To Evaluate Anti-Human Leukocyte Antibodies Sensitization in Pre- and Post-renal Transplant Patient's Serum: A Retrospective Case Series

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Abstract

Introduction: In India, patients of renal failure are dependent on live-related or unrelated donor. Due to poor financial condition, patients do not go for donor-specific antibody (DSA) detection using Luminex. In the absence of screening of *de novo* production of DSA and does not get proper management. In the absence of pre-transplant screening and post transplant care, patients experiences acute graft rejection. **Materials and Methods:** Here, we are presenting five acute rejection cases comparing there DSA in pre-transplant and post-transplant sera using solid-phase assays. Five renal transplanted patients undergone acute and hyperacute rejection (Banff classification) were considered for presented case series. Collected serum (pre- and post-transplant on day of rejection) from each patient was subjected to the detection of anti-human leukocyte antibodies (HLAs) using Luminex panel-reactive antibody. **Conclusion:** The presence of donor-specific anti-HLA antibody with their titer was detected in pre- and post-transplant serum. It is found that the strength of DSA is directly proportional to graft life.

Keywords: Anti-human leukocyte antibodies, Donor-specific antibody, Renal transplant

Introduction

In 1966, correlation of preformed anti-human leukocyte antibodies (HLA) was introduced as an important clinical factor for transplants.[1] Since the past decayed, many studies have shown that the presence of donor-specific anti-HLA (donor-specific antibody [DSA]) in patients is responsible for the graft survival. In India, living-related or unrelated donors are the main sources of graft for patients with end-stage renal disease (ESRD). Conventionally, complement-dependent cytotoxicity (CDC) cross-match and CDC panel-reactive antibody (CDC-PRA) are in use to detect the presence of an antibody against the donor. Other methods to detected anti-HLA are enzyme-linked immunosorbent assay (ELISA) and flow cytometry.[2] Unfortunately, these tests are unable to tell the exact titer value of antibodies. CDC test is also not able to indicate if the present antibody is DSA; therefore, it is very difficult to predict the grafted life.[3] As a result of which, even after prescribing immunosuppressant patients undergo graft rejection.[3] Studies conducted in case of other solid organ transplants had proved that the presence of anti-HLA antibody is responsible for the reduced graft life.[4]

Luminex-PRP is a new technique now available to detect the presence of DSA with its strength and titer.[5] Luminex with single bead antigen assay is much sensitive variant of Luminex-PRP. It is also known solid-phase assay where polystyrene beads are coated with different HLA antigens further bound with a fluorochrome.[6]

Our aim of presenting following case series to analyze whether the detection of donor-specific anti-HLA antibody in patient's sera using solid-phase assay (Luminex) can be used as a parameter to predict graft life so that graft failure can be prevented in time. In this article, we are discussing

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five cases of renal transplant who underwent acute rejection.

Materials and Methods

All five cases presented in this study are renal transplanted patients with live-related or unrelated donors and undergone graft rejection. HLA typing of recipients and their donors was obtained from serology (Class I typing) and molecular methods (Class II typing). In the present study, all patients underwent a CDC cross-match test for the determination of anti-donor antibodies. The transplant was done only when the immediate pre-transplant CDC cross-match was negative. A retrospective evaluation of serum samples from transplant recipients who were otherwise CDC cross-match negative was performed to confirm the presence of anti-donor antibodies directed against specific cell types (namely, T-cell, B-cells, and monocytes) and to determine the isotypes of these immunoglobulins (whether immunoglobulin G/immunoglobulin M or immunoglobulin A).[7] At the time of transplant, all the patients were negative for HIV, HBsAg, and HCV infection as pre-transplant screening. Serum samples for pre-transplant and post-transplant time point were collected and stored. These samples were retrospectively analyzed for the presence of donor-specific anti-HLA using solid-phase assay (Luminex). Color key to interpret Luminex result is shown in Table 1.

Table 1: The color key to interpreting graphs generated for Luminex assay

Color code	Interpretation
Maroon	>2500 MFI – strong positive
Red	1000–2500 MFI – positive
Yellow	500–1000 MFI – weak positive
Green	Up to 500 MFI – negative

MFI: Medium fluorescent intensity

Table 2: Demographic data of subjects

Case no	Age	Gender	Relation	Blood group
Case 1				
Patient	38	Female		O+
Donor	56	Male	Father	O+
Case 2				
Patient	48	Male		B+
Donor	45	Female	Wife	B+
Case 3				
Patient	14	Female		A+
Donor	36	Female	Mother	O+
Case 4				
Patient	44	Male		B+
Donor	40	Female	Wife	B+
Case 5				
Patient	28	Female		O+
Donor	52	Female	Mother	O+

Results

Demographic data for all patients are presented in Table 2.

