

# Significance of Assay of Tacrolimus and Estimation of Serum Creatinine in Renal Transplantation

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## Abstract

Kidney transplantation is the treatment of choice for most patients with end-stage renal disease (ESRD). Strategies to increase donor organ availability and to prolong the transplanted kidney's survival have become primary in kidney transplantation. Standard immunosuppressive therapy consists of initial treatment and maintenance regimens to prevent rejection and short courses of more intensive immunosuppressive therapy to treat episodes of acute rejection. The calcineurin inhibitors, cyclosporine and Tacrolimus, provide mainstay immunosuppression after renal transplantation. Studies have shown that Tacrolimus is crucially more effective than both standard and microemulsion formulations of cyclosporine in preventing acute rejection of renal allografts, but as yet, the relative impact of both drugs on long-term renal function remains undetermined. Moreover there is a lot of inter-individual variation in tacrolimus uptake and efficacy as indicated by many studies.

## INTRODUCTION

Kidney transplantation or renal transplantation is the organ transplant of a kidney into a patient with end-stage renal disease. Kidney transplantation is typically classified as deceased-donor (formerly known as cadaveric) or living-donor transplantation depending on the source of the donor organ. Living-donor renal transplants are further characterized as genetically related (living-related) or non-related (living-unrelated) transplants, depending on whether a biological relationship exists between the donor and recipient. Renal transplantation is the treatment of choice for most of these patients, but the number of kidneys available for transplantation is limited<sup>(1)</sup>. Since 1988, there has been a growing discrepancy between the number of transplantations performed and the number of patients awaiting transplantation, underscoring the need to maximize graft survival<sup>(1)</sup>. The term "renal failure" means failures of renal excretory function owing to depression of the glomerular filtration rate<sup>2</sup>. It can occur abruptly, as in acute renal failure (ARF), or over a long period, as in chronic renal failure (CRF)<sup>(3)</sup>. Renal failure causes alterations in electrolytes, acid-base, and water balance, and accumulation of substances normally excreted by the body. Such alteration can result in uremia, a toxic condition that affects all body systems<sup>3</sup>. Acute renal failure means abrupt deterioration in renal function which is usually, but not invariably, reversible over a period of days or weeks<sup>2</sup>. ARF is characterized by azotemia, the accumulation of nitrogenous waste products in the blood, oliguria (urine volume < 400 ml / 24h) or anuria (no urine production) occurs in about half of patients. *Chronic Renal Failure (CRF)* is defined as progressive and irreversible loss of renal function<sup>(3)</sup>. It is a major public health problem, with increasing incidence and prevalence, poor outcomes, and high costs<sup>(4,5)</sup>. CRF frequently leads to end stage renal disease (ESRD), which without renal replacement therapy would lead to death<sup>(3,6,7)</sup>.

CRF may be caused by any condition which destroys the normal structure and function of the kidney<sup>(8)</sup>. Wide geographical variations in the incidence of disorders causing CRF exist. For example, the most common cause of glomerulonephritis in sub-Saharan Africa is malaria. In

part of the Middle East, including southern Iraq, Schistosomiasis is a common cause of renal failure due to urinary tract obstruction<sup>(2)</sup>. The short-term outcome of renal transplantation has improved substantially in the past 15 years. The introduction of cyclosporine for the prevention of acute and chronic rejection in the early 1980s and the introduction of muromonab-CD3 (OKT3 monoclonal antibody) for the treatment of acute rejection in the early 1980s have increased the rate of graft survival at one year<sup>(9)</sup>. In recent years, use of newer immunosuppressive drugs such as mycophenolatemofetil and tacrolimus has been associated with further reduction in the incidence of acute rejection episodes<sup>10</sup>. However, there has not been a noticeable improvement in long-term graft survival<sup>(11)</sup>. Long-term graft failure is usually due to death with a functioning graft, chronic rejection, or recurrent kidney disease<sup>(12)</sup>. Cyclosporine is nephrotoxic and has been thought to aggravate graft failure. Among these causes of long-term graft failure, the most important and the most potentially remediable is chronic rejection<sup>13</sup>. Renal transplantation has become the treatment of choice for most patients with end-stage renal disease (ESRD). Before the advent of immunosuppression, renal transplantation was limited to human leukocyte antigen (HLA)-identical (HLA-ID) siblings and was not applicable to the vast majority of patients with ESRD. The introduction of combined azathioprine-steroid therapy in 1963 produced encouraging results and became the mainstay of immunosuppression. Although this therapy improved the results of transplantation, acute rejection and complications associated with steroid therapy persisted.

