EARLY DIAGNOSIS IN POST RENAL TRANSPLANT OPPORTUNISTIC INFECTIONS: A FRESH LOOK

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ABSTRACT

A total of 86 renal transplant patients who were transplanted with live related donor (LRD) and live unrelated donor (LURD) kidneys were studied for opportunistic infections. Immune diagnosis of Toxoplasma, Cytomegalovirus (CMV), Herpes-simplex virus type II (HSV-2), Aspergillosis and Tuberculosis was carried out in these patients along with sputum examination, CSF studies and biopsy of lymphnode and other tissues in few cases. A high degree of Toxoplasma, CMV & HSV-2 positivity was seen in transplanted patients. However sensitivity of serological diagnosis of tuberculosis was found to be low with standard criteria, which increased significantly when modified criteria were used. It is concluded that regular immunological monitoring should be carried out in transplanted patients so as to reach an early diagnosis and management of opportunistic infections.

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KEY WORDS: Immune diagnosis; Opportunistic infections; Transplantation.

Introduction

Despite the discovery of newer immunosuppressive drugs, improvement in diagnostic techniques and availability of better antimicrobial agents, infections remain a major cause of both early and later mortality after renal transplantation. The important infections encountered among the renal allograft recipients in the western world are those caused by opportunistic organisms such as CMV and *Pneumocystis carinii* [1], fungal infections like cryptococcus, mucoraceae, candida, aspergillosis and systemic mycosis with histoplasmosis and others [2]. Other not so common infections are toxoplasmosis [3], *Strongyloides stercoralis* [4] and nontyphoid salmonellosis [5]. The two most common opportunistic bacterial infections in renal transplant patients are those due to *Listeria monocytogens* and *Nocardia asteroides* [2]. Tuberculosis has been reported uncommonly in post transplant patients from developed countries. However in developing countries like India, the incidence is expected to be high [6]. All these infections account for major cause of morbidity and mortality in transplant patients [7]. So awareness, early detection and quick management of these infections is very essential. Keeping these points in view and to detect opportunistic infections at an early stage, a prospective study was carried out in renal transplant patients of our centre.

Materials and Methods

A total of 88 transplant patients were studied. Out of this, 57 patients were transplanted in Army Hospital (R & R) and AIIMS New Delhi where only live related donor (LRD) transplantation is being carried out. Rest of the 31 patients were transplanted in other authorised transplant centres of the country conducting LRD and live unrelated donor (LURD) transplantation. The patients were in the range of 14 to 51 years age (mean 32.5 ± 11.5 years). There were 77 males and 11 females in the study.

Collection of serum samples

Serum samples were collected both at pre and post transplant periods and kept frozen at -20° till assay time. Repeated thawing was avoided. Serum samples were also collected from 20 healthy controls. These serum samples were tested for the antibodies against following infectious disorders using imported kits as mentioned below:

1. Toxoplasma IgG & IgM, CMV IgM, HSV-2 IgG & IgM from Melotest, Spain.
2. Aspergillus IgG & IgM from IBL Germany.
3. Tuberculosis IgG, IgA, IgM from ANDA Biologicals France.

Test procedure

For detection of anti IgM Toxoplasma, CMV and HSV-2, the serum samples were treated with an absorbent solution initially so as to prevent interference from nonspecific auto-antibodies like rheumatoid and antinuclear antibodies. Serum samples and controls obtained from manufacturers, were treated to the precoated plates wells against Toxoplasma, CMV, HSV-2 and Aspergillus. After a defined incubation and automatic washing, the complexes were treated with an antihuman antibody conjugated with horseradish peroxidase enzyme solution. These complexes were again washed and a substrate solution (TMB) was added resulting in

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colour development. Final results were obtained using computerised ELISA data analysis system.

For detection of antimycobacterial antibodies, the serum samples were treated with precoated A60 antigen wells and a total of 83 serum samples from 49 transplant patients were analysed.

The normal range of IgG and IgA was taken as < 225 u/ml and < 330 u/ml respectively as was directed by the manufacturers. Since all the transplant patients studied were immunosuppressed, a new normal cut off range for these patients was taken as < 150 u/ml and < 200 u/ml for IgG and IgA respectively. The IgM positivity was taken as .60 of normal IgM control.

