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Original Research Article

Post kidney transplant infections with special reference to cytomegalovirus

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ABSTRACT

Background: The burden of infectious disease is high among kidney transplant recipients because of concomitant immunosuppression.
Methods: Study was a retrospective and prospective cohort study. The study was conducted for a period of 12 months and 30 transplant recipients were included.
Results: Males comprised 86.66% of the study population. The mean age of the recipients was 37.96 years. UTI was the most common post-transplant infection observed in 15 patients (50%) of 30 patients. *E. Coli* infection was most predominant (50%) causing UTI. 5 patients (16.66%) in this study developed tuberculosis after renal transplantation. Two patients (6.66%) developed CMV infection in the study. Two patients (6.66%) developed COVID-19 infection in the study. Two patients (6.66%) developed herpes infection post-transplant. One patient (3.33%) in the study developed cryptococcal meningitis. One patient (3.33%) developed hepatitis C after 18 years of transplant. One patient (3.33%) developed hepatitis B after 10 years of transplant. Three patients (10%) developed lower respiratory tract infection and developed acute respiratory distress. Three patients (10%) had developed acute graft rejection within first year after transplantation. There are total of 13 deaths (43.33%) among 30 patients all of which were secondary to infections. Total of 4 patients among the 13 deaths had chronic graft rejection. Most common cause of death was tuberculosis (38.46%) followed by UTI (23%) and Lower respiratory tract infection (23%).
Conclusions: The incidence of infections is relatively higher in kidney transplant recipients when compared to general populations due to immunosuppression.

Keywords: Cytomegalovirus, Infections, Post kidney transplant, Tuberculosis

INTRODUCTION

Immunosuppression comes with a heavy price in the form of infection, which also has an elevated mortality rate. In order to prevent the occurrence of infection, one should know the commonest types of infection in that particular group of patients.[1] Interventions such as changes to pre-transplant screening of recipient and donor, vaccination and prophylaxis, and post-transplant surveillance may effectively decrease infection rates following kidney transplantation.[2] It is known that graft loss increases by three times the mortality risk when compared with patients with a functioning kidney, and it is estimated that the patient's survival 5 years after graft loss is less than 40%.[3]

Advanced donor age, deceased donor status, cytomegalovirus (CMV) positive donor, recipient age <18 or >50 years, female sex of recipient, number of years on dialysis, and systemic lupus erythematosis or diabetes mellitus as the cause of kidney disease have been identified as factors that increase the risk of post-transplant infection.

The traditional paradigm has noted that early infections (within the first month) are more likely to be due to nosocomially acquired pathogens, surgical issues, and some donor-derived infections. Opportunistic pathogens occur later, often during the subsequent 5 months, reflecting the greater impact of immunosuppressive therapies (Figure 5).[4]

Late infections may be secondary to opportunistic pathogens or conventional ones; opportunistic pathogens are more frequently seen in patients who require greater immunosuppression or who have specific environmental exposures.[4] Urinary tract infection (UTI) is the most common infection in the post-transplant period followed by candidiasis and tuberculosis.[7] Hepatitis B and C viral (HBV and HCV) infection, cytomegalovirus (CMV), and pneumocystis are also common infections that are encountered in renal transplant recipients.

Recent trends in kidney transplantation have included a dramatic enhancement in short- term issues, reflecting better understanding of immune mechanisms leading to allograft rejection and the development of potent new regimes of immunosuppressive medicines that effectively obviate and treat rejection. The mechanism of action of various immunosuppressant shown in Figure 6. Concurrent surgical inventions have included new ways for harvesting multiple organs, laparoscopic organ removal, and new strategies for surgical operation of borderline donors and recipients who would have been rejected in an earlier period. Attention has turned increasingly to strategies that optimize long-term allograft survival by minimizing the major factors contributing to post transplant mortality.

Worldwide, an estimated 119,873 solid organ transplants were performed in 2014. Renal transplants were the most usual, followed by those of the liver, heart, lung, and others, including binary organ, pancreatic, and intestinal transplantation. Over the last several decades, the field of solid organ transplantation (SOT) science and practice has advanced significantly, only to be continually challenged by the threats for infection in SOT donors.