Case 1

Subject experienced contrast-induced nephropathy in mid-2008. The patient underwent a renal transplant (live-related donor) with her father as the donor. The patient was on hemodialysis since 2008 (42 times) and 6 units of blood were transfused before transplant. She had a history of two pregnancies. Demographic details are given in Table 2. HLA typing of patient and donor found to be as follows:

Patient: A28, A31; B8, B55; Bw6; DRB1 * 14, DRB1 * 15; DRB3*, DRB5*

Donor: A1, A31; B52, B55; Bw4, Bw6, Cw1; DRB1 * 14, _; DRB3*

The patient shared two of six antigens at HLA Class-I and II loci with the donor. The first CDC cross-match was weak positive. CDC cross-match was repeated twice at 1-month interval. The third cross-match was found to be negative with the final PRA below 20.0%. Flow cytometry and ELISA found to be negative for the presence of DSA.

Therefore, the patient was subjected to transplant. One month post-transplant, the patient was suspected to be undergoing acute rejection. Pre- and post-transplant (day 27) serum were analyzed. The antibodies detected by Luminex and their medium fluorescent intensity (MFI) values are summarized in Figure 1.

Luminex analysis revealed that pre-transplant serum had DSA against A1 having MFI in strong range of 1895.5. In addition to A1, there was the presence of antibodies against A11 and A80 with MFI values of 1985.03 and 1300.97, respectively. According to cross-reactivity group charts, antibody against A11 and A80 can cross-react with A1 antigen. In post-

transplant serum collected on day 27, persistence of antibody against A1 (1794.8) and A11 (1595.78) can be seen easily.

Case 2

A patient diagnosed with chronic glomerulonephritis at the end of 2007. He underwent for transplantation with his wife as a live unrelated donor. He was on hemodialysis since January 2008 for 58 times and 15 units of blood were transfused. HLA typing revealed expression (Class I and Class II) of HLA antigen as follows. Demographic details are given in Table 2. HLA typing of patient and donor found to be as follows:

Patient: A28, A33; B44, _; Bw4; DRB1 * 01, DRB1 * 07; DRB4*

Donor: A3, A11; B52, B35; Bw4, Bw6, Cw6; DRB1 * 14, _; DRB3*

The patient did not share any antigen out of six Class I and Class II HLA antigen. CDC cross-match was negative and PRA was 2.5%.

The patient experienced acute rejection on day 29, pre-transplant and post-transplant serum were analyzed. The results are summarized in Figure 2.

There was no DSA detected in either pre-transplant serum or post-transplant serum. However, there were other anti-HLA antibodies in both sera with MFI belong to the strong positive range (>2500).

Case 3

The patient was diagnosed with ESRD in early 2007. She underwent renal transplant with a live-related donor (mother). The patient was on dialysis since March 2007. Demographic details are given in Table 2. HLA typing of patient and donor found to be as follows:

Patient: A11, A68; B44, B62; Cw4, Cw7, Bw4, Bw6; DRB1 * 07, DRB1 * 15; DRB4*, DRB5*

Donor: A26, A68; B44, B57; Cw4, Cw7, Bw4, Bw4; DRB1 * 07, DRB1 * 15; DRB4*, DRB5*

Patient shares two of six Class I and Class II HLA antigen. CDC cross-match was weak positive. Therefore, the CDC cross-match was repeated after 2 weeks. For pre-transplant testing, flow cytometry cross-match was negative.

The patient experienced hyperacute rejection within 3 days of post-transplant. Hence, patient's pre-transplant and post-transplant (day 3) sera

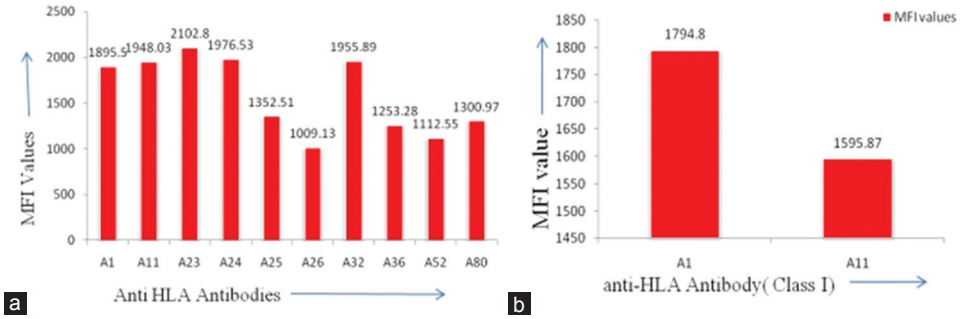

Figure 1: (a) Anti-human leukocyte antibodies (HLA) present in pre-transplant sera with their medium fluorescent intensity (MFI) (b) Anti-HLA present in post-transplant sera with their MFI

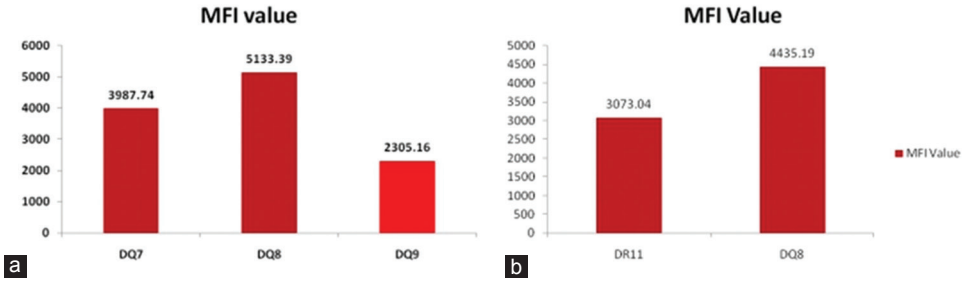

Figure 2: Anti-human leukocyte antibodies present in sera (a) in pre-transplant (b) in post-transplant

were analyzed. Moreover, results are noted in Figure 3.

On analysis, the presence of donor-specific anti-HLA antibody against Class I antigen B57 with MFI value 1123.84 and a cross-reacting antibody against B58 with MFI 1126.07 was found in the pre-transplant sera. In addition to it, there was no DSA persisting in the serum collected on day 3 post-transplant. However, in the case of HLA Class II, the patient did not contain any DSA in either pre-transplant or post-transplant.

Case 4

Subject 4 was diagnosed for diabetic nephropathy in mid of 2005. He underwent a renal transplant with his wife (live-unrelated donor). The patient underwent 12 hemodialysis and had 12 units of blood transfusion before transplant. Demographic details are given in Table 2. HLA typing of patient and donor found to be as follows:

Patient: A2, A31; B8, B60; Cw7, _, Bw6, _.

Donor: A24, A11; B57, B55; Cw1, Cw3; Bw4, Bw6.

The patient does not share any of the HLA antigens with a donor. CDC cross-match found to be negative and PRA was 0%. For pre-transplant testing, the cross-match was negative.

However, the patient experienced acute rejection. Then, pre- and post-transplant serum day 173 were analyzed. Results are shown in Figure 4.

After Luminex analysis, it revealed that pre-transplant serum contains antibody against Class I B55 antigen having MFI value 2513.4 and also antibody against antigen B54 (MFI value 2852.23).

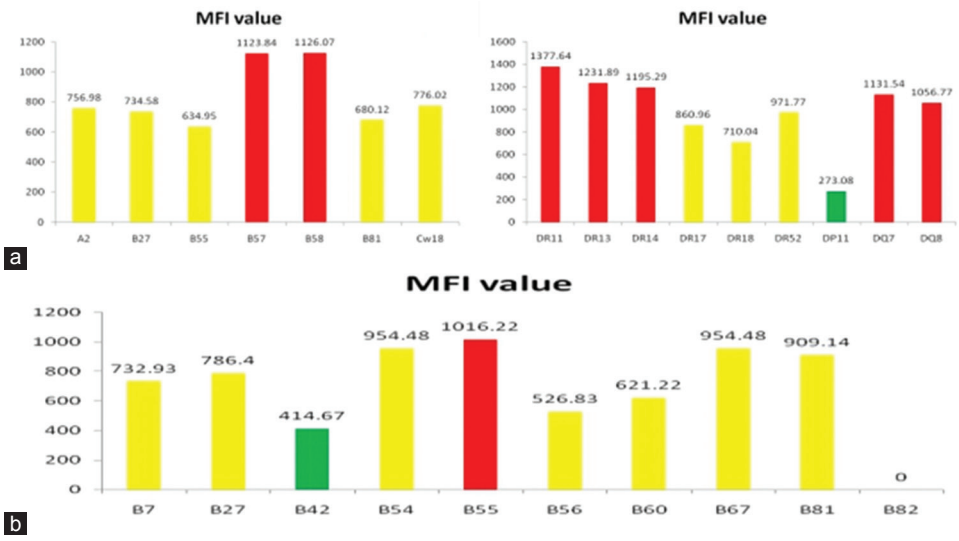

Figure 3: Anti-human leukocyte antibodies and their medium fluorescent intensity present in sera (a) Class I antibody in pre-transplant (b) Class II antibody in pre-transplant (c) antibody in post-transplant sera