In addition to the immune diagnosis, CSF samples from six patients who were suspected to be cases of meningitis, were analysed for bacterial and fungal infections. Similarly sputum samples from suspected cases were checked by AFB and fungal stains. Biopsy samples from 11 patients were taken for histopathological examination. During the period of study, three patients died of post transplant complications. Their autopsies were performed and findings noted down.

Results

Pre transplant samples from 42 patients and post transplant samples from 68 patients were analysed for the presence of anti-Toxoplasma and CMV antibodies. In the pretransplant samples only 5 patients were found to be positive for IgG and three for IgM antitoxoplasma antibodies (Table 1). Similarly 5 patients were found to be positive for IgM CMV antibodies. However at post transplant period which ranged from 3 months to 4 years, high rate of positivity was seen for antitoxoplasma IgG (27 out of 68, 39.7%), IgM (19 out of 68, 27.9%) antibodies (Table 1). 11 patients showed positivity for both antitoxoplasma IgG and IgM antibodies. In addition to this, 12 patients were also found to be positive for CMV IgM antibodies (Table 2). When pre transplant samples from 42 patients were analysed for HSV-2 antibodies only 3 patients were positive for both IgG and IgM. However in the post transplant serum samples from 51 patients, 13(25.4%) were found to be positive for anti HSV-2 IgG and 9 (17.6%) for IgM antibodies (Table 3). One of the healthy controls was also found to be positive for both these antibodies.

TABLE 1

Anti toxoplasma antibodies

	No.	IgG		IgM	
		+ve	%	+ve	%
Healthy controls	20	2	10	0	0
Pre Tx patients	42	5	11.9	3	7.1
Post Tx patients	68	27	39.7%	19	27.9

p value for IgG < 0.01, for IgM < 0.05 (pre Tx vs post Tx)

TABLE 2

Anti CMV antibodies

	No.	IgM	
		Positive	%
Healthy control	30	1	5
Pre Tx patients	42	5	11.9
Post Tx patients	68	12	17.6

p value > 0.05 (pre Tx vs post Tx)

TABLE 3

Anti HSV-2 antibodies

	No.	IgG+	%	IgM+	%
Healthy control	20	1	5	1	5
Pre Tx	42	3	7.1	3	7.1
Post Tx	51	13	25.4	9	17.6

p value for IgG < 0.05 for IgM > 0.05 (pre Tx vs post Tx)

In the pre transplant sera of 41 patients, only 2 were positive for anti IgG Aspergillus antibodies. However in the 57 post transplant samples, 7 patients showed positivity for these antibodies. This figure rose from 3 to 11 for IgM antibodies in pre and post transplant samples (Table 4). Out of the three post mortem cases, two showed aspergillosis in lungs. Both these patients were retrospectively found to be positive for aspergillosis by serological methods.

TABLE 4

Anti aspergillosis antibodies

	Number	IgG+	%	IgM+	%
Healthy control	20	0	0	0	0
Pre Tx	41	2	4.8	3	7.3
Post Tx	57	7	12.2	11	19.2

p value IgG & IgM > 0.05 (pre Tx vs post Tx)

Anti mycobacterial antibodies

34 pretransplant serum samples and 49 post transplant samples were analysed for antimycobacterial antibodies. Out of these 49 patients, 18 patients were later on proved as cases of mycobacterial infection by clinical/laboratory investigations or by drug response. The remaining 31 patients did not have any symptoms and signs of mycobacterial infection. The positivity rate for anti A60 antibodies in all these patients along with healthy controls is shown in Table 5.

However when the new cut off lines were taken the positive results increased and are shown in Table 6.

TABLE 5

Anti mycobacterial antibodies

	Number	IgG+ (> 225 u/ml)	IgA+ (> 350 u/ml)	IgM+ 1 : 1
Healthy controls	20	1	0	2
+Pre Tx samples	34	3	2	6
Post Tx samples	49			
1. Mycobacterial infection	18	4	1	10
2. No mycobacterial infection	31	3	1	3

TABLE 6

Anti mycobacterial antibodies

	Number	IgG+ (> 150 u/ml)	IgA+ (> 200 u/ml)	IgM+ 0.6 : 1
Healthy controls	20	1	1	3
Pre Tx samples	34	4	3	6
Post Tx samples	49			
1. Mycobacterial infection	18	9	6	15
2. No mycobacterial infection	31	5	3	6

The six CSF samples from suspected cases of meningitis showed *Cryptococcus neoformans* in two and *Streptococcus pneumoniae* in one patients. 38 patients where sputum was examined for AFB and fungal stains, showed AFB positivity in seven cases, 4 cases showed candidiasis & 2 were positive for aspergillus. Lymphnode biopsy from 11 patients showed granulomatous lesions in 3 cases. Rest of the 8 patients showed reactive changes. Out of the three patients whose post mortem examination was carried out, two showed *Cryptococcus* and two *Aspergillus* infection.

