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Research Article

Need for Screening Presence/Absence of HLA Antibodies Before and/or After Transplantation

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Abstract

Anti HLA antibodies are significant impediments in kidney transplantation. In 1969, Terasaki and Patel [1] reported association of hyper-acute renal allograft rejection with preformed lymphocytotoxic antibodies. Since then, various methodologies were introduced and adopted to study the role of HLA antibodies in acceptance/rejection mechanism of allograft Transplantation. Aim of the present study is to detect, analyse and evaluate anti HLA antibodies, pre and post transplantation in sensitised patients, using Solid Phase LABSCan3D (Luminex) Technology.

The present study comprised 12 clinical cases and 10 normal subjects for detection of HLA antibodies class I and II IDs PRA and L-SBA Cl I and CLII antigens, using LABSCan3D Luminex solid phase technology and CDC (T and B cells) XM.

Out of 12 cases having clinical history, 4 were females (parous), age between 43 -57 years and 8 males, age 35- 56 years. 3 females and one male who had a H/O Chronic rejection, were studied and found highly sensitised, cPRA >90%; MFI >21,150, L- SBA> 20,000 and CDC (T and B cell) XM was strongly positive. It was found that after 2 years of Tx, 2 patients were positive for HLA antibodies and had cPRA 66%, MFI > 1600, L-SBA > 1500 and another patient was weakly positive for IDs class I and II, and L-SBA Cl II. 1 patient, after one year of cadaver Tx and 3 patients before Tx, all were negative for antibodies.

Out of 2 transfused patients, one was weakly positive for ID Cl I, MFI 1225-1415, positive for Cl II MFI 1163-8136 and also positive for L-SBA Cl II, MFI 2000-4200. However, this patient was negative for Cl I SBA MFI <1000.

Total 7 patients were positive and 5 were negative for HLA antibodies. The results were compared with (CDC) XM. Only 2 patients were studied for Luminex XM (DSA) with Lysate, and results were found comparable.

Availability of solid-phase HLA antibody testing enhanced renal Tx outcome, especially monitoring Pre and Post Tx HLA antibodies, selection of donors and suitable choice of drug regimes. However, in the present study, due to non- availability of HLA typing, it was not possible to rule out and correlate Donor Specific Antibody (DSA) or production of *De Novo* antibodies or both in positive cases.

Keywords: *De Novo*; Panel Reactive Antibodies; Screening

Abbreviations

H/O: History of; PRA: Panel Reactive Antibodies; cPRA: % of PRA; Tx: Transplant; XM: Cross Match; DSA: Donor Specific Antibody; SAB: Single Antigen Antibody

Introduction

Kidney transplantation is the best option for the treatment of End Stage Renal Disease. The science of transplantation has evolved over time and helped us understand the immunological

factors involved in the acceptance or rejection of an allograft. It has been observed that, the precise and timely detection of Human Leukocyte Antigen (HLA) Donor Specific Antibodies (DSAs) is vital for evaluating humoral immune status of the patient pre- and Post-Transplantation.

The complement-dependent lympho-cytotoxicity (CDC) XM method has been the Classical Technique to detect leukocyte cytotoxic antibodies in sera of patients who are listed for Tx. But because of drawbacks of CDC, such as low sensitivity and low resolution in characterizing antibody specificities, the more specific ELISA technique was introduced in 1990s which utilizes solubilised HLA molecules instead of lymphocytes.

Since, last more than 15 years, the availability of solid phase HLA antibody testing has revolutionized our ability to detect HLA Donor Specific Antibody (DSA) and to appropriate their significance in Kidney Transplantation outcome. Donor Specific Antibodies (DSA) testing is in fact considered far more advanced than the so- called "Gold Standard" CDC' antibody assay. Combined with new immunological research data, the increased sensitivity and specificity of these assays have allowed for more advanced interpretation of Donor Specific Antibodies and impact in organ transplant recipients before and after Tx [3].

Hence, accurate assignment of a patient's antibody signature allows for a standardized approach to determine the frequency of incompatible donors in a particular population by establishing cPRA (calculated Panel Reactive Antibody levels) [8]. Moreover, using this information in non-invasive virtual cross-matching (VXM) one could provide reasonable prediction of the likelihood of a positive cross-match and of AMR risk stratification [8-10].