Chronic kidney disease is characterized by an irreversible retrogradation of renal function that progressively progresses to end-stage renal disease (ESRD). Chronic kidney disease has appeared as a serious public health problem. Data from the United States Renal Data System (USRDS) show that happening of kidney failure is rising among adults and is commonly associated with poor outcomes and high cost. Moreover, in the past 2 decades, the incidence of the chronic kidney disease in children has steadily increased. The indication for kidney transplantation is end-stage renal disease (ESRD), regardless of the

primary cause. This is defined as a glomerular filtration rate <15ml/min/1.73 sq.m. Common diseases leading to ESRD include malignant hypertension, infections, diabetes mellitus, and focal segmental glomerulosclerosis; genetic causes include polycystic kidney disease, a number of inborn errors of metabolism, and autoimmune conditions such as lupus. Diabetes is the most common cause of kidney transplantation, accounting for approximately 25% of those in the US. Organ transplantation elicits a complex series of immunologic processes. These processes are generally categorized as inflammation, immunity, and tissue repair and structural reinforcement of damaged tissues. Immunosuppression can be achieved by depleting lymphocytes, conversion lymphocyte traffic, or blocking lymphocyte response pathways. Immunosuppressive drugs have three effects: the therapeutic effect (suppressing rejection), undesired immunosuppressive consequences of immunodeficiency (infection or cancer), and no immune toxicity to other tissues.

### Classification of Immunosuppressive Drugs

Immunosuppressive drugs can be classified into five groups:

1. Glucocorticoids
2. Cytostatics
3. Antibodies
4. Drugs acting on immunophilins
5. Other drugs.

### Tacrolimus

Tacrolimus is used as a major immunosuppressive agent to prevent acute rejection in renal transplantation. Renal transplant recipients are treated with a combination of Tacrolimus, glucocorticoid, and mycophenolatemofetil (MMF), with or without basiliximab. Therapeutic drug monitoring (TDM) of Tacrolimus is generally performed to examine the dose of the drug that should be used to optimize the immunosuppressive efficacy. However, estimation of the efficacy of immunosuppressive agents should include pharmacokinetics and pharmacodynamics based on the sensitivity of patient-derived cells to the immunosuppressive agents. The lymphocyte immunosuppressant sensitivity test (LIST) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay procedure can evaluate the pharmacological efficacy of immunosuppressive drugs using peripheral blood mononuclear cells (PBMCs) of transplant recipients<sup>(14)</sup>. Tacrolimus was discovered in 1984; it was among the first macrolide immunosuppressant discovered, preceded by the discovery of rapamycin (sirolimus) on Rapa Nui (Easter Island) in 19758). It is produced by a type of soil bacterium, *Streptomyces tsukubaensis*. The name Tacrolimus is derived from 'Tsukubamacrolide immunosuppressant'. Tacrolimus was first approved by the Food and Drug Administration (FDA) in 1994 for use in liver transplantation; this has been extended to include kidney, heart, small bowel, pancreas, lung, trachea, skin, cornea, bone marrow, and limb transplants<sup>15</sup>.

### Glucocorticoids

(GC) are a class of steroid hormones that bind to the glucocorticoid receptor (GR), which is present in almost every vertebrate animal cell. The name glucocorticoid (pertaining to **glucose** + **cortex**) derives from its role in the regulation of the metabolism of glucose, its synthesis in the adrenal cortex

GCs cause their effects by binding to the glucocorticoid receptor (GR). The activated GR complex, in turn, up-regulates the expression of anti-inflammatory proteins in the nucleus (a process known as transactivation) and represses the expression of proinflammatory proteins in the cytosol by preventing the translocation of other transcription factors from the cytosol into the nucleus<sup>(16)</sup>.

### Cytostatics

Cytostatic drugs have the ability to prevent the growth and proliferation of cells. They often are used to prevent growth and development of malignant cells and neoplasms and in this case named antineoplastic drugs.

### Antibodies

Antibodies are sometimes used as a quick and potent immunosuppressive therapy to prevent the acute rejection reactions as well as a targeted treatment of lymphoproliferative or autoimmune disorders (e.g., anti-CD20 monoclonals).

### Polyclonal Antibodies

Heterologous polyclonal antibodies are obtained from the serum of animals (e.g., rabbit, horse), and injected with the patient's thymocytes or lymphocytes. The antilymphocyte (ALG) and antithymocyte antigens (ATG) are being used. They are part of the steroid-resistant acute rejection reaction and grave aplastic anemia treatment

### Monoclonal antibodies

Monoclonal antibodies are directed towards exactly defined antigens. Therefore, they cause fewer side-effects. Especially significant are the IL-2 receptor- (CD25-) and CD3-directed antibodies. They are used to prevent the rejection of transplanted organs, but also to track changes in the lymphocyte subpopulations