The positive effects of the immunosuppressive agents, obligatory for the prevention of organ rejection, have been tempered by the negative effects of these same therapies, leading to various infections that range in both frequency and severity.

One limitation to transplantation also, as now, was the lack of suitable donor organs. The first pioneers had used animal organs or organs from long departed humans. In the 1950s, there came a consummation of the need to avoid excessive ischemic injury and kidneys from live donors began to be utilized. Some of these were from the relations of the recipient; others were unconnected cases having a good kidney removed for other reasons. The surgical technique also demanded refinement; while a kidney based on the thigh or arm vessels might be technically straightforward, and conceivably acceptable for the short- term treatment of acute renal failure, it wasn't a realistic result for the long term.

Immunosuppression is needed for as long as the graft functions; if it's stopped, then rejection occurs and the graft is lost. However, the intensity of immunosuppression isn't constant. High levels of immunosuppression are needed soon after transplant, but later doses can be reduced to a lower maintenance level.

In addition to individual medicine side- effects, cases who are immunosuppressed have an advanced threat of infection and malignancy. Generally encountered infections include pneumocystis jiroveci and cytomegalovirus, although other unusual pathogens such as aspergillus are also more common in transplant recipients. Cases are generally given antimicrobial prophylaxis for the first 3-6 months, after which the effects of the induction immunosuppression have worn off and the baseline immunosuppression has been reduced.

Urinary tract infections

Bacterial UTIs are mainly caused by Enterobacteriaceae of the recipient or nosocomial pseudomonas or Enterobacter species.[8]

Cytomegalovirus infection

Cytomegalovirus infection is a frequent complication after transplantation. It can affect allograft function and increase patient morbidity and mortality through a number of direct and indirect effects.[9] CMV infection in a kidney transplant recipient is mostly asymptomatic. Still it should be considered in a patient presenting with unexplained rise in serum creatinine, low grade fever, diarrhoea or unexplained anaemia.[10] T cell-mediated immune response (CMI) has a significant part in protection against CMV infection.[11] Recent research suggests that the humoral response, particularly neutralising antibodies, may also be required for defence against CMV infection.[21] The donor who has CMV-IgG (D+) with a receptor (R) that is negative (R-) marked as D+/R-, as well as those receivers who have received anti-lymphocyte anti-body therapy, are now the groups with the highest risk of developing CMV infection and illness.[22]

According to current guidelines, universal prophylaxis is recommended in patients with high risk (i.e., those who have D+/R− CMV IgG or who have received T-cell depletion for induction prior to transplantation.[12] The most commonly used medication for prophylaxis is oral valganciclovir with dose adjustment according to kidney function. In D+/R−, prophylaxis should last for 3-6 months. In D+/R+ or D−/R+, prophylaxis should last for 3 months.

Detection of CMV includes IgM ELISA, IgG avidity testing, pp65 antigenemia assay, PCR for CMV DNA.

Serology

These are complement fixation, enzyme-linked immunosorbent assay (ELISA), anticomplement immunofluorescence, radioimmunoassay, and indirect hemagglutination.

Cell culture

This approach utilizes clinical specimens which are inoculated onto human fibroblast cells and incubated and observed for a period of time ranging from 2 to 21 days**.** CMV exhibits a typical cytopathic effect (CPE).

Antigenemia

This assay depends on the use of monoclonal antibodies that detect the viral pp65 antigen, a structural late protein expressed in blood leukocytes during the early phase of the CMV replication cycle.

Polymerase chain reaction amplification

Polymerase chain reaction (PCR) is a widely available rapid and sensitive method of CMV detection based on amplification of nucleic acids.

Immunohistochemistry

Immunohistochemistry is performed primarily on tissue or body fluid samples. Slides are made from frozen sections of biopsy tissue samples (liver, lung) or by centrifuging cells onto a slide. The stained slides are then examined by fluorescent or light microscopy.

