

Overcoming the Immunological Barrier in Highly HLA Sensitized Renal Transplant Recipients - A Desensitization Experience from a Transplant Center in Pakistan

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Abstract

Highly Human Leukocyte antigen sensitized patients have relatively fewer chances of being transplanted successfully and may remain dialysis dependent for a long time. In the last few years with the development of immunomodulatory therapies and advancements in immunological investigations, the chances of transplantation in these sensitized patients have improved. Desensitization therapies in these patients include plasma exchange, intravenous immune globulins and immunomodulatory agents such as rituximab and bortezomib. These agents used together in desensitization protocols across the world have shown encouraging results in highly Human Leukocyte Antigen sensitized recipients awaiting renal transplant. We used a desensitization protocol using rituximab followed by bortezomib with concurrent plasma exchange sessions and Intravenous Immune Globulins. Our aim was to assess improvement in renal function and quality of life in these patients after desensitization and renal transplantation. To the best of our knowledge, this is the first account of desensitization prior to renal transplantation from Pakistan.

Keywords: Renal Transplantation, HLA sensitized, Desensitization.

Introduction

Renal transplantation is the treatment of choice for patients with end stage renal disease (ESRD). It confers improved patient survival and a better quality of life.¹ Human leukocyte antigen (HLA) sensitization may trigger the production of donor specific antibodies (DSA) in the recipient. This can occur because of previous renal transplantation, blood transfusions, and pregnancies. It predisposes to antibody mediated rejection (ABMR), leading to early or late graft loss. With technological advancements, a better assessment of the specificity and strength of anti-HLA antibodies can now be made.^{1,2}

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Over the last two decades, desensitization strategies have been developed and practiced successfully to overcome this problem. These employ immunomodulatory therapies which either remove DSA or inhibit their synthesis. We report the successful desensitization and renal transplantation of three highly HLA sensitized recipients. Their renal function is stable 3 months after treatment and surveillance renal biopsies reveal no evidence of rejection. To the best of our knowledge, this is the first account from Pakistan of such a desensitization protocol.