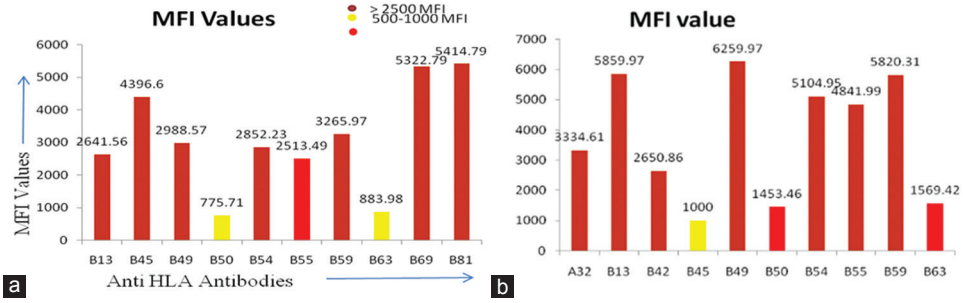

Figure 4: Anti-human leukocyte antibodies present in sera from patient 4 (a) in pre-transplant status (b) post-transplant status

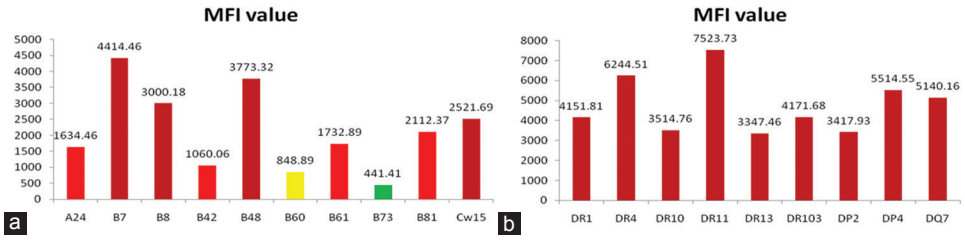

Figure 5: Patient 5 is showing the presence of anti-human leukocyte antibodies against (a) Class I and (b) Class II, only in post-transplant sera

Furthermore, post-transplant serum collected on day 173 contains DSA against B55 with MFI value 4841.99 (strongly positive) and also contain antibody against B54 with MFI value 5104.95. Strength of both the antibodies is amplified by the factor of 2 in post-transplant sera when compared to pre-transplant sera.

Case 5

Subject 5 was diagnosed for ESRD in mid-2009 and underwent renal transplant with her mother. The patient was on hemodialysis since June 2009 and there was no blood transfusion. The patient has two children. Demographic details are given in Table 2. HLA typing of patient and donor found to be as follows:

Patient: 24, A26; B7, B8; Cw7, _,Bw6, _; DRB1 * 03, DRB1 * 15; DRB3*, DRB5*

Donor: A11, A26; B8, B62; Cw7, _; Bw6, _; DRB1 * 03, DRB1 * 15; DRB3*, DRB5*

Out of six, patient and donor share four antigens. CDC cross-match found to be negative and CDC PRA was 64%.

The patient experienced hyperacute rejection. Pre- and post-transplant sera were analyzed. The results of the Luminex analysis are summarized in Figure 5.

There was no DSA found in pre-transplant serum (results not shown). Analysis of post-transplant serum revealed the presence of antibody against the antigen B8 having MFI value 3000.18. Furthermore, the antibody against B42 (1060.60 MFI) and other cross-reacting antibodies were present. Therefore, antibodies in pre-transplant sera must be some cross-reacting antibodies.

Conclusion

From the above case series, we can conclude that the patients who experienced acute or hyperacute rejection found to have DSA with MFI in strong range in their serum. We also found that a greater number of mismatched HLA antigen or difference in the age of donor and recipient can lead to the production of anti-HLA antibodies of higher MFI. Patient with positive cross-match should undergo the screening for the presence of DSA.

Anti-HLA antibody other than DSA with positive or strong positive MFI values should be considered.

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Contribution

Nishtha Agarwal: Luminex assay carried out for the patient serum and preparation of manuscript.

Sanjeev Goswami: Idea of the project and procurement of kit and guidance.

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