistical.
tools such as SAS, SPSS , ANOVA and MANOVA

RESEARCH METHODOLOGY: SAMPLES AND PROTOCOLS

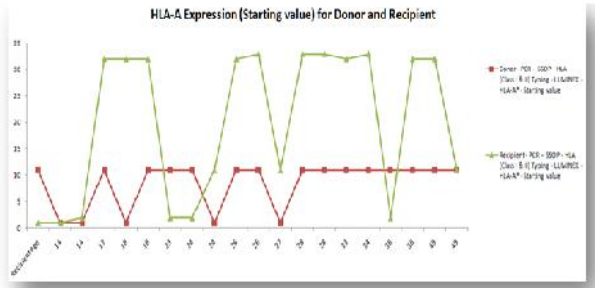
Forty two individual patients paired by D/R(Donor- Recipient with 20 kidney donors and recipient; 10 liver transplants; 10 lung transplant ; 2 heart transplant subjects were considered from MMC/ Rajive Gandhi Government General Hospital and other city hospitals and after obtaining respective institutional ethical clearance experimental samples were obtained and subjected to HLA typing and allied as depicted under and sequenced using blood cell, leucocyte DNA (vide schematic work flow design, 4.0)

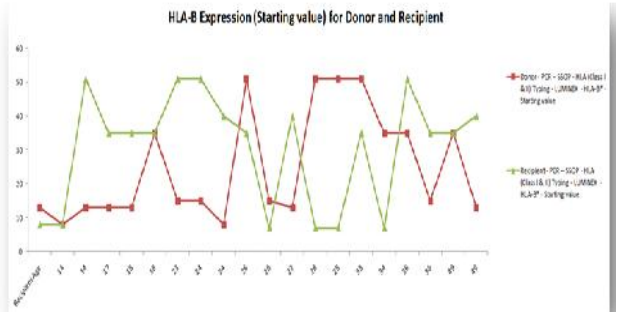


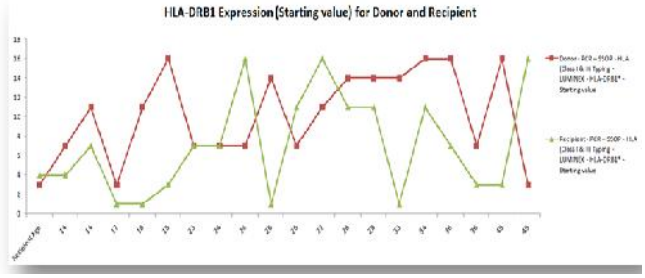
RESULTS

TABLES

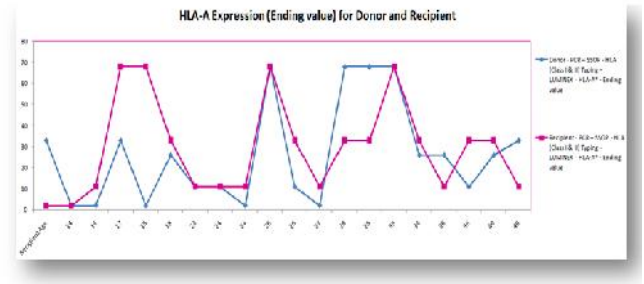
	Donor	Recipient
Age	27 to 59 years	14 to 54 years
HLA-A Expression – Starting value	1 to 11 minutes	1 to 33 minutes
HLA-B Expression – Starting value	8 to 51 minutes	7 to 51 minutes
HLA-DRB1 Expression – Starting value	3 to 16 minutes	1 to 16 minutes
HLA-A Expression – Ending value	2 to 68 minutes	2 to 68 minutes
HLA-B Expression – Ending value	37 to 58 minutes	37 to 58 minutes
HLA-DRB1 Expression – Ending value	7 to 16 minutes	7 to 16 minutes

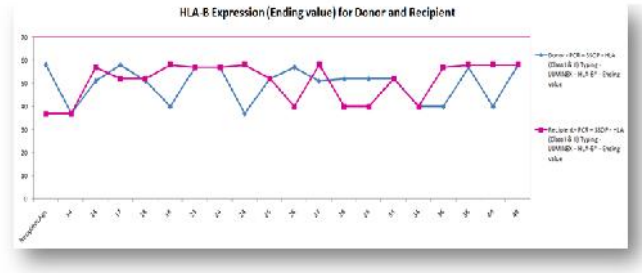






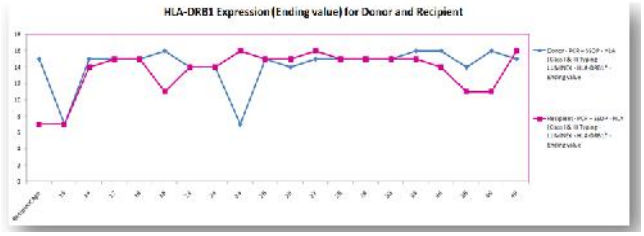
DONOR - RECIPIENT HLA EXPRESSIONS (Ending values)