Nucleic acid sequence-based amplification (NASBA)

The assay allows the specific nucleic assay sequence-based amplification of unspliced viral mRNAs (late pp67 mRNA expression).

Treatment is always indicated in case of active CMV infection (CMV viral syndrome) or in the presence of tissue-invasive CMV disease.[12] Intravenous ganciclovir is a gold standard for the treatment of CMV disease. In mild to moderate cases of the disease, oral valganciclovir was found to be non- inferior to intravenous ganciclovir.

BK virus nephropathy

BK virus nephropathy is the most common manifestation of BKV reactivation after renal transplantation, leading to loss of renal grafts in roughly 43% of patients. BKV viruria and viremia can be seen without renal injury and viral nephropathy, so renal biopsy remains the gold standard for definite BKN diagnosis.[4,12]

Varicella infection

Varicella presents as primary Chicken pox or as secondary reactivation- herpes zoster. VZV infection in KTR is an indicator of degree of immunosuppression.[5] Treatment is with oral Val acyclovir/acyclovir for mild cases.

Hepatitis C

The frequency of HCV antibody positivity in RTR is about 10.3%.

Hepatitis B

Hepatitis B virus infection can occur in transplant cases as a result of primary infection, reactivation, or donor transmission.

Pneumocystis carinii infection

PCP presents with a broad alveolar-arterial PO_2 gradient, elevated serum lactic dehydrogenase (>300 IU/ml), and frequently elevated beta-1,3, glucan levels.[6] Antibody staining reveals both cysts and trophozoites.

Tuberculosis

The incidence of infection with *Mycobacterium tuberculosis* among kidney transplant recipients in north America, Europe, and India/Pakistan is 0.5-1.0%, 0.7-5%, and 5-15%, respectively.[4]

Dengue

Dengue is endemic in tropical and subtropical regions, such as Brazil, the Caribbean, and Southeast Asian countries. Dengue occurs both as an endemic disease and as epidemic outbreaks. Dengue diagnosis is grounded on clinical and laboratory findings, including antibodies, by using a commercial immunoglobulin M (IgM) capture enzyme-linked immunosorbent assay (ELISA).

METHODS

This study was descriptive with both retrospective and prospective study. Retrospective study period was from February 1996 to November 2019. Prospective study period was from December 2019 to December 2020. Sample size of 30 patients was considered.

Study place

Study took place at the department of nephrology, Goa medical college, Goa, India.

Duration of study is for 12 months. Mean±standard deviation was calculated for quantitative data. Numbers

and percentage for qualitative data for categorical valuation.

Ethical committee permission was obtained from institutional ethical committee (Goa medical college).

Inclusion and exclusion criteria

Patients of any age after kidney transplantation following up in nephrology department of Goa medical college was the inclusion criteria and patients with CKD stage 5 who have not undergone kidney transplantation was the exclusion criteria

Data for the study was collected from patient's inpatient and follow up records from medical records division of Goa medical college. Patients were evaluated with detailed history of transplant including preoperative period and postoperative period. Details of all the in-hospital admission, including fever, burning micturition, turbid urine, cough with expectoration, abdominal pain, yellowish discolouration of eyes, skin rashes, loose motion, swelling of legs and the treatment history for the same will be collected from admission papers. Detailed physical examination including general and systemic examination, and relevant laboratory and radiological assessment data are collected.

Full details of the patient have been taken including age, sex, education, occupation, native kidney disease. Detailed clinical history was taken like fever, burning micturition, turbid urine, cough with expectoration, abdominal pain, yellowish discoloration of eyes and any other relevant clinical history if required.

Past history of the patient like diabetes, hypertension, tuberculosis, HIV, ADPKD, CMV serostatus, hepatitis B, hepatitis C, chicken pox or history of graft rejection was taken. Treatment history including induction immunosuppression details, post-transplant immunosuppression details, post-transplant prophylaxis details for fungal, viral and pneumocystis infections were taken.

Patient examination was carried out in detail including blood pressure, JVP, respiratory system, central nervous system, per abdomen and cardiovascular system.

