3. MATERIALS AND METHODS

3.1 Retrospective Study

Study Site

A retrospective study was conducted at Muljibhai Patel Urological Hospital, Nadiad, Gujarat, India. Though not involved in direct care of the patients, I assessed the patients by review of clinical records maintained by Muljibhai Patel Urological Hospital. For conduction of this study, permission was taken by the hospital authorities as per letter appended as an appendix 9.1.

Study Duration and Patient Selection

Patients who underwent renal transplant between July 2004 and June 2011 at Muljibhai Patel Urological Hospital were enrolled in the study. All enrolled patients were followed up till their last followed up visit on or before June 2012. Patients were selected on the bases of bellow mention inclusion and exclusion criteria.

Inclusion Criteria


Exclusion Criteria

1. Non-Indian renal transplant recipients.
2. Patients whose data were not available.

Patients Visit and Follow-up

As per study center protocol, renal transplant patients were discharged from renal transplant unit usually on a 10th postoperative day in the uneventful postoperative course and then followed up every alternate day in the first month and for twice weekly for next one month and every monthly till six-month post transplant. After six months, they were followed every three-month interval. Data was collected on 0 (date of transplant), 1, 3, 6, 12, 18, 24, 30, 36, 42, 48, 60, 72 and 84 months after transplant. Each patient was followed up for duration mentioned above from the day of transplant till June 2012.
Data Collection

Following data were collected by reviewing the patient's file. Performa for the retrospective study is appended as Appendix 9.5.

Basic Information

Basic details of recipients and donor were collected which includes recipient and donor identification number and demographic data at the time of transplant, recipient-donor HLA mismatch number, recipient’s pre-transplant HCV and HBV infection status, recipient’s native kidney disease and type of donor (Cadaver or Living).

Drug Information

As per study center protocol, all transplant recipients started calcineurin inhibitor (CNIs) (TAC or CyA) and antiproliferative agents (Mycophenolate or Azathioprine), two days before scheduled transplant day except patients who underwent cadaver kidney transplant. All recipients administered a higher dose of steroids as an induction immunosuppressant on the day of transplant. Apart from steroids, few recipients were also administered antibody as an inducing agent on the day of transplant. Patients were also treated with other concomitant condition based on their status.

Patients started three drug regimens from the day of transplant which includes steroids, CyA/TAC and AZA/MMF as a maintenance drug regimen. The decision for selection of drug regimen was taken by treating nephrologists. Drug class, formulation or brand administered was maintained same throughout follow-up period except for changes required on medical or financial grounds.

Kidney transplant patients have also started antibiotics from first post-transplant day to prevent or to treat the infection. Transplant recipients were also treated with other drugs based on their physical and biological condition.

Data was collected which included induction therapy and maintenance drug regimen or changes of drug at various interval following transplant [0 (day of transplant), 1, 3, 6, 12, 18, 24, 30, 36, 42, 48, 60, 72 and 84 month post transplant] on or before June 2012. Following parameters were analyzed at interval mentioned above.
Patient Survival and Graft Survival

Graft loss was defined as graft nephrectomy, re-transplantation, or a return to dialysis for at least six consecutive weeks. Graft and patient survival were recorded and analyzed at 0, 1, 3, 6, 12, 18, 24, 30, 36, 42, 48, 60, 72 and 84 month for entire follow-up period.

Rejection and Calcineurin Toxicity

Recipient’s creatinine level was measured at the time of transplant, during hospitalization stay and each visit to the hospital and various intervals as mentioned above.

Recipient underwent kidney graft biopsy on suspicion for rejection based on more than 20% rise in creatinine or clinical grounds. Patients with biopsy-confirmed rejection were treated with steroid with or without antibody. Some patients were given anti-rejection therapy on suspicion of rejection of clinicopathological grounds. This was defined as presumptive rejection. All data of rejection episode was analyzed during study period follow-up.

Data of those patients who developed calcineurin inhibitor toxicity confirmed on clinicopathologically was also collected for each patient during the follow-up period.

New Onset of Diabetes after Transplant (NODAT)

NODAT was defined requirement of the antidiabetic drug for more than one month. Patients who had diabetes at the time of transplant were excluded from the statistical analysis.

Infection

Enrolled transplant patients were closely monitored for development of infection based on clinical signs, symptoms, and appropriate laboratory tests were performed during the hospital stay and at each visit to the study center. Infections were broadly categorized to viral (HCV, HBV, CMV, Herpes), Fungus, TB, Malaria, UTI, URTI and other infections.

Others

Patient’s blood pressure, hemoglobin level, leucocytes count, urine proteins were noted down at interval mentioned above.
Parameters for Analysis

Parameters measured in this study were mention as below.

- Effect of patient’s age on the various parameters.
- Usage of induction therapy and its effect on rejection episode, patient and graft survival.
- Trends of immunosuppressant drug prescription pattern.
- Effect of immunosuppressant drugs on rejection episode, patient and graft survival, and requirement of anti-rejection therapy.
- Effect of CNI agent on the development of CNI toxicity.
- Effects of immunosuppressant drugs on one year mean creatinine level.
- Effect of immunosuppressant drugs on hematological side effects.
- Effect of immunosuppressant drugs on blood pressure.
- The prevalence rate of NODAT and effect of immunosuppressant drugs and various parameters on the prevalence rate of NODAT.
- Effect of NODAT on rejection episode, patient and graft survival.
- Prevalence of various infections and effect of immunosuppressant drugs and various parameters on the prevalence rate of infection.
- Effect of infection on rejection episode, patient and graft survival.