KIDNEY (DESCRIPTIVE STATISTICS)

The donor and recipient data were tabulated and the ranges were noted. Overall, the ranges in age and HLA expression did not show sharp differences between the donor and the recipient groups. The range of HLA-B expression was higher than the expression of HLA-A and HLA-B expressions.



DATA REPRESENTATIONS

DONOR - Recipient HLA Expressions (Starting values).

DONOR - Recipient HLA Expressions (Ending values).

The data for PCR-SSOP assay was mostly negatively skewed. The expression ranged between 11 and 68 for Donors and 1 and 37 for Recipients.

PAIRED TTEST: Paired t test was done for the PCR –SSOP assay and the value was 0.007362. This shows that the data accepts the null hypothesis and explains that there is no significant difference in the data across the Donor and the Recipient groups.

DISCUSSION

Despite improvements in patient selection and management, every *transplant* carries some risk of organ or graft loss. Donor-specific alloantibody (DSA) either present at the time of transplantation or arising de novo post-transplant is a risk factor for antibody mediated rejection (AMR) and potentially allograft loss in almost all types of *organ* transplants (12-16). Ideally, all DSA would be avoided, but this is often impractical in the setting of *organ* scarcity and recipient sensitization. Instead, the clinician must estimate the risk of AMR in each situation, while considering the consequences of remaining on dialysis. Understanding the complexities and limitations of DSA detection techniques is the key for making an accurate risk assessment while improving access to transplantation. The prime aim of this study is to provide a practical guidelines for using solid phase assays and crossmatch (XM) testing. It also provide possible explanations for ambiguous test results and recommendations for further investigation. A major emphasis is made on pretransplant alloantibody assessment in kidney *transplant* candidates, but the basic principles apply posttransplantation and to other solid *organ* transplants such as liver, heart and lung as well. In the present investigation an assessment was made on the potential renal *transplant* subjects involving HLA *typing* of the donor and recipient (Table 5.1 - 5.10) , screening for alloantibody against HLA, and obtaining a history of sensitizing events. HLA *typing* ideally includes A; B; C; DRB1; DRB3,4,5; DQB1; DQA; DPB1; and DPA; but this is not always performedhttps://journals.lww.com/ transplant journal/Fulltext/2016/08000/Interpreting_Anti_HLA_Antibody _Testing_Data__A.14.aspx#R7-14 (17-18). The single antigen bead (SAB) solid-phase assay is most commonly used as the first line of screening for alloantibody, but multi-antigen screening beads can also be used. In the tables mentioned above, 5.1 - 5.5 imply live donors, while the tables 5.6 - 5.10 are based on cadaveric donors. Furthermore, the criteria such as CDC HLA crossmatch, PRA Class - I and II HLA, PCR-SSOP are pre transplant data, while the remaining such as DSA IgG

PCR – SSOP - HLA (Class I & II) Typing – LUMINEX	MIN	MAX	MEAN	STD DEV	SKEW	KURT
Donor - HLA-A* - Starting value	1	11	8.5	4.442617	-1.25051	-0.49673
Donor - HLA-B* - Starting value	8	51	24.9	16.13398	0.718417	-1.17273
Donor - HLA-DRB1* - Starting value	3	16	10.2	4.652108	-0.18289	-1.37176
Donor -HLA-A* - Ending value	2	68	26.45	24.03829	0.841759	-0.52281
Donor - HLA-B* - Ending value	37	58	49.85	7.761409	-0.61737	-1.23067
Donor - HLA-DRB1* - Ending value	7	16	14.2	2.546411	-2.54326	5.77462
Recipient - HLA-A* - Starting value	1	33	19.95	14.40934	-0.34709	-1.93721
Recipient - HLA-B* - Starting value	7	51	30.65	16.72463	-0.4927	-1.24093
Recipient - HLA-DRB1* - Starting value	1	16	7.05	5.216119	0.517324	-0.94714
Recipient - HLA-A* - Ending value	2	68	29.2	22.851	0.742905	-0.64348
Recipient - HLA-B* - Ending value	37	58	50.9	8.328139	-0.75837	-1.25792
Recipient - HLA-DRB1* - Ending value	7	16	13.55	2.723678	-1.57128	1.648031

HLA Class-I and II, SAB-FCXM HLA Class - I and II , NGS - Illumina MiniSeq are post transplant data. Besides in the current pursuit, more importance was given to renal transplant data, than liver, heart and lung. The data were also analysed in the light of advanced statistical and computational tools and expressed as graphics and tables (5.21 - 5.24).