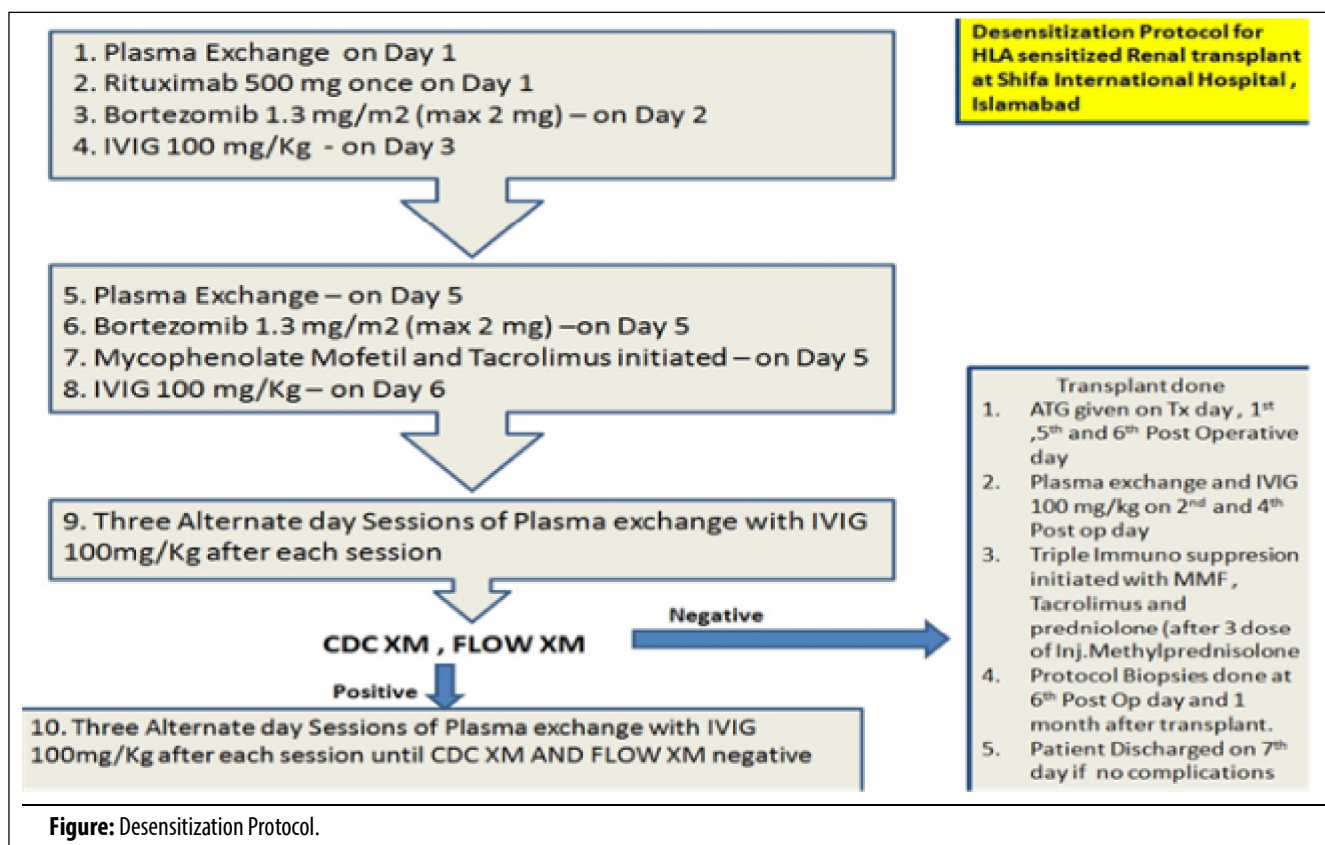
Case Presentation

Case 1

A 50 years old female patient with end stage renal disease (ESRD) secondary to Autosomal dominant polycystic kidney disease (ADPKD) was seen in September 2016 for renal transplant workup. She was on Hemodialysis for 3 years and had a history of multiple blood transfusions. On transplant work up, her blood group was B positive which matched with her sister (donor). Tissue typing revealed an HLA B35:02 mismatch. The Luminex Single antigen bead (SAB) test revealed a significant level of donor specific antibodies (DSA) against the mismatched antigen. These values are depicted in table. The Complement dependent cellular cross match (CDCXM) and Flow cross match (FCXM) test were positive against both B and T lymphocytes. The percentage cell deaths on CDCXM before and after desensitization for T cells were 40% and 0% (negative) respectively. Those for B

Table: Comparison of Immunological testing before and after Desensitization. (CDCXM- complement-dependent cytotoxicity cross match, FCXM- Flow cytometry crossmatch, SAB - single antigen bead test).

Case	Investigation	Before Desensitization	After Desensitization
1.	CDC XM	+ for both B and T Cells	Negative for T cells + for B cells
	FCXM		
	SAB	18600 MFI	600 MFI
	CDC XM	+ for both B and T cells	Negative for T cells + for B cells
2.	FCXM		
	SAB	17000 MFI	490 MFI
3.	CDC XM	+ for both B and T cells	Negative for both B and T cells
	FCXM		
	SAB	1691 MFI	652 MFI



cells were 40% and 20% respectively.

The desensitization protocol was then administered as shown in figure until the CDCXM and FCXM tests were negative prior to transplant. Sixteen sessions of plasma exchange (PLEX) sessions were required to achieve this. She was transplanted successfully in January 2017.

Case 2

A 30-year-old gentleman with ESRD secondary to Chronic Membranoproliferative Glomerulonephritis and was on Haemodialysis for the past 2 years. He was seen in December 2016 for renal transplant workup. He had his first renal transplant in 2001 and the donor was his mother; the graft rejected due to humoral rejection and chronic allograft nephropathy. His second donor was his brother whose blood group was B positive which matched his blood group. CDCXM and FCXM were positive against T and B lymphocytes. Luminex SAB detected high-level antibodies against an HLA 02:01 mismatch. The percentage cell deaths on CDCXM before and after desensitization for T cells were 40% and 0% (negative), respectively. Those for B cells were 20% on

both occasions.

The Desensitization protocol illustrated in figure was performed. Three sessions of plasma exchange were needed prior to transplant. He was transplanted successfully in February 2017.

Case 3

A 27 years lady with ESRD secondary to chronic vesicoureteric reflux was on twice weekly haemodialysis. She was seen in December 2016 for renal transplant work up. Her blood group was B positive, and her donor was her first cousin. CDCXM was positive for both T and B cells. FCXM was positive for IgG against T lymphocytes and B lymphocytes. The Luminex screen showed low-level IgG antibodies detected against 3/5 groups of HLA class II antigens. The percentage cell deaths on CDCXM before and after desensitization for T cells were 40% and 0% (negative), respectively. Those for B cells were 20% and 0% (negative), respectively. The desensitization protocol illustrated in figure was used and 16 sessions of PLEX were required prior to transplant. She was transplanted successfully in March 2017.