Routine investigations including complete blood count, renal function test, liver function test, urine routine, HBA1C, complete hemogram, chest x-ray, ECG, ABG, amylase, lipids, blood culture, urine culture, abdomen ultrasound including graft ultrasound, urine routine including fungal and AFB smear, ESR, PPD reports were collected. Radiological investigations like chest x-ray, abdominal ultrasound with graft ultrasound, Doppler of the graft kidney, CT scan of the abdomen and thorax data had been taken as and when required.

Kidney biopsy of the graft kidney was done under ultrasound guidance with prior PT/INR if required and the sample sent for histopathology was analysed using light microscopy, electron microscopy and immunofluorescence staining to rule out graft rejection and ATN or recurrence of the disease.

Statistical analysis was conducted using SPSS 10.0 software package. Statistical significance was determined using Student's t-test and Anova test for covariance and $p<0.05$ was considered significant.

CMV infection

IgM and IgG CMV antibodies and CMV PCR has been used in our study.

BK virus nephropathy

Urine examination for decoy cells and BKV DNA PCR and graft kidney biopsy when indicated was employed in our study for diagnosis of BK nephropathy.

Tuberculosis

Sputum microscopy, sputum culture, PPD, ESR, CT thorax and abdomen, urine smear, tissue fluid aspirate smear and culture, CBNAAT of sputum/urine vis used for diagnosis of tuberculosis as indicated clinically in our study.

Hepatitis C

We have used anti-HCV antibodies and HCV RNA levels in our study as clinically indicated. 5 ml of serum of the patient was sent for laboratory for the same.

Hepatitis B

We have used serological tests like HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc IgM and IgG and HBV DNA quantitative load as and when required in our study. 5 ml of serum sample was sent for the test.

Dengue

IgM dengue ELISA and NS1 antigen detection has been used in our study for diagnosis. Serial rise in IgM titres was used for confirmation.

COVID-19 infection

Detection of the virus

Reverse transcription polymerase chain reaction

RT-PCR first uses reverse transcription to obtain DNA, followed by PCR to amplify that DNA, creating enough to be analysed.

Isothermal amplification assays

Isothermal nucleic acid amplification tests also amplify the virus's genome. This test detects DNA using fluorescent tags.

Antigen test

It looks for antigen proteins from the virus surfaces spikes. Specificity of test is 99.5% and average sensitivity is 56.8% (ranging from 0-94%).

Imaging test-CT scan of lung initially include bilateral multinodular ground glass opacities with peripheral or posterior distribution, sub pleural dominance, crazy paving and consolidation may develop as the disease evolves.

RT PCR, antigen, imaging tests were used in our study.

Urinary tract infection

We have used urine routine microscopy and urine culture for diagnosis of urinary tract infection in our study.

Pneumocystis jiroveci

Sputum routine and microscopy, examination of broncho alveolar lavage, chest x-ray and CT thorax was used for diagnosis

Varicella-zoster

Tzanc smear of the vesicle fluid and VZV PCR was used in our study for diagnosis of varicella zoster infection.

Cryptococcus neoformans infection

Cryptococcal antigen latex agglutination test, CSF India ink preparation and CSF culture was used in our study. Around 10 ml of CSF was used for laboratory immediately for analysis.

Epstein Barr virus

EBV PCR was assayed in the first week post-transplant and thereafter at least monthly for the first 6 months and three monthly to the end of the first year in high risk KTRs (D+/R−). EBV viral load was to be monitored after treatment.

RESULTS

This study comprised of 30 live donor renal transplants who were followed for a variable period post-transplant ranging from 1 year to 25 years. Main findings of the study are:

Males comprised 86.66% of the study population. The mean age of the recipients was 40.13±14.26 years. Mean age of donors was 44.05±7.373 years (Table 1). Total of 25 people (83.33%) developed one or other infections among 30 people. Chronic interstitial nephritis (56.6%), diabetic nephropathy (13.7%) and ADPKD (6%) were the three most common causes of ESRD accounting for 90 percent of cases in our study (Table 1, Figure 1). The probability of being infection free at the end of 1 year was 65%.

Table 1: Result.