Significance of Kidney transplant: Renal transplantation has transformed the life of patients with end-stage renal disease and other chronic kidney disorders by returning endogenous kidney function and enabling patients to cease dialysis. Several clinical indicators of graft outcome and long-term function have been established. Although rising creatinine levels and graft biopsy can be used to determine graft loss, identifying early predictors of graft function will not only improve our ability to predict long-term graft outcome but importantly provide a window of opportunity to therapeutically intervene to preserve graft function before graft failure has occurred. Since understanding the importance of matching genetic variation at the HLA region between donors and recipients and translating this into clinical practise to mprove *transplant* outcome, much focus has been placed on trying to identify additional genetic predictors of *transplant* outcome/function.

Discussion is also made on the challenges faced by candidate gene studies, such as differences in donor and recipient selection criteria and use of small data sets, which have led to many genes failing to be consistently associated with ***transplant*** outcome. This review will also look at how recent advances in our understanding of and ability to screen the genome are starting to provide new insights into the mechanisms behind long-term graft loss and with it the opportunity to target these pathways therapeutically to ultimately increase graft lifespan and the associated benefits to patients. Improvements in induction/immunosuppressant regimes, sharing of technical expertise between centers, the establishment of *organ* allocation networks and increased numbers of *transplant* centers have enabled kidney transplantation to become a successful treatment for patients with end-stage renal failure. Establishment of the *Organ* Procurement and Transplantation Network/United Network for *Organ* Sharing, Euro transplant and other databases have lead the way in identifying donor and recipient features and measures of renal function which act as indicators of long-term *transplant* success, including cold ischemia time, deceased versus living donor and body mass index (19) Although donors and recipients are matched for clinical features shown to maximize *transplant* success, some immediate and early complications can occur after transplantation, including hemorrhage, thrombosis, intra-abdominal infection, and acute rejection) (20).

Many of these are treatable with surgery or changes in immunosuppressant/induction regimes, leading to 90% to 95% of cadaveric donor organs and approximately 100% of living donor organs still functioning 1 year after transplantation. Over time however kidney graft function declines, with greater than 50% of deceased donor transplanted kidneys failing within 10 years and greater than 50% of living related donor *transplant* kidneys failing within 17 to 18 years(21). Although kidney biopsy and creatinine levels can determine graft failure, usually this is after substantial graft damage has occurred. Because of the importance of providing equity of access to kidney transplants across different ethnic and social-economical groups and with waiting lists outstripping *organ* supply, it is not always possible to match purely on the best clinical indicators of long-term graft survival. It is also important to bear in mind that although clinical features provide indicators of *transplant* survival and long-term function, they are not definitive predictors of *transplant* longevity. One key feature of transplantation is that the donor and recipient, except between identical twins, differ in their genetic makeup. Utilization of HLA matching has enabled transplantation to become one of the first fields to translate genetic information into improved graft outcome (22) The use of genetic markers to predict disease outcome in common diseases has however been questioned as although genetic factors can predict disease outcome as well as clinical features, when added to well-established clinical predictors in common autoimmune and metabolic diseases, they do not improve disease prediction (23-24). In transplantation, where we do not have good clinical predictors of long-term graft survival/function, identifying genetic predictors of graft dysfunction could provide a window of opportunity to intervene therapeutically to prevent *organ* loss early on extending the benefits to patients of having a functioning graft.

HLA antibodies represent a significant risk factor for hyperacute rejection and can contribute to chronic rejection (25).

The HLA region encodes numerous molecules involved in presentation of exogenous and endogenous antigens for recognition by the immune system (8). The immune system determines if antigens presented are self, triggering no response, or nonself, causing an immune response to be triggered. If the donor *organ* encodes different HLA molecules to those recognized by the recipient's immune system, when encountered by the immune system alloantibodies against the donor *organ* will be generated. Understanding that matching donors and recipients for *HLA-DRB1*, *HLA-A*, and *HLA-B* would lead to reduced alloantibody production and improved *transplant* outcome is a keystone of most clinical *transplant* protocols.