Material and Methods

Total 12 clinically referred cases and 10 normal subjects were selected for proposed study. These were categorised in 5 groups as per availability. The detection of HLA Antibodies, Class I and II IDs (PRA) and Single Antigen (L- SBA) using LABSCan3D, Luminex solid - phase technology was carried out. Serological testing using CDC XM (NIH Complement dependent Cytotoxicity)[1] was carried out with aliquots of sera of these patients to compare the result for specificity and sensitivity by both the methods.

The 10 control serum samples were procured from normal population, included in the present study. Serum sample of each patient was separated. Aliquots of each serum sample were stored at -86^{0}C till the use. Random/prospective donors were included in the present study.

Lymphocytes were separated on density gradient (sp.1.07) and T and B cells were separated using nylon wool columns for CDC XM. Positive and Negative controls were used with each tests.

For separation of T and B cells, another method of Stem cells was used. This method gives purity of cells.

Sr. No.	Types of Patient	Sample Type	Class I ID Results	Class II ID Results	SBA Cl I Results	LSA Cl II Results	CDC XM T and B cell
1	Category I 4 Patient of chronic rejection						
	1st pt PC1	Serum	Strongly positive	Strongly positive	Strongly positive	Strongly positive	Positive
	2nd pt.PC2	Serum	Positive	Positive	Positive	Positive	Positive
	3rd pt P13	Serum	Strongly positive	Strongly positive	Strongly positive	Strongly positive	Positive
	4th pt P16	Serum	Positive	Positive	positive	positive	Not done
Category II 2 Transfused Patients							
2.	1st pt 2nd pt.	Serum	Wkly. Positive Negative	Positive Negative	Negative Negative	Negative Positive	Negative Negative
Category III 1 Cadaver Patient							
3.	Pt.14 1 year aft Tx	Serum	Negative	Negative	Negative	Negative	Negative
Category IV 3 Patient Before Tx							

4.	1st.pt.17 2nd.pt.19 3rd. Pt.15	Serum	Not done Not done Not done	Not done Not done Not done	Not done Not done Not done	Not done Not done Not done	Not done
Category IV 2 Patient After Tx (Two Years)							
5.	1st pt P1 2nd pt P5	Serum	Positive wkly pos	Positive wkly pos	Positive wkly pos	Positive Negative	Positive Negative
6.	Normal 10 Subjects	Serum	Negative	Negative	Negative	Negative	Negative

Table 1: ID CL I and Cl II and SBA Cl I and CL II.

Results

It is absolutely essential to perform cross match before transplantation, to detect pre-formed antibodies that may cause hyper-acute rejection of the graft. For many years, the basic NIH and the Amos modified versions of the complement mediated cytotoxicity technique were being widely used for this purpose [13]. However, in cases where the presence of low level of antibodies or of low memory cells, the conventional CDC method does have limited sensitivity for reflecting sensitization status of the recipient accurately. But, the use of solid phase assay has characterised HLA antibody profile more clearly and to large extent, correct specificities.

First category: (Sensitized Patients)

Out of 12 cases, 4 cases had H/O of chronic rejection. Again 3 out of these were females. The 3 females, 1st, 2nd and 3rd (PC1, P2 and P13) patients had H/O of having 3-4 blood transfusions and had 2 or 3 pregnancies (Parous) each.

Patient P13 (3rd) was transplanted 12 year back with one Haplotype HLA antigen matched related donor and went in chronic rejection after 8 years of successful graft functioning. Since then, the patient has been showing strongly positive CDC XM all the time with near related donors as well as panel of random donors. She was also positive for Class I cPRA 96% and ID MFI 5000-23,000 with most of the panel of donors. Also her Class II cPRA 95%, ID II, MFI 1056-25520 and Single Antigen (L-SBA) Class I and II, MFIs were ranging between 11,000-23,500.

This patient was found strongly positive in LABSCan3D Screening (Luminex) Class I and Class II test (class I all 7 Beads were positive, MFIs 9288-12928 and Class II 5 Beads positive MFI 17373-26786) and LABSCan3D Luminex XM (DSA) using Lysate with random donors was Positive, MFIs 14000-18000.