Variables	Percentage
Recipient sex	
Male	86.6
Female	13.4
Mean recipient age	40.13±14.26 years
Donor sex	
Male	10.8
Female	89.2
Mean donor age	44.05±7.37 years
Cause of end stage renal disease	
Chronic interstitial nephritis	56.60
Diabetic nephropathy	13.70
ADPKD	6
Other	23.70
Acute graft rejection	10.70
Chronic allograft nephropathy	4.00

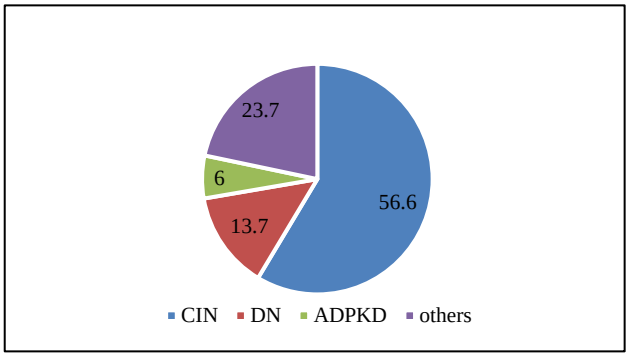

Figure 1: Native kidney disease etiology before transplant.

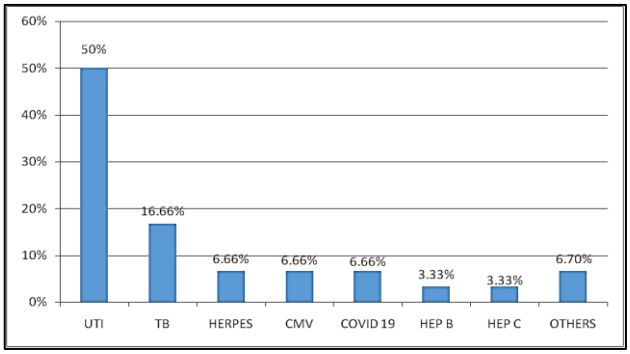

Figure 2: Prevalence of infections.

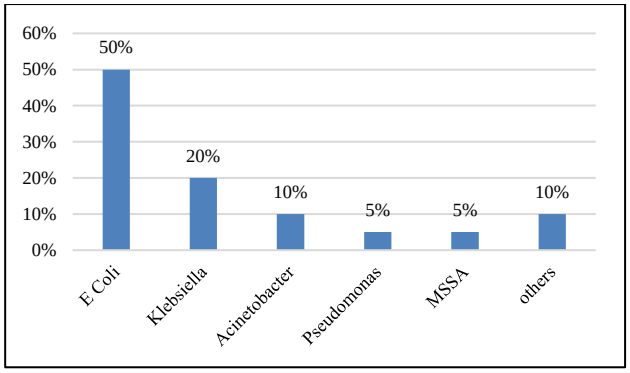

Figure 3: Prevalence of organisms causing UTI in present study.

Bacterial infections were more common than viral and parasitic infections.13 out of 24 patients (54.16%) who developed one or the other infections were given induction with basiliximab and methyl prednisolone where as others received only methyl prednisolone. 6 patients among the 9 patients (66.6%) who developed post-transplant infections within 1 year of the transplant had received induction with basiliximab.

In this study, UTI was the most common post-transplant infection observed in 15 patients (50 %) of 30 patients followed by tuberculosis 5 patients (16.66%), herpes infection 2 patients (6.66%), CMV infection 2 patients (6.66%), COVID 19 infection in 2 patients (6.66%), hepatitis B 1 patient (3.33%), hepatitis C 1 patient (3.33%). A total of 5 patients (16.66%) developed multiple infections. Among the patients who developed infections 36.66% of the patients developed infections in <1 year following transplant and 63.34% of the patients developed infection after more than one year of transplant (Figure 2).