Discussion

The evaluation of infections in transplant recipients involves consideration of both the donor and the recipient of transplanted organs. Infections following the transplantation are complicated by the use of drugs that are necessary to enhance the likelihood of survival of the transplanted organ, but also cause the host to be immunocompromised. Thus what might have been a latent or asymptomatic infection in an immunocompetent donor becomes a life threatening problem for the immunocompromised recipient [8]. Many centres have thus developed specific protocol for screening opportunistic infections in transplanted organs.

During the early post transplant period, infections are most often caused by extracellular bacteria like *Staphylococci*, *E-coli* and other gram negative organisms. However in the subsequent weeks, consequence of suppression of cell mediated immunity, some viral infections like CMV & parasitic infection are encountered [8]. In addition some fungal infections like candida & mycobacterial diseases may also be associated.

CMV infection continues to be a major cause of morbidity and mortality in transplant recipients, yet prompt diagnosis remains a problem [9]. Fifty percent of all renal transplant recipients, presenting with fever in the first 4 months after transplantation have evidence of CMV disease. CMV disease is frequently severe, specially when the donor is seropositive and recipient seronegative [7]. In a recent study [10] the incidences of CMV infection and disease were 56% and 23% respectively during the first 6 months following renal transplantation. Majority of the patients (93%) developing CMV disease had seropositive donors. In our study of 42 pretransplant samples, there were only five patients (11.9%) who were CMV (IgM) seropositive. This however increased to 12(17.6%) in the 68 post transplant serum samples. The increase in seropositivity appears to be due to reactivation of the disease due to immunosuppression. There may also be few donors who were CMV seropositive causing infection in the recipient. So all the donors should be screened for CMV before transplantation.

Toxoplasmosis infection is a frequent event in most

of the developing and developed countries. When this occurs in immunocompetent individuals it is rarely a matter of concern, the contrary occurs with immunosuppressed patients or in pregnancy [3]. Transplant patients are treated with immunosuppressive drugs which mainly disturb their mechanisms of cellular immunity, thus opening the way to infection to intracellular organisms. Most of the times toxoplasmosis occurs in seropositive patients usually in the first few months after transplantation. In our study we demonstrated a steep rise in antitoxoplasma IgG and IgM antibodies in post transplant patients, necessitating regular monitoring and management.

Other common infections seen in transplanted patients are by nocardia, aspergillus, mucor, candida, herpes and cryptococcus in which T cell/macrophage axis plays an important role. We encountered a significant higher rate of HSV-2 and aspergillosis in our transplanted patients as compared to healthy controls and pretransplant samples. Similarly *Cryptococcus* infection was seen in both antemortem and post mortem specimens.

Tuberculosis is encountered more frequently in renal transplant recipients than in the general population in all parts of the world [11]. The incidence of post transplant tuberculosis varies from 0.5% to 4% in USA and upto 12% in India [6]. A careful pretransplant evaluation of all recipients for evidence of tuberculosis and close follow up in post transplant period to facilitate early detection is therefore essential.

In our study of serological evaluation of A 60 antibodies by standard cut off lines, we found no useful purpose of this test in detection of tuberculosis in transplanted patients and also in differentiating them from healthy and non tuberculosis patients. This is probably due to decreased formation of A60 antibodies in immunosuppressed patients. However when new cut off lines were applied, the test was positive in almost all confirmed cases of tuberculosis and could differentiate them from most of the healthy and non tubercular cases. Similarly it showed increase in tuberculosis cases in post transplant period as compared to pre transplant patient samples, which correlated clinically.

It is concluded that in post transplant period, there is a significant risk of opportunistic infections necessitating early detection and management. Regular serological monitoring of various disease processes at different pre-and post-transplant periods is found to be very useful and is recommended for better patient care by decreasing morbidity and mortality. It is also rec-

ommended that prospective donors should be serologically screened for infections like toxoplasmosis, CMV and tuberculosis, apart from others which are already being carried out.

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