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Post Transplant Human Cytomegalovirus Infection Can Provoke Acute Kidney Allograft Rejection

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ABSTRACT: *Human Cytomegalovirus (HCMV) infection can trigger immunological cascades leading to kidney allograft rejection. In this study we have selected 17 post renal transplant patients with negative Luminex Donor Specific Antibody (DSA) crossmatch and Complement Dependent Cytotoxicity (CDC) crossmatch with Anti Human Globulin (AHG) augmentation. All the 17 patients were detected with post transplant HCMV infection. 4 of the 17 patients were recognized with rejection symptoms identified histologically and among them 1 patient died. The patient who died was co- infected with blood stream Staphylococcus aureus.*
Keywords: *Acute Rejection, Complement Dependent Cytotoxicity, Donor Specific Antibody, Human Cytomegalovirus, Kidney Allograft Transplantation.*

I. Introduction

Recent periodicals suggest strong interrelation between Human Cytomegalovirus (HCMV) infection and donor allograft rejection[1]. HCMV, a member of Herpesvirus group has direct effect on the target tissue causing irreversible damage or the immune system responding to viral sequence present in transplanted cells thus plays an important role in graft survival[2]. HCMV infection could promote allograft rejection through different mechanisms including the production of several pro-inflammatory cytokines, increased expression of major histocompatibility complex (MHC), adhesion molecules and molecular mimicry[1, 3, 4, 5]. It has been reported that Cytomegalovirus is a persistent virus that induces a permanent increase of highly differentiated IFN-γ secreting effector T cells, and also induces a systemic inflammatory response[3]. Primary CMV infection induces a clear pro-inflammatory response that is maintained during latency. HCMV infection has also been shown to increase intracellular adhesion molecule-1 (ICAM-1) expression and thus frequently associated with vasculopathy and transplant rejection[4]. HCMV infection can also lead to increased MHC class I and class II expression in cultured human endothelial cells[2]. Transcription of HCMV can be divided into three separate phases, early (E), immediate early (IE), and late (L) [6, 7]. IE genes are transcribed in the absence of viral protein synthesis and are located in restricted areas of the viral genome[6, 7]. Recent evidences indicate that IE products are recognized by HCMV specific cytomegalovirus toxic T lymphocytes[8, 9, 10, 11]. IE2 protein of HCMV and the HLA chain share a common epitope, therefore antibody produced against the HCMV peptide binds to the β chain of HLA DR Antigen thus present a cross-reactive epitope. The sharing of a microbial epitope with a host "self" epitope from two dissimilar proteins has been termed molecular mimicry[12, 13]. Therefore, the immune response would be generated against viruses also react with host self determinants as viral IE2 and 29-kDa HLA DR β sequences are antigenically identical[1], thus initiate immune activation which could lead to rejected allograft. HCMV is also associated with the inhibition of the up regulation of MHC antigens due to increased IFN-γ production during infection[14].

II. Method

2.1 Patient: 17 post transplant patients have been included in this study between November 2012 to July 2013 in Medica Superspecialty Hospital, Kolkata, India.

2.2 DSA crossmatch: To determine specificity of HLA antibodies tests has been performed using Lifecodes LSA class I and / or class II, phycoerithrin conjugated goat anti human IgG antibody was used as secondary antibody. Samples were analyzed on a life match fluroanalyser using Luminex 1001S v.2.3 as software for data acquisition and Quicktype for Lifematch as analysis software. The manufacturer provides positive and negative control sera and beads that were included in each test. To determine if an individual bead is recognized by the anti-HLA antibodies present in serum, only the Mean Fluorescent Intensity (MFI) raw value was considered. A serum sample was considered to be positive to a specific bead when the MFI raw value for this bead was < 500.

2.3 Lymphocyte crossmatch (CDC): Pre transplant Lympho Cytotoxicity Test was performed using both direct complement-dependent cytotoxicity (CDC) crossmatch and CDC crossmatch with added anti-human

globulin (AHG-CDC). Incubation was conducted using 1 microliter of donor lymphocyte suspension and 1 microliter of recipient sera in a Terasaki plate (Nunc, Roskilde, Denmark) at room temperature for 30 min in the AHG-CDC crossmatch, AHG (Goat IgG k and 1 light chains) was added and incubated at room temperature for 1-2 min. 5 microliters of rabbit complement was added to each well and the mixture was incubated at room temperature for 60 min. 2 microliters of 5% eosin solution were added and the mixture was examined using phase-contrast microscopy (Leica DM IL LED, Germany). The results were considered positive when more than 20% of the donor lymphocytes were killed by the recipient's serum in either test. Dithiothreitol was not used for the inactivation of IgM antibodies.

2.4 Real Time PCR: Viral DNA from patient sera has been extracted using QIAmp blood mini kit 51104. Real Time PCR was performed using Rotor Gene Q 50512241 using artus CMV RG PCR kit 4503263. To generate the standard curve, positive and negative controls were run in parallel along with the patient's sample.

2.5 Co-infection: Co-infection has been studied after picking a pure isolated colony from the culture plate and diluting it in inoculum water and then transferring it in the Microscan combo panel. It is incubated upto 16 hrs and then read into the instrument Microscan Autoscan 4, Siemens. The software used for the analysis is Labpro V3.0.

2.6 Detection of Fungus and Yeast: Presence of Fungus and Yeast has been confirmed microscopically.

2.7 Kidney Biopsies: Patient underwent protocol biopsies post transplant to determine allograft dysfunction and rejection.