1st patient(43 years old) - PC1 having H/O chronic rejection, was Positive for all above mentioned Tests, Class I ID was positive (MFI 4080-21150), Class II ID was also positive (MFI 1606-21954) and L-SBA Class I positive (MFI 3393-20096) and Class II positive (MFI 5000-19,000).

2nd patient(57 years old) - PC2, also had H/O chronic rejection, was positive for all the above mentioned tests parameters. This patient was 100% cPRA positive, Class I ID (MFI 5,000-21,000) and Class II ID 4078-22521). Single Bead Antigen (L-SBA) Class I MFI 1026-10631) and Class II (MFI 4000-18149) were also positive.

4th patient (16 years) was transplanted in 2006 and his allo-graft was rejected after 10 years. Now he is posted for second transplant and was referred for HLA screening test only. His sample was found strongly positive for both Class I beads (MFI 2036-11,404) and Class II beads (MFI12,394-24,700) and was also positive for IDs Class I and II, (MFI >15,000) when his serum was tested for IDs. However, Single Bead Assay was not carried out. Further, this sample was tested for XM onLABSCan3D (Luminex) for detection of donor specific antibody (DSA) with random donor and was found strongly reactive for class I (MFI 13070) and Class II (MFI 6174).

Second category: (Transfused Patients)

Out of 2 Transfused Patients (with unknown clinical History randomly selected), of which, patient No. 2 was negative for all tests except L-SBA Cl II (border line positive).

Patient No.1 was positive for Class I ID (MFI 1225 - 1415), Class II ID (MFI 1163-8136) and L-SAB Class II positive (MFI 2000-4200) but was negative for Class I L- SAB, (MFI < 1000).

This patient was positive for CL I and II XM on LABSCan3D (Luminex) (DSA). He was negative for Class I Screening and positive for Class II Beads- No.2, 4 and 5 (MFI 1648-3396).

Third category: (Cadaver Tx Patients)

Pt. 14 who received Cadaver Tx, tested after one year and was found negative for CDC XM, Class I and Class II IDs and also both Class I and II single Bead Assay (L-SBA).

Further, this patient was found weakly positive (MFI 1391) in screening class II. In Luminex XM (DSA) test, he was weakly positive for Class II, MFI 1224.

Fourth category: (Patients Before Tx)

Patients studied under 4th category, out of these 3 patients, pt. 17 and pt. 19 were negative for CDC XM and Class I and II IDs.

However, in screening test, the pt.17, was found in grey zone with Bead 6, MFI 1115 in Class I and Bead No. 1 and 2, MFIs 1353 and 1471 respectively.

The pt. 15 and 19 were totally negative for all the parameters taken for present study.

Fifth category: (2 Years after)

Patient P1 was negative for CDC XM, PRA (4%) with panel of donors for Class I ID. However, he was positive with one donor (MFI 2502) from class I. While Class II ID PRA, he was 66% positive with 6 donors of the panel, MFI 1478-16760. With Class I Single Antigen was positive 4%, MFI 1070-2772 and Class II, also 4%, MFI 1059-1556. Screening test was negative for class I and II. Perhaps these are weak antibodies.

Patient P5 was weakly positive, PRA 34% with panel of donors in class I ID, MFI 1051-3295 while Class II ID was positive 83%, MFI 1236-12803. Class I L-SBA was weakly positive 4%, MFI1538-2225 and L- SBA Class II positive 4%, MFI 1059-1736. In the Screening test this patient was negative for Class I and positive for Class II Bead 5, MFI 1716. CDC XM was weakly positive 30%.

Negative Controls were negative in all the tests and values of MFIs were within the limit of negative ranges of kit i.e. < 800. Cut off value was standardized in the lab. Values of positive control were compatible with the values of positive control provided with the kit.

Discussion

The availability of clinically relevant theories, its developments, methodologies and applications of HLA-DSAs testing in organ transplantation has opened a new chapter and, the availability of solid phase technology of HLA antibody testing has revolutionized our ability to detect HLA donor specific antibodies and to appreciate their significance in kidney transplant outcome, though the best approach to determine the strength, immunogenicity and pathogenicity of HLA antibodies is still remains controversial subject of discussion.

The presence of pre-transplant antibodies against antigens encoded by the HLA (human leukocyte antigen) complex, and specifically those antigens that are expressed by the donor's organ [donor specific antibodies (DSA)] in kidney recipients, is strongly associated with hyper-acute and accelerated acute rejection [1-3]. Likewise, the production of *de novo* or anamnestic DSA in kidney recipients post-transplant is often indicative of the onset of antibody-mediated rejection (AMR) [4]. Ample clinical data have supported the importance of HLA-DSAs monitoring for risk assessment in recipients pre- and post-transplant [4,5].

Though false-positivity of L-SAB reactions may occur and, hence, to overcome this, the immunological sensitization history of the patient, e.g. transfusions, mismatches from previously failed grafts, or HLA typing of the husband in case of pregnancy-induced sensitization, should be documented. Besides, combination of L-SBA with other forms of Luminex testing, such as LABSCan3D (Luminex) PRA or Screening, could be helpful.

It has been shown that the MFI often does not correlate with strength of CDC or flow cross-match, suggesting that the immunological risk cannot be determined based on this parameter alone and additional tools to individualize approaches to rejection treatment, as well as gauging their effectiveness [5,13,14] state-of-the-art developments in antibody analysis and their utilization in clinical practice.

In the present study, first 4 (3 female and 1 male) patients were highly sensitised due to multiple pregnancies, allograft rejection, and transfusion episodes. Besides, they had high titres of MFI against the panel of donors in general population. However, we could not get the HLA typing of both recipients and donors to rule

out and interpret results and to correlate with donors specific antigens. This could be due to antibody sensitization. However, patients P13 and PC2 were positive for CDC XM for a long period of time. Hence, our CDC XM results were compatible, especially in the case of highly sensitised patients.

The LABSCan3D Luminex-based Single Bead Assay (L-SBA) technique, used is a recombinant single HLA molecules detection and characterization of HLA antibodies at greater sensitivity than CDC and ELISA. A drawback associate with this technique is the interpretation of results is demanding and requires comprehensive experience in HLA antibody diagnosis. Current role and value of L-SBA technology is appears to be an excellent method for monitoring *de novo* DSA/and or both development in its earliest phases. This technique is also being used as an alternative search for a compatible donor, especially in the case of living-donor kidney transplantation patients with DSA against the donor for desensitization by lowering DSA levels prior to transplantation and in the immediate postoperative period by means of plasmapheresis with or without low-dose intravenous immune-globulins or immune-adsorption, together with potent immunosuppression to prevent an antibody rebound. The L-SBA method allows the precise monitoring of antibody levels; in general, if pre-transplant DSA levels can be decreased below 1,000 MFI, the transplantation may be performed.

However, in some patients, observed that no harm to the graft was witnessed in presence of DSA. Possibly, it could be of accommodation phenomenon or weak expression of antigens.

In the present study, patient P 5 who had low expression of DSA even at single antigen level, had no problem after 2 years of transplantation.

Conclusions

Pre and post Tx donor specific antibodies have to be considered a risk factor more than a contraindication, though this will require further study.

Though methodology of detecting DSA is a rapid evolving field, but characterization and identification of subgroup of DSA positive patients may differ in prognosis. However, these assays have had a major influence in both allocation and outcome of transplanted kidneys.

The availability of these sensitive assays has enabled clinicians to perform calculated panel reactive antibody and virtual cross-match which has led to a more accurate assessment of immunological risk of transplants.

Hence, it is important to have precise and timely detection of human leukocyte antigen (HLA) donor-specific antibodies (DSAs) which are vital to monitor for evaluating immune status of patients pre- and post-transplantation.

Important suggestions

- Must determine most accurate way to establish Antibody strength
- Must establish ranges for Borderline, Low, Moderate, and High Positive MFI value
- Clinical correlation must be based on uniform definitions.
- Recommendation for listing unacceptable Antigens Assessment of antibody strength.
- Candidates for desensitization protocols can be determined based on antibody strength.
- Virtual cross match can be based on antibody strength that accurately predicts a negative cross match (XM).

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