Among the urinary tract infections majority of them were bacterial (90%) followed by fungal (10%). Among the bacterial infections *E. coli* infection was most predominant (50%) followed by klebsiella (20%), acinetobacter (10%), pseudomonas (5%), methicillin sensitive staphylococcus (5%). Among the fungal UTI majority of them were due to non-albicans candida. The prevalence of organisms was similar to the study by Olenski et al who found is *E. coli* accounted for 53% followed by klebsiella which accounted for 29% (Figure 3).[17]

There were total of 13 deaths (43.33%) among 30 patients all of which have happened because of infections. Total of 4 patients among the 13 deaths had chronic graft rejection. Most common cause of death was tuberculosis (38.46%) followed by UTI (23%) and lower respiratory tract infection (23%). This data was correlating with the data published by Chan et al who found the incidence of pulmonary cause for mortality in transplant recipients as 38% followed by septicemia as 32%.[16] All the deaths in kidney transplant recipients were after many years of transplant except one patient who expired after 1 year of transplant (Figure 4).

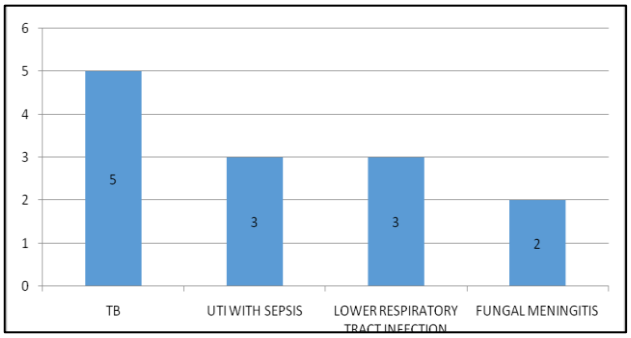

Figure 4: Cause of death.

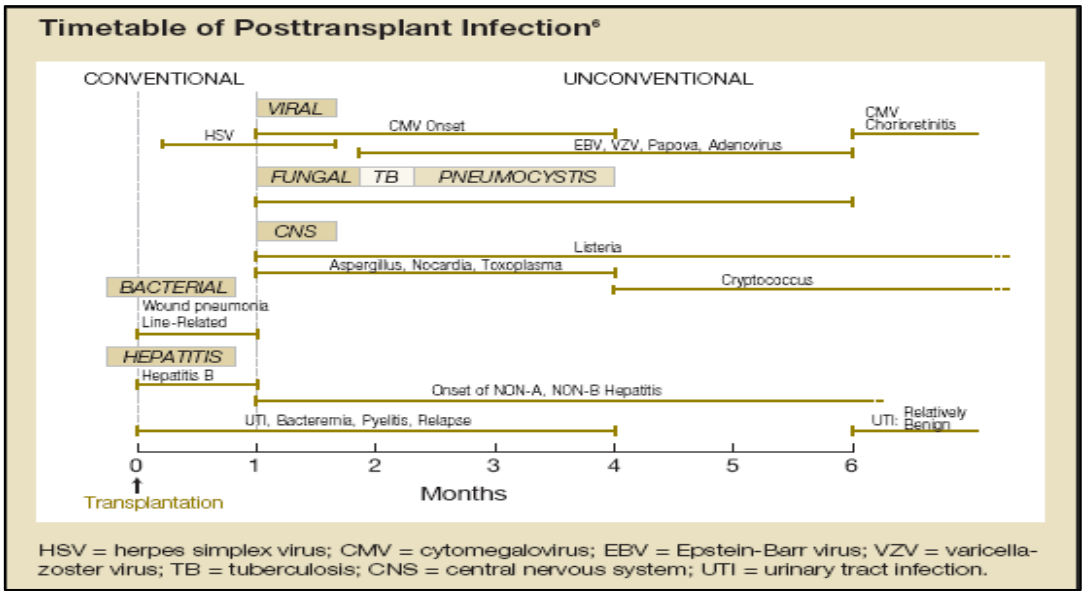

Figure 5: Timetable of post transplant infection.