2.8 Statistical analysis: All statistical analysis has been done using Prism 4.1 and Microsoft Excel 2007.

III. Results

We had selected 17 HCMV infected kidney allograft recipients (35.42%) among 48 HCMV suspected post transplant cases (Fig.1). DSA and CDC crossmatch report showed negative in all cases for both class I and class II (Table.1). 4 recipients (23.53%) among 17 had been detected with acute rejection of the allograft and 1 died (5.88%) (Fig.2). Among the 4 rejected cases 2 had been detected with *Klebsiella pneumoniae* infection from wound swab (Table.2). 3 of the allograft rejected recipients had also been detected with Gram negative ESBL strain in urine specimen (Table.2). 1 of the allograft recipient detected with associated fungal infection in urine and wound swab (Table.2). The recipient who died was detected with blood stream *Staphylococcus aureus* infection (Table.2). Real time PCR quantification of HCMV viral load had shown a mean viral peak of 5312 copies/ml. All of the rejected transplant recipients exhibit Gram positive, Gram negative and fungal co-infections. Rejection symptoms were established histologically.

IV. Figures and Tables

Table 1. Patients information including number of patients (n=17), mean age, sex, donor's information, transplant information, HLA match, DSA and CDC crossmatch.

Characteristic:		
Recipient's information :		
	Age (mean) yrs	43
	Male	11
	Female	06
Donor's information :		
	Deceased	0
	Living	17
Transplantation:		
	First Transplantation	17
	Second Transplantation	0
HLA -A,B and DR mismatches		
	Matched	0
	Mismatched	17
DSA Crossmatch:		
	Positive	0
	Negative	17
CDC Crossmatch:		
	Positive	0
	Negative	17

Table 2. Co-infections in HCMV infected patients with allograft rejection.

Patient	Sample	Organism
P1	Wound Swab	*Klebsiella pneumoniae*
	Urine	*Klebsiella pneumoniae* ESBL, Budding Yeast, *Candida* sp.
P2	Blood	*Staphylococcus aureus*
	Wound Swab	*Klebsiella pneumoniae*
P3	Urine	*Klebsiella pneumoniae* ESBL, *Enterococcus faecalis*
P4	Urine	*Escherichia coli* ESBL

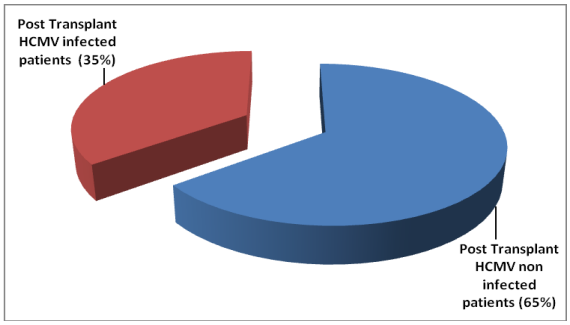

Fig. 1. Total number of post transplant HCMVsuspected but non infected patients (65%) and post transplant HCMV infected patients (35%).

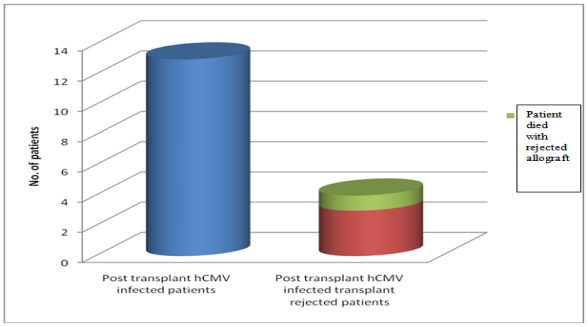

Fig. 2. Total number of post transplant HCMV infected patients (first bar) and total number of post transplant HCMV infected transplant rejected patients (second bar) with the number of patient who died (fraction of second bar). Y-axis depicts the number of patients.

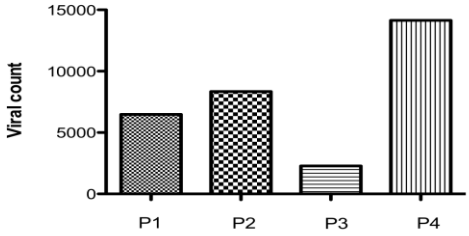

Fig. 3. HCMV viral load (copies/ml) for all the 4 patients who developed post transplant infection with acute rejection symptoms.

V. Discussion

With the help of recent research we already know that HCMV infection can generate a worst scenario among kidney allograft recipients. On one hand the specific region of viral genome can mimic MHC class II DR β chain and on the other can induce increased IFN-γ secretion and promotes the expression of other adhesion molecules which in turn can potentially intensify the severity of the immune reaction[1, 4, 5]. Thus extremity of the immune response generated among post transplant HCMV infected recipients is directly proportional to the magnitude of the infection.

Among the 17 HCMV infected patients (mean age 43 yrs) 4 were presented with the clear histological evidence of vasculopathy and acute rejection. Luminex based Donor Specific Antibody crossmatch for all the 17 transplant recipients were negative with the MFI <500. Therefore chances of donor specific antibody mediated immune activation were abrogated. AHG augmented extended complement dependent cytotoxicity crossmatch report were also indicate clear negative result for the transplant rejected recipients. To that end low titre complement fixing antibodies were also not involved in the immunogenic trigger. The rejection symptoms for all the 4 cases appeared only after the detection of HCMV infection. On this ground the appearance of the rejection symptoms could be very evidently correlated with the HCMV infection. The patient who died was also detected with blood stream *Staphylococcus aureus* infection.

VI. Conclusion

From the above clinical study and the recent findings in the field of transplantation research it is very clear there is significant correlation between post transplant HCMV infection and acute kidney allograft rejection.

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