### T-cell receptor directed antibodies

Muromonab-CD3 is a murine anti-CD3 monoclonal antibody of the IgG2a type that prevents T-cell activation and proliferation by binding the T-cell receptor complex present on all differentiated T cells. As such it is one of the most potent immunosuppressive substances and is administered to control the steroid- and/or polyclonal antibodies-resistant acute rejection episodes. As it acts more specifically than polyclonal antibodies it is also used prophylactically in transplantations

### IL-2 receptor directed antibodies

Interleukin-2 is an important immune system regulator necessary for the clone expansion and survival of activated lymphocytes T. Its effects are mediated by the trimer cell surface receptor IL-2a, consisting of the  $\alpha$ ,  $\beta$ , and  $\gamma$  chains. The IL-2a (CD25, T-cell activation antigen, TAC) is expressed only by the already-activated T lymphocytes. Therefore, it is of special significance to the selective immunosuppressive treatment, and research has been

focused on the development of effective and safe anti-IL-2 antibodies.

## Immunophilins

### Cyclosporine

Like tacrolimus, cyclosporine is a calcineurin inhibitor (CNI). It has been in use since 1983 and is one of the most widely used immunosuppressive drugs. It is a cyclic fungal peptide, composed of 11 amino acids. Cyclosporine is thought to bind to the cytosolic protein cyclophilin (an immunophilin) of immunocompetent lymphocytes, especially T-lymphocytes. This complex of cyclosporine and cyclophilin inhibits the phosphatase calcineurin, which under normal circumstances induces the transcription of interleukin-2. The drug also inhibits lymphokine production and interleukin release, leading to a reduced function of effector T-cells. Cyclosporine is used in the treatment of acute rejection reactions, but has been increasingly substituted with newer and less nephrotoxic, immunosuppressant<sup>(17)</sup>.

### Aims and Objectives

The central issue in organ transplantation is allograft rejection. Thus, development of immunosuppressive drugs is the key to successful allograft function. Immunosuppressive agents are used for induction (intense immunosuppression in the initial days after transplantation), maintenance, and reversal of established rejection.

Maintenance of immunosuppressive therapy is administered to almost all renal transplant recipients to help prevent acute rejection and the loss of the renal allograft. Although an adequate level of immunosuppression is required to dampen the immune response to the allograft, the level of chronic immunosuppression is slowly decreased over time.

The optimal maintenance immunosuppressive therapy in renal transplantation is not established. The major immunosuppressive agents that are currently being used in various combination regimens are corticosteroids (primarily oral prednisone), azathioprine, mycophenolate mofetil (MMF), mycophenolate sodium (myFortic), cyclosporine (in standard form or microemulsion), tacrolimus, everolimus, and rapamycin (sirolimus).

Thus the objectives of present studies were:

1. Collection of the blood samples from the patient suffering with renal disease.
2. To estimate the optimum Tacrolimus dosage on the patients undergone renal transplantation.
3. To estimate the serum creatine level of these patients.
4. Comparative study of the significance of both Tacrolimus assay and the estimation of the serum creatine in the patients undergone the renal transplantation

5. To study the improvement of the long-term outcomes of renal transplantation by combining Tacrolimus with two novel immunosuppressive agents, mycophenolate mofetil (MMF) and sirolimus.

## MATERIALS AND METHODS

In the course of our work in the GCRL we have performed ELISA to assay the amount of tacrolimus in blood of six renal transplant patients along with the estimation of serum creatinine and creatinine clearance test. The blood samples from the six patients who undergone the renal transplantation were collected in the Heparinized vial from the Global Hospital, Hyderabad. These samples were then immediately brought and analysed.

### Collection of the sample

The blood samples from the six patients undergone the renal transplantation were collected in the Heparinized vial from the Global Hospital, Hyderabad. These samples were then immediately brought to GCRL and maintained at 40C for future research work.

### Tacrolimus ELISA Assay in Whole Blood

The samples were then analyzed for Tacrolimus assay with the help of DiaSorin PRO-Trac™II Tacrolimus ELISA kit.

### Serum Creatinine Level

The blood creatinine level shows how well the kidneys are working. A high creatinine level may mean the kidneys are not working properly. The amount of creatinine in the blood depends partly on the amount of muscle tissue you have; men generally have higher creatinine levels than women.

### Creatinine Clearance Test

#### Principle

The creatinine clearance is a method of assessing renal function. This clearance test provides an estimate of the amount of plasma that must have flowed through the kidney glomeruli per minute with complete removal of its content of creatinine to account for the creatinine per minute actually appearing in the urine. The creatinine clearance is practically the same as the glomerular filtration rate. The test requires the complete collection of the urine formed in an accurately measured period of time in addition to the analyses of the creatinine concentrations in the urine and in the serum. The excretion of creatinine is dependent on muscle mass and the calculation for the clearance is corrected using a formula for body surface area.