None of our patients received vaccinations in the past 12 months. Our patients were screened with their family members who wished to donate.

Discussion

Kidney donors are often considered unsuitable if the recipient has pre conditioning DSA against them and this may limit the number of available donors.^{3,4} Solutions to these problems include paired donor exchange or desensitization of the recipient. Most transplants in Pakistan involve living donors and there is an absence of cadaveric transplants or paired donor exchange programmes. Thus, desensitization is a viable and attractive option for such patients who otherwise, may have to remain on dialysis and may never receive a transplant.

Desensitization protocols have been in practice for the past two decades and numerous studies have shown good short and long term renal function as well as improved quality of life, although the long term graft survival is less as compared to non HLA sensitized recipients.⁵ The British Transplant Society (BTS) describes a lower risk of rejection when transplanting in HLA sensitized patients with a negative CDCXM and FCXM, and a DSA level of <5000 MFI.⁶ All our patients achieved negative T cell cross match tests and low amounts of DSA. Dithiothreitol (DTT) is routinely used in our immunology testing; it breaks IgM antibodies rendering positive cross matches due to them negative; they have limited significance in transplantation.⁵ The two cross matches are capable of detecting antibodies against B and T cells. IgG antibodies against the latter are of profound importance due to their association with hyper acute rejection. B cell cross match may remain positive post desensitization due to the effects of Rituximab which itself, is a monoclonal antibody. These are false positive results as evident in our cases 1 and 2.

All patients in our series had positive CDCXM and FCXM initially. The former detects complement binding antibodies. These tests are cell based assays utilizing a mixture of donor lymphocytes and recipient serum; in addition, CDCXM uses complement and FCXM, fluoresceinated Anti Human Globulin (AHG). CDCXM is reported as percentage cell death caused by complement fixation and cell lysis. FCXM gives a mean channel shift reading corresponding to the intensity of the fluorescence caused by AHG binding to their targets. In

the presence of a negative CDCXM, FCXM positivity usually indicates a non complement binding, non anti HLA or low level antibody.⁵

Our patients underwent desensitization until the cross matches against T cells became negative and DSA levels declined. A positive CDCXM or FCXM is considered a contraindication to transplant; the former depicts the presence of complement fixing DSA which can cause acute, accelerated or chronic graft rejection. Studies have repeatedly shown that high levels of DSA are associated with graft rejection.⁷

Most desensitization protocols use either high dose IVIG or low dose IVIG in combination with plasma exchange as the major components. IVIG inhibits B and T lymphocytes, up regulates anti-inflammatory T-Helper 2 cytokines and neutralizes alloantibodies. It also enhances removal of pathogenic IgG.⁸ We used the low dose IVIG (100mg/kg/dose) and PLEX strategy and found it to be effective. Rituximab binds to CD20 receptors on pre-B and mature B lymphocytes essentially depleting them. Studies have shown that IVIG and Rituximab prevent a rebound in DSA, thus preventing acute humoral rejection and transplant glomerulopathy.^{9,10} Various doses have been used; we used a dose of 500 mg/m² in our patients.

PLEX can remove a lot of antibodies from the circulation; however, there may be a rebound. The number and frequency of the PLEX sessions are dependent on the donor specific antibody titer. None of our patients suffered any complications related to PLEX.⁶

Bortezomib is a proteasome inhibitor and targets mature plasma cells. Plasma cells are not affected by rituximab since they lack the CD20 receptors. Together, rituximab and bortezomib have been used to successfully desensitize kidney transplant candidates who have a positive cross match and are highly sensitized.^{9,10} The dose used was 1.3 mg/m², corresponding with past studies.

Conclusion

We used low dose IVIG, Plasma exchange, Rituximab and Bortezomib to successfully desensitize and transplant three Highly HLA sensitized recipients at our center. The short term follow up of these patients was unremarkable with no evidence of graft dysfunction/rejection. Desensitization is an attractive option for such patients

in Pakistan, where cadaveric and paired donor exchange programmes are nonexistent.

Disclaimer: None

Conflict of Interest: None

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Patient Consent: Consent from all three patients was taken prior to the writing of this manuscript.

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