SUMMARY AND CONCLUSION

POWERFUL POINTS

- Transplantation is the process of moving cells, tissues or organs from one site to another for the purpose of replacing or repairing damaged or diseased organs and tissues. It saves thousands of lives each year. However, the immune system poses a significant barrier to successful organ transplantation when tissues/organs are transferred from one individual to another.
- Rejection is caused by the immune system identifying the transplant as foreign, triggering a response that will ultimately destroy the transplanted organ or tissue. Long term survival of the transplant can be maintained by manipulating the immune system to reduce the risk of rejection.
- Donor and recipient are carefully matched prior to transplantation to minimise the risk of rejection. They are matched based on their blood group, tissue typing, and how the recipient's blood serum reacts to donor cells.
- Immunosuppressive drugs are used to prevent and to treat transplant rejection by dampening the overall immune response. However, immunosuppressive drugs are non-specific and leave patients more susceptible to disease as well as being associated with numerous unwanted side effects.
- Further research on the immunological mechanisms of rejection will help improve cross matching, diagnosis and treatment, as well as facilitating the discovery of novel strategies for preventing.

The alloimmune response is initiated by T-cell recognition of alloantigens through direct or indirect pathways. Three signal models have been established during T-cell activation, which subsequently produces various effector T-cells and antibody production. Sensitive crossmatch is routinely performed before kidney transplant to detect any significant DSA, so that hyperacute rejection can be eliminated. Solid phase based Luminex assay can further characterize HLA antibodies before and after kidney transplant to guide our clinical practice. In addition to the traditional anti-HLA antibodies, alloreactive and autoreactive antibodies against non-HLA antigens have now been increasingly recognized to play an important role in humoral rejection of allograft

CONCLUSION

Understanding of the immunology related to RT, advances in the techniques of detection and characterisation of antibodies before and after RT and the crossmatch techniques have significantly improved the outcomes of RT over last two decades. A late allograft loss from chronic antibody-mediated rejection still remains a major problem, which needs further research to advance our understandings of the immunological process involved that would help to reduce the transplant losses.

KIDNEY: The HLA expression started from the first minute for HLA-A, and from the Eighth minute for HLA-B. The ending time of HLA expression extended up to 68 minutes for HLA-A, whereas it was as low as 16 minutes for HLA-DRB1. The data was negatively skewed. The ranges and the paired t test value (0.007362) between the donor and recipient groups showed that there's not much difference in expression data of HLA-A, HLA-B and HLA-DRB1.

LIVER: In liver, the expression of HLA-A, HLA-B and HLA-DRB1 was studied. The deviation was low for HLA-DRB1 but higher for HLA-A and HLA-B.
For liver data, the null hypothesis was accepted implying that there was no variation in the HLA expression data.

HEART: Overall, expression of HLA-B was more deviated than the expression of HLA-A and HLA-DRB1. Various statistics calculated on the HLA expression data in heart showed that the null hypothesis could be rejected but the effects contribute less to the model. The F statistic was as low as 0.32 for HLA-DRB1 signifying that the variance is less in the group. Residual analysis showed the data points randomly dispersed around the horizontal axis suggesting that the data might not fit in to a linear model. These observations possibly suggest that the contribution of HLA-A, HLA-B and HLA-DRB1 expression to transplantation acceptance (or rejection) by the recipient in heart is equal.

Heart transplant subjects with OHT assessment will grow dramatically over the next few decades. OHT is a component of a multifaceted advanced heart failure strategy and should be provided by an expertise in all areas to identify and manage the anatomical and physiological challenges. Recognition of the different sub-groups of ACHD heart failure patients should lead the medical community to develop novel astrategies for a death-free survival at long term follow-up. There are some powerful insights to be known in this conext: Adults with moderate and complex congenital heart disease (ACHD) have higher mortality than the general cardiovascular causes; Cardiac transplant for ACHD has a relatively high early mortality; Underlying congenital diagnosis has no influence on subsequent mortality or chance of transplantation.

LUNG: In lung, the expression of HLA-A, HLA-B, HLA-C, HLA-DPB1, HLA-DQB1 and HLA- DRB1was studied. Overall, in kidney, the HLA expressions across donor and recipient have been significantly matched.

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