## RESULTS AND DISCUSSION

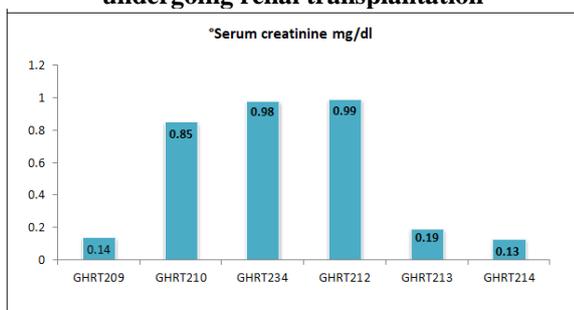
Our work consisted of the assay of the immunosuppressant drug Tacrolimus and estimation of serum creatinine along with creatinine clearance test in the six patients who had undergone renal transplants at Global Hospitals. The results are indicated in the table below.

**Table 1 Results of the tests carried out in six renal transplant patients.**

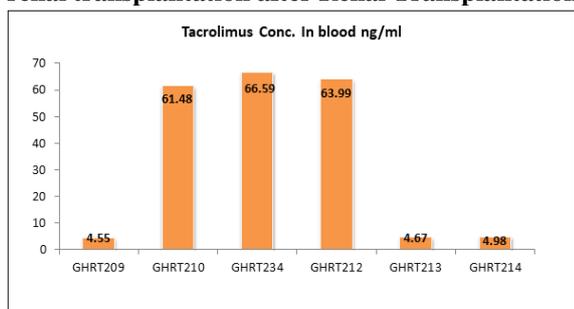
Sr. No.	Sample No	Age /sex Patient	Condition	°Serum creatinine mg/dl	°Tacrolimus conc in blood ng/ml	Creatinine clearance ml/min
1	GHRT209	48/f	Renal Transplant done	0.14	4.55	80
2	GHRT210	43m	Renal Transplant done	0.85	61.48	67
3	GHRT234	66/m	Renal Transplant done	0.98	66.59	65
4	GHRT212	69/f	Renal Transplant done	0.99	63.99	68
5	GHRT213	53/f	Renal Transplant done	0.19	4.67	72
6	GHRT214	48/m	Renal Transplant done	0.13	4.98	80

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The values presented are the average of three values taken on 15th, 30th and 45th day after transplant.

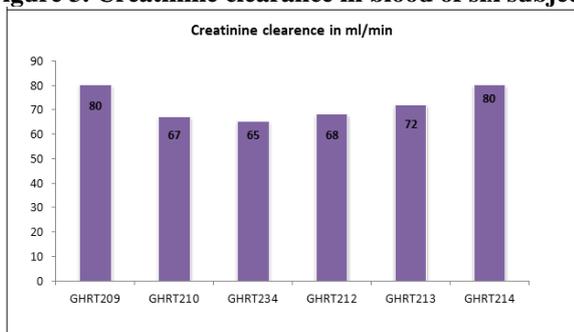
**Figure 1 Serum creatinine mg/dl in blood of patients undergoing renal transplantation**



**Figure 2 Tacrolimus conc. ng/ml in blood undergone renal transplantation after Renal Transplantation**



**Figure 3. Creatinine clearance in blood of six subjects**



The results indicate that if the Tacrolimus level in blood of patient is 2-6 ng/ml then it is being actively metabolized by liver so that it can show immunosuppressant activity. If the Tacrolimus level is more than 50 ng/ml it is not being metabolized by liver consequently no immune suppression and this may lead to rejection of the kidney. This is further exemplified by serum creatinine levels. Patients in whom the Tacrolimus levels are higher than 50 ng/ml then it is not metabolized and used for immunosuppression and these patients showed high levels of serum creatinine indicating episodes of possible rejection of the transplant and depletion of renal functions. It seems that because of this reason two patients (GHRT 234,212) who showed high conc. of Tac in blood along with high levels of serum creatinine showed severe rejection symptoms of acute nephrotoxicity one month after the transplantation.

**CONCLUSION**

The use of Tacrolimus has increased steadily, and the drug is now the predominant calcineurin inhibitor, but most transplantation programs utilize the vigor of both Tacrolimus and cyclosporine, depending on the risks in individual patients. Studies have shown that the extent of acute rejection is reduced by Tacrolimus use over cyclosporine. Although short-term immunosuppression in connection with patient and graft survival is found to be similar between the two drugs, Tacrolimus results in a more suitable lipid profile and this may have important long-term implications given the asserted influence of rejection on inaly survival. The results of Tacrolimus assay showed that, if the Tacrolimus level is more than 50 ng/ml it is not being metabolized by liver consequently no immune suppression and this may lead to rejection of the kidney. Hence the study concluded that the estimation of the Tacrolimus level and the serum creatinine in patients with End Stage renal Disease (ESRD) was useful for prevention of the patients from the dangerous hyper allergic responses of immune system

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