Statistical Analysis

Demographic and Baseline Characteristics

Demographic and baseline characteristics of recipients and donors were summarized descriptively by mean and standard deviation (SD) for continuous variables and frequency distribution for discrete variables.

Statistical Method

The results expressed as mean ± SD were determined by t-test and expressed as contingency table was determined by chi-square test by using SPSS (Statistical package for social sciences) version 15.0, statistical computer software. Only those values are showing statistical difference P ≤ 0.05, were considered as statistically significant.
3.2 Prospective Study

Study Center and Study Design

This study was an observational prospective study and conducted on patients who underwent kidney transplantation between January 2013 and August 2013 at Muljibhai Patel Urological Hospital, Nadiad, Gujarat, India. In this study, I was not directly associated with the patient’s drug therapy and testing, but I observed the treatment given and its impact on infection episode. This study was planned to identify actual infection rate in transplant patients, to identify infectious agent responsible for infection, to find out the effect of the various parameter on infection and to effect of infection on rejection episode, graft survival and patient survival. All patients were followed up till one year after transplant or till graft loss or death before one year of transplantation.

Inclusion and Exclusion Criteria

Inclusion Criteria

➢ The subjects who willing to participate in the study and provide signed written informed consent

Exclusion Criteria

➢ Patients with kidney retransplantation.
➢ Recipients of other solid organ transplants.
➢ Patients who lost to follow up
➢ Pregnant and nursing women.
➢ Known carriers of hepatitis B, Hepatitis C or human immunodeficiency viruses at the time of transplant.

Ethics

The study was conducted in accordance with the ethical principles that have their origin in the current version of Declaration of Helsinki and consistent with International Conference on Harmonization, Good Clinical Practice (ICH-GCP). Prior approval was taken from the ethics committee of Muljibhai Patel Urological Hospital, Nadiad, Gujarat. Ethics committee approval letter is appended as Appendix 9.2.
Inform Consent Process

Transplant patients were given a copy of inform consent document approved by the ethics committee. Study related information (Aim of the study, methodology, benefits to the subject, side effect and right to withdraw from the study) in detail were explaining to each transplant patients before signing of inform consent document. All patients were given sufficient time before signing in the informed consent form and signing of inform consent document shows their willingness to participate in the study. Specimen copy of inform consent document in English and vernacular language (Gujarati) are appended as Appendix 9.3 and 9.4.

Patients Follow-up Visits

Patients were discharged from renal transplant unit usually on a 10th postoperative day in uneventful postoperative course and followed up in outpatient department (OPD) every alternate day except Sundays for first one month and for twice weekly for next one month. During this period patient stayed near hospital premises. Patient with stable graft function was allowed to return to their native place at the end of 2 months and asked to follow up in outpatient department every monthly at scheduled date till six-month post transplant. After six months they were followed every third month. Hence, we followed up patients at the time of transplant and 1, 3, 6 and 12-month post-transplant.

Drug Therapy

All enrolled transplant patients were treated with immunosuppressant drug based on their protocol which was prepared by nephrologists of the study center. All recipients were administered a higher dose of steroids as an induction immunosuppressant on the day of transplant. Among this, few recipients were also administered antibody as an inducing agent on the day of transplant.

All transplant patients were started one of the Calcineurin inhibitor (CyA or TAC) and one of the antiproliferative agents (Mycophenolate or Azathioprine), two days before scheduled day of transplant except patients who underwent cadaver kidney transplant. Then after patients were treated with steroids, CyA/TAC, and AZA/MMF drug regimen as a maintenance regimen and same drug was continued throughout the study period. Patients
who unable to take drug orally immediately after transplant were administered the drug orally through Ryle's tube. Patients were also treated with antibody to prevent and to cure rejection episode. Patients were given intravenous third-generation cephalosporin during first ten days followed by antibiotic prophylaxis with sulfamethoxazole and trimethoprim combination for six-month post transplant. Appropriate antimicrobial agents were given to treat infection episode as per the decision of treating physician.

**Data Collection**

Data was collected about patient’s demographical profile including recipient’s age, sex, type of donor and donor sex at the time of transplant. Performa for the prospective study is appended as Appendix 9.6.

During indoor and follow up visit, infection was actively looked for with all available clinical, laboratory and imaging tools and were treated promptly by a physician. Infection information was also recorded at the time of transplant and at 1, 3, 6, 12-month post transplant. If any infection was reported between these intervals, then it was included at nearest next visit. Data about the type of infection and infectious agent was also collected.

Data were also collected about various parameters to know the impact of infection at different intervals as mentioned above. These parameters included graft and patient survival, serum creatinine, maintenance drug regimen, induction therapy, rejection episode and anti-rejection treatment. Graft function following renal transplant was monitored by serum creatinine level and was noted down at the above-mentioned interval.

**Parameters for Analysis**

- Prevalence rate and timing of various infections.
- Infectious agent responsible for infection.
- Effect of immunosuppressant drug on infection.
- The prevalence rate of infection in various groups.
- Influence of infection on graft function, graft survival, and patient survival.
- Prevalence of UTI, CMV and URTI in various groups.
Statistical Analysis

Demographic and Baseline Characteristics

Demographic and baseline characteristics of recipients and donors will be summarized descriptively by mean and standard deviation (SDs) for continuous variables, and frequency distribution for discrete variables.

Statistical Methods

The results expressed as mean ± SD were determined by t-test and expressed as contingency table was determined by chi-square test by using SPSS (Statistical package for social sciences) version 15.0, statistical computer software. Only those values showing statistical difference P ≤ 0.05 were considered as statistically significant.