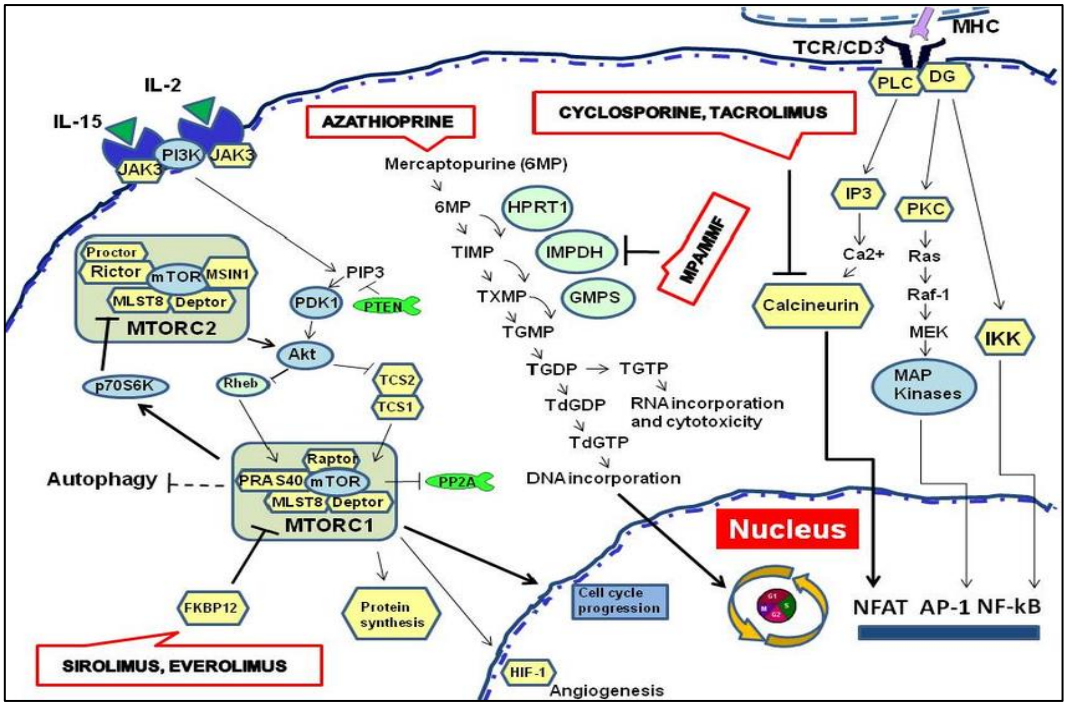

Figure 6: Mechanism of action of various immunosuppressants.[23]

DISCUSSION

Compared to other similar studies conducted in India the present study has attained similar results. The incidence of UTI in patients who are not receiving antimicrobial prophylaxis has been reported to vary from 5-36%.[14] This study showed infection rate of 35% in the first year after transplant which is slightly lower than the study conducted by Cowan et al 54% possibly due to lower sample size in our study and difference in the induction immunosuppression compared to their study.[2] Our Incidence of UTI in present study was 50 percent compared to 37.1% in Umesh et al study and 45.4% in Kumar et al.[1,7] Most common infection in the study conducted by Umesh et al and Kumar et al was also UTI.[1,7] Similar study conducted by Cowan et al in Canada showed most common infection post-transplant was UTI i.e. 49.3% comparable to the present study.[2] UTI is the most common infection in the post-transplant period. UTI occurred predominantly in the females affecting (100%) of the recipients correlating with the previous studies. Among the bacterial infections *E. coli* infection was most predominant (50%) followed by klebsiella (20%), acinetobacter (10%) correlating the study conducted by Kumar et al.[7] UTI was more common in the first month of the transplant before stent removal correlating with the study conducted by Kumar et al and Cowan et al.[2,7]

Reported prevalence of post-transplant tuberculosis varies between 2% and 15% in Asia and other countries.[7] In our study, the occurrence rate of post-transplant tuberculosis was 16.66% correlating with the study conducted by Kumar et al.[7] The prevalence noted in our study is higher when compared to study conducted by Lezaic et al where the prevalence was 3.13%, this is probably due to lower incidence of tuberculosis in Serbia as compared to India.[15] The high prevalence of tuberculosis could be because India is the world capital of tuberculosis with maximum risk of exposure. A similar observation was reported from Indian authors, who demonstrated a 51% of tuberculosis infection in RTR. The use of modern, potent immunosuppressive agents such as tacrolimus or mycophenolate might increase the risk of tuberculosis, when compared to older immunosuppressant drugs such as azathioprine. In our study, all patients of tuberculosis were using mycophenolate.

Table 2: Comparison with other studies.

Study	UTI	TB	Herpes	CMV	COVID-19	Hep B	Hep C	Others
Present study	50%	16.66%	6.66%	6.66%	6.66%	3.33%	3.33%	6.7%
Umesh et al[1]	37.1%	16.1%	12%	8.1%	-	-	6.5%	20%
Kumar et al[7]	45.4%	17.8%	-	13.3%	-	11.1%	6%	22%
Cowan et al[2]	49.3%	-	-	12%	-	-	1.3%	-

Incidence of CMV infection in renal transplant recipients was 6.66% in present study compared to 8.1% in Umesh et al study and 13.3% in Kumar et al study conducted on Indian population. Incidence of CMV in study conducted by Cowan et al in Canada was 12% almost similar to present study.[2] Naraqi et al and Brennan et al estimated that symptomatic CMV infection occurs in 20-60% of all transplant recipients and about 8-32% in renal transplant recipient.[26-28] Mostly the lower prevalence of CMV infections in Indian population is secondary to higher sero prevalence of cmv infection in Indian population i.e. 80-90% compared to 70% in western population (Table 2).

Patients with HBV infection had significant graft dysfunction and mortality in our study. Similarly, Lee et al have reported an inferior 10-year graft survival for HBV or HCV infected RTRs.[13]

Limitations of present study should be noted, First, the majority of infections were identified by positive microbiology data that likely resulted in an underestimation of infection rate. Nevertheless, based on our medical record system, we could identify hospitalization due to lower respiratory tract infection even though microbiological data were not available or were negative.

Second, infections and hospitalizations that occurred outside of our centre were not captured. However, we estimate this number to be small as our transplant patients are rarely, if ever, treated at other institutions.

Third, the study results cannot be generalized because this is a single-centre study. Finally, patients who had undergone transplant in other centres were also included in our study and their complete medical records including perioperative records were not available which could under estimate the prevalence.

CONCLUSION

In conclusion, infection related complications in kidney transplant recipients are an important issue. The incidence of infections is relatively higher in kidney transplant recipients when compared to general populations due to immunosuppression. Compared to older times the incidence of infection is low due to proper titration of immunosuppressants.

The incidence of infections in kidney transplant recipients in decreasing order of frequency are UTI, tuberculosis, CMV, COVID-19, herpes and fungal meningitis. Bacterial infections are more common than fungal and viral infections similar to all the western studies. The probability of being infection free at the end of 1 year was 65% which was comparable to western literature.

Urinary tract infection is the most commonly encountered infection however has relatively low mortality. Among the urinary tract infections majority of them were bacterial (90%) followed by fungal (10%). *E. coli* is the most common culprit. These findings are similar to studies done in western countries.

Tuberculosis is the 2nd most common infection in present study and is associated with high mortality and morbidity. The incidence of tuberculosis is relatively high in the present study due to higher prevalence of tuberculosis in Asian countries.

Although CMV being the most common infection in kidney transplant recipients in first year of transplant, in our study the incidence as well as mortality is relatively lower compared to western studies due higher seropositivity rate of recipients and higher IgG titer for CMV and chemoprophylaxis with valganciclovir according to KDIGO guidelines.

Although the mortality related to infections has reduced over time, approximately 43% of kidney transplant recipients died from infection after a transplant and the majority of these patients died with a functioning graft, indicating majority of infections increased mortality in kidney transplant recipients without affecting functioning of the graft. All the deaths in kidney transplant recipients were after many years of transplant except one patient who expired after 1 year of transplant.

However, in view of the studies that show that post-transplant infections have deleterious effect on graft function, it is necessary to design standard protocols for surveillance, prevention and management of infections in renal transplant recipients for a particular institution in lines with standard